DETERIORATION OF MALE REPRODUCTIVE FUNCTIONS IN HYPERPROLACTINAEMIA

Achteruitgang van mannelijke voortplantingsfuncties bij hyperprolactinemie

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. Dr. C.J. Rijnvos
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 14 november 1990 om 15.45 uur.

door

ADRIAAN KOOY

geboren te Voorburg

universiteits
Erasmus
DRUKKERIJ
1990

PROMOTIECOMMISSIE

Promotor:	Prof. Dr. J.J. van der Werff ten Bosch
Co-promotor:	Dr. J.T.M. Vreeburg
Overige leden:	Prof. Dr. J.C. Birkenhäger Prof. Dr. S.W.J. Lamberts Prof. Dr. G.H. Zeilmaker

Dit proefschrift werd bewerkt binnen de afdeling Endocrinologie, Groei en Voortplanting van de Faculteit der Geneeskunde, Erasmus Universiteit Rotterdam.

Deze promotie werd mede ondersteund door Organon Nederland B.V. en Gist-brocades Farma B.V.

Omslag: C. de Vries en A. Kooy; interferentiepatronen van prolactine met mannelijke voortplantingsfuncties.

Observation shows, experiment teaches

To my parents
To Patricia and
the children

CONTENTS pp.

Chapter V

Effects of the new PRL-producing tumour 7315b on gonadotrophin secretion in adult male and female rats

A. Kooy, R.F.A. Weber, M.P. Ooms and J.T.M. Vreeburg
Journal of Endocrinology 120, 261-268 (1989) 79

LIST OF ABBREVATIONS

ACTH	adrenocorticotrophic hormone
BW	body weight
CA	corticosterone acetate
CL	contact latency
CNS	central nervous system
CRF	corticotrophic hormone-releasing factor
CSF	cerebrospinal fluid
DA	dopamine
DES	diethylstilbestrol
EL	ejaculation latency
GABA	gamma-amino-butyric acid
GAP	gonadotrophin-releasing hormone associated peptide
hyperPRLaemia	hyperprolactinaemia
FSH	follicle-stimulating hormone
IF	intromission frequency
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
MF	mount frequency
MIR	mount plus intromission rate
POMC	pro-opiomelanocortin
PEI	post ejaculatory interval
PRL	prolactin
RIA	radioimmunoassay
SEM	standard error of the mean
TIDA neurons	tuberoinfundibular dopaminergic neurons
TRH	thyreotrophin-releasing hormone
VIP	vasoactive intestinal peptide

CHAPTER I

THE PHYSIOLOGICAL SIGNIFICANCE OF PROLACTIN

1.1. The natural occurrence of prolactin in vertebrates

Prolactin (PRL) is a single-chained protein that after its release from the pituitary gland enters the blood stream by which it is carried to the target organs to rouse to diverse biological activities in different animal species (for review: Nicoll, 1974). Already in 1933 the term "prolactin" had been given to the hormone inducing milk secretion by the crop glands of the pigeon (Riddle et al., 1933). In this bird, glandular structures within a bilateral outpouching of the oesophagus become stimulated during brooding to produce a thick secretion, the crop milk, for feeding the young. For many years the bio-assay for PRL was based on this phenomenon (Nicoll et al., 1985).

Evidence exists that in mammals PRL-like molecules are secreted under physiological conditions not only by the pituitary gland but also by other tissues, like endometrium (Maslar et al., 1980), decidua (Bigazzi et al., 1979), myometrium (Riddick, 1985), connective tissue (Riddick, 1985), jejunal mucosa (McFarland 1979) and neurons in hypothalamic nuclei (Emanuele et al., 1986; Fuxe et al., 1977; Toubeau et al., 1979). Accordingly, PRL is ubiquitously present in humans and many other vertebrates. The hormone has been demonstrated by radio-immunologic determination in peripheral blood, umbilical cord blood, portal vessel blood, cerebrospinal fluid, milk, amniotic fluid, ovarian follicular fluid, uterine cervical mucus and semen in man and animals (Table 1.1.). These data suggest a physiological role of this hormone related to reproductive functions in vertebrates. Indeed, functions of PRL in favour of the young offspring, like the induction and maintenance of lactation and maternal behaviour, are established (Bridges et al., 1985; Hutchison, 1978;

Nicoll, 1974). In many other aspects, however, the relevance of PRL in reproduction is still controversial, and remains to be elucidated. The diversity of its actions among different animal species hinders a unifying concept about the physiological role of PRL in reproductive endocrinology.

Table 1.1. The occurrence of PRL in body fluids

Body fluid	Author
peripheral blood	Chirito et al., 1972
umbilical cord blood	Gluckman et al., 1978
portal vessel blood	Olivier et al., 1977
cerebrospinal fluid	Assies et al., 1978
	Clemens and Sawyer, 1974
	Kendall and Orwoll, 1980
	Logan and MacLeod, 1977
milk	Grosvenor and Whitworth, 1976
amniotic fluid	Riddick, 1985
ovarian follicular fluid	McNatty, 1979
uterine cervical mucus	Sheth et al., 1976
semen	Sheth et al., 1975
urine	Gala et al., 1975
	Sinha et al., 1973

1.2. The structure of PRL: its origin and polymorphism

In order to understand the biological actions of PRL it is important to elucidate its molecular structure. In the late twenties Stricker and Grueter (1928) discovered lactogenic hormone activity in bovine pituitary extracts. In the years thereafter the lactogenic hormone, called PRL, has been demonstrated in pituitary extracts of almost all vertebrates studied. The bioassay-supported identification of human PRL was brought about much later, in 1970, partly due to the fact that human growth

hormone (hGH), in contrast to GH of other species, has pronounced PRL-like activity. Moreover, the concentration of hGH in pituitary extracts is a hundred times as high (10 mg/gland) as that of hPRL (0.1 mg/gland, Friesen and Hwang, 1973). The entire linear amino acid sequence of hPRL has been reported by Shome and Parlow (1977). The single-chained molecule consists of 198 amino acid residues including three disulfide bridges. Further research on the PRL gene family revealed that PRL is related to GH, to placental lactogen and to placental proliferin on the basis of the amino acid sequences and gene structures. Therefore, these hormones, defined as members of the PRL-GH family, are thought to be derived from a common ancestral gene (Miller and Eberhardt, 1983; Nicoll et al., 1986).

Using gel filtration techniques, such as Sephadex column chromatography, different molecular sizes of hPRL, ranging from 23 kD (small) via 40-50 kD (big) to 60 kD (big-big), have been demonstrated in body fluids and pituitary extracts (Rogol and Rosen, 1974; Suh and Frantz 1974; Fang et al., 1979). The majority of immunoreactive hPRL (about 70 percent) elutes as small PRL, the native hormone, while 8 to 24 percent of total PRL immunoreactivity elutes as big PRL. A minute fraction of immunoreactive PRL, the so-called "big-big" PRL, is thought to represent an artefact of PRL bound to polysaccharides in the void volume of the Sephadex column (Fang et al., 1979; Von Werder and Clemm, 1974).

The molecular polymorphism of PRL is not unique for the human species (Asawaroengchai et al., 1978). The significance and origin of the different PRL moieties in the serum is still not fully understood. The finding that in patients with pituitary tumours with suprasellar extension big and small PRL were found in the serum, while only small PRL was found in the cerebrospinal fluid, may suggest that big PRL results from aggregation of small PRL in the presence of serum (Jordan and Kendall, 1978). The percentual distribution of the different PRL molecules in humans varies with different physiological and pathophysiological conditions; the highest amounts of big PRL have been found in pregnant females (Suh and Frantz, 1974). Freezing and thawing of serum leads to conversion of big into small PRL, suggesting that big PRL may be a non-covalently bound dimer of monomeric small PRL (Suh and Frantz, 1974). No difference could be demonstrated in immunoreactivity of the different PRL fractions (Guyda, 1975; Rogol, 1975; Suh and Frantz, 1974; Von Werder and Clemm, 1974). On the contrary, pronounced differences in bioactivity of

these fractions have been found (Guyda, 1975; Jackson et al., 1985). Big-big PRL shows reduced mitogenic activities in the Nb-2 rat lymphoma bioassay (Jackson et al., 1985) and diminished receptor binding (Guyda, 1975), as compared with the smaller PRL moieties. Interestingly, hyperPRLaemic women with predominant increases of circulating big-big PRL exhibit a relative lack of clinical symptoms, and still have normal menses with minimal galactorrhoea (Jackson et al., 1985). On the other hand, women with marked rises of "small PRL" in serum, however, usually present with galactorrhoea, menstrual irregularities and infertility (Bohnet et al., 1976).

1.3. Factors regulating pituitary PRL secretion

There is growing evidence that multiple (neuro-)endocrine systems are involved in the regulation of PRL release by the pituitary gland. Since these regulating systems in turn are influenced by PRL itself, understanding of the complex regulation of PRL release may contribute to our knowledge about the possible mechanisms involved in PRL-induced actions within the area of reproductive endocrinology.

1.3.1. Hypothalamic factors

The hypothalamic regulation of pituitary PRL secretion is predominantly inhibitory, since severance of the hypothalamic-hypophysial connections results in an increased PRL release, while the secretion of other pituitary hormones diminishes (Everett, 1954). Dopamine, released by hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons into the hypophysial portal blood, is principally responsible for this inhibitory tone on PRL secretion (Gibbs and Neil, 1978). This is supported by the stimulating action of dopamine antagonists on pituitary PRL release (Barbieri and Ryan, 1983). PRL itself, in turn, influences the dopaminergic tone generated by the TIDA neurons under several endocrine conditions (Arita and Porter, 1984; Ben-Jonathan et al., 1980; Cramer et al., 1979; Demarest et al., 1984, De Greef and Visser, 1981; Pilotte

and Porter, 1981; Weber et al., 1983), which suggests that PRL-induced actions on the CNS may be effected through the dopaminergic system.

The neuronal control of PRL secretion involves not only dopamine. Other inhibitory factors, that have been reported, are GABA (gamma-amino-butyric acid; Gudelsky et al., 1983; Mitchell et al., 1983), somatostatin (Enjalbert et al., 1982) and GAP (gonadotrophin-releasing hormone associated peptide; Nikolics et al., 1985).

In 1972, the classical dogma that PRL release is only under inhibitory hypothalamic control, was shaded by the finding that hypothalamic extracts contained factors able to stimulate PRL release (Valverde et al., 1972). Since then, many hypothalamic factors have been identified, which stimulate pituitary PRL secretion directly, like TRH (thyreotrophin-releasing factor; Haug and Gautvik, 1976; Leong et al., 1983), VIP (vasoactive intestinal peptide; Malarkey et al., 1981), angiotensine II (Schramme and Denef, 1984) and oxytocin (Lumpkin et al., 1983; Mori et al., 1990). Furthermore, other substances of hypothalamic origin stimulate pituitary PRL release indirectly, through effects on dopaminergic and other neuronal systems. Endorphins (Arita and Kimura, 1988; Arita and Porter, 1984; Delitala et al., 1983; Enjalbert et al., 1979) and substance P (Rivier et al, 1977) are likely to fit in the latter category.

1.3.2. Other factors

Not only hypothalamic dopamine, but also dopamine of the posterior pituitary lobe is able to powerfully inhibit PRL release (Murai and Ben-Jonathan, 1986). These investigators also demonstrated that posterior pituitary lobectomized rats were not able to exhibit the classical increase in plasma PRL during the suckling stimulus (Murai and Ben-Jonathan, 1987). This suggests that a PRL-releasing factor from the posterior pituitary lobe mediates the suckling-induced increase in PRL secretion. In vitro studies seem to support this finding (Hyde et al., 1987). Recently, Mori and colleagues chemically identified the neurohypophysial PRL-releasing factor using porcine and rat posterior pituitary lobe extracts (Mori et al., 1990). They found that oxytocin is the major PRL-releasing factor in the posterior pituitary lobe.

Since ectopic pituitary glands disconnected from the hypothalamic-hypophysi-

al stalk are able to secrete PRL, it seems probable that an autocrine 'drive' within the lactotroph and/or a paracrine 'stimulus' is responsible for the high rate of basal PRL release by those ectopic glands. Indeed, local autocrine and paracrine factors acting on the level of the pituitary interfering with PRL release have been reported. Recently, VIP has been shown to be produced by the adenohypophysis and to have a direct action on pituitary PRL release (Arnaout et al., 1986; Hagen et al., 1986). Moreover, VIP antibodies and antagonists greatly suppress basal PRL secretion in vitro from individual lactotrophs in a reverse haemolytic plaque assay that precluded cell-cell interaction (Nagy et al., 1988), pointing to an autocrine role for VIP in PRL release. In addition, accumulation of PRL in medium (1-4 mg/l; Bentley and Wallis, 1987) surrounding lactotrophs in vitro is able to suppress PRL secretion. The physiological significance of such extremely high PRL concentrations surrounding the lactotrophs is doubtful. Paracrine interaction between LHRH-stimulated gonadotrophs and lactotrophs has appeared to elicit PRL secretion (Denef, 1985; Denef and Andries; 1983). This suggests that gonadotrophs can release a substance with PRL releasing activity. On the other hand some evidence exists that PRL itself in turn may directly influence gonadotrophin release (Cheung, 1983; Marchetti and Labrie, 1982).

Many peripheral organs, like the thymus, adrenals, thyroid and ovaries are known to secrete substances interfering with PRL release (Table 1.2.). Little is known about their physiological relevance in the regulation of PRL release.

In conclusion, many substances are involved in the regulation of PRL release. To which extent individual factors contribute to the PRL secretion may vary with different physiological conditions (suckling, stress). The complexity of the regulation of PRL release and the feedback interactions involved may suggest that PRL itself physiologically affect many other (neuro-)endocrine systems, resulting in a broad spectrum of actions.

1.4. Spectrum of actions

PRL has a wide variety of physiological effects throughout the vertebrate animal kingdom (Nicoll, 1974). The diversity of actions and the involvement of many diffe-

Table 1.2. Factors involving pituitary PRL release

Sources of factors	*Factors*	*Action*	*Authors*
Hypothalamus	dopamine	–	Gibbs and Neil, 1978
	GABA	–	Gudelsky et al., 1983
	somatostatin	–	Enjalbert et al., 1982
	GAP	–	Nikolics et al., 1985
	TRH	+	Ching and Utiger, 1983
		+	Haug and Gautvik, 1976
		+	Leong et al., 1983
	VIP	+	Malarkey et al., 1981
	angiotensin II	+	Schramme and Denef, 1984
	oxytocin	+	Lumpkin et al., 1983
	endorphins	+	Arita and Porter, 1984
		+	Enjalbert et al., 1979
	substance P	+	Rivier et al., 1977
	cholecystokinin	+	Malarkey et al., 1981
	bombesin	+	Westendorf et al., 1982
	neurotensin	+	Rivier et al., 1977
Posterior pituitary gland	dopamine	–	Murai and Ben-Jonathan, 1986 & 1987
	oxytocin	+	Mori et al., 1990
Anterior pituitary gland	ACTH	+	Leung et al., 1980
	angiotensin II	+	Canonico and MacLeod, 1986
		+	Denef, 1985
	VIP	+	Hagen et al., 1986
		+	Nagy et al., 1988
Thymus	thymus factor 5	+	Spangelo et al., 1985
Gonads	estradiol	+	West and Dannies, 1980
	progesteron	–/+	Caligaris et al., 1974
Adrenals	corticosteroids	–	Fang and Shian, 1981

Note. Factors stimulating (+) and inhibiting (–) PRL release are given. See text for abbrevations.

rent target organs hinder any unifying concept about the physiological role of PRL. However, one intriguing conformity of many of PRL's actions, from electrolyte metabolism to galactopoiesis, is the finding that PRL acts in concert with steroid hormones. Moreover, evidence is growing that other factors, like synlactins, known as insulin-like growth factors, are involved in the growth-promoting effects of PRL on its target tissues (Nicoll et al., 1985). A number of well-known actions of PRL are listed in Table 1.3.

The increasing concentration of blood PRL during pregnancy is required for *growth and development of the mammary glands* in preparation for breast feeding post partum. Additionally, other hormones, like estrogens, progestagens, placental lactogen, insulin and cortisol, are synergistically involved in these mammotrophic effects (Nicoll, 1974; Vonderhaar and Greco, 1979). Subsequent *lactogenesis*, i.e. the initiation of milk formation and secretion, starts under the influence of PRL after its prepartum rise when serum levels of progesterone (Kann et al., 1978) and estrogen (Brun del Re et al., 1973) decrease. Maintenance of milk secretion, finally, depends on regular emptying of the mammary gland by nipple stimulation, and can be inhibited by suppression of PRL secretion in the post-partum woman, in the rat, rabbit and other mammals (Fluckiger, 1978; Kann et al., 1978). Hence PRL is necessary to be secreted in sufficient amounts for normal *galactopoiesis*, i.e. for physiological maintenance of established milk secretion.

During milk production adaptive changes in *calcium metabolism* are needed. Increased plasma concentrations of calcium and calciuria can be provoked by PRL infusions in the rat (Mahajan et al., 1974). This action of PRL is even more marked in parathyroid hormone- and calcitonin-deprived animals, which suggests a mediating role for vitamine D_3 (Horrobin, 1974). In the human, serum levels of $1,25(OH)_2$-vitamine D_3 increases during late pregnancy, and subsequently declines post partum, in parallel with the changes in serum concentrations of PRL (Lund and Selnes, 1979). Interestingly, in a woman with surgical hypoparathyroidism, increases in serum levels of $1,25(OH)_2$-vitamine D_3 were measured during lactation. These increases were closely related to the fluctuations in serum concentrations of PRL, suggesting that PRL stimulates the activation of vitamine D_3 by 1α-hydroxylase in the kidney (Cundy et al., 1987).

Osmoregulation has been described as the major function of PRL in fish

Table 1.3. Physiological functions of PRL in vertebrates

Growth-promoting effects on:	
- mammary gland	Nicoll, 1974
- accessory sex glands	Bartke, 1974 Thomas and Manandhar, 1975
- lymphoid tissue	Russel, 1988 Spangelo et al., 1985
- crop sac of pigeons	Riddle et al., 1933
Effects relating to reproductive functions on:	
- ovary : luteotrophic	Everett et al., 1954 Zeilmaker and Carlsen, 1962
luteolytic	Grandison and Meites, 1972
- testis	Bartke, 1966 Bartke and Lloyd, 1970
- gametes	Hoshino, 1988
- mammary gland	Nicoll, 1974
- lactation	Brun del Re, 1973 Fluckiger, 1978 Kann et al., 1978
- maternal behaviour	Bridges et al., 1985 Hutchison, 1978
Other effects, concerning:	
- calcium and vitamine D_3 metabolism	Cundy et al., 1987 Lund and Selnes, 1979 Mahajan et al., 1974
- salt and water metabolism	Nicoll, 1974
- carbohydrate and fat metabolism	Landgraf et al., 1977 McGarry and Beck, 1972
- immunomodulation	Russel et al., 1985 Spangelo et al., 1985

(Nicoll, 1974). In rats PRL is able, like morphine, to initiate a small dose-related reduction in urine volume and in electrolytes excretion, which can be antagonized by naloxone, suggesting an opioid-dependant action of PRL (Ramaswamy and Bapna, 1987). In man, the anti-diuretic actions by PRL have appeared to be of minor physiological relevance (Baumann et al., 1977; Horrobin et al., 1971; Vorherr, 1979).

Evidence is accumulating that PRL has an *immunomodulatory role* in mammals (Russel, 1988; Spangelo et al., 1985). Regression of lymphoid tissue and impaired antibody formation have been found to occur after hypophysectomy and bromocriptine treatment, while immunocompetence can be restored by administration of PRL. The presence of PRL receptors on T- and B-lymphocytes may suggest that immunomodulation can occur by a direct action of PRL on these immunologic cells (Russel et al., 1985). A bioassay for PRL has recently been developed on the basis of such action (Friesen et al., 1985). Interestingly, the immune system itself, especially the thymus, secretes factors able to stimulate PRL release under physiologial conditions (Spangelo et al., 1985). This may be a mechanism by which the immune system regulates itself through the immunomodulatory function of PRL.

Only some aspects of PRL function in reproduction have been elucidated. In mammals *lactation* by well-prepared mammary glands and *maternal behaviour* in favour of the young are established being PRL-dependent (Bridges et al., 1985; Hutchison, 1978; Nicoll, 1974). Much controversy, however, still exists about the relevance of PRL in *gametogenesis*, *sexual behaviour* and *conception*.

In vitro PRL appears to have positive effects on motility, capacitation and subsequent acrosomal reaction of spermatozoa in mice (Hoshino et al., 1988). Convincing evidence is still lacking that these phenomena are of physiological relevance, since in vivo experiments in male mice failed to reveal favourable effects of PRL on spermatogenesis, rates of motility and fertilization (Hoshino et al., 1988).

The effects of PRL on the ovary are species dependent. In the female rat PRL can have *luteotrophic effects* (Everett, 1954), associated with increased progesterone production. In a variety of other species, however, PRL is not able to stimulate progesterone biosynthesis by corpora lutea (Dorfman, 1972). In the rat, prolonged luteotrophic stimulation by PRL, induced by pituitary transplants, results in pseudopregnancy, which can be interrupted by suppressing PRL secretion resulting in new ovarian cycles (Zeilmaker and Carlsen, 1962). In this animal pregnancy can be

interrupted by inhibition of PRL release on day 7 or earlier, but from day 8 of pregnancy, PRL seems to be no longer critical for corpus luteum function in the rat (Morishige and Rothchild, 1974). PRL has also been shown to exert *luteolytic effects* on the non-functional corpora lutea of the rat and the mouse (Grandison and Meites, 1972). A physiological significance of PRL in human corpus luteum function remains to be established.

In infertile PRL-deficient mice, administration of PRL results in increased testicular activity with improvement of *spermatogenesis* (Bartke, 1966; Bartke and Lloyd, 1970). However, supraphysiological levels of PRL have been shown to deteriorate testicular function, male sexual behaviour and fertility, in rats as well as in humans (Buvat et al., 1985; Doherty et al., 1986; Drago, 1984; Schwartz et al., 1982). On the contrary, male sexual behaviour in other species, like hamsters and mice, has appeared to be maintained or even stimulated during hyperPRLaemia (Bartke, 1980; Bartke et al., 1975; Klemcke and Bartke, 1981; Shrenker and Bartke, 1987). Because of the resemblance between the effects of hyperPRLaemia observed in rats and humans, in this thesis the rat is used as an animal model to study the hyperPRLaemia-induced effects on *male reproductive functions* in an attempt to unravel the mechanisms involved.

1.5. Mechanisms of PRL action

The diverse functions of PRL described are brought about by an action of the hormone on molecular processes within the target cells. In most studies focussing on intracellular molecular processes influencable by PRL, the mammary gland (Rillema et al., 1977 & 1985), the pigeon crop-sac (Pukac and Horseman, 1984; Nicoll et al., 1985) or the Nb2 node lymphoma cells (Gertler et al., 1985; Ofenstein et al., 1985) have been used as target tissues. In these tissues mechanisms of PRL action have been studied at different levels:

1. PRL receptors

2. Second messengers

3. Gene expression and mitogenesis

1.5.1. PRL receptors

Pituitary PRL is a straight-chained peptide hormone with a molecular weight of about 24,000. It consists of about 200 amino acid residues and three intrachain disulphide bridges (Nicoll et al., 1986; Shome and Parlow, 1977). Similar to other polypeptide hormones, PRL binds to its specific receptors on the plasma membrane of target cells to initiate its biological effects (Amit et al., 1984; Shiu et al., 1973). PRL-binding sites have also been identified on intracellular organelles, like the endoplasmic reticulum, lysosomes and the golgi apparatus (Costlow, 1987; Josefsberg et al., 1979). Moreover, PRL can be internalized into several types of cells (Costlow, 1987; Kelly et al., 1984). These findings can be explained by the fact that the assemblage of membrane receptor proteins and the bio-degradation of PRL are carried out within the cell. An intracellularly initiated action of PRL is unlikely, since antibodies to the PRL receptor on the plasma membrane abolish or mimic PRL responses, depending on the antibody preparations employed (Costlow, 1987; Kelly et al., 1984; Shiu et al., 1983; Shiu and Friesen, 1980). Moreover, PRL bound to large particles, such as Sephadex beads, can bring about the normal action of the hormone, while in this form it can not be internalized (Turkington, 1970).

Efforts to characterize the structure of the PRL receptor have resulted in the discovery of a diversity of isolated molecular entities - M_r ranging from 28 upto 320 kilodaltons - with binding characteristics allowing them to be classified as PRL receptors (Amit et al., 1984; Costlow, 1987; Webb and Wallis, 1988; Ymer and Herington, 1986). The variation in molecular weights reported does not necessarily reflect a wide spectrum of receptors, since the isolation procedures employed may have profound effects on the molecular structure that is isolated. High molecular weight estimates can be attributed to aggregration of receptor-units. Moreover, it is not precluded that PRL receptors consist of several peptide-units linked to lipid and sugar moieties, possibly disintegrating during isolation. Davis and Linzer isolated very recently three distinct cDNA clones for the PRL receptor in mouse liver, indicating that the expression and function of this receptor is likely to be complex (Davis and Linzer, 1989). They revealed that the PRL receptor is actually a family of proteins with common signal sequences, extracellular domains and transmembrane domains.

The terminal regions of the cytoplasmic domains, however, exhibited a marked variability, suggesting that multiple signalling mechanisms may be evoked by PRL, even within a single tissue. Thus a possible diversity in modes of PRL action within the cell may serve to explain the unusually varied effects that PRL exerts on cell function. However, such diversity in mechanisms of PRL action has currently not been proven.

The numbers of PRL receptors in target tissues are subject to regulation by a variety of hormonal and other factors (Hughes et al., 1985). PRL receptors are known to be internalized following binding of the hormone, consequently leading to down-regulation (Posner et al., 1981). In the longer term, however, PRL leads to up-regulation of its receptors, as has been demonstrated in a range of tissues (Hughes et al., 1985; Posner et al., 1979 & 1981). The physiological significance of this receptor-regulation is fully dependent on whether the receptor numbers are rate limiting for the PRL responses in a particular physiological state. The relatively rapid turnover rate of the PRL receptor, having a $t_{1/2}$ of 40-50 min in the rat liver (Baxter,1985), may suggest an important role for the regulation of PRL action at the level of the receptor. However, our understanding about this is limited at the present time: the molecular mechanisms involved remain to be elucidated.

1.5.2. Second messengers

Considerable effort has been devoted to investigating possible second messengers for PRL (Hughes et al., 1985; Shiu and Friesen, 1980), but evidence is still lacking which intracellular mediator primarily transduces the signal of the PRL-receptor complex resulting in a cascade of processes in the target cells leading to biological effects. The mechanism of PRL action has been investigated most extensively in the mammary gland system. Based on such studies, cyclic nucleotides, prostaglandins, polyamines, small peptides and calcium have been proposed as intracellular mediators in PRL action (Matusik and Rosen, 1980; Rillema, 1976; Rillema et al., 1977).

Cyclic nucleotides have been subjected to investigation in studies on PRL action. Since definitive changes in cyclic AMP levels in response to PRL have not

been consistently observed (Shiu and Friesen, 1980; Rillema, 1980), cyclic AMP is unlikely to be involved in the mechanism of PRL action. Moreover, cAMP-derivatives do not induce PRL-like actions in vitro, but they are able to inhibit some actions of the hormone, including stimulation of synthesis of milk proteins, fatty acids, RNA and DNA, in cultured mammary cells and tissues from pregnant and lactating animals (Loizzi, 1978; Loizzi et al., 1975; Sapag-Hagar et al., 1974; Speake et al., 1976). On the contrary, cGMP appears to enhance these lactogenic processes by stimulation of RNA synthesis and elevation of casein mRNA, however, to a lesser extent as PRL (Matusik and Rosen, 1980). Thus, the magnitude of these responses to cGMP is too small to indicate cGMP as being the main second messenger for PRL action. Moreover, a direct stimulation of guanylate cyclase in mammary tissues by PRL could not be demonstrated (Rillema, 1987).

In mammary gland explants, prostaglandins B_2, E_2 and $F_{2\alpha}$ exert PRL-like stimulatory effects on uridine incorporation into RNA (Rillema, 1975 & 1976) and on ornithine decarboxylase activity, an enzyme involved in polyamine synthesis (Wing and Rillema, 1983). Interestingly, prostaglandins can only stimulate the production of milk proteins in the presence of polyamines, especially spermidine (Rillema, 1976), of which the biosynthetic pathways are presented in Figure 1.1.

Already in 1972 the polyamine spermidine has been demonstrated in mammary gland cells of lactating rats in concentrations up to 5mM (Russel and McVicker, 1972), and, in later studies, it has been proposed as a possible regulator of lactational processes (Oka and Perry, 1976; Rillema et al., 1977). Ornithine decarboxylase activity, which is believed to be rate-limiting for the biosynthesis of polyamines (Janne et al., 1978; Williams-Ashman et al., 1972), is elevated in response to PRL in mammary gland explants of mice, with a delay of action varying from 0.5 to 1.0 h (Rillema et al., 1977). Since ornithine decarboxylase, a cytoplasm-soluble enzyme not bound to the plasma membrane, becomes stimulated after a significant time delay from the onset of PRL stimulation, elevation of ornithine decarboxylase activity may be mediated by PRL-activated factors associated with the plasma membrane.

Two different mechanisms initiated in the plasma membrane are well known to transduce signals of membrane-bound peptide hormones into the target cells: transduction by activation of adenylate cyclase with an enhanced rate of cAMP for-

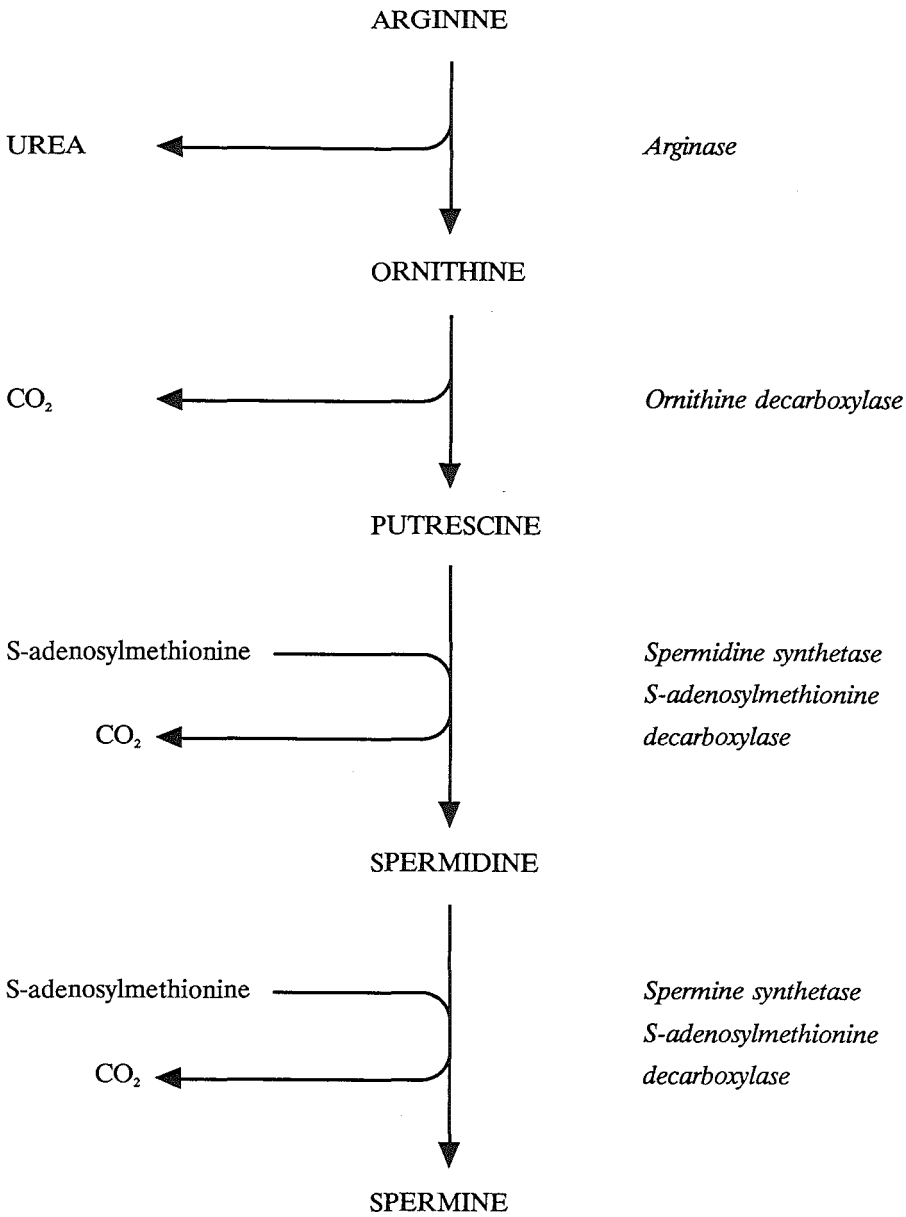

Figure 1.1. *Biosynthetic pathways of the polyamines. Ornithine decarboxylase activity is stimulated by PRL in mouse mammary explants, and rate-limiting for the synthesis of spermidine which is a possible regulator of lactational processes.*

mation, and transduction by activation of phospholipases with changes in phospholipid metabolism, increased intracellular calcium and altered prostaglandine synthesis. Most findings suggest that the latter mechanism is involved in PRL action.

PRL enhances membrane fluidity and prostaglandin synthesis in membrane preparations from mouse liver and rat prostate gland (Dave, 1987), while phospholipase A_2 has similar effects on fluidity of mouse hepatic membranes (Dave et al., 1981). Phospholipase A_2 as well as phospholipase C have PRL-like effects in cultured mouse mammary tissues: they stimulate the rate of ^{3}H-uridine incorporation in RNA and activate ornithine decarboxylase; moreover, phospholipase C appeared to be as potent as PRL itself in the stimulation of ornithine decarboxylase activity (Rillema et al., 1976, 1983 & 1986). Interestingly, PRL actions on DNA, RNA, casein and lipid biosynthesis and on ornithine decarboxylase in mouse and rabbit mammary gland explants can be abolished by phospholipase inhibitors (Rillema 1979a & 1979b; Rillema et al., 1983 & 1986), indicating that PRL can only exert its action in the presence of phospholipase activity, but it remains unproven whether activation of phospholipases mediates PRL action.

1.5.3. Gene expression and mitogenesis

PRL can induce the production and secretion of casein, a milk protein in the well-primed mammary gland (Topper and Freeman, 1980) and in mouse mammary gland explants (Rillema et al., 1986).

Using a sensitive cDNA hybridization probe specific for casein mRNA, Matusik and Rosen revealed that PRL can induce casein mRNA accumulation and increased casein gene transcription (Matusik and Rosen, 1978; Guyette et al., 1979), which appeared not to be essentially mediated by cyclic nucleotides or polyamines (Matusik and Rosen, 1980). Recently, Doppler and co-workers (1989) demonstrated that the milk protein ß-casein is regulated at the level of transcription and requires the synergistic action of PRL and glucocorticoid hormones. However, the molecular mechanism by which PRL regulates gene transcription is unclear at the present time.

The mitogenic action of PRL has been studied in different models. PRL is

known to stimulate epithelial cell proliferation in mammary tissues from several species in vivo and in vitro (Topper and Freeman, 1980; Rudland et al., 1980). In mouse mammary gland explants, PRL can stimulate the rate of thymidine incorporation into DNA, 24-48 h after adding PRL to the culture medium. Both quinacrin, a phospholipase inhibitor, and indomethacin, an inhibitor of prostaglandin synthesis, are able to abolish this action (Rillema and Foley, 1983). Thus, the proliferative effects of PRL on mammary cells may be mediated by an activation of phopholipases and ongoing synthesis of prostaglandins.

The rat Nb2 lymphoma cell line, a T-cell derived cell line, can be grown in vitro indefinitely. Since under appropriate conditions lactogenic hormones act as specific mitogens, this cell line has been used as a sensitive and specific biological assay for lactogenic hormones (Tanaka et al., 1980). The mitogenic action of PRL on the Nb_2 cells is mediated by a surface receptor (Shiu et al., 1983), and appears to involve a receptor-activated Ca^{2+}-influx (Murphy et al., 1988) and a stimulation of polyamine metabolism (Ofenstein et al., 1985).

Already in 1933, Riddle and associates introduced the pigeon crop sac as a "milk"-secreting organ sensitive to PRL. The growth-promoting action of lactogenic hormones on this gland is potentiated by a liver factor called "synlactin", at present known as "insulin-like growth factor", the secretion of which is stimulated by PRL (Nicoll et al., 1985). Despite its sensitivity to PRL, the crop has not been extensively used for studies on PRL's molecular mechanisms.

In summary, most findings from studies on molecular mechanisms of PRL action on cell function are in favour of a plasma membrane transduced mode of action with activation of phospholipases and subsequent changes in phospholipid metabolism, intracellular Ca^{2+}, prostaglandine and polyamine synthesis. Modulation of gene-expression at the level of transcription and increased DNA-synthesis dependent on other factors have been established. But, at the present moment, the complete cascade of molecular processes by which the biological effects of PRL are brought about is far from clear. Whether there is a common mechanism whereby PRL can induce its broad spectrum of actions is currently not known.

1.6. References

Amit,T., Barkey,R.J., Gavish,M., and Youdim,M.B.H. (1984). Induction of prolactin (PRL) receptors by PRL in the rat lung and liver. Demonstration and characterization of a soluble receptor. Endocrinol. 114, 545-552.

Arita,J., and Kimura,F. (1988). Enkephalin inhibits dopamine synthesis in vitro in the median eminence portion of rat hypothalamic slices. Endocrinol. 123, 694-699.

Arita,J., and Porter,J.C. (1984). Relationship between dopamine release into hypophysial portal blood and prolactin release after morphine treatment in rats. Neuroendocrinol. 38, 62-67.

Arnaout,M.A., Garthwaite,T.L., Martinson,D.R., and Hagen,T.C. (1986). Vasoactive intestinal polypeptide is synthesized in anterior pituitary tissue. Endocrinol. 1986, 2052-2057.

Asawaroengchai,H., Russel,S.M., and Nicoll,C.S. (1978). Electrophoretically separable forms of rat prolactin with different bioassay and radioimmunoassay activities. Endocrinol. 102, 407-414.

Assies,J., Schellekens,A.P.M., and Touber,J.L. (1978). Prolactin in human cerebrospinal fluid. J. Clin. Endocrinol. Metab. 46, 576-586.

Barbieri,R.L., and Ryan,K.J. (1983). Bromocriptine: endocrine pharmacology and therapeutic applications. Fert. Steril. 39, 727-741.

Bartke,A. (1966). Influence of prolactin on male fertility in dwarf mice. J. Endocrinol. 35, 419-420.

Bartke,A. (1974). Effects of inhibitors of pituitary prolactin on testicular cholesterol stores, seminal vesicle weight, fertility and lactation in mice. Biol. Reprod. 11, 319-325.

Bartke,A. (1980). Role of prolactin in reproduction in male mammals. Fed. Proc. 39, 2577-2581.

Bartke,A., Croft,B.T., and Dalterio,S. (1975). Prolactin restores plasma testosterone levels and stimulates testicular growth in hamsters exposed to short day-length. Endocrinol. 97, 182-186.

Bartke,A., and Lloyd,C.W. (1970). Influence of prolactin and pituitary isografts on spermatogenesis in dwarf mice and hypophysectomized rats. J. Endocrinol. 46, 321-329.

Baumann,G., Marynick,G.F., Winters,S.J., and Loriaux,D.L. (1977). The effects of osmotic stimuli on prolactin secretion and renal water excretion in normal man and in chronic hyperprolactinemia. J. Clin. Endocrinol. Metab. 44, 199-202.

Baxter,R.C. (1985). Measurement of growth hormone and prolactin receptor turnover in rat liver. Endocrinol. 117, 650-655.

Ben-Jonathan,N., Neill,M.A., Arbogast,L.A., Peters,L.L., and Hoefer,M.T. (1980). Dopamine in hypophysial portal blood: relationship to circulating prolactin in pregnant and lactating rats. Endocrinol. 106, 690-696.

Bentley,A.M., and Wallis,M. (1987). In-vitro evidence for the autoregulation of prolactin secretion at the level of the pituitary gland in the rat. J. Endocrinol. 115, 13-18.

Bigazzi,M., Pollicino,G., and Nardi,E. (1979). Is human decidua a specialized endocrine organ ? J. Clin. Endocrinol. 49, 847-850.

Bohnet,H.G., Dahlen,H.G., Wultke,W., and Schneider,H.P. (1976). Hyperprolactinemic anovulatory syndrome. J. Clin. Endocrinol. Metab. 42, 132-143.

Bridges,R.S., Loundes,D.D., DiBiase,R., and Tate-Ostroff,B.A. (1985). Prolactin and pituitary involvement in maternal behavior in the rat. In: MacLeod,R.M., Thorner, M.O., and Scapagnini,U., eds. Prolactin. Basic and clinical correlates. Fidia Res. Series vol. I, Liviana Press, Padova, pp. 591-599.

Brun del Re,R., del Pozo,E., de Grandi,O., Friesen,H.G., Hinselmaan,M., and Wyss,H. (1973). Prolactin inhibition and suppression of puerperal lactation by 2-Br-a-ergocryptin (CB154). A comparison with estrogen. Obstet. Gynecol. 41, 884-887.

Buvat,J., Lemaire,A., Buvat-Herbaut,M., Fourlinnie,J.C., Racadot,A., and Fossati,P. (1985). Hyperprolactinemia and sexual function in men. Horm. Res. 22, 196-203.

Caligaris,L., Astrada,J.J., and Taleisnik,S. (1974). Oestrogen and progesterone influence on the release of prolactin in ovariectomized rats. J. Endocrinol. 60, 205-215.

Canonico,P.L., and MacLeod,R.M. (1986). Angiotensin peptides stimulate phosphoinositide breakdown and prolactin release in anterior pituitary cells in culture. Endocrinol. 118, 233-238.

Cheung,C.Y. (1983). Prolactin suppresses luteinizing hormone secretion and pituitary responsiveness to luteinizing hormone releasing hormone by a direct action at the anterior pituitary. Endocrinol. 113, 632-638.

Ching,M.C.H., and Utiger,R.D. (1983). Hypothalamic portal blood immunoreactive TRH in the rat: lack of effect of hypothyroidism and thyroid hormone treatment. J. Endocrinol. Invest. 6, 347-352.

Chirito,E., Gonda,A., and Friesen,H. (1972). Prolactin in renal failure. Clin. Res. 20, 423 (Abstract).

Clemens,J.A., and Sawyer,B.D. (1974). Identification of prolactin in cerebrospinal fluid. Exp. Brain Res. 21, 399-402.

Costlow,M.E. (1987). Prolactin interaction with its receptors and the relationship to the subsequent regulation of metabolic processes. In: Actions of prolactin on molecular processes, edited by J.A. Rillema, pp.5-26, CRC Press, Boca Raton, Fla.

Cramer,O.M., Parker,C.R.,Jr., and Porter,J.C. (1979). Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinol. 105, 636-640.

Cundy,T., Haining,S.A., Guilland-Cumming,D.F., Butler,J. and Kanis,J.A. (1987). Remission of hypoparathyroidism during lactation: evidence for a physiological role for prolactin in the regulation of vitamin D metabolism. Clin. Endocrinol. 26, 667-674.

Dave,J.R. (1987). Prolactin regulation of membrane fluidity and prostaglandin formation. In: actions of prolactin on molecular processes, J.A. Rillema, pp. 69-106. CRC Press, Boca Raton, Fla.

Dave,J.R., Knazek,R.A., and Liu,S.C. (1981). Arachadonic acid, bradykinin and phospholipase A_2 modify both prolactin binding capacity and fluidity of mouse hepatic membranes. Biochem. Biophys. Res. Comm. 103, 727-738.

Davis,J.A., and Linzer,D.I.H. (1989). Expression of multiple forms of the prolactin receptor in mouse liver. Mol. Endocrinol. 3, 674-680.

Delitala,G., Grossman,A., and Besser,M. (1983). Differential effects of opiate peptides and alkaloids on anterior pituitary hormone secretion. Neuroendocrinol. 37, 275-279.

Demarest,K.T., Riegle,G.D., and Moore,K.E. (1984). Prolactin-induced activation of tuberoinfundibular dopaminergic neurons: evidence for both a rapid "tonic" and delayed "induction" component. Neuroendocrinol. 38, 467-475.

Denef,C., and Andries,M. (1983). Evidence for paracrine interaction between gonadotrophs and lactotrophs in pituitary cell aggregates. Endocrinol. 112, 813-822.

Denef,C. (1985). Paracrine interaction in anterior pituitary. In: MacLeod,R.M., Thorner,M.O., and Scapagnini,U., eds. Prolactin. Basic and clinical correlates. Fidia Research Series vol. I, Liviana Press, Padova, pp. 53-57.

Doherty,P.C., Baum,M.J. and Todd,R.B. (1986). Effects of chronic hyperprolactinemia on sexual arousal and erectile function in male rats. Neuroendocrinol. 42, 368-375.

Doppler,W., Groner,B., and Ball,R.K. (1989). Prolactin and glucocorticoid hormones synergistically induce expression of transfected rat ß-casein gene promotor constructs in a mammary epithelial cell line. Proc. Natl. Acad. Sci. USA 86, 104-108.

Dorfman,R.I. (1972). Mechanism of action of gonadotropins and prolactin. In: Biochemical Actions of Hormones. Vol. II. Litwack,G., ed., Academic Press Inc., New York, pp. 295-316.

Drago,F. (1984). Prolactin and sexual behavior: A review. Neurosc. Biobeh. Rev. 8, 433-439.

Emanuele,N.V., Metcalfe,L., Wallock,L., Tentler,J., Hagen,T.C., Beer,C.T., Martinson,D., Gout,P.W., Kirsteins,L., and Lawrence,A.M. (1986). Hypothalamic prolactin: characterization by radioimmunoassay and bioassay and response to hypophysectomy and restraint stress. Neuroendocrinol. 44, 217-221.

Enjalbert,A., Epelbaum,J., Arancibia,S., Tapia-Arancibia,L., Bluet-Pajot,M., and Kordon,C. (1982). Reciprocal interactions of somatostatin with thyrotropin-releasing hormone and vasoactive intestinal peptide on prolactin and growth hormone secretion in vitro. Endocrinol. 111, 42-47.

Enjalbert,A., Ruberg,M., Arancibia,S., Priam,M., and Kordon,C. (1979). Endogenous opioids block dopamine inhibition of prolactin secretion in vitro. Nature, 280, 595-596.

Everett,J.W. (1954). Luteotrophic function of autografts of the rat hypophysis. Endocrinol. 54, 685-690.

Fang,V.S., and Shian,L.R. (1981). Adrenal influence on pituitary secretion of thyrotropin and prolactin in rats. Endocrinol. 108, 1545-1551.

Fang,V.S., Shian,L.R., and Worsley,I.G. (1979). Generation of the big prolactin by sephadex column chromatography. In: MacIntyre,I., and Szelke,M., eds. Molecular Endocrinology. Elseviers/North-Holland Biomedical Press, Amsterdam, pp. 133-140.

Fluckiger,E. (1978). Lactation inhibition by ergot drugs. In: Yokoyama,A., Mizuno,H., and Nagasawa,H., eds. Physiology of mammary glands. Japan Scientific Societies Press, Tokyo, pp. 71-82.

Fluckiger,E., Marko,M., Doepfner,W., and Niederer,W. (1976). Effects of ergot alkaloids on the hypothalamo-pituitary axis. Postgrad. Med. J. (Suppl. 1) 52, 57-61.

Friesen,H.G., Gertler,A., Walker,A., and Elsholtz,H. (1985). Mechanism of action of prolactin in stimulating cell growth. In: MacLeod,R.M., Thorner,M.O., and Scapagnini,U., eds. Prolactin. Basic and clinical correlates. Fidia Research Series vol. I, Liviana Press, Padova, pp. 315-326.

Friesen,H.G., and Hwang,P. (1973). Human prolactin. Annu. Rev. Med. 24, 251-270.
Fuxe,K., Hökfelt,T., Eneroth,P., Gustafson,J.A., and Skett,P.P. (1977). Prolactin-like immunoreactivity: localization in nerve terminals of rat hypothalamus. Science 196, 899-900.
Gala,R.R., Singhakowinta,A., and Brennan,M.J. (1975). Studies on prolactin in human serum, urine and milk. Horm. Res. 6, 310-320.
Gertler,A., Walker,A., and Friesen,H.G. (1985). Enhancement of human growth-hormone-stimulated mitogenesis of the Nb_2 node lymphoma cells by 12-O-tetradecanoyl-phorbol-13-acetate. Endocrinol. 116, 1636-1644.
Gibbs,D.M., and Neil,J.D. (1978). Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinol. 102, 1895-1900.
Gluckman,P.D., Ballard,P.L., Kaplan,S.L., Liggins,G.C., and Grumbach, M.M. (1978). Prolactin in umbilical cord blood and the respiratory distress syndrome. J. Pediatr. 93, 1011-1014.
Grandison,L., and Meites,J. (1972). Luteolytic action of prolactin during oestrus cycle of the mouse. Proc. Soc. Exp. Biol. Med. 140, 323-325.
Greef,W.J.de, Plotsky,P.M., and Neill,J.D. (1981). Dopamine levels in hypophysial stalk plasma and prolactin levels in peripheral plasma of the lactating rat: effects of a simulated suckling stimulus. Neuroendocrinol. 32, 229-233.
Greef,W.J.de, and Schoot,P.vander. (1985). Some recent developments in the study of prolactin in mammals. In: Wimersma Greidanus, Tj.B. van, ed. Frontiers of Hormone Research, Karger, Basel, vol. 14, pp. 70-99.
Greef,W.J.de, and Visser,T.J. (1981). Evidence for the involvement of hypothalamic dopamine and thyrotrophin-releasing hormone in suckling-induced release of prolactin. J. Endocrinol. 91, 213-233.
Grosvenor,C.E., and Whitworth, N.S. (1976). Incorporation of rat prolactin into rat milk in vivo and in vitro. J. Endocrinol. 70, 1-9.
Gudelsky,G.A., Apud,J.A., Masotto,C., Locatelli,V., Cocchi,D., Racagni, G., and Mueller,E.E. (1983). Ethanolamine-O-sulfate enhances gamma-aminobutyric acid secretion into hypophysial portal blood and lowers serum prolactin concentrations. Neuroendocrinol. 37, 397-399.
Guyda,H.J. (1975). Heterogeneity of human growth hormone and prolactin secreted in vitro: Immunoassay and radioreceptor assay correlations. J. Clin. Endocrinol. Metab. 41, 953-967.
Guyette,W.A., Matusik,R.J., and Rosen,J.M. (1979). Prolactin-mediated transcriptional and post-transcriptional control of casein gene expression. Cell 17, 1013-1023.
Hagen,T.C., Arnaout,M.A., Scherzer,W.J., Martinson,D.R., and Garthwaite,T.L. (1986). Antiserum to vasoactive intestinal polypeptide inhibit basal prolactin release from dispersed anterior pituitary cells. Neuroendocrinol. 43, 641-645.
Haug,E., and Gautvik,K.M. (1976). Radioimmunoassay of rat prolactin and its use in measuring prolactin production by cultured pituitary cells. Acta Endocrinol. 82, 282-297.
Horrobin,D.F. (1974). Prolactin 1974. Medical and technical publishing, Lancaster, p. 96.
Horrobin,D.F., Lloyd,I.J., Lipton,A., Burstyn,P.G., Durkin,N., and Muiruri,K.L. (1971). Actions of prolactin on human renal function. Lancet 2, 352-354.
Hoshino,K. (1988). Biological effects of prolactin on spermatozoa, oocytes and fertilization. In: Hoshino,K., ed. Prolactin gene family and its receptors. Excerpta Medica 819, Kyoto, pp. 1-12.

Hughes,J.P., Elsholtz,H.P., and Friesen,H.G. (1985). Growth hormone and prolactin receptors. In: Posner,B.I.,ed., Polypeptide hormone receptors, Dekker, New York and Basel, pp. 157-199.

Hutchison,R.E. (1978). Prolactin and parental behaviour in birds and mammals. In: Robyn,C., and Harter,M., eds. Progress in prolactin physiology and pathology. Elsevier/North Holland Biomedical Press, pp. 243-251.

Hyde,J.F., Murai,I., and Ben-Jonathan,N. (1987). The rat posterior pituitary contains a potent prolactin-releasing factor: studies with perifused anterior pituitary cells. Endocrinol. 121, 1531-1539.

Jackson,R.D., Wortsman,J., and Malarkey,W.B. (1985). Characterization of a large molecular weight prolactin in women with idiopathic hyperprolactinemia and normal menses. J. Clin. Endocrinol. Metab. 61, 258-264.

Janne,J., Poso,H., and Raina,A. (1978). Polyamines in rapid growth and cancer. Biochim. Biophys. Acta 474, 241-246.

Jordan,R.M., and Kendall,J.W. (1978). Dissociation of plasma and CSF prolactin heterogeneity. Acta Endocrinol. 89, 38-47.

Josefsberg,Z., Posner,B.I., Patel,B., and Bergeron,J.J.M. (1979). The uptake of prolactin in female rat liver: Concentration of intact hormone in the Golgi apparatus. J. Biol. Chem. 254, 209-214.

Kann,G., Carpentier,M.C., Fevre,J., Martinet,J., Maubon,M., Meusnier,C., Paly,J., and Vermeire,N. (1978). Lactation and prolactin in sheep, role of prolactin in initiation of milk secretion. In: Robyn,C., and Harter,M., eds. Progress in prolactin physiology and pathology. Elsevier/North Holland Biomedical Press, Amsterdam, pp. 201-212.

Kelly,P.A., Djiane,J., Katoh,M., Ferland,L.H., Houdebine,L-M., Teyssot,B., and Dusanter-Fourt,L. (1984). The interaction of prolactin with its receptors in target tissues and its mechanism of action. Rec. Progr. Horm. Res. 40, 379-439.

Kendall,J., and Orwoll,E. (1980). Anterior pituitary hormones in the brain and other extrapituitary sites. In: Martini,L., and Ganong,W.F., eds. Frontiers in Neuroendocrinology 6, 33-65. Raven, New York.

Klemcke,H.G., and Bartke,A. (1981). Effects of chronic hyperprolactinemia in mice on plasma gonadotropin concentrations and testicular human chorionic gonadotropin binding sites. Endocrinol. 108, 1763-1768.

Landgraf,R., Landgraf-Leurs,M.M.C., Weissman,A., Hörl,R., Werder,K.von, and Scriba,P.C. (1977). Prolactin: a diabetogenic hormone. Diabetol. 13, 99-104.

Leong,D.A., Frawley,L.S., and Neill,J.D. (1983). Neuroendocrine control of prolactin secretion. Ann. Rev. Physiol. 45, 109-127.

Leung,F.C., Chen,H.T., Verkaik,S.J., Steger,R.W., Peluso,J.J., Campbell,G.A., and Meites,J. (1980). Mechanism(s) by which adrenalectomy and corticosterone influence prolactin release in the rat. J. Endocrinol. 87, 131-140.

Logan,I.S., and Macleod,R.M. (1977). Prolactin in human and rat serum and cerebrospinal fluid. Brain Res. 132, 477-483.

Loizzi,R.F. (1978). Cyclic AMP inhibition of mammary gland lactose synthesis specificity and potentiation by 1-methyl-3-isobutylxanthine. Horm. Metab. Res. 10, 415-419.

Loizzi,R.F., dePont,J.J.H.H.M., and Bonting,S.L. (1975). Inhibition by cyclic AMP of lactose production in lactating guinea pig mammary gland slices. Biochem. Biophys. Acta 392, 20-25.

Lumpkin,M.D., Samson,W.K., and McCann,S.M. (1983). Hypothalamic and pituitary sites of action of oxytocin to alter prolactin secretion in the rat. Endocrinol. 112, 1711-1717.
Lund,B., and Selnes,A. (1979). Plasma 1,25-dihydroxyvitamin D levels in pregnancy and lactation. Acta Endocrinol. 92, 330-335.
Mahajan,K.K., Robinson,C.J., and Horrobin,D.F. (1974). Actions of prolactin on plasma calcium and calcium excretion in normal, parathyroidectomised and thyroparathyroidectomised rats. Lancet 1, 1237-1238.
Malarkey,W.B., O'Dorisio,T.M., Kennedy,M., and Cataland,S. (1981). The influence of vasoactive intestinal polypeptide and cholecystokinin on prolactin release in rat and human monolayer cultures. Life Sci. 28, 2489-2495.
Marchetti,B., and Labrie,F. (1982). Prolactin inhibits pituitary luteinizing hormone-releasing hormone receptors in the rat. Endocrinol. 11, 1209-1216.
Maslar,I.A., Kaplan,B.M., Luciano,A.A., and Riddick,D.H. (1980). Prolactin production by the endometrium of early human pregnancy. J. Clin. Endocrinol. Metab. 51, 78-83.
Matusik,R.J., and Rosen,J.M. (1978). Prolactin induction of casein mRNA in organ culture. J. Biol. Chem. 253, 2343-2347.
Matusik,R.J., and Rosen,J.M. (1980). Prolactin regulation of casein gene expression: possible mediators. Endocrinol. 106, 252-259.
McFarland,R.J. (1979). Gastrointestinal hormones and disease of the gastrointestinal tract. Clin. Endocrinol. Metab. 8, 331-347.
McGarry,E.E., and Beck,J.C. (1972). Biological effects of nonprimate prolactin and human placental lactogen. In: Wolstenholme,G.E.W., and Knight,J., eds. Lactogenic hormones. Ciba foundation symposium. Livingstone, London, pp. 361-383.
McNatty,K.P. (1979). Relationship between plasma prolactin and the endocrine microenvironment of the developing human antral follicle. Fert. Steril. 32, 433-438.
Miller,W.L., and Eberhardt,N.L. (1983). Structure and evolution of the growth hormone gene family. Endocr. Rev. 4, 97-130.
Mitchell,R., Grieve,G., Dow,R., and Fink,G. (1983). Endogenous GABA receptor ligands in hypophysial portal blood. Neuroendocrinol. 37, 169-176.
Mori,M., Vigh,S., Miyata,A., Yoshihara,T., Oka,S., and Arimura,A. (1990). Oxytocin is the major prolactin releasing factor in the posterior pituitary. Endocrinol. 125, 1009-1013.
Morishige,W.K., and Rothchild,I. (1974). Temporal aspects of the regulation of corpus luteum function by luteinizing hormone, prolactin and placental luteotrophin during the first half of pregnancy in the rat. Endocrinol. 95, 260-274.
Murai,I., and Ben-Jonathan,N. (1986). Chronic posterior pituitary lobectomy: prolonged elevation of plasma prolactin and interruption of cyclicity. Neuroendocrinol. 43, 453-458.
Murai,I., and Ben-Jonathan,N. (1987). Posterior pituitary lobectomy abolishes the suckling-induced rise in prolactin (PRL): evidence for a PRL-releasing factor in the posterior pituitary. Endocrinol. 121, 205-211.
Murphy,P.R., DiMattia,G.E., and Friesen,H.G. (1988). Role of calcium in prolactin-stimulated c-myc gene expression and mitogenesis in Nb_2 lymphoma cells. Endocrinol. 122, 2476-2485.
Nagy,G., Mulchahey,J.J., and Neill,J.D. (1988). Autocrine control of prolactin secretion by vasoactive intestinal peptide. Endocrinol. 112, 364-366.

Nicoll,C.S. (1974). Physiological actions of prolactin. In: Knobil,E., and Sawyer,W.H., eds. The pituitary gland and its neuroendocrine control IV. American Physiological society, Washington, pp. 253-292.

Nicoll,C.S., Anderson,T.R., Hebert,N.J., and Russel,S.M. (1985). Comparative aspects of the growth-promoting actions of prolactin on its target organs: evidence for synergism with an insulin-like growth factor. In: MacLeod,R.M., Thorner,M.O., and Scapagnini,U., eds. Prolactin. Basic and clinical correlates. Fidia Research Series vol. I, Liviana Press, Padova, pp. 393-410.

Nicoll,C.S., Mayer,G.L., and Russel,S.M. (1986). Structural features of prolactins and growth hormones that can be related to their biological properties. Endocr. Rev.7, 169-203.

Nikolics,K., Mason,A.J., Szönyi,E., Ramachandran,J., and Seeburg,P. (1985). A prolactin-inhibiting factor within the precursor for human gonadotropin-releasing hormone. Nature 316, 511-517.

Ofenstein,J.P., Rillema,J.A., and Lawson,D.M. (1985). Prolactin stimulation of Nb_2 node lymphoma cell division is inhibited by polyamine biosynthesis inhibitors. Proc. Soc. Exp. Biol. Med. 180, 236-239.

Oka,T., and Perry,J.W. (1976). Studies on the regulatory factors of ornithine decarboxylase activity during the development of mouse mammary gland epithelium in vitro. J. Biol. Chem. 251, 1738-1744.

Olivier,C., Mical,R.S., and Porter,J.C. (1977). Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. Endocrinol. 101, 598-604.

Pilotte,N.S., and Porter,J.C. (1981). Dopamine in hypophysial portal plasma and prolactin in systemic plasma of rats treated with 5-hydroxytryptamine. Endocrinol. 108, 2137-2141.

Posner,B.I., Bergeron,J.J.M., Josefsberg,Z., Khan,M.N., Kahn,R.J., Patel,B.A., Sikstrom,R.A., and Verma,A.K. (1981). Polypeptide hormones: intracellular receptors and internalization. Rec. Progr. Horm. Res. 37, 539-582.

Posner,B.I., Josefsberg,Z., and Bergeron,J.J.M. (1979). Intracellular polypeptide hormone receptors; characterization and induction of lactogen receptors in the golgi apparatus of rat liver. J. Biol. Chem. 254, 12494-12499.

Pukac,L.A., and Horseman,N.D. (1984). Regulation of pigeon crop gene expression by prolactin. Endocrinol. 114, 1718-1724.

Ramaswamy,S., and Bapna,J.S. (1987). A study of the possible mechanism of antidiuretic activity of prolactin in rats. Med. Sci. Res. 15, 1387-1388.

Riddick,D.H. (1985). Regulation and physiological relevance of nonpituitary prolactin. In: MacLeod,R.M., Thorner,M.O., and Scapagnini,U., eds. Prolactin. Basic and clinical correlates. Fidia Research Series vol. I, Liviana Press, Padova, pp. 463-473.

Riddle,O., Bates,R.W., and Dykshorn,S.W. (1933). The preparation, identification and assay of prolactin, a hormone of the anterior pituitary. Am. J. Physiol. 105, 191-216.

Rillema,J.A. (1987). Actions of prolactin on molecular processes. CRC Press, Boca Raton, Fla.

Rillema,J.A. (1980). Calcium requirement for prolactin actions on ribonucleic acid and casein synthesis in mouse mammary gland explants. Endocrinol. 106, 1360-1364.

Rillema,J.A. (1979a). Actions of quinacrine on RNA and casein synthesis in mouse mammary gland explants. Prostagl. Leukotr. Med. 2, 155-164.

Rillema,J.A. (1979b). Inhibitions of prolactin actions in mouse mammary gland explants by p-bromphenacyl bromide, a phospholipase A_2 inhibitor. Proc. Soc. Exp. Biol. Med. 161, 355-357.

Rillema,J.A. (1976). Effects of prostaglandins on RNA and casein synthesis in mammary gland explants of mice. Endocrinol. 99, 490-495.

Rillema,J.A. (1975). Possible role of prostaglandin $F_{2\alpha}$ in mediating the effect of prolactin on RNA synthesis in mammary gland explants of mice. Nature 253, 466-467.

Rillema,J.A., Etindi,R.N., and Cameron,C.M. (1986). Prolactin actions on casein and lipid biosynthesis in mouse and rabbit mammary gland explants are abolished by p-bromphenacyl bromide and quinacrine, phospholipase A_2 inhibitors. Horm. Metabol. Res. 18, 672-674.

Rillema,J.A., and Foley,K.A. (1983). Characteristics of the action of prolactin on ^{3}H-thymidine incorporation into DNA in mammary gland explants from virgin mice. Horm. Metabol. Res. 15, 385-388.

Rillema,J.A., Foley,K.A., and Etindi,R.E. (1985). Temporal sequence of prolactin actions on phospholipid biosynthesis in mouse mammary gland explants. Endocrinol. 116, 511-515.

Rillema,J.A., Linebaugh,B.E., and Mulder,J.A. (1977). Regulation of casein synthesis by polyamines in mammary gland explants of mice. Endocrinol. 100, 529-536.

Rillema,J.A., Wing,L.C., and Foley K.A. (1983). Effects of phospholipases on ornithine decarboxylase activity in mammary gland explants from midpregnant mice. Endocrinol. 113, 2024-2028.

Rivier,C., Brown,M., and Vale,W. (1977). Effect of neurotensin, substance P and morphine sulfate on the secretion of prolactin and growth hormone in the rat. Endocrinol. 100, 751-754.

Rogol,A.D. (1975). Three components of immunoreactive and receptor active plasma prolactin in man. Univ. Milan. Ric. Sci. Educaz Permamente (Suppl.) 1, 139-144.

Rogol,A.D., and Rosen,S.W. (1974). Prolactin of apparent large molecular size: The major immunoactive prolactin component in plasma of a patient with a pituitary tumor. J. Clin. Endocrinol. Metab. 38, 714-717.

Rudland,P.S., Bennett,D.C., and Warburton,M.J. (1980). Growth and differentiation of cultured rat mammary epithelial cells. In: Hormones and cancer; ed. Iacobelli et al., Raven Press, New York, 255-269.

Russel,D.H. (1988). Prolactin and immunomodulation. In: Hoshino,K., ed., Prolactin gene family and its receptors. Excerpta Medica 819, Kyoto, pp. 155-166.

Russel,D.H., Kibler,R., Matrisian,L., Larson,D.F., Poulos,B., and Magun,B,E. (1985). Prolactin receptors on rat lymphoid tissues and on human T- and B-lymphocytes: antagonism of prolactin binding by cyclosporine. In: MacLeod, R.M., Thorner,M.O., and Scapagnini,U., eds. Prolactin. Basic and clinical correlates. Fidia Research Series vol. I, Liviana Press, Padova, pp. 375-384.

Russel,D.H., and McVicker,T.A. (1972). Polyamine biogenesis in the rat mammary gland during pregnancy and lactation. Bioch. J. 130, 71-76.

Sapag-Hagar,M., Greenbaum,A.L., Lewis,D.J., and Hallowes,R.C. (1974). The effects of di-butyryl cAMP on enzymatic and metabolic changes in explants of rat mammary tissues. Biochem. Biophys. Res. Commun. 59, 261-268.

Schramme,C., and Denef,C. (1984). Stimulation of spontaneous and dopamine inhibited prolactin release from anterior pituitary reaggregate cell cultures by angiotensin peptides. Life Sci. 34, 1651-1658.

Schwartz,M.F., Bauman,J.E., and Masters,W.H. (1982). Hyperprolactinemia and sexual disorders in men. Biol. Psychiatry 17, 861-876.
Sheth,A.R., Mugatwala,P.P., Shah,G.V., Rao,S.S. (1975). Occurence of prolactin in human semen. Fert. Steril. 26, 905-907.
Sheth,A.R., Vaidya,R.A., Raiker,R.S. (1976). Presence of prolactin in human cervical mucus. Fert. Steril. 27, 397-398.
Shiu,R.P.C., Elsholtz,H.P., Tanaka,T., Friesen,H.G., Gout,P.W., Beer,C.T., and Noble,R.L. (1983). Receptor-mediated mitogenic action of prolactin in a rat lymphoma cell line. Endocrinol. 113, 159-165.
Shiu,R.P.C., and Friesen,H.G. (1973). Blockade of prolactin action by antiserum to its receptors. Science 192, 259-261.
Shiu,R.P.C., and Friesen,H.G. (1980). Mechanism of action of prolactin in the control of mammary gland function. Annu. Rev. Physiol. 42, 83-102.
Shome,B., and Parlow,A.F. (1977). Human pituitary prolactin: The entire linear amino acid sequence. J. Clin. Endocrinol. Metab. 45, 1112-1119.
Shrenker,P., and Bartke A. (1987). Effects of hyperprolactinaemia on male sexual behaviour in the golden hamster and mouse. J. Endocrinol. 112, 221-228.
Sinha,Y.N., Selby,F.W., and Vanderlaan,W.P. (1973). Radioimmunoassay of prolactin in the urine of mouse and man. J. Clin. Endocrinol. Metab. 36, 1039-1042.
Spangelo,B.L., Hall,N.R., and Goldstein,A.L. (1985). Evidence that prolactin is an immunomodulatory hormone. In: MacLeod,R.M., Thorner,M.O., and Scapagnini, U., eds. Prolactin. Basic and clinical correlates. Fidia Research Series vol. I, Liviana Press, Padova, pp. 343-349.
Speake,B., Dils,R., and Mayer,R.J. (1976). Interactions of insulin, prolactin and cortisol in controlling the turnover of fatty acid synthetase in rabbit mammary gland in organ culture. Biochem. J. 154, 359-370.
Stricker,P., and Grueter,F. (1928). Action du lobe antérieur de l'hypophyse sur la montée laiteuse. CR Soc. Biol., Paris, 99, 1978-1980.
Suh,H.K., and Frantz,A.G. (1974). Size heterogeneity of human prolactin in plasma and pituitary extracts. J. Clin. Endocrinol. Metab. 39, 928-935.
Tanaka,T., Shiu,R.P.C., Gout,P.W., Beer,C.T., Noble,R.L., and Friesen,H.G.(1980). A new sensitive and specific bioassay for lactogenic hormones: measurement of prolactin and growth hormone in human serum. J. Clin. Endocrinol. Metab. 51, 1058-1065.
Thomas,J.A., and Manandhar,M. (1975). Effects of prolactin and/or testosterone on nucleic acid levels in prostate glands of normal and castrated rats. J. Endocrinol. 65, 149-150.
Topper,Y.J., and Freeman,C.S. (1980). Multiple hormone interactions in the developmental biology of the mammary gland. Physiol. Rev. 60, 1049-1106.
Toubeau,G., Desclin,J., Parmentier,M., and Pasteels,H. (1979). Cellular localization of a prolactin-like antigen in the rat brain. J. Endocrinol. 83, 261-266.
Turkington,R.W. (1970). Stimulation of RNA synthesis in isolated mammary cells by insulin and prolactin bound to sepharose. Biochem. Biophys. Res. Commun. 41, 1362-1367.
Valverde,C.R., Chieffo,V., and Reichlin,S. (1972). Prolactin-releasing factor in porcine and rat hypothalamic tissue. Endocrinol. 91, 982-993.
Vonderhaar,B.K., and Greco,A.E. (1979). Lobuloalveolar development of mouse mammary glands is regulated by thyroid hormones. Endocrinol. 104, 409-418.
Vorherr,H. (1979). Renal and vascular activity of prolactin preparations. Klin. Wochenschr. 57, 101-109.

Webb,C.F., and Wallis,M. (1988). A comparison of lactogenic receptors from rat liver and Nb_2 rat lymphoma cells by using cross-linking techniques. Biochem. J. 250, 215-219.

Weber,R.F.A., Greef,W.J.de, Koning,J.de, and Vreeburg,J.T.M. (1983). LH-RH and dopamine levels in hypophysial stalk plasma and their relationship to plasma gonadotrophins and prolactin levels in male rats bearing a prolactin- and adrenocorticotrophin-secreting pituitary tumor. Neuroendocrinol. 36, 205-210.

Werder,K.von, and Clemm,C. (1974). Evidence of "big" and "little" components of circulating immunoreactive prolactin in humans. FEBS Lett. 47, 181-184.

West,B., and Dannies,P.S. (1980). Effects of estradiol on prolactin production in primary cultures of rat pituitary cells. Endocrinol. 106, 1108-1113.

Westendorf,J.M., and Schonbrunn,A. (1982). Bombesin stimulates prolactin and growth hormone release by pituitary cells in culture. Endocrinol. 110, 352-358.

Williams-Ashman,H.G., Janne,J., Coppoc,G.L., Geroch,M.E., and Schenone,A. (1972). New aspects of polyamine biosynthesis in eukaryotic organisms. Adv. Enzyme Regul., 10225-232.

Wing,L-Y,C., and Rillema,J.A. (1983). Prostaglandin stimulation of ornithine decarboxylase activity in mammary gland explants from midpregnant mice. Prostaglandins 25, 321-333.

Ymer,S.I., and Herington,A.C. (1986). Binding and structural characteristics of a soluble lactogen binding protein from rabbit mammary gland cytosol. Biochem. J. 237, 813-820.

Zeilmaker,G.H., and Carlsen,R.A. (1962). Experimental studies on the effect of ergocornine methanesulphonate on the luteotrophic function of the rat pituitary gland. Acta Endocrinol. 41, 321-35.

CHAPTER II

EXPERIMENTAL MODELS EMPLOYED TO STUDY THE EFFECTS OF HYPERPROLACTINAEMIA ON MALE REPRODUCTIVE FUNCTIONS

Early during the 1970s it became clear that PRL is a distinct pituitary hormone in humans (Guyda et al., 1971; Hwang et al., 1972). Subsequent clinical investigations revealed an unexpectedly frequent association of hyperPRLaemia with sexual dysfunctions, like decreased libido and potency (Modebe, 1989; Perryman and Thorner, 1981; Schwartz et al., 1982; Thorner et al., 1974). Since PRL can similarly suppress male sexual behaviour in the rat, copulatory behaviour of this animal has been extensively studied during hyperPRLaemia (for reviews: Adler, 1986; Drago, 1984). In addition, elevated levels of PRL have been found to suppress the secretion of gonadotrophic hormones leading to varying degrees of gonadal dysfunctions (Bartke et al., 1977 & 1985; Fang et al., 1974; Katovich et al., 1985; Sharpe et al., 1980). Attempting to study the endocrine mechanisms underlying these deleterious effects on male reproductive functions, investigators have used different experimental models of hyperPRLaemia in the rat. In this animal high serum levels of PRL have been experimentally induced and maintained during a long period of time by pituitary grafts, drugs and PRL-secreting tumours.

2.1. Pituitary grafts

Already in 1939 Loeb and Kirtz introduced the pituitary grafted rat as an endocrine model for studies on hormonally mediated mammary tumours (Loeb and Kirtz, 1939).

They described that multiple ectopic pituitary grafts increased the incidence of mammary tumours in mice. During the early fifties, Everett reported luteotrophic effects in rats after autotransplantation of the anterior pituitary under the kidney capsule (Everett, 1954). Many years later, serum levels of PRL appeared to be increased in hypophysectomized rats bearing ectopic pituitary grafts (Chen et al., 1970). This rise in serum PRL was found to be positively correlated with the number of the pituitaries implanted, about 80-100 ng PRL/ml plasma for each gland (De Greef and Zeilmaker, 1978). Such levels could be maintained for a period of at least 6 months (Bartke et al., 1977). Several authors reported that after the transplantation procedure an immediate but transient rise occurred in the serum concentrations of LH, FSH and other pituitary hormones during the first hours or days after transplantation, while hyperPRLaemia arose gradually as the grafted pituitary gland became revascularized and started the secretion almost exclusively of PRL (Advis and Ojeda, 1979; De Jong and Van der Schoot, 1979). Thus, effects attributed to pituitary grafts are likely to be due to the hyperPRLaemia induced.

Intrasellar "eutopic" pituitary PRL concentration is decreased in rats bearing pituitary grafts under the kidney capsule (Adler and Sokol, 1983). An increase of dopamine, known as the PRL inhibiting factor, however, could not be demonstrated in pituitary stalk plasma of such rats (Cramer et al., 1979). Apparently moderately elevated PRL levels do not markedly influence dopamine release by the hypothalamic tubero-infundibular dopaminergic neurons into the pituitary portal plexus.

In pituitary grafted rats a mild reduction of gonadotrophin secretion has been reported without affecting testosterone secretion and testicular size (Bartke et al., 1977). In such rats, moreover, minor effects on copulatory behaviour have been described (Bailey and Herbert, 1982; Drago, 1984). It is possible that in the rat the PRL levels obtained with pituitary grafts are too low to affect sexual behaviour distinctly. In hyperPRLaemic men with sexual dysfunctions, PRL levels are much higher than those gained in these rats. In such men serum PRL at the time of initial diagnosis is usually in the range of hundreds and not infrequently thousands of ug/l (Buvat et al., 1985; Perryman and Thorner, 1981; Spark et al., 1982; Modebe, 1989).

In other experimental models, to be described, much higher serum levels of PRL have been obtained, and these are usually associated with more marked deleterious effects on male sexual behaviour and on gonadotrophin release and testicular function.

2.2. Drugs

2.2.1. Estrogens

In man estrogen medication for female hypogonadism and prostatic carcinoma induces moderate hyperPRLaemia within a short period of time (Frantz et al., 1972; Robyn and Vekemans, 1976; Yen et al., 1974). Experimental estrogen treatment in rats results in hyperplasia of mammotrophic cells in the pituitary and PRL-secreting adenomata (Phelps and Hymer, 1983; Sarkar et al., 1982; Wiklund and Gorski, 1982). Male rats show an elevation of plasma PRL levels approximately 100-fold within 15 weeks after subcutaneous insertion of capsules filled with diethylstilbestrol (DES, Bartke et al., 1984). After subsequent removal of the capsules, increased plasma PRL levels remained in the range of 1-15 ug/ml for at least 3 months. During this period the DES-treated animals exhibited a temporary state of hypogonadotrophic hypogonadism, while an unexpectedly mild suppression of male sexual behaviour was observed.

Despite the extremely high serum PRL levels reached after estrogen treatment, this experimental model shows shortcomings for studying the effects of hyperPRLaemia on male sexual behaviour and gonadotrophin secretion in several respects. Since exogenous estrogens are able to activate and maintain male sexual behaviour (Baum and Vreeburg, 1973; Södersten, 1973), it can be argued that suppressive effects of hyperPRLaemia on male copulatory behaviour may be obscured during the DES-treatment and a certain period thereafter. Moreover, the accompanying pituitary enlargement may disrupt pituitary activity and disconnect the gland from its hypothalamic regulation by displacement of surrounding tissue. Thus antigonadotrophic effects observed in estrogen-treated animals may not be attributable to the resultant hyperPRLaemia alone.

2.2.2. Other drugs

The loss of libido and potency observed during the treatment of psychotic patients with neuroleptics has been associated with the neuroleptic-induced hyperPRLaemia (Malmnas, 1973). In the rat, chronic administration of the dopamine antagonist domperidon results in serum PRL levels between 200 and 500 ng/ml, and in suppression of male sexual behaviour (Bailey and Herbert, 1982). The effects of such treatment on copulatory behaviour were mild, similar to those found in pituitary grafted rats. All animals remained sexually active, but displayed prolonged latencies to mount and to ejaculate. In another study, the anti-dopaminergic drugs haloperidol and bromperidol also raised serum PRL levels to a comparable degree as pituitary grafts, but had different effects on copulatory behaviour as had excess PRL from pituitary grafts (Drago and Scapagnini, 1985). It is quite possible that individual drugs may affect male sexual behaviour independently of their hyperPRLaemia-inducing capacity, thereby hampering the interpretation of the effects observed.

2.3. PRL-secreting tumours

An experimental approach other than those previously described, inducing severe hyperPRLaemia without mechanical or drug-induced side-effects on hypothalamic-adenohypophysial function, would allow investigation of the effects of hyperPRLaemia on male sexual function more reliably. Subcutaneously transplantable PRL-secreting tumours are likely to meet these requests.

2.3.1. Tumours used in other studies

Transplantable pituitary tumours cause a rapid elevation of the PRL levels in blood. Within a few weeks after tumour inoculation, serum PRL levels may rise to micrograms

per milliliter (Hodson et al., 1980; Kalra et al., 1983; Weber et al., 1982a & 1982b). In rats bearing the PRL- and growth hormone-secreting tumour MtTW15, the markedly increased concentrations of serum PRL are associated with a pronounced suppression of gonadotrophin secretion and decreased testicular functions (Clark and Kalra, 1985; Hodson et al., 1980; Katovich et al., 1985). In such rats, there is almost complete loss of male sexual behaviour (Kalra et al., 1983).

In previous studies with the PRL- and ACTH-secreting tumour 7315a, antigonadotrophic effects and inhibition of male copulatory behaviour were found to be minor and mainly dependent on the presence of the adrenals (Weber et al., 1982a & 1982b). It can be speculated upon that these discrepancies with observations made by other investigators (Hodson et al., 1980; McNeilly et al., 1980; Shrenker and Bartke, 1985) may be due to bioactive products secreted by tumour 7315a in addition to PRL.

2.3.2. Tumour 7315b: a new model of hyperPRLaemia

Thus, so far, both ectopic transplantation of pituitaries, drug treatment and inoculation of transplantable PRL-secreting tumours, devised as models for hyperPRLaemia in the rat, have appeared not to be completely satisfactory for studying male reproductive functions in the hyperPRLaemic rat. As far as reported, investigators using PRL-secreting tumours could not preclude effects of factors, co-secreted in addition to PRL by the tumour inoculated, obscuring or enhancing the actions of hyperPRLaemia studied.

Recently, we obtained a cell line derived from tumour 7315a, which has been originally characterized as a purely PRL-secreting cell line, and was called 7315b (Lamberts et al., 1984)[1]. This new experimental model is introduced in this thesis on the study of chronic hyperPRLaemia and reproductive functions in the male rat.

[1]*Note: At a later stage of the investigations, evidence emerged that tumour 7315b has begun to secrete some immuno-assayable ACTH (cf. chapters VII and VIII). Experimental data presented in this thesis should be interpreted in light of this recent observation.*

2.4. References

Adler,R.A. (1986). The anterior pituitary-grafted rat: A valid model of chronic hyperprolactinemia. Endocr. Rev. 7, 302-313.

Adler,R.A., and Sokol,H.W. (1983). Studies of anterior pituitary-grafted rats: I. Abnormal prolactin response to thyrotropin releasing hormone, clonidine, insulin, and fasting. Life Sciences 32, 2949-2956.

Advis,J.P., and Ojeda,S.R. (1979). Acute and delayed effects of anterior pituitary transplants in inducing precocious puberty in the female rat. Biol. Reprod. 20, 879-887.

Bailey,D.J., and Herbert,J. (1982). Impaired copulatory behavior of male rats with hyperprolactinemia induced by domperidone or pituitary grafts. Neuroendocrinol. 35, 186-193.

Bartke,A., Doherty,P.C., Steger,R.W., Morgan,W.W., Amador,A.G., Herbert,D.C., Siler-Khodr,T.M., Smith,M.S., Klemcke, H.G., and Hymer,W.C.H. (1984). Effects of estrogen-induced hyperprolactinemia on endocrine and sexual functions in adult male rats. Neuroendocrinol. 39, 126-135.

Bartke,A., Klemcke,H., and Matt,K. (1985). Effects of physiological and abnormally elevated prolactin levels on the pituitary-testicular axis. Med. Biol. 63, 264-272.

Bartke,A., Smith,M.S., Michael,S.D., Peron,F.G., and Dalterio,S. (1977). Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinol. 100, 182-186.

Baum,M.J., and Vreeburg,J.T.M. (1973). Copulation in castrated male rats following combined treatment with estradiol and dihydrotestosterone. Science 182, 283-285.

Buvat,J., Lemaire,A., Buvat-Herbaut,M., Fourlinnie,J.C., Racadot,A., and Foscati P. (1985). Hyperprolactinemia and sexual function in men. Horm. Res. 22, 196-203.

Chen,C.L., Amenomori,Y., Lu,K.H., Voogt,J.L., and Meites,J. (1970). Serum prolactin levels in rats with pituitary transplants or hypothalamic lesions. Neuroendocrinol. 6, 220-226.

Clark,J.T., and Kalra,P.S. (1985). Effects on penile reflexes and plasma hormones of hyperprolactinemia induced by MtTW15 Tumors. Horm. Beh. 19, 304-310.

Cramer,O.M., Parker,C.R., and Porter,J.C. (1979). Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinol. 105, 636-640.

Drago,F. (1984). Prolactin and sexual behavior: a review. Neurosc. Biobeh. Rev. 8, 433-439.

Drago,F., and Scapagnini,U. (1985). Side effects of drugs stimulating prolactin secretion on the behavior of male rats. Arch. Int. Pharmacodyn. and Therapeut. 276, 271-278.

Everett,J.W. (1954). Luteotrophic function of autografts of the rat hypophysis. Endocrinol. 54, 685-690.

Fang,V.S., Refetoff,S., and Rosenfield,R.L. (1974). Hypogonadism induced by a transplantable, prolactin-producing tumor in male rats: Hormonal and morphological studies. Endocrinol. 95, 991-998.

Frantz,A.G., Kleinberg,D.L., and Noel,G.L. (1972). Studies on prolactin in man. Rec. Prog. Horm. Res. 28, 527-590.

Greef,W.J.de, and Zeilmaker,G.H. (1978). Regulation of prolactin secretion during the luteal phase in the rat. Endocrinol. 102, 1190-1195.

Gudelsky,G.A., and Porter,J.C. (1980). Release of dopamine from tubero-infundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. Endocrinol. 106, 526-529.
Guyda,H., Hwang,P., and Friesen,H.G. (1971). Immunologic evidence for monkey and human prolactin. J. Clin. Endocrinol. Metab. 32, 120-123.
Hodson,C.A., Burden,H.W., Louis,T.M., Poole,M., and Lawrence,I.E.,Jr.(1981). Inhibition of hypothalamic LHRH depletion after ovariectomy by transplantable prolactin- and growth-hormone-secreting tumors. Proc. Soc. Exp. Biol. Med. 167, 369-373.
Hodson,C.A., Simpkins,J.W., Pass,K.A., Aylsworth,C.F., Steger,R.W., and Meites,J. (1980). Effects of a prolactin-secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in malerats. Neuroendocrinol. 30, 7-10.
Hwang,P., Guyda,H., and Friesen,H.G. (1972). Purification of human prolactin. J. Biol. Chem. 247, 1955-1958.
Jong,R.A.P.de, and Schoot,P.van der. (1979). Advancement of sexual maturation in male rats by pituitary transplants. Biol. Reprod. 21, 1263-1271.
Kalra,P.S., and Kalra,S.P. (1987). The effect of hyperprolactinemia produced by transplantable pituitary MtTW15 tumor cells in male rats on hypothalamic luteinizing hormone-releasing hormone release in vitro: effects of naloxone and K^+. Endocrinol. 121, 310-315.
Kalra,P.S., Simpkins,J.W., Luttge,W.G., and Kalra,S.P. (1983). Effects on male sexual behavior and preoptic dopamine neurons of hyperprolactinemia induced by MtTW15 pituitary tumors. Endocrinol. 113, 2065-2071.
Katovich,M.J., Cameron,D.F., Murray,F.T., and Gunsalus,G.L. (1985). Alterations of testicular functions induced by hyperprolactinemia in the rat. J. Androl. 6, 179-189.
Lamberts, S.W.J., Uitterlinden,P., Bons,E.G., Zuiderwijk,J.M., Verleun,T., Oosterom,R., and Hackeng,W.H.L. (1984). Hyperprolactinemia exerts a negative effect on the ß-endorphin content of the rat neurointermediate pituitary lobe. Endocrinol. 114, 2349-2353.
Loeb,L., and Kirtz,M.M. (1939). The effects of transplants of anterior lobes of the hypophyses on the growth of the mammary gland and on the development of mammary gland carcinoma in various strains of mice. Am. J. Cancer 36, 56-60.
Malmnas,C.O. (1973). Monoaminergic influence on testosterone activated copulatory behavior in the castrated male rat. Acta Physiol. Scand. Suppl. 395.
McNeilly,A.S., Sharpe,R.M., and Fraser,H.M. (1980). Effect of adrenalectomy or castration on the inhibition of gonadotrophin secretion induced by hyperprolactinaemia in the adult male rat. J. Endocrinol., 85, 83-92.
Modebe,O. (1989). Serum prolactin concentration in impotent african males. Androl. 21, 42-47.
Perryman,R.L., and Thorner,M.O. (1981). The effects of hyperprolactinemia on sexual and reproductive function in men. J. Androl. 5, 233-242.
Phelps,C., and Hymer,W.C. (1983). Characterization of estrogen-induced adenohypophysial tumors in the Fisher 344 rat. Neuroendocrinol. 37, 23-31.
Robyn,C., and Vekemans,M. (1976). Influence of low-dose estrogen on circulating prolactin, LH and FSH levels in post-menopausal women. Acta Endocrinol. 83, 9-14.
Sarkar,D.K., Gotschall,P.E., and Meites,J. (1982). Damage to hypothalamic dopaminergic neurons is associated with development of prolactin-secreting pituitary tumors. Science 218, 684-686.
Schwartz,M.F., Bauman,J.E., and Masters,W.H. (1982). Hyperprolactinemia and sexual disorders in men. Biol. Psychiatry 17, 861-876.

Sharpe,R.M., and McNeilly,A.S., Davidson,D.W., and Swanston,I.A. (1980). Leydig cell function in hyperprolactinemic adult rats. J. Endocrinol. 87, 28-35.
Shrenker,P., and Bartke,A. (1985). Adrenalectomy does not prevent the hyperprolactinemic induced sexual behavior deficits in CDF male rats. Life Sciences 36, 1881-1888.
Spark,R.F., Wills,C.A., O'Reilly,G., Ransil,B.J., and Bergland,R. (1982). Hyperprolactinemia in males with and without pituitary macroadenomas. Lancet i, 129-132.
Södersten,P. (1973). Estrogen activated sexual behavior in male rats. Horm. Beh. 4, 247-253.
Thorner,M.O., McNeilly,A.S., Hagan,C., and Besser,G.M. (1974). Long term treatment of galactorrhoea and hypogonadism with bromocriptine. Brit. Med. J. ii, 419-422.
Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982a). Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex organ weights in adult male rats: A possible role of the adrenals. Endocrinol. 111, 412-417.
Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982b). Effects of a prolactin-secreting tumour on copulatory behaviour in male rats. J. Endocrinol. 93, 223-229.
Wiklund,J.A., Gorski,J. (1982). Genetic differences in estrogen-induced deoxyribonucleic acid synthesis in the rat pituitary: correlations with pituitary tumor susceptibility. Endocrinol. 111, 1140-1149.
Yen,S.S.C., Ehara,Y., and Siter,T.M. (1974). Augmentation of prolactin secretion by estrogen in hypogonadal women. J. Clin. Invest. 53, 652-655.

CHAPTER III

EFFECTS OF HYPERPROLACTINAEMIA ON MALE REPRODUCTIVE FUNCTIONS

Chronic excess of serum PRL has been associated with disturbance of reproductive functions both in women (Jacobs, 1976) and men (Bancroft et al., 1984; Perryman and Thorner, 1981; Spark et al., 1982). The prevalence of hyperPRLaemia among men who present with impotence ranges between 1 and 26.5 % (Ambrosi et al., 1980; Miller et al., 1980; Modebe, 1989; Schwartz et al., 1982). As discussed in chapter II, the male rat provides a suitable model to study sexual behaviour during hyperPRLaemia. Generally accepted standards used for such studies are briefly described in chapter IV (for review: Van der Schoot and Kooy, 1988).

3.1. PRL and male sexual behaviour

Most investigators studying effects of PRL on male sexual behaviour have utilized intact or castrated testosterone-treated male rats with one or more isogeneic pituitary glands transplanted under the renal capsule. Both increased (Drago et al., 1981) and decreased (Doherty et al., 1986) sexual activity have been recorded at 5-7 days after pituitary transplantation. Such early occurring effects, however, may not be attributable to hyperPRLaemia alone, since various other pituitary hormones are released during the first days after grafting (Advis and Ojeda, 1979; De Jong and Van der Schoot, 1979).

The chronic presence of pituitary grafts affects copulatory behaviour in several respects. Effects that have been described are generally small (Bailey and Herbert, 1982). It has been reported that pituitary-grafted rats showed reduced mounting and

intromission rates and, therefore, increased ejaculation latencies (Bailey et al., 1984; Doherty et al., 1985a, 1985b & 1986). Also during genital anaesthesia mounting rates appeared reduced in animals bearing pituitary grafts (Doherty et al., 1986). Observations with pituitary-grafted rats injected with the dopamine agonist bromocriptine, to suppress PRL secretion, or rats injected with ovine PRL, confirmed that hyperPRLaemia was indeed causally related to the pituitary graft-induced effects on copulatory behaviour (Doherty et al., 1981 & 1985b). In tests of penile activity ex copula (Hart, 1968), pituitary-grafted rats displayed fewer erections but no other differences from controls (Doherty et al., 1986). Erectile function thus examined in rats with pituitary transplants was not different from that of controls after spinal transection. From the combined in copula and ex copula tests it was concluded that hyperPRLaemia, established through pituitary grafts, depressed sexual motivation and erectile function. The latter effect seemed dependent on supraspinal influences of PRL.

Further investigations revealed that pituitary grafts did not affect testicular hormonal activity (Doherty et al., 1986). Also possible effects of PRL on testosterone metabolism seemed to play no critical role, since copulatory behaviour deficiencies remained when, after castration, testosterone was replaced by combined treatment with dihydrotestosterone and estradiol to induce copulation (Doherty et al., 1985a). However, a recent in vitro study concerning the formation of dihydrotestosterone from precursor testosterone has shown a reduction of this conversion in the amygdala of hyperPRLaemic rats, but not in the hypothalamus or caudal spinal cord (Bailey et al., 1984). Thus, local activities of testosterone-metabolizing enzymes in certain areas of the central nervous system may be decreased in hyperPRLaemia.

While PRL acts in concert with corticosteroids on different target tissues, experiments with adrenalectomized rats indicated that adrenal glands played no critical role in the occurrence of the effects of the pituitary grafts on copulatory behaviour and gonadotrophin release (Bailey et al., 1984; Shrenker and Bartke, 1985).

Estrogen treatment of rats is well known to rapidly induce pituitary enlargement and increased secretion of PRL. One study examined sexual behaviour in rats which had been treated for 15-20 weeks with a high dose of DES (diethylstilbestrol; Bartke et al., 1984). The DES-induced elevation of PRL (more than 1 μg PRL/ml plasma) was retained during the period with sexual behaviour tests (7-14 weeks after cessation of DES treatment) allowing for the study of effects of chronic hyperPRLaemia on sexual

behaviour. Two tests with eleven rats revealed relatively minor effects on copulatory behaviour. Latencies to the onset of copulation were prolonged and the intromission rates were reduced, while the numbers of intromissions prior to ejaculation, the intromission efficiency (= the percentage of the mountings resulting in intromissions) and the duration of the post ejaculatory interval were not affected in these severely hyperPRLaemic animals. In view of the much longer existence and much higher blood concentrations of PRL in these rats, compared with the pituitary-graft-bearing rats mentioned above, copulatory behaviour deficiencies were not impressive. Further study in these animals revealed normal testicular hormonal activity despite persistent severe hyperPRLaemia (Bartke et al., 1984). As discussed in chapter II, the effects found in estrogen-treated animals may not be attributable to the resultant hyperPRLaemia alone.

Chronic treatment of male rats with the dopamine antagonist domperidone resulted in a moderate elevation of plasma PRL and in minor effects on sexual behaviour, similar to those described in pituitary-grafted animals (Bailey and Herbert, 1982; Bailey et al., 1984).

Transplantable pituitary tumours can secrete massive amounts of PRL into the bloodstream, resulting in serum PRL levels of 1 μg /ml and more within a few weeks after tumour inoculation. Most of these tumours, however, have been demonstrated to secrete other products into the circulation, some of them with unequivocal biological effects such as adrenal gland enlargement in rats inoculated with MtTW15 (Hodson et al., 1980) and 7315a (Weber et al., 1982a). In the latter study, despite the rapid and non-suppressible outgrowth of the tumour, tumour-bearing rats adrenalectomized before tumour inoculation did not show an evident deterioration of their sexual behaviour, while their non-adrenalectomized counterparts displayed marked copulatory deficiencies during the tumour-induced hyperPRLaemia. Consequently, these suppressive effects on male sexual behaviour can not be claimed to be due to the debilitating effects induced by the fast outgrowth of the tumour itself. In the non-adrenalectomized tumour-bearing rats increased numbers of mounts without intromission and prolonged ejaculation latencies were found, while the numbers of intromissions prior to ejaculation remained unchanged. These findings seem to indicate that a relative inability to intromit contributes to the copulatory deficits in hyperPRLaemia. Interestingly, a failure of penile reflexes has been found in intact male rats bearing the PRL-secreting tumour MtTW15 within 35 days after inoculation (Clark and Kalra, 1985).

The above investigations clearly demonstrate that high serum levels of PRL are generally related to a suppression of male sexual function in the rat. However, little is known about the mechanisms by which PRL exerts its effects. A possible role of the adrenals as an intermediate to PRL-induced effects on copulatory behaviour has been claimed by Weber et al. (1982b). The possible ways in which PRL might affect adrenal function have not been further evaluated in that study, but may involve increases of adrenocortical steroidogenesis.

HyperPRLaemia resulting from pituitary grafts occurs without pronounced effects on testicular testosterone production. However, some other actions on the CNS-hypophysial-testicular axis were recorded, such as an increase in the density of testicular LH/HCG receptors (Bartke et al., 1984; Doherty et al., 1982), elimination of stress-induced testosterone secretion (Bartke et al., 1983) and altered testosterone metabolism in the amygdala, but not in the hypothalamus (Bailey et al., 1984). In contrast, tumour-induced hyperPRLaemia may lead to hypogonadotrophic hypogonadism (Fang et al., 1974; Hodson et al., 1980 & 1981; Clark and Kalra, 1985), which disappears after surgical removal of the tumour (Katovich et al., 1985). Hypogonadism, as seen in rats with PRL-secreting tumours, can be the explanation for hyperPRLaemia-induced effects on penile reflexes in view of their dependence on androgens (Hlíñak et al., 1979; Meisel et al., 1984). However, hypogonadism cannot completely account for hyperPRLaemia-induced effects on copulation, since treatment with testosterone does not normalize the altered copulatory behaviour (Bailey et al., 1984; Doherty et al. 1985a).

3.2. PRL and organs involved in the regulation of male sexual functions

HyperPRLaemic men with sexual dysfunctions have been found to have hypogonadism and decreased pituitary sensitivity to LHRH (Winters and Troen, 1984). The mechanisms through which PRL affects male reproductive functions have not yet been clarified. In order to investigate these mechanisms many studies have been carried out in experimental animals of different species (for review: Drago, 1984). From studies in the rat it has become clear that PRL can affect the neuroendocrine regulation of male sexual functions. In this section the sites of PRL action possibly involved are discussed.

3.2.1. Effects of PRL on the hypothalamic-hypophysial axis

PRL is known to exert a negative feedback effect on its own secretion by the pituitary gland (for review: De Greef and Van der Schoot, 1985). This autoregulation is not likely to occur at the pituitary level (Vician et al., 1982), but probably at different sites in the hypothalamus resulting in an increased activity of tubero-infundibular dopaminergic neurons (Demarest et al., 1984; Gudelsky and Porter, 1980; Kishi et al. 1984; Selmanoff, 1985). Indeed, dopamine levels increase in hypophysial stalk plasma of rats inoculated subcutaneously with a PRL-secreting tumour (Cramer et al., 1979; Weber et al., 1983) and in animals which had been injected with PRL into the lateral ventricles of the brain (Gudelsky and Porter, 1980). It is of interest that dopaminergic neurons have their highest density in the lateral palisade zone where axo-axonic apposition with LHRH-containing terminals has been demonstrated (Ajika, 1979; Selmanoff, 1985). This suggests that dopaminergic activity may play a neuromodulatory role in LHRH release, which is perhaps of importance for the suppressive effects of hyperPRLaemia on gonadotrophin secretion.

Another hypothalamic system known to be influenced by hyperPRLaemia is the opioid system (Sarkar and Yen, 1985a; Sweeney et al. 1985). Both during lactation and after inoculation of a PRL-secreting tumour, hyperPRLaemia in rats induces a decrease of ß-endorphin and met-enkephalin concentrations in the hypothalamus (Panerai et al., 1980). In view of the inhibiting action exerted by opioids on LH secretion (Cicero et al., 1979; Spencer and Whitehead, 1986), and the fact that opioid antagonists like naloxone can counteract the anti-gonadotrophic effects of hyperPRLaemia (Carter et al., 1984), increased release of opioids from hypothalamic stores may be involved in the reduction of serum LH and FSH during hyperPRLaemia. Indeed, Sarkar and Yen (1985a) found a significant increase of the ß-endorphin-like immunoreactivity in pituitary portal plasma of rats bearing ectopic pituitary glands. Since much evidence exists that ß-endorphin is inhibitory to LHRH neurons (Carter et al., 1984; Sarkar and Yen, 1985b), high levels of PRL are likely to suppress gonadotrophin release by increasing the ß-endorphin inhibitory tone on LHRH-secreting neurons in the hypothalamus. However, it still remains unclear whether PRL acts mainly directly or indirectly on the opioid system (Rasmussen et al., 1988).

In addition to the hypothalamic mechanisms involved in PRL-induced effects on gonadotrophin secretion, PRL may also exert effects on the gonadotroph proper. Pituitary responsiveness to LHRH stimulation (Kalra and Kalra, 1987; McNeilly et al., 1978; Winters and Loriaux, 1978) and pituitary LHRH-receptor content (Clayton and Bailey, 1982; Marchetti and Labrie, 1982; Fox et al., 1987) have been shown to be decreased in the presence of high blood levels of PRL. In most in vitro studies, however, it has proven impossible to demonstrate that these functional changes in the gonadotrophs are caused by a direct action of PRL at the pituitary cell level (Bennet and Sundberg, 1983; Hetzel et al., 1987). On the other hand, extremely high levels of PRL (40.000 - 80.000 ng/ml) in an incubation medium appeared to inhibit LHRH-stimulated LH secretion by isolated rat pituitaries (Cheung, 1983). The latter unusual findings, however, are probably due to aspecific effects of the extremely high PRL concentrations in the medium, since otherwise these effects should also have been observed by other workers using PRL levels ranging from 50 up to 750 ng/ml (Bennet and Sundberg, 1983; Hetzel et al., 1987), enough to suppress gonadotrophin secretion in vivo (Adler, 1986).

3.2.2. Effects of PRL on adrenal function

Already in 1973 Piva and others described that PRL stimulates adrenal progesterone secretion (Piva et al., 1973). In the years thereafter, the existence of specific PRL-binding sites in the adrenal (Calvo et al., 1981) and the ability of PRL to influence adrenocortical activity have been reported with increasing frequency (Colby, 1979; Eldridge and Lymangrover, 1984; Mann et al., 1977; Mazzocchi et al., 1986). The role of PRL as an adrenocortical secretagogue is of great interest beyond the scope of this thesis, since adrenal steroids can act to modify pituitary gonadotrophin release (Ringstrom and Schwartz, 1985; Vreeburg et al., 1984). To which extent changes in adrenal steroid output may actually contribute to the suppression of pituitary gonadotrophin release in hyperPRLaemic rats, will be discussed later, in light of the experiments reported in chapter V.

In addition to the adrenocortical effects, chronic increase of the peripheral PRL

concentrations in pituitary grafted female rats has also been associated with adrenomedullar activation, reflected by increased plasma levels of catecholamines (Fernandez-Ruiz et al., 1987). This phenomenon has also been observed in chicks injected with ovine PRL (Maiti and Bose-Mitra, 1980). Recently, direct effects of PRL on catecholamine synthesis and release in rat adrenal medulla have been suggested (Fernandez-Ruiz et al., 1988). This latter study indicated that PRL affects adrenomedullary activity in vitro, depending on the previous plasma levels of this hormone. In addition, PRL may also exert an adrenomedullary effect by activation of the hypothalamic-adenohypophysial-adrenal axis (Adler, 1986), through an enhanced secretion of ACTH due to an increased synthesis and secretion of CRF by hypothalamic neurons. Such a mechanism would account for the observation that both PRL (Clayton and Bailey, 1982; Grandison et al., 1977; Hodson et al., 1981) and CRF (Gambacciani et al., 1986; Gindoff and Ferin, 1987; Rivier and Vale, 1984) inhibit the release of LHRH and gonadotrophins and exert an inhibitory influence on sexual behaviour (Sirinathsinghji, 1987). Further experiments focussed on this subject are described later (chapter VI).

3.2.3. Effects of PRL on gonadal function

PRL has been demonstrated to bind specifically to Leydig cells in the rat testis (Aragona et al., 1977; Barkey et al., 1977; Morris and Saxena, 1980) as well as in the human tesis (Bouhdiba et al., 1989). Furthermore, in the rat, hyperPRLaemia has been related to altered testicular morphology (Fang et al., 1974; Hodson et al., 1980), altered spermatogenic function (Sharpe and McNeilly, 1979), and decreased serum levels of androgen binding protein (Katovich et al., 1985). It may, therefore, be postulated that hyperPRLaemia directly affects testicular functions, in addition to indirect effects through the hypothalamic-hypophysial and adrenal pathways described.

PRL is known to raise the number of testiculair LH/HCG receptors in the rat (Aragona et al., 1978; Bartke et al., 1985; Zipf et al., 1978), thereby augmenting the ability of the testes to respond to LH stimulation (Bartke and Dalterio, 1976; Purvis et al., 1979). Interestingly, moderately decreased serum levels of LH and FSH in pituitary

grafted rats do not affect testosterone secretion by the testes (Bartke et al., 1977; McNeilly et al., 1983; Weber et al., 1987), possibly because of the increased sensitivity of the testes in these animals to LH stimulation. However, when a more pronounced suppression of plasma gonadotrophins is reached, as in rats inoculated with a PRL-secreting tumour, testicular androgen production is inhibited (Clark and Kalra, 1985; Hodson et al., 1980; Katovich et al., 1985). An augmented testicular sensitivity to LH stimulation, claimed to be present in hyperPRLaemia, cannot compensate for the lack of stimulation in these animals with markedly decreased plasma gonadotrophins, and, therefore, cannot prevent a state of hypogonadotrophic hypogonadism in the severely hyperPRLaemic rat.

Many morphological testicular alterations have been described in hyperPRLaemic rats, such as seminiferous epithelial disorganization, germ cell exfoliation, increased tubule wall thickness, abnormal Leydig cell lipid content, structural disruption of Sertoli-germ cell junctional complexes and apical Sertoli cell cytoplasmic degeneration (Fang et al., 1974; Hodson et al., 1980; Katovich et al., 1985; Wilson, 1971).

The alterations in testicular secretory functions and testicular morphology reported can be due both to direct PRL effects on the testis and to indirect PRL-induced changes in the control of gonadotrophin release. To which extent alterations in testicular functions actually contribute to sexual dysfunctions in the hyperPRLaemic male rat is discussed in light of the experiments on male sexual behaviour, described in chapter IV.

3.2.4. Effects of PRL on the accessory sex glands

A well-known property of PRL in male mammals, especially during sexual maturation, is its ability to potentiate the effects of androgens on the growth of the ventral prostate and seminal vesicles (Baranao et al., 1981; Bartke, 1980; Coert et al., 1985; Holland and Lee, 1980; Moger and Geschwind, 1972; Negro-Vilar et al., 1977). In several species, like the mouse (Bohnet and Friesen, 1976), the hamster (Bex and Bartke, 1977) and the rat (Zipf et al., 1978), this action may be augmented by a stimulatory effect of PRL on testicular testosterone release. This effect may be due to increased sensitivity of the

testis to LH stimulation and/or caused by a direct stimulatory action of PRL on the Leydig cells (Bartke, 1980). In castrated animals PRL can synergize with exogenous androgens and enhance the growth of male accessory reproductive organs (Thomas and Keenan, 1976). Moreover, PRL alone also has direct trophic effects on the ventral prostate and seminal vesicles in castrated male rats (Bartke and Lloyd, 1970; Negro-Vilar et al., 1977). The finding that these purely PRL-induced stimulating effects on accessory sex organs are relatively small in the absence of endogenous or exogenous androgens may be partially explained by the demonstration that PRL binding to prostatic membranes and cytosol is androgen dependent (Charreau et al., 1977; Kledzik et al., 1976). In addition to these synergistic trophic effects of PRL with androgens, PRL has recently been reported also to act in concert with testosterone and dihydrotestosterone on the prostatic secretion of secretory acid phosphatase and citric acid in the adult rat (Srinivasan et al., 1987).

Effects of pathologically raised serum levels of PRL on the organ weights of the accessory sex glands in the rat are discussed later in this thesis (cf. chapter V).

3.3. Aims and experimental designs of studies to be reported in this thesis

In this thesis the results are described of experiments designed to obtain answers to the following questions, which are dealt with in the chapters indicated:

Chapter IV:

1. Does a low serum testosterone concentration contribute to the deterioration of male sexual behaviour in the hyperPRLaemic male rat ?
2. Which is the role played by the adrenals in the suppression of male copulatory behaviour during hyperPRLaemia ?
3. Is hyperPRLaemia able to suppress androgen-induced male copulatory behaviour in female rats ?

Chapter V:

1. To what extent do testicular and adrenal functions participate in the suppression of gonadotrophin secretion by severe hyperPRLaemia ?
2. Are the effects of elevated serum PRL levels on gonadotrophin secretion mainly determined by an action on the central nervous system ?
3. Are effects of PRL on gonadotrophin secretion in gonadectomized and adrenalectomized rats sex-dependent ?

Chapter VI:

1. Does PRL activate CRF-secreting neurons to exert its deleterious effects on reproductive functions in rats bearing pituitary grafts or tumour 7315b ?
2. Does both models of hyperPRLaemia enhance hypothalamic CRF release into hypophysial stalk plasma and consequently pituitary ACTH secretion ?

Chapter VII:

1. Are the adrenocorticotrophic effects in hyperPRLaemia due to a centrally activated CRF-ACTH-adrenal axis ?
2. Can increased ACTH levels in pituitary-grafted rats and in rats bearing tumour 7315b be suppressed by treatment with high doses of corticosteroids ?
3. Can PRL stimulate adrenal growth independently of pituitary function ?

3.4. References

Adler,R.A. (1986). The anterior pituitary-grafted rat: A valid model of chronic hyperprolactinemia. Endocr. Rev. 7, 302-313.

Advis,J.P., and Ojeda,S.R. (1979). Acute and delayed effects of anterior pituitary transplants in inducing precocious puberty in the female rat. Biol. Reprod. 20, 879-887.

Ajika,K. (1979). Simultaneous localization of LHRH and catecholamines in rat hypothalamus. J. Anatomy 128, 331-347.

Ambrosi,B., Gaggini,M., Moriondo,P., and Faglia,G. (1980). Prolactin and sexual function. J.A.M.A. 244, 2608.

Aragona,C., Bohnet,H.G., and Friesen,H.G. (1977). Localization of prolactin binding in prostate and testis. The role of serum prolactin concentration on the testicular LH receptor. Acta Endocrinol. 84, 402-409.

Bailey,D.J., Dolan,A.L., Pharaoh,P.D.P., and Herbert,J. (1984). Role of gonadal and adrenal steroids in the impairment of the male rat's sexual behavior by hyperprolactinemia. Neuroendocrinol. 39, 555-562.

Bailey,D.J., and Herbert,J. (1982). Impaired copulatory behavior of male rats with hyperprolactinemia induced by domperidone or pituitary grafts. Neuroendocrinol. 35, 186-193.

Bancroft,J., O'Carroll,R., McNeilly,A, and Shaw,R.W. (1984). The effects of bromocriptine on the sexual behavior of hyperprolactinemic man: a controlled case study. Clin. Endocrinol. 21, 131-138.

Baranao,J.L.S., Legnani,B., Chianzzi,V.A., Bertini,L.M., Suescun,M.O., Calvo., J.C., Charreau,E.H., and Calandra,R.S. (1981). Effects of prolactin on androgen metabolism in androgen target tissue of immature rats. Endocrinol. 109, 2188-2195.

Barkey,R.J., Shani,J., Amit,T., and Barzilai,D. (1977). Specific binding of prolactin to seminal vesicle, prostate and testicular homogenates of immature, mature and aged rats. J. Endocrinol. 74, 163-173.

Bartke,A. (1980). Role of prolactin in reproduction in male mammals. Fed. Proc. 38, 2577-2581.

Bartke,A., and Dalterio,S. (1976). Effects of prolactin on the sensitivity of the testis to LH. Biol. Reprod. 15, 90-93.

Bartke,A., Doherty,P.C., Steger,R.W., Morgan,W.W., Amador,A.G., Herbert,D.C., Siler-Khodr,T.M., Smith,M.S., Klemcke, H.G., and Hymer,W.C.H. (1984). Effects of estrogen-induced hyperprolactinemia on endocrine and sexual functions in adult male rats. Neuroendocrinol. 39, 126-135.

Bartke,A., Klemcke,H., and Matt,K. (1985). Effects of physiological and abnormally elevated prolactin levels on the pituitary-testicular axis. Med. Biol. 63, 264-272.

Bartke,A., and Lloyd,C.W. (1970). The influence of pituitary homografts on the weight of sex accessories in castrated male mice and rats and on mating behaviour in male mice. J. Endocrinol. 46, 313-320.

Bartke,A., Smith,M.S., Michael,S.D., Peron,F.G., and Dalterio,S. (1977). Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinol. 100, 182-186.

Bartke,A., Svare,B.B., Doherty,P.C., Smith,M.S., and Klemcke,H.G. (1983). Effects of hyperprolactinaemia on male reproductive functions. In: Negro-Vilar A., ed., Reproduction and Andrology, Raven Press, New York, p. 1.

Bennet,B.A., and Sundberg,K.D. (1983). Hypothalamic catecholamine biosynthesis and pituitary gonadotropin secretion in vitro: effect of hyperprolactinemia. Mol. Cell. Endocrinol. 30, 149-160.
Bex,F.J., and Bartke,A. (1977). Testicular LH-binding in the hamster: modifications by photoperiod and prolactin. Endocrinol. 100, 1223-1226.
Bohnet,H.G., and Friesen,H.G. (1976). Effect of prolactin and growth hormone on prolactin- and LH-receptors in the dwarf mouse. J. Reprod. Fertil. 48, 307-311.
Bouhdiba,M., Leroy-Martin,B., Peyrat,J.Ph., Saint Pol,P., Dijane,J., and Leonardelli,J. (1989). Immunohistochemical detection of prolactin and its receptors in human testis. Androl. 21, 223-228.
Buvat,J., Lemaire,A., Buvat-Herbaut,M., Fourlinnie,J.C., Racadot,A., and Foscati P. (1985). Hyperprolactinemia and sexual function in men. Horm. Res. 22, 196-203.
Calvo,J.C., Finocchiaro,L., Luthy,I., Charreau,E.H., Callandra,R.S., Engstrom,B., and Hansson,V. (1981). Specific prolactin binding in the rat adrenal gland: its characterization and hormonal regulation. J. Endocrinol. 89, 317-321.
Carter,D.A., Cooper,J.S., Inkster,S.E., and Whitehead,S.A. (1984). Evidence for an increased opioid inhibition of LH secretion in hyperprolactinaemic ovariectomized rats. J. Endocrinol. 101, 57-61.
Charreau,E.H., Attramadal,A., Torjesen,P.A., Purvis,K., Calandra,R., and Hansson,V. (1977). Prolactin binding in rat testis: specific receptors in interstitial cells. Mol. Cell. Endocrinol. 6, 303-307.
Cheung,C.Y. (1983). Prolactin suppresses luteinizing hormone secretion and pituitary responsiveness to luteinizing hormone releasing hormone by a direct action at the anterior pituitary. Endocrinol. 113, 632-638.
Cicero,T.J., Schainker,B.A., and Meyer,E.R. (1979). Endogenous opioids participate in the regulation of the hypothalamic-pituitary-luteinizing hormone axis and testosterone's negative feedback control of luteinizing hormone. Endocrinol. 104, 1286-1291.
Clark,J.T., and Kalra,P.S. (1985). Effects on penile reflexes and plasma hormones of hyperprolactinemia induced by MtTW15 Tumors. Horm. Behav. 19, 304-310.
Clayton,R.N., and Bailey,L.C. (1982). Hyperprolactinaemia attenuates the gonadotrophin releasing hormone receptor response to gonadectomy in rats. J. Endocrinol. 95, 267-274.
Coert,A., Nievelstein,H., Kloosterboer,H.J., Loonen,P., and Van der Vies,J. (1985). Effects of hyperprolactinemia on the accessory sexual organs of the male rat. The Prostate 6, 269-276.
Colby,H.D. (1979). Mechanism of action of prolactin on adrenocortical steroid secretion in hypophysectomized female rats. Endocrinol. 104, 1299-1303.
Cramer,O.M., Parker,C.R., and Porter,J.C. (1979). Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinol. 105, 636-640.
Damassa,D.A., Smith,E.R., Tennent,B., and Davidson,J.M. (1977). The relationship between circulating testosterone levels and male sexual behavior in rats. Horm. Behav. 8, 275-286.
Demarest,K.T., Riegle,G.D. and Moore,K.E. (1984). Prolactin-induced activation of tubero-infundibular dopaminergic neurons: evidence for both a rapid 'tonic' and delayed 'induction' component. Neuroendocrinol. 38, 467-475.
Doherty,P.C., Bartke,A., Hogan,M.P., Klemcke,H., and Smith,M.S. (1982). Effects of hyperprolactinemia on copulatory behavior and testicular human chorionic gonadotropin binding in adrenalectomized rats. Endocrinol. 111, 820-826.

Doherty,P.C.,Bartke,A., and Smith,M.S. (1981). Differential effects of bromocriptine treatment on LH release and copulatory behavior in hyperprolactinemic male rats. Horm. Behav. 15, 436-450.
Doherty,P.C., Bartke,A., and Smith,M.S. (1985a). Hyperprolactinemia and male sexual behavior: Effects of steroid replacement with estrogen plus dihydrotestosterone. Phys. Behav. 35, 99-104.
Doherty,P.C., Bartke,A., Smith,M.S., and Davis,S.L. (1985b). Increased serum prolactin levels mediate the suppressive effects of ectopic pituitary grafts on copulatory behavior in male rats. Horm. Behav. 19, 111-121.
Doherty,P.C., Baum,M.J., and Todd,R.B. (1986). Effects of chronic hyperprolactinemia on sexual arousal and erectile function in male rats. Neuroendocrinol. 42, 368-375.
Drago,F. (1984). prolactin and sexual behavior: a review. Neurosc. Biobeh. Rev. 8, 433-439.
Drago,F., Pellegrini-Quarantotti,B., Scapagnini,U., and Gessa,G.L. (1981). Short-term endogeneous hyperprolactinemia and sexual behavior of male rats. Phys. Behav. 26, 277-281.
Eldridge,J.C., and Lymangrover,J.R. (1984). Prolactin stimulates and potentiates adrenal steroid secretion in vitro. Horm. Res. 20, 252-255.
Fang,V.S., Refetoff,S., and Rosenfield,R.L. (1974). Hypogonadism induced by a transplantable, prolactin-producing tumor in male rats: Hormonal and morphological studies. Endocrinol. 95, 991-998.
Fernandez-Ruiz,J.J., Cebeira,M., Agrasal,C., Tresguerres,J.A., Esquifino,A.I., and Ramos,J.A. (1987). Effect of elevated prolactin levels on the synthesis and release of cathecholamines from the adrenal medulla in female rats. Neuroendocrinol. 45, 208-212.
Fernandez-Ruiz,J.J., Martinez-Arrieta,R., Hernandez,M.L., and Ramos,J.A. (1988). Possible direct effect of prolactin on catecholamine synthesis and release in rat adrenal medulla: in vitro studies. J. Endocrinol. Invest. 11, 603-608.
Fox,S.R., Hoefer,M.T., Bartke,A., and Smith,M.S. (1987). Suppression of pulsatile LH secretion, pituitary GnRH receptor content and pituitary responsiveness to GnRH by hyperprolactinemia in the male rat. Neuroendocrinol. 350-359.
Gambacciani,M., Yen,S.S.C., and Rasmussen,D.D. (1986). GnRH release from the mediobasal hypothalamus: in vitro inhibition by corticotropin-releasing factor. Neuroendocrinol. 43, 533-536.
Gindoff,P.R., and Ferin,M. (1987). Endogenous opioid peptides modulate the effects of corticotropin-releasing factor on gonadotropin release in the primate. Endocrinol. 121, 837-842.
Grandison,L., Hodson,C., Chen,H.T., Advis,J.,Simpkins,J., and Meites,J. (1977). Inhibition by prolactin of postcastration rise in LH. Neuroendocrinol. 23, 312-322.
Greef,W.J.de, and Schoot,P.van der. (1985). Some recent developments in the study of prolactin in mammals. In: Wimersma Greidanus,Tj.B.v., ed., Front. Horm. Res., Karger, Basel, vol. 14, pp. 70-99.
Greef,W.J.de, and Zeilmaker,G.H. (1978). Regulation of prolactin secretion during the luteal phase in the rat. Endocrinol. 102, 1190-1195.
Gudelsky,G.A., and Porter,J.C. (1980). Release of dopamine from tubero-infundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. Endocrinol. 106, 526-529.
Hart,B.L. (1968). Sexual reflexes and mating behavior in the male rat. J. Comp. Phys. Psychol. 65, 453.

Hetzel,W.D., Schneider,P.M. and Pfeiffer,E.F. (1987). Further evidence that prolactin does not affect gonadotropin release at pituitary level. Horm. Metabol. Res. 19, 555-562.

Hlíñak,Z., Madlafousek,J., and Mohapelová,A. (1979). Initiation of copulatory behavior in castrated male rats injected with critically adjusted doses of testosterone. Horm. Behav. 13, 9-20.

Hodson,C.A., Burden,H.W., Louis,T.M., Poole,M., and Lawrence,I.E.,Jr. (1981). Inhibition of hypothalamic LHRH depletion after ovariectomy by transplantable prolactin and growth-hormone-secreting tumors. Proc. Soc. Exp. Biol. Med. 167, 369-373.

Hodson,C.A., Simpkins,J.W., Pass,K.A., Aylsworth,C.F., Steger,R.W., and Meites,J. (1980). Effects of a prolactin secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in malerats. Neuroendocrinol. 30, 7-10.

Holland,J.M., and Lee,C. (1980). Effects of pituitary grafts on testosterone stimulated growth of rat prostate. Biol. Reprod. 22, 351-355.

Jacobs,H.S. (1976). Prolactin and amenorrhea. New Engl. J. Med. 295, 954-956.

Jong,R.A.P.de, and Schoot,P.van der. (1979). Advancement of sexual maturation in male rats by pituitary transplants. Biol. Reprod. 21, 1263-1271.

Kalra,P.S., and Kalra,S.P. (1987). The effect of hyperprolactinemia produced by transplantable pituitary MtTW15 tumor cells in male rats on hypothalamic luteinizing hormone-releasing hormone release in vitro: effects of naloxone and K^+. Endocrinol. 121, 310-315.

Kalra,P.S., Simpkins,J.W., Luttge,W.G., and Kalra,S.P. (1983). Effects on male sexual behavior and preoptic dopamine neurons of hyperprolactinemia induced by MtTW15 pituitary tumors. Endocrinol. 113, 2065-2071.

Katovich,M.J., Cameron,D.F., Murray,F.T., and Gunsalus,G.L. (1985). Alterations of testicular functions induced by hyperprolactinemia in the rat. J. Androl. 6, 179-189.

Kishi,K., and Kobayashi,F. (1984). Role of prolactin in controlling prolactin surges in pseudopregnant rats. Biol. Reprod. 30, 879-885.

Kledzik,G.S., Marshall,S., Campbell,G.A., Gelato,M., and Meites,J.(1976). Effects of castration, testosterone, estradiol and prolactin on specific prolactin-binding activity in ventral prostate of male rats. Endocrinol. 98, 373-379.

Maiti,B.R., and Bose-Mitra,K. (1980). Adrenomedullary and glycemic response to prolactin in chicks. Mikroskopie 36, 107-112.

Mann,D.R., Cost,M.G., Jacobson,C.D., and MacFarland,L.A. (1977). Adrenal gland rhythmicity and pituitary regulation of adrenal steroid secretion. Proc. Soc. Exp. Biol. Med. 156, 441-445.

Marchetti,B., and Labrie,F. (1982). Prolactin inhibits pituitary luteinizing hormone-releasing hormone receptors in the rat. Endocrinol. 111, 1209-1216.

Mazzocchi,G., Robba,C., Rebuffat,P., and Nussdorfer,G.G. (1986). Effects of prolactin administration on the zona glomerulosa of the rat adrenal cortex: stereology and plasma hormone concentrations. Acta Endocrinol. 111, 101-105.

McNeilly,A.S., Sharpe,R.M., Davidson,D.W., and Fraser,H.M. (1978). Inhibition of gonadotrophin secretion by induced hyperprolactinaemia in the male rat. J. Endocrinol. 79, 59-63.

McNeilly,A.S., Sharpe,R.M., and Fraser,H.M. (1983). Increased sensitivity to the negative feedback effects of testosterone induced by hyperprolactinemia in the adult male rat. Endocrinol. 112, 22-28.

Meisel,R.L., O'Hanlon,J.K., and Sachs,B.D. (1984). Differential maintenance of penile responses and copulatory behavior by gonadal hormones in castrated male rats. Horm. Behav. 18, 56.
Miller,J.B., Howards,S.S., and Macleod,R.M. (1980). Serum prolactin in organic and psychogenic impotence. J. Urol. 123, 862.
Moger,W.H., and Geschwind, I.U. (1972). The action of prolactin on the sex accessory glands of the male rat. Proc. Soc. Exp. Biol. Med. 141, 1017-1021.
Modebe,O. (1989). Serum prolactin concentration in impotent african males. Androl. 21, 42-47.
Morris,P.L., and Saxena,B.B. (1980). Dose- and age-dependent effects of prolactin (PRL) on luteinizing hormone- and PRL-binding sites in rat Leydig cell homogenates. Endocrinol. 107, 1639-1645.
Negro-Vilar,A., Saad,W.A., and McCann,S.M. (1977). Evidence for a role of prolactin in prostate and seminal vesicle growth in immature male rats. Endocrinol. 100, 729-737.
Panerai,A.E., Petraglia,F., Sacerdote,P., and Genazzani,A.R. (1985). Mainly mu-opiate receptors are involved in luteinizing hormone and prolactin secretion. Endocrinol. 117, 1096-1099.
Panerai,A.E., Sawynok,J., LaBella,F.S., and Friesen,H.G. (1980). Prolonged hyperprolactinemia influences ß-endorphin and met-enkephalin in the brain. Endocrinol. 106, 1804-1808.
Perryman,R.L., and Thorner,M.O. (1981). The effects of hyperprolactinemia on sexual and reproductive function in men. J. Androl. 5, 233-242.
Piva,F.P., Gagliano,M.M., and Martini,L. (1973). Adrenal progesterone: factors controlling its secretion. Endocrinol. 93, 1178-1182.
Purvis,K., Claussen,O.P.F., Olsen,A., Haug,E., and Hansson,V. (1979). Prolactin and Leydig cell responsiveness to LH/HCG in the rat. Arch. Androl. 3, 219-230.
Rasmussen,D.D., Kennedy,B.P., Ziegler,M.G., and Nett,T.M. (1988). Endogenous opioid inhibition and facilitation of gonadotropin-releasing hormone release from the median eminence in vitro: potential role of catecholamines. Endocrinol. 123, 2916-2921.
Ringstrom,S.J. and Schwartz,N.B. (1985). Cortisol suppresses the LH, but not the FSH, response to gonadotropin-releasing hormone after orchidectomy. Endocrinol. 116, 472-474.
Rivier,C., and Vale,W. (1984). Influence of corticotropin-releasing factor on reproductive functions in the rat. Endocrinol. 114, 914-921.
Sarkar,D.K., and Yen,S.C.C. (1985a). Hyperprolactinemia decreases the luteinizing hormone-releasing hormone concentration in pituitary portal plasma: a possible role for ß-endorphin as a mediator. Endocrinol. 116, 2080-2084.
Sarkar,D.K., and Yen,S.C.C. (1985b). Changes in ß-endorphin-like immunoreactivity in pituitary portal blood during the estrous cycle and after ovariectomy in rats. Endocrinol. 116, 2075-2079.
Schoot,P. van der, and Kooy,A. (1988). Current topics in the study of sexual behavior in rats. In: Sitsen,J.M.A., ed., Handbook of Sexology. vol. 6: The pharmacology and endocrinology of sexual function, Elsevier, Amsterdam - New York - London, pp. 145-192.
Schwartz,M.F., Bauman,J.E., and Masters,W.H. (1982). Hyperprolactinemia and sexual disorders in men. Biol. Psychiatry 17, 861-876.

Selmanoff,M. (1985). Rapid effects of hyperprolactinemia on basal prolactin secretion and dopamine turnover in the medial and lateral median eminence. Endocrinol. 116, 1943-1952.

Sharpe,R.M., and McNeilly,A.S. (1979). The effects of induced hyperprolactinemia on Leydig cell function and LH-induced loss of LH receptors in the rat testis. Mol. Cell. Endocrinol. 16, 19-27.

Sharpe,R.M., and McNeilly,A.S., Davidson,D.W., and Swanston,I.A. (1980). Leydig cell function in hyperprolactinaemic adult rats. J. Endocrinol. 87, 28-35.

Shrenker,P., and Bartke,A. (1985). Adrenalectomy does not prevent the hyperprolactinemic, induced sexual behavior deficits in CDF male rats. Life Sciences 36, 1881-1888.

Sirinathsinghji,D.J.S. (1987). Inhibitory influence of corticotropin releasing factor on components of sexual behavior in the male rat. Brain Res. 407, 185-190.

Spark,R.F., Wills,C.A., O'Reilly,G., Ransil,B.J., and Bergland,R. (1982). Hyperprolactinemia in males with and without pituitary macroadenomas. Lancet i, 129-132.

Spencer,G.M. and Whitehead,S.A. (1986). A comparison of the effects of gonadal steroids on naloxone-induced LH secretion in gonadectomized rats. J. Endocrinol. 110, 327-334.

Srinivasan,N., Aruldhas,M.M., and Govindarajulu,P. (1987). Interaction of sex steroids and prolactin on phosphatases, transaminases, and citric acid in the ventral prostate of male albino rats. The Prostate 11, 23-31.

Suter,D.E., and Schwartz,N.B. (1985). Effects of glucocorticoids on responsive-ness of luteinizing hormone and follicle-stimulating hormone to gonadotropin releasing hormone by male rat pituitary cells in vitro. Endocrinol. 117, 855-859.

Svare,B., Bartke,A., Doherty,P.C., Mason,I., Michael,S.D., and Smith,M.S. (1979). Hyperprolactinemia suppresses copulatory behavior in male rats and mice. Biol. Reprod. 21, 529-535.

Sweeney,C.A., Morgan,W.W., Smith,M.S., and Bartke,A. (1985). Altered sensitivity to an opiate antagonist, naloxon, in hyperprolactinemic male rats. Neuroendocrinol. 41, 1-6.

Thomas,J.A., and Keenan,E.J. (1976). Prolactin influences upon androgen action in male accessory sex organs. In: Singhal,R.I., and Thomas, J.A., eds., Cellular mechanisms modulating gonadal hormone action, Baltimore, University Park Press, pp. 425-470.

Vician,L., Lieberman,M.E., and Gorski,J. (1982). Evidence that autoregulation of prolactin production does not occur at the pituitary level. Endocrinol. 110, 722-726.

Voogt,J.L., De Greef,W.J., Visser,T.J., De Koning,J., Vreeburg,J.T.M., and Weber,R.F.A. (1987). In vivo release of dopamine, LHRH and TRH in male rats bearing a prolactin-secreting tumor. Neuroendocrinol., 46, 110-115.

Vreeburg,J.T.M., De Greef,W.J., Ooms,M.P., Van Wouw,P., and Weber,R.F.A (1984). Effects of adrenocorticotropin and corticosterone on the negative feedback action of testosterone in the adult male rat. Endocrinol. 115, 977-983.

Weber,R.F.A., De Greef,W.J., De Koning,J., and Vreeburg,J.T.M. (1983). LH-RH and dopamine levels in hypophysial stalk plasma and their relationship to plasma gonadotropins and prolactin levels in male rats bearing a prolactin- and adrenocorticotropin-secreting pituitary tumor. Neuroendocrinol. 36, 205-210.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982a). Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex organ weights in adult male rats: A possible role of the adrenals. Endocrinol. 111, 412-417.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982b). Effects of a prolactin-secreting tumour on copulatory behaviour in male rats. J. Endocrinol. 93, 223-229.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1987). The contribution of corticosterone and testosterone to the suppression of serum LH in hyperprolactinaemic adult male rats with pituitary transplants. J. Endocrinol. 113, 111-116.

Wilson,J.T. (1971). Altered rat hepatic drug metabolism after implantation of a pituitary mammotropic tumor (MtT), Walker carcinosarcoma or adenocarcinoma and after removal of the MtT. Endocrinol. 88, 185-194.

Winters,S.W., and Loriaux,D.L. (1978). Suppression of plasma luteinizing hormone by prolactin in the male rat. Endocrinol. 102, 864-868.

Winters,S.J., and Troen,P. (1984). Altered pulsatile secretion of luteinizing hormone in hypogonadal men with hyperprolactinemia. Clin. Endocrinol. 21, 257-263.

Zipf,W.B., Payne,A.H., and Kelch,R.P. (1978). Prolactin, growth hormone and luteinizing hormone in the maintenance of testicular luteinizing hormone receptors. Endocrinol. 103, 595-600.

Horm. Behav. 22, 351-361 (1988)

CHAPTER IV

DETERIORATION OF MALE SEXUAL BEHAVIOUR IN RATS BY THE NEW PROLACTIN-SECRETING TUMOUR 7315B

4.1 Abstract

The effects of hyperPRLaemia on male copulatory behaviour in adult male and female rats were studied. HyperPRLaemia was induced by the transplantable purely PRL-secreting tumour 7315b[1]. Male rats were castrated and received testosterone-filled capsules of different sizes which induced normal and subnormal testosterone levels. After sexual training the rats of the experimental groups were inoculated with tumour 7315b. Three weeks after tumour inoculation high PRL levels (2000-3000 ng/ml) were found. During this hyperPRLaemia ejaculation latency (EL) increased significantly, while the mount frequency and intromission frequency (IF) remained unchanged. Only 9 out of 22 rats ejaculated 19 days after inoculation. Moreover, it appeared that the inhibitory effect of the tumour was as strong in the presence of normal (2.33 ± 0.07 ng/ml) as in the presence of low (0.35 ± 0.01 ng/ml) testosterone levels. The inhibitory effect of tumour 7315b on copulatory behaviour was not influenced by adrenalectomy. In gonadectomized female rats bearing testosterone-filled capsules, tumour 7315b induced PRL levels of about 2000 ng/ml and an almost complete cessation of mounts and intromission patterns 4 weeks after tumour inoculation. It was concluded that tumour 7315b causes a strong inhibitory effect on male copulatory behaviour in male and female rats and that this effect is not

[1]*Note: At a later stage of the investigations, evidence emerged that tumour 7315b has begun to secrete some immuno-assayable ACTH (cf. chapters VII and VIII). Experimental data presented in this thesis should be interpreted in light of this recent observation.*

influenced by the presence of normal or low testosterone levels or removal of the adrenals, suggesting a direct effect of PRL on brain functions.

4.2. Introduction

It is well established that hyperPRLaemia in male rats results in a deterioration of their masculine sexual behaviour (Bailey and Herbert, 1982; Doherty et al., 1986; Shrenker and Bartke, 1985; Weber et al., 1982b). Nevertheless, it is still unclear how hyperPRLaemia affects male copulatory behaviour.

In most investigations high serum PRL was induced by grafting pituitaries (Adler 1986), resulting in a moderate hyperPRLaemia and mostly in relatively slight effects on copulatory behaviour (Drago 1984; Shrenker and Bartke, 1985). Higher levels of PRL, induced by the growth hormone(GH)- and PRL-secreting tumour MtTW15, however, caused an almost complete suppression of male sexual behaviour (Kalra et al., 1983). In contrast, the hyperPRLaemia induced by the adrenocorticotrophin (ACTH)- and PRL-secreting tumour 7315a had only minor effects on male sexual behaviour (Weber et al., 1982b). Despite very high levels of PRL, the rats showed active mounting behaviour, but they had a long ejaculation latency or did not ejaculate at all, since their ability to intromit was severely impaired. Moreover, the inhibitory effects of tumour 7315a were completely dependent on the presence of the adrenals. The absence of a suppressive action of tumour 7315a in adrenalectomized rats is difficult to reconcile with the finding that the inhibitory effects of hyperPRLaemia, induced by pituitary grafts, were not affected by adrenalectomy (Shrenker and Bartke, 1985; Doherty et al. 1982).

Recently, a new tumour derived from tumour 7315a has been characterized by a pure PRL secretion, and was called 7315b (Lamberts et al, 1984). In light of the controversial results obtained with tumour 7315a, we studied the effects of tumour 7315b on mating behaviour of gonadectomized male rats bearing testosterone-filled capsules. The size of the capsules was varied in order to explore whether the effects of hyperPRLaemia were related to serum testosterone levels. In an additional experiment, we investigated whether the effects of tumour 7315b were dependent on the presence of the adrenals.

It is well known that administration of testosterone propionate to gonadectomized female rats induces high levels of mounting behaviour and a limited number of intromission patterns (Södersten, 1972). Since much evidence exists that PRL suppresses male mating behaviour in male rats through direct effects on the central nervous system (Drago, 1984), and since the responsiveness of tuberoinfundibular dopaminergic neurons to the action of PRL is different between male and female rats (Demarest and Moore, 1981), we decided to investigate the effects of tumour 7315b on mounting behaviour in gonadectomized testosterone-treated female rats.

4.3. Materials and methods

4.3.1. Animals

Male and female rats of the Buffalo strain were housed under controlled conditions of temperature (20 °C) and light (14h light, 10h dark). The lights were on between 18.30 and 08.30 h. Standard food and water were always accessible. Adrenalectomized rats received 0.9 % NaCl (w/v) solution for drinking.

Before surgery each male was placed with a gonadectomized female made sexually receptive as previously described (Weber et al., 1982b) for at least 30 minutes in a cage to obtain sexual experience. For each animal to be studied this procedure was carried out 5 times. All male rats ejaculated at least during the last two training-sessions. The female rats of experiment 3 showed active mounting behaviour during the last two pre-experimental tests.

4.3.2. Effects of tumour 7315b on male copulatory behaviour

In order to study the effects of hyperPRLaemia in male rats with subnormal and normal levels of testosterone, 16 (experiment 1a), 14 (experiment 1b) and 14

(experiment 1c) adult male rats were gonadectomized and received a 0.5 cm, 1 cm and 3 cm testosterone-filled silastic capsule (inner diameter: 0.8 mm; outer diameter: 1.4 mm; Talas, Zwolle, The Netherlands), respectively. Four days later (day 0) half of the rats in each experiment were inoculated with tumour 7315b. Behavioral testing took place in the 1st, 2nd and 3rd week after inoculation.

In experiment 2, 15 adult male rats were gonadectomized, adrenalectomized, and received a 1 cm testosterone-filled silastic capsule (inner diameter: 0.8 mm; outer diameter: 1.4 mm) and a corticosterone pellet (40 mg), made as previously described (Weber et al., 1987). Four days later (day 0) tumour inoculation took place in 7 of the 15 rats. Sexual behaviour was tested in the 2nd, 3rd and 4th week after inoculation.

Finally, in experiment 3, 16 adult female rats were gonadectomized and treated with a 1.5 cm testosterone-filled silastic capsule (inner diameter: 1.5 mm; outer diameter: 2.1 mm). Beginning 1 week after surgery, the females underwent 5 preliminary tests for mounting behaviour in order to allow for adaptation to the experimental situation. Subsequently, half of the rats were inoculated with tumour 7315b (day 0). Mounting behaviour was tested in the 1st, 2nd, 3rd and 4th week after inoculation. In all experiments the inoculation of tumour 7315b was carried out with 0.4 ml of tumour suspension, administered subcutanuously at the dorsal side of the neck.

4.3.3. Behavioral testing

All the behavioral tests were carried out weekly during the afternoon in a dimly lit room. After adaptation of the animal to the test-cage for 5 minutes, a receptive female was introduced and the following parameters of male sexual behaviour were scored:

(1) *Contact latency* (CL), i.e. the period between the introduction of the receptive female and the first mount or intromission. If such a sexual contact was not achieved within 15 minutes after introduction of the female, a CL of 900 seconds was scored, and the test was ended.

(2) *Mount frequency* (MF), i.e. the number of mounts preceding ejaculation. A mount has been defined as a climbing with pelvic thrusting without an intromission.
(3) *Intromission frequency* (IF), i.e. the number of intromissions preceding ejaculation.
(4) *Ejaculation latency* (EL), i.e. the period between the initial sexual contact and the first ejaculation.
(5) *Post ejaculatory interval* (PEI), i.e. the time elapsing between ejaculation and the first subsequent mount or intromission. After the first PEI or maximal testing period the test was ended. The animals in experiment 1 and 2 were tested until the PEI was scored, provided ejaculation took place within 30 minutes. Rats who did not achieve an ejaculation within 30 minutes were assigned the maximal EL of (1800-CL) seconds, if they have had sexual contact before, during the behavioral test.

Moreover, data are expressed as the *mount plus intromission rate* (MIR), defined as the MF plus IF divided by the EL. In the female rats of experiment 3, during a testing period of 15 minutes, the CL, the MF, the *intromission pattern frequency* (IF) were scored. The *mount plus intromission pattern rate* (MIR) was calculated by dividing the MF plus IF by the testing period.

4.3.4. Blood sampling and hormone determinations

One or two days after each test blood was taken by puncturing one orbital plexus under ether anaesthesia. Serum PRL was determined by a double-antibody radio-immunoassay (RIA) as described earlier (Weber, et al., 1983) using materials and protocols supplied by the NIADDK. The results are expressed in terms of NIADDK-rat-PRL-RP-1. Serum testosterone was measured by RIA, using the method previously described by Verjans and colleagues (1973). Serum corticosterone was measured by RIA using highly specific antiserum provided by Bioclinical Services Ltd, Cardiff, South Glamorgan (Weber et al., 1987). The interassay coefficients of variation for PRL, testosterone and corticosterone were 15%, 15% and 12%, respectively.

4.3.5. Statistical analysis

The results are presented as means ± S.E.M. The data obtained from our behavioral studies were statistically analyzed with the non-parametric Mann-Whitney-U test and the Kruskal-Wallis one-way analysis of variance by ranks (Siegel, 1956). The serum levels of hormones, were analyzed with the two-way analysis of variance (ANOVA); statistically significant differences were further evaluated by the Duncan's new multiple range test (Kirk, 1968).

4.4. Results

4.4.1. Hormone levels

Inoculation of the tumour resulted in a significant increase in serum PRL in all tumour-bearing rats of experiment 1, 2 and 3 (Figure 4.1.). No significant differences were found between the increments of PRL measured in the male rats of experiment 1a, 1b, 1c and 2. At the end of the experiments high levels of PRL (2533 ± 234 ng/ml) were measured in the tumour-bearing rats. Compared with experiment 1 and 2 serum PRL increased rather slowly in the tumour-bearing female rats (Figure 4.1.c.). Nevertheless, very high levels were reached in these animals during the 3rd (862 ± 141 ng/ml) and 4th (1842 ± 452 ng/ml) week after inoculation.

The serum levels of the administered hormones were not significantly different either between or within the experimental and control group in all experiments. Testosterone levels in experiment 1b (0.81 ± 0.03 ng/ml) were significantly ($p<0.01$) higher than in experiment 1a (0.35 ± 0.01 ng/ml), but significantly ($p<0.01$) lower than in experiment 1c (2.33 ± 0.07 ng/ml). Testosterone and corticosterone levels in the animals of experiment 2 were 0.53 ± 0.04 ng/ml and 56.7 ± 2.3 ng/ml, respectively. Testosterone treatment in the female rats of experiment 3 resulted in serum levels of 3.84 ± 0.11 ng/ml.

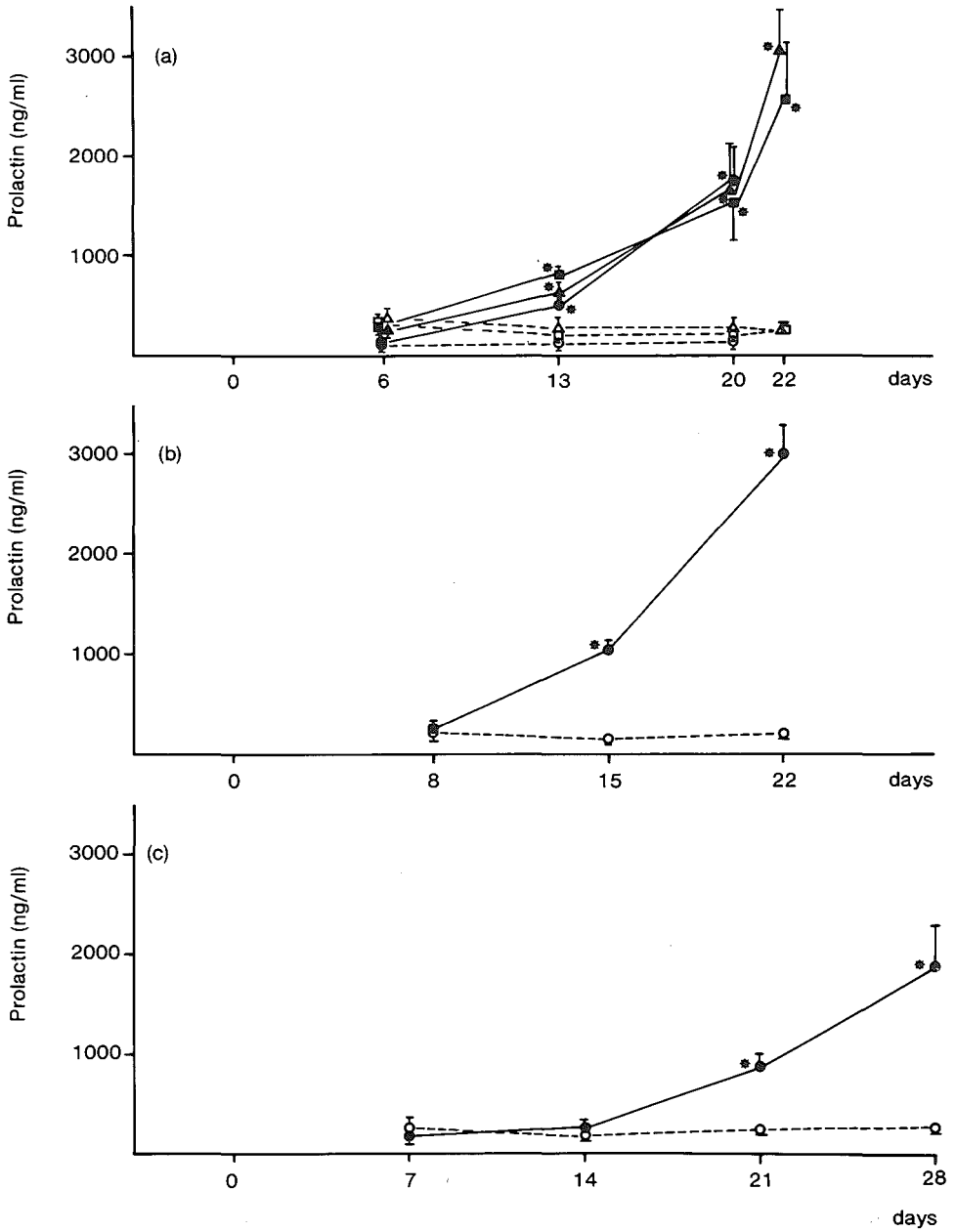

*Figure 4.1. Serum concentrations of PRL in (a) gonadectomized male rats implanted with 0.5 cm (● ○), 1 cm (■ □) and 3 cm (▲ △) capsules filled with testosterone, in (b) gonadectomized, adrenalectomized and testosterone- plus corticosterone-treated male rats and in (c) gonadectomized, testosterone-treated female rats; day 0: inoculation of tumour 7315b; the levels in tumour-bearing (solid lines) and control (broken lines) rats are shown. *)$p < 0.01$ compared with control rats (ANOVA, Duncan's new multiple range test).*

4.4.2. Effects of tumour 7315b on male copulatory behaviour

Parameters of male sexual behaviour tested are shown in Figure 4.2. (experiment 1 and 2) and Figure 4.3. (experiment 3). Male sexual behaviour in control rats did not change during the experiments, and sexual performance did not significantly differ between the tumour-bearing and control rats in the 1st week after tumour inoculation.

In experiment 1a, 1b and 1c, the EL increased ($p<0.01$) and the MIR decreased ($p<0.01$) significantly in tumour-bearing rats, within 12 to 22 days after inoculation, while the MF, IF and PEI (data not shown) remained unchanged in those animals. The number of tumour-bearing rats, which ejaculated, decreased significantly ($p<0.05$) in all these experiments; at day 19, only 9 out of 22 rats ejaculated. At the end of experiment 1a, 1b and 1c, a few hyperPRLaemic rats did not perform any sexual contact. In the rats which were sexually active, no effects of the tumour on the CL were found (data not shown).

Also in experiment 2, the EL increased ($p<0.01$) and the MIR decreased ($p<0.01$) significantly in the hyperPRLaemic animals, while the number of ejaculating tumour-bearing rats was reduced from 7 to 2 at the end of the experiment (Figure 4.2.). Significant differences were not found in the CL, MF, IF and PEI (data not shown) either within or between the tumour-bearing and control group.

The results of experiment 3 (Figure 4.3.) showed that tumour 7315b significantly ($p<0.01$) reduced the MF and IF in testosterone-treated, gonadectomized female rats. As a consequence the MIR decreased in the hyperPRLaemic animals. Moreover, a significant increase of the CL was found in these rats 22 days after inoculation.

4.5. Discussion

The present study confirms that tumour 7315b induces very high levels of serum PRL in male (Voogt et al., 1987) and female rats (Lamberts et al., 1984).

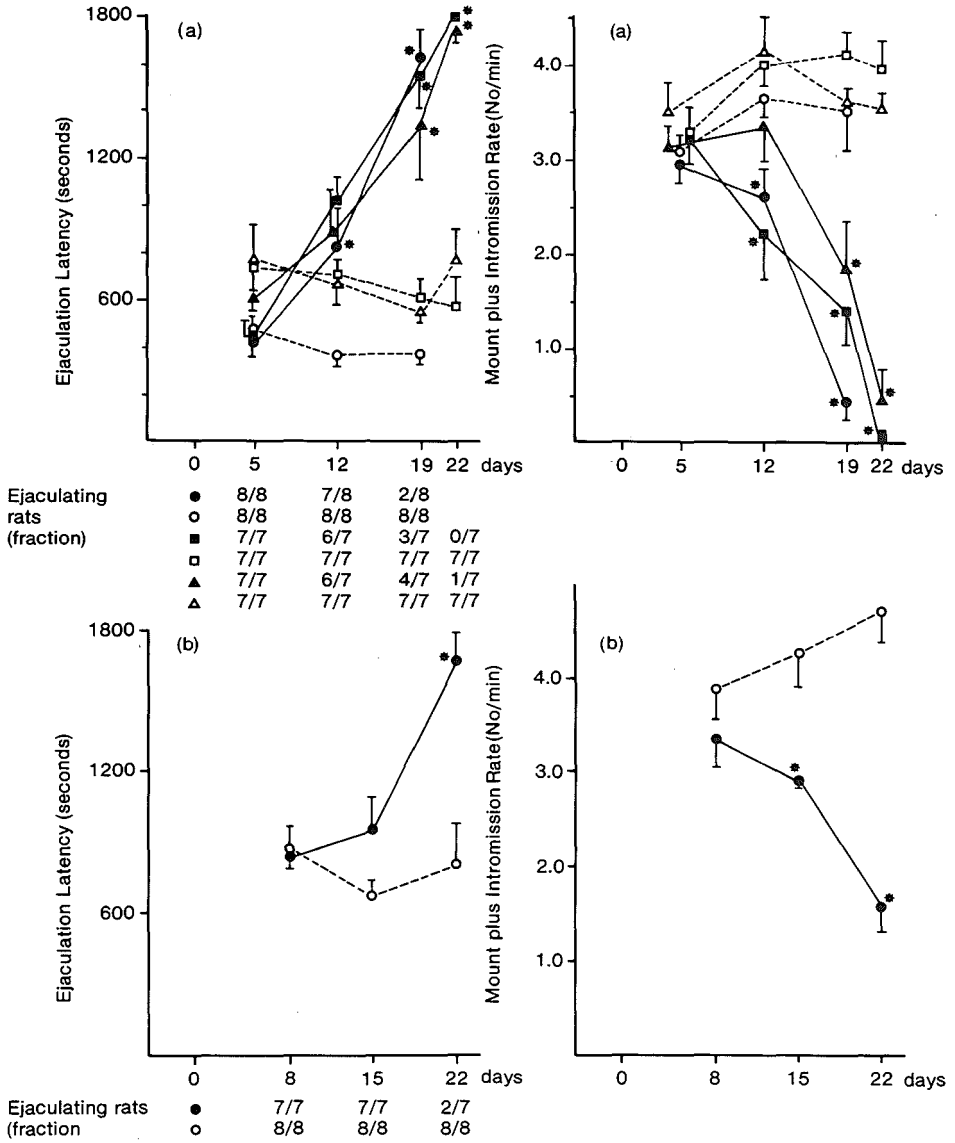

*Figure 4.2. Ejaculation latency and mount plus intromission rate in (a) gonadectomized male rats implanted with 0.5 cm (● ○), 1 cm (■ □) and 3 cm (▲ △) capsules filled with testosterone, and in (b) gonadectomized, adrenalectomized, testosterone- plus corticosterone-treated male rats; day 0: inoculation of tumour 7315b; results of tumour-bearing (solid lines) and control (broken lines) rats are shown; *)$p < 0.05$ compared with control rats (Mann-Whitney-U test).*

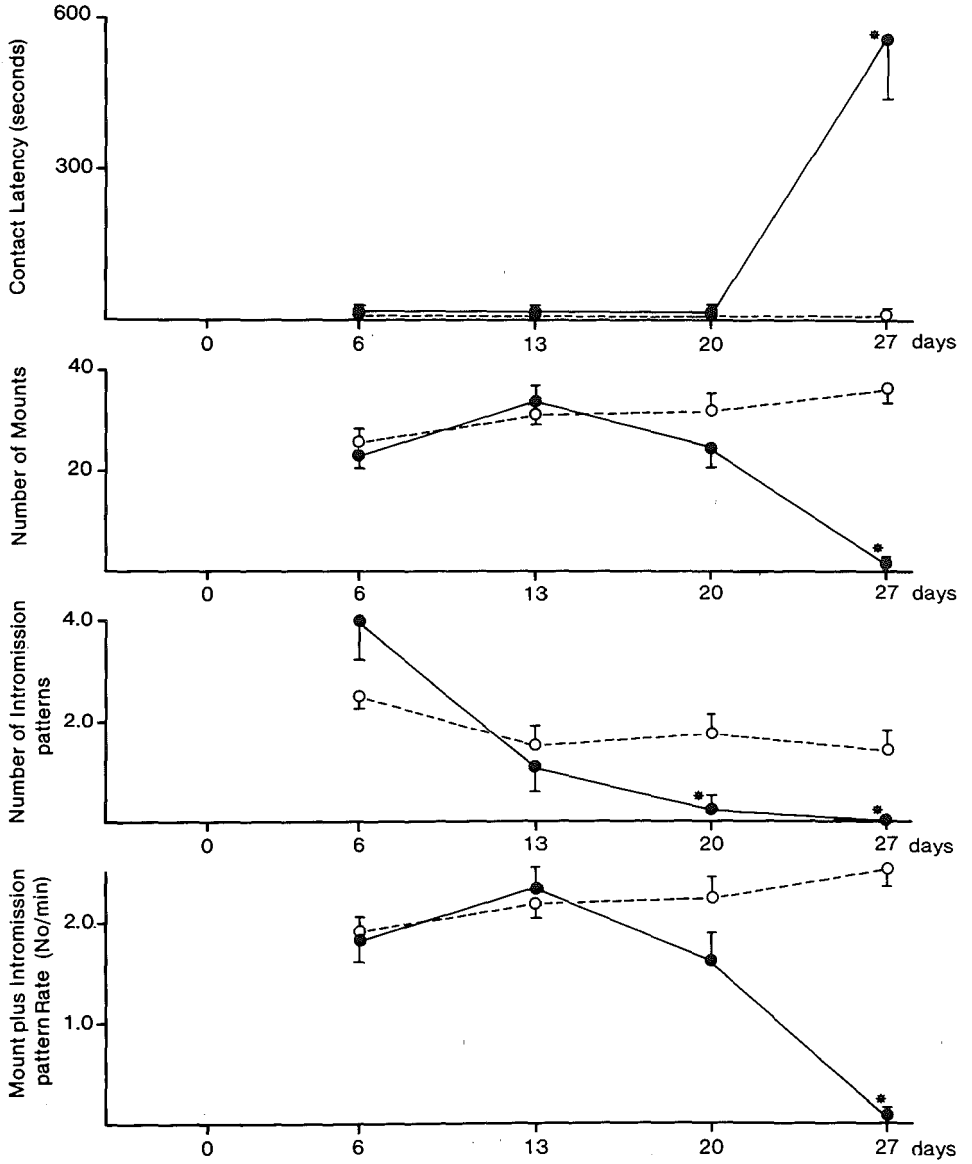

*Figure 4.3. Parameters of male copulatory behaviour in gonadectomized, testosterone treated female rats; day 0: inoculation of tumour 7315b; results of tumour-bearing (solid lines) and control (broken lines) rats are shown; *)p < 0.05 compared with control rats (Mann-Whitney-U test).*

Intact male rats, rendered hyperPRLaemic by PRL-producing tumours, develop a state of hypogonadotrophic hypogonadism (Fang et al., 1974; Hodson et al., 1980; Weber et al., 1982a). Therefore, in most studies investigating effects of hyperPRLaemia on copulatory behaviour, rats were gonadectomized and treated with testosterone. Our data demonstrate, that hyperPRLaemia induced by tumour 7315b has markedly inhibitory effects on testosterone induced copulatory behaviour. The effects found in rats with a normal serum testosterone were as strong as those observed in animals with a serum testosterone probably just enough to maintain masculine copulatory behaviour (±0.4 ng/ml; Damassa et al., 1977). One possible mechanism for PRL to inhibit copulatory behaviour has suggested to be by lowering the availability of bioactive testosterone metabolites in target tissues (Bailey et al., 1984; Doherty et al, 1985). With respect to our data, however, it is highly unlikely that a PRL-induced reduction in the concentration of testosterone metabolites in target organs contributes to the effects of hyperPRLaemia on male copulatory behaviour, since this mechanism would have more profound effects in the presence of low than of normal testosterone levels.

It has been established that hyperPRLaemia suppresses male copulatory behaviour by inhibiting both sexual arousal and erectile function (Kalra et al. 1983; Doherty et al. 1986). In order to measure solely the effect of hyperPRLaemia on sexual arousal, male copulatory behaviour was studied in hyperPRLaemic male rats after genital anaesthetization (Doherty et al., 1986). In these animals an almost 50% reduction in the mount rate was found. In the present study, testosterone-treated, hyperPRLaemic female rats displayed almost a complete loss of sexual arousal as indicated by the strong reduction of the MF and the IF. These findings demonstrate that the suppressive effects of hyperPRLaemia, induced by tumour 7315b, on mounting behaviour are at least as strong in female rats as in male rats.

In a previous investigation of copulatory behaviour in male rats bearing the ACTH- and PRL-secreting tumour 7315a only a minor effect was found (Weber et al., 1982b); despite PRL levels of more than 4000 ng/ml the animals maintained a high mount rate, suggesting that their sexual arousal was hardly affected. The fact that the animals had an increased EL or did not ejaculate at all could be explained by the increased secretory activity of the adrenals, since tumour 7315a did not inhibit sexual behaviour in adrenalectomized animals. In contrast to tumour 7315a, the

present tumour 7315b exerted a strong inhibition of male copulatory behaviour. Moreover this inhibition was fully independent of the presence of the adrenals. The cause of the discrepancy between the effect of tumour 7315a and 7315b can only be speculated upon. Different effects on dopaminergic neurons which are thought to mediate PRL effects on sexual behaviour (Drago, 1984) are unlikely, since the dopamine secretion into the hypophysial portal blood was as strongly increased by tumour 7315a (Weber et al., 1983) as by tumour 7315b (Voogt et al., 1987). It is more conceivable that ACTH or related peptides inhibit the suppressive action of PRL on male copulatory behaviour, since intracerebroventricular administration of ACTH (1-24) is able to induce an increase in sexual excitement (Mann et al., 1986).

In conclusion, the purely PRL-producing tumour 7315b has been shown to be a suitable model for studying effects of hyperPRLaemia on male sexual behaviour, and to inhibit important components of that behaviour in the rat, independently of gonadal and adrenal function, probably by a direct action on certain brain functions.

4.6. References

Adler,R.A. (1986). The anterior pituitary-grafted rat: A valid model of chronic hyperprolactinemia. Endocr. Rev. 7, 302-313.

Bailey,D.J., Dolan,A.L., Pharaoh,P.D.P., and Herbert,J. (1984). Role of gonadal and adrenal steroids in the impairment of the male rat's sexual behavior by hyperprolactinemia. Neuroendocrinol. 39, 555-562.

Bailey,D.J., and Herbert,J (1982). Impaired copulatory behavior of male rats with hyperprolactinemia induced by domperidone or pituitary grafts. Neuroendocrinol. 35, 186-193.

Damassa,D.A., Smith,E.R., Tennent,B., and Davidson,J.M. (1977). The relationship between circulating testosterone levels and male sexual behavior in rats. Horm. Behav. 8, 275-286.

Demarest,K.T., and Moore,K.E. (1981). Sexual differences in the sensitivity of tuberoinfundibular dopamine neurons to the actions of prolactin. Neuroendocrinol. 33, 230-234.

Doherty,P.C., Bartke,A., Hogan,M.P., Klemcke,H., and Smith,M.S. (1982). Effects of hyperprolactinemia on copulatory behavior and testicular human chorionic gonadotropin binding in adrenalectomized rats. Endocrinol. 111, 820-826.

Doherty,P.C., Bartke,A., and Smith,M.S. (1985). Hyperprolactinemia and male sexual behavior: Effects of steroid replacement with estrogen plus dihydrotestosterone. Physiol. Behav. 35, 99-104.

Doherty,P.C., Baum,M.J., and Todd,R.B. (1986). Effects of chronic hyperprolactinemia on sexual arousal and erectile function in male rats. Neuroendocrinol. 42, 368-375.

Drago,F. (1984). Prolactin and Sexual Behavior: A Review. Neurosc. Biobehav. Rev. 8, 433-439.
Fang,V.S., Refetoff,S., and Rosenfield,R.L. (1974). Hypogonadism induced by a transplantable, prolactin-producing tumor in male rats: Hormonal and morphological studies. Endocrinol. 95, 991-998.
Hodson,C.A., Simpkins,J.W., Pass,K.A., Aylsworth,C.F., Steger,R.W., and Meites,J. (1980). Effects of a prolactin secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats. Neuroendocrinol. 30, 7-10.
Kalra,P.S., Simpkins,J.W., Luttge,W.G., and Kalra,S.P. (1983). Effects on male sexual behavior and preoptic dopamine neurons of hyperprolactinemia induced by MtT-W15 pituitary tumors. Endocrinol. 113, 2065-2071.
Kirk,R.E. (1968). Experimental design: Procedures for the behavioral sciences. Belmont, California: Brooks/Cole.
Lamberts,S.W.J., Uitterlinden,P., Bons,E.G., Zuiderwijk,J.M., Verleun,T., Oosterom, R., and Hackeng,W.H.L. (1984). Hyperprolactinemia exerts a negative effect on the ß-endorphin content of the rat neurointermediate pituitary lobe. Endocrinol. 114, 2349-2353.
Mann,D.R., Evans,D.C., Jacobs, V.L. and Collins,D.C. (1986). Influence of acute intracerebroventricular (i.c.v.) administration of adrenocorticotrophin (ACTH) on LH secretion in male rats: effect of pretreatment (i.c.v.) with ACTH antiserum on the LH response to an acute etherstress. J. Endocrinol. 108, 275-280.
Shrenker,P., and Bartke,A. (1985). Adrenalectomy does not prevent the hyperprolactinemic, induced sexual behavior deficits in CDF male rats. Life Sciences 36, 1881-1888.
Siegel,S. (1956). Nonparametric statistics for the behavioral sciences. McGraw-Hill Kogakusha, Ltd., Tokyo.
Södersten,P. (1972). Mounting behavior in the female rat during the estrous cycle, after ovariectomy, and after estrogen or testosterone administration. Horm. Behav. 3, 307-320.
Verjans,H.L., Cooke,B.A., De Jong,F.H., De Jong,C.M.M., and Van der Molen,H.J. (1973). Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid Bioch. 4, 665-676.
Voogt,J.L., de Greef,W.J., Visser,T.J., de Koning,J., Vreeburg J.T.M., and Weber R.F.A. (1987). In vivo release of dopamine, LHRH and TRH in male rats bearing a prolactin-secreting tumor. Neuroendocrinol. 46, 110-116.
Weber,R.F.A., de Greef,W.J., de Koning,J., and Vreeburg,J.T.M. (1983). LRH levels and dopamine levels in hypophysial stalk plasma and their relationship to plasma gonadotropins and PRL levels in male rats bearing a PRL- and ACTH-secreting pituitary tumor. Neuroendocrinol. 36, 205-210.
Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982a). Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex organ weights in adult male rats: A possible role of the adrenals. Endocrinol. 111, 412-417.
Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982b). Effects of a prolactin-secreting tumour on copulatory behaviour in male rats. J. Endocrinol. 93, 223-229.
Weber,R.F.A., Ooms,M.P. and Vreeburg,J.T.M. (1987). The contribution of corticosterone and testosterone to the suppression of serum LH in hyperprolactinaemic adult male rats with pituitary transplants. J. Endocrinol. 113, 111-116.

J. Endocrinol. 120, 261-268 (1989)

CHAPTER V

EFFECTS OF THE NEW PROLACTIN-PRODUCING TUMOUR 7315B ON GONADOTROPHIN SECRETION IN ADULT MALE AND FEMALE RATS

5.1. Abstract

The effects of the transplantable purely PRL-secreting tumour 7315b[1] on serum gonadotrophins were studied in adult rats. Possible contributions of the adrenals to the tumour-induced inhibition of serum LH and FSH concentrations were evaluated. The suppressive actions of tumour 7315b on serum gonadotrophins in gonadectomized plus adrenalectomized male and female rats were compared.

Within 4 weeks after inoculation of tumour 7315b in intact male rats very high levels of PRL and decreased serum concentrations of gonadotrophins and testosterone were recorded. At autopsy reduced weights of testes and accessory sex organs and slightly increased adrenal weights were found. Also in animals treated with a small testosterone-filled capsule after castration, tumour 7315b reduced serum concentrations of LH and FSH. Adrenalectomy did not prevent this suppressive action of the tumour on the postcastration rise of serum gonadotrophins. Suppression of serum concentrations of gonadotrophins during hyperPRLaemia was more pronounced in gonadectomized plus adrenalectomized female rats than in male rats, indicating that the degree of the tumour-induced suppression of LH and FSH after castration is determined to a large extent by the sex of the animal.

[1]*Note: At a later stage of the investigations, evidence emerged that tumour 7315b has begun to secrete some immuno-assayable ACTH (cf. chapters VII and VIII). Experimental data presented in this thesis should be interpreted in light of this recent observation.*

The purely PRL-secreting tumour 7315b has therefore been shown to be a suitable model for studying the effects of severe hyperPRLaemia on the adenohypophysial-gonadal axis in rats.

5.2. Introduction

It is well established that a decrease in gonadotrophin secretion and inhibition of reproductive functions occurs in male rats rendered hyperPRLaemic by PRL-secreting tumours (Fang et al., 1974; Weber et al., 1982a & 1982b; Kalra, 1983) or pituitary grafts (Bartke et al., 1977; Bailey and Herbert 1982; Doherty et al., 1986; Weber et al., 1987). Nevertheless it remains unclear how PRL exerts its effects on the neuroendocrine regulation of gonadal function.

Moderate hyperPRLaemia (200-600 μg PRL/l) induced by pituitary grafts in the intact male rat results in a reduction of gonadotrophin secretion, without affecting testosterone secretion by the testes (Bartke et al., 1977; Mcneilly et al.,1983; Weber et al., 1987), and without increasing dopamine levels in pituitary stalk blood (Cramer, et al., 1979). Increased concentrations of PRL such as those in rats bearing the PRL- and growth hormone-secreting tumour MtTW15, lead to a greater suppression of plasma gonadotrophins and an inhibition of testicular function (Hodson et al., 1980; Clark and Kalra, 1985; Katovich et al., 1985). In a previous study in our laboratory, suppression of serum gonadotrophins and testosterone was found in male rats with hyperPRLaemia induced by the PRL- and adrenocorticotrophin-secreting tumour 7315a (Weber et al., 1982a), which appeared to be associated with an increase in dopamine and a decrease in luteinizing hormone releasing hormone (LHRH) in pituitary stalk blood (Weber et al., 1983). After adrenalectomy, however, the inhibitory effects of tumour 7315a on LHRH and serum gonadotrophins were totally absent, while dopamine levels in portal vessel blood were still high. This finding is at variance with observations made by other investigators (Hodson et al.,1980; McNeilly et al., 1980), and may be due to bioactive products secreted by tumour 7315a in addition to PRL. Thus, so far, both the transplantation of pituitaries and the inoculation of pituitary tumours, devised as models for hyperPRLaemia in the rat, are not completely satisfactory for investigating the

effects of hyperPRLemia on the adenohypophysial-testicular axis.

Recently, however, we obtained the tumour 7315b, which has been considered as a purely PRL-secreting tumour (Lamberts et al., 1984); the present study was designed to explore the effects of severe hyperPRLaemia induced by tumour 7315b on the adenohypophysial-testicular axis in adult male rats, and to evaluate the possible contributions of the adrenals to the effects of tumour 7315b on serum gonadotrophins. In an additional experiment these effects were studied in both male and female rats, which had been gonadectomized and adrenalectomized.

5.3. Materials and methods

5.3.1. Animals

Adult male and female rats (3-4 months of age) of the Buffalo strain were housed under controlled conditions of temperature (20 °C) and light (14h light; 10h darkness). The lights were on between 18.30 and 08.30 h. Standard food and water were always available. HyperPRLaemia was induced in the experimental animals by inoculation of tumour 7315b, as described previously (Kooy et al., 1988). Preceding inoculation tumour tissue was minced into small pieces, which were suspended in an equal volume of 0.9% NaCl (w/v) solution. Subsequently 0.4 ml of the tumour suspension was subcutaneously injected into the experimental animals on the dorsal side of the neck. Ten days later, a palpable mass with a diameter of ± 0.5 cm could be found at the place of injection. Three to four weeks after inoculation tumours reached diameters ranging from 4 to 5 cm. All animals were in good condition during the experiments.

5.3.2. Experiment 1: *Effects of tumour 7315b on serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone and on organ weights in intact male rats*

On day 0, six male rats were inoculated with tumour 7315b, while another six animals remained untreated and served as controls. Blood was sampled from all rats on days 11 and 18 and directly preceding decapitation on day 26. After death, organs were removed and weighed.

5.3.3. Experiment 2: *Effects of tumour 7315b on serum concentrations of LH and FSH in gonadectomized testosterone-treated male rats*

Seventeen male rats were gonadectomized and treated with a 0.5 cm testosterone-filled silicone elastomer implant (inner diameter: 0.8 mm; outer diameter: 1.4 mm; Talas, Zwolle, The Netherlands), leading to serum concentrations of testosterone being too low to prevent the postcastration rise of LH and FSH (Weber et al., 1982a). Two days later (day 0) 9 of 17 rats were inoculated with tumour 7315b. Blood was sampled from all animals on days 7, 13 and 21.

5.3.4. Experiment 3: *Effects of tumour 7315b on serum concentrations of LH and FSH in gonadectomized plus adrenalectomized male rats treated with testosterone and corticosterone*

In order to ascertain whether changes in adrenal steroid output are essential for the effects of tumour 7315b on serum concentrations of LH and FSH after castration, 15 male rats were gonadectomized, adrenalectomized and treated with a 1 cm testosteron-filled silicone elastomer implant and with a 40 mg corticosterone pellet, made as described previously (Weber et al., 1987). Seven days later, on day 0, the tumour was injected into seven of the animals. Blood was taken from all rats on days 8, 15 and 22.

5.3.5. Experiment 4: *Effects of adrenalectomy on serum concentrations of LH and FSH after castration in hyperPRLaemic male rats*

The influence of the presence of the adrenals on the serum concentrations of gonadotrophins was evaluated in 14 hyperPRLaemic rats, gonadectomized 6 days before injection of the tumour 7315b. Half of the animals had been adrenalectomized 6 days before inoculation on day 0. Blood was collected from all rats on days 0, 7, 13 and 20.

5.3.6. Experiment 5: *Effects of tumour 7315b on serum concentrations of LH and FSH in gonadectomized plus adrenalectomized male and female rats*

In order to compare the effects of tumour 7315b on serum gonadotrophins in male and female rats, 11 male rats, and 11 female rats were gonadectomized and adrenalectomized, without subsequent treatment with steroids. Eight days later, on day 0, six of the male and five of the female rats were inoculated. Blood was collected from all animals on days 0, 7 and 14. Additionally, blood was sampled on day 21 in the male rats.

5.3.7. Blood sampling and hormone determinations

Blood was taken under ether anaesthesia, by puncturing the orbital plexus or following decapitation preceding autopsy. Serum concentrations of PRL, LH and FSH were determined by a double-antibody radioimmunoassay (RIA) as described previously (Weber et al., 1983) using materials and protocols supplied by the NIADDK (National Hormone and Pituitary Program, Baltimore, MD, USA). The intra- and interassay coefficients of variation were between 15 and 8%. The results are expressed in terms of NIADDK-rat-PRL/LH/FSH-RP-1. Testosterone was measured by RIA, using the method previously described by Verjans and colleagues (1973). The interassay coefficient of variation was 15%, and the intra-assay coefficient of variation was 7%. Corticosterone

was measured by RIA using highly specific antiserum provided by Bioclinical Services Ltd, Cardiff, South Glamorgan, U.K. (Weber et al., 1987). The interassay coefficient of variation was 12%, and the intra-assay coefficient of variation was 8%.

5.3.8. Statistical analysis

The results are presented as means ± S.E.M. Serum concentrations of hormones were analysed by two-way analysis of variance; statistically significant differences were further evaluated by the Duncan's new multiple range test (Kirk, 1968). Effects of tumour 7315b on body and organ weights in experiment 1 were analysed by Student's t-test.

5.4.Results

5.4.1. Experiment 1

Serum concentrations of PRL, LH, FSH and testosterone are presented in Figure 5.1. Inoculation induced a significant increase in serum concentrations of PRL in all tumour-bearing rats within 11 days, resulting in very high levels of PRL (4428 ± 162 ng/ml) at the end of the experiment, while serum concentrations of PRL in the control rats remained unchanged (163 ± 46 ng/ml).

Serum concentrations of LH and FSH did not differ between the hyperPRLaemic rats and control animals on day 11. On days 18 and 26, however, a significant decrease of serum LH was found in the hyperPRLaemic rats. FSH levels were significantly reduced on day 26. The reduction in serum LH was accompanied by significantly lower levels of serum testosterone, and reduced weights of testes and accessory sex organs at autopsy. The adrenal weights of the tumour-bearing rats were increased, as compared with controls (Table 5.1).

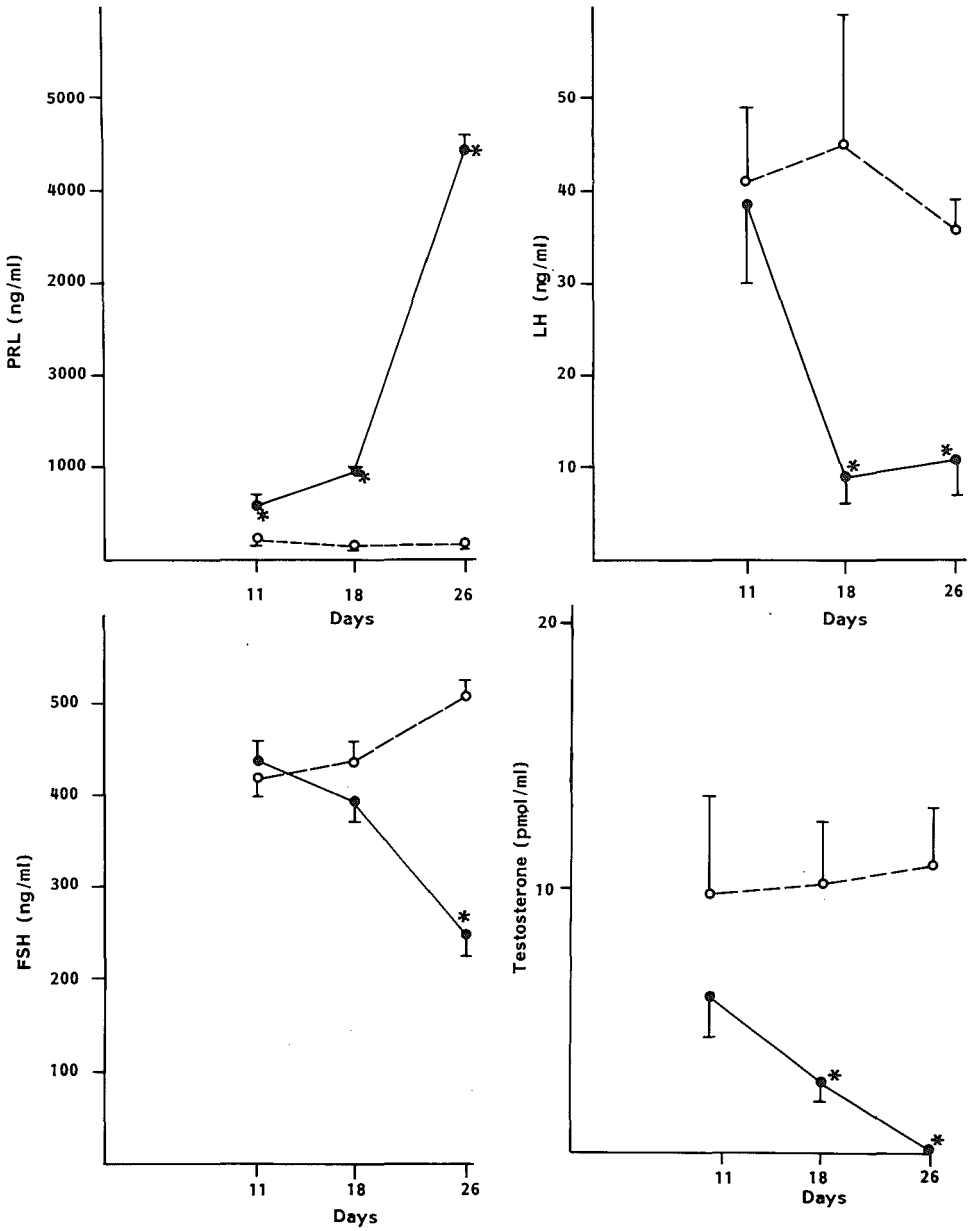

*Figure 5.1. Serum concentrations of PRL, LH, FSH and testosterone in intact tumour-bearing (●) and control (○) male rats; rats were inoculated with tumour 7315b on day 0; *)p<0.01, compared with controls (ANOVA, Duncan's new multiple range test).*

Table 5.1. Body and organ weights of intact male rats with or without the prolactin-secreting tumour 7315b

	Body (g)	*Testes (mg)#*	*Seminal vesicles (mg)#*	*Ventral prostate (mg)*	*Adrenal (mg)*
Intact controls	312±9	3199±63	279±10	413±28	20±1
Intact + 7315b	321±9	2706±107*	181±9**	244±15**	26±2**

*Values are means ± SEM for six rats; *)p<0.05, **)p<0.01 compared with controls (Student's t-test); # sum of two organs*

5.4.2. Experiment 2

On day 7, there were no significant differences in serum concentrations of PRL, LH and FSH between the control and the tumour-bearing rats (Figure 5.2). On day 13, serum PRL levels were significantly higher in tumour-bearing rats than in control rats, while the rise in LH after castration was significantly inhibited in the hyperPRLaemic animals. On day 21, serum concentrations of both LH and FSH were significantly suppressed in the hyperPRLaemic animals. During this experiment, testosterone concentrations (1.21 ± 0.03 nmol/l) in castrated rats were not affected by the presence of tumour 7315b.

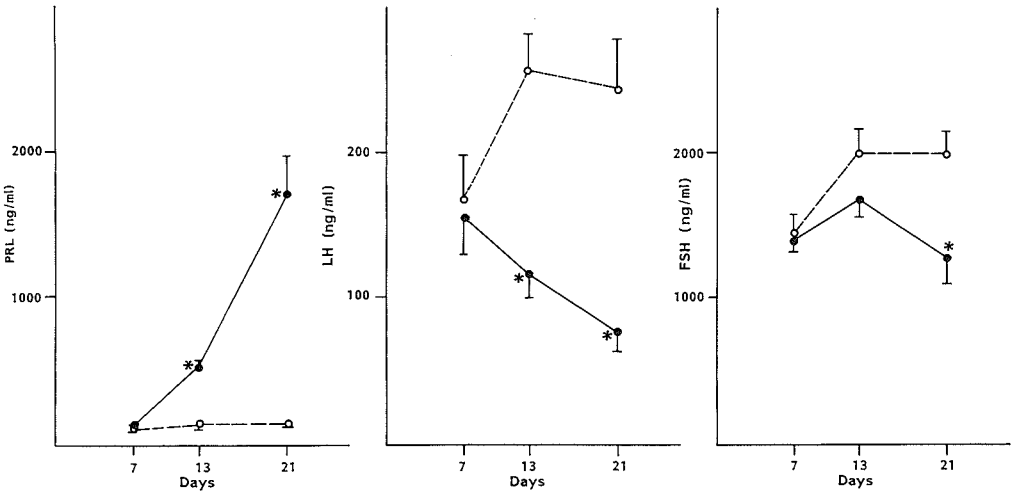

*Figure 5.2. Serum concentrations of PRL, LH and FSH in gonadectomized tumour-bearing (●) and control (○) male rats; castration was followed by treatment with a 0.5 cm testosterone-filled silicone elastomer implant; rats were inoculated with tumour 7315b on day 0, 2 days after castration; *)p < 0.01,compared with controls (ANOVA, Duncan's new multiple range test).*

5.4.3. Experiment 3

Eight days after inoculation, serum concentrations of PRL, LH and FSH were not significantly different between the control and tumour-bearing rats (Figure 5.3). On day 15, the serum concentration of PRL was high in the tumour-bearing animals, while serum concentrations of LH and FSH remained unchanged. Coincident with a further rise of serum concentrations of PRL by day 22, serum concentrations of LH and FSH had significantly decreased. No significant differences were found in the serum levels of testosterone (1.84 ± 0.14 nmol/l) and corticosterone (177.3 ± 7.1 nmol/l) of the hyperPRLaemic and control animals.

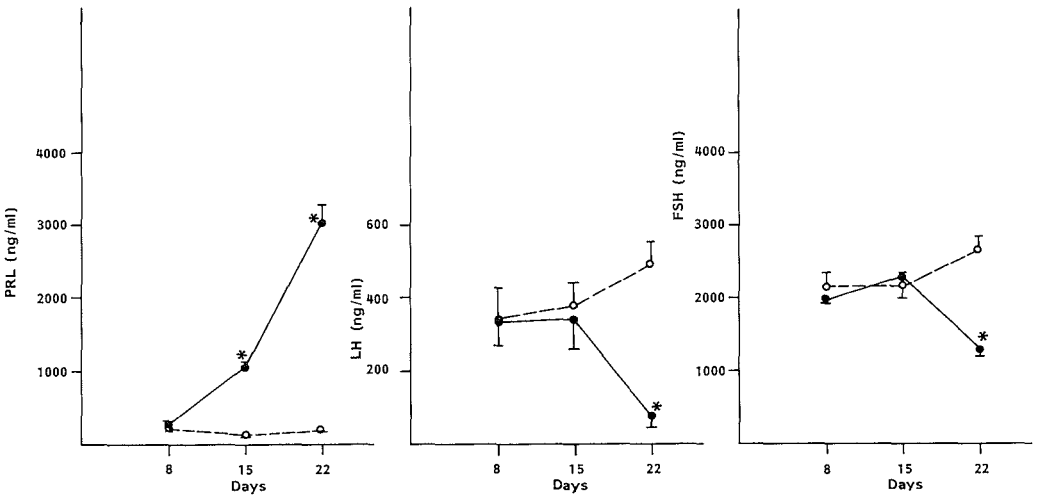

*Figure 5.3. Serum concentrations of PRL, LH and FSH in gonadectomized adrenalectomized testosterone- plus corticosterone-treated tumour-bearing (●) and control (○) male rats. Rats were inoculated with tumour 7315b on day 0, 7 days after surgery; *p<0.01, compared with controls (ANOVA, Duncan's new multiple range test).*

5.4.4. Experiment 4

During the whole experiment no significant differences in serum concentrations of PRL were found between the adrenalectomized and non-adrenalectomized animals, rendered hyperPRLaemic by tumour 7315b (Figure 5.4). In both groups serum PRL had risen by day 7, reaching levels of 1982 ± 155 ng/ml on day 20. While the hyperPRLaemia was associated with a significant reduction in serum concentrations of LH, the concentrations of FSH remained unchanged in both groups after castration (2450 ± 62 ng/ml).

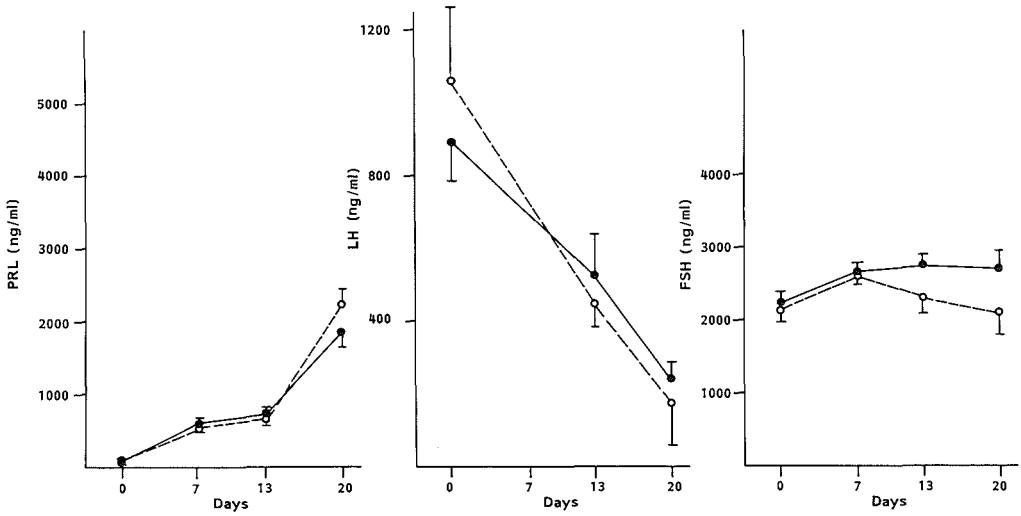

Figure 5.4. Serum concentrations of PRL, LH and FSH in gonadectomized, tumour-bearing male rats, with (○) and without (●) adrenalectomy. Animals were inoculated with tumour 7315b on day 0, 15 days after surgery. No significant differences in hormone levels could be demonstrated between the adrenalectomized and non adrenalectomized rats (ANOVA, Duncan's new multiple range test).

5.4.5. Experiment 5

On day 0, no significant differences were found in serum concentrations of PRL, LH and FSH between experimental and control rats (data not shown). During the experiment PRL levels remained unchanged in the controls. By day 14, however, serum PRL had risen in the tumour-bearing male and female rats to high levels (Table 5.2). In the female rats this was accompanied by a significant ($p<0.01$) reduction in the concentrations of LH and FSH unto $11\pm2\%$ and $14\pm3\%$ of the control values,

respectively, while in the hyperPRLaemic male rats no significant decrease of these levels could be demonstrated. Even after a prolonged time of hyperPRLaemia, at day 21, the male rats exhibited a slighter reduction in the concentrations of gonadotrophins (LH unto 44±8% and FSH unto 70±12% of the control values) as compared with the earlier effects in the female rats at day 14. The levels of PRL measured in the female rats on day 14 did not significantly differ from those in the male rats on day 21, but were just significantly ($p<0.05$) higher than those in the male animals on day 14.

Table 5.2. Effects of tumour 7315b on serum gonadotrophins in control and tumour-bearing (7315b) gonadectomized plus adrenalectomized male and female rats

			PRL	*LH*	*FSH*
Males	*day 14*	*control (5)*	130±43	940±107	2818±96
		7315b (6)	1799±715*	773±54	2608±142
	day 21	*control (5)*	209±40	1150±113	3239±145
		7315b (6)	3994±464**	504±89*	2247±372*
Females	*day 14*	*control (6)*	215±33	345±127	2225±245
		7315b (5)	3177±328**	36±16**	316±64**

*Values are given in ug/l (means ± SEM); numbers of animals are given in parentheses; rats were inoculated with the tumour on day 0, 8 days after gonadectomy and adrenalectomy; *)$p<0.05$, **)$p<0.01$ compared with controls (ANOVA, Duncan's new multiple range test).*

5.5. Discussion

As in our previous study (Kooy et al., 1988) inoculation of tumour 7315b in male and female rats resulted in very high serum concentrations of PRL, which were comparable to those found with tumour 7315a (Weber et al., 1982a & 1982b), but were reached sooner. A more important difference between the tumours was the finding that the adrenotrophic effects of tumour 7315b were slight in comparison to those of tumour 7315a. Since tumour 7315b has been qualified as a purely PRL-secreting tumour (Lamberts et al., 1984), and since adrenal growth induced by pituitary transplants (McNeilly et al., 1978) was comparable to that in rats bearing tumour 7315b, the slight growth of the adrenals in these animals must be due to the increased PRL levels, and not to other secretory products, such as those described in studies using other PRL-secreting tumours (Weber et al., 1982a; Clark and Kalra, 1985).

In both intact and gonadectomized tumour-bearing male rats, serum concentrations of LH and FSH were significantly suppressed. The reduction in serum LH in the intact animals was clearly accompanied by a decrease in the serum concentration of testosterone, confirming the finding that a state of hypogonadotrophic hypogonadism can be induced by severe hyperPRLaemia (Fang et al., 1974). Since increased adrenal weights were found at autopsy in animals rendered hyperPRLaemic by tumour 7315b, an alteration in adrenal steroid output might be involved in the reduction in gonadotrophin secretion during hyperPRLaemia. In adrenalectomized and gonadectomized animals, however, tumour 7315b greatly reduced serum concentrations of gonadotrophins. This finding is in marked contrast with our previous results with tumour 7315a; in rats bearing this tumour, adrenalectomy could completely prevent the suppression of serum gonadotrophins and male sexual behaviour (Weber et al., 1982a & 1982b). The cause of these differences is unknown, but must be related to the fact that tumour 7315a secretes PRL, ACTH and several other derivatives of the proöpiomelanocortin molecule (Lamberts et al., 1984). It is tempting to speculate that one of these compounds can prevent the inhibitory action of PRL, since it has been found that intracerebroventricular administration of ACTH (1-24) can stimulate LH release and sexual behaviour (Mann et al., 1986). The above mentioned findings, however, do not exclude a contribution of the adrenals to the suppression of serum concentrations of LH and FSH in

hyperPRLaemic animals after castration. Therefore, we studied the effects of tumour 7315b on serum LH and FSH after gonadectomy in adrenalectomized and non-adrenalectomized male rats, not treated with testosterone or corticosterone. During this experiment, the rise in PRL levels was accompanied by a suppression of serum LH, which was as marked in the adrenalectomized as in the non-adrenalectomized animals. Concentrations of FSH after castration remained unchanged in both groups, which may reflect a tumour-induced inhibition of a further increase after castration, independent of adrenal factors. The suppression of serum concentrations of gonadotrophins by severe hyperPRLaemia in castrated rats was not therefore potentiated by adrenal factors. This experiment demonstrates also that sex steroids are not required for the inhibition of gonadotrophin secretion in severe hyperPRLaemia. This observation is at variance with the finding that PRL-induced suppression of gonadotrophin secretion in male rats requires the presence of testosterone, suggesting that PRL inhibits gonadotrophin secretion by sensitizing the hypothalamus to the negative feedback action of gonadal steroids (McNeilly et al., 1980 &'1983; Weber et al., 1987). It appears that PRL inhibits gonadotrophin release both by sensitizing the hypothalamus to the negative feedback effects of testosterone and by a direct suppressive action of PRL itself. It is not clear, however, whether both inhibitory actions on gonadotrophin release are mediated by the same or different neuroendocrine mechanisms.

The suppressive action of hyperPRLaemia induced by tumour 7315b on serum concentrations of LH and FSH has found to be due to a suppression of the LHRH release into hypophysial stalk plasma (Voogt et al., 1987). The present study indicates that the inhibition of serum gonadotrophins by tumour 7315b is much stronger in adrenalectomized plus gonadectomized female rats than in their male counterparts, suggesting that PRL suppresses LHRH release more easily in female rats. Another sexually dimorph PRL effect has been reported by Demarest and Moore (1981), who found that tuberoinfundibular dopaminergic neurons are more sensitive to PRL in female rats than in male rats. These findings and the hypothesis that the tuberoinfundibular dopaminergic neurons terminating in the lateral aspects of the median eminence function as inhibitory modulators of LHRH release (Selmanoff, 1981) might explain why in the presence of tumour 7315b the serum gonadotrophins are more reduced in female rats than in male rats. We believe, however, that the higher activity of the dopaminergic neurons are not involved in the suppression of LHRH during hyperPRLaemia, since,

in the presence of high dopamine levels, high LHRH concentrations are found in pituitary stalk blood sampled from adrenalectomized rats inoculated with tumour 7315a (Weber et al., 1983).

In conclusion, the purely PRL-secreting tumour 7315b has been shown to be a suitable model for studying the effects of severe hyperpPRLaemia on the adenohypophysial-testicular axis. The present study revealed that the tumour-induced hyperPRLaemia exerts inhibitory influences on serum concentrations of gonadotrophins in intact as well as in castrated male rats. Adrenalectomy could not prevent the suppressive effect of hyperPRLaemia on serum concentrations of gonadotrophins after castration. Moreover, the central inhibitory action of hyperPRLaemia on serum concentrations of LH and FSH appeared to be greater in female than in male rats.

5.6. References

Bailey,D.J., and Herbert,J. (1982). Impaired copulatory behavior of male rats with hyperprolactinemia induced by domperidone or pituitary grafts. Neuroendocrinol. 35, 186-193.

Bartke,A., Smith,M.S., Michael,S.D., Peron,F.G. and Dalterio,S. (1977). Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinol. 100, 182-186.

Clark,J.T., and Kalra,P.S. (1985). Effects on penile reflexes and plasma hormones of hyperprolactinemia induced by MtTW15 Tumors. Horm. Behav. 19, 304-310.

Cramer, O.M., Parker,C.R., and Porter,J.C. (1979). Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinol. 105, 636-640.

Demarest,K.T., and Moore,K.E. (1981). Sexual differences in the sensitivity of tuberoinfundibular dopamine neurons to the actions of prolactin. Neuroendocrinol. 33, 230-234.

Doherty,P.C., Baum,M.J., and Todd,R.B. (1986). Effects of chronic hyperprolactinemia on sexual arousal and erectile function in male rats. Neuroendocrinol. 42, 368-375.

Fang,V.S., Refetoff,S., and Rosenfield,R.L. (1974). Hypogonadism induced by a transplantable, prolactin-producing tumor in male rats: Hormonal and morphological studies. Endocrinol. 95, 991-998.

Hodson,C.A., Simpkins,J.W., Pass,K.A., Aylsworth,C.F., Steger,R.W., and Meites,J. (1980). Effects of a prolactin secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats. Neuroendocrinol. 30, 7-10.

Kalra,P.S., Simpkins,J.W., Luttge,W.G., and Kalra,S.P. (1983). Effects on male sexual behavior and preoptic dopamine neurons of hyperprolactinemia induced by MtTW15 pituitary tumors. Endocrinol. 113, 2065-2071.

Katovich,M.J., Cameron,D.F., Murray,F.T., and Gunsalus,G.L. (1985). Alterations of testicular functions induced by hyperprolactinemia in the rat. J. Androl. 6, 179-189.

Kirk,R.E. (1968). Experimental design: Procedures for the behavioral sciences. Belmont, California: Brooks/Cole.

Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1988). Deterioration of male sexual behavior in rats by the new prolactin-secreting tumor 7315b. Horm. Behav. 22, 351-361.

Lamberts,S.W.J., Uitterlinden,P., Bons,E.G., Zuiderwijk,J.M., Verleun, T., Oosterom,R., and Hackeng,W.H.L. (1984). Hyperprolactinemia exerts a negative effect on the ß-endorphin content of the rat neurointermediate pituitary lobe. Endocrinol. 114, 2349-2353.

Mann,D.R., Evans,D.C., Jacobs,V.L. and Collins,D.C. (1986). Influence of acute intracerebroventricular (i.c.v.) administration of adrenocorticotrophin (ACTH) on LH secretion in male rats: effect of pretreatment (i.c.v.) with ACTH anti-serum on the LH response to an acute etherstress. J. Endocrinol. 108, 275-280.

McNeilly,A.S., Sharpe,R.M., Davidson,D.W., and Fraser,H.M. (1978). Inhibition of gonadotrophin secretion by induced hyperprolactinemia in the male rat. J. Endocrinol. 79, 59-68.

McNeilly,A.S., Sharpe,R.M., and Fraser,H.M. (1980). Effect of adrenalectomy or castration on the inhibition of gonadotrophin secretion induced by hyperprolactinemia in the adult male rat. J. Endocrinol. 85, 83-87.

McNeilly,A.S., Sharpe,R.M., and Fraser,H.M. (1983). Increased sensitivity to the negative feedback effects of testosterone induced by hyperprolactinemia in the adult male rat. Endocrinol. 112, 22-28.

Selmanoff,M. (1981). The lateral and medial median eminence: distribution of dopamine, norepinephrine, and luteinizing hormone-releasing hormone and the effect of prolactin on catecholamine turnover. Endocrinol. 108, 1716-1722.

Verjans,H.L., Cooke,B.A., De Jong,F.H., De Jong,C.M.M., and Van der Molen,H.J. (1973). Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid Bioch. 4, 665-676.

Voogt,J.L., de Greef,W.J., Visser,T.J., de Koning,J., Vreeburg,J.T.M, and Weber,R.F.A. (1987). In vivo release of dopamine, LHRH and TRH in male rats bearing a prolactin-secreting tumor. Neuroendocrinol. 46, 110-116.

Weber,R.F.A., de Greef,W.J., de Koning,J., and Vreeburg,J.T.M. (1983). LRH levels and dopamine levels in hypophysial stalk plasma and their relationship to plasma gonadotropins and PRL levels in male rats bearing a PRL- and ACTH-secreting pituitary tumor. Neuroendocrinol. 36, 205-210.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982a). Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex organ weights in adult male rats: A possible role of the adrenals. Endocrinol. 111, 412-417.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982b). Effects of a prolactin-secreting tumour on copulatory behaviour in male rats. J. Endocrinol. 93, 223-229.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1987). The contribution of corticosterone and testosterone to the suppression of serum LH in hyperprolactinaemic adult male rats with pituitary transplants. J. Endocrinol. 113, 111-116.

Neuroendocrinol. 51, 261-266 (1990)

CHAPTER VI

EVIDENCE FOR THE INVOLVEMENT OF CORTICOTROPHIN-RELEASING FACTOR IN THE INHIBITION OF GONADOTROPHIN RELEASE INDUCED BY HYPERPROLACTINAEMIA

6.1. Abstract

The hypothesis was tested that corticotrophin-releasing factor (CRF) is involved in the inhibition of gonadotrophin secretion during chronic hyperPRLaemia. Two models of hyperPRLaemia were used, namely inoculation with the PRL-secreting tumour 7315b[1] and implantation of isogeneic pituitary glands.

Gonadectomized, adrenalectomized male rats received a testosterone capsule and a corticosterone pellet and were inoculated subcutaneously with tumour 7315b. Similar rats without tumour served as controls. The rats were studied 3-4 weeks later while anaesthetized with urethane. Plasma levels of testosterone and corticosterone were similar in the two groups of rats. Compared to controls, the tumour-bearing rats had significantly higher plasma levels of PRL (PRL; 100-fold increase) and adrenocorticotrophin (ACTH; 3-fold increase), whereas plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) had significantly decreased to 15 and 40%, respectively. CRF release into hypophysial stalk plasma was higher in rats with tumour 7315b than in controls (298±23 vs 197±28 pg/h), and hypothalamic CRF content had increased from 3.0±0.3 to 4.3±0.3 ng.

Male rats received 3 pituitary glands under the kidney capsule.

[1]*Note: At a later stage of the investigations, evidence emerged that tumour 7315b has begun to secrete some immuno-assayable ACTH (cf. chapters VII and VIII). Experimental data presented in this thesis should be interpreted in light of this recent observation.*

Sham-operated rats served as controls. They were studied 5-7 weeks later while anaesthetized with urethane. Compared to controls, pituitary-grafted rats had larger adrenals (49±4 vs 34±2 mg), higher plasma PRL (156±18 vs 52±8 ng/ml), ACTH (0.46±0.05 vs 0.22±0.02 ng/ml) and corticosterone (455±39 vs 268±14 ng/ml), and lower plasma levels of LH (21±2 vs 41±6 ng/ml). Plasma FSH and testosterone, and hypothalamic CRF content were similar in both groups of rats. CRF release into hypophysial stalk plasma was somewhat, but not significantly, higher in pituitary-grafted rats than in control rats (102±32 vs 82±37 pg/h).

Thus, both models of chronic hyperPRLaemia seem to activate the hypothalamic-adenohypophysial-adrenal axis. It is suggested that at least part of the action of PRL is due to an activation of CRF-containing neurons, which causes inhibition of hypothalamic luteinizing hormone-releasing hormone secretion and consequently pituitary gonadotrophin release.

6.2. Introduction

In the rat, pituitary gonadotrophin secretion and male copulatory behaviour is usually suppressed during chronic hyperPRLaemia (Bartke et al., 1977; Kooy et al., 1988 & 1989; Lamberts et al., 1981; McNeilly et al., 1978). These reduced gonadotrophin levels are probably due to an effect of PRL on pituitary gonadotrophin-secreting cells (Cheung, 1983; Vasquez et al., 1980; Winters and Loriaux, 1978) and on hypothalamic luteinizing hormone-releasing hormone (LHRH) secretion (Clayton and Bailey, 1982; Grandison et al., 1977; Hodson et al., 1981; Sarkar and Yen 1985; Voogt et al., 1987; Weber et al., 1983). Recent evidence suggests that a reduction in hypothalamic LHRH release contributes to deficits in male copulatory behaviour (Bain et al., 1987).

In contrast to the inhibition of reproductive functions, hyperPRLaemia seems to activate the hypothalamic-adenohypophysial-adrenal axis (Adler, 1986). Thus, chronic hyperPRLaemia may induce an enhanced synthesis and secretion of corticotrophin releasing factor (CRF) by hypothalamic neurons. Such a mechanism would account for the observation that both PRL (Clayton and Bailey, 1982;

Grandison et al., 1977; Hodson et al., 1981; Voogt et al., 1987; Weber et al., 1983) and CRF (Gambacciani et al., 1986; Gindoff and Ferin, 1987; Petraglia et al., 1987; Rivier and Vale, 1984) inhibit the release of LHRH and gonadotrophins and exert an inhibitory influence on sexual behaviour (Sirinathsinghji, 1987).

To test the hypothesis that the inhibitory effect of PRL on gonadotrophin secretion is mediated by CRF, we investigated the effect of hyperPRLaemia on hypothalamic CRF content and release into hypophysial portal blood in male rats. Two models of hyperPRLaemia were used, namely subcutaneous inoculation with the PRL-secreting tumour 7315b (Lamberts et al., 1984; Voogt et al., 1987) and implantation of 3 pituitary glands under the kidney capsule.

6.3. Materials and methods

6.3.1. Animals

Adult male rats of the Buffalo strain, weighing 320-360 g, were used. They had free access to food and tap water, and were kept under controlled conditions (20-24 °C, lights on 07.00-19.00 h).

6.3.2. Experiment 1: *HyperPRLaemia induced with tumour 7315b*

Gonadectomized, adrenalectomized rats were used for this experiment, since high PRL levels influence both testicular and adrenal secretory activity (Adler, 1986). After removal of adrenals and testes from ether-anaesthetized animals, a 1-cm-long Silastic implant (inner diameter: 1.5 mm; outer diameter: 2.1 mm) filled with testosterone and a 50 mg corticosterone pellet were implanted subcutaneously. Both steroids were purchased from Steraloids Inc. (Wilton, NH, USA). HyperPRLaemia

was induced by subcutaneous injection with 0.4 ml suspension of 7315b tumour (Kooy et al., 1988 & 1989; Voogt et al., 1987). Rats not inoculated with tumour 7315b were used as controls. The adrenalectomized rats received NaCL (0.9% w/v) solution for drinking. Three to four weeks later, the rats were anaesthetized with urethane (1.2 g/kg body weight, i.p.) and a polyethylene cannula (inner diameter: 0.58 mm; outer diameter: 0.96 mm) was inserted in the right femoral arteria. The hypophysial stalk was exposed via a parapharyngeal approach (Gibbs and Neil, 1978; De Greef and Visser, 1981; Porter and Smith, 1967). After exposure of the hypophysial stalk, heparin (500 IU; Thromboliquine, Organon, Oss, The Netherlands) was given via the arterial cannula and then approximately 2 ml peripheral blood was collected. Hypophysial stalk blood was collected for 1 h in a tube kept in melting ice. Immediately after collection, stalk blood was centrifuged for 10 min at 4°C, and the volume of plasma obtained was measured. Part of stalk plasma was mixed with an equal volume of 0.2 M $HClO_4$ for dopamine determination. The rest of the stalk plasma was used for CRF measurement. After collection of stalk blood, the rats were killed and the hypothalamus, excluding the preoptic area, was excised. The tissue was placed in ice-cold phosphate buffered saline (pH 7.0), homogenized with a glass tissue grinder and centrifuged for 5 min at 4 °C. Part of the supernatant was mixed with an equal volume of 0.2 M $HClO_4$ and was used for determination of dopamine. In the rest of the supernatant CRF was estimated. Plasma and hypothalamic samples were stored at -20 °C until assayed.

6.3.3. Experiment 2: *HyperPRLaemia induced with pituitary transplants*

Male rats were anaesthetized with ether and 3 isogeneic pituitary glands were implanted under the left kidney capsule. Controls received a similar treatment, but were not implanted with pituitary glands. Five to seven weeks later, portal blood was collected from these rats. Hypophysial portal blood was also collected from rats which had been adrenalectomized two weeks earlier. Before cutting the hypophysial stalk, approximately 2 ml peripheral blood was collected. Stalk blood was collected for 1 h to measure hypothalamic CRF release. Then, the rats were killed and the

hypothalamus was removed to measure the CRF content. Plasma and hypothalamic samples were kept at -20 °C until assayed.

6.3.4. Hormone determinations

PRL, LH and FSH were estimated by RIA in at least 2 volumes of plasma as described before (PRL: De Greef and Zeilmaker, 1978; LH and FSH: Welschen et al., 1975) using NIADKK RP-1 as standards. Intra- and interassay coefficients of variation for these assays were between 7 and 14%. ACTH was measured by RIA in unextracted plasma using a commercial kit (Byk-Mallinckrodt, Dietzenbach, Federal Republic of Germany). Inter-assay variation was 10% and intra-assay variation for plasma ACTH between 0.6 and 6 ng/ml was 12%. Testosterone was measured by RIA as described by Verjans et al. (1973), and intra- and interassay coefficients of variation were 7 and 15%, respectively. Corticosterone was determined by RIA (Weber et al., 1987) using antiserum purchased from Bioclinical Services Ltd. (Cardiff, South Glamorgan, United Kingdom). Interassay coefficient of variation was 12%, and intra-assay coefficient of variation was 8%.

Dopamine was determined by an isocratic high-pressure-liquid-chromatographic-electrochemical method (De Greef and Visser, 1981). ESA Coulochem Model 5100A with conditioning cell 5021 and analysis cell 5011 (ESA, Bedford, MA, USA) was used as detector. The minimal detectable amount of dopamine was about 5 pg. CRF was measured by RIA using antiserum K8659 which was raised in rabbits against CRF1-41 coupled with 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide-HCl (Biorad, Richmond, CA, USA) to bovine thyroglobulin (Sigma, St. Louis, MO, USA). Synthetic CRF1-41 (Peninsula Labs Europe, Merseyside, UK) was used as standard, and ^{125}I-Tyr-CRF1-41 (NEN Research Products, Boston, MA, USA) as label. Native CRF and standard CRF (added to charcoal-treated serum) were extracted with acetone, with a recovery of more than 95%. The sensitivity of this assay, defined as the amount of hormone that reduced binding to 90% of that in absence of unlabeled hormone, is approximately 30 pg. A dilution curve of hypothalamic extract was found to be parallel to synthetic CRF.

Cross-reactivity with a number of peptides, including TRH, LHRH and somatostatin, was less than 5%. Intra- and interassay coefficients of variation were 8 and 11%, respectively.

6.3.5. Statistical Analysis

Results are presented as means ± SEM. Student t-tests or analysis of variance followed by Duncan's multiple range tests were used to establish significant differences. Differences were considered to be significant at $p \leq 0.05$.

6.4. Results

6.4.1. Experiment 1

Hormone levels 3-4 weeks after inoculation with tumour 7315b and just before the hypophysial stalk was cut are presented in Table 6.1. Compared with controls, tumour-bearing rats had higher plasma PRL and ACTH levels, but lower plasma LH and FSH. Plasma testosterone and corticosterone were similar in both groups of rats. Immunoreactive CRF in peripheral plasma was not significantly different between control and tumour-bearing rats.

In hypophysial stalk plasma of tumour-bearing rats dopamine levels increased from 5.8±0.8 to 15.3±1.5 ng/ml, and those of immunoreactive CRF from 380±44 to 617±61 pg/ml (Table 6.2.). The hypothalamic content of dopamine did not change by tumour 7315b but hypothalamic CRF content significantly increased from 3.0±0.3 to 4.3±0.3 ng.

Table 6.1. Effect of chronic hyperprolactinaemia induced by tumour 7315b on plasma levels (ng/ml) of corticosterone (CORT), testosterone (TEST), LH, FSH, PRL and ACTH in adrenalectomized, gonadectomized male rats (n=16)

Treatment	*CORT*	*TEST*	*PRL*	*LH*	*FSH*	*ACTH*
Controls	42±8	0.7±0.1	72± 23	305±77	941±122	0.9±0.3
Tumour 7315b	51±7	0.9±0.1	7615±960*	43±14*	333±35*	3.1±0.6*

*The rats had been inoculated with 7315b tumour and had received a testosterone capsule and a corticosterone pellet 3-4 weeks earlier. Blood was collected just before the hypophysial stalk was cut to collect hypophysial portal blood. Results are given as means ± SEM. *)$p \leq 0.05$ compared with controls.*

Table 6.2. Effect of chronic hyperPRLaemia induced by tumour 7315b (7315b) on hypothalamic content (ng/hypothalamus) and on levels and release in hypophysial stalk plasma of CRF and dopamine (DA) in adrenalectomized, gonadectomized male rats (n=16)

Treatment	*Hypophysial portal plasma*				*Hypothalamic*	
	CRF		*DA*		*CRF*	*DA*
	pg/ml	*pg/h*	*ng/ml*	*ng/h*		
Controls	380±44	197±28	5.8±0.8	2.9±0.4	3.0±0.3	2.0±0.2
7315b	617±61*	298±23*	15.3±1.5*	7.4±1.0*	4.3±0.3*	2.1±0.3

*The rats had been inoculated with 7315b tumour and had received a testosterone capsule and a corticosterone pellet 3-4 weeks earlier. Results are given as means ± SEM. *)$p \leq 0.05$ compared with controls.*

6.4.2. Experiment 2

HyperPRLaemia induced by implantation of 3 pituitary glands under the kidney capsule caused an increase in the adrenal weight (49 ± 4 mg vs 34 ± 2 mg for controls). Pituitary-grafted rats had higher plasma PRL, ACTH and corticosterone levels, and lower LH levels than controls (Table 6.3.). Plasma testosterone and FSH were similar in pituitary-grafted and control rats. Adrenalectomized rats had higher ACTH and lower corticosterone levels than controls. Hypothalamic CRF-content was not different between control and experimental animals (Table 6.4.). Release of CRF into hypophysial stalk blood was somewhat, but not significantly, higher in pituitary-grafted rats than in control rats (102 vs 82 pg/h). Hypothalamic CRF secretion was increased in adrenalectomized rats (Table 6.4.).

Taken together the data of both experiments, a positive correlation was found between plasma PRL and plasma ACTH (coefficient of correlation 0.89, $p \leq 0.005$).

Table 6.3. Effect of adrenalectomy (ADX) or chronic hyperPRLaemia induced by pituitary grafts (GRAFTS) on plasma levels (ng/ml) of corticosterone (CORT), testosterone (TEST), LH, FSH, PRL and ACTH in male rats

Treatment	*CORT*	*TEST*	*PRL*	*LH*	*FSH*	*ACTH*
Controls (8)	268±14	1.7±0.3	52±8	41±6	393±26	0.22±0.02
GRAFTS (8)	455±39*	1.5±0.4	156±18*	21±2*	335±29	0.46±0.05*
ADX (6)	15± 1*	2.6±0.6	39±12	30±3	410±49	1.02±0.23*

*Three pituitary glands had been inserted under the left kidney capsule 5-7 weeks earlier, while adrenalectomy was performed 2 weeks earlier. Blood was collected just before the hypophysial stalk was cut to collect hypophysial portal blood. Results are means ± SEM, and the number of rats is in parentheses. *)$p \leq 0.05$ compared with controls.*

Table 6.4. Effect of adrenalectomy (ADX) or chronic hyperPRLaemia induced by pituitary grafts (GRAFTS) on hypothalamic content (ng/hypothalamus) and on levels and release of CRF in hypophysial portal plasma of male rats

Treatment	*Hypophysial portal plasma*		*Hypothalamus*
	pg/ml	*pg/h*	
Controls (8)	127±45	82±37	5.8±1.0
GRAFTS (8)	170±34	102±32	6.3±0.9
ADX (6)	477±48*	242±26*	6.3±1.4

*Three pituitary glands had been inserted under the kidney capsule 5-7 weeks earlier, whereas adrenalectomy was performed 2 weeks earlier. Results are means±SEM. Number of rats is in parentheses. *)$p \leq 0.05$ compared with controls.*

6.5. Discussion

The present study confirms that severe hyperPRLaemia induced by the transplantable PRL-secreting tumour 7315b is associated with a marked reduction in plasma gonadotrophins (Kooy et al., 1989; Voogt et al., 1987). Previously, it was observed that the inhibitory action of hyperPRLaemia on gonadotrophin levels is related to a suppression of LHRH release into hypophysial stalk plasma (Voogt et al., 1987). The mechanism by which high PRL levels reduce hypothalamic LHRH release is, however, unknown. One hypothesis states that PRL inhibits LHRH secretion by stimulation of the activity of the tubero-infundibular dopaminergic neurons terminating in the lateral aspects of the median eminence (Selmanoff, 1981). Indeed, hyperPRLaemia induces a marked rise of dopamine in hypophysial stalk plasma (Voogt et al., 1987; Weber et al., 1983; present study). We believe, however, that an increased activity of dopaminergic neurons is not a major factor involved in the suppression of LHRH since raised LHRH levels in pituitary stalk blood can occur in

the presence of increased dopamine levels (Weber et al., 1983).

The present study also demonstrates that chronic hyperPRLaemia induced by tumour 7315b increases hypothalamic content and release of immunoreactive CRF. This increased release of CRF in tumour-bearing rats was associated with a 3-fold rise in plasma ACTH. The immuno-reactive CRF concentrations measured in portal blood in the present study were highly comparable to those measured by Plotsky and Sawchenko (1987). These investigators reported values in intact and adrenalectomized urethane-anaesthetized male rats of about 0.2 and 0.4 ng/ml, respectively. In our adrenalectomized, corticosterone-treated rats plasma ACTH levels were, however, as high as those found in adrenalectomized rats (Akana et al., 1985, present study). This was probably due to the fact that the levels of corticosterone achieved by the corticosterone pellet were just too low to suppress ACTH secretion (Akana et al., 1985).

It could be argued that the increased ACTH level in tumour-bearing rats is not due to the induced hyperPRLaemia, but is caused by CRF release from tumour 7315b. However, it is not likely that tumour 7315b secretes CRF since levels of CRF in peripheral plasma of tumour-bearing rats did not differ significantly from those in control animals. Similarly, it is improbable that the raised levels of ACTH in tumour-bearing rats are due to ACTH secretion by tumour 7315b, since adrenal glands of intact tumour-bearing rats are only slightly enlarged (J.T.M. Vreeburg, unpublished results). Moreover, tumour 7315b does not contain ACTH (S.W.J. Lamberts, unpublished results). It was also found that mild chronic hyperPRLaemia, induced by implantation of three pituitary glands, led to higher ACTH and corticosterone levels, and to increased adrenal weight. Furthermore, plasma LH was reduced in the pituitary-grafted rats. Thus, this model of hyperPRLaemia also seems to activate the hypothalamic-hypophysial-adrenal-axis. However, hypothalamic CRF content and release was not significantly increased in pituitary-grafted rats, although CRF release tended to increase in these animals. Perhaps, the stimulus provided by the pituitary transplants was too weak to overcome the variability in the CRF levels. Nevertheless, the finding that hyperPRLaemia induced by pituitary grafts activates the hypothalamic-hypophysial-adrenal-axis, provides further evidence that the enhanced hypothalamic CRF release in rats with tumour 7315b is caused by PRL, and not by other secretory products of this tumour.

The increased hypothalamic immunoreactive CRF release observed in tumour-bearing rats is interesting since intracerebroventricular administration of CRF has been found to suppresse the hypothalamic release of LHRH, the concentrations of LH in plasma and components of male copulatory behaviour (Gambacciani et al.,1986; Gindoff and Ferin, 1987; Petraglia et al., 1987; Rivier and Vale, 1984; Sirinathsinghji, 1987). These effects are highly similar to those found during chronic hyperPRLaemia. Moreover, both the effects of hyperPRLaemia (Carter et al., 1984; Sarkar and Yen, 1985) and of CRF (Almeida et al., 1985; Sirinathsinghji; 1987) on the release of LHRH and LH and on male copulatory behaviour can be antagonized with naloxone. Thus, it is tempting to suggest that the negative effect of hyperPRLaemia on the release of gonadotrophins from the pituitary gland and on male sexual behaviour is caused by activation of hypothalamic CRF neurons, which act via endogenous opioids on the LHRH-secreting neurons.

The suggestion that CRF mediates the effect of hyperPRLaemia would also explain previous results obtained with the PRL- and ACTH-secreting tumour 7315a and the PRL-secreting tumour 7315b, which was derived from tumour 7315a (Lamberts et al., 1984). Whereas tumour 7315b markedly reduced gonadotrophin secretion and sexual behaviour in adrenalectomi-zed male rats (Kooy et al., 1988 & 1989; Voogt et al., 1987), tumour 7315a had no negative effects in the adrenalectomized male rat (Weber et al., 1982 & 1983). Thus, ACTH secreted by tumour 7315a seems to prevent the negative effect of PRL, probably because high levels of ACTH can inhibit the secretory activity of CRF neurons. Support for this concept is that intracerebroventricular administration of ACTH has been found to induce a dose-related rise in serum LH levels (Mann et al., 1986). Further research is necessary to prove that CRF and endogenous opioids are indeed involved in the negative effect of hyperPRLaemia on the release of gonadotrophins and male sexual behaviour.

In conclusion, we have provided evidence that chronic hyperPRLaemia induced by the PRL-secreting tumour 7315b as well as by implantation of 3 pituitary glands under the kidney capsule causes activation of the hypothalamic-hypophysial-adrenal-axis. Furthermore, we have postulated that hypothalamic CRF is a factor mediating the negative effects of chronic hyperPRLaemia on gonadotrophin release.

6.6. References

Adler,R.A. (1986). The anterior pituitary-grafted rat: A valid model of chronic hyperprolactinemia. Endocr. Rev. 7: 302-313.

Akana,S.F., Cascio,C.S., Shinsako,J., and Dallman,M.F. (1985). Corticosterone: narrow range required for normal body and thymus weight and ACTH. Am.J.Physiol. 249 (Regulatory Integrative Comp. Physiol. 18), R527-R532.

Almeida,O.F.X., Nikolarakis,K.E., and Herz,A. (1988). Evidence for the involvement of endogenous opioids in the inhibition of luteinizing hormone by corticotropin-releasing factor. Endocrinol. 122, 1034-1041.

Bain,P.A., Shrenker,P., and Bartke,A. (1987). The effect of luteinizing hormone releasing hormone on the copulatory behavior of hyperprolactinemic male rats. Horm. Behav. 21, 430-439.

Bartke,A., Smith,M.S., Michael,S.D., Peron,F.G., and Dalterio,S. (1977). Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotrophin levels in male rats and mice. Endocrinol. 100, 182-186.

Carter,D.A., Cooper,J.S., Inkster,S.E., and Whitehead,S.A. (1984). Evidence for an increased opioid inhibition of LH secretion in hyperprolactinaemic ovariectomized rats. J. Endocr. 101, 57-61.

Cheung,C.Y. (1983). Prolactin supresses luteinizing hormone secretion and pituitary responsiveness to luteinizing hormone-releasing hormone by a direct action at the anterior pituitary. Endocrinol. 113, 632-638.

Clayton,R.N., and Bailey,L.C. (1982). Hyperprolactinaemia attenuates the gonadotrophin releasing hormone receptor response to gonadectomy in rats. J. Endocr. 95, 267-274.

Gambacciani,M., Yen,S.S.C., and Rasmussen,D.D. (1986). GnRH release from the mediobasal hypothalamus: in vitro inhibition by corticotropin-releasing factor. Neuroendocrinol. 43, 533-536.

Gibbs,D.M., and Neill,J.D. (1978). Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinol. 102, 1895-1900.

Gindoff,P.R., and Ferin,M. (1987). Endogenous opioid peptides modulate the effect of corticotropin-releasing factor on gonadotropin release in the primate. Endocrinol. 121, 837-842.

Grandison,L., Hodson,C., Chen,H.T., Advis,J., Simpkins,J., and Meites,J. (1977). Inhibition by prolactin of post-castration rise in LH. Neuroendocrinol. 23, 312-322.

Greef,W.J.de, and Visser,T.J. (1981). Evidence for the involvement of hypotha-lamic dopamine and thyrotrophin-releasing hormone in suckling-induced release of prolactin. J. Endocr. 91, 213-223.

Greef,W.J.de, and Zeilmaker,G.H. (1978). Regulation of prolactin secretion during the luteal phase in the rat. Endocrinol. 102, 1190-1198.

Hodson,C.A., Burden,H.W., Louis,T.M., Poole,M., and Lawrence, I.E.,Jr. (1981). Inhibition of hypothalamic LHRH depletion after ovariectomy by transplantable prolactin and growth-hormone-secreting tumors. Proc. Soc. Exp. Biol. Med. 167, 369-373.

Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg, J.T.M. (1988). Deterioration of male sexual behavior in rats by the new prolactin-secreting tumor 7315b. Horm. Behav. 22, 351-361.

Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1989). Effects of the new prolactin-producing tumour 7315b on gonadotrophin secretion in adult male and female rats. J. Endocrinol. 120, 261-268.

Lamberts,S.W.J., Uitterlinden,P., Bons,E.G., Zuiderwijk,J.M., Verleun,T., Oosterom,R., and Hackeng,W.H.L. (1984). Hyperprolactinemia exerts a negative effect on the ß-endorphin content of the rat neurointermediate pituitary lobe. Endocrinol. 114, 2349-2353.

Lamberts,S.W.J., Zuiderwijk,J.M., Bons,E.G., Uitterlinden,P., and De Jong,F.H. (1981). Gonadotropin secretion in rats bearing a prolactin-secreting pituitary tumor: effects of naloxon administration. Fert. Steril. 35, 557-562.

Mann,D.R., Evans,D.C., Jacobs,V.L., and Collins,D.C. (1986). Influence of acute intracerebroventricular (i.c.v.) administration of adrenocorticotrophin (ACTH) on LH secretion in male rats: effect of pretreatment (i.c.v.) with ACTH antiserum on the serum LH response to an acute ether stress. J. Endocrinol. 108, 275-280.

McNeilly,A.S., Sharpe,R.M., Davidson,D.W., and Fraser,H.M. (1983). Inhibition of gonadotrophin secretion by induced hyperprolactinaemia in the male rat. J. Endocrinol. 79, 59-68.

Petraglia,F., Sutton,S., Vale, W., and Plotsky,P.M. (1987). Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation. Endocrinol. 120, 1083-1088.

Plotsky,P.M., and Sawchenko,P.E. (1987). Hypophysial-portal plasma levels, median eminence content, and immunohistochemical staining of corticotropin-releasing factor, arginine vasopressin, and oxytocin after pharmacological adrenalectomy. Endocrinol. 120, 1361-1369.

Porter,J.C. and Smith,K.R. (1967). Collection of hypophysial stalk blood in rats. Endocrinol. 81, 1182-1185.

Rivier,C., and Vale,W. (1984). Influence of corticotropin-releasing factor on reproductive functions in the rat. Endocrinol. 114, 914-921.

Sarkar,D.K., and Yen,S.S.C. (1985). Hyperprolactinemia decreases the luteinizing hormone-releasing hormone concentration in pituitary portal plasma: a possible role for ß-endorphin as a mediator. Endocrinol. 116, 2080-2084.

Selmanoff,M. (1981). The lateral and medial median eminence: distribution of dopamine, norepinephrine, and luteinizing hormone-releasing hormone and the effect of prolactin on catecholamine turnover. Endocrinol. 108, 1716-1722.

Sirinathsinghji,D.J.S. (1987). Inhibitory influence of corticotropin-releasing factor on components of sexual behaviour in the male rat. Brain Res. 407, 185-190.

Vasquez,J.M., Ellegood,J.O., Nazian,S.J., and Mahesh,V.B. (1980). Effect of hyperprolactinemia on pituitary sensitivity to luteinizing hormone-releasing hormone following manipulation of sex steroids. Fert. Steril. 33: 543-549.

Verjans,H.L., Cooke,B.A., de Jong,F.H., de Jong,C.M.M., and Van der Molen,H.J. (1973). Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid Biochem. 4, 665-676.

Voogt,J.L., de Greef,W.J., Visser,T.J., de Koning,J., Vreeburg,J.T.M., and Weber,R.F.A. (1987). In vivo release of dopamine, luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in male rats bearing a prolactin-secreting tumor. Neuroendocrinol.46, 110-116.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982). Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex organ weights in adult male rats: a possible role of the adrenals. Endocrinol. 111, 412-417.

Weber,R.F.A., de Greef,W.J., de Koning,J., and Vreeburg,J.T.M. (1983). LH-RH and dopamine levels in hypophysial stalk plasma and their relationship to plasma gonadotropins and prolactin levels in male rats bearing a prolactin- and adrenocorticotrophin-secreting pituitary tumor. Neuroendocrinol. 36, 205-210.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1987). The contribution of corticosterone and testosterone to the suppression of serum LH in hyperprolactinaemic adult male rats with pituitary transplants. J. Endocrinol. 113, 111-116.

Welschen,R., Osman,P., Dullaart,J., de Greef,W.J., Uilenbroek,J.Th.J., and de Jong,F.H. (1975). Levels of follicle-stimulating hormone, luteinizing hormone, oestradiol-17ß and progesterone, and follicular growth in the pseudopregnant rat. J. Endocrinol. 64, 37-47.

Winters,S.J., and Loriaux,D.L. (1978). Suppression of plasma luteinizing hormone by prolactin in the male rat. Endocrinol. 102, 864-868.

CHAPTER VII

EFFECTS OF HYPERPROLACTINAEMIA ON THE ADENOHYPOPHYSIAL-ADRENAL AXIS IN MALE RATS BEFORE AND DURING GLUCOCORTICOID TREATMENT

7.1. Abstract

HyperPRLaemia induced by the transplantable PRL-secreting tumour 7315b as well as by pituitary grafts has been associated with an activation of the (hypothalamic)-adenohypophysial-adrenal axis in intact male rats. Since pituitary grafts have been demonstrated to cause an increase in adrenal weight in rats even during treatment with high doses of corticosterone acetate, the hypothesis was tested that hyperPRLaemia is able to activate the adenohypophysial-adrenal axis in the presence of glucocorticoid excess. Two models of hyperPRLaemia were used, namely inoculation with the PRL-secreting tumour 7315b and implantation of three isogeneic pituitary glands.

Fourteen days after pituitary grafting or tumour inoculation, an increase in serum ACTH concentrations was found in intact pituitary-grafted (ACTH: 111±15 pg/ml; PRL: 329±36 ng/ml) and tumour-bearing (ACTH: 195±24 pg/ml; PRL: 964±77 ng/ml) animals, as compared with their controls (ACTH: 73±7 pg/ml; PRL: 9±1 ng/ml). Subsequent treatment of control animals with corticosterone acetate (daily 10 mg subcutaneously, during 9-10 days) resulted in a marked decrease of serum ACTH levels down to the level of detection (ACTH: 10±2 pg/ml; PRL: 11±2 ng/ml), while the effect of this treatment on serum ACTH levels appeared to be inhibited in the pituitary-grafted (ACTH: 49±12 pg/ml; PRL: 212±20 ng/ml) and tumour-bearing (ACTH: 257±46 pg/ml; PRL: 2624±240 ng/ml) rats; from these

data, PRL appeared to be highly correlated with serum ACTH (r=0.88) and with adrenal weight (r=0.94), despite the severe glucocorticoid excess induced.

In an additional experiment, pituitary grafting resulted again in a significant rise of serum ACTH concentrations (529±113 pg/ml in grafted animals vs 306±42 pg/ml in controls). However, in this experiment the increase of serum PRL levels induced by pituitary grafts was less marked (132±27 ng/ml), and not associated with a rise of serum ACTH concentrations during glucocorticoid excess. Probably, the hyperPRLaemia induced may have been too low to provide an adequate stimulus to overcome the inhibitory action of glucocorticoid excess on ACTH release. So the origin of the rise in ACTH levels, as previously found in pituitary-grafted animals during glucocorticoid excess (experiment 1), could not be established in this experiment, but evidence exists that ectopic pituitary glands are not likely to secrete significant amounts of ACTH.

Finally, the tumour-induced increase of serum levels of immuno-active ACTH could not be prevented by hypophysectomy. Therefore, ectopic production of ACTH and/or other POMC-derived molecules by tumour 7315b is probable. Nevertheless, tumour 7315b has been shown to stimulate hypothalamic CRF-release into pituitary stalk blood, indicating that at least part of the rise in serum ACTH originates from the eutopic pituitary gland.

It is concluded, that different models of hyperPRLaemia are able to activate the adenohypophysial-adrenal axis in adult male rats, confirming the findings presented in chapter VI. The height of the PRL levels induced after pituitary grafting seems to determine to what extent a hyperPRLaemia-associated rise of serum ACTH can be suppressed by high doses of corticosterone acetate. Part of the tumour-induced increase in serum concentrations of immunoactive ACTH may originate from the tumour itself, which does not preclude a stimulatory action of PRL on the hypothalamic-adenohypophysial-adrenal axis.

7.2. Introduction

Decrease of gonadotrophin secretion and inhibition of male sexual behaviour occur

in male rats rendered hyperPRLaemic by PRL-secreting tumours (Fang et al., 1974; Kalra et al., 1983; Kooy et al., 1988 & 1989) or pituitary grafts (Bailey and Herbert, 1982; Bartke et al., 1977; Doherty et al., 1986; Shrenker and Bartke, 1985). Although it is obvious that hyperPRLaemia directly affects certain brain functions, such as hypothalamic secretion of dopamine and LHRH into pituitary portal vessel blood (Carter et al., 1984; Hodson et al., 1981; Voogt et al., 1987), it is not clear how hyperPRLaemia interferes with the regulation of male reproductive functions. Recently, we have provided evidence that at least part of the action of PRL on male reproductive functions may be mediated by an activation of CRF-containing neurons in the hypothalamus (Kooy et al., 1990). Since CRF can markedly decrease LHRH concentrations in pituitary portal vessel blood (Petraglia et al., 1987), and inhibit male sexual behaviour (Sirinathsinghji, 1987), stimulated CRF-secreting neurons may bring about both the inhibition of reproductive functions and the activation of the hypothalamic-adenohypophysial-adrenal axis observed in hyperPRLaemic rats. In these animals, elevated serum levels of corticosterone and increased adrenal weights are common findings (Colby, 1979; Kooy et al., 1989; Mann et al., 1977; Mazzocchi et al., 1986; Piva et al., 1973). Even in rats treated with high doses of corticosterone acetate (7-10 times substitution dose) the adrenal weights are increased after pituitary grafting (Weber et al., 1987). To explain the latter finding several mechanisms of PRL action can be proposed. Firstly, PRL may induce adrenal growth independently of ACTH secretion. Secondly, PRL may stimulate ACTH release even when extremely high serum levels of corticosterone are induced. The present study was performed to investigate to what extent the raised serum concentrations of ACTH during hyperPRLaemia are affected by daily treatment with high doses of corticosterone acetate (experiment 1). In this experiment, the finding of increased levels of ACTH in the corticosterone-treated hyperPRLaemic animals suggested that PRL may stimulate the secretion of ACTH from the eutopic pituitary in the presence of glucocorticoids. However, secretion of ACTH by the ectopic pituitary glands or tumour 7315b could not be excluded. Therefore, we decided to hypophysectomize tumour-bearing and pituitary-grafted rats (experiment 2). The pituitary-grafted rats and their controls were treated with high doses of corticosterone acetate (CA) to exclude activation of the pituitary grafts by uninhibited hypothalamic CRF release.

7.3. Materials and methods

7.3.1. Animals

Adult male rats (2.5-3.5 months of age) of the Buffalo strain were used and housed under controlled conditions of temperature (20°C) and light (14 h light : 10 h darkness). The lights were on between 18.30 and 8.30 h. Standard food and water were always available. HyperPRLaemia was induced by either transplanting 3 isogeneic pituitary glands under the left kidney capsule or subcutaneous inoculation of tumour 7315b, as previously described (Kooy et al., 1988). Preceding inoculation tumour tissue was minced into small pieces, which were suspended in an equal volume of 0.9% NaCl(w/v) solution. Subsequently, 0.4 ml of this suspension was subcutaneously injected into the experimental animals on the dorsal side of the neck. Ten days later, a palpable mass with a diameter of ± 0.5 cm could be found at the place of injection. Three to four weeks after inoculation tumours reached diameters ranging from 4 to 5 cm. All animals remained in good condition during the experiments.

7.3.2. Experiment 1: *Effects of pituitary grafts and tumour 7315b on adrenal weight and serum levels of PRL, ACTH, LH, and testosterone in male rats during daily treatment with high doses of CA*

On day 0, ten male rats received three isogeneic pituitary grafts under the left kidney capsule (group I, n=10), while another eleven animals were inoculated with tumour 7315b (group II, n=11). A third group of nine rats was not subjected to one of these treatments and served as controls (group III, n=9). From day 14 until the day of autopsy (day 22-23), all animals were subcutaneously injected with CA, daily 10 mg (7-10 times substitution dose). Blood was sampled from all rats on day 14, just before the first treatment with CA, and directly preceding autopsy on day 22-23.

7.3.3. Experiment 2: *Effects of hypophysectomy on organ weights and serum concentrations of PRL and ACTH in hyperPRLaemic male rats*

In order to ascertain whether the presence of the eutopic pituitary gland contributes to the adrenocorticotrophic effects of pituitary grafts during glucocorticoid excess, three isogeneic pituitary glands were successfully transplanted under the left kidney capsule of ninteen rats on day 0 (group I, n=7; group II, n=12). Twelve of these rats were hypophysectomized on day 22 (group II), five of which were excluded from the experiment because of incompleteness of hypophysectomy. A third group of intact animals served as controls (n=7). All rats were daily treated with 10 mg CA subcutaneously, from day 22 until day 36. High doses of CA were given to suppress hypothalamic CRF release in the hypophysectomized and non-hypophysectomized animals. Blood was taken from all animals on day 14 and in the last week before autopsy on days 29 and 36. After decapitation on day 36, organs were removed and weighed. The sella turcica was inspected in the hypophysectomized rats.

Additionally, fourteen rats were inoculated with the tumour on day 0. Eight of them had been hypophysectomized two days before. Another seven control animals had been hypophysectomized on the same day. In the latter two groups five and four rats, respectively, were excluded because of incompleteness of hypophysectomy. On day 25, the rats were killed and blood was sampled for assaying PRL and ACTH; adrenals were removed and weighed.

7.3.4. Blood sampling and hormone determinations

During the experiments blood was sampled by orbital venous puncture under light halothane anaesthesia between 11.00 and 16.00 h. Since PRL, LH, FSH and testosterone levels in serum are unaffected by halothane (Cameron et al., 1983; Subramanian and Gala, 1977), we preferred halothane narcosis above the stress-inducing ether narcosis (Krulich et al., 1974).

PRL and LH were estimated by RIA in at least 2 duplicate samples of serum as described before (PRL: De Greef and Zeilmaker, 1978; LH: Welschen et al., 1975) using NIADKK RP-1 as standards. Intra- and interassay coefficients of variation for these assays were between 7 and 14%. ACTH was measured by RIA in unextracted serum using a commercial kit (Sa IRE Medgenix ACTH-RIA-100, code 30.006.60, B-6220 Fleurus, Belgium). Inter-assay variation was 10% and intra-assay variation for serum ACTH between 0.6 and 6 ng/ml was 12%. Testosterone was measured by RIA as previously described (Verjans et al., 1973), and intra- and interassay coefficients of variation were 7 and 15%, respectively. Corticosterone was determined by RIA (Weber et al., 1987) using antiserum purchased from Bioclinical Services Ltd. (Cardiff, South Glamorgan, UK). Interassay coefficient of variation was 12%, and intra-assay coefficient of variation was 8%.

7.3.5. Hypophysectomy

The bottom of the sella turcica was exposed and drilled via a parapharyngeal approach. After exposure of the hypophysial gland, the total gland was drained off, as previously described (Van Straalen et al., 1981). At autopsy in all hypophysectomized rats the sella was inspected under a dissection microscope to verify whether the pituitary gland had been removed. The animals with visible pituitary remnants were excluded from the experiments.

7.3.6. Statistical analysis

Results are presented as means±SEM. Student t-tests or analysis of variance followed by Duncan's multiple range tests were used to establish significant differences. Differences were considered to be significant at $p \leq 0.05$.

7.4. Results

7.4.1. Experiment 1

Serum concentrations of PRL, ACTH and testosterone before and during CA treatment are presented in Table 7.1. Pituitary grafting as well as tumour inoculation resulted in a significant increase of serum PRL on day 14 and days 22-23, as compared with controls. Moreover, before and during glucocorticoid excess, serum concentrations of ACTH from both pituitary grafted and tumour-bearing animals were significantly raised, as compared with controls.

Table 7.1. Effects of pituitary grafts (GRAFTS) and tumour 7315b (7315b) on serum concentrations of PRL(ng/ml), ACTH(pg/ml) and testosterone (TEST,ng/ml) in intact male rats before and during daily treatment with corticosterone acetate

Group			*PRL*	*ACTH*	*TEST*
I.	GRAFTS (n=10)	day 14	329±36a	111±15a	2.9±0.5
		day 22-23	212±20ac	49±12ac	0.9±0.2ac
II.	7315b (n=11)	day 14	964±77ab	195±24ab	2.8±0.2
		day 22-23	2624±240abc	257±46ab	<0.1abc
III.	controls (n=9)	day 14	9±1	73±7	2.6±0.1
		day 22-23	11±2	10±2c	0.3±0.2c

Pituitary grafting and tumour inoculation had been carried out on day 0. All rats had been sc treated with corticosterone acetate, daily 10 mg, from day 14, subsequently to the first blood sampling, until the day of autopsy, day 22/23. Numbers of animals within each group are presented between parentheses. Results are given as means ± SEM. a)$p<0.05$, compared with corresponding values in group III; b)$p<0.05$, compared with corresponding values in group I; c)$p<0.05$, compared with values at day 14 within the same group.

The positive correlation found between serum concentrations of PRL and ACTH during glucocorticoid excess is illustrated in Figure 7.1.a. In all groups serum LH became suppressed below the level of detection during CA treatment (data not shown), leading to a marked decrease of serum testosterone at the end of the experiment. On days 22-23, testosterone levels in pituitary-grafted rats were less reduced than those in control rats, while serum testosterone in tumour-bearing animals became suppressed below the level of detection.

Body and adrenal weights, are presented in Table 7.2. In both pituitary-grafted and tumour-bearing animals adrenal weights were significantly increased. The positive correlation between serum PRL concentrations and adrenal weights during CA treatment is illustrated in Figure 7.1.b.

Table 7.2. Body (BW, g) and adrenal (ADRENALS, mg) weights in intact pituitary-grafted (GRAFTS), tumour-bearing (7315b) and control male rats treated with corticosterone acetate

Group		*BW*	*ADRENALS#*
I.	GRAFTS (n=10)	244±8	16±2a
II.	7315B (n=11)	248±4	39±4ab
III.	controls (n=9)	252±3	11±1

Pituitary grafting and tumour inoculation had been carried out on day 0. All rats had been sc treated with corticosterone acetate, daily 10 mg, from day 14, subsequently to the first blood sampling, until the day of autopsy, day 22/23. Numbers of animals within each group are presented between parentheses. Results are given as means ± SEM. a)$p<0.05$, compared with group III; b)$p<0.05$, compared with group I; #) weight of two organs.

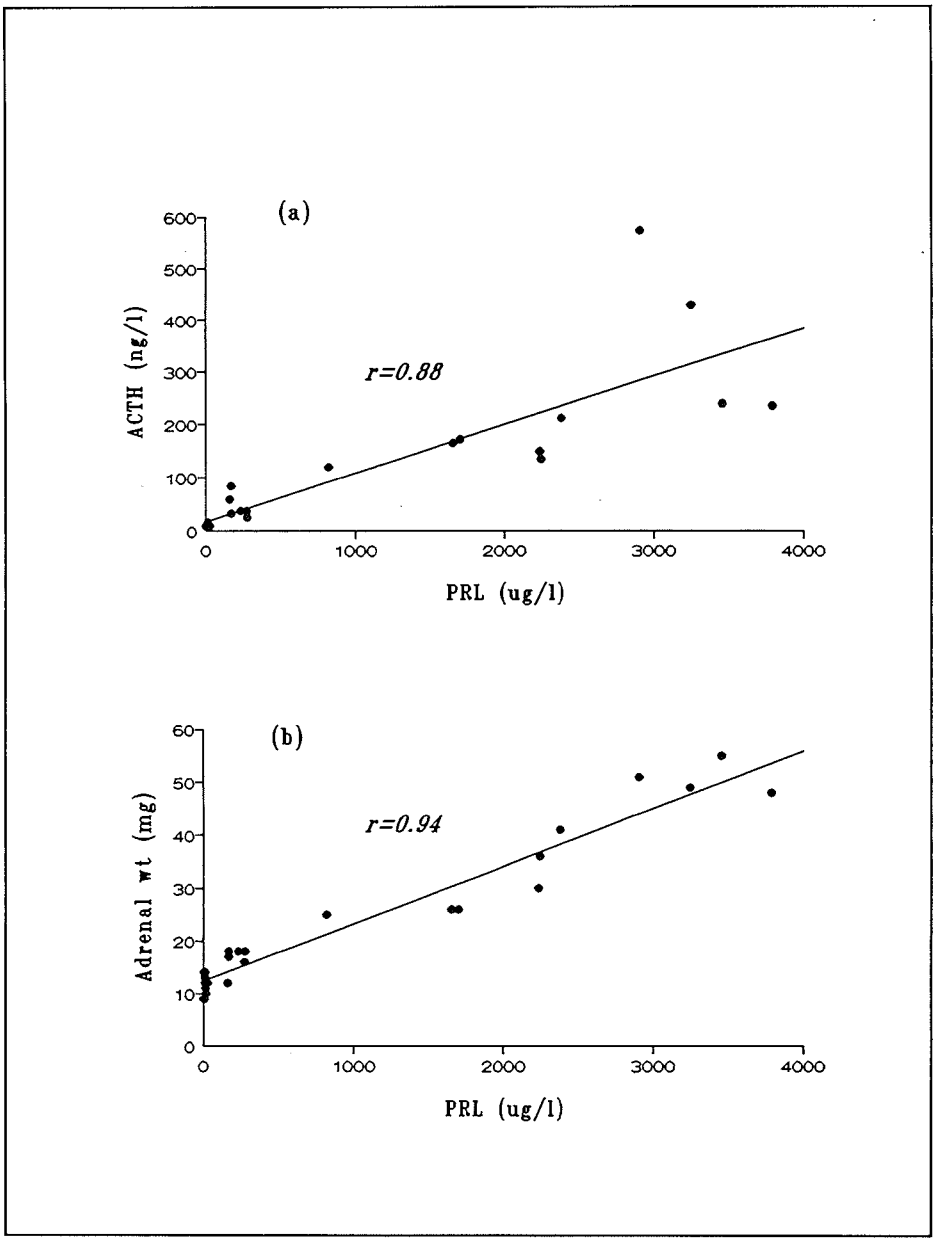

Figure 7.1. Correlations of serum PRL concentrations with serum ACTH levels and adrenal weights in normoPRLaemic and hyperPRLaemic animals treated with high doses of corticosterone acetate

7.4.2. Experiment 2.

Serum concentrations of PRL and ACTH of the pituitary-grafted rats and their controls are shown in Table 7.3. In all animals bearing pituitary transplants increased levels of PRL were found on days 14 and 29. On day 36, serum concentrations of PRL in the control rats were markedly elevated (128±29 ng/ml) indicating that the animals were stressed during sampling preceding autopsy. Therefore, serum concentrations of PRL and ACTH on day 36 are not given.

As in experiment 1, increased serum levels of ACTH were found in the hyperPRLaemic animals (Group I and II) on day 14. Contrary to experiment 1, no difference was found in the levels of ACTH between the intact pituitary-grafted rats

Table 7.3. Effects of pituitary grafts (GRAFTS) on serum concentrations of PRL (ng/ml) and ACTH (pg/ml) in intact and hypophysectomized (hypox) male rats before and during treatment with corticosterone acetate

Group			*PRL*	*ACTH*
I.	GRAFTS (n=7)	day 14	180±44a	529±113a
		day 29	132±27a	30±6b
II.	GRAFTS + hypox (n=7)	day 14	250±42a	544±93a
		day 29	196±42a	22±6b
III.	controls (n=7)	day 14	59±13	306±42
		day 29	18±6b	28±4b

Transplantation of pituitary grafts and hypophysectomy were carried out on day 0 and day 22, respectively. All rats had been sc treated with corticosterone acetate, daily 10 mg, from day 22 until autopsy. Numbers of animals within each group are presented between parentheses. Results are given as means ± SEM. a)$p<0.05$, compared with group III; b)$p<0.05$, compared with values on day 14 within the same group.

and the control animals after CA treatment. Hypophysectomy on day 22 did not affect serum ACTH in the animals bearing pituitary transplants during CA treatment (Group II, day 29).

Body and organ weights of the pituitary-grafted rats and their controls are presented in Table 7.4. At the beginning of the experiment the body weights did not differ between the groups (group I: 241±4 g; group II: 249±5 g; group III: 247±8 g). In the hypophysectomized rats a 15-20% decrease of body weight was observed at the end of the experiment. In these animals (group II), markedly decreased weights of testes, ventral prostate and seminal vesicles were found at autopsy, as compared with the non-hypophysectomized rats of groups I and III. A higher adrenal weight was observed in the intact pituitary-grafted rats (group I) than in the control group (III). This effect was diminished, but not abolished by hypophysectomy (group II).

Table 7.4. Effects of pituitary grafts (GRAFTS) on body (BW, g) and organ weights (mg) of adrenals (ADL), testes, prostate (PROST) and seminal vesicles (SV) in intact and hypophysectomized (hypox) male rats treated with corticosterone acetate

	Group	*BW*	*ADL#*	*TESTES#*	*PROST*	*SV#*
I.	GRAFTS (n=7)	217±6	24±1a	2147±81a	127±13	118±8
II.	GRAFTS + hypox (n=7)	187±6ab	21±1ab	906±44ab	60±3ab	87±3ab
III.	controls (n=7)	233±8	17±1	2555±73	154±18	121±7

Transplantation of pituitary grafts and hypophysectomy were carried out on day 0 and day 22, respectively. All rats had been sc treated with corticosterone acetate, daily 10 mg, from day 22 until autopsy. Numbers of animals within each group are presented between parentheses. Results are given as means ± SEM. a)$p<0.05$, compared with group III; b)$p<0.05$, compared with group I; #)weight of two organs.

Adrenal weights and serum concentrations of PRL and ACTH in tumour-bearing rats with and without hypophysectomy and in hypophysectomized control rats are presented in table 7.5. BW did not differ between the groups at the beginning of the experiment (group I: 268±13 g; group II: 253±6 g; group III: 257±19 g). Tumour inoculation resulted in a comparable elevation of serum PRL in the hypophysectomized and non-hypophysectomized animals. Hypophysectomy did not prevent the tumour-induced increase of serum ACTH and adrenal growth. Adrenal weights can be related to those found in untreated animals amounting 22±1 mg (n=6, BW: 273±13 g).

Table 7.5. Effects of tumour 7315b (7315b) on adrenal weight and serum concentrations of PRL (ng/ml) and ACTH (pg/ml) in intact and hypophysectomized (hypox) male rats

Group		*ADRENALS#*	*PRL*	*ACTH*
I.	7315b (n=6)	30±1	2397±312a	541±75a
II.	hypox + 7315b (n=3)	20±1ab	2580±241a	476±92a
III.	hypox (n=3)	9±0	<1.0	28±5

Inoculation of tumour 7315b took place on day 0. Rats of group II and III had been hypophysectomized 2 days before. On day 25, blood was sampled and adrenals were removed from all animals. Numbers of animals within each group are presented between parentheses. Results are given as means ± SEM. a)p<0.05, compared with group III; b)p<0.05, compared with group I; #)weight of two organs.

7.5. Discussion

In accordance with previous findings, in the present study inoculation of tumour 7315b induced markedly elevated levels of serum PRL within two weeks (2-3 ug/ml; Kooy et al., 1988 & 1989), while implantation of three pituitary glands resulted in a moderate hyperPRLaemia (200-400 ng/ml; Adler, 1986). Deterioration of male sexual behaviour and suppression of gonadotrophin release have been demonstrated in rats bearing tumour 7315b (Kooy et al., 1988 & 1989) or other PRL-secreting tumours (Fang et al., 1974; Kalra et al., 1983). These effects were more pronounced than those described in animals rendered moderately hyperPRLaemic by pituitary grafts (Adler, 1986; Drago, 1984). Thus, the inhibitory action of PRL on male reproductive functions appears to be dose-dependent. Evidence exists that at least part of this PRL action is centrally mediated by CRF, and that hypothalamic CRF release in hyperPRLaemia is dose-dependent (Kooy et al., 1990). The results of the first experiment confirm that hyperPRLaemia induced by pituitary grafts and by tumour 7315b is associated with an increase of serum ACTH. Subsequent treatment of control animals with high doses of CA resulted in a marked decrease of serum ACTH unto the level of detection. This dramatic decrease was expected since similar serum concentrations of ACTH have been found in adrenalectomized corticosterone-treated animals with circulating corticosterone levels of 80 ng/ml or more, indicating that only a moderate increase of serum corticosterone is sufficient to strongly suppress ACTH secretion (Akana et al., 1985). In the hyperPRLaemic animals of experiment 1, however, the suppressive action of the treatment with CA (7-10 times substitution dose, Weber et al., 1987) on serum concentrations of ACTH seemed to be inhibited by PRL in a dose-dependent manner; PRL was found to be highly correlated with serum ACTH ($r=0.88$) and with adrenal weight ($r=0.94$), despite the severe glucocorticoid excess induced. However, the conclusion that in the presence of high levels of corticosterone PRL stimulates ACTH release from the in situ pituitary cannot be drawn from these results for several reasons. Firstly, the moderately increased levels of ACTH in the pituitary-grafted rats during CA treatment could not be confirmed in the second experiment. This discrepancy might be due to the differences in the levels of PRL between the grafted animals of experiment 1 and 2

(212±20 and 132±27 ng/ml, respectively), as stimulation of the hypothalamic-adenohypophysial-adrenal axis in hyperPRLaemia is dose-dependent (Kooy et al., 1990). The concentrations of PRL in the pituitary-grafted rats of experiment 2 may have been too low to provide an adequate stimulus to overcome the inhibitory action of CA on ACTH release. Secondly, we could not demonstrate that the tumour-induced increase of serum levels of immunoactive ACTH is prevented by hypophysectomy. Therefore, ectopic production of ACTH and other POMC-derived molecules by tumour 7315b is probable, and must be established by further molecular characterization of the substances secreted by the tumour. Nevertheless, the increase of serum ACTH in rats bearing tumour 7315b has been previously associated with locally increased levels of CRF in hypothalami and hypophysial stalk serum (Kooy et al., 1990). Thus, it seems likely that tumour 7315b stimulates ACTH release by the eutopic pituitary gland through an action on CRF-secreting neurons. Some ACTH release by the tumour does not preclude, but may mask a PRL-induced stimulation of the (hypothalamic-) adenohypophysial-adrenal axis through feedback mechanisms activated by increased serum levels of ACTH and corticosterone. This may explain why no difference could be demonstrated in the serum concentration of ACTH between the tumour-bearing rats with and without hypophysectomy (476±92 and 541±75 pg/ml, respectively).

Just as in tumour-bearing rats, it can be argued that ectopic ACTH release may also be present in pituitary-grafted animals. However, since in hypophysectomized male rats with two transplanted pituitaries, corticosterone secretion into adrenal vein blood is not different from that of hypophysectomized control animals, even during traumatic stress (Greer et al., 1963), ectopic pituitary glands are not likely to secrete significant amounts of ACTH. Indeed, ten heterotopic pituitary grafts are necessary to maintain nearly normal adrenal weight in hypophysectomized male rats, but are not able to normalize corticosterone secretion (3.1±0.3 versus 19.0±1.6 ug/hr in intact animals; Kendall et al., 1966). As the contribution of each ectopic pituitary to the serum concentrations of ACTH is apparently very small, the significant increase of serum ACTH in the pituitary-grafted animals in the present study is thought to be due to the increased production of ACTH by the eutopic pituitary gland. In these animals, the stimulated adrenal growth was diminished, but not abolished by hypophysectomy, which may be due to the

relatively short period of time between hypophysectomy and autopsy (Kendall et al., 1966; Singer et al., 1955). Moreover, PRL itself may also directly exert adrenocorticotrophic effects (Colby, 1979).

The stimulatory action of PRL in vivo on ACTH release in intact animals (present study; Kooy et al., 1990) is in favour of a releasing capacity of PRL on CRF and/or ACTH containing cells. Indeed, very recently, it has been shown in in vitro studies that PRL (10^{-7} M), in contrast with growth hormone, is able to increase CRF release from hypothalamic tissue cultures, and to directly enhance pituitary ACTH secretion (Weber et al., 1990). Thus, the in vivo effects of hyperPRLaemia on serum concentrations of ACTH may be the result of a combined action on hypothalamic CRF-secreting neurons and adenohypophysial adrenocorticotrophic cells.

In conclusion, we have confirmed our previous findings that different models of hyperPRLaemia are able to activate the adenohypophysial-adrenal axis in adult male rats. This activation appeared to be markedly suppressed during treatment with high doses of CA in pituitary-grafted rats. Part of the tumour-induced increase in serum concentrations of immunoactive ACTH, however, may originate from the tumour itself.

7.6. References

Adler,R.A. (1986). The anterior pituitary-grafted rat: A valid model of chronic hyperprolactinemia. Endocr. Rev. 7, 302-313.

Akana,S.F., Cascio,C.S., Shinsako,J., and Dallman,M.F. (1985). Corticosterone: narrow range required for normal body and thymus weight and ACTH. Am. J. Physiol. 249 (Reg. Integr. Comp. Physiol. 18), R527-R532.

Bailey,D.J., and Herbert,J (1982). Impaired copulatory behavior of male rats with hyperprolactinemia induced by domperidone or pituitary grafts. Neuroendocrinol. 35, 186-193.

Bartke,A., Smith,M.S., Michael,S.D., Peron,F.G., Dalterio,S. (1977). Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinol. 100, 182-186.

Cameron,A.M., Dale,O., Haug,E., and Nilsen,O.G. (1983). Inhalation of enflurane and halothane at subanesthetic concentrations and effects on circulating serum testosterone, luteinizing hormone and follicle-stimulating hormone in the male rat. Toxicol. Lett. 19, 333-338.

Carter,D.A., Cooper,J.S., Inkster,S.E., and Whitehead,S.A. (1984). Evidence for an increased opioid inhibition of LH secretion in hyperprolactinaemic ovariectomized rats. J. Endocr. 101, 57-61.

Colby,H.D. (1979). Mechanism of action of prolactin on adrenocortical steroid secretion in hypophysectomized female rats. Endocrinol. 104, 1299-1303.
Doherty,P.C., Baum,M.J., and Todd,R.B. (1986). Effects of chronic hyperprolactinemia on sexual arousal and erectile function in male rats. Neuroendocrinol. 42, 368-375.
Drago,F. (1984). Prolactin and Sexual Behavior: A Review. Neurosc. Biobehav. Rev. 8, 433-439.
Fang,V.S., Refetoff,S., and Rosenfield,R.L. (1974). Hypogonadism induced by a transplantable, prolactin-producing tumor in male rats: Hormonal and morphological studies. Endocrinol. 95, 991-998.
Greef,W.J.de, Zeilmaker,G.H. (1978). Regulation of prolactin secretion during the luteal phase in the rat. Endocrinol. 102, 1190-1198.
Greer,M.A., Kendall,J.W.Jr., and Duyck,C. (1963). Failure of heterotopic rat pituitary transplants to maintain adrenocortical secretion. Endocrinol 72, 499-501.
Hodson,C.A., Simpkins,J.W., Pass,K.A., Aylsworth,C.F., Steger,R.W., and Meites,J. (1980). Effects of a prolactin secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats. Neuroendocrinol. 30, 7-10.
Kalra,P.S., Simpkins,J.W., Luttge,W.G., and Kalra,S.P. (1983). Effects on male sexual behavior and preoptic dopamine neurons of hyperprolactinemia induced by MtTW15 pituitary tumors. Endocrinol. 113, 2065-2071.
Kendall,J.W., Stott,A.K., Allen,C., and Greer,M.A. (1966). Evidence for ACTH secretion and ACTH suppressibility in hypophysectomized rats with multiple heterotopic pituitaries. Endocrinol. 78, 533-537.
Kooy,A., De Greef,W.J., Vreeburg,J.T.M., Hackeng,W.H.L., Ooms,M.P., Lamberts, S.W.J., and Weber,R.F.A. (1990). Evidence for the involvement of corticotropin-releasing factor in the inhibition of gonadotropin release induced by hyperprolactinemia. Neuroendocrinol. 51, 261-266.
Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1988). Deterioration of male sexual behavior in rats by the new prolactin-secreting tumor 7315b. Horm. Behav. 22, 351-361.
Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1989). Effects of the new prolactin-producing tumour 7315b on gonadotrophin secretion in adult male and female rats. J. Endocrinol. 120, 261-268.
Krulich,L., Hefco,E., Illner,P., and Read,C.B. (1974). The effects of acute stress on the secretion of LH, FSH, prolactin and GH in the normal male rat, with comments on their statistical evaluation. Neuroendocrinol. 16, 293-311.
Mann,D.R., Cost,M.G., Jacobson,C.D., and MacFarland,L.A. (1977). Adrenal gland rhythmicity and pituitary regulation of adrenal steroid secretion. Proc. Soc. Exp. Biol. Med. 156, 441-445.
Mazzocchi,G., Robba,C., Rebuffat,P., and Nussdorfer,G.G. (1986). Effects of prolactin administration on the zona glomerulosa of the rat adrenal cortex: stereology and plasma hormone concentrations. Acta Endocrinol. 111, 101-105.
Petraglia,F., Sutton,S., Vale,W., and Plotsky,P. (1987). Corticotropin-releasing factor decreases plasma LH levels in female rats by inhibiting GnRH release into hypophysial portal circulation. Endocrinol. 120, 1083-1088.
Piva,F.P., Gagliano,M.M., and Martini,L. (1973). Adrenal progesterone: factors controlling its secretion. Endocrinol. 93, 1178-1182.
Shrenker,P., and Bartke,A. (1985). Adrenalectomy does not prevent the hyperprolactinemic, induced sexual behavior deficits in CDF male rats. Life Sciences 36, 1881-1888.

Singer,B., and Stack-Dunne,M.P. (1955). The secretion of aldosterone and corticosterone by the rat adrenal. J. Endocrinol. 12, 130-145.
Sirinathsinghji, D.J.S. (1987). Inhibitory influence of corticotropin releasing factor on components of sexual behaviour in the male rat. Brain Res. 407, 185-190.
Subramanian,M.G., and Gala,R.R. (1977). The effects of continuous chloroform and halothane anesthesia on plasma prolactin levels in ovariectomized estrogen-treated rats. Experientia 33, 1245-1246.
Van Straalen,R.J.C., Leemborg,F.G., Vreeburg,J.T.M., and G.H. Zeilmaker (1981). Prolonged steroidogenesis in luteinized ovaries of hypophysectomized rats. Acta Endocrinol. 98, 437-440.
Verjans,H.L., Cooke,B.A., de Jong,F.H., de Jong,C.M.M., van der Molen,H.J. (1973). Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid Biochem. 4, 665-676.
Voogt,J.L., de Greef,W.J., Visser,T.J., de Koning,J., Vreeburg,J.T.M., and Weber,R.F.A. (1987). In vivo release of dopamine, luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in male rats bearing a prolactin-secreting tumor. Neuroendocrinol. 46, 110-116.
Vreeburg,J.T.M., Samaun,K., Verkade,H.J., Verhoef,P., Ooms,M.P., and Weber,R.F.A. (1988). Effects of corticosterone on the negative feedback action of testosterone, 5α-dihydrotestosterone and estradiol in the adult male rat. J. Steroid. Biochem. 29, 93-98.
Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1987). The contribution of cortico-sterone and testosterone to the suppression of serum LH in hyperprolactinaemic adult male rats with pituitary transplants. J. Endocrinol. 113, 111-116.
Weber,R.F.A., and Calogero,A.E. (submitted). Prolactin stimulates rat hypothalamic corticotrophin-releasing hormone and pituitary adrenocorticotrophin secretion in vitro.
Welschen,R., Osman,P., Dullaart,J., de Greef,W.J., Uilenbroek,J.Th.J., and de Jong,F.H. (1975). Levels of follicle-stimulating hormone, luteinizing hormone, oestradiol-17ß and progesterone, and follicular growth in the pseudopregnant rat. J. Endocrinol. 64, 37-47.

CHAPTER VIII

GENERAL DISCUSSION

8.1. Introduction

Since chronic hyperPRLaemia can deteriorate male sexual behaviour and suppress gonadotrophin release in the rat independently of gonadal and adrenal activity (chapters IV and V), an action of PRL on brain functions interfering with reproduction is conceivable. Indeed, peripherally induced hyperPRLaemia is able to influence hypothalamic LHRH, DA and CRF release and pituitary ACTH secretion possibly interfering with male reproductive functions (chapters VI and VII). With respect to the effects of PRL on neuronal processes, however, several questions may arise. Firstly, how can PRL, originating from peripheral blood, reach the brain ? Secondly, does any evidence exist that PRL can have electrophysiological effects on neuronal processes ? Thirdly, by which putative mediators does PRL exert its effects on the neuroendocrine regulation of male reproductive functions ?

8.2. The presence of PRL in the brain

PRL has been found by immunocytochemical and radioimmunological techniques in several brain sites (DeVito, 1989; Emanuele et al., 1986 & 1987; Fuxe et al., 1977; Kendall and Orwoll, 1980) and cerebrospinal fluid (CSF) (Emanuele et al., 1989; Login and MacLeod, 1979), but the routes by which PRL enters the CSF are not completely clear. Several ways by which PRL may have access to hypothalamic neurons have been

proposed. Positive correlations between PRL levels in peripheral blood and CSF with a relatively low plasma/CSF ratio, as compared with proteins of similar molecular size and configuration, suggest that PRL can be transported across the blood-CSF barrier at specific sites in the choroid plexus (Assies et al., 1978a & 1978b; Schroeder et al., 1976). Evidence exists that PRL is also able to circumvent the blood-brain barrier by retrograde transport through the portal vessels from the pituitary to the brain (Assies et al., 1978b; Bergland and Page, 1978; Oliver et al., 1977; Paradisi et al., 1989).

Although PRL in the brain may originate from peripheral blood, PRL-immunoreactivity in the rat brain and CSF is maintained after hypophysectomy, while PRL concentrations in serum decrease to undetectable levels (DeVito, 1988; Emanuele et al., 1986 & 1989). Therefore, PRL may also be locally produced by neurons in several brain sites. The idea that PRL is synthesized in the brain is supported by the observation that in the rat hypothalamus PRL mRNA has been found (Schachter et al., 1984). Since PRL-like immunoreactivity has been characterized within neuronal structures, like cell bodies, dendrites, axons and synaptosomes (DeVito et al., 1987; Emanuele et al., 1987; Harlan et al., 1989; Nisizuka et al., 1990), locally synthesized PRL may play a neuromodulatory role. Indeed, several authors have reported electrophysiological effects of PRL on neurons in the ventromedial (Moss et al., 1985) and arcuate (Nishihara and Kimura, 1989) nucleus of the rat hypothalamus. The physiological significance, however, of these electrophysiological actions is currently not understood.

HyperPRLaemia can be experimentally induced in several ways (cf. chapter II), and may exert its effects on the cerebrum by passing the blood-brain barrier via the choroid plexus and/or via a retrograde flow of blood into the pituitary portal vessels (Bergland et al., 1978; Oliver et al., 1977; Walsh et al., 1978 & 1984). Specific PRL-binding sites have been demonstrated and characterized on ependyma of the rat choroid plexus by in vivo radioautography (Walsh et al. 1978) and in vitro competitive binding methods (Posner et al., 1983; Muccioli et al., 1988a). These binding sites for PRL, which are also present in human choroid plexus (Muccioli et al., 1988b), may play a role in transporting PRL across the blood-CSF barrier, since a transmembranous passage by a pinocytotic-vesicular mode has been demonstrated for the choroid plexus with regard to PRL (Walsh et al., 1984). In the latter study, ^{125}I-oPRL administered in the external jugular vein, was found to be bound to the basal and lateral surfaces of choroid plexus epithelial cells. Subsequently, ^{125}I-oPRL was internalized and preferentially translocated

into non-lysosomal (i.e. acid phosphatase-negative) vesicles within the cell. These vesicles are likely to be involved in a transcellular transport of PRL. The earlier finding that the CSF concentration of PRL in rats reaches an upper limit in spite of progressively elevating blood concentrations of the hormone (Login and MacLeod, 1977) indicates that the process of PRL uptake from blood into the CSF is saturable, which is consistent with a receptor-mediated uptake mechanism. Such a mechanism has been recently confirmed, since unlabeled lactogenic hormones (ovine PRL and human GH) compete with the uptake of ^{125}I-PRL from blood into CSF as well as with the binding of ^{125}I-PRL to the choroid plexus (Walsh et al., 1987). Therefore, through a PRL-receptor mediated mode of entry, peripherally induced hyperPRLaemia may have access to hypothalamic neurons, resulting in a decline of LHRH release into pituitary portal blood (chapter VI). Evidence is growing that several mediators are involved in this central action of PRL.

8.3. Mechanism of PRL action in the brain: putative mediators

Several neurotransmitters and/or neurohormones have been suggested to participate in the central action of PRL on hypothalamic LHRH release and behaviour related to reproduction. The sites and systems in the brain upon which PRL may act to impair male sexual behaviour have received very little attention. Therefore, the following putative mediators involved in the neuroendocrine mechanisms of PRL action on gonadotrophin release will be discussed in light of the experimental results presented in this thesis:

1. *Dopamine*
2. *Opioids*
3. *Corticotrophin-releasing factor*
4. *Adrenocorticotrophic hormone*

8.3.1. Dopamine

As activation of the tuberoinfundibular dopaminergic (TIDA) neurons and increased levels of dopamine in pituitary stalk blood are consistently found in hyperPRLaemic rats (chapter VI, Cramer et al., 1979; Voogt et al., 1987; Weber et al., 1983), it might be speculated upon that dopamine mediates PRL-induced decrease of LHRH release. Such a mechanism would be in line with the sex differences described in the PRL-induced inhibition of gonadotrophin release (chapter V), since TIDA neurons of the female brain are more sensitive to PRL than those in the male brain (Barton et al., 1989; Demarest and Moore, 1981). Effects of dopamine on LHRH- and gonadotrophin release have been extensively studied. The strategically located TIDA-neurons terminate in the lateral aspects of the median eminence, and may have functional connections with LHRH-secreting axons (Selmanoff, 1981). In several in vivo studies increased dopamine turnover in the terminals of the TIDA system was associated with suppressed LH release in castrated rats treated with physiological concentrations of testosterone (Füxe et al., 1969; Simpkins et al., 1980 & 1983). However, Schneider and McCann (1969) found that dopamine stimulates pituitary LH release in a dose-related fashion in vitro, when hypothalamic fragments were coincubated with pituitaries from male rats. These findings have been confirmed by those of other in vitro studies, despite variations in the in vitro procedures employed (Negro-Vilar et al., 1979; Rotsztejn et al., 1977). More recently, Jarjour and colleagues reported a dopamine-induced LHRH release from isolated mediobasal rat hypothalamus (Jarjour et al., 1986). This may explain the earlier observed stimulatory action of dopamine on LH release in vivo, when dopamine is intracerebroventricularly injected (Kamberi et al., 1969; Schneider and McCann, 1970). Thus, although the literature is not conclusive about the effects of dopamine on gonadotrophin release, most studies are in favour of a stimulatory action of dopamine on the secretion of LH and FSH. Therefore, PRL-stimulated dopamine release is unlikely to mediate the suppression of LHRH release in hyperPRLaemia. This conclusion is in agreement with the observation that PRL-stimulated hypothalamic dopamine release is not necessarily associated with decreased levels of LHRH in pituitary stalk blood (Weber et al., 1982).

8.3.2. Opioids

An opioid-dependent mechanism has been claimed in PRL action, since opiate antagonists are able to antagonize PRL-induced suppression of gonadotrophin release (Carter et al., 1984; Sarkar and Yen, 1985) and PRL-induced analgesia (Scapagnini et al., 1985). Opioids, especially ß-endorphin, are frequently reported to inhibit LH-release in gonadectomized rats (Cicero et al., 1979; Kalra and Kalra, 1983; Panerai et al., 1985). The ß-endorphin producing neurons are located in the arcuate region and project into the lateral aspects of the median eminence, where ß-endorphin is secreted into the hypophysial portal veins (Finley et al., 1981). At the level of the hypothalamus these endorphinic neurons innervate most pronouncedly the nucleus paraventricularis (Barden and Dupont, 1982) an area which contains a high density of CRF-secreting neurons (Palkovits et al., 1985).

In several experiments, not reported in this thesis, we were not able to block the suppressive action of severe tumour-induced hyperPRLaemia on gonadotrophin release by the opiate antagonist naloxone. This does not exclude an opioid-dependent mechanism, since PRL can markedly inhibit naloxone binding in the rat hypothalamus (Sweeney et al., 1985; Weiland and Wise, 1989); thus, severe hyperPRLaemia like induced by tumour 7315b may prevent any antagonistic action of naloxone.

8.3.3. Corticotrophin-releasing factor

As CRF, administered intracerebroventricularly, is able to exert PRL-like effects on gonadotrophin release and sexual behaviour (Petraglia et al., 1987; Sirinathsinghji et al., 1987), and since stimulation of adrenal growth is a common finding in hyperPRLaemia (Colby et al., 1979), the experiments described in chapters VI and VII, were undertaken to investigate CRF as a putative mediator in PRL action. HyperPRLaemia induced by pituitary-grafting as well as by inoculation of tumour 7315b resulted in increased plasma levels of ACTH in intact male rats. Strong positive correlations were found between the plasma concentrations of PRL and ACTH in these

animals, even during treatment with high doses of corticosterone acetate. Hypothalamic CRF-content and CRF-release into pituitary portal vessel blood were significantly increased in the tumour-bearing rats, and tended to rise in the pituitary-grafted animals. These findings are consistent with recent experiments demonstrating that PRL can stimulate rat hypothalamic CRF release and pituitary ACTH secretion in vitro (Weber et al., 1990). The activation of the hypothalamo-adenohypophysial-adrenal axis may explain the adrenocorticotrophic effects in hyperPRLaemia in vivo. However, PRL alone may also be able to stimulate adrenal growth since pituitary grafts and implanted tumour 7315b are able to increase adrenal weight in hypophysectomized animals (chapter VII). With regard to the tumour-bearing rats, however, it is not clear to what extent these direct PRL-induced adrenocorticotrophic effects are enhanced by ACTH originating from tumour 7315b. However, the increased hypothalamic CRF content and release into pituitary portal blood in tumour-bearing rats are not in favour of a substantial ACTH release by the tumour.

In rats bearing pituitary grafts, hyperPRLaemia-associated ACTH release became markedly suppressed by CA-treatment (chapter VII). Since this feedback action is known to occur not only at the level of the corticotroph (Roberts et al., 1987) but also by actions on several brain sites (Levin et al., 1988; Plotsky et al., 1986), it may be hypothesized that high doses of glucocorticoids also prevent the inhibitory CRF-mediated action of hyperPRLaemia on gonadotrophin release and sexual behaviour. Such a mechanism is difficult to study, because glucocorticoids themselves can affect gonadotrophin secretion by increasing the hypothalamic-adenohypophysial sensitivity to the negative feedback effects of testosterone (Vreeburg et al., 1984), and by diminishing the pituitary gonadotrophin response to LHRH (Ringstrom and Schwartz, 1985). Interestingly, pretreatment with glucocorticoids prevents the rise in ACTH following CRF administration (Britton et al., 1985), as well as the inhibitory effect of CRF on gonadotrophin release in the primate (Gindoff et al., 1989). Since this CRF action can also be antagonized by naloxone (Almeida et al., 1986; Carter et al., 1984; Gindoff and Ferin, 1987), it is probable that glucoccorticoids may overcome the CRF inhibitory action on gonadotrophin release by preventing CRF-induced endorphin release. In fact, glucocorticoids are known to inhibit POMC-derived endorphinic expression in the pituitary (Boscaro et al., 1990; Vale et al., 1983) as well as in the hypothalamus (Beaulieu, 1986). Therefore, glucocorticoids may counterbalance the PRL-induced

inhibition of gonadotrophin release as far as mediated by CRF-stimulated opioid release. Such an action of glucocorticoids, however, is not likely to be of (patho)physiological significance, since no difference could be demonstrated in the PRL-induced antigonadotrophic effects between adrenalectomized and non-adrenalectomized animals bearing tumour 7315b (chapter V).

In addition to an opioid dependent mechanism, CRF may directly influence LHRH release, for direct synaptic connections between CRF and LHRH secreting neurons have been demonstrated in the preoptic area of the rat (MacLusky et al., 1988). Functional aspects of these connections remain to be established, since an opioid-independent action of CRF on LHRH release could not be demonstrated in the naloxone treated rat (Sarkar and Yen, 1985) and primate (Gindoff and Ferin, 1987).

8.3.4. Adrenocorticotrophic hormone

In addition to CRF mediated actions at several brain sites, increased ACTH release may contribute to the inhibition of gonadotrophin release and reproductive functions in hyperPRLaemia through different mechanisms. First, ACTH(1-24) can increase adrenal steroid output resulting in increased serum levels of corticosterone able to decrease pituitary responsiveness to LHRH (Ringstrom and Schwartz, 1985) and to increase hypophysial sensitivity to the feedback action of testosterone (Vreeburg et al., 1984), leading to suppressed pituitary gonadotrophin release. However, the inhibition of gonadotrophin release in rats bearing tumour 7315b appeared to be independent of adrenal function (chapter V). Thus, the central action of PRL during severe hyper-PRLaemia may overrule any contribution of the adrenals. Additionally, during hyperPRLaemia, antigonadotrophic effects of glucocorticoids at the pituitary level may be neutralized by a suppression of PRL-induced CRF release at the hypothalamic level (Beaulieu, 1986; Levin et al., 1988).

Second, ACTH has been found in the central nervous system, and may act directly on several brain sites (Krieger and Liotta 1979). Indeed, local administration of ACTH into the brain can elicit certain elements of sexual behaviour in the rodent (Bertolini et al., 1969; Haun and Haltmeyer, 1975). Studies about the central effects of

ACTH on gonadotrophin release, however, are rather conflicting, since stimulating (Haun and Haltmeyer, 1975; Mann et al., 1986) as well as inhibiting (Ortega et al., 1988) effects of i.c.v. ACTH on LH release have been reported.

8.4. Tentative concept of PRL action

In light of the experimental data discussed in this thesis, a concept of PRL action is presented in figure 8.1. A key role is attributed to CRF-producing neurons and the opioidergic system. At present, however, other mechanisms of PRL action are not excluded. Further research focussed on direct PRL action on neuronal processes may contribute to unravel the neuroendocrine events responsible for the inhibition of male reproductive functions in hyperPRLaemia.

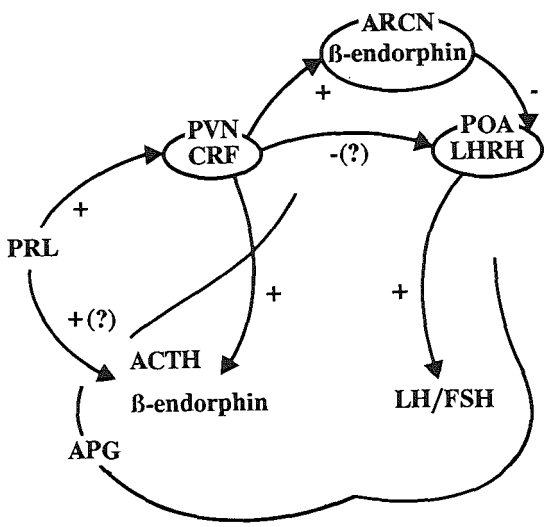

Figure 8.1. Concept of PRL action on the regulation of gonadotrophin release during hyperPRLaemia. APG = anterior pituitary gland; ARCN = arcuate nucleus; POA = preoptic area; PVN = paraventricular nucleus.

8.5. Proposals for further research

As far as is known, in all investigations studying the effects of chronic hyperPRLaemia on male reproductive functions high levels of PRL were achieved peripherally by drugs, pituitary grafts or PRL-secreting tumours (cf. chapter II). The latter are known to induce severe hyperPRLaemia resulting in marked suppression of male reproductive functions. The tumour employed in this thesis is unique, in that it has been originally qualified as being a purely PRL-secreting tumour. At present, however, since the tumour might secrete not only PRL and we know that PRL acts centrally to modify hypothalamic neuronal activity related to LHRH release and copulatory behaviour probably mediated by CRF and opioids, a more local approach is recommended to further unravel the mechanisms of PRL action. Such an approach can be employed by, for instance, microinfusions of PRL in small brain areas.

8.6. References

Almeida,O.F.X., Nikolarakis,K.E., Herz,A. (1988). Evidence for the involvement of endogenous opioids in the inhibition of luteinizing hormone by corticotropin-releasing factor. Endocrinol. 122, 1034-1041.

Assies,J., Schellekens,A.P.M., and Touber,J.L. (1978a). Prolactin in human cerebrospinal fluid. J. Clin. Endocrinol. Metab. 46, 576-586.

Assies,J., Schellekens,A.P.M., and Touber,J.L. (1978b). Protein hormones in cerebrospinal fluid: evidence for retrograde transport of prolactin from the pituitary to the brain in man. Clin. Endocrinol. 8, 487-491.

Barden,N., and Dupont,A. (1982). Distribution and changes in peptides in the brain. In: McKerns,K., and Pantic,V., eds, Hormonally active brain peptides, Plenum Press, New York, p. 307.

Barton,A.C., Demarest,K.T., Lookingland,K.J., and Moore,K.E. (1989). A sex difference in the stimulatory afferent regulation of tubero-infundibular dopaminergic neuronal activity. Neuroendocrinol. 49, 361-366.

Beaulieu,S., Gagne,B., and Barden,N. (1986). Glucocorticoid regulation of pro-opiomelanocortin (POMC) in RNA content of rat hypothalamus and amygdala. 1st Int. Congr. Neuroendocrinology, San Francisco, abstract 196.

Bennett,G.W., Edwardson,J.A., Holland,S., Jeffcoate,S.L., and White,N. (1975). Release of immunoreactive luteinizing hormone and thyrotrophin releasing hormone from hypothalamic synaptosomes. Nature 257, 323.

Bergland,R.M., and Page,R.B. (1978). Can the pituitary secrete directly to the brain ? (Affirmative anatomical evidence) Endocrinol. 102, 1325-1338.

Bertolini,A., Vergoni,W., Gessa,G.L. and Ferrari,W. (1969). Induction of sexual excitement by the action of adrenocorticotrophic hormone in brain. Nature 221, 667-669.

Boscaro,M., Paoletta,A., Giacomazzi,P., Fallo,F., and Sonino,N. (1990). Inhibition of pituitary ß-endorphin by ACTH and glucocorticoids. Neuroendocrinol. 51, 561-564.

Britton,D.R., Varela,M., Garcia,A., and Rosenthal,M. (1985). Dexamethasone suppresses pituitary-adrenal but not behavioral effects on centrally administered CRF. Life Sci. 38, 211-216.

Carter,D.A., Cooper,J.S., Inkster,S.E., and Whitehead,S.A. (1984). Evidence for an increased opioid inhibition of LH secretion in hyperprolactinaemic ovariectomized rats. J. Endocrinol. 101, 57-61.

Cicero,T.J., Schainker,B.A., and Meyer,E.R. (1979). Endogenous opioids participate in the regulation of the hypothalamic-pituitary luteinizing hormone axis and testosterone's negative feedback control of luteininzing hormone. Endocrinol. 104, 1286-1291.

Colby,H.D. (1979). Mechanism of action of prolactin on adrenocortical steroid secretion in hypophysectomized female rats. Endocrinol. 104, 1299-1303.

Costlow,M.E., and McGuire,W.L. (1977). Autoradiographic localization of the binding of ^{125}I-labelled prolactin to rat tissues in vitro. J. Endocrinol. 75, 221-225.

Cramer,O.M., Parker,C.R., and Porter,J.C. (1979). Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinol. 105, 636-640.

Demarest,K.T., and Moore,K.E. (1981). Sexual differences in the sensitivity of tuberoinfundibular dopaminergic neurons to the actions of prolactin. Neuroendocrinol., 33, 230-234.

DeVito,W.J. (1989). Comparison of brain and pituitary immunoreactive prolactin by peptide mapping and lectin affinity chromatography. Endocrinol. 125, 2439-2444.

DeVito,W.J. (1988). Distribution of immunoreactive prolactin in the male and female rat brain: effects of hypophysectomy and intraventricular administration of colchicine. Neuroendocrinol. 47, 284-289.

DeVito,W.J., Connors,J.M., and Hedge,G.A. (1987). Immunoreactive prolactin in the rat hypothalamus: In vitro release and subcellular localization. Neuroendocrinol. 46, 155-161.

Emanuele,N.V., Azad,N., Luckey,J.P., Engel,J., Melone,G., Metcalfe,L., Gout,P.W., Beer,C.T., Kirsteins,L., and Lawrence,A.M. (1989). Presence of prolactin-like immunoreactivity and bioactivity in rat spinal cord. Neuroendocrinol. 49, 331-335.

Emanuele,N.V., Metcalfe,L., Lubrano,T., Rubinstein,H., Kirsteins,L., and Lawrence,A.M. (1987). Subcellular distribution of hypothalamic prolactin-like immunoreactivity. Brain Res. 407, 223-229.

Emanuele, N.V., Metcalfe, L., Wallock, L., Tentler, J., Hagen, T.C., Beer, C.T., Martinson,D., Gout,P.W., Kirsteins,L., and Lawrence,A.M. (1986). Hypothalamic prolactin: characterization by radioimmunoassay and bioassay and response to hypophysectomy and restraint stress. Neuroendocrinol. 44, 217-221.

Finley,J., Lindstrom,P., and Petrusz,P. (1981). Immunocytochemical localization of ß-endorphin-containing neurons in the hypothalamus. Neuroendocrinol. 33, 28-42.

Fuxe,K., Hökfelt,T., and Nilsson,O. (1969). Castration, sex hormones and tuberoinfundibular dopamine neurons. Neuroendocrinol. 5, 107-120.

Fuxe,K., Hökfelt,T., Eneroth,P., Gustafson,J.A., and Skett,P.P. (1977). Prolactin-like immunoreactivity: localization in nerve terminals of rat hypothalamus. Science 196, 899-900.

Gindoff,P.R., and Ferin,M. (1987). Endogenous opioid peptides modulate the effect of corticotropin-releasing factor on gonadotropin release in the primate. Endocrinol. 121, 837-842.
Gindoff,P.R., Xiau,E., Luckhaus,J., and Ferin,M. (1989). Dexamethasone treatment prevents the inhibitory effect of corticotropin-releasing hormone on gonadotropin release in the primate. Neuroendocrinol. 49, 202-206.
Harlan,R.E., Shivers,B.D., Fox,S.R., Kaplove,K.A., Schachter,B.S. and Pfaff,D.W. (1989). Distribution and partial chatacterization of immunoreactive prolactin in the rat brain. Neuroendocrinol. 49, 7-22.
Haun,C.K., and Haltmeyer,G.C. (1975). Effects of an intraventricular injection of synthetic ACTH on plasma testosterone, progesterone and LH levels and on sexual behavior in male and female rabbits. Neuroendocrinol. 19, 201-213.
Jarjour,L.T., Handelsman,D.J., Raum,W.J., and Swerdloff,R.S. (1986). Mechanism of action of dopamine on the in vitro release of gonadotropin-releasing hormone. Endocrinol. 119, 1726-1732.
Kalra,S.P., and Kalra,P.S. (1983). Neural regulation of luteinizing hormone secretion in the rat. Endocr. Rev. 4, 311-351.
Kamberi,I., Mical R., and Porter,J.C. (1969). Luteinizing hormone-releasing activity in hypophysial stalk blood and elevation by dopamine. Science 166, 388.
Kendall,J., and Orwoll,E. (1980). Anterior pituitary hormones in the brain and other extrapituitary sites. In: Martini,L., and Ganong,W.F., eds., Frontiers in Neuroendocrinology, vol. 6, Raven Press, New York, p. 33.
Krieger,D.T. and Liotta,A.S. (1979). Pituitary hormones in brain: Where, how and why? Science 205, 366.
Levin,N., Shinsako,J., and Dallman,M.F. (1988). Corticosterone acts on the brain to inhibit adrenalectomy-induced adrenocorticotropin secretion. Endocrinol. 122, 694-701.
Login,I.S., and Macleod,R.M. (1977). Prolactin in human and rat serum and cerebrospinal fluid. Brain Res. 132, 477-483.
MacAdams,M.R., White,R.H., and Chipps,B.E. (1986). Reduction of serum testosterone levels during chronic glucocorticoid therapy. Ann. Intern. Med. 104, 648-651.
MacLusky,N.J., Naftolin,F., and Leranth,C. (1988). Immunocytochemical evidence for direct synaptic connections between corticotropin-releasing factor (CRF) and gonadotropin-releasing hormone (GnRH)-containing neurons in the preoptic area of the rat. Brain Res. 439, 391-395.
Mann,D.R., Evans,D.C., Jacobs,V.L. and Collins,D.C. (1986). Influence of acute intracerebroventricular (i.c.v.) administration of adrenocorticotrophin (ACTH) on LH secretion in male rats: effect of pre-treatment (i.c.v.) with ACTH antiserum on the serum LH response to an acute ether stress. J. Endocrinol. 108, 275-280.
Marchetti,B., and Labrie,F. (1982). Prolactin inhibits pituitary luteinizing hormone-releasing hormone receptors in the rat. Endocrinol. 11, 1209-1216.
McNeilly,A.S., Sharpe,R.M., & Fraser,H.M. (1980) Effect of adrenalectomy or castration on the inhibition of gonadotrophin secretion induced by hyperprolactinaemia in the adult male rat. J. Endocrinol. 79, 59-68.
Moss,R.L., Chan,A., and Dudley,C.A. (1985). Hyperprolactinemia: its electrophysiologic and pharmacologic effect on neurons of the ventromedial nucleus of the hypothalamus. Brain Res. 346, 301-309.
Muccioli,G., Bellussi,G., Ghé,C., Pagnini,G., Di Carlo,R. (1988a). Regional distribution and species variation of prolactin binding sites in the brain. Gen. Comp. Endocrinol. 69, 399-405.

Muccioli,G., Genazzani,E., Papotti,M., Di Carlo,R. (1988b). Prolactin receptors in human choroid plexus. In: Hoshino,K., ed., Prolactin gene family and its receptors; molecular biology to clinical problems. Proceedings of the fifth international congress on prolactin, Kyoto, Japan, Excerpta Medica, Amsterdam - New York - Oxford. pp. 167-174.

Negro-Vilar,A., Ojeda,S.R., and McCann,S.M. (1979). Catecholaminergic modulation of luteinizing hormone-releasing hormone release by median eminence terminals in vitro. Endocrinol. 104, 1749-1757.

Nishihara,M., and Kimura,F. (1989). Postsynaptic effects of prolactin and estrogen on arcuate neurons in rat hypothalamic slices. Neuroendocrinol. 49, 215-218.

Nishizuka,M., Shivers,B.D., Leranth,C. and Pfaff,D.W. (1990). Ultrastructural characterization of prolactin-like immunoreactivity in rat medial basal hypothalamus. Neuroendocrinol. 51, 249-254.

Oliver,C., Mical,R.S., and Porter,J.C. (1977). Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. Endocrinol. 101, 598-604.

Olster,D.H., and Ferin,M. (1987). Corticotropin-releasing hormone inhibits gonadotropin secretion in the ovariectomized rhesus monkey. J. Clin. Endocrinol. Metab. 65, 262-267.

Ortega,E., Frias,J., Rodriguez,E., and Ruiz,E. (1988). Influence of intracerebroventricular (i.c.v.) injection of ACTH (1-24) on plasma gonadotropin in female rats: dose-response study. Life Sc. 43, 1349-1354.

Palkovits,M., Brownstein,M.J., and Vale,W. (1985). Distribution of corticotropin releasing factor in rat brain. Fed. Proc. 44, 215-219.

Panerai,A.E., Petraglia,F., Sacerdote,P. and Genazzani,A.R. (1985). Mainly μ-opiate receptors are involved in luteinizing hormone and prolactin secretion. Endocrinol. 117, 1096-1099.

Paradisi,R., Frank,G., Magrini,O., Venturoli,S., Porcu,E., and Flamigni,C. (1989). Prolactin in hypothalamic-hypophysial blood. Lancet 1989, 1034.

Petraglia,F., Sutton,S., Vale,W., Plotsky,P.M. (1987). Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation. Endocrinol. 120, 1083-1088.

Petraglia,F., Vale,W., and Rivier,C. (1986). Opioids act centrally to modulate stress-induced decrease in luteinizing hormone in the rat. Endocrinol. 119, 2445-2450.

Plotsky,P.M., Otto,S., and Sapolsky,R.M. (1986). Inhibition of immuno-reactive corticotropin-releasing factor secretion into the hypophysial-portal circulation by delayed glucocorticoid feedback. Endocrinol. 119, 1126-1130.

Posner,B.I., Van Houten,M., Patel,B., and Walsh,R.J. (1983). Characterization of lactogen binding sites in choroid plexus. Exp. Brain Res. 49, 300-306.

Ringstrom,S.J. and Schwartz,N.B. (1985). Cortisol suppresses the LH, but not the FSH, response to gonadotropin-releasing hormone after orchidectomy. Endocrinol. 116, 472-474.

Rivier,C., Rivier,J., and Vale,W. (1986). Stress-induced inhibition of reproductive functions: role of endognous corticotropin-releasing factor. Science 231, 607-609.

Rivier,C., and Vale,W. (1985). Effect of the long-term administration of corticotropin-releasing factor on the pituitary-adrenal axis and pituitary-gonadal axis in the male rat. J. Clin. Invest. 75, 689-694.

Rivier,C., Vale,W. (1984). Influence of corticotropin-releasing factor on reproductive functions in the rat. Endocrinol. 114, 914-921.

Roberts,J., Lundblad,J., Eberwine,J. et al. (1987). Hormonal regulation of POMC gene expression in pituitary. Ann. NY. Acad. Sci. 512, 275-285.
Rotsztejn,W., Charli,J., Pattou,E., and Kordon,C. (1977). Stimulation by dopamine of luteinizing hormone releasing hormone (LHRH) release from the mediobasal hypothalamus in male rats. Endocrinol. 101, 1475-1483.
Sarkar,D.K., and Yen,S.S.C. (1985). Hyperprolactinemia decreases the luteinizing hormone-releasing hormone concentration in pituitary portal plasma: a possible role for ß-endorphin as a mediator. Endocrinol. 116: 2080-2084.
Scapagnini,U., Drago,F., Continella,G., Spadaro,F., Pennisi,G., and Gerendai,I. (1985). Experimental and clinical effects of prolactin on behavior. In: Prolactin, Basic and clinical correlates, MacLeod,R.M., Thorner,M.O., and Sacapagnini,U. (eds.), Fidia Research Series vol. I, Liviana Press, Padova.
Schachter,B.S., Durgerian,S., Harlan,R.E., Pfaff,D.W., and Shivers,B.D. (1984). Prolactin mRNA exists in rat hypothalamus. Endocrinol. 114, 1947-1949.
Schneider,H.P.G., and McCann,S.M. (1969). Possible role of dopamine as transmitter to promote discharge of LH-releasing factor. Endocrinol. 85, 121-132.
Schneider,H.P.G., and McCann,S.M. (1970). Mono- and indolamines and control of LH secretion. Endocrinol. 86, 1127-1133.
Schroeder,L.L., Johnson,J.C., and Malarkey,W.B. (1976). Cerebrospinal fluid prolactin: a reflection of abnormal prolactin secretion in patients with pituitary tumors. J. Clin. Endocr. Metab. 43, 1255-1260.
Selmanoff,M. (1981). The lateral and medial median eminence: distribution of dopamine, norepinephrine, and luteinizing hormone-releasing hormone and the effect of prolactin on catecholamine turnover. Endocrinol. 108: 1716-1722.
Shivers,B.D., Harlan,R.E., and Pfaff,D.W. (1989). A subset of neurons containing immunoreactive prolactin is a target for estrogen regulation of gene expression in rat hypothalamus. Neuroendocrinol. 49, 23-27.
Simpkins,J.W., Kalra,P.S., and Kalra,S.P. (1980). Effects of testosterone on catecholamine turnover and LHRH contents in the basal hypothalamus and preoptic area. Neuroendocrinol. 30, 94-100.
Simpkins,J.W., Kalra,S.P., and Kalra,P.S. (1983). Variable effects of testosterone on dopamine activity in several microdissected regions in the preoptic area and medial basal hypothalamus. Endocrinol. 112, 665-669.
Sirinathsinghji, D.J.S. (1987) Inhibitory influence of corticotropin-releasing factor on components of sexual behaviour in the male rat. Brain Res. 407, 185-190.
Sirinathsinghji,D.J.S., Rees,L.H., Rivier,J., and Vale,W. (1983). Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat. Nature 305, 232-235.
Suter,D.E., and Schwartz,N.B. (1985). Effects of glucocorticoids on secretion of luteinizing hormone and follicle-stimulating hormone by female rat pituitary cells in vitro. Endocrinol. 117, 849-854.
Sweeney,C.A., Morgan,W.W., Smith,M.S., and Bartke,A. (1985). Altered sensitivity to an opiate antagonist, naloxone, in hyperprolactinemic male rats. Neuroendocrinol. 41, 1-6.
Vale,W., Vaughan,J., Smith,M., Yamamoto,G., Rivier,J., and Rivier,C. (1983). Effects of synthetic ovine CRF, glucocorticoids, catecholamines, neurohypophysial peptides, and other substances on cultured corticotropic cells. Endocrinol. 113, 1121-1131.

Voogt,J.L., de Greef,W.J., Visser,T.J., de Koning,J., Vreeburg,J.T.M., and Weber,R.F.A. (1987). In vivo release of dopamine, luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in male rats bearing a prolactin-secreting tumor. Neuroendocrinol. 46, 110-116.

Vreeburg,J.T.M., De Greef,W.J., Ooms,M.P., Van Wouw,P., and Weber,R.F.A. (1984). Effects of adrenocorticotropin and corticosterone on the negative feedback action of testosterone in the adult male rat. Endocrinol. 115, 977-983.

Vreeburg,J.T.M., Samaun,K., Verkade,H.J., Verhoef,P., Ooms,M.P., and Weber,R.F.A. (1988). Effects of corticosterone on the negative feedback action of testosterone, 5α-dihydrotestosterone and estradiol in the adult male rat. J. Steroid. Biochem. 29, 93-98.

Walsh,R.J., Posner,B.I., Kopriwa,B.M., and Brawer,J.R. (1978). Prolactin binding sites in the rat brain. Science 201, 1041-1043.

Walsh,R.J., Posner,B.I., and Patel,B. (1984). Binding and uptake of (^{125}I)iodoprolactin by epithelial cells of the rat chorioid plexus: An in vivo autoradiographic analysis. Endocrinol. 114, 1496-1505.

Walsh,R.J., Slaby,F.J., and Posner,B.I. (1987). Receptor-mediated mechanism for the transport of prolactin from blood to cerebrospinal fluid. Endocrinol. 120, 1846-1850.

Weber,R.F.A., and Calogero,A.E. (submitted). Prolactin stimulates rat hypothalamic corticotrophin-releasing hormone and pituitary adrenocorticotrophin secretion in vitro.

Weber,R.F.A., de Greef,W.J., de Koning,J., and Vreeburg,J.T.M. (1983). LH-RH and dopamine levels in hypophysial stalk plasma and their relationship to plasma gonadotrophins and prolactin levels in male rats bearing a prolactin- and adrenocorticotrophin-secreting pituitary tumor. Neuroendocrinol. 36: 205-210.

Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1982). Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex orgn weights in adult male rats: a possible role of the adrenals. Endocrinol. 111, 412-417.

Weiland,N.G., and Wise,P.M. (1989). Hyperprolactinemia decreases naloxone binding in the arcuate nucleus of ovariectomized rats. Neuroendocrinol. 50, 667-672.

Xiao,E., Luckhaus,J., Niemann,W., and Ferin,M. (1989). Acute inhibition of gonadotropin secretion by corticotropin releasing hormone in the primate: are the adrenal glands involved ? Endocrinol. 124, 1632-1637.

SUMMARY

HyperPRLaemia and male reproductive functions: introduction

Since the early seventies, chronic hyperPRLaemia due to hypothalamic-pituitary disorders, drug abuse, hypothyroidism or other causes, has been recognized as a cause of reproductive dysfunctions in both women and men. Possible mechanisms by which PRL exerts its effects on male reproductive functions have been extensively studied during the last two decades, but the mode of PRL action on reproductive functions has not been clarified by these studies.

After an introduction on the physiological significance of PRL in vertebrates and the possible molecular processes involved in PRL action (chapter I), experimental models are discussed to study the effects of hyperPRLaemia on male reproductive functions in the rat (chapter II). This animal was used in the experiments presented in this thesis, because of the resemblance between the effects of hyperPRLaemia observed in rats and humans. During the experiments, the transplantable PRL-secreting tumour 7315b was employed as an experimental model to induce high serum PRL levels (2000-5000 ng/ml), comparable to those found in humans with hyperPRLaemia-associated impotence.

Preceding the presentation of the data obtained from the experiments discussed in this thesis (chapters IV, V, VI, VII and VIII), male reproductive dysfunctions during hyperPRLaemia in the rat are reviewed (chapter III). Subsequently, aims and questions which are dealt with in this thesis are described.

Effects of hyperPRLaemia on male copulatory behaviour and gonadotrophin release

Effects of tumour-induced hyperPRLaemia on male copulatory behaviour in male and female rats are presented in chapter IV. Inoculation of the tumour resulted in high PRL levels (2000-3000 ng/ml) within three weeks. During this hyperPRLaemia a suppression of copulatory behaviour was observed in castrated male rats treated with subcutaneous testosterone-filled silastic capsules of different sizes. The inhibitory effect of the tumour was as strong in the presence of normal (2.33 ± 0.07 ng/ml) as in the presence of low (0.35 ± 0.01 ng/ml) testosterone levels. During behavioural testing the hyperPRLaemic animals exhibit prolonged latencies to ejaculation and decreased numbers of mounts and intromissions per minute, independently of the testosterone levels induced. The inhibitory effect of tumour 7315b on copulatory behaviour was not affected by adrenalectomy. In gonadectomized female rats, testosterone-induced male sexual behaviour performed, such as mounts and intromission-patterns, was almost completely suppressed by the tumour within four weeks after inoculation. Thus, hyperPRLaemia induced by tumour 7315b is able to deteriorate male copulatory behaviour in male as well as female rats. No evidence exists that gonadal and adrenal functions are essentially involved, suggesting a direct effect on brain functions.

Analogously to the inhibition of male copulatory behaviour in tumour-bearing rats, tumour 7315b appeared to suppress pituitary gonadotrophin release independently of gonadal and adrenal function, and apparently by a central action. This central action was clearly more pronounced in female rats than in male rats (chapter V).

Mechanisms involved in PRL action on male reproductive functions

On the basis of the experimental data discussed in chapters IV and V, an action of PRL on brain functions was postulated to interfere with the regulation of male reproductive functions. Since CRF locally administered in the brain is able to suppress male copulatory behaviour, as well as hypothalamic LHRH release, and since adrenocorticotrophic effects have been consistently found in hyperPRLaemia, subsequent experiments

are focussed on CRF as a potential mediator in PRL action on male reproductive functions (chapter VI). Two different models of hyperPRLaemia were used, namely inoculation with the PRL-secreting tumour 7315b and implantation of isogeneic pituitary glands. Within four weeks after tumour inoculation, severe hyperPRLaemia resulted in an increase of hypothalamic CRF content and release into pituitary portal vessel blood in male rats previously gonadectomized and adrenalectomized, and treated with corticosterone plus testosterone; simultaneously, an increase of plasma levels of ACTH and a decrease of plasma concentrations of LH and FSH were observed in the hyperPRLaemic animals as compared with the values in the control rats. Also in intact pituitary-grafted male rats, a significant activation of the adenohypophysial-adrenal axis was observed, while the increase in hypothalamic CRF release into pituitary portal vessel blood just did not reach statistical significance.

Thus, both models of chronic hyperPRLaemia are likely to activate the hypothalamic-adenohypophysial-adrenal axis. It was postulated that at least part of PRL action on male reproductive functions is due to an activation of CRF-secreting neurons, which causes inhibition of hypothalamic LHRH secretion and consequently pituitary gonadotrophin release, and probably suppression of male copulatory behaviour. Moreover, a mediating role for CRF in PRL action is supported by the finding that CRF- as well as PRL-induced suppression of gonadotrophin release and copulatory behaviour can be antagonized by the opiate antagonist naloxone.

Since pituitary grafts have been demonstrated to increase adrenal weight even in rats treated with high doses of glucocorticoids, it was investigated whether the activation of the adenohypophysial-adrenal axis in hyperPRLaemia is still present during glucocorticoid excess (chapter VII). The two models of hyperPRLaemia mentioned above were used. Fourteen days after pituitary grafting or tumour inoculation, a rise of serum ACTH levels was found in intact hyperPRLaemic animals, which appeared to be related to the height of the PRL concentrations obtained. Subsequent treatment of control animals with corticosterone acetate (daily 10 mg s.c., during 9-10 days) resulted in a marked decrease of serum ACTH levels down to the level of detection, while the effect of this treatment on serum ACTH levels seemed to be inhibited in the pituitary-grafted and tumour-bearing rats. From these data, PRL appeared to be highly correlated with serum ACTH ($r=0.88$) and with adrenal weight ($r=0.94$), despite the severe glucocorticoid excess induced.

In an additional experiment, pituitary grafting resulted again in a significant rise of serum ACTH concentrations. However, in this experiment, the increase of serum PRL levels induced by pituitary grafting was less marked, and not associated with a rise of serum ACTH during glucocorticoid excess. Probably, the hyperPRLaemia induced may have been too low to provide an adequate stimulus to overcome the inhibitory action of glucocorticoid excess on ACTH release. So the origin of the rise in ACTH levels as previously found in pituitary-grafted animals during glucocorticoid excess could not be established in this experiment, but evidence exists that ectopic pituitary glands are not likely to secrete significant amounts of ACTH.

The tumour-induced increase of serum levels of immunoactive ACTH was not prevented by hypophysectomy. Therefore, ectopic production of ACTH and/or other POMC-derived molecules by tumour 7315b is probable. Nevertheless, tumour 7315b has been shown to stimulate hypothalamic CRF-release into pituitary stalk blood, indicating that at least part of the rise in serum ACTH originates from the eutopic pituitary gland.

It is concluded, that differènt models of hyperPRLaemia are able to activate the adenohypophysial-adrenal axis in adult male rats, confirming the findings presented in chapter VI. The height of the PRL levels induced after pituitary grafting seems to determine to what extent a hyperPRLaemia-associated rise of serum ACTH can be suppressed by high doses of corticosterone acetate. Part of the tumour-induced increase in serum concentrations of immunoactive ACTH may originate from the tumour itself, which does not preclude a stimulatory action of PRL on the hypothalamic-adenohypophysial-adrenal axis.

Concluding remarks

Finally, in the general discussion (chapter VIII), ways of entry are discussed by which peripherally induced hyperPRLaemia may have access to the brain. In light of the experimental data presented in this thesis PRL is likely to activate hypothalamic CRF-secreting neurons resulting in a decrease of LHRH release and inhibition of male copulatory behaviour through an opioid-dependent mechanism. Further research, however, is needed to substantiate and extend this tentative concept of PRL action.

SAMENVATTING

Hyperprolactinemie en mannelijke voortplantingsfuncties: inleiding

Vanaf de zeventiger jaren wordt chronische hyperprolactinemie, ontstaan door ondermeer hypothalame en/of hypofysaire stoornissen, medicatie en hypothyreoidie, herkend als oorzaak van voortplantingsstoornissen bij zowel de man als de vrouw. Gedurende de laatste twee decennia is veel onderzoek verricht naar de mogelijke mechanismen waardoor de remmende effecten van hoge PRL spiegels op de mannelijke voortplantingsfuncties tot stand komen. In die studies is echter nog niet duidelijk geworden hoe PRL interfereert met deze voortplantingsfuncties.

Na een inleiding over de functionele betekenis van PRL voor gewervelde dieren en over de mogelijke moleculaire processen betrokken bij de werking van PRL (hoofdstuk I), worden experimentele modellen besproken die toegepast kunnen worden om de effecten van hyperprolactinemie op de mannelijke voortplantingsfuncties bij de rat te bestuderen (hoofdstuk II). De rat werd verkozen als proefdier in dit promotie-onderzoek op basis van de overeenkomst tussen mens en rat t.a.v. de effecten van hyperprolactinemie op de mannelijke voortplantingsfuncties. Tijdens de experimenten werd de transplanteerbare PRL secernerende tumor 7315b toegepast als een experimenteel model ter inductie van hoge PRL spiegels in het bloed (2000-5000 ng/ml), vergelijkbaar met die bij mannen met potentiestoornissen bij hyperprolactinemie.

Voorafgaande aan de presentatie en bespreking van de experimentele gegevens verkregen tijdens dit onderzoek (hoofdstukken IV, V, VI, VII en VIII), wordt een overzicht gegeven van mannelijke voortplantingsstoornissen tijdens hyperprolactinemie bij de rat (hoofdstuk III). Vervolgens worden de vraagstellingen geformuleerd die ten grondslag liggen aan de experimenten die gepresenteerd worden in dit proefschrift.

Effecten van hyperprolactinemie op het mannelijk paringsgedrag en de afgifte van gonadotrope hormonen

De effecten van hyperprolactinemie op copulatiegedrag bij mannelijke en vrouwelijke ratten worden beschreven in hoofdstuk IV. Inoculatie van de PRL secernerende tumor resulteerde binnen drie weken in hoge PRL spiegels in het serum (2000-3000 ng/ml). Tijdens deze hyperprolactinemie werd een remming van mannelijk paringsgedrag waargenomen bij gecastreerde mannelijke ratten die behandeld werden met (sub)fysiologische hoeveelheden testosteron middels subcutane implantaten van verschillende lengtes. Het remmende effect van de tumor bleek even duidelijk in de aanwezigheid van normale (2,33±0,07 ng/ml) als in de aanwezigheid van lage (0,35±0,01 ng/ml) testosteronconcentraties in het serum. Gedurende de gedragstesten vertoonden de hyperprolactinemische dieren toegenomen latenties tot ejaculatie en afgenomen aantallen beklimmingen en intromissies per tijdseenheid, ongeacht de testosteronspiegels die werden geïnduceerd middels de subcutane implantaten. Het onderdrukkende effect van de tumor op het copulatiegedrag werd niet beïnvloed door verwijdering van de bijnieren.

Bij vrouwelijke ratten waarvan de ovaria verwijderd zijn, kan door behandeling met testosteron mannelijk paringsgedrag worden geïnduceerd, zoals beklimmingen en "intromissie-patronen". Dergelijk gedrag werd bij de aldus behandelde vrouwtjes bijna volledig onderdrukt door de tumor. Hyperprolactinemie geïnduceerd door tumor 7315b bleek dus gepaard te gaan met een verstoring van mannelijk paringsgedrag bij zowel mannelijke als vrouwelijke dieren. Bovendien zijn er geen aanwijzingen dat de activiteit van de gonaden of die van de bijnieren betrokken is in voornoemde effecten. Hieruit volgt dat de effecten van hyperprolactinemie waarschijnlijk tot stand komen via een directe werking van PRL op hersenfuncties.

Overeenkomstig de remming van mannelijk paringsgedrag bij tumordragende ratten, bleek tumor 7315b de hypofysaire afgifte van gonadotrope hormonen te verminderen, onafhankelijk van de activiteit van gonaden en bijnieren, en waarschijnlijk via een effect op de hersenen. Deze centrale werking van PRL bleek duidelijk meer uitgesproken bij vrouwelijke dan bij mannelijke ratten (hoofdstuk V).

Mechanismen van werking betrokken bij de effecten van PRL op mannelijke voortplantingsfuncties

Op basis van de experimentele gegevens besproken in de hoofdstukken IV en V werd aangenomen dat PRL via directe effecten op hersenfuncties mannelijke voortplantingsfuncties beïnvloedt. Omdat CRF locaal toegediend in de hersenen in staat is mannelijk paringsgedrag te onderdrukken en de hypothalame afgifte van LHRH te verminderen, en omdat vergroting van de bijnieren wordt gevonden bij hyperprolactinemie, werd vervolgens onderzocht of CRF een "mediator" zou kunnen zijn in het werkingsmechanisme van PRL op mannelijke voortplantingsfuncties (hoofdstukken VI en VII). Twee verschillende modellen van hyperprolactinemie werden toegepast, namelijk inoculatie met de PRL-secernerende tumor 7315b en implantatie van isogene hypofysen onder het nierkapsel. Binnen vier weken na inoculatie met de tumor, resulteerde de ernstige hyperprolactinemie in een toename van de hoeveelheid CRF in de hypothalamus en de afgifte van CRF aan het hypofysesteelbloed bij mannelijke ratten; tegelijkertijd werden bij deze dieren een toename van ACTH spiegels en een afname van LH en FSH concentraties in het bloed waargenomen. Ook bij de mannelijke ratten met ectopische hypofysen werd een significante activering van de hypofyse-bijnieras waargenomen, terwijl de toename in de hypothalame afgifte van CRF aan het hypofysesteelbloed net geen statistische significantie bereikte.

Beide toegepaste experimentele modellen ter inductie van een chronische hyperprolactinemie zijn kennelijk in staat de (hypothalamus-) hypofyse-bijnieras te activeren. Gepostuleerd werd dat tenminste een deel van de effecten van PRL op mannelijke voortplantingsfuncties tot stand komt via een activering van CRF-secernerende neuronen, leidende tot een remming van de hypothalame afgifte van LHRH en dientengevolge tot een verminderde hypofysaire secretie van gonadotrope hormonen. Ook de remmende effecten van locaal toegediend CRF in de hersenen op het mannelijk paringsgedrag passen bij een CRF-afhankelijk werkingsmechanisme van PRL. Een dergelijk mechanisme wordt bovendien ondersteund door de bevinding dat de remmende effecten van zowel CRF als PRL op de afgifte van gonadotropinen en op het mannelijk paringsgedrag kunnen worden tegengegaan door de opioïde antagonist naloxon.

Omdat reeds eerder is aangetoond dat hypofyse-transplantaten bijniergewichten doen toenemen, zelfs bij ratten behandeld met hoge doseringen glucocorticoïden, werd in vervolgexperimenten onderzocht of de activering van de hypofyse-bijnieras door hyperprolactinemie nog steeds aanwezig is bij dieren behandeld met een overmaat aan glucocorticoïden (hoofdstuk VII). De twee voornoemde experimentele modellen ter inductie van een chronische hyperprolactinemie werden toegepast. Bij intacte dieren werd veertien dagen na transplantatie van isogene hypofysen onder het nierkapsel of na inoculatie van de tumor een toename van ACTH spiegels waargenomen, die positief gecorreleerd bleken met de hoogte van de verkregen PRL concentraties. Daaropvolgende behandeling van de controle dieren met een suprafysiologische dosering corticosteron acetaat (dagelijks 10 mg s.c., gedurende 9 tot 10 dagen) resulteerde in een markante afname van de ACTH concentraties in het serum tot onder het detectieniveau. Daarentegen bleek het effect van deze behandeling op de ACTH spiegels geremd bij de dieren met hypofyse-transplantaten of PRL-secernerende tumoren. Ook in aanwezigheid van een overmaat glucocorticoïden bleken de PRL concentraties in hoge mate positief gecorreleerd met ACTH spiegels in het serum ($r=0,88$) en met bijniergewichten ($r=0,94$).

In een aanvullend experiment resulteerde implantatie van hypofysen wederom in een significante toename van ACTH concentraties in het serum. In dit experiment bleek echter de toename van de PRL spiegels bij dieren met hypofyse-implantaten minder uitgesproken, en niet gepaard te gaan met een stijging van de ACTH concentraties tijdens een overmaat glucocorticoïden. Mogelijk is de hoogte van de geïnduceerde PRL concentraties in dit laatste experiment ontoereikend geweest om een stimulerend effect te kunnen hebben op de hypofysaire ACTH afgifte bij dieren behandeld met een suprafysiologische dosering glucocorticoïden. Dus de oorsprong van de toename in ACTH spiegels tijdens een overmaat aan glucocorticoïden zoals deze eerder werd waargenomen gedurende hyperprolactinemie, kon in dit experiment niet worden achterhaald. Evenwel bestaan er duidelijke aanwijzingen dat ectopische hypofysen slechts geringe hoeveelheden ACTH secerneren.

De toename van de concentraties van immunologisch actief ACTH in het serum onder invloed van tumor 7315b werd niet voorkomen door verwijdering van de eutopische hypofyse. Daarom kan ectopische productie van ACTH en/of andere brokstukken van het pro-opiomelanocortine (POMC) molecuul door tumor 7315b niet

worden uitgesloten. Aangezien echter werd aangetoond (hoofdstuk VI), dat tumor 7315b de hypothalame afgifte van CRF aan het hypofysesteelbloed kan stimuleren, moet worden aangenomen dat ten minste een deel van de toename in de ACTH concentraties in het perifere bloed tijdens hyperprolactinemie afkomstig is van de eutopische hypofyse.

Concluderend kan gesteld worden dat verschillende modellen van hyperprolactinemie in staat blijken de (hypothalamus-) hypofyse-bijnieras te activeren bij volwassen mannelijke ratten (hoofdstukken VI en VII). De hoogte van de geïnduceerde PRL concentraties na hypofyse-transplantatie lijkt van kritische waarde voor de stimulerende werking van de hyperprolactinemie op de hypofysaire ACTH-afgifte tijdens een overmaat glucocorticoïden. Bij het tumormodel moet de kanttekening geplaatst worden dat de toename in de concentraties van immunologisch actief ACTH onder invloed van de tumor ten dele afkomstig kan zijn van de tumor zelf, wat overigens een activerende werking van PRL op de hypothalamus-hypofyse-bijnieras niet uitsluit, blijkens de toename in de hypothalame CRF-afgifte aan het hypofysesteelbloed bij dieren geïnoculeerd met tumor 7315b.

Concluderende opmerkingen

Tenslotte wordt in de algemene discussie (hoofdstuk VIII) besproken via welke toegangsmogelijkheden een perifeer geïnduceerde hyperprolactinemie de hersenen kan bereiken. In het licht van de experimentele gegevens gepresenteerd in dit proefschrift is het aannemelijk dat PRL hypothalame CRF-secernerende neuronen activeert, leidende tot een afname van de hypothalame LHRH-afgifte en een remming van mannelijk paringsgedrag via een opioid-afhankelijk mechanisme. Voortgezet onderzoek is echter nodig dit voorlopig concept van de werking van PRL uit te breiden.

DANKWOORD

Velen hebben een zodanige bijdrage geleverd aan het wordingsproces van dit proefschrift, dat ik de dank aan hen verschuldigd niet onbetuigd wil laten.

Mijn ouders hebben mij een plezierige en onvergetelijke jeugd bezorgd, en gedurende vele jaren in de gelegenheid gesteld om onder prettige omstandigheden te studeren. Hun bijdrage in deze is moeilijk in woorden te vangen. Waarvoor mijn dank.

Co-promotor Jan Vreeburg wist mij met een groot enthousiasme en een opmerkelijk gevoel voor humor op directe, plezierige en doortastende wijze te begeleiden. Tijdens onze werkbesprekingen heb ik veel mogen leren van zijn heldere inzichten en "no-nonsense-attitude". Zijn "knedende" invloed is evenwel niet beperkt gebleven tot mijn wetenschappelijke vorming.

Mijn promotor Koos van der Werff ten Bosch ben ik hoogst erkentelijk voor zijn gastvrijheid en voor zijn optimale bereidheid de manuscripten kritisch door te nemen. De overige leden van de promotie-commissie, Prof. Dr. J.C. Birkenhäger, Prof. Dr. Steven W.J. Lamberts en Prof. Dr. Gerard H. Zeilmaker, dank ik voor de bereidwilligheid dit proefschrift te beoordelen.

De enthousiaste, kritische en daardoor vaak stimulerende inbreng van Rob Weber tijdens onze discussies heb ik als een extra stimulans mogen ervaren. Zijn opbouwende kritiek en inspirerende "prolactinerge" belangstelling heb ik altijd zeer gewaardeerd.

De dank verschuldigd aan Marja Ooms voor haar deskundige technische hulp is groot. Zij bleek van een niet te onderschatten waarde bij de tot stand koming van de meeste experimentele gegevens. Ook bedank ik Paula van der Vaart voor het uitvoeren van de hypofyse-transplantaties. Gert van Capellen ben ik dank verschuldigd voor de adviezen bij het vervaardigen van de "lay out" van dit proefschrift.

De samenwerking met Wim de Greef maakte metingen in het hypofysesteelbloed

mogelijk. Hij leverde daarmee een belangrijke bijdrage aan dit proefschrift.

Gerard Zeilmaker ben ik dankbaar voor zijn opbouwende kritiek en prettige samenwerking, onder meer tijdens de hypofysectomieën en onze schaakpartijen, hoewel ik bij deze laatste eerder tegenwerking ondervond.

Ook wil ik mijn dank niet onbetuigd laten aan Steven Lamberts om zijn gastvrijheid op zijn laboratorium en Piet Uitterlinden om zijn technische steun, waardoor de ACTH-bepalingen konden worden gerealiseerd. De CRF-metingen werden mogelijk gemaakt door Dr. W.H.L. Hackeng, waarvoor mijn dank.

Tenslotte, noem ik Patricia en onze geliefde dochters Esther en Elvira, aan wie dit proefschrift opgedragen is. Het is moeilijk in te schatten hoe waardevol de stimulerende en prettige sfeer thuis met hen tijdens het promotie-onderzoek is geweest.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 25 oktober 1960 te Voorburg. Aansluitend op het behalen van het diploma Gymnasium-ß aan het lyceum "Overvoorde" te 's-Gravenhage vond in 1979 inschrijving plaats aan de Erasmus Universiteit te Rotterdam voor de studie Geneeskunde. Tijdens de kandidaatsfase van deze studie werd onderzoek verricht op het Laboratorium voor Obstetrische en Gynaecologische Research (hoofd: Prof.Dr. H.C.S. Wallenburg) naar de haemodynamische effecten van prostaglandine-syntheseremmers bij de zwangere cavia. Na het behalen van het doctoraal examen in 1984 (oud curriculum), werd het artsexamen afgelegd op 20 december 1985.

Vanaf 1 januari 1986 tot 1 oktober 1990 was de auteur werkzaam als wetenschappelijk onderzoeker op de afdeling Endocrinologie, Groei en Voortplanting (hoofd: Prof. Dr. J.J. van der Werff ten Bosch) van de Erasmus Universiteit te Rotterdam, alwaar het hier gepresenteerde onderzoek werd uitgevoerd. Tijdens voornoemd onderzoek was hij betrokken bij het fysiologie onderwijs aan tweedejaars medische studenten (blok Endocrinologie, Groei en Voortplanting). Tevens was hij sedert 1 juni 1987 verbonden aan de polikliniek Andrologie van de afdeling Interne Geneeskunde III (hoofd: Prof. Dr. J.C. Birkenhäger) in het Academisch Ziekenhuis Rotterdam (spreekuren voor mannen met fertiliteitsstoornissen; begeleiding van artsassistenten).

Op 1 oktober 1990 werd een aanvang gemaakt met de opleiding tot internist (opleiders: Prof.Dr. J.C. Birkenhäger, Dr. B.P. Hazenberg).

LIJST VAN PUBLICATIES

Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1988).
Deterioration of male sexual behavior in rats by the new prolactin-secreting tumor 7315b.
Horm. Behav. 22, 351-361.

Van der Schoot,P., and Kooy,A. (1988).
Current topics in the study of sexual behavior in rats.
In: Handbook of Sexology, volume 6: The Pharmacology and Endocrinology of Sexual Function; Sitsen,J.M.A., editor; Elsevier, Holland; pp. 145-192.

Kooy,A., Weber,R.F.A., Ooms,M.P., en Vreeburg,J.T.M. (1988).
Onderdrukking van mannelijk paringsgedrag bij de rat door de prolactine producerende tumor 7315b.
Ned. T. Geneesk. 132, 1555 (abstract).

Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1988).
Deterioration of male sexual behavior in rats by the new prolactin-secreting tumor 7315b.
8th International Congress of Endocrinology, Kyoto, Japan (abstract).

Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1988).
Reduction of plasma LH in adult male rats by the new prolactin-secreting tumor 7315b.
5th International Congress on Prolactin, Kyoto, Japan (abstract).

Eikenboom,H.C.J., Weber,R.F.A., Vreeburg,J.T.M., en Kooy,A. (1988).
Het effect van clomifeen behandeling bij idiopathische oligozoöspermie op de transferrineconcentratie in het semen.
Annalen Vereniging Fertiliteitsstudie, pp. 56-57.

Kooy,A., en Grootegoed,J.A. (1988).
Nieuwe mogelijkheden bij het semenonderzoek.
14e Nascholingscursus Endocrinologie Noordwijkerhout, pp. 107-111. ISBN 90-71636-05-4.

Kooy,A., Weber,R.F.A., Ooms,M.P., and Vreeburg,J.T.M. (1989).
Effects of the new prolactin-producing tumour 7315b on gonadotrophin secretion in adult male and female rats.
J. Endocrinol. 120, 261-268.

Kooy,A., en Zeilmaker,G.H. (1989).
Klinische en technologische aspecten van mannelijke infertiliteit.
15e Nascholingscursus Endocrinologie Noordwijkerhout, pp. 121-124. ISBN 90-71636-06-2.

Kooy,A., De Greef,W.J., Vreeburg,J.T.M., Hackeng,W.H.L., Ooms,M.P., Lamberts, S.W.J., and Weber,R.F.A. (1990).
Evidence for the involvement of corticotropin-releasing factor in the inhibition of gonadotropin release induced by hyperprolactinemia.
Neuroendocrinol. 51, 261-266.

Kooy,A. (1990).
Nieuwe inzichten in de werking van prolactine.
16e Nascholingscursus Endocrinologie Noordwijkerhout, ter perse.

Vreeburg,J.T.M., Kooy,A., Ooms,M.P., De Greef,W.J., and Weber, R.F.A. (1990).
Effects of hyperprolactinemia on gonadotropin release.
In: Psychoneuroendocrinology of growth and development in animals and man, Rotterdam, pp. 32-34.

Kooy,A., Sneller,W., Vreeburg,J.T.M., en Weber,R.F.A. (1990).
Diagnostische en therapeutische mogelijkheden bij mannelijke infertiliteit.
Urologische brief, ter perse.