EXTINCTION OF CONFLICT BEHAVIOUR IN RATS, A MODEL WHICH MAY HAVE PREDICTIVE VALUE FOR DRUGS ACTIVE IN ANXIETY DISORDERS

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Extinctie van conflictgedrag in ratten, mogelijk een model met voorspellende waarde wat betreft angstdempende middelen

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CONTENTS

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CHAPTER I

RELEVANT NEUROTRANSMITTER SYSTEMS IN ANXIETY; A SURVEY OF THE LITERATURE

1.1. INTRODUCTION

1.2. THE GABA-ERGIC SYSTEM

1.2.1. The neurotransmitter GABA
1.2.2. GABA receptors
1.2.2.A The $GABA_A$/benzodiazepine chloride channel complex
1.2.2.B The $GABA_B$ receptor
1.2.3. GABA-induced modulation of activity of other neurotransmitters

1.3. EFFECT OF LONG-TERM ADMINISTRATION OF ANTI-DEPRESSANTS ON $GABA_A$, BENZODIAZEPINE, $GABA_B$, 5-HT AND NORADRENERGIC RECEPTOR BINDING

1.4. THE 5-HT SYSTEM

1.4.1. The neurotransmitter 5-HT
1.4.2. 5-HT receptor types
1.4.3. 5-HT reuptake inhibitors
1.4.4. Effect of 5-HT modulating drugs on anxiety in man

1.5. OTHER NEUROTRANSMITTER SYSTEMS AND ANXIETY

1.5.1. Noradrenergic system
1.5.2. Dopaminergic system
1.5.3. Cholinergic system, glutaminergic system and ethanol

1.6. ANIMAL MODELS FOR ANXIETY

1.6.1. Conflict tests
1.6.2. Neophobia
1.6.3. Social interation
1.6.4. Other animal models for anxiety
1.6.5. Short comment on extinction of conflict behaviour

1.7. PANIC DISORDER

1.1. INTRODUCTION

The anxiety syndrom "panic disorder" is at the moment subject of intensive biological psychiatrical research. The syndrom consists of panic attacks (intense fear) with several somatic symptoms (dizziness, palpitations, hyperventilation). Most patients develop some degree of anticipatory anxiety (i.e. fear of having another panic attack). A number of panic disorder patients develop agoraphobic symptoms. These symptoms may be described as fear to be in a situation in which no help is present if something (e.g. a panic attack) happens to the patient.

It has been described that panic anxiety is benzodiazepine resistant (Sheehan (1982), McNair and Kahn (1981)), but anti-depressants have been shown to have anti-panic properties (Klein (1967), Zitrin et al (1978)). The therapeutic efficacy seems to correlate with the reuptake inhibition of serotonin (5-hydroxytryptamine (5-HT)) (Den Boer (1988)). The triazolo-benzodiazepine alprazolam has also a beneficial effect in panic disorder (Ballenger et al (1988)). It is less known that the $GABA_B$ receptor agonist baclofen also possesses anti-panic properties (Pepplinkhuizen and Bruinvels (1978), Breslow et al. (1989)).

In order to investigate the mechanism responsible for the anxiolytic effect of baclofen, an animal behavioural model has been developed in which this compound is effective. Chapter I reviews the literature on the GABA-ergic system, 5-HT system, the involvement of several neurotransmitters in anxiety, various animal models for anxiety and panic disorder. The aim of the experiments was to investigate the relationship between baclofen on one hand, and the $GABA_A$/benzodiazepine receptor chloride channel complex and the 5-HT system on the other hand, using extinction of conflict behaviour as a tool. The Chapters II to V describe the experiments which try to shed more light on two possible mechanisms, namely $GABA_A$ and/or 5-HT involvement, by which baclofen might mediate its effect on extinction of conflict behaviour.

1.2. THE GABA-ERGIC SYSTEM

1.2.1 The neurotransmitter GABA.

The amino acid τ-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the central nervous system. Anatomical studies suggest that GABA is localized primarily in small interneurons throughout the central nervous system but there are also GABA-ergic projections linking the corpus striatum and nucleus accumbens to other brain regions like the globus pallidus, substantia nigra and interpeduncular nucleus (Scheel-Kruger (1983)). It is estimated that 10 to 40 % of all terminals in the cerebral cortex, hippocampus and substantia nigra are GABA-ergic (Iversen and Bloom (1972), Schon and Iversen (1974)). In the neocortex it is shown that the GABA containing neurons are almost exclusively local neurons and are distributed through all layers (Ottersen and Storm-Mathisen (1984), Reiffenstein and Neil (1974), Ribak (1978)). The hippocampus also contains local GABA-ergic neurons, and is most densely present in the pyramidal/granular layer and the molecular layer (Storm-Mathisen and Fonnum (1971)). The Purkinje, stellate, basket and Golgi cells in the cerebellum are identified as being GABA-ergic (Ottersen and Storm-Mathisen (1984), Fonnum et al. (1970), McLaughlin et al. (1974)). In the medulla the evidence of the existence of GABA-ergic neurons is most impressive in the raphe nuclei (Belin et al. (1979), Vincent et al. (1980)). It has been reported that the neurotransmitters serotonin (5-hydroxytryptamine, 5-HT) and GABA coexist in some raphe neurons (Belin et al. (1983)).

1.2.2. GABA receptors

GABA receptors were initially defined on the basis of their sensitivity to inhibition by bicuculline and picrotoxin (Curtis et al. (1971)). More recently, it has been shown that receptors for GABA in the central nervous system are not of an homogenous type. Three types of GABA receptors have been identified: $GABA_A$ receptors are bicuculline sensitive and baclofen (Lioresal, β-(p-chlorophenyl)GABA) insensitive while $GABA_B$ receptors are bicuculline insensitive and baclofen sensitive (Bowery et al. (1980)). A third type, $GABA_C$, has been proposed which is both bicuculline and baclofen insensitive (Johnston (1986)).

Radioligand binding studies show that receptors for GABA are present on virtually all neurons of the central nervous system (Fonnum (1987)). On the whole, the $GABA_A$ receptors outnumber $GABA_B$ receptors in the rat central nervous system (Bowery et al. (1987)). However, autoradiographic studies indicate the reverse in some specific areas such as the interpeduncular nucleus, fasciculus retroflexus, medial habenular nucleus, substantia gelatinosa, superior colliculus, globus pallidus, lateral amygdaloid nucleus, pontine nucleus, raphé magnus and molecular layer of the cerebellum. (Bowery et al. (1987)).

1.2.2.A The $GABA_A$/benzodiazepine chloride channel complex.

The $GABA_A$ receptor has been extensively investigated. $GABA_A$ receptors can be subdivided in a receptor with a high (dissociation constant 3-10 nM) and a low (dissociation constant 50-120 nM) affinity for GABA (Enna and Karbon

(1986)). It is known that $GABA_A$ receptor activation increases chloride channel conductance which results in an influx of chloride ions in most of the cases (postsynaptic hyperpolarization), but sometimes in an outward flux (presynaptic depolarization) (review: Enna (1981)). The $GABA_A$ receptor can be subdivided in a population which is associated with the benzodiazepine receptor and a population which is not (Palacios et al. (1981), Unnerstal (1981)). It is the low affinity GABA receptor which has been shown to be linked with the benzodiazepine receptor (Tallman et al. (1978), Karobath and Sperk (1979), Skerritt et al. (1982)). On the other hand, it has been suggested that high and low affinity $GABA_A$ receptors represent in fact a single site that can exists interconvertibly in an agonist or an antagonist state (Maksay and Ticku (1984)).

It appears that all $GABA_A$ receptors are associated with the chloride ionophore (Enna and Karbon (1986)), while most postsynaptic $GABA_A$ receptors form a part of the $GABA_A$/benzodiazepine receptor chloride channel complex. The three components of this complex can be characterized in vitro by radioligand binding assays using [^{3}H]GABA for the $GABA_A$ receptor sites, [^{3}H]benzodiazepines for the benzodiazepine receptors and [^{3}H]DHP (α-dihydropicrotoxinin) for the chloride ion site (review: Ticku (1983)). It has been shown that these three sites interact with eachother; the binding characterics of each site are under the influence of the degree of activation of the other two receptor sites (Enna and Karbon (1986)).

Three kinds of benzodiazepine receptor ligands are reported: agonists (e.g. diazepam, chlordiazepoxide), antagonists (e.g. RO15-1788 (Hunkeler et al. (1981)) and inverse agonists (e.g. methyl β-carboline-3-carboxylate (βCCE) and methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) (Oakley and Jones (1980), Braestrup et al. (1982)). Benzodiazepine receptors can be subdivided into 2 types on basis of the affinity for the triazolopyridazine CL218,872; Type 1 benzodiazepine receptors have a higher affinity for this compound than type 2 receptors while both types have a high affinity for benzodiazepines (Squires et al. (1979)). It was postulated that activation of type 1 benzodiazepine receptors mediates anxiolytic effects, and activation of type 2 benzodiazepine receptors mediates the sedative effect of these compounds (Lippa et al. (1979)). However, Oakley et al. (1984) reported sedative effects of CL 218,872 in the same dose range as its anxiolytic effects. Receptors for benzodiazepines are found in the highest concentrations in the cerebral cortex, cerebellum and amygdala, and in intermediate concentrations in the hippocampus, striatum and spinal cord. Type 2 receptors have a relatively higher density in the amygdala and hippocampus (50 %), a relatively lower density in the cortex (25 %), and were virtually absent in the cerebellum (Braestrup and Nielsen (1980), Young et al. (1981)).

The behavioural effects resulting from $GABA_A$/benzodiazepine chloride channel complex activation are extensively documented. They concern anxiolytic, anti-convulsive, sedative, muscle relaxant, anti-aggressive and (anterograde) amnestic actions (review: File and Cooper (1985)). Clinical use of benzodiazepines, barbiturates, anti-epileptics etc. are a reflection of the properties mentioned above, e.g. anxiolysis, anti-convulsive action, hypnotic and spasmolytic action etc. In practice, benzodiazepines are used in all kinds of anxiety, however, it appears that they are most rightfully indicated in acute stress situations and generalized anxiety disorder. It was initially reported that benzo-diazepines (administered in low doses) were not active in panic disorder (McNair and Kahn (1981)), but recent data suggest that the high affinity benzodiazepine receptor agonist alprazolam, a triazolobenzodiazepine, and high doses of diazepam, lorazepam, bromazepam and clonazepam have

benificial effects in panic disorder (Ballenger et al. (1988), Beaudry et al. (1984), Spier et al. (1986), Noyes et al. (1984,1988)).

1.2.2.B The $GABA_B$ receptor

The $GABA_B$ receptor site has been characterized more recently (Bowery et al. (1982)). The concept of baclofen being a selective $GABA_B$ receptor agonist came from studies on the peripheral nervous system, where GABA and the (-)-isomer of baclofen were shown to have a bicuculline-insensitive inhibitory action on the release of noradrenaline from atria and vas deferens (Bowery and Hudson (1979), Bowery et al. (1980), review: Bowery (1983)). Baclofen was designed as a lipophilic derivative of GABA, able to cross the blood-brain barrier. The localization of the $GABA_B$ receptor in comparison with the $GABA_A$ receptor distribution is described in 1.2.2.. The $GABA_B$ receptor is not linked to the chloride channel and its binding is not modulated by benzodiazepines (Bowery et al. (1984)). In analogy with the $GABA_A$ receptors, high and low affinity $GABA_B$ receptors are described (Bowery and Hill (1981)). Early reports on the second messenger of the $GABA_B$ receptor indicate a blockade of calcium influx (Dunlap (1981)). However, later on it has also been reported that $GABA_B$ receptor activation causes a hyperpolarization via an increase in potassium conductance in the hippocampus (Newberry and Nicoll (1984)) and substantia nigra (Pinnock (1984)) and that this phenomenon might underly the observed decreased calcium influx (Ghäwiler and Brown (1985)). On its turn, also the latter notion has been challenged in the sense that the decrease in calcium influx is reported to be a potassium independent phenomenon (Diesz and Lux (1985)). Baclofen is shown to inhibit basal adenylate cyclase activity in the presence and absence of forskolin in several areas of the brain, including hippocampus, striatum and cerebellum of rat brain membrane preparations (Wojcik and Neff (1983)). It is reported that $GABA_B$ receptor activation inhibits forskolin-stimulated adenylate cyclase activity but, in contrast, enhances the calcium-dependent stimulation of cAMP accumulation induced by adenosine, histamine, β-adrenoceptor agonists and vasoactive intestinal peptide (Hill and Dolphin (1984), Enna and Karbon (1987)). This synergistic interaction is region-specific: it occurs in cerebral cortex, hippocampus and striatum, but not in spinal cord, cerebellum and pons-midbrain. The β-adrenoceptor-induced increase of cyclic AMP is enhanced by $GABA_B$ receptor activation via a GTP regulatory protein. This might also be the case concerning the $GABA_B$ effect on potassium channels (Asano et al. (1985), Andrade et al. (1986), Hill et al. (1984)). No antagonist of $GABA_B$ receptors is available for in vivo studies, but now reports of Bonnano et al. (1988) and Kerr et al. (1987) exist of in vitro antagonism by the compound phaclofen (β-(p-chlorophenyl)-3 amino propyl phosphonic acid). The reported $GABA_B$ receptor activation-induced changes of neurotransmitter release (see paragraph 1.2.3.) might be mediated via the effects of $GABA_B$ receptor activation on potassium channels, calcium channels, adenylate cyclase activity or a combination of these factors (Dolphin (1984)).

Several effects of $GABA_B$ receptor activation are observable in vivo: muscle relaxation, sedation, hypothermia (Gray et al. (1987)), anti-convulsive effects (Ault et al. (1986), Petersen (1983)), anti-nociception (Cutting and Jordan (1975)) and disruption of passive avoidance retention, (which was interpreted as a baclofen-induced memory consolidation deficit) (Swartzfelder et al. (1987)). On the other hand, it was shown by Nabeshima et al. (1988) that baclofen antagonized the memory consolidation deficits induced by several

GABA receptor antagonists. (For the effect of $GABA_B$ receptor activation in animal models for anxiety: see discussion).

A number of reports exist on the effect of baclofen in humans. The only clinical use on which there is a consensus appears to be spasticity (Jones et al. (1970), Young and Delwaide (1981)). Other reports concern a treatment for restless legs (Guilleminault and Flagg (1984), convulsant and (seldom) anti-convulsant effects (Terrence et al. (1983), Nugent et al. (1986)). Abrupt withdrawal of baclofen has been reported to induce dyskinisias and psychosis (Kirubakaran et al. (1984)). Administration of baclofen to patients treated with anti-depressants can induce memory deficits (Sandyk and Gillman (1985)). The latter effect might be related to $GABA_B$ receptor upregulation (see paragraph 1.3.). Finally, baclofen has been reported to have beneficial effects in panic disorder (Pepplinkhuizen and Bruinvels (1978), Breslow et al. (1989).

1.2.3. Modulation of activity of other neurotransmitter systems.

A number of neurotransmitter systems are reported to be affected by activation of GABA receptors. The best documented interactions concern the effect of GABA receptor activation on release of GABA itself, glutamate and aspartate, dopamine, 5-HT and noradrenaline.

A: GABA: It has been suggested that both $GABA_A$ and $GABA_B$ receptors are simultaneously involved in modulating the activity of the GABAergic system (Anderson & Mitchell (1985)). It is shown that $GABA_A$ autoreceptor activation inhibits release (Mitchell and Martin (1978), Brennan et al. (1981)) while reports on the effect of $GABA_B$ receptor activation are conflicting. It has been reported that $GABA_B$ receptor activation increases release of GABA (Kerwin & Pycock (1978), Roberts et al. (1978)), while other reports state that baclofen does not affect or even decreases GABA release (Anderson & Mitchell (1985), Potashner (1979), Collins et al. (1982), Johnston et al. (1980), Pittaluga et al. (1987) Maurin (1988) and Bonanno et al. (1988)). Thus, it appears that the release of GABA can be modulated by activation of both $GABA_A$ and $GABA_B$ type receptors, depending on the brain region explored and experimental conditions used.

B: Glutamate and aspartate: It has been shown that benzodiazepines inhibit release of glutamate and aspartate (Baba et al. (1983), Collins (1981)). However, this action might be GABA independent (Stone (1981)). A bicuculline-sensitive GABA-induced inhibition of excitatory amino acid release is reported by Davies (1981) but a GABA-induced increase in glutamate release in cerebellar tissue is also reported (Levi et al. (1981)). In contrast, several reports exist on a baclofen-induced inhibition of glutamate and aspartate release (Potashner (1979), Fox et al. (1978), Davies (1981) and Collins et al. (1982)).

C: Dopamine: A bicuculline-sensitive GABA-induced inhibition of dopaminergic neurons in the substantia nigra and ventral tegmental area is reported by Waszczak and Walters (1979) and Wolf et al. (1978). However, other studies indicate that systemic administration of muscimol (a selective $GABA_A$ agonist) increases firing of dopaminergic neurons in the pars compacta of the substantia nigra (Waszczak and Walters (1979)), and increases dopamine turnover in the caudate nucleus (Fuxe et al. (1979)). The effect of benzodiazepines om dopamine turnover are mostly described as inhibitory, while the interaction between the GABA and dopamine system is quite complex with region-dependent stimulatory and inhibitory effects (review: Taylor (1982)).

Baclofen inhibits substantia nigra cell firing (Olpe et al. (1977)) and decreases turnover in mesolimbic dopamine neurons (Fuxe et al. (1975)). In vivo experiments -e.g. neuroleptic-induced hyperactivity and catalepsy, rotarod performance- indicate an effect of l-baclofen on muscle hypotonia and sedation, while d-baclofen causes an impairment of the dopaminergic system (Kerwin et al. (1979). It is suggested by Delini-Stula (1977) that the effect of baclofen on conditioned avoidance behaviour and controversive turning is mediated by an inhibition of acetylcholine release, which on its turn decreases the inhibitory input on GABA-ergic neurones and thus increases the inhibitory influence on dopaminergic cells. Psychosis and dyskinesias have been reported after abrupt withdrawal of baclofen in humans (Kirubakaran et al. (1984)). Additional support for the suggestion of a link between baclofen and the dopaminergic system consists of a report by Mereu et al. (68), who suggest a supersensitivity of the dopamine autoreceptor after a single dose of baclofen. The same author tentatively suggested a similar mechanism for the 5-HT and noradrenergic system.

D: 5-HT: It was shown by Chase et al. (1970) that diazepam decreases the turnover of the 5-HT system. It was postulated by Wise et al. (1972) and Stein et al. (1973) that the anxiolytic action of benzodiazepines is caused by an inhibition of the 5-HT system. This effect of the benzodiazepines might be mediated via the $GABA_A$-benzodiazepine receptor chloride channel complex since it has been shown that activation of GABA and benzodiazepine receptors exert an inhibitory effect on 5-HT neurones in vivo (Gallager (1978), Thiébot et al. (1980)) and in vitro (Balfour (1980)). On the other hand, some evidence exists that this effect of benzodiazepines might be $GABA_A$-independent (Soubrié (1981)) and there are also reports which state that GABA has no effect on 5-HT transmission (Reubi et al. (1978), Starr (1979)). In addition, if present, it is not clear in what proportion the GABA-induced impairment of the 5-HT system is mediated via $GABA_A$ or via $GABA_B$ receptors. Baclofen has been shown to decrease the release of 5-HT from rat cortical tissue in vitro via activation of presynaptically located $GABA_B$ receptors (Schlicker et al. (1984), Gray and Green (1987) and Bowery et al. (1980)). Furthermore, activation of the $GABA_B$ receptor and of the $5\text{-}HT_{1A}$ (auto)receptor have both been shown to increase potassium conductance in a shared potassium channel in the hippocampus and the raphe nuclei (Innis and Aghajanian (1987), Andrade et al. 1986)). Further evidence of $GABA_B$ receptor linkage with the 5-HT system will be described in the section of 5-HT receptor types. Some studies are worthwhile mentioning in the context of the relationship between GABA and 5-HT: It is reported by Handley and Singh (1985) that a certain type of 5-HTP-induced behaviour (head-twitch) is enhanced by $GABA_A$ receptor activation (this was also shown in a $GABA_A$ independent way for the benzodiazepines (Moser and Redfern (1986)) but inhibited by $GABA_B$ activation. The $GABA_A$ and $GABA_B$ mediated effect on head-twitch response was dependent on an intact noradrenergic system (Singh et al. (1986)). Two mechanisms were suggested to be responsible for this effect of baclofen: an inhibition of 5-HT release (Metz et al. (1985)), and/or a baclofen-induced postsynaptic antagonism which is modulated by the noradrenergic system (Singh et al. (1986)).

E: Noradrenaline: It is reported that benzodiazepines impaired activity of the noradrenergic system, e.g. by means of inihibitory influence on the locus ceroeleus (Taylor and Laverty (1969), Grant et al. (1980)), while it is also shown that GABA enhances potassium-evoked release of noradrenaline from the rat occipital lobe (Langer and Arbilla (1979)). Fung and Fillenz (1983) reported that $GABA_A$ receptor activation or a low concentration of chlordiazepoxide enhance noradrenaline release from hippocampal tissue. The

reverse is shown for $GABA_B$ receptor activation and a high concentration of the benzodiazepine. From this results it has been suggested that chlordiazepoxide enhances the noradrenaline release via the $GABA_A$-benzodiazepine receptor complex but decreases release in a high concentration. The latter effect is mimicked by alprazolam but not by diazepam, since only alprazolam inhibits the MHPG (3-methoxy-4-hydroxy-phenylethyleneglycol, a metabolite of noradrenaline) increase after yohimbine (Charney and Heninger (1985a). $GABA_B$ receptor activation inhibits the activity of the locus ceroeleus via an increase in potassium conductance at this region. Suzdak and Gianutsos (1985b) reported an increase and a decrease of noradrenaline release in rat cortex and hippocampus by $GABA_A$ and $GABA_B$ receptor activation respectively. The authors suggest a relationship between the noradrenaline system and the $GABA_B$ receptor at two levels: Firstly: since chronic administration of baclofen prevented a DSP_4 (a neurotoxin that abolishes the presynaptic noadrenergic neuron) -induced increase in Bmax of the β-adrenoceptor, it was suggested that a relationship exists between the β-adrenoceptor and the $GABA_B$ receptor at a postsynaptic level. Secondly: since the Bmax of the low affinity $GABA_B$ receptors decreases after DSP_4 treatment (Suzdak and Gianutsos (1986a)), and the fact that a decrease in release of noradrenaline is observed, it was suggested that $GABA_B$ receptors are also located on the nerve terminal. On the other hand, it was reported by Bonanno and Raiteri (1987) that $GABA_B$ receptor activation did not affect noradrenaline release from rat hippocampus synaptosomes. The existence of an interaction between the noradrenergic system and the GABA system is supported by Suzdak and Gianutsos (1985b), who report that long-term treatment of baclofen and THIP (a selective $GABA_A$ agonist) decrease the Bmax of β-adrenoceptors in mouse cerebral cortex and hippocampus. A more precise description of the interaction between the β-adrenoceptor and the $GABA_B$ receptor comes from Karbon and Enna (1983); Baclofen potentiates the norepinephrine-stimulated adenylate cyclase activity, presumably through a postsynaptic mechanism.

1.3. EFFECT OF LONG-TERM ADMINISTRATION OF ANTI-DEPRESSANTS ON $GABA_A$, $GABA_B$, BENZODIAZEPINE, 5-HT AND NORADRENERGIC RECEPTOR BINDING

Since long-term administration of anti-depressants has anti-panic properties, it is of interest to review its effect on receptor binding of the neurotransmitters involved in anxiety.

GABA: Pilc and Lloyd (1984) described a $GABA_B$ receptor number increase in mouse frontal cortex after long-term treatment (18 days) with amitryptiline, desipramine, viloxazine, citalopram and a MAO inhibitor pargyline. The binding parameters of the $GABA_A$ receptor were unchanged. In contrast to these results, Suzdak and Gianutsos (1985a) reported that long-term (two week) treatment with the anti-depressants imipramine and nomifensine decreased Bmax of β-adrenoceptors and $GABA_A$ receptors in the cortex and hippocampus of mice. The same group replicated the results of Pilc and Lloyd (increased $GABA_B$ binding) and reported an increase of the baclofen-induced potentiation of cAMP production (Suzdak and Gianutsos (1986b)). Gray and Green (1987) reported that the $GABA_B$-induced inhibition of potassium-evoked 5-HT release from mouse frontal cortex is increased after chronic (14 days) treatment with amitryptiline, desipramine, mianserin or zimeldine. This was also shown after repeated electroconvulsive shock treatment (ECS). The same investigators found that the baclofen-induced hypothermia increased after treatment with the above-mentioned anti-depressants (Gray et al. (1987)). However, Cross and Horton (1986) reported no change in $GABA_B$ receptor binding after long-term treatment with desipramine or zimeldine.

The mechanism by which anti-depressants increase $GABA_B$ receptor binding is not clarified. It is observed that GABA levels increase and GABA uptake sites decrease during treatment with these compounds (Pilc and Lloyd (1984)). It would implicate an unusual finding in pharmacology if the increase in $GABA_B$ receptors results from an enhanced receptor activation. However, there are precedents since $GABA_A$ receptors are reported to be increased after treatment with $GABA_A$ agonists (Beart et al. (1985)), and 5-HT_2 receptors are reported to decrease in number after treatment with antagonists (Leysen et al. (1986)). On the other hand, $GABA_B$ receptor binding is decreased after long-term administration of baclofen (Suzdak and Gianutsos (1986b)). This makes an activation-induced upregulation of receptor sites rather unlikely, unless GABA regulates $GABA_B$ receptor number in an opposite way as baclofen does.

Benzodiazepine: Reports considering the effect of anti-depressants on benzodiazepine receptor binding are very mixed. Thus, an increase (Rozhanets et al. (1983)), decrease (Suranyi-Cadotte et al. (1985), Barbaccia et al. (1986)) and no change (Przegalinski et al. (1987)) has been reported.

Adrenoceptors: It has been reported that long-term administration of anti-depressants decreases α2-adrenoceptor binding (Crews and Smith (1978)), β-adrenoceptor binding (Sulser and Mobley (1981)), and norepinephrine-stimulated adenylate cyclase activity (Sulser (1979)). The α2-adrenoceptor agonist clonidine induces a sedation response and hypothermia which are both attenuated after repeated administration of ECS or anti-depressant drugs (Heal et al. (1981,1983), Von Voigtlander et al. (1978), Pilc and Vitulani (1982)).

5-HT: Long-term treatment with anti-depressants affects also 5-HT receptor binding (Maggi et al. (1981)). It was reported by Peroutka and Snyder (1980) that several anti-depressants downregulate 5-HT_2 receptors. 5-HT_2 receptor activation-induced head-twitch in the mouse was reported to be reduced after

long-term administration with anti-depressants (Friedman et al. (1983)), but ECS increased 5-HT_2 binding in the rat (Kellar et al. (1981)) and a report exists of increased head-twitch after repeated ECS (Green et al. (1983)). The opposing effects of ECS and antidepressant drug treatment on binding and behaviour in mice were confirmed by Goodwin et al. (1984). In contrast, two reports state that 5-HT_1 binding sites are reduced after chronic 5-HT reuptake blockade (Wong and Bymaster (1981), Dumbrill-Ross and Tang (1983)). This is supported by the observation that repeated ECS and long-term (14 days) administration of zimeldine and desipramine attenuates the 8-OH-DPAT-induced hypothermia and 5-HT syndrom (Goodwin et al. (1987b)). Hypothermia is a presynaptically 5-HT_{1A} induced effect, while 5-HT behaviour is a postsynaptically evoked effect (Goodwin et al. (1987a))). The attenuation of the 5-HT syndrom after anti-depressants was also reported by Stolz et al. (1983) but was only partly replicated by Lucki and Fraser (1982). The behavioural effects of 5-HT_{1B} receptor activation after chronic administration was studied by Aulakh et al. (1987). It was reported that the locomotor and food intake suppressant effect of mCPP are enhanced after 21 days of treatment with imipramine. These results made the authors suggest that $5HT_{1B}$ receptors become supersensitive after antidepressant pretreatment. However, it was reported by the same group that the MAO inhibitor clorgyline attenuates the above mentioned effects of mCPP (Cohen et al. (1983)).

1.4. THE 5-HT SYSTEM

1.4.1. The neurotransmitter 5-HT.

The cellbodies of 5-HT neurones are largely confined to the seven defined raphe nuclei of the rat brain stem. From these cell groups three major ascending projections to the forebrain are observed. They project to the a: caudate/putamen, b: substantia nigra and c: interpeduncular nucleus, ventral tegmentum, thalamus/hypothalamus, amygdala, cortex and hippocampus (Steinbusch and Nieuwenhuys (1983)).

1.4.2. The 5-HT receptor types.

Due to the development of selective 5-HT ligands, three types of 5-HT receptors have been identified: 5-HT_1, 5-HT_2 and 5-HT_3 receptors. The 5-HT_1 receptor has been further subclassified into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptor types (Bradley et al. (1986), Heuring and Peroutka (1987)). The classification is based on a high or low receptor affinity for 5-HT and the various receptor ligands.

5-HT_{1A}: 5-HT_{1A} receptors are localized in the raphe nuclei and limbic terminal regions (hippocampus, septum) of the rat (Pazos and Palacios (1985), Glaser et al. (1985)). 8-hydroxy-2-(dl-n-propylamino)tetralin) (8-OH-DPAT) is a selective agonist for this receptor type (Middlemiss and Fozard (1983)). Some caution has to be garded in interpreting 8-OH-DPAT effects as being mediated only via the 5-HT_{1A} receptor, since an agonistic-like interaction with α2-adrenoceptors is also described (Marsden (1989)). Other 5-HT_{1A} ligands are buspirone, ipsapirone and gepirone. Buspirone is a 5-HT_{1A} mixed agonist/antagonist which also blocks dopamine receptors while ipsapirone and gepirone are mixed agonists/antagonists without dopamine blocking action. All three compounds are metabolized to 1-pyrimidinilpiperazine, which blocks α2-adrenoceptors (Assié and Briley (1987), Giral et al. (1987)). No selective antagonist for the 5-HT_{1A} receptor is available, (cyano)pindolol and propranolol act as antagonists of 5-HT_{1A} as well as 5-HT_{1B} receptors (Middlemiss et al. (1977), Green et al. (1983)).

It has been reported that activation of 5-HT_{1A} receptors results in both augmentation and inhibition of adenylate cyclase activity. The direction of the induced effect might depend on the pre-existing state of the enzyme activity or on which subclass of 5-HT_{1A} receptors is activated (Roth and Chuang (1987)). 5-HT_{1A} receptors and $GABA_B$ receptors increase conductance in shared potassium channels via a pertussis toxin-sensitive G protein as has been shown in the hippocampus of the rat (Andrade et al. (1986)). In addition, Innis and Aghajanian (1987) reported that 5-HT_{1A} and $GABA_B$ receptor activation inhibits 5-HT neuron activity in the rat dorsal raphe. This inhibition was also sensitive to pertussis toxin.

Behavioural, or in vivo observable, effects of 5-HT_{1A} receptor activation are hypothermia and hyperphagia, mediated via activation of presynaptically localized 5-HT_{1A} receptors (Goodwin et al.

(1987a), Dourish et al. (1986)). In addition, 5-HT_{1A} receptor activation by 8-OH-DPAT induces signs of 5-HT behaviour, this appears to be mediated via postsynaptically localized 5-HT_{1A} receptors (Tricklebank et al. (1984), Goodwin et al. (1987b)). The effects of 5-HT_{1A} agonists are anxiolytic-like in some, but not all animal models for anxiety (Riblet et al. (1982), Traber et al. (1984), Eison et al. (1986), Engel (1986) and McMillen et al. (1987), (see paragraph 1.6.)).

The observed anxiolysis of 5-HT_{1A} agonists in man is mostly ascribed to the decrease in 5-HT neuron firing rate after activation of somatodendritic autoreceptors, however, it is possible that the postsynaptically mediated inhibitory action in the hippocampus also exerts an anxiolytic-like action (Marsden (1989).

5-HT_{1B}: This receptor type appears to be present in rat and mouse brain only (Heuring et al. (1986)). The highest densities of 5-HT_{1B} receptors are found in the caudate, superior colliculus, lateral geniculate, subiculum, globus pallidus and substantia nigra (Pazos and Palacios (1985)). The most selective 5-HT_{1B} agonist is 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)1H indole succinate (RU24969). However, this compound and also 1-[3-(Trifluoromethyl)-phenyl]piperazine (TFMPP) and 1-(3-chloro-phenyl)piperazine (mCPP) are not completely selective for 5-HT_{1B} receptors since all three drugs have also some affinity for the 5-HT_{1A} receptor (Peroutka (1986)).

It is not known which second messenger is linked to the 5-HT_{1B} receptor. The functional correlate of the 5-HT_{1B} receptor appears to be the 5-HT terminal autoreceptor and a postsynaptic receptor. An interaction between the 5-HT reuptake site and the 5-HT_{1B} receptor is also described since 5-HT reuptake inhibitors acutely reduce the efficacy and potency of 5-HT_{1B} agonists (Langer (1982)).

The effects of postsynaptic activation (e.g. anorexia, hyperlocomotion) prevail in vivo after administration of 5-HT_{1B} agonists, it appears that there are no drugs available which selectively activate pre- or postsynaptic 5-HT_{1B} receptors (Kennett et al. (1987)).

5-HT_{1C} and 5-HT_2: The 5-HT_{1C} receptor has been localized in the choroid plexus, frontal cortex and hippocampus (Pazos et al. (1984), Pazos and Palacios (1985)). The 5-HT_2 receptors are mainly found in the IIIth and IVth layer of the cerebral cortex, claustrum, olfactory tubercle, caudate nucleus and nucleus accumbens of human and rat brain (Luabeya et al. (1984), Leysen et al. (1982), Hoyer et al. (1986)). Selective ligands for the 5-HT_{1C} receptor are not available. Mianserine, mesulergine and metergoline are antagonists with a high affinity for the 5-HT_{1C} receptor. There is a considerable overlap of ligand-binding profile with the 5-HT_2 receptor. Thus it is shown that 5-HT_2 antagonists like ritanserin, cyproheptadine and pizotifen are also 5-HT_{1C} blockers (Hoyer (1988)). The compound 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) is an example of a quite selective 5-HT_2 agonist (Glennon et al. (1986)).

5-HT_{1C} and 5-HT_2 receptor activation leads to stimulation of phospholipase C and to the breakdown of inositolphospholipids (Conn et al. (1986)). This may on its turn affect ion channel activity, e.g. the calcium activated potassium channels (VanderMaelen and Aghajanian (1982)).

The 5-HT_{1C} receptors in the choroid plexus may be involved in the composition and volume of the cerebro-spinal fluid (Moskowitz et al. (1979)). The 5-HT_2 receptor displays a high affinity for a number of neuroleptics (Peroutka and Snyder (1979)). Effects of 5-HT_2 receptor activation are for instance head twitching in mice and wet-dog shakes in rats (Leysen et al. (1984)). In man, 5-HT_2 receptor stimulation appears to play a role in the effects of hallucinogenic compounds like LSD (Glennon et al. (1984)). In addition, it is postulated that 5-HT_2 blockade exerts an improving effects in dysthymia, aggression and anxiety.

5-HT_{1D}: This binding site has been identified recently. Regional studies in bovine brain indicate that the site exists in all regions of the brain but most densely in the basal ganglia. Nothing is known about the existence of a second messenger or a functional correlate linked to this site. No selective ligand is available, but tryptamines, ergotamines, RU24969 and yohimbine display high affinity for the 5-HT_{1D} site (Heuring and Peroutka (1987)).

5-HT_3: The 5-HT_3 receptor has initially been identified in the peripheral nervous system (Bradley et al. (1986)). However, binding sites are now shown in membrane preparations of entorhinal cortex and cortical and limbic regions (Kilpatrick et al. (1988)). It is not clear whether these receptors are localized pre- or postsynaptic to the neuron cell body. Several selective 5-HT_3 ligands are available: 2-methyl-5-HT (agonist), GR38032F, ICS 205-930 and MDL 72222 (antagonists). It is speculated that 5-HT_3 antagonists may have a beneficial effect in migraine (Loisy et al. (1985)), anxiety and psychosis (Tyers et al. (1987)).

For reviews, see Fozard (1987), Roth and Chuang (1987), Heuring and Peroutka (1987) and Conn and Sanders-Bush (1987).

1.4.3. 5-HT reuptake inhibitors.

On the way to find equipotent but better tolerated anti-depressants, and to find drugs with more specific and selective activity, the group of selective 5-HT reuptake inhibitors has been developed. Among the best known and clinically used are fluvoxamine, fluoxetine, trazodone and clomipramine. The binding sites for these compounds are assumed to be localized presynaptically in the raphe nuclei and on the 5-HT nerve terminals of the projection sites (review: Chesselet (1984)). Fluvoxamine belongs to the most selective drugs within this group, since this compound has not only no effect on reuptake of noradrenaline or dopamine, but has also no affinity for $\alpha 1$, $\alpha 2$, $\beta 1$, dopamine$_2$, histamine$_1$, muscarinic, 5-HT_1 and 5-HT_2 receptors (Benfield and Ward (1986)).

It has been reported that selective 5-HT reuptake inhibitors have not only a beneficial effect on depression and suicidal ideation, but also on panic disorder with or without agoraphobia, obsessive compulsive disorder, posttraumatic stress disorder, obesity, alcohol abuse, impulsivety and aggression (Van der Kolk (1987), Westenberg and den Boer (1988), Linnoila (1988)).

The mechanism behind the therapeutic action of selective 5-HT reuptake inhibitors is not fully clarified. For instance, several

hypotheses concerning the anti-panic efficay of anti-depressants exist: Desensitization of postsynaptic $5HT_1$ receptors (Den Boer (1988)), an enhanced $GABA_{(B)}$ receptor activation (Breslow et al. (1989)), or downregulation of adrenoceptor sites (Charney and Heninger (1985b)). The latter effect has also been observed with the selective 5-HT reuptake inhibitor fluvoxamine (Racagni (1984)).

It is shown that selective 5-HT reuptake inhibitors have different effects on the 5-HT system after acute or chronic administration. The functional consequences after acute administration are generally believed to be a facilitation of serotonin neurotransmission (Schipper and Berkelmans (1988)). However, there are some conflicting data on this matter: investigations on a neurochemical level have shown that 5-HT turnover, synthesis rate and firing rate are decreased after a selective 5-HT reuptake inhibitor (Schipper and Berkelmans (1988), Dresse and Scuvee-Moreau (1984)). It has to be noted however, that these decreases might be caused by a feed-back mechanism induced via postsynaptic 5-HT activation at the projection sites. Thus, behavioural studies and electro-fysiological studies indicate a potentiation of 5-HTP effects (Claassen et al. (1977)) and postsynaptic fysiologic responses (de Montigny et al. (1984)).

Besides the presynaptic $5\text{-}HT_{1A}$ receptor and the postsynaptic $5\text{-}HT_1$ and $5\text{-}HT_2$ desensitisation (receptor binding experiments and behavioural studies, see paragraph 1.3.), Chaput et al. (1988) described in an electrophysiological study a desensitization of cell body and terminal autoreceptors.

1.4.4. Effect of 5-HT modulating drugs on human anxiety.

It has been hypothesized by the group of Wise and Stein that the anxiolytic effect of the benzodiazepines is mediated via an impairment of the 5-HT system function (Wise et al. (1972), Stein et al. (1973)). As stated already in paragraph 1.2.3., this effect of the benzodiazepines might be mediated on its turn via the $GABA_A$/benzodiazepine receptor chloride channel complex. Benzodiazepines are often prescribed for generalized anxiety disorder. This is consistent with the fact that it has been shown that $5HT_{1A}$ autoreceptor agonists like buspirone, gepirone and ipsapirone (Ortiz et al. (1987), Csanalosi et al. (1987), Traber and Glaser (1987)), have a similar beneficial effect in this anxiety disorder. Buspirone has some beneficial effect on anticipatory anxiety in panic disorder, it has no effect on panic attacks (Robinson et al. (1989)). The anxiolytic effect of $5\text{-}HT_{1A}$ receptor agonists might be due to activation of the presynaptic (somatodendritic) autoreceptor (see also paragraph 1.4.2.), and/or to activation of the postsynaptic $5HT_{1A}$ receptor in the hippocampus, which inhibits firing at that locus (Andrade et al. (1986), Marsden (1989)). It has been claimed that $5\text{-}HT_2$ receptor blockade has beneficial effects in generalized anxiety disorder (Ceulemans et al. (1985), Bressa et al. (1987)), but it has no effect on any of the components of panic disorder (Den Boer (1988). The effect of 5-HT reuptake inhibitors on anxiety is mentioned in paragraph 1.4.3. The most recent claim is the putative anxiolytic effect of $5\text{-}HT_3$

antagonists (e.g. GR38032F) by Tyers et al. (1987). It is not known yet what anxiety syndromes are affected by the latter compounds.

The hypothesis that the 5-HT system has an important role in panic disorder is supported by a number of observations: The treatment of patients with 5-HTP (in combination with peripheral decarboxylase inhibition) exerts an anti-panic effect (Kahn and Westenberg (1985), Kahn et al. (1987)), it was noted that anxiety was slightly elevated in the first treatment week. The latter effect was also noted by Den Boer, using fluvoxamine (1988). In addition, mCPP, a 5-HT_1 receptor agonist, causes anxiety in panic disorder patients (Charney et al. (1987), Kahn et al. (1988)). The latter two groups reported contrasting data on the effect of mCPP on healthy subjects. Fenfluramine, a 5-HT releaser, has been reported to increase anxiety selectively in panic disorder patients, as well as inducing a greater cortisol and prolactine response in patients than in controls (Targum and Marshall (1989)). It has been reported that selective 5-HT reuptake inhibitors have anti-panic efficacy (Evans et al. (1986), Kahn et al. (1987), Den Boer (1988)). Several challenge tests are performed in order to further investigate the 5-HT system involvement in panic disorder. Kahn et al. (1988) reported an augmented cortisol release after mCPP administration in panic disorder patients, and suggested a 5-HT_1 receptor supersensitivity in this group of patients. Taken together, these data suggest an important role of the 5-HT system in anxiety and in panic disorder.

1.5. OTHER NEUROTRANSMITTER SYSTEMS AND ANXIETY

1.5.1. Noradrenergic system.

There is not much doubt that the noradrenergic system is in some way involved in the pathogenesis of anxiety (review: Redmond and Huang (1979)). There is evidence that adrenoceptor ligands such as adrenaline, noradrenaline, yohimbine (α2 autoreceptor antagonist) and isoproterenol (β-adrenoceptor agonist) induce anxiety- and panic-like symptoms in man and animals (Garfield et al. (1967), Charney and Heninger (1982), Frohlich et al. (1969)). In addition, some indication exists that clonidine (α2 autoreceptor agonist) and propranolol (β-adrenoceptor antagonist) have a weak anxiolytic effect (Hoehn-Saric (1982), Noyes et al. (1984)). Furthermore, it is postulated that the downregulation of α2- and β-adrenoceptor number and function are responsible for the anxiolytic effects of anti-depressants (Charney and Heninger (1985b)).

The specific involvement of the noradrenergic system in the symptoms of patients suffering from panic disorder is supported by a number of data: Yohimbine elecits panic attacks in 50 % of panic disorder and agoraphobic patients, while this was seldom seen in control groups. MHPG, cortisol and blood pressure increases after administration of yohimbine were larger in panic disorder patients than in healthy controls. Also, the decrease of MHPG and blood pressure, and the attenuation of anxiety is greater in panic disorder patients than in controls after clonidine administration (Charney and Heninger (1986), Nutt (1989)). In contrast to these increased effects of clonidine, other effects of α2-adrenoceptor activation like sedation and growth hormone release are smaller in patients than in controls. This suggests a regional difference of α2-adrenoceptor sensitivity alterations in panic disorder (Nutt (1989)). Panic attacks are reported after isoproterenol (Pohl et al. (1985)), while, in addition, a chronic general increase in sympathetic tone has been found in patients suffering from panic disorder (Villacres et al. (1987)). Inhibition of the reuptake of noradrenaline induces an α2-adrenoceptor subsensitivity, this might be related to the anti-panic effects of these drugs. However, it has been reported that imipramine decreased the number of the panic attacks of patients while the yohimbine-induced changes (MHPG, blood pressure and anxiety) in the same patients were unaltered (review: Heninger (1988)). Clonidine has anxiety reducing properties in panic disorder, but a fast tolerance (approximately 3 days) develops, and its effects differs considerably per patient (Uhde et al. (1989)). Two groups reported that propranolol has a modest effect on panic anxiety, but two other groups reported that the drug was not effective (review: Munjack et al. (1989)).

When the role of the noradrenergic (or 5-HT) system in anxiety is discussed, it must be realized that the noradrenergic system is functionally linked with the 5-HT system, e.g. the locus coeruleus receives projections from the nucleus raphe and vice versa (Descarrier and Leger (1978), Leger et al. (1979), Fuxe et al. (1978)). An increased activity of the raphe nuclei has an inhibitory effect on the locus coeruleus, but an increased activity of the latter increases the firing rate of neurons in the raphe nuclei (Segal (1979), Aghajanian (1980)). Furthermore, the anti-depressant-induced downregulation of β-adrenoceptors in animal studies requires an intact 5-HT system (Brunello et al. (1982)). In addition, the existence of functional α2-adrenoceptors on 5-HT terminals has been reported (Feuerstein et al. (1985)), several β-adrenoceptor

antagonists are effective 5-HT_1 blockers (Green and Graham-Smith (1976), Costain and Green (1978)), and in addition to its α2-adrenoceptor blockade, yohimbine has a high affinity to 5-HT_{1D} recognition sites (Heuring and Peroutka (1987).

Taken together, this suggests that it is impossible to postulate a specific and selective role of the noradrenergic or serotonergic system in the pathofysiology of panic disorder.

1.5.2. Dopaminergic system.

Dopamine receptor blockers may be prescribed as anxiolytics in borderline and prepsychotic patients (Brinkley et al. (1979)). They may also be effective in benzodiazepine resistant anxiety (Rickels (1978)). On the other hand, the dopamine receptor agonist apomorphine has also been prescribed as an anxiolytic (Medan (1979)).

1.5.3. Cholinergic system, glutaminergic system and ethanol.

Belladonna (atropine) was used as an anti-anxiety agent before the barbiturates were introduced in 1903 (Harvey (1975)). In addition to atropine, the muscarinic receptor blocker scopolamine has been used as an anxiolytic lateron (Rickels (1978)). However, no large studies investigating specific anxiolytic effects of anti-cholinergics have been performed.

It is postulated recently that excitatory amino acid (glutamate, aspartate) receptor antagonists may have anxiolytic activity since it has been shown that these compounds are active in several animal models for anxiety (Stephens et al. (1986), Bennett and Amrick (1986), Clineschmidt et al. (1982) and Dunn et al. (1989)).

Ethanol was and is still used as a self-prescribed anxiolytic; its action is probably mediated by the $GABA_A$/benzodiazepine receptor chloride channel complex (Burch and Ticku (1980)).

1.6. ANIMAL MODELS FOR ANXIETY

Numerous behavioural models have been developed in order to be used as a screening instrument for the detection of anxiolytic drugs. Animal models for anxiety have to meet to at least a number of criteria as specified recently by Shephard (1984): 1/ Specificity: Anxiolytic effects in an animal model should be induced by more than one compound from one class of drugs with similar pharmacological actions (e.g. benzodiazepines, GABA agonists, barbiturates, 5-HT antagonists etc). 2/ Sensitivity: The model is expected to be sensitive for a low dose of a benzodiazepine. 3/ Correlation with clinical potency: There ought to be a correlation between clinical potency of drugs and drug potency in the animal model. 4/ Absence of tolerance: Anxiolytic action of a drug should still be present after a few days of daily administration of the drug. 5/ Validity: The action of drugs in a model should not be based on another motivational system like feeding or anti-nociception. These criteria receive some criticism since the present tests rely heavily on the effects of benzodiazepines. It is conceivable that the present models are therefore more or less "suited" to the anxiolytic profile of the benzodiazepines (Abbott (1987)), which might be incorrect since the latter compounds are not effective in all forms of anxiety in man.

Most animal models for anxiety make use of punished operant behaviour (e.g. Geller-Seifter test, punished drinking (Vogel's test)), neophobia (shock prod burying, marble burying), social interaction and innate behaviour (e.g. aversion for open field, brightly illuminated areas). Some presently used models and the models used in the experiments described in chapter II to V (conflict behaviour and extinction of conflict behaviour) are discussed below.

1.6.1. Conflict models.

The Geller-Seifter conflict test (Geller and Seifter (1960)) is a procedure in which food-deprived rats follow a multiple schedule in which periods of partial reinforcement (random interval (RI) schedule) alternate with periods in which every response is rewarded with food (fixed ratio (FR) 1 schedule), but in which the latter is also accompanied by electric shock. The desired degree of behavioural suppression can be obtained by appropiate adjustment of shock severity. It is shown that barbiturates, meprobamate and benzodiazepines enhance the rate of punished responding (FR 1 schedule) (Geller and Seifter (1960), Geller et al. (1962), Geller (1964)). This effect is attributed to an anxiolytic effect of the drugs since this effect would resolve the "conflict" between fear for the foot-shock on one hand, and desire for feeding on the other hand. The inhibition of conflict behaviour is also called "anti-conflict effect". The RI schedule is designed as a control for adverse reactions, like sedation, ataxia, muscle relaxation etc, and to maintain "ongoing" behaviour. In a part of the experiments described in chapter II, III and IV, the Geller-Seifter conflict test was used with some adjustments (see method sections). The Geller-Seifter test receives criticism on the point that drugs may exert a seemingly anti-conflict effect via an increase in other drives (e.g. feeding, anti-nociception, general rate-enhancing effects), the time-consuming aspect of the procedure, and the for the animal unnatural behaviour (in comparison with social interaction for instance). Several drugs that impair activity of the 5-HT system (e.g. PCPA, 5-HT_{1A} agonists, 5-HT_2 antagonists) have been

reported to exert an anti-conflict effect in the conflict procedures, but the effects are weak in comparison with the benzodiazepines and a number of negative results are also reported. 5-HT reuptake inhibitors (acute administration) were inactive or even enhanced shock-induced suppression. (Reviews: Chopin and Briley (1987), Broekkamp et al. (1989)).

Vogel's test: This conflict test procedure uses water deprived animals, of which punished (electric shock) drinking is monitored. No training is necessary in this procedure. Benzodiazepines exert an anti-conflict effect since they enhance punished drinking (Vogel et al. (1971)). Criticism on this procedure is mainly centered around effects of drugs on drinking per sé and anti-nociception. The missing of monitoring the motor performance during this test may also be an uncertain factor in this procedure. The effect of drugs which modulate the 5-HT system are largely the same in Vogel's test and in the Geller-Seifter conflict test.

1.6.2. Neophobia.

Procedures based on the fear for novelty were initially called conflict tests, since it was felt that animals were in conflict between their desire to feed themselves and fear for the novel environment in which they were placed or to novel food which was presented. Administration of benzodiazepines enhances feeding in these procedures (Poschel (1971)). In analogy with the above-mentioned tests, also here criticism is focussed on a possible drug-induced enhancement of food intake.

Other tests which belong at least partly in this group are the tests using the placement of unfamiliar objects in a novel environment of the rats and observe behaviour afterwards (e.g. defensive burying test, shock probe conflict test (Broekkamp et al. (1986), Meert and Colpaert (1986a)). Marble burying is decreased after administration of benzodiazepines and 5-HT reuptake inhibitors, but not affected by 5-HT antagonists, 5-HT_{1A} agonists or a 5-HT_{1B} agonist (Broekkamp et al. (1986,1989)). In the shock probe conflict procedure rats are placed in an unfamiliar environment where a shock probe is present. Exploration of the probe is enhanced after administration of benzodiazepines. A number of 5-HT antagonists and 8-OH-DPAT are weakly active, ipsapirone and buspirone have no effect (Meert and Colpaert (1986a, 1986b)).

1.6.3. Social interaction.

This paradigm assesses interactions between the test animal and an undrugged animal in a novel environment (File and Hyde (1978)). Like neophobia, it utilizes the aversion of rats to novelty as an inhibitory behavioural influence. It is proposed that this test is a better reflection of clinical anxiety since it does not use predictable aversive experience (i.e. there is more "uncertainty" in the rat than in the conflict models). The use of the model does not need training and starvation. Social interaction is increased by benzodiazepines (File and Hyde (1978)). The reports on 5-HT modulating drugs and social interaction are mixed: Methysergide, metergoline, clomipramine are not active (File (1981,1985)). Buspirone, ipsapirone, PCPA and GR38032F are reported to be active (File and Hyde (1977), Schuurmans and

Spencer (1987), Gardner (1985), Jones et al. (1987)). A negative result on buspirone is reported by File (1985).

1.6.4. Other animal models for anxiety.

Four Plate: This test is based on the innate behaviour of an animal to explore a novel environment. Crossings from one part to the other part of a test floor are foot shock punished. Benzodiazepines, fluvoxamine and ipsapirone enhance crossings, but PCPA, methysergide, buspirone and clomipramine do not (Boissier et al. (1968), Aron et al. (1971), Molewijk and Van der Heijden (1988)).

Elevated plus maze: The elevated plus maze test is based on the fact that rats tend to avoid exposure to an elevated and open maze alley. This tendency is opposed by the innate drive of the animal to explore its environment. The animals may choose between an open and a closed alley, the increase in percentage of open arm entries provides a measure for anxiolytic activity of drugs like the benzodiazepines (Pellow et al. (1985)). Metergoline, ketanserine, ritanserine have anxiolytic activity, but 5-HT_{1A} agonists, RU 24969 and quipazine were not active or seem to act as anxiogenic compounds in this test. Propranolol, fenfluramine, paroxetine and indalpine were reported to be anxiogenic (Pellow et al. (1987), Critchley and Handley (1986), Handley and Mithani (1984), Chopin and Briley (1987)).

One trial passive avoidance: This test depends on a conflict between the rat's aversions for a brightly illuminated area on one hand, and a dark compartment in which the animal received a foot-shock 24 hours before the test on the other hand (Ader et al. 1972)). Benzodiazepines shorten the latency for reëntry in the dark compartment while also the anxiolytic ipsapirone decreases passive avoidance (Gray (1982), Traber et al. (1984)).

One should be careful with interpreting the effects of anxiolytic drugs in this procedure, since effects on memory and locomotion might affect the results of the test. In fact, this paradigm is used as a measure for drug-induced changes in consolidation, retention and retrieval of memory processes. Research on memory retrieval revealed that pirenperone, ketanserin, mianserin, methysergide, metergoline, alaproclate and zimeldine increased latency in a passive avoidance task procedure (Altman and Normile (1986), Altman et al. (1984)). It is conceivable that the latter effect might be explained by an enhanced retrieval of memory and/or an anxiogenic effect per sé.

Aversive brain stimulation: Electrical stimulation of the dorsal central grey area holds an aversive effect for the rat. The aversive threshold can be determined by measuring the current at which stimulation induces flight behaviour. This threshold is increased after the administration of $GABA_A$ agonists or benzodiazepines (Audi and Graeff (1984), Brandao et al. (1982)). 5-HT, clomipramine and 5-HTP have an anti-aversive action (Schutz et al. (1985), Kiser et al. (1978)). The anti-aversive action of 5-HT was blocked by metergoline or ketanserin (Schutz et al. (1985)). Pirenperone and ketanserine were found to have an anti-aversive action, 5-HT_3 blockade did not show any efficacy, metergoline and mianserine facilitate aversive stimulation (Jenck et al. (1989a,1989b)).

1.6.5. Short comment on extinction of conflict behaviour.

The adjusted Geller-Seifter conflict test without foot-shock punishment in the test situation was used as the procedure for extinction of conflict behaviour (see method sections). It has been shown by Cook and Davidson (1973) that FR 1 responding increases after omitting foot shock in the Geller-Seifter test. In untreated rats it takes three days before the original level of responses has been reached again. This process of returning to the pre-existing level of FR 1 responding may be explained as an "extinction of aversive experience", or as "extinction of conflict behaviour". This model is based on the tentative hypothesis that extinction of conflict behaviour of the rat may be close to the anxiety experienced by man after the occurrance of an aversive event. Therefore, extinction of conflict behaviour might be a model with predictive value for drugs which affect phobic and/or anticipatory anxiety symptoms in man (see paragraph 1.7.).

1.7. PANIC DISORDER

Panic disorder is an anxiety disorder that generally consists of two components: panic attacks and anticipatory anxiety. A case-history nearly always starts with a spontaneous panic attack in persons who feel quite well, although there are indications that an imminent threat of separation (e.g. from closely-related members of the family) triggers the first attack(s). The anticipatory anxiety is characterized by the fear of having (another) panic attack. Along with this, the patients develop a general chronic increase in the level of anxiety, which has the characteristics of a generalized anxiety disorder (Uhde et al. (1985)). In addition, phobic avoidance (agoraphobia), which can be described as a fear for particular places at which no help will be available in case of a sudden incapacitation, often accompanies the panic disorder. The exact etiology and pathogenesis of an attack has not been clarified yet. There seems to be a genetic predisposition for developing panic disorder (Crowe et al. (1980), Torgersen (1983)). In addition, several methods to provoke panic-like anxiety have been described, e.g. lactate infusion (Pitts and McClure (1967)), caffeine (Boulenger et al. (1984)), yohimbine or isoproterenol administration (Charney and Heninger (1985), Rainey et al. (1984)), mCPP or other compounds that increase postsynaptic 5-HT function (Kahn and Westenberg (1985), Kahn et al. (1988)), and CO_2 administration (Van den Hout and Griez (1984)). These observations formed the basis for several hypotheses about the pathogenesis of panic disorder, of which the serotonergic and noradrenergic theory seem the most attractive ones (see also discussion and appendix).

It is postulated by Klein (1964) that patients couple the environment in which the panic attacks occurred to the cause of the panic attack itself. In this way, phobic avoidance behaviour develops secondary to the panic attacks and might be considered as a learned response to the aversive event which is represented by the panic attack. This concept is supported by a large number of investigations but is still a matter of contradiction since several reports seem difficult to fit in with this hypothesis: Firstly, the existence of a very small group of patients with phobic avoidance behaviour without concomitant panic attacks. It is possible however that these patients have a very adequate phobic avoidance and/or still show phobic avoidance after a long forgotten panic attack (Marks (1987)). Secondly: phobic behaviour (school-phobia and/or separation anxiety) in children shows a remarkable similarity with agoraphobia of adults (Perugi et al. (1988)). Although sometimes present in adolescents, panic attacks are generally absent in children. However, it is interesting to note that schoolphobic children often develop panic disorder at an older age and/or have parents with panic disorder. Thirdly: Phobic avoidance, generalized anxiety and hypochondriacal ideas are reported to be present before the onset of panic attacks (Fava et al. (1988). This is supported by a study of Thompson et al. (1989), who report an earlier onset of agoraphobia (low teens) than panic disorder (about 20).

Taken together, the present reports make it doubtful that phobic avoidance and general anxiety disorder occurring in patients suffering from panic disorder, are solely the result of panic attacks. It is not unlikely that at least a subgroup of the patients shows avoidance behaviour without a causal relationship with the (preceding) panic attacks.

As stated before (par. 1.4.4. and 1.5.1.), theories concerning the neurochemical mechanism behind the pathofysiology of panic disorder mainly concern the function of the 5-HT system and/or the noradrenergic system in

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Allikmets and Rago (1988) abstract Capo Boi conference

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CHAPTER II

GABA-B RECEPTOR ACTIVATION AND CONFLICT BEHAVIOUR.

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Summary

Baclofen and oxazepam enhance extinction of conflict behaviour in the Geller-Seifter test while baclofen and diazepam release punished behaviour in Vogel's conflict test. In order to investigate the possibility that the effect of the selective GABA-B receptor agonist baclofen is mediated indirectly via the GABA-A/benzodiazepine receptor complex, the effect of pretreatment of rats with baclofen on [^{3}H]-diazepam binding to washed and unwashed cortical and cerebellar membranes of rats has been studied. Baclofen pretreatment increased Bmax in washed cerebellar membranes when bicuculline was present in the incubation mixture. No effect was seen in cortical membranes. The present results render it unlikely that the effect of baclofen on extinction of conflict behaviour and punished drinking is mediated via the GABA-A/benzodiazepine receptor complex.

Introduction

The receptors for τ-aminobutyric acid (GABA) and the benzodiazepines in the central nervous system are closely associated (1,2,3,4). The extent in which the anxiolytic, anti-convulsant, sedative and muscle relaxant effects of the benzodiazepines are mediated via the GABA-ergic system has been studied extensively. The results of these investigations are more equivocal concerning anxiolysis than for the other effects (5,6,7,8).
GABA receptors in rat brain are subdivided according to their sensitivity to the GABA receptor antagonist bicuculline and the GABA-analog baclofen (β-(p-chlorophenyl)-GABA). GABA-A receptors are sensitive to bicuculline and not to baclofen, while GABA-B receptors are sensitive to baclofen and not to bicuculline (9,10). Although evidence exists that the GABA-benzodiazepine receptor interaction **in vitro** applies only to the GABA-A receptor and not to the GABA-B receptor (11,12), it is still conceivable that activation of the GABA-B receptor **in vivo** may affect GABA-benzodiazepine receptor interaction. Thus it has been reported that GABA-B receptor activation increases release of GABA (13,14), which may subsequently increase the affinity of benzodiazepine receptors (1,2). Other reports however state that baclofen does not affect or even decreases GABA release (15-18). Furthermore, it has been reported that GABA-B receptor activation **in vivo** increased benzodiazepine receptor binding **in vitro** (19,20).
On the behavioural level, baclofen predominantly has muscle relaxant, sedative and anti-convulsant properties (21,22). Baclofen as well as the benzodiazepines have been shown to interfere with aquisition of a conditioned emotional response (23,24). In contrast with the benzodiazepines, baclofen has

no anti-conflict effect in the Geller-Seifter test (25). However, the results of only one type of test should not be taken as decisive evidence. It is also possible to study effects of drugs on the behaviour of rats in the Geller-Seifter test in the absence of foot shock (26). So far, effects of benzodiazepines and other drugs on the extinction of conflict behaviour have not been studied.
In the present studies it was shown that baclofen and a benzodiazepine increased extinction of conflict behaviour and had an anti-conflict effect in Vogel's conflict test.
To establish whether baclofen exerts its action via the GABA-A/benzodiazepine receptor complex, the effect of baclofen on benzodiazepine receptor binding was studied.

Methods

Behavioural studies.
In order to study extinction of conflict behaviour, the Geller-Seifter test (27) was used with some small modifications. The effects of baclofen and oxazepam were measured separately in two groups of 12 rats.
Procedure: After being deprived of water for 16 hours, male Wistar rats (starting weight: 80 g, training was completed at 300 ± 50 g) were placed in an experimental chamber measuring 20 x 20 x 20 cm, containing a response lever, milk delivery apparatus, an electrifiable grid floor and a stimulus light. The rats were trained to press a lever in order to obtain milk and were subsequently trained once a day for a duration of 42 minutes. In these training periods, two schedules were used in alternation. In 6 periods of 5 minutes a 5 % chance of reward was offered, resulting in a random interval schedule. The 5 % chance intervals were generated with the aid of a random generator based on electric noise. In 6 periods of 2 minutes a fixed ratio 1 (FR 1) schedule was used in which a steady light signal served as a discriminative stimulus. After stabilization of performance, response-contingent foot shock was given during the FR 1 periods. The shock level was individually adjusted until cumulative lever pressings during the FR 1 periods were below 5 per 12 minutes, which is about 5 % of the number of lever pressings before foot shock was applied. Extinction of conflict behaviour was measured on test days using the same set-up, except that foot shock was turned off. Between testdays training continued, and shock levels were adjusted when the animals took more than 5 shocks per 12 minutes. After 20 training sessions, spontaneous extinction was virtually non-existent in saline-injected rats ; i.e. these animals responded with an average of 2 to 5 pressings per 12 minutes. Drug effects on extinction of conflict behaviour were measured each time after at least 2 days of training in order to achieve a stable "base line" of conflict behaviour. Oxazepam was used in doses that did not release punished behaviour in the presence of foot shock. Drugs were administered once a week to allow for a sufficiently long wash out period.
In order to study punished licking, Vogel's conflict test was used (28) with some modifications, as described by Wren et al. (29). Male Wistar rats (170-250 g) were only allowed to drink for 45 min per day 2 hours after the test. After two days of acclimatization sessions in which the rats accustom to drinking from the waterspout, the third day a shock (1 mA) was delivered through the spout every 20th lick. The number of licks per 15 min were counted. Baclofen was tested in comparison with diazepam.
Drugs: All drugs used in the extinction experiments were administered intra-peritoneally in a volume of 1 ml/kg, 20 minutes before the behavioural

experiments started. (±)-Baclofen was dissolved in saline, oxazepam was injected as a sonicated suspension in distilled water with a drop of Tween 80 added. Haloperidol was dissolved in acidified saline (pH 5), other drugs were dissolved in a saline/propyleneglycol 1:1 mixture. In Vogel's conflict test, drugs were administered subcutaneously 45 min before the behavioural experiment. Baclofen was dissolved in a saline/polypropyleneglycol 9:1 mixture, while diazepam was administered as a suspension in a mulgofen 5 % -saline mixture. The control rats were injected with the saline/poly-propyleneglycol vehicle.

Binding studies. Baclofen pretreatment: The effect of pretreatment of rats with baclofen on [^{3}H]-diazepam binding, using a brain membrane preparation, was assessed by comparing baclofen with saline injected rats. Doses of 1.5 or 3 mg/kg of baclofen in a volume of 1 ml/kg were administered intraperitoneally 30 min before decapitation.

Preparation of rat brain membranes: Male Wistar rats (200-250 g) were decapitated and brains were dissected immediately. Cortex and cerebellum were isolated and stored at -70°C. Homogenization was carried out 1 to 4 days later. The cortex of each rat was thawed and homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose, using a glass-teflon homogenizer (clearance 0.15 mm, 5 strokes). The suspension was centrifuged for 10 min at 1000 x g. The resulting supernatant was divided over 2 centrifuge tubes and was centrifuged again for 20 min at 40.000 x g. Of the resulting two membrane pellets, one was immediately stored at -70°C and will be referred to as the unwashed cortical membrane pellet. The other pellet was resuspended and washed 4 times with 10 volumes (v/v) of 50 mM Tris-HCl buffer (pH:7.4) before storage at -70°C and will be referred to as the washed cortical membrane pellet. The homogenization procedure for the cerebellar tissue is actually the same as described for the cortical tissue, with the exception that the supernatant was not divided into two portions, so that one pellet per cerebellum was obtained after the first centrifugation.

Binding assay: Membrane pellets were thawed and resuspended in a 50 mM Tris-HCl buffer (pH:7.4). Binding studies were performed using 5 concentrations of [^{3}H]-diazepam (specific activity: 90 Ci/mmole, Amersham) ranging from 2.5 to 20 nM. Incubation was performed at 4°C for 30 min in a total volume of 1 ml. Specific [^{3}H]-diazepam binding was determined as the difference in membrane bound radioactivity measured in the absence and presence of 20 µM unlabelled diazepam. Incubation was terminated by rapid filtration through Whatman GF/C glass fiber filters. Filters were washed 4 times with 3 ml of the Tris-HCl buffer and subsequently transferred to scintillation vials containing 7 ml of scintillator 299^{tm} (Packard). Vials were kept overnight at room temperature. The next day radioactivity was measured in a TRI-CARB Scintillation Spectrometer with an efficiency of 32 %. Kd and Bmax were calculated from Scatchard plots (30) with the aid of the computerized program "LIGAND" (31). Protein concentrations of the membrane suspensions per incubation tube were 250-350 µg/ml for cerebellar membranes and 500-800 µg/ml for cortical membranes and were determined according to Bradford (32).

Statistical analysis. Data were analysed using Student's t-test, parametric or non-parametric one way analysis of variance (ANOVA) followed by the Student-Newman-Keuls'-test (SNK) or Mann-Whitney U test and Wilcoxon's signed rank test.

Results

Behavioural studies:

Intraperitoneal injection of baclofen or oxazepam resulted in a dose dependent increase of extinction of conflict behaviour (Fig. 1A). From the log dose-response curve, it appears that baclofen is about twice as active as oxazepam in enhancing the rate of extinction. Baclofen and diazepam both have an anti-conflict effect in Vogel's conflict test (Table I). Baclofen, oxazepam and diazepam did not affect responding during unpunished periods in both tests, with the exception of 2.5 mg/kg baclofen, where a decrease of responding occurred in the extinction experiments (Fig. 1B). Higher doses of baclofen (data not shown) led to muscle relaxation and sedation in both tests which interfered with responding.

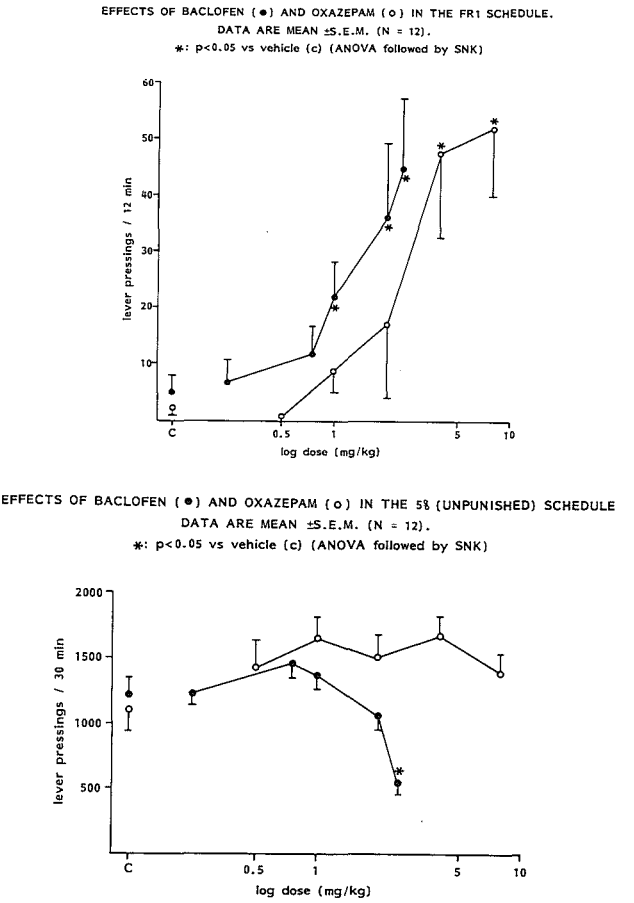

FIG. 1

A: Effects of baclofen (●) and oxazepam (o) in the FR 1 schedule. B: Effects of baclofen (●) and oxazepam (o) in the 5% (unpunished) schedule. Data are means ± s.e.m. (N = 12). * : $P < 0.05$ vs vehicle (c) (ANOVA followed by SNK).

Table I
Effects of Baclofen and Diazepam in Vogel's Conflict Test.

Drug	Dose (mg/kg)	n	Number of licks per rat per 15 min punished	n	unpunished
Vehicle		(20)	76 ± 12	(20)	1100 ± 65
Diazepam	0.46	(10)	215 ± 108*	(10)	1309 ± 78
Diazepam	1.00	(10)	485 ± 134*	(9)	1139 ± 109
Diazepam	4.60	(10)	829 ± 188*	(10)	1319 ± 70
Baclofen	0.46	(10)	158 ± 33*	(10)	1008 ± 80
Baclofen	1.00	(10)	217 ± 61*	(9)	1281 ± 113

* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test).

TABLE II
Extinction of Conflict Behaviour.

	Dose (mg/kg)	5 % schedule	FR 1 schedule	n
Neuroleptics.				
Haloperidol	0.0	1043 ± 146	2.7 ± 0.5	(6)
	0.1	1158 ± 121	2.5 ± 0.4	(6)
	0.3	859 ± 172	8.7 ± 1.7*	(6)
	0.5	1198 ± 148	21.8 ± 3.9*	(6)
	1.0	55 ± 20	0.6 ± 0.4*	(6)
Chlorpromazine	0.0	963 ± 57	1.3 ± 0.3	(4)
	3.0	970 ± 56	2.3 ± 0.6	(4)
Thioridazine	0.0	909 ± 134	0.8 ± 0.5	(4)
	5.0	520 ± 131	0.8 ± 0.3	(4)
Antidepressants.				
Imipramine	0.0	1202 ± 141	1.3 ± 0.5	(4)
	10.0	628 ± 40	2.3 ± 0.9	(4)
Amitryptiline	0.0	1290 ± 114	2.8 ± 1.0	(8)
	1.0	890 ± 101	4.1 ± 1.2	(8)
Protryptiline	0.0	1290 ± 114	2.8 ± 1.0	(8)
	0.05	1217 ± 122	3.4 ± 0.7	(8)
	0.1	830 ± 104	13.8 ± 5.4	(8)
	0.5	1043 ± 95	5.4 ± 1.9	(8)
	1.0	657 ± 98	13.5 ± 3.5*	(8)
Mianserine	0.0	1396 ± 191	1.5 ± 0.3	(4)
	1.0	552 ± 140	0.7 ± 0.3	(3)

*: $P < 0.05$ vs vehicle. (Wilcoxon's signed rank test).

In order to test the specificity of the effect of baclofen on extinction of conflict behaviour, effects of several other psycho-active drugs were tested. Each drug was tested in 3 to 4 doses of which the maximal dose is shown in Table II. The results indicated that none of the neuroleptics or anti-depressants, with the exception of haloperidol and protryptiline had a significant effect on extinction. The doses of haloperidol used, had no effect on punished behaviour in the presence of foot shock. Protryptiline was not tested in the latter situation.

Binding studies:

Binding was determined in unwashed and washed membranes to investigate a possible effect of a baclofen induced change in the level of GABA or another factor **in vitro**. In one series of experiments (±)-bicuculline was added to the incubation mixture (concentration per incubation tube: 100 µM) in order to prevent GABA-A mediated effects on the benzodiazepine receptor (1,2). As shown in Table III, washing of the cortical membranes significantly increased Kd as well as Bmax of the benzodiazepine receptors. The presence of bicuculline did not affect Bmax significantly, while Kd increased in washed as well as unwashed membranes to a value of about 15 nM. Pretreatment of the rats with baclofen was without effect on Bmax and Kd.

In analogy with the cortical tissue, the Kd and the Bmax of cerebellar membranes for diazepam were also significantly increased after washing (Table IV). However, in these preparations bicuculline did not further increase the Kd of washed membranes. Another difference with cortical tissue, was that bicuculline addition decreased the Bmax significantly. A third difference was the small but significant increase of Bmax in washed, bicuculline containing membrane preparations, obtained from brains of baclofen pretreated rats.

Incubation of washed and unwashed cortical and cerebellar membranes in the presence of baclofen (25-400 µM) did not affect [^{3}H]-diazepam binding (data not shown).

TABLE III

[^{3}H]-Diazepam Binding to Cortical Membranes.

				+ BICUCULLINE.		
bacl. mg/kg	Kd:	Bmax:		Kd:	Bmax:	
UNWASHED						
0	6.1 ± 0.2	0.92 ± 0.03	(8)	15.9 ± 1.1**	0.89 ± 0.02	(4)
1.5	6.6 ± 0.3	0.94 ± 0.03	(8)	15.5 ± 1.7**	0.94 ± 0.05	(4)
3	5.4 ± 0.5	0.86 ± 0.04	(4)	14.3 ± 0.4**	0.91 ± 0.03	(4)
WASHED						
0	9.3 ± 0.5*	1.41 ± 0.06*	(6)	14.3 ± 0.5**	1.25 ± 0.05*	(4)
1.5	11.3 ± 1.9*	1.46 ± 0.04*	(4)	15.4 ± 1.4	1.34 ± 0.03*	(4)
3	7.9 ± 0.7*	1.31 ± 0.09*	(4)	13.3 ± 0.5**	1.28 ± 0.03*	(4)

Kd: nM. Bmax: pmol/mg protein. Bicuculline conc.: 100 µM.
Number of animals are given in parentheses.
* : $P < 0.05$ vs unwashed (Student's t-test).
** : $P < 0.05$ vs without bicuculline addition (Student's t-test).

TABLE IV

[^{3}H]-Diazepam Binding to Cerebellar Membranes.

bacl. mg/kg	Kd:	Bmax:		+ BICUCULLINE. Kd:	Bmax:	
UNWASHED						
0	4.8 ± 0.3	0.70 ± 0.03	(8)	15.0 ± 1.8**	0.61 ± 0.01**	(4)
1.5	5.9 ± 0.2	0.70 ± 0.02	(4)	14.4 ± 1.4**	0.64 ± 0.06	(4)
3	5.4 ± 0.4	0.75 ± 0.03	(4)	13.7 ± 1.8**	0.61 ± 0.03**	(4)
WASHED						
0	15.2 ± 1.6*	0.99 ± 0.06*	(7)	12.1 ± 1.0	0.81 ± 0.01**	(4)
1.5	13.5 ± 1.7*	1.04 ± 0.02*	(4)	15.0 ± 1.2	0.92 ± 0.04**+	(4)
3	14.2 ± 1.4*	0.99 ± 0.03*	(4)	15.4 ± 0.9	0.93 ± 0.04+	(4)

Kd: nM. Bmax: pmol/mg protein. Bicuculline conc.: 100 µM.
Number of animals are given in parentheses.
\+ : $P < 0.05$ vs saline (ANOVA followed by SNK).
* : $P < 0.05$ vs unwashed (Student's t-test).
** : $P < 0.05$ vs without bicuculline addition (Student's t-test).

Discussion

It has been shown by Quintero et al. (25,33,34) and Buckland et al. (35) that baclofen affects behaviour of rats in several tests in a complicated manner, suggesting anxiolytic but also anxiogenic activity.
The present results show that administration of baclofen to rats enhanced extinction of conflict behaviour in a dose dependent manner. Baclofen did not affect punished behaviour when foot shock was activated in the Geller-Seifter test (unpublished results), which is in agreement with the results obtained by Quintero et al. (25). However, the dose range of baclofen used had to be limited because doses above 2.5 mg/kg caused sedation and paresis of the hind legs and thus interfered with responding. Low doses of oxazepam (without effect on punished behaviour in the Geller-Seifter test), were used in the extinction experiments. In this dose range oxazepam enhanced extinction of conflict behaviour in a dose dependent manner. Baclofen seemed to be about twice as active as oxazepam in enhancing extinction. It is however difficult to state this with certainty since baclofen was administered as a solution while oxazepam was administered as a suspension. Observation of the animals during the tests on extinction of conflict behaviour revealed that after presentation of the discriminative stimulus, the rats displayed "conflict behaviour" before their first lever pressing, i.e. they approached to- and backed away from- the lever several times without pushing it. Saline injected rats only sporadically pushed the lever during the test session, while baclofen and oxazepam treated rats showed extinction of conflict behaviour after a short response latency. Once the first responses were made, the rate of lever pressings accelerated.
In order to further evaluate the effect of baclofen on punished behaviour, Vogel's conflict test was used. In contrast with the Geller-Seifter conflict

test, baclofen displayed anti-conflict activity. The results obtained with control (unshocked) rats in the latter test indicate that baclofen affected only conflict behaviour, which makes it unlikely that an increased locomotor activity or a dipsogenic effect is playing a role in the observed effects. In order to show the specificity of a drug-induced increase of extinction of conflict behaviour, other psycho-active drugs were tested. Of the neuroleptics only haloperidol had an effect comparable to baclofen. Of the anti-depressants tested, only protryptiline increased extinction. Haloperidol (0.1-1.0 mg/kg) did not affect responding when foot shock was activated (data not shown) as has also been shown previously by Cook and Davidson (27). Protryptiline was not tested in the latter situation. Therefore the effect of baclofen on extinction of conflict behaviour was rather specific since anti-depressants and neuroleptics were ineffective, while a similarity in effect exists between baclofen and oxazepam. A second similarity in effect between baclofen and a benzodiazepine is observed in Vogel's conflict test. If extinction of conflict behaviour and an increase in punished licking form aspects of the anxiolytic activity of the benzodiazepines, one may suggest that also GABA-B receptor activation will have anxiolytic activity.

The behavioural results obtained may be explained by the following actions: (a) baclofen and the benzodiazepines both act via the GABA-A/benzodiazepine receptor/chloride channel complex, (b) baclofen and the benzodiazepines both act via the GABA-B system, or (c) the mode of action is different for both drugs. Considering the first possibility, it has been shown that in contrast with activation of the GABA-A receptor, GABA-B receptor activation **in vitro** does not increase the affinity of benzodiazepine receptors. (11,12). Also under our experimental conditions baclofen (25-400 µM) did not affect Kd or Bmax of [^{3}H]-diazepam binding (data not shown). Thus **in vitro** experiments do not support a direct effect of baclofen on the GABA-A/benzodiazepine receptor/chloride channel complex. Although the evidence is equivocal, there are indications that baclofen may cause GABA release (13,14) and this may explain the common effect of the benzodiazepines and baclofen on conflict behaviour. One therefore may expect that **in vivo** administration of behaviourally active doses of baclofen will increase the affinity of benzodiazepine receptors **in vitro** by increasing GABA release **in vivo**. However, no effect on Kd and Bmax of [^{3}H]-diazepam binding in unwashed cortical and cerebellar membranes, obtained from baclofen pretreated rats, could be shown and therefore no support for an enhanced release of GABA **in vivo** by baclofen was obtained.

The increase in Kd in washed preparations and in the presence of the GABA-A antagonist bicuculline is probably due to the partial removal of GABA from the membrane preparations (36). This interpretation is supported by bicuculline's effect on the affinity of diazepam for the benzodiazepine receptor in crude membrane preparations (37). The significant decrease in affinity in 4x washed cortical membranes as compared to unwashed membranes is similar to that found by Chiu and Rosenberg (37). This effect has been well documented (2).

While pretreatment of rats with baclofen did not affect [^{3}H]-diazepam binding in a cortical membrane preparation, in cerebellar membranes a small but significant Bmax increase was found using washed membrane preparations incubated in the presence of bicuculline. These results suggest that baclofen is able to increase the Bmax of benzodiazepine receptors probably in a limited number of sites in cerebellar tissue since the increase was small (13 %). Furthermore, only after inactivation of the GABA-A receptor a GABA-B interaction on benzodiazepine receptor sites could be demonstrated and, secondly, the increase in Bmax could only be shown after washing and not in an

unwashed preparation in the presence of bicuculline. The mechanism behind this effect of baclofen remains to be established.
The increase in Bmax observed in cortical and cerebellar membranes after washing with Tris buffer has been reported previously (37,38). The removal of a diazepam binding inhibitor, possibly acting as an endogenous ligand for the benzodiazepine receptor has been suggested as one of the mechanisms responsible for this effect (39-45). Therefore the effect of baclofen treatment on Bmax in washed cerebellar membrane preparations and the absence of effect in unwashed preparations indicates an opposite effect of baclofen treatment and the putative endogenous ligand; removing the binding inhibitor will unmask the effect of baclofen. But it is, however, unlikely that the behavioural effects of baclofen can be ascribed to the mechanisms discussed above.
The second possibility, a common effect of baclofen and benzodiazepines via the GABA-B system, is also not likely as the benzodiazepines enhance the effects of the neurotransmitter GABA at the level of the GABA-A/benzodiazepine receptor complex, and not via altered GABA release, uptake or catabolism. Thus, benzodiazepines do not affect the level of GABA-B receptor activation in an indirect way (review: 46). In addition, it has been reported that benzodiazepines do not affect binding of GABA to the GABA-B receptor (11).
Final proof for the non-involvement of the GABA-A/benzodiazepine receptor complex has to come from behavioural experiments investigating whether GABA-A/benzodiazepine receptor antagonists affect the observed baclofen effects. Finally, it is possible that benzodiazepines and baclofen both affect the function of another neurotransmitter involved in punished behaviour. Among the possible candidates are the serotonergic and dopaminergic systems, which are both related to the GABA-A/benzodiazepine receptor complex and the GABA-B receptor and are involved in behavioural suppression induced by aversive stimuli (47-50).

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CHAPTER III

THE ANTI-CONFLICT EFFECT OF CYPROHEPTADINE IS NOT MEDIATED BY ITS 5-HYDROXYTRYPTAMINE ANTAGONISTIC PROPERTY.

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Summary

Cyproheptadine, a $5HT_2$ receptor antagonist with prominent anti-muscarinic and anti-histaminic properties, was shown to have an anti-conflict effect in rats using a modified Geller-Seifter test and also enhanced extinction of conflict behaviour. The selective $5HT_2$ receptor antagonist ritanserin, however, had neither an anti-conflict effect nor an effect on extinction of conflict behaviour. The muscarinic receptor antagonist scopolamine, on the other hand, was active in both paradigms. The effect of cyproheptadine on extinction of conflict behaviour was decreased by co-administration of physostigmine, an acetylcholinesterase inhibitor, but not affected by the concomitant administration of the muscarine receptor agonist oxotremorine. The results suggest that the anti-conflict effect of cyproheptadine has to be ascribed to its anti-muscarinic activity and is not due to its $5HT_2$ antagonism.

Introduction

The action of benzodiazepines in rat conflict models has been ascribed to a facilitation of the GABA-ergic system (1). In addition, it has been suggested that this anti-conflict effect occurs via modulation of the serotonergic (5HT) system (2). In agreement with this, it has been shown that activation of the GABA-A/benzodiazepine receptor complex exerts an inhibitory effect on 5HT neurones (3). Investigations using drugs that alter 5HT activity, have indeed been shown to change behaviour in animal anxiety models (4,5). Cyproheptadine has been shown to be active in animal studies on conflict behaviour (6,7,8,9,10,11). This effect has been ascribed to its 5HT antagonistic property (7,8,9,10,11).

As proposed by Bradley et al (12), central 5HT receptors can be subdivided into $5HT_{1A}$, $_B$ and $_C$, $5HT_2$ and $5HT_3$ receptors. Cyproheptadine has $5HT_2$, muscarine and histamine blocking properties (13,14,15). The present availability of the highly selective $5HT_2$ antagonist ritanserin (16,17) makes it now possible to compare the behavioural effects of cyproheptadine with those of ritanserin in order to relate the effects of cyproheptadine to its $5HT_2$ blocking action or to one of its other properties.

Two aspects of conflict behaviour were studied; anti-conflict effect, (i.e. increase of punished (foot shock) responding) and extinction of conflict behaviour, (i.e. responding after disconnection of the foot shock generator (18)). Extinction of conflict behaviour was chosen as an additional model for detecting anxiolytic activity of drugs. This model is based on the idea that anxiety neurosis in patients often occurs without the presence of a conflict situation. It was therefore assumed that these patients may suffer from an impaired extinction of a previously experienced anxiety-provoking event. As

shown previously, extinction of rat conflict behaviour has been shown to be a more sensitive behavioural test for detecting drugs with anxiolytic properties than conflict behaviour itself (18).

Methods

In order to study conflict behaviour the Geller-Seifter test (19) was used as has been described previously (18,20). In brief: After being deprived of water for 16 hours, male Wistar rats (starting weight: 70 - 90 g; after training was completed: 250 - 350 g) were placed in an experimental chamber measuring 20 x 20 x 20 cm, containing a response lever, milk delivery apparatus, an electrifiable grid floor and a stimulus light. The rats were trained to press a lever in order to obtain milk and were subsequently trained once a day for a duration of 42 minutes. Within these training (and test) periods, two schedules were used in alternation. During 5 minutes a 5 % random interval (RI) schedule was offered. This schedule was alternated with 2 minutes of a fixed ratio 1 (FR 1) schedule. A steady light signal during the FR 1 schedule served as the stimulus by which the animals were able to discriminate between both schedules. This combination of RI and FR 1 schedules was presented 6 times to each rat per session. After stabilization of performance, response-contingent foot shock was given during the FR 1 periods. Shock levels (100-160 V, 2 - 3.3 mA) were individually adjusted until cumulative lever pressings during the FR 1 periods were below 5 per 12 minutes, which represents about 5 % of the number of lever pressings before foot shock was applied. Drug effects on conflict behaviour were measured each time after at least 2 days of training in order to achieve a stable "base line" level of conflict behaviour. Extinction of conflict behaviour was measured in the same way using the same procedure except that foot shock was turned off. Between test days training continued in the presence of foot shock, and shock levels were adjusted when the animals took more than 5 shocks per 12 minutes. After at least 20 training sessions, spontaneous extinction was virtually non-existent in control rats during the trial period; i.e. these animals responded with an average of 0 to 5 pressings per 12 minutes. Four groups of 12 rats were used. Two groups were used in the conflict test and two in the extinction experiments. Vehicle and different doses of each drug were administered to each rat of a group using a latin square design. The effects of drugs were tested once a week to allow for a sufficiently long wash out period.

Drugs: All drugs used were administered intraperitoneally in a volume of 1 ml/kg. Cyproheptadine-HCl (MSD), scopolamine-HBr (Sigma), oxotremorine-sesquifumarate (Aldrich) and physostigmine-salicylate (Sandoz) were dissolved in saline and injected 10 minutes before the behavioural experiments started. Doses are expressed as the salt in mg/kg. Oxazepam and ritanserin were administered 20 minutes before the experiment as a sonicated suspension in distilled water with a drop of Tween 80 added. Doses were expressed as the base in mg/kg. Oxazepam was kindly donated by Wyeth, ritanserin was kindly donated by Janssen Pharmaceutica.

Statistical analysis. Data were analysed using Student's t-test or by parametric or non-parametric one way analysis of variance (ANOVA) followed by Student-Newman-Keuls'-test or the Mann-Whitney U test respectively.

Results

Administration of 7 or 10 mg/kg cyproheptadine to the rats resulted in an increase in responding during the FR 1 schedules in both conflict and extinction tests (Table I). No effect occurred on the level of responding during the random interval schedule.
When ritanserin (20 mg/kg i.p.) was used instead of cyproheptadine, no effect could be found in both behavioural tests (Table II). Lower doses of ritanserin (2.5 and 10 mg/kg i.p.) were also ineffective as well as an oral dose of 10 mg/kg (data not shown). Oxazepam (15 mg/kg) was active under both conditions.

TABLE I
Effects of Cyproheptadine on Conflict Behaviour.

		Conflict behaviour			Extinction of conflict behaviour		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n	RI schedule	FR 1 schedule	n
	0.0	991 ± 299	0.6 ± 0.2	(7)	994 ± 175	12.4 ± 7.8	(7)
Cyproh	7.0	1384 ± 334	2.4 ± 0.7*	(7)	1057 ± 221	49.4 ± 13.3*	(7)
	0.0	1225 ± 145	1.3 ± 0.2	(12)	1381 ± 180	27.5 ± 10.6	(12)
Cyproh	10.0	1164 ± 199	5.2 ± 0.7*	(12)	964 ± 231	70.3 ± 9.8*	(12)

*: $P < 0.05$ vs vehicle (Student's t test). Cumulative level of responding per session during random interval (RI) and fixed ratio 1 (FR 1) schedule per 30 and 12 min respectively. Data are mean ± s.e.m.

TABLE II
Effects of Oxazepam and Ritanserin on Conflict Behaviour.

		Conflict behaviour			Extinction of conflict behaviour		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n	RI schedule	FR 1 schedule	n
	0.0	1893 ± 149	1.1 ± 0.4	(8)	1871 ± 215	16.5 ± 10.8	(8)
Oxaz	15.0	1792 ± 195	9.1 ± 5.4*	(8)	1660 ± 157	165.0 ± 40.7*	(8)
Ritans	20.0	1522 ± 126	2.0 ± 0.4	(8)	1616 ± 154	2.5 ± 0.8	(8)

* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). Cumulative level of responding per session during RI and FR 1 schedule per 30 and 12 min respectively. Data are mean ± s.e.m.

Scopolamine, a muscarinic receptor antagonist, was administered to rats and its effect on conflict behaviour recorded. As shown in table III, scopolamine had an anti-conflict effect and enhanced extinction of conflict behaviour. The highest dose of scopolamine (0.2 mg/kg) also caused a significant decrease of responding during the random interval schedule. Gross behavioural disturbances were not observed.
In order to see whether an increased availability of acetylcholine could antagonize the effect of cyproheptadine, the acetylcholinesterase inhibitor physostigmine was administered in a dose of 0.25 mg/kg immediately followed by

cyproheptadine. The drugs were administered 10 minutes before the start of the experiment. The effect of cyproheptadine on extinction of conflict behaviour was counteracted when the rats were treated simultaneously with physostigmine, while the number of responses during the random interval schedule was not affected (Table IV). Administration of physostigmine alone resulted in a significant decrease in the number of responses during FR 1 and RI schedules. Under this condition pronounced chewing and licking, mild tremor and occasional stretching was noted.

TABLE III
Effects of Scopolamine on Conflict Behaviour.

		Conflict behaviour			Extinction of conflict behaviour		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n	RI schedule	FR 1 schedule	n
Scopol	0.0	1095 ± 252	1.2 ± 0.4	(9)	1250 ± 267	1.0 ± 0.3	(5)
	0.05	1029 ± 332	2.9 ± 0.7	(7)	1444 ± 473	0.8 ± 0.6	(5)
	0.1	450 ± 110	2.9 ± 0.6*	(9)	1262 ± 465	8.4 ± 6.4	(5)
	0.2	285 ± 164*	9.3 ± 6.1*	(8)	284 ± 123	43.8 ± 12.7*	(5)

* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). Cumulative level of responding per session during RI and FR 1 schedule per 30 and 12 min respectively. Data are mean ± s.e.m.

TABLE IV
Effects of combined administration of Cyproheptadine and Physostigmine on Extinction of Conflict Behaviour.

		Extinction of conflict behaviour.		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n
Cyproheptadine	0.0	1152 ± 328	1.3 ± 0.7	(8)
	10.0	1190 ± 255	42.4 ± 12.2*	(8)
Cyproh/physos	10.0/0.25	872 ± 254	12.0 ± 6.2	(7)
Physostigmine	0.0	1216 ± 165	1.6 ± 0.4	(5)
	0.25	14 ± 10$	0.0 ± 0.0$	(5)

* : $P < 0.05$ vs vehicle and vs cyproheptadine/physostigmine (Kruskal-Wallis ANOVA followed by Mann-Whitney U test).
$: $P < 0.05$ vs vehicle (Student's t test).
Cumulative level of responding per session during RI and FR 1 schedule per 30 and 12 min respectively. Data are mean ± s.e.m.

Administration of 0.08 mg/kg oxotremorine, a muscarinic (M_2) agonist, increased responding during the FR 1 schedule in the conflict as well as in the extinction experiments while a significant decrease of responses during the random interval schedules occurred (Table V). After administration of 0.08

mg/kg oxotremorine, the following behavioural signs were observed: flat body posture, abducted hindlimbs, piloerection, chewing, hyperlocomotion and tremors. Oxotremorine was not able to counteract the effect of cyproheptadine on extinction of conflict behaviour. However, the behavioural syndrom observed after oxotremorine (0.08 mg/kg) was not present when cyproheptadine was administered immediately after administration of oxotremorine.

TABLE V
Effects of Oxotremorine on Conflict Behaviour.

		Conflict behaviour			Extinction of conflict behaviour		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n	RI schedule	FR 1 schedule	n
	0.0	1100 ± 274	1.3 ± 0.4	(10)	975 ± 151	2.9 ± 1.5	(9)
Oxotr	0.04	613 ± 153	2.0 ± 0.5	(10)	476 ± 214	30.2 ± 12.1	(9)
	0.08	24 ± 5*	3.7 ± 1.0*	(9)	80 ± 23*	38.8 ± 12.5*	(8)

* : $P < 0.05$ vs vehicle parametric one way ANOVA followed by Student-Newman-Keuls' test. Cumulative level of responding per session during RI and FR 1 schedule per 30 and 12 min respectively. Data are mean ± s.e.m.

TABLE VI
Effects of combined administration of Cyproheptadine and Oxotremorine on Extinction of Conflict Behaviour

		Extinction of conflict behaviour		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n
	0.0	1100 ± 280	0.7 ± 0.3	(6)
Cyproheptadine	10.0	555 ± 138	39.9 ± 15.1*	(8)
Cyproh/oxotr	10.0/0.04	854 ± 342	58.5 ± 19.0*	(6)
Cyproh/oxotr	10.0/0.08	848 ± 365	41.2 ± 12.0*	(5)
Cyproh/oxotr	10.0/0.16	610 ± 241	70.8 ± 18.2*	(4)

* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). Cumulative level of responding per session during RI and FR 1 schedule per 30 and 12 min respectively. Data are mean ± s.e.m.

Discussion

The effects of cyproheptadine observed in several animal models for anxiety have been ascribed to its 5HT receptor blocking action (7,8,9,10,11). Since at present more selective 5HT antagonists are available and the receptor binding profile of cyproheptadine has meanwhile been established, it is now possible to determine in a more definitive way whether the anti-conflict effect of cyproheptadine is caused by its $5HT_2$ antagonistic property. Behavioural experiments and receptor binding studies indicate that cypro-heptadine,

besides its $5HT_2$ antagonism, also blocks histamine and muscarinic receptors (13,14,15). The present results indicate that cyproheptadine, like benzodiazepines (18), released punished behaviour and increased extinction of conflict behaviour. However, this effect did not seem to be related to its $5HT_2$ receptor blocking property since the specific $5HT_2$ antagonist ritanserin was without effect. This failure of ritanserin to enhance extinction of conflict behaviour is in agreement with other reports showing that ritanserin, in doses comparable to those used in the present study, is not active in three other conflict paradigms (7,17,21). Binding data indicate that ritanserin is a highly selective ligand for 5HT2 receptors (16,17,22). It is reported that 0.63 mg/kg given subcutaneously results in a full occupation of $5HT_2$ receptors in the central nervous system, while doses up to 160 mg/kg (s.c.) do not affect binding of ligands to adrenoceptors or dopamine receptors (22). Although ritanserin has been reported to possess some affinity to the histamine (H_1) receptor, the IC50 of ritanserin for this receptor is 35 times higher (35 nM) than that for the $5HT_2$ (1 nM) receptor (22). In the present experiments, ritanserin was used in a dose (20 mg/kg) which blocks $5HT_2$ receptors completely (22), and which is unlikely to affect H_1 receptors. The results therefore indicate that the $5HT_2$ blocking property of cyproheptadine can not be held responsible for the anti-conflict effects. This conclusion is supported by the lack of effect of mianserin, another $5HT_2$ antagonist, on extinction of conflict behaviour (18).

The doses of cyproheptadine used in the present experiments make it very unlikely that the weak histamine receptor blocking property of this compound is responsible for the anti-conflict effect. This inference is supported by Graeff (11) who was not able to demonstrate the release of punished behaviour by a number of anti-histaminics.

The only property left to explain the anti-conflict effects of cyproheptadine is therefore its muscarinic receptor blocking property. Administration of scopolamine, a selective muscarinic receptor antagonist (23), was shown to increase responding during punishment and enhanced the extinction of conflict behaviour. These effects indeed point to the possibility that the anti-cholinergic property of cyproheptadine was responsible for the observed behavioural effect. However, in contrast to cyproheptadine, scopolamine increased responding during the FR 1 schedule at doses which decreased unpunished responding. Nevertheless, supporting evidence for a role of the muscarinic receptor blocking property of cyproheptadine was obtained by co-administration of the cholinesterase inhibitor physostigmine which counteracted the enhanced extinction of conflict behaviour evoked by cyproheptadine. It is well established that muscarinic receptor subtypes M_1 and M_2 exist (24). Muscarinic receptor antagonists generally are non-selective or have been shown to act preferentially as M_1 antagonists (25). Since the muscarinic agonist oxotremorine preferentially binds to M_2 receptors which in part may function as autoreceptors on cholinergic nerve fibers (25), it is conceivable that oxotremorine will impair acetylcholine release. This mechanism may explain the results of oxotremorine on the release of punished behaviour and on the increased extinction of conflict behaviour. The M_1 blocking activity of cyproheptadine and the decreased release of acetylcholine by the M_2 agonist oxotremorine are both responsible for an impaired cholinergic transmission which may thus explain the observed anti-conflict effects of both drugs. However, in contrast to cyproheptadine, administration of oxotremorine induced a number of cholinergic effects (tremor, chewing and piloerection) which were not seen after the admini-stration of cyproheptadine. The finding that these effects of oxotremorine did not occur after the

concomitant administration of cyproheptadine suggest that these cholinergic effects of oxotremorine may be caused by its M_1 agonistic action. However, further experiments are needed to support this latter explanation.
In conclusion, the present results indicate that the anti-conflict effects of cyproheptadine can not be ascribed to its well known $5HT_2$ antagonistic property but seem to be the result of its M_1 blocking activity.

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CHAPTER IV

IMPAIRED 5-HT FUNCTION ENHANCES EXTINCTION OF CONFLICT BEHAVIOUR

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submitted for publication

Summary

In order to investigate whether a decreased function of the serotonergic (5-HT) system affects extinction of conflict behaviour in a way similar to baclofen, the effect of PCPA, 8-OH-DPAT, ICS 205-930 and propranolol on extinction was studied. PCPA, 8-OH-DPAT and propranolol enhanced extinction while ICS 205-930 was not effective. The selective β-adrenoceptor blockers butoxamine and metoprolol did not enhance extinction. The doses of propranolol which enhanced the extinction of conflict behaviour were in the same dose range as those used to inhibit the 5-HT syndrome induced by 8-OH-DPAT. In PCPA pretreated rats, baclofen and 8-OH-DPAT were not any more able to enhance extinction. The present results suggest that an impaired 5-HT system induced an enhancement of extinction of conflict behaviour and may be involved in the previously described effect of baclofen on extinction.

Introduction

The anti-conflict effect of the benzodiazepines has been ascribed to a facilitation of the GABA-ergic system (Haefely 1978; Sepinwall & Cook 1984; Quintero et al. 1985; Zakusov et al. 1977; Scheel-Kruger and Petersen 1982; Velluci and Webster 1984). Besides, it has been proposed that the benzodiazepines might affect conflict behaviour via modulation of the 5-HT system (Wise et al. 1972; Stein et al. 1973). Both these theories may be valid since activation of the $GABA_A$-benzodiazepine receptor chloride channel complex has been shown to exert an inhibitory effect on 5-HT neurons (Wise et al. 1972; Gallager 1978).

Baclofen has been shown to decrease the release of 5-HT from rat cortical tissue via activation of presynaptically located $GABA_B$ receptors (Schlicker et al. 1984; Gray and Green 1987). Furthermore, activation of the $GABA_B$ receptor and of the $5\text{-}HT_{1A}$ (auto)receptor have both been shown to inhibit the opening of a shared potassium-channel in the hippocampus and the raphe nuclei (Innis and Aghajanian 1987; Andrade et al. 1986).

However, in contrast to the benzodiazepines, baclofen does not cause an anti-conflict effect in the Geller-Seifter test, but both increase extinction of conflict behaviour (Ketelaars et al. 1988).

It is therefore conceivable that baclofen and the benzodiazepines both exert their effect on the extinction of conflict behaviour by a modulation of the 5-HT system. A reduction in 5-HT neurotransmission has been suggested to decrease anxiety while stimulation of the 5-HT system may evoke anxiety (see review by Chopin and Briley (1987). Several compounds which modulate the activity of the 5-HT system have indeed been shown to have an effect in some animal models for anxiety (Chopin and Briley 1987).

The aim of the present study was to investigate whether modulation of the 5-HT system affects extinction of conflict behaviour, an experimental situation which has been shown to be more sensitive than anti-conflict effect itself, and whether a relationship exists between the 5-HT system and baclofen with regard to this behaviour.

Methods

Conflict behaviour and extinction of conflict behaviour. In order to study conflict behaviour the Geller-Seifter test (Geller and Seifter 1960) was used as described previously (Ketelaars et al. 1988). In brief: After being deprived of water for 16 hours, male Wistar rats (starting weight: 70 - 90 g; after training was completed: 250 - 350 g) were placed in an experimental chamber measuring 20 x 20 x 20 cm, containing a response lever, milk delivery apparatus, an electrifiable grid floor and a stimulus light. The rats were trained to press a lever in order to obtain milk and were subsequently trained once a day for a duration of 42 minutes. Within these training (and test) periods, two schedules were used in alternation. During 5 minutes a 5 % random interval (RI) schedule was offered. This schedule was alternated with 2 minutes of a fixed ratio 1 (FR 1) schedule. A steady light signal during the FR 1 schedule served as the discriminative stimulus. This combination of RI and FR 1 schedules was presented 6 times to each rat per session. After stabilization of performance, response-contingent foot shock was given during the FR 1 periods. Shock levels (100-160 V, 2 - 3.3 mA) were individually adjusted until cumulative lever pressings during the FR 1 periods were below 5 per 12 minutes, which represents about 5 % of the number of lever pressings before foot shock was applied. Drug effects on conflict behaviour were measured on the third day, always after 2 days of training in order to achieve a stable "base line" level of conflict behaviour.
Drug effects on extinction of conflict behaviour were measured in the same way using the same procedure except that foot shock was turned off. During the 2 days before the test days training continued in the presence of foot shock, and shock levels were adjusted when the animals took more than 5 shocks per 12 minutes. After at least 20 training sessions, spontaneous extinction was non-existent in control rats during the trial period; i.e. these animals responded with an average of 0 to 5 pressings per 12 minutes.
Eight groups of 12 rats were used. Four groups were used in the conflict test and four in the extinction experiments. Vehicle and different doses of each drug were administered to each rat of a group using a latin square design. The effects of drugs were tested once a week to allow for a sufficiently long wash out period.

5-HT behaviour. The 5-HT behaviour was measured as described by Deakin and Green (1978) and Kennett et al. (1985). Male Wistar rats weighing 250 ± 50 grams were used. After administration of 1.0 mg/kg 8-OH-DPAT, 5-HT behaviour was evaluated after 6, 12, 18, 24 and 30 minutes. The four components of the syndrome were scored each time by counting piano playing and head weaving for a period of 1 minute per animal. Flat body posture and hind limb abduction were scored by assessment of intensity (0-1-2-3 for absent-just present-present-extreme). Propranolol was injected 25 minutes before administration of 8-OH-DPAT. The scorer was blind to the treatment.

Drugs. All drugs used were administered intraperitoneally in a volume of 1 ml/kg, except p-chlorophenylalanine (PCPA) which was administered in 2 ml/kg. 8-OH-DPAT was dissolved in saline and injected immediately before the start of the experiment, ICS 205-930, propranolol-HCl, butoxamine-HCl, metoprolol tartrate and baclofen were dissolved in saline and injected 20 minutes before the experiments started. PCPA was dissolved in saline (acidified with HCl and brought to pH 6 with NaOH) and administered 17 hours before the experiments. The time between treatment and test procedure was chosen in accordance with that reported by Koe and Weissman (1966), who have shown an 80 % decrease in brain 5-HT level after administration of comparable doses of PCPA. It was not possible to test at the time of the maximum effect of PCPA (90 % decrease after 72 hours), because the PCPA treatment might then interfere with the training-phase of the animals. Control animals were injected with the proper vehicle. (±)-Baclofen was kindly donated by Ciba-Geigy, ICS 205-930 was kindly donated by Sandoz. Butoxamine-HCl was kindly donated by Wellcome Nederland B.V. Other drugs were obtained from commercial sources.

Statistical analysis. Data were analysed using (Kruskal-Wallis) non-parametric one way analysis of variance (ANOVA) followed by the Mann-Whitney U test.

Results

Administration of graded doses of 8-OH-DPAT resulted in a dose dependent increase in responding during the FR 1 schedule in the extinction experiments (Table 1). However, no effect of 8-OH-DPAT could be detected during the FR1 schedule in the conflict experiments (left column table 1). When the highest dose of 8-OH-DPAT was used (0.1 mg/kg), a significant decrease in the number of responses was observed during the random interval schedule. Since some signs of 5-HT behaviour (hyperactivity, flat body posture) were observed after administration of this dose of 8-OH-DPAT, it could not be excluded that these signs were interfering with the level of responding during the RI schedule. Administration of the tryptophan hydroxylase inhibitor PCPA also caused a dose related increase in extinction of conflict behaviour during the FR1 schedule without affecting conflict behaviour itself (Table 2).

Table 1
Effect of 8-OH-DPAT on conflict behaviour and extinction of conflict behaviour

8-OH-DPAT Dose (mg/kg)	Conflict behaviour: RI schedule	Conflict behaviour: FR 1 schedule	n	Extinction of conflict behaviour: RI schedule	Extinction of conflict behaviour: FR 1 schedule	n
0.0	988 ± 241	1.0 ± 0.3	(5)	1129 ± 170	2.0 ± 0.9	(9)
0.0125	1313 ± 294	1.0 ± 0.3	(5)	1162 ± 135	2.2 ± 1.5	(9)
0.025	1626 ± 237	1.0 ± 0.6	(5)	1200 ± 185	9.7 ± 3.7	(9)
0.05	1088 ± 234	1.2 ± 0.4	(5)	1073 ± 182	24.9 ± 7.4*	(9)
0.1	406 ± 237*	0.6 ± 0.4	(5)	545 ± 203*	13.0 ± 7.5	(9)

8-OH-DPAT or vehicle were administered immediately before the start of the experiment.
* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). RI schedule: amount of lever pressings/30 minutes during the random interval schedule. FR 1 schedule: amount of lever pressings/12 minutes during the fixed ratio 1 schedule. (Data are means ± s.e.m.)

Table 2
Effect of PCPA on conflict behaviour and extinction of conflict behaviour

PCPA Dose (mg/kg)	Conflict behaviour: RI schedule	Conflict behaviour: FR 1 schedule	n	Extinction of conflict behaviour: RI schedule	Extinction of conflict behaviour: FR 1 schedule	n
0.0	1283 ± 126	3.2 ± 0.6	(6)	1310 ± 194	1.6 ± 0.8	(8)
200.0	1216 ± 74	3.5 ± 0.9	(6)	1484 ± 296	23.9 ± 6.5*	(8)
300.0	1142 ± 150	6.3 ± 1.7	(6)	962 ± 250	57.5 ± 16.0*	(8)

PCPA or vehicle were administered 17 hours before the start of the experiments.
* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). RI schedule: amount of lever pressings/30 minutes during the random interval schedule. FR 1 schedule: amount of lever pressings/12 minutes during the fixed ratio 1 schedule. (Data are means ± s.e.m.)

Administration of graded doses of ICS 205-930, a 5HT3 antagonist, was without effect in both paradigms (Table 3).

Table 3
Effect of ICS 205-930 on conflict behaviour and extinction of conflict behaviour

ICS 205-930 Dose (mg/kg)	Conflict behaviour: RI schedule	Conflict behaviour: FR 1 schedule	n	Extinction of conflict behaviour: RI schedule	Extinction of conflict behaviour: FR 1 schedule	n
0.0	1376 ± 205	2.4 ± 0.8	(5)	1106 ± 191	2.2 ± 0.8	(9)
0.0001	1211 ± 220	1.2 ± 0.4	(5)	1147 ± 157	4.6 ± 4.3	(7)
0.0005	1082 ± 226	1.5 ± 0.7	(4)	1256 ± 242	0.4 ± 0.2	(7)
0.0025	648 ± 174	2.0 ± 0.7	(5)	1521 ± 126	2.5 ± 1.0	(6)
0.0125	1093 ± 282	1.8 ± 0.7	(5)	1256 ± 194	12.5 ± 10.2	(6)
0.0625	-			1309 ± 156	0.8 ± 0.4	(5)

ICS 205-930 or vehicle were admnistered 20 minutes before the start of the experiments.
RI schedule: amount of lever pressings/30 minutes during the random interval schedule. FR 1 schedule: amount of lever pressings/12 minutes during the fixed ratio 1 schedule. (Data are means ± s.e.m.)

Administration of propranolol, a non-specific 5-HT_1 type receptor blocker and a β-adrenoceptor antagonist led to an enhanced extinction of conflict behaviour without affecting conflict behaviour itself (Table 4). When using doses of 20 or 40 mg/kg, the number of responses during the random interval schedule was decreased. After administration of 40 mg/kg the number of responses during the FR1 schedule was lower as compared with the effect of 20 mg/kg. Observation of the behaviour of the propranolol treated rats did not indicate that the animals were sedated.

Table 4
The effect of propranolol on conflict behaviour and extinction of conflict behaviour

Propanolol Dose (mg/kg)	Conflict behaviour: RI schedule	Conflict behaviour: FR 1 schedule	n	Extinction of conflict behaviour: RI schedule	Extinction of conflict behaviour: FR 1 schedule	n
0.0	1469 ± 294	5.8 ± 1.2	(5)	1711 ± 194	3.2 ± 1.7	(6)
5.0	1230 ± 139	6.6 ± 0.9	(5)	1041 ± 246	27.3 ± 11.6	(6)
10.0	1041 ± 184	4.8 ± 0.9	(5)	911 ± 204	23.7 ± 9.2	(7)
20.0	595 ± 171	8.6 ± 2.9	(5)	698 ± 128*	64.9 ± 15.0*	(7)
40.0	755 ± 183	5.2 ± 1.4	(5)	581 ± 232*	31.0 ± 10.9	(6)

Propranolol or vehicle were administered 20 minutes before the start of the experiments.
* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). RI schedule: amount of lever pressings/30 minutes during the random interval schedule. FR 1 schedule: amount of lever pressings/12 minutes during the fixed ratio 1 schedule. (Data are means ± s.e.m.)

In order to investigate whether the β1/β2-adrenoceptor blocking property of propranolol might be responsible for the enhancement of extinction of conflict behaviour, the selective β1-adrenoceptor antagonist metoprolol and the selective β2-adrenoceptor antagonist butoxamine were tested. Both drugs were used in dose ranges which are known to block central β-adrenoceptors (Handley and Singh 1986). While butoxamine was not effective, metoprolol did induce a small increase in the number of responses using a dose of 4 mg/kg, but due to the large variation, statistical significance was not reached in these experiments. Metoprolol decreased the level of responding slightly during the random interval schedule (Table 5).

Table 5
The effect of butoxamine and metoprolol on conflict behaviour and extinction of conflict behaviour

	Extinction of conflict behaviour.		
Dose (mg/kg)	RI schedule	FR 1 schedule	n
Butoxamine			
0.0	1747 ± 184	6.1 ± 3.1	(8)
5.0	1750 ± 214	11.1 ± 6.6	(8)
10.0	1625 ± 278	9.5 ± 4.0	(8)
Metoprolol			
0.0	1786 ± 66	6.3 ± 2.6	(9)
2.0	1470 ± 133*	14.2 ± 6.2	(9)
4.0	1210 ± 160*	24.6 ± 12.4	(9)
8.0	1411 ± 184	8.2 ± 3.6	(9)

Butoxamine, metoprolol or vehicle were administered 20 minutes before the start of the experiments.
* : $P < 0.05$ vs vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). RI schedule: amount of lever pressings/30 minutes during the random interval schedule. FR 1 schedule: amount of lever pressings/12 minutes during the fixed ratio 1 schedule. (Data are means ± s.e.m.)

Pretreatment of the rats with 20 or 40 mg/kg propranolol showed an overall tendency to decrease 5HT behaviour evoked by 1.0 mg/kg 8-OH-DPAT (Table 6) but statistical significance was only reached for piano-playing and head weaving when using the dose of 40 mg/kg.

Table 6
The effect of propranolol on the 8-OH-DPAT -induced 5HT behaviour

	dose mg/kg	piano-playing	head weaving	flat body-posture	hind limb abduction
8-OH-DPAT	1.0	2.2 ± 0.9	15.3 ± 2.4	5.5 ± 1.4	6.5 ± 0.9
8-OH-DPAT/propr	1.0/20.0	1.3 ± 0.4	17.2 ± 3.2	4.2 ± 1.1	5.7 ± 0.8
8-OH-DPAT/propr	1.0/40.0	0.2 ± 0.2*	2.8 ± 0.5*	3.8 ± 1.1	3.0 ± 1.1

5HT behavioural symptoms (piano playing, head weaving, flat body posture and abducted hind limbs). Sum of scores over 6,12,18,24 and 30 minutes after injection of 1.0 mg/kg 8-OH-DPAT. Propranolol or vehicle were injected 25 minutes before administration of 8-OH-DPAT.
*: $P < 0.05$ vs 8-OH-DPAT alone (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). Data are means ± s.e.m., $n = 6$.

In order to investigate whether baclofen and 8-OH-DPAT were still able to enhance the extinction of conflict behaviour after impairment of the serotonergic system, one active dose of each compound was administered to rats pretreated with 200 mg/kg PCPA. As shown in table 7 administration of baclofen or 8-OH-DPAT to rats pretreated with PCPA did not further increase the enhanced extinction caused by the pretreatment.

Table 7
The effect of baclofen or 8-OH-DPAT on extinction of conflict behaviour after pretreatment with PCPA

		Extinction of conflict behaviour.		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n
Vehicle pretreated 17 hours before experiment:				
	0.0	1272 ± 179	1.7 ± 0.7	(6)
Baclofen	2.0	975 ± 151	33.8 ± 12.0*	(6)
8-OH-DPAT	0.03	881 ± 168	34.3 ± 8.3*	(6)
PCPA 200 mg/kg pretreated 17 hours before experiment:				
	0.0	1218 ± 341	26.2 ± 7.3*	(6)
Baclofen	2.0	702 ± 195	20.7 ± 7.9*	(6)
8-OH-DPAT	0.03	1076 ± 254	32.2 ± 18.0*	(6)

The rats were pretreated with PCPA or vehicle 17 hours before the experiments. The animals were subsequently injected with baclofen or vehicle 20 minutes- or with 8-OH-DPAT or vehicle immediately before- the start of the experiments.
* : $P < 0.05$ vs vehicle pretreated vehicle (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). RI schedule: amount of lever pressings/30 minutes during the random interval schedule. FR 1 schedule: amount of lever pressings/12 minutes during the fixed ratio 1 schedule. (Data are means ± s.e.m.)

Discussion

The present results indicate that low doses of 8-OH-DPAT, a 5-HT_{1A} agonist (Middlemiss and Fozard 1983), enhanced the extinction of conflict behaviour without affecting the conflict behaviour itself, an effect shared with the $GABA_B$ agonist baclofen (Ketelaars et al. 1988). In fact it is not unlikely that the effect of baclofen occurs via an impairment of the activity of the serotonergic system. An impairment of the 5-HT system has been suggested to be responsible for the anxiolytic effects in certain animal models (see Iversen 1984; Chopin and Briley 1987).

A functional decrease in the activity of the 5-HT system can be accomplished in three ways. 1. A blockade of the 5-HT biosynthesis; 2. activation of autoreceptors located on 5-HT neurons and 3. postsynaptic 5-HT antagonism. The present results show that PCPA, an inhibitor of tryptophan hydroxylase, increased the extinction of conflict behaviour without affecting conflict behaviour itself and thus caused an effect as has been described previously for baclofen (Ketelaars et al. 1988). Although the lack of effect of PCPA on conflict behaviour is in agreement with results of Blakely and Parker (1973) and those by Cook and Sepinwall (1975), others have reported a weak effect (Kilts et al. 1982) or marked effects on conflict behaviour (Geller and Blum 1970; Robichaud and Sledge 1969; Stein et al. 1973; Tye et al. 1979; Engel et al. 1986). Also anti-conflict effects of 8-OH-DPAT have been reported (Engel et al. 1986; Carli and Samanin 1988). However, other reports on the anti-conflict effect of $5HT_{1A}$ receptor agonists are less consistent (Deacon and Gardner 1986; Chopin and Briley 1987). It is postulated that the effect of 8-OH-DPAT is dependent on the state of arousal of the rat and thus on the type of animal model of anxiety used (Carli and Samanin 1988). These differences in anti-conflict effect, obtained after pretreatment of the rats with PCPA or after treatment with 8-OH-DPAT, may be explained by differences in the training procedure, schedules or degree of suppression. In the present experiments, the use of rats trained on a conflict schedule resulting in 0-5 responses during the whole session (6 times 2 min sessions alternated by 6 times a random interval schedule, see Method section) can clearly differentiate between benzodiazepines (Ketelaars et al. 1988 and Ketelaars and Bruinvels 1989) on one hand and drugs that impair serotonergic function on the other by measuring both the anti-conflict effect and the extinction of conflict behaviour. The effect of low doses of 8-OH-DPAT which enhanced extinction of conflict behaviour without affecting conflict behaviour itself, may also be the result of an impaired serotonergic function by activation of 5-HT_{1A} autoreceptors decreasing impulse-flow-induced release of serotonin (Dourish et al. 1986), while the use of higher doses of 8-OH-DPAT may activate also postsynaptically located 5-HT_{1A} receptors evoking the 5-HT syndrom (Goodwin et al. 1987; Tricklebank et al. 1984). The involvement of the 5-HT system in enhancing extinction of conflict behaviour was further supported by the effect of propranolol, a non-specific 5-HT_1 receptor antagonist (Middlemiss et al. 1977; Green et al. 1983; Nahorski and Willcocks 1983), which also increased the extinction of conflict behaviour without affecting conflict behaviour by blocking postsynaptically located 5-HT_1 receptors. Although it could not be concluded whether the antagonism of 5-HT_{1A} or 5-HT_{1B} receptors or both receptor types are responsible for the effect of propranolol, the doses of propranolol used seem at least to be appropiate for blocking postsynaptic 5-HT_{1A} receptors since these doses were capable to antagonize the 5-HT syndrome induced by a high dose (1 mg/kg) of 8-OH-DPAT. That the β_1 and β_2 blocking properties were not responsible for the effect of

propranolol was shown by the lack of effect of the specific β_1 and β_2 antagonists metoprolol and butoxamin respectively. Both drugs were administered in doses shown to be behaviourally active (Handley and Singh 1986). The effect of propranolol to antagonize the 5-HT syndrome is in agreement with results obtained by Tricklebank et al. (1984) who used 8-OH-DPAT and with Green and Graham-Smith (1976) who used L-tryptophan combined with tranylcypromine to induce the 5-HT syndrome.
The lack of effect of the 5-HT_3 antagonist ICS 205-930 on extinction of conflict behaviour indicated that the enhancement of extinction may be specifically mediated by blockade of 5-HT_1 receptors since, as has been previously shown, also 5-HT_2 receptor antagonists were ineffective (Ketelaars et al. 1988; Ketelaars and Bruinvels 1989).
Since the effect of 8-OH-DPAT, PCPA or propranolol is identical to that obtained with baclofen (Ketelaars et al. 1988), it is not unlikely that the latter exerts its effect via the 5-HT neuronal system. Indeed it has been shown that baclofen decreases 5-HT activity by inhibition of 5-HT release (Schlicker et al. 1984; Gray and Green 1987). Supporting evidence for an intermediary role of the 5-HT system in the effect of baclofen on extinction of conflict behaviour was obtained by pretreating rats with PCPA. In these rats both baclofen and 8-OH-DPAT were without effect.
In conclusion, the present results show that impairment of the 5-HT neuronal activity by three different procedures increased extinction of conflict behaviour without affecting conflict behaviour itself. The latter is in contrast to the action of benzodiazepines (Ketelaars et al. 1988). A combination of increased extinction of conflict behaviour and a lack of effect on conflict behaviour itself has also been shown to occur after administration of baclofen to rats (Ketelaars et al. 1988). This effect did not occur in rats depleted of 5-HT by pretreatment with PCPA, suggesting that the effect of baclofen could only be evoked through an intact 5-HT system.

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BACLOFEN-INDUCED EXTINCTION OF CONFLICT BEHAVIOUR, RELATIONSHIP WITH THE GABAERGIC AND SEROTONERGIC SYSTEM.

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Summary

The mechanism behind the effect of the baclofen-induced extinction of conflict behaviour might be mediated via two mechanisms; an indirect $GABA_A$ receptor activation or an impairment of serotonergic system function. The present results showed that bicuculline antagonized the effect of oxazepam on extinction, but the $GABA_A$ antagonist did not counteract the baclofen-induced extinction. The selective serotonine (5-HT) reuptake inhibitor fluvoxamine counteracted the effect of both oxazepam and baclofen on extinction, but not the 8-OH-DPAT-induced extinction. Finally, the effect of long-term administration of fluvoxamine and its interaction with the effect of baclofen and 8-OH-DPAT is described. The present results support the suggestion that the effect of baclofen on extinction of conflict behaviour is mediated via an impaired serotonergic system, and not via an indirect effect via the $GABA_A$/benzodiazepine receptor chloride channel complex. The results obtained during and after long-term fluvoxamine administration seem to agree with the suggestion that anti-depressants induce a $GABA_B$ receptor upregulation, but do not support the reports that these compounds induce a desensitization of pre- and postsynaptic $5\text{-}HT_{1A}$ receptors.

Introduction

GABA receptors in rat brain are subdivided into two subtypes depending on their affinity for the GABA receptor antagonist bicuculline or for the GABA-analog baclofen (β-(p-chlorophenyl)-GABA). $GABA_A$ receptors are sensitive to bicuculline and not to baclofen, while $GABA_B$ receptors are sensitive to baclofen and not to bicuculline (Hill and Bowery (1981), Bowery et al. (1983)). It is assumed that the anxiolytic effects of the benzodiazepines are mediated via the $GABA_A$/benzodiazepine receptor chloride ionophore complex. Thus it has been reported that $GABA_A$ antagonists antagonize the action of benzodiazepines in rat conflict models, but the reports are equivocal and no consensus about the exact mode of action has been reached (Costa et al. (1975), Sepinwall and Cook (1980), Sanger (1985)). As shown previously, benzodiazepines and baclofen both enhance extinction of conflict behaviour (Ketelaars et al. 1988). On the level of receptor binding no interrelationship between benzodiazepine and $GABA_B$ receptors could be shown (Majewska and Chuang (1984), Karobath and Sperk (1979), Ketelaars et al. (1988)). However, it can not be ruled out that the effect of baclofen on extinction of conflict behaviour is mediated **in vivo** via $GABA_A$ receptors. It is still possible that baclofen may exert its effect via presynaptic activation of GABA-ergic neurones, resulting in an enhanced release of GABA (Kerwin and Pycock (1978), Roberts et al. (1978)).

Another possibility for a mediator responsible for the effect of baclofen on extinction may be decrease of activity of the serotonergic system. It has been shown that a reduction in 5-HT neurotransmission has anxiolytic effects in

animal models for anxiety, while the reverse is true for a stimulation of the 5-HT system (review: Chopin and Briley (1987)). The 5-HT theory (i.e. a decreased 5-HT activity) related to the anti-conflict effect of benzodiazepines was first postulated by the group of Wise and Stein (1972 and 1973). It has also been shown by Sclicker et al. (1984) and Gray and Green (1987a) that baclofen decreases the release of 5-HT in a synaptosomal fraction obtained from rat cortical tissue. Moreover, in a previous study, it has been shown that impairment of the 5-HT function by PCPA, 8-OH-DPAT or propranolol (inhibition of 5-HT biosynthesis, inhibition of 5-HT release or post-synaptic 5-HT_1 antagonism respectively) enhances extinction of conflict behaviour (Ketelaars and Bruinvels, submitted).
In clinical literature, it is reported that baclofen (Pepplinkhuizen and Bruinvels (1978)), Breslow et al. (1989)) and the selective 5-HT reuptake inhibitor fluvoxamine (Den Boer et al. (1987)) both possess anti-panic properties. Several anti-depressants, which also have anti-panic activity, increase the Bmax of $GABA_B$ receptors after long-term administration via a still unknown mechanism, but it is shown that the latter mechanism is not caused by a decreased GABA concentration in the synaptic cleft (Pilc and Lloyd (1984), Suzdak and Gianutsos (1986)). It has been suggested that anti-depressants may exert their anti-panic effect via $GABA_B$ receptor upregulation and function (Breslow et al. (1989)).
The aim of the present experiments was twofold: 1/ To investigate further the possibilities that the effect of baclofen on extinction of conflict behaviour is mediated by an enhanced $GABA_A$ receptor activation or by an impairment of 5-HT function (or both). Therefore, the effect of the $GABA_A$ antagonist bicuculline and the effect of the 5-HT reuptake inhibitor fluvoxamine on the baclofen-induced increase in extinction of conflict behaviour were investigated. Oxazepam was used as an additional control group in the experiments with bicuculline. 2/ The second aim was to see whether long-term administration of fluvoxamine was active in enhancing extinction of conflict behaviour. It is shown by several groups that long-term administration of anti-depressants increases $GABA_B$ receptor number and function, (Pilc and Lloyd (1984), Suzdak and Gianutsos (1986), Gray and Green (1987), Gray et al. (1987)) and decreases pre- and postsynaptic $5HT_{1A}$ receptor function (Goodwin et al. (1987), Stolz et al. (1983)) during or after 2 to 3 weeks of treatment. In order to find out whether these observations would also agree with the anxiolytic effects induced by $GABA_B$- and 5-HT_{1A}- receptor activation, the effect of long-term administration of fluvoxamine alone and its effect on the baclofen- and 8-OH-DPAT-induced extinction was investigated.

Methods

Extinction of conflict behaviour. In order to study extinction of conflict behaviour the Geller-Seifter test (Geller and Seifter 1960) was used as described previously (Ketelaars et al. 1988). In brief: After being deprived of water for 16 hours, male Wistar rats (starting weight: 70 - 90 g; after training was completed: 250 - 350 g) were placed in an experimental chamber measuring 20 x 20 x 20 cm, containing a response lever, milk delivery apparatus, an electrifiable grid floor and a stimulus light. The rats were trained to press a lever in order to obtain milk and were subsequently trained once a day for a duration of 42 minutes. Within these training (and test) periods, two schedules were used in alternation. During 5 minutes a 5 % random interval (RI) schedule was offered. This schedule was alternated with 2 minutes of a fixed ratio 1 (FR 1) schedule. A steady light signal during the

FR 1 schedule served as the discriminative stimulus. This combination of RI and FR 1 schedules was presented 6 times to each rat per session. After stabilization of performance, response-contingent foot shock was given during the FR 1 periods. Shock levels (100-160 V, 2 - 3.3 mA) were individually adjusted until cumulative lever pressings during the FR 1 periods were below 5 per 12 minutes, which represents about 5 % of the number of lever pressings before foot shock was applied. Drug effects on extinction of conflict behaviour were measured on the third day, always after 2 days of training in order to achieve a stable "base line" level of conflict behaviour. On the test days, foot shock was turned off. During the 2 days before the test days training continued in the presence of foot shock, and shock levels were adjusted when the animals took more than 5 shocks per 12 minutes. After at least 20 training sessions, spontaneous extinction was non-existent in control rats during the trial period; i.e. these animals responded with an average of 0 to 5 pressings per 12 minutes.
In the extinction test with the low level of conflict behaviour, training was stopped and test were started when spontaneous extinction was at the level of 50 % (± 50 lever presses per 12 minutes). Six groups of 12 rats and two groups of 18 rats were used. Five groups of 12 rats were used in the extinction test with a high level of conflict and one group of 12 rats was used in the extinction test with a low level of conflict. The two groups of 18 rats were used in the extinction tests with long-term fluvoxamine or its vehicle administration. Vehicle and different doses of each drug were administered to each rat of a group using a latin square design. The effects of drugs were tested once a week to allow for a sufficiently long wash out period. During the training, before the extinction experiments were performed, the chronic administration of fluvoxamine caused no need for extra adjustments of shock levels.

Drugs:
The doses of baclofen, oxazepam and 8-OH-DPAT were active doses taken from previously established dose-response curves (Ketelaars et al. (1988), Ketelaars and Bruinvels, submitted). As specified later in this section, bicuculline was administered in a dose of 0.5 mg/kg twice; 20 minutes and immediately before the start of the experiments. As indicated in the result section, some rats showed signs of 5-HT behaviour when the highest dose (20 mg/kg) of fluvoxamine was used. Since this might disturb performance of the rats, a dose of 10 mg/kg was used in the acute experiments. All drugs used were administered intraperitoneally in a volume of 1 ml/kg. Baclofen was dissolved in saline and injected 20 minutes before the behavioural experiments started. Oxazepam was administered 20 minutes before the experiment as a sonicated suspension in distilled water to which a drop of Tween 80 had been added. Bicuculline was dissolved in acidified saline (pH 4.0). A pilot study showed that higher doses than 0.5 mg/kg could not be used since the rats started to show convulsions. In order to prevent this seizure activity and to prolong the duration of the $GABA_A$ antagonistic action, bicuculline was injected twice in a dose of 0.5 mg/kg. The first dose was administered 20 minutes before and the second dose immediately before the start of the experiments. 8-OH-DPAT was dissolved in saline and injected immediately before the experiments started. Fluvoxamine was dissolved in distilled water and injected 30 minutes before the start of the experiments. Every control rat received the appopriate vehicle. Separate injections of drugs were given in the combination experiments. For the long-term administration of fluvoxamine (14 days), Alzet mini pumps were used. In these experiments, fluvoxamine was

dissolved in DMSO/distilled water (1:1 v/v). Fluvoxamine was dissolved in such a concentration that the mini pumps delivered a dose of 20 mg/kg/day to the rats. Mini pumps with vehicle were implanted in control rats. Oxazepam was kindly donated by Wyeth, baclofen was kindly donated by Ciba-Geigy. Alzet mini pumps and fluvoxamine were kindly donated by Duphar. 8-OH-DPAT was obtained from RBI. Bicuculline was obtained from Sigma.

Statistical analysis. Data were analysed using non-parametric (Kruskal Wallis) one way analysis of variance (ANOVA) followed by the Mann-Whitney U test.

Results

As shown in Table 1A, bicuculline antagonized the effect of oxazepam on extinction of conflict behaviour. Both drugs given alone or in combination did not affect the level of responding during the RI schedule. The same dose-regimen of bicuculline was used in the extinction experiments using baclofen instead of oxazepam. In contrast to the effect of oxazepam, the effect of baclofen on extinction of conflict behaviour was not antagonized by bicuculline (Table 1B). A small decrease in responses during the RI schedule occurred after baclofen administration. This effect was also not affected by bicuculline. Like in the experiments with oxazepam, bicuculline itself did not affect the number of responses during both schedules.

Table 1 Effects of bicuculline on oxazepam- and baclofen-induced extinction of conflict behaviour.

	Dose (mg/kg)	Extinction of conflict behaviour. RI schedule	FR 1 schedule	n
A				
Control		1372 ± 223	3.9 ± 1.7	(10)
Oxazepam	10.0	1335 ± 242	72.8 ± 23.2$	(10)
Oxaz/bic*	10.0/0.5	1631 ± 242	14.2 ± 5.2	(9)
Bicuculline*	0.5	1127 ± 286	6.6 ± 3.5	(9)
B				
Control		1774 ± 189	4.3 ± 1.8	(8)
Baclofen	2.0	1029 ± 191*	37.9 ± 12.2*	(8)
Bac/bic*	2.0/0.5	1088 ± 291*	47.7 ± 15.6*	(6)
Bicuculline*	0.5	1685 ± 156	13.2 ± 8.5	(6)

$: $P < 0.05$ vs control and vs oxaz/bic (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). *: $P < 0.05$ vs control (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). Cumulative level of responding per session during random interval (RI) and fixed ratio 1 (FR 1) schedule per 30 and 12 min respectively. Data are mean ± s.e.m. *: Bicuculline was administered twice as described in the method section.

Administration of fluvoxamine did not affect responding in the FR 1 or RI schedule (Table 2A). Using the highest dose (20 mg/kg), some mild signs of 5HT behaviour were observed (flat body posture, abducted hindlimbs). Since fluvoxamine administration will increase serotonergic activity, an anxiogenic effect may occur resulting in a decreased extinction. Therefore a lower level of punishment (50 % decrease of preexisting level of responding) was used. However, as shown in Table 2B, also under these circumstances fluvoxamine administration was without effect.
The effect of fluvoxamine on the baclofen-, 8-OH-DPAT- and oxazepam- induced extinction was investigated. As shown in Table 3A, fluvoxamine (10 mg/kg) counteracted the oxazepam- and baclofen-induced enhancement of extinction of conflict behaviour, while fluvoxamine did not affect the action of 8-OH-DPAT on extinction (Table 3B). The animals treated with baclofen in combination with fluvoxamine were sedated.

Table 2 Effect of fluvoxamine on extinction of conflict behaviour.

		Extinction of conflict behaviour.		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n
A				
Control		1402 ± 186	6.6 ± 4.2	(9)
Fluvoxamine	5.0	1715 ± 312	6.4 ± 2.3	(8)
	10.0	1275 ± 379	7.4 ± 5.6	(7)
	20.0	1558 ± 238	16.9 ± 10.2	(8)
B				
Control		1019 ± 127	45.6 ± 8.2	(12)
Fluvoxamine	10.0	1110 ± 167	37.5 ± 8.0	(12)
	20.0	815 ± 98	50.8 ± 7.6	(12)

Cumulative level of responding per session during random interval (RI) and fixed ratio 1 (FR 1) schedule per 30 and 12 min respectively. Data are mean ± s.e.m.. In order to allow the possibility of a decrease in extinction, a lower conflict level was realized by training the animals to a 50% decrease from pretraining FR 1 responding (see method section).

Table 3 Effect of fluvoxamine on the baclofen-, oxazepam- and 8-OH-DPAT -induced extinction of conflict behaviour.

		Extinction of conflict behaviour.		
	Dose (mg/kg)	RI schedule	FR 1 schedule	n
A				
Control		1361 ± 318	2.5 ± 2.1	(6)
Baclofen	1.5	1276 ± 277	34.3 ± 14.1$	(6)
	2.0	1088 ± 242	26.0 ± 10.7$	(6)
	2.5	431 ± 222*	27.0 ± 10.4*	(6)
Oxazepam	10.0	1456 ± 398	77.3 ± 36.9$	(6)
Fluvoxamine	10.0	1435 ± 391	5.7 ± 3.7	(6)
Flu/baclofen	10.0/1.5	461 ± 172+	7.3 ± 6.9	(6)
	10.0/2.0	68 ± 49+	2.2 ± 2.2	(5)
Flu/oxazepam	10.0/10.0	1532 ± 487	7.2 ± 6.4	(6)
B				
Control		1520 ± 259	1.5 ± 0.4	(10)
8-OH-DPAT	0.03	1165 ± 201	55.1 ± 14.9*	(10)
Fluvoxamine	10.0	1037 ± 199	5.8 ± 3.3	(10)
Flu/8-OH-DPAT	10.0/0.03	1063 ± 226	69.7 ± 17.9*	(9)

*: $P < 0.05$ vs control $: P < 0.05$ vs control and vs fluvoxamine/oxazepam or fluvoxamine/baclofen +: $P < 0.05$ vs control and fluvoxamine and baclofen alone. (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). Cumulative level of responding per session during random interval (RI) and fixed ratio 1 (FR 1) schedule per 30 and 12 min respectively. Data are mean ± s.e.m.

As shown in fig 1A, administration of 20 mg/kg fluvoxamine 17 hours before baclofen or 8-OH-DPAT did not affect the action of the latter two drugs significantly. It must be noted however, that the mean number of responses of the baclofen-induced extinction was nearly doubled. The effect of administration of fluvoxamine (20 mg/kg/day) by mini-pump alone and its effect on the baclofen- and 8-OH-DPAT-induced extinction is also described in figure 1A and B. The number of responses during the RI schedule tended to be lower in the fluvoxamine treated group during the whole experiment. In analogy with the acute experiments, the baclofen-induced decrease in responding during the RI schedule was augmented in the fluvoxamine treated group of rats. In contrast, but again in analogy with the results shown in Table 3, fluvoxamine tended to counteract the effect of baclofen on extinction. On the 16th day, i.e. two days after terminating the administration of fluvoxamine, the extinction was enhanced in the fluvoxamine treated control group. The baclofen-induced extinction tended to be enhanced. The sedative effect of baclofen during the RI schedule was significantly increased. The effect of 8-OH-DPAT on extinction was not affected by pretreatment of fluvoxamine, while in this group the number of responses during the RI schedule was decreased in the fluvoxamine pretreated group. On the 19th day, the fifth day after terminating the fluvoxamine administration, the effect of fluvoxamine on extinction in the control group had disappeared. The enhanced sedative effect of baclofen, as shown by the decrease in number of responses during the RI schedule, was still present, as well as the decreased number of responses in the RI schedule in

the 8-OH-DPAT group. The tendency for an enhanced baclofen -induced extinction was also still present. The responses during the RI schedule seemed to normalize on the last test day, 2 weeks after terminating administration.

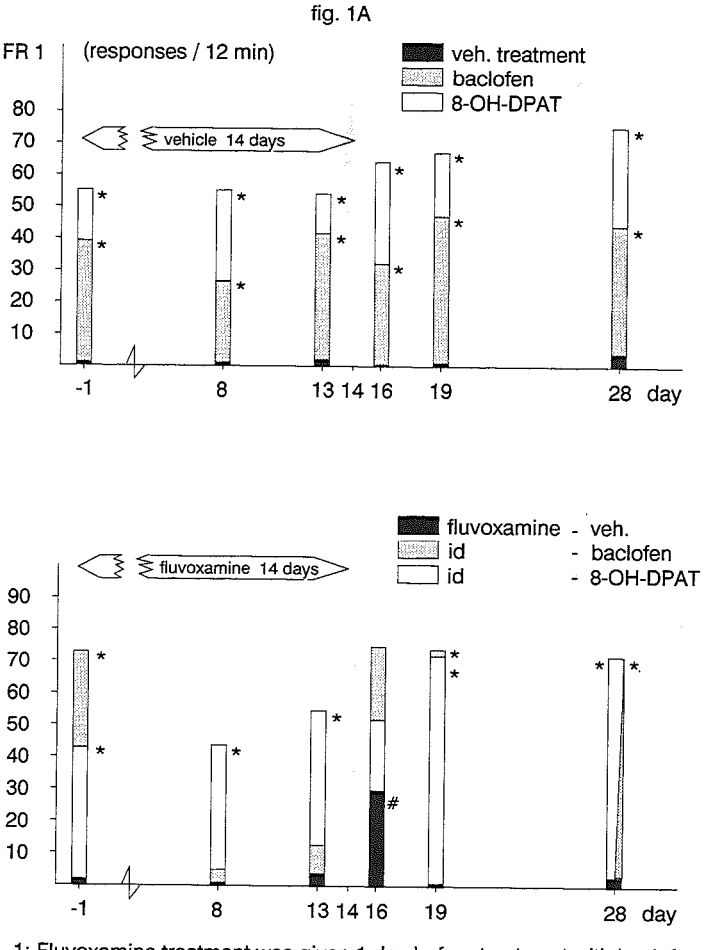
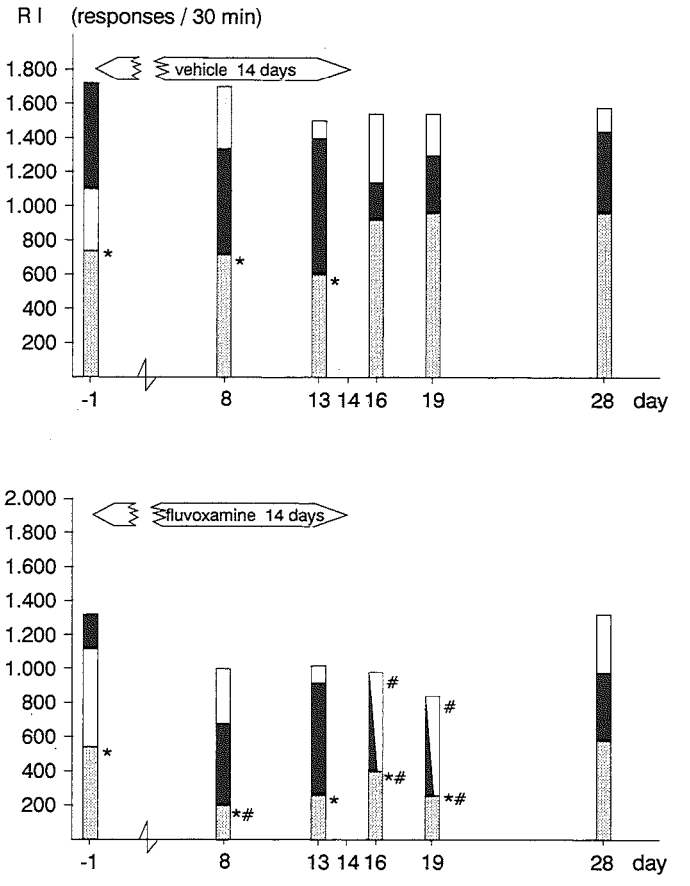


FIGURE 1A.
Cumulative level of responding per session during Fixed Ratio 1 (FR 1) schedule per 12 minutes. Data are mean, s.e.m. was in the similar range as in Table 1 to 3.
*: $P < 0.05$ vs control within same mini-pump administered treatment.
#: $P < 0.05$ vs same drug/vehicle mini-pump treated.
Statistical significance is after Kruskal-Wallis ANOVA followed by Mann-Whitney U test.

fig. 1B



-1: Fluvoxamine treatment was given 1 day before treatment with baclofen or 8-OH-DPAT

FIGURE 1B.
Cumulative level of responding per session during random interval (RI) schedule per 30 minutes. Data are mean, s.e.m. was in the similar range as in Table 1 to 3.
*: $P < 0.05$ vs control within same mini-pump administered treatment.
#: $P < 0.05$ vs same drug/vehicle mini-pump treated.
Statistical significance is after Kruskal-Wallis ANOVA followed by Mann-Whitney U test.

Discussion

The assumption that the oxazepam- or baclofen- induced extinction of conflict

behaviour in rats is mediated via the $GABA_A$ system, (oxazepam via its well known $GABA_A$ facilitating effect and baclofen indirectly by causing release of GABA (Kerwin and Pycock (1978), Roberts et al. (1978)) seems valid for oxazepam, but could not be proven for baclofen. The present experiments clearly showed that the baclofen-induced enhancement of extinction of conflict behaviour could not be antagonized by the $GABA_A$ antagonist bicuculline, while the increase in extinction induced by oxazepam could be antagonized by this $GABA_A$ antagonist. Therefore, $GABA_A$ receptor activation can be excluded as a common pathway for oxazepam and baclofen in vivo, a conclusion which is supported by previous results showing that baclofen administration to rats does not affect GABA release as measured by binding properties of benzodiazepines in vitro (Ketelaars et al. (1988)). The action of bicuculline to antagonize the anxiolytic effect of benzodiazepines has been shown previously (Quintero et al. (1985), Zakusov et al. (1977), Scheel-Kruger and Petersen (1982), Velluci and Webster (1984)). However, some of these reports are open to criticism, e.g. due to an inadequate description of the method used (Zakusov et al. (1977)), or the occurrance of seizure activity in the rats treated with bicuculline (Quintero et al. (1985)). Besides, negative results on the anxiolytic effect of diazepam after co-administration with bicuculline have also been reported (Liljequist & Engel 1984). The present experiments rule out the possibility that baclofen in vivo indirectly activates $GABA_A$ receptors and so mediates its effect on extinction.
This leads to a second possibility, namely that baclofen enhances the extinction of conflict behaviour by impairment of serotonergic activity. This would be supported by the fact that inhibition of 5-HT synthesis by PCPA also results in an increase of extinction and prevented a further increase by baclofen and 8-OH-DPAT (Ketelaars and Bruinvels, submitted). It was therefore decided to use the specific 5-HT reuptake inhibitor fluvoxamine to see whether an increased availability of 5-HT could counteract the effect of baclofen. Since it is has been suggested that the effects of 8-OH-DPAT and benzodiazepines in animal models for anxiety are mediated by a decrease of 5-HT function (Traber and Glaser (1987), Wise et al. (1972), Stein et al. (1973)), 8-OH-DPAT and oxazepam were taken as additional control groups in the experiments with baclofen and fluvoxamine. Fluvoxamine itself was without effect on the extinction of conflict behaviour. Also when a lower level of conflict was used, to detect an anxiogenic action, fluvoxamine was ineffective. However, administration of fluvoxamine counteracted the effect on extinction of both baclofen and oxazepam, an effect which can not be ascribed to a sedative action since the RI response obtained after the concomitant administration of fluvoxamine and 1.5 mg/kg baclofen is comparable to the RI response obtained after 2.5 mg/kg baclofen. Thus it may be concluded that administration of fluvoxamine counteracted the increased extinction induced by baclofen or oxazepam independent of the presence of sedation. This effect may be caused by a increased accumulation of 5-HT in the synaptic cleft resulting in an augmented interaction with 5-HT receptors. Although this agrees very well with the proposed anxiogenic effect of 5-HT in animal models for anxiety (Chopin and Briley (1987), Broekkamp et al. (1989)), and may thus explain this action of fluvoxamine, the failure to detect in the present experiments an anxiogenic effect of this drug when administered alone does not support this explanation. However, this may depend on the model used, since anxiogenic responses in animal models of anxiety have been reported after administration of several selective 5-HT reuptake inhibitors (Chopin and Briley (1987)) while, on the other hand, it is also shown that acute administration of fluvoxamine has an anxiolytic action in the defensive burying test and the

four plates test (Broekkamp et al (1989), Molewijk and Van der Heijden (1988)).
Like baclofen the 5-HT_{1A} agonist 8-OH-DPAT also increases extinction of conflict behaviour without affecting conflict behaviour itself (Ketelaars and Bruinvels, submitted). However, in contrast to baclofen fluvoxamine did not affect the 8-OH-DPAT-induced increase in extinction. This lack of effect of fluvoxamine was unexpected since the action of 8-OH-DPAT is ascribed to an activation of somatodendritic autoreceptors resulting in a decreased 5-HT release (Traber and Glaser (1987)). If this would be the mechanism responsible for the enhanced extinction by 8-OH-DPAT, fluvoxamine should have counteracted the effect. This lack of effect of fluvoxamine might be explained by an anxiolytic effect of 8-OH-DPAT mediated via postsynaptic 5-HT_{1A} receptors. This would implicate that activation of postsynaptic 5-HT receptors may not only exert anxiogenic effects but also anxiolytic effects. This latter effect might be evoked by the 5-HT_{1A} receptors which exert an inhibitory effect on hippocampal activity (Andrade et al. (1986), Newberry and Nicoll (1984)). A mixture of anxiogenic and anxiolytic postsynaptic 5-HT receptors might also explain the reported different effects of 5-HT reuptake inhibitors, since the net effect of such a drug would than be dependent on the balance of stimulation of various 5-HT receptors. Although a dose of 10 mg/kg fluvoxamine counteracted the effects of oxazepam and baclofen, another explanation for the lack of effect of fluvoxamine on the 8-OH-DPAT-induced extinction might be that a higher dose of fluvoxamine is needed to antagonize the effect of 8-OH-DPAT.
Several hypotheses about the mechanism behind the anxiolytic effects of anti-depressants exist: Desensitization of postsynaptic $5HT_1$ receptors (Den Boer (1988)), an enhanced $GABA_{(B)}$ receptor activation (Breslow et al. (1989)) or downregulation of adrenoceptors (Charney and Heninger (1985)).
The present results showed that extinction of conflict behaviour was enhanced on the third day after terminating long-term treatment of fluvoxamine. The latter effect indeed suggests an adaptive process induced by this compound and supports the findings of Fontana et al. (1989), who have shown an anxiolytic effect in an animal model after long-term treatment with an anti-depressant. The results obtained with baclofen after long-term administration of fluvoxamine, suggest the existence of $GABA_B$ receptor upregulation. Although the baclofen-induced extinction was counteracted during administration of fluvoxamine (this is in analogy with the acute experiments), it was indeed increased after terminating the fluvoxamine administration. Interestingly, the baclofen-induced extinction was of the same magnitude one day after a single injection of 20 mg/kg fluvoxamine. This suggests a fast process after an injection, but a slow process using a mini-pump. This fast process was already described by Pilc and Lloyd (1984), since they showed a consistent tendency for an increased binding after one day pretreatment with anti-depressants. However, Gray and Green (1987) and Gray et al. (1987) did not confirm this result in their behavioural studies. The long-term administration of fluvoxamine did not affect the 8-OH-DPAT-induced extinction. This is in contrast with the reports of Goodwin et al. (1987), who have shown a decrease of pre- and postsynaptic $5HT_{1A}$ receptor activation-induced effects after two weeks of anti-depressant treatment. Chaput et al. (1988) reported that within 14 days of treatment with selective 5-HT reuptake inhibitors both somatodendritic and terminal autoreceptors were desensitized. However, this group reported that postsynaptic-mediated electrophysiological responses to 5-HT were not changed. Since the effect of 8-OH-DPAT on extinction was not affected, the likelyhood of the earlier suggestion that the 8-OH-DPAT-induced

extinction may not be mediated via the presynaptic 5-HT_{1A} autoreceptor increases. It is tentative to suggest that the postsynaptic 5-HT_{1A} receptor by which 8-OH-DPAT may exert its effect on extinction does not desensitize after fluvoxamine treatment.

The results obtained in the RI schedule deserve a separate discussion; In the acute and long-term experiments, combined administration of fluvoxamine and baclofen caused an excessive sedation of the rats. This suggests that fluvoxamine and baclofen interact with eachother in two opposite directions: a synergistic-like effect on sedation, and an antagonistic effect on extinction. The baclofen-induced decrease in the number of responses during the RI schedule was not only augmented during fluvoxamine treatment, but also after the termination of the fluvoxamine administration, the latter effect supports the suggestion of $GABA_B$ upregulation.

The present experiments support the suggestion that baclofen enhances extinction via an impairment of the serotonergic system. The results obtained after long-term administration of fluvoxamine seem to agree with the reported $GABA_B$ receptor upregulation but do not support the reported pre- and postsynaptic 5-HT_{1A} receptor downregulation.

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CHAPTER VI

GENERAL DISCUSSION

Introduction.

Baclofen has a beneficial effect on all components of panic disorder, i.e. panic attacks, anticipatory anxiety and phobic symptoms. This was shown by Pepplinkhuizen and Bruinvels (1978) in an open trial with 9 panic disorder patients (than called patients with an anxiety neurosis) which were compared with 10 patients diagnosed as having anxiety hysteria, neurasthenia and depressive neurosis with anxiety. While all panic disorder patients showed great improvement, no effect was shown in the control group. The drug doses (10 mg three times per day) used does not cause muscle relaxation. The efficacy of baclofen in panic disorder has also been investigated by Breslow et al. (1988), who used the same dose-regimen as Pepplinkhuizen and Bruinvels, and reported in a pilot study with 9 patients that the panic attack frequency and anxiety decreases during 4 weeks of treatment. The observations of Pepplinkhuizen and Bruinvels formed the basis for the present investigation into the mechanism responsible for the anxiolytic effect of baclofen. A new animal model for anxiety (extinction of conflict behaviour) was conceptualized in which baclofen might be active since baclofen is without effect in a conventional animal model for anxiety, the Geller-Seifter conflict test (see paragraph 1.6.1.). However, the anxiety of a patient suffering from panic disorder does not resemble an actual conflict situation. After a panic attack, the patients tend to anticipate attacks and/or tend to avoid situations in which an attack might occur (resp. anticipatory anxiety and phobic avoidance). The idea behind the model of extinction of conflict behaviour is that passive avoidance, which might be defined as an inhibition of a behaviour caused by the memory of an aversive experience, resembles closely the human behaviour after panic attacks.

Extinction of conflict behaviour.

Extinction of conflict behaviour fulfills a number of criteria mentioned in paragraph 1.6., it is sensitive to low doses of oxazepam and baclofen (chapter II), chlordiazepoxide (unpublished observations) and 5-HT modulating drugs (chapter IV and V). In addition, haloperidol (chapter II) and anticholinergics (chapter III) are active. Baclofen does not induce an increase in drinking of rats while staying in their home cage (unpublished observations), or in the unpunished control group of the Vogel test (chapter II). Baclofen shows habituation of its effect on extinction after 3 to 4 days of daily administration (unpublished observations). A similar finding (loss of action after 5 days of baclofen administration) concerning baclofen-induced hypothermia in mice is reported by Gray et al. (1987), and $GABA_B$ receptor downregulation after 14 days of baclofen administration is reported by Suzdak and Gianutsos (1986)).

The behaviour of the rats during the FR 1 schedule has been observed frequently. Generally, the rats showed an approach-avoidance conflict, i.e. they approached and backed away from the lever with occasionally pushing it. In other words: The fear for the foot-shock was present while the foot-shock itself was not. Although it is not allowed to think about animal models for anxiety in antropomorphic terms, it is tentative to suggest that this

expectation of an aversive event may reflect anticipatory anxiety in man, and the passive avoidance behaviour of the rats may reflect phobic avoidance.

In addition to the anxiolytic effect of baclofen in the extinction model and in Vogel's test (chapter II), baclofen is active in the four plate test (pers. comm. J. van der Heyden, Duphar BV Weesp). It has been reported by Allikmets and Rago (1988) that baclofen has an anxiolytic action in the elevated plus maze test. It is shown in a pilot study that baclofen exerted a strong tendency to shorten the latency for re-entering the dark compartment in the one-trial passive avoidance test at doses that did not affect open field behaviour (pers. comm. D. de Wied, Rudolf Magnus Institute Utrecht). In addition, baclofen tends to increase exploration in the shock probe conflict test (pers. comm. T.F. Meert, Janssen Research Foundation Beerse). Finally, Audi and Graeff (1987) reported that baclofen does not increase the threshold for aversive brain stimulation. In conclusion, these data indicate that baclofen is effective in several animal models for anxiety, this is in agreement with the clinically observed anxiolytic effect of this compound.

Extinction of conflict behaviour seems a sensitive paradigm for detecting anxiolytic drugs. This is especially of importance concerning the 5-HT modulating agents, which are weakly or not active in the classical tests using conflict behaviour. An interesting feature is that it is now possible to separate drugs into two groups: Those that have both anti-confict efficacy and enhance extinction of conflict behaviour, and those drugs that show selective efficacy, i.e. only enhance extinction of conflict behaviour. These separate groups may reflect different anxiolytic profiles in man.

The fact that haloperidol is active in enhancing extinction might be explainable by the fact that neuroleptics possess anxiolytic efficacy in man (paragraph 1.5.2.). Both dopamine receptor blockade and/or 5-HT receptor blockade (Wander et al. (1987)) might be responsible for its effect on extinction. The action of anti-cholinergic agents might also be due to an anxiolytic action (paragraph 1.5.3.). On the other hand, the effect on extinction might also be partly caused by a decreased ability to retrieve memory for aversive experiences like foot shock punishment (review: Bartus et al. (1987)). Since passive (or active) avoidance is based on memory for aversive experiences (paragraph 1.6.4.), it is conceivable that these kind of tests are open for anxiolytic and amnestic effects.

Relationship with $GABA_A$/benzodiazepine receptors.

After establishing the model of investigation, the mechanism by which baclofen exerts its effect was investigated. Benzodiazepines mediate their anxiolytic effect via facilitating the interaction of GABA with the $GABA_A$ receptor. Therefore, if a benzodiazepine and a $GABA_B$ agonist are active in the same model, three possibilities exist: a/ A shared pathway via the $GABA_A$ receptor, b/ via the $GABA_B$ receptor or, c/ two differents mechanisms with the same behavioural result. The investigation of the first possibility, to establish whether the effect of baclofen on extinction is related with the $GABA_A$/benzodiazepine receptor chloride channel complex, is described in chapter II and V. This possibility had to be considered because of a number of reasons: 1/ Benzodiazepines are indirect $GABA_A$ agonists and also enhance extinction. 2/ $GABA_B$ receptor activation is reported to modulate the release of GABA itself (for references see chapter V), and some groups reported an increase of release which would mean that baclofen is also an indirect $GABA_A$ agonist. 3/ Baclofen is reported to enhance binding of benzodiazepine receptors ex vivo (Gallager et al. (1978), Rago et al. (1986)).

In vitro investigations of Majewska and Chuang (1984), Karobath and Sperk

(1979) and Ketelaars et al. (chapter II) have shown that $GABA_B$ receptor activation does not affect binding to the benzodiazepine receptors, which makes a direct interaction between baclofen and the $GABA_A$/benzodiazepine receptor chloride channel complex unlikely. Also, a clear indirect ex vivo effect of baclofen on benzodiazepine receptor binding could not be shown (chapter II). Although an increase in number of cerebellar benzodiazepine receptors was found in the presence of bicuculline, no effect was present in cortical tissue and in cerebellar tissue without bicuculline addition to the incubation media. The second possibility, an effect of benzodiazepine receptor activation on $GABA_B$ receptors, is highly unlikely since it is reported that benzodiazepines do not affect binding of GABA to the $GABA_B$ receptor, (Majewska and Chuang (1984)), and it is firmly established that benzodiazepines affect $GABA_A$ receptor activation via an allosteric modulation, and not via GABA release, uptake or catabolism (review: Tallman et al. (1980)).

Definite evidence for the non-involvement of the $GABA_A$ receptor in the effect of baclofen on extinction is presented in the in vivo study described in chapter V. The oxazepam-induced extinction of conflict behaviour was antagonized by the $GABA_A$ antagonist bicuculline. This was also shown for the anti-conflict effect of oxazepam, using the same dose regimen for bicuculline (unpublished results). In contrast, the effect of baclofen on extinction was not blocked by bicuculline. Taken together, it can be concluded that benzodiazepines enhance extinction via an enhanced function of $GABA_A$ receptors, while baclofen acts in a $GABA_A$-independent way via activation of $GABA_B$ receptors.

5-HT relationship.

There are numerous thinkable mechanisms by which baclofen may enhance extinction of conflict behaviour. For instance, it is known that $GABA_B$ receptor activation modulates the activity of several neurotransmitter systems (chapter I). It is of interest to investigate whether the anxiolytic effect of $GABA_B$ receptor stimulation is exerted via modulation of other systems, since this would add to the knowledge about the possible role of various neurotransmitter systems in (panic) anxiety. The present investigation has focussed on the 5-HT system. The reasons for this choice have been described in chapter III, IV and V, they concern the anxiolytic properties of 5-HT modulating compounds and the relationship between the $GABA_B$ receptor and the 5-HT system.

In animal models for anxiety, it is generally assumed that a decreased function of the 5-HT system exerts anxiolytic effects and an increased activity exerts an anxiogenic effect. $GABA_B$ receptor activation decreases the firing rate of 5-HT neurons and reduces the release of 5-HT (see paragraph 1.2.3.). If it is postulated that baclofen affects extinction of conflict behaviour via an impaired 5-HT function, a number of experiments can be performed to support this hypothesis. a: Inhibition of biosynthesis, b: activation of autoreceptors, as well as c: postsynaptic 5-HT receptor antagonism should mimic the effect of baclofen on extinction. PCPA (p-chlorophenylalanine, an inhibitor of biosynthesis of 5-HT) and 8-OH-DPAT (autoreceptor agonist) indeed enhance extinction of conflict behaviour (chapter IV). Additional evidence for the suggestion that baclofen and 8-OH-DPAT enhance extinction via a decrease in 5-HT release is provided by the finding that PCPA pretreatment prevents an additional increase of extinction induced by baclofen and 8-OH-DPAT (chapter IV). The observation that fluvoxamine (a selective 5-HT reuptake inhibitor) counteracts the baclofen-induced extinction also supports the 5-HT hypothesis. However, the lack of

effect of fluvoxamine on the 8-OH-DPAT-induced extinction needs explanation (see below). An interesting synergism on sedation (in contrast to the blocked extinction) is observed after combined treatment of rats with baclofen and fluvoxamine (chapter V). As far as it is known at the moment, postsynaptically localized receptors are of the 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 type. The localization of 5-HT_{1C}, 5-HT_{1D} and 5-HT_3 receptors in the synaps is unknown (see paragraph 1.4.2.). In chapter II, III and IV it is shown that 5-HT_{1C}, 5-HT_2 and 5-HT_3 blockade does not enhance extinction of conflict behaviour. In contrast, 5-HT_1 blockade by propranolol enhances extinction, while the selective β-adrenoceptor blockers butoxamine and metoprolol were not active (chapter IV). 5-HT_1 receptors are present on pre- and postsynaptical sites. Since propranolol was shown to antagonize the postsynaptically 5-HT_{1A} receptor-induced 5-HT behaviour in the same dose range as the range in which this drug enhanced extinction (chapter IV), a decreased activation of postsynaptic 5-HT_1 receptors might explain the observed effects of propranolol and baclofen on extinction of conflict behaviour. This hypothesis receives support from other findings: Den Boer (1988) suggested that desensitization of postsynaptic 5-HT_1 receptors underlies the effect of fluvoxamine on panic disorder. In addition, it is reported by Moser et al. (1988) that MDL 73005EF, a selective 5-HT_{1A} antagonist, exerted anxiolytic effects in several animal models for anxiety. It is tentative to suggest that this antagonist mediates its anxiolytic effect via blockade of postsynaptic 5-HT_{1A} receptors. This is supported by the finding that the described anxiolytic effect could be antagonized by relatively high doses of 8-OH-DPAT (Moser et al. (1988)). This implicates that activation of at least a subgroup of postsynaptic 5-HT_1 receptors should exert an anxiogenic effect. This has actually been described for several 5-HT_1 receptor agonists like RU 24969, mCPP and TFMPP in animal models for anxiety (reviews: Broekkamp and Jenck (1989), Chopin and Briley (1987)). Furthermore, it is shown by Engel et al. (1984) that 8-OH-DPAT has an anxiogenic effect in an animal model of anxiety after pretreatment of the rats with PCPA. The latter treatment was suggested to cause a supersensitivity of postsynaptic (anxiogenic) 5-HT_{1A} receptors. Finally, administration of the 5-HT_1 receptor agonist mCPP is reported to elicit anxiety and panic in panic disorder patients (Kahn et al. (1988a)).

Although conflicting data exist, it is assumed that acute administration of a 5-HT reuptake inhibitor like fluvoxamine increases postsynaptic 5-HT receptor activation (see paragraph 1.4.3.). If this holds truth, such a drug should evoke an anxiogenic effect after acute administration, as has been shown to occur in patients suffering from panic disorder. It is shown in chapter V that fluvoxamine does not enhance extinction after acute administration, nor does it decrease extinction in rats with a lower level of conflict. The failure to detect an anxiogenic effect on extinction may depend on the model used, since anxiogenic responses in animal models of anxiety have been reported after administration of several selective 5-HT reuptake inhibitors (Chopin and Briley (1987)). However, fluvoxamine has an anxiolytic action in the defensive burying test and the four plates test (see paragraph 1.6.).

If the effect of baclofen on extinction of conflict behaviour is somehow a reflection of its anti-panic efficacy, long-term administration of fluvoxamine, another anti-panic drug, should also enhance extinction of conflict behaviour. It is shown in chapter V that enhancement of extinction is not accomplished on the 8th and 13th day, but is indeed present at the 16th day, 2 days after terminating the fluvoxamine treatment. Since it is postulated that the anxiolytic effect of fluvoxamine is caused by an adaptive

mechanism after long-term administration, it is conceivable that this adaptive mechanism only occurred after a longer period than 13 days, and is still present 2 days after terminating the fluvoxamine administration. This would be in accordance with the findings of Fontana et al. (1989), who reported a delay in anxiolytic action during treatment with anti-depressants of 2 to 4 weeks.

It is reported that long-term treatment with anti-depressants causes an upregulation of $GABA_B$ receptor number and function. In contrast, 5-HT_{1A} receptor binding and function are decreased after the same treatment (see paragraph 1.3.). In order to find out whether these findings can be generalized to the anxiolytic effect of $GABA_B$ and 5-HT_{1A} receptor activation, the effect of baclofen and 8-OH-DPAT on extinction were monitored during and after fluvoxamine treatment (chapter V). As in the acute experiments, an increased sedation could be observed with combined treatment of baclofen and fluvoxamine in the first two weeks. After termination of fluvoxamine administration, the baclofen-induced sedation was again increased, the latter effect might reflect $GABA_B$ receptor upregulation. It was shown that the effects of baclofen on extinction were counteracted on the 8th and 13th day (again in analogy with the acute experiments), but showed a sharp increase after termination of fluvoxamine. Unfortunately, since the level of statistical significance was not reached in the extinction experiments with baclofen, definite conclusions about $GABA_B$ upregulation after long-term fluvoxamine can not be drawn at this moment.

The reported results on desensitization of presynaptic 5-HT_{1A} receptor number and function (paragraph 1.3.) could not be shown using 8-OH-DPAT-induced extinction of conflict behaviour. As in the acute experiments using fluvoxamine and 8-OH-DPAT, this lack of effect of chronic fluvoxamine on the 8-OH-DPAT induced extinction needs explanation. Several possible explanations can be brought forward: An anxiolytic effect of 8-OH-DPAT via a subgroup of postsynaptic 5-HT_{1A} receptors which do not desensitize after fluvoxamine. This would implicate that activation of postsynaptic 5-HT_{1A} receptors may not only exert anxiogenic effects, but also anxiolytic effects. This latter effect might be exerted by the 5-HT_{1A} receptors with an inhibitory effect on hippocampal activity (see paragraph 1.4.2. and 1.4.4.). Neurochemical data indicate that 5-HT_{1A} receptor activation has two opposite effects on the activity of adenylate cyclase in the rat hippocampus (Devivo and Maayani (1986)). It is tentative to suggest that these neurochemical effects underly an anxiogenic and anxiolytic effect induced by 5-HT_{1A} receptor stimulation. Other explanations for the lack of effect of fluvoxamine on the 8-OH-DPAT-induced extinction are also possible: Although a dose of 10 mg/kg counteracted the effects of oxazepam and baclofen, it is conceivable that higher acute and chronic doses of fluvoxamine were necessary in order to counteract the effect of 8-OH-DPAT. Finally, administration of fluvoxamine by mini-pump might exert different effects on receptor number and/or function than the more usual twice or once daily injections.

In conclusion, the present experiments strongly suggest that the impairment of the 5-HT system, (more precisely, a decreased activation of postsynaptic 5-HT_1 receptors) plays a role in the effect of baclofen on extinction of conflict behaviour. However, this does not implicate that there are no other neurotransmitters systems (e.g. noradrenergic system, glutaminergic system) by which baclofen might affect anxiety.

APPENDIX

AN INTEGRATION OF THE PRESENT INVESTIGATIONS INTO THE EXISTING CONCEPTS OF ANXIETY DISORDERS.

In a review on the phenomenology of panic disorder, Mavissakalian (1988) tentatively postulates that a panic attack is partly a cognitive phenomenon, which is triggered in anxiety prone subjects by aspecific increases in physiological arousal. On its turn, the anxiety proneness is for a great part a genetically transferred phenomenon. The view of Mavissakalian on anxiety disorders is characterized by the notion that the various DSM based syndromes are gradual manifestations of the same constitutional "anxious" predisposition. Gray (1988) and Gorman et al. (1989) describe the neurochemical and neuroanatomical substrate of this "anxious" predisposition and its resulting anxiety disorders; they postulate that the key structure is formed by the septohippocampal system, in which the noradrenergic and 5-HT system play the most important role. The onset of panic attacks is provoked by hyperactivity in the brain stem, where the cell bodies of the 5-HT and noradrenergic neurons originate. The anxiolytics exert their action by impairing the function of these structures, (i.e. reduction of input from locus ceroeleus or raphe nuclei to their projection sites). The septo-hippocampal system functions as a "comparator", it receives sensory input and compares it with memory of equivalent situations. If input from the locus ceroeleus and raphe nuclei is increased, the capacity of the septohippocampal system as a comparator is increased. It might be this area which produces the anticipatory anxiety, since the "scanning" which results from this "comparing" activity seems alike with the symptoms of the patient in this particular form of anxiety. It is reasoned that the anticipatory anxiety would than persist after the panic attack as a result of a kindling phenomenon. The cognitive value which is adhered to the sensory input lies more beyond the septohippocampal system, e.g. the prefrontal cortex. It is postulated that avoidance behaviour has its root in these areas. It has to be remarked here that this theory considers avoidance behaviour as a cognitive/learned response on the panic attacks. However, opposing views on the latter notion exist, as is pointed out in paragraph 1.7.. In a further attempt to synthetize the concepts of Mavissakalian, Gorman and Gray on anxiety, it might be postulated that subjects are more prone to become anxious if their constitutional factors somehow cause an hyperfunction of the septohippocampal system (e.g. supersensitive 5-HT_1 receptors, subsensitive α2-adrenoceptors and subsensitive $GABA_B$ receptors).

Several drugs of different classes are reported to be active on all components of panic disorder. This is shown for anti-depressants, (of which the 5-HT reuptake inhibition seems to correlate with the anxiolytic action), baclofen, alprazolam, or high doses of more current benzodiazepines. In addition, several classes of pharmaca are reported to elicit (panic) anxiety: lactate infusion, caffeine, β-carbolines (benzodiazepine inverse agonists), isoproterenol, yohimbine, CO_2 inhalation and 5-HT system activation.

If one assumes that the neurochemically-based "key" component in the pathophysiology of (panic) anxiety is related with the septohippocampal system, all the above-mentioned therapeutic drugs shoulf affect this "key" component. On first sight, all drugs affect the function of the 5-HT system

and the noradrenergic system (see chapter I). It has to be noted that a reduction in activity of only one system does not induce a complete relief for the panic disorder patient, since the three 5-HT autoreceptor agonists buspirone, ipsapirone and gepirone only affect anticipatory anxiety (paragraph 1.4.4.), and clonidine has only transient and weak anxiolytic effects (paragraph 1.5.1.). The same picture arises for blockade of postsynaptic receptors of one neurotransmitter system: It is shown that antagonism of 5-HT_2 receptors by the selective antagonist ritanserin does not reduce anxiety in panic disorder (Den Boer (1988)). No data are available yet about the specific anxiolytic effects of 5-HT_3 antagonists. Den Boer (1988) has postulated that desensitization of postsynaptic 5-HT_1 receptors may play a role in the anti-panic effects of fluvoxamine. This is supported by a few reports of positive effects of propranolol on panic anxiety but conflicting reports exist, and the consensus is that propranolol has no anti-panic properties (Munjack et al. (1989)). Still, propranolol is an interesting agent since it is a blocker of both β-adrenoceptors and 5-HT_1 receptors although it is not clear whether propranolol is used in patients at doses which indeed block 5-HT_1 receptors. A putative anti-panic effect of postsynaptic blockade of both β-adrenoceptors and 5-HT_1 receptors remains an interesting hypothesis until selective (postsynaptic) 5-HT_1 antagonists are available for clinical studies.

Since impairment of function of one of the two neurotransmitter systems provides no complete relief for the patient suffering from panic disorder, it might be suggested that the more complete anti-panic properties of the drugs mentioned below result from a combined effect on both the noradrenergic and 5-HT system:

Baclofen: Baclofen decreases noradrenergic system activity and also modulates the β-adrenoceptor-coupled adenylate cyclase system. In addition, it inhibits release at the projection sites of the 5-HT system and is closely related to the 5-HT_{1A} receptor at presynaptic and postsynaptic sites. The effect of baclofen on anticipatory anxiety might be explained by its effect on the 5-HT system function while the action of baclofen in the locus ceroeleus, its "fine tuning" of the β-adrenoceptor function in the frontal cortex, and its postsynaptic inhibitory effect in the hippocampus might play an important role in the effect of baclofen on panic attacks and avoidant behaviour.

Alprazolam and high doses of 1,4 benzodiazepines: The benzodiazepines decrease the activity of the 5-HT and noradrenergic system in the raphe nuclei and locus ceroeleus respectively (paragraph 1.2.3.). High affinity benzodiazepines such as alprazolam have a more powerful effect on these systems. The decrease in activity of the 5-HT system may account for the described effects on anticipatory anxiety. The effect of alprazolam and high doses of other benzodiazepines on panic attacks and avoidant behaviour might be explainable by several observations: It has been reported that high doses of benzodiazepines decrease release of noradrenaline in the hippocampus and so mimicked the effect of baclofen in the same experiment (Fung and Fillenz (1983)). Secondly: Alprazolam appears to be linked to the α2-adrenoceptor (Eriksson et al. (1986)).

Selective 5-HT reuptake inhibitors: These compounds modulate the number and function of pre- and postsynaptic 5-HT receptors, β-adrenoceptors and $GABA_B$ receptors. It is not inconceivable that the antidepressant-induced postsynaptic 5-HT_1 receptor desensitization accounts for their effect on anticipatory anxiety. The effect of anti-depressants on panic attacks and avoidant behaviour might be mediated via the combined effect of $GABA_B$ receptor upregulation, 5-HT receptor downregulation and adrenoceptor downregulation.

In conclusion, data derived from the present experiments combined with existing concepts of anxiety disorders lead to the suggestion that the total effect on panic disorder of the above-mentioned agents might be caused by a summation of their effects on both the 5-HT and noradrenergic system.

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SUMMARY

The observation that the $GABA_B$ receptor agonist baclofen (Lioresal) has anxiolytic properties in man formed the basis of the present investigations into the mechanism responsible for the anxiolytic effect of this drug. Baclofen is not active in the Geller-Seifter conflict test, which is a classic animal model for anxiety. Therefore a model had to be developed in order to study this compound. Since baclofen has a beneficial effect not only on panic attacks, but also on anticipatory anxiety and phobic avoidance, the Geller-Seifter test was modified in such a way that the new test might be a putative animal model for the latter two forms of anxiety in man. It is described that conflict behaviour in the Geller-Seifter test shows a slow extinction after removal of foot-shock punishment, enhancement of this process in rats might reflect the disappearance, or decrease, of anticipatory anxiety and phobic avoidance in man.

The first chapter reviews the literature on the neurotransmitters which are somehow connected with anxiety. The emphasis lies on the GABAergic, serotonergic and noradrenergic system. Receptor types, second messengers, neurotransmitter interrelationships, effect of anti-depressants, animal models for anxiety and panic disorder are described.

Chapter II describes extinction of conflict behaviour as an animal model for anxiety. Baclofen, benzodiazepines and haloperidol enhance extinction of conflict behaviour while only the benzodiazepines have an anti-conflict action. Baclofen enhances punished drinking in Vogel's test. It is shown via in vitro and ex vivo receptor binding experiments that the anxiolytic effect of baclofen is probably not mediated via the $GABA_A$/benzodiazepine receptor chloride channel complex.

In the third chapter, it is shown that the 5-HT_2 antagonist ritanserin is not active on extinction and conflict behaviour with foot shock punishment. In contrast, anti-cholinergic compounds have an anti-conflict effect and increase extinction. It is not inconceivable that a mixture of anxiolytic effects and effects on retrieval of memory for aversive events are responsible for the activity of these compounds.

Chapter IV describes the experiments which investigate the activity of several 5-HT modulating compounds on extinction of conflict behaviour. It is shown that impairment of the 5-HT function via biosynthesis inhibition (PCPA), autoreceptor activation (8-OH-DPAT) and postsynaptic 5-HT_1 receptor blockade (propranolol) enhance extinction of conflict behaviour without affecting conflict behaviour itself. Blockade of 5-HT_3 receptors and β-adrenoceptors does not affect extinction of conflict behaviour or conflict behaviour with foot shock. In addition, it is shown that in PCPA pretreated rats, baclofen and 8-OH-DPAT do not enhance extinction above the level of extinction induced by PCPA alone. These data suggest that impairment of the 5-HT system, or more precisely: reduced postsynaptic 5-HT_1 receptor stimulation, plays a role in the baclofen-induced extinction.

In the fifth chapter, conclusive evidence is shown that baclofen enhances extinction in a $GABA_A$-independent way, since the effect of baclofen on

extinction is not antagonized by the $GABA_A$ antagonist bicuculline. In contrast, bicuculline antagonizes the oxazepam-induced anti-conflict effect and the oxazepam-induced extinction of conflict behaviour. Acute administration of the selective 5-HT reuptake inihibitor fluvoxamine counteracts both the oxazepam and baclofen-induced extinction. This supports the suggestion that the latter effect of baclofen is caused by an impairment of the 5-HT system. However, the 8-OH-DPAT-induced extincion was not counteracted with the same dose of fluvoxamine. Long-term administration of fluvoxamine enhances extinction of conflict behaviour. Since it is reported that long-term administration of anti-depressants results in an upregulation and downregulation in number and function of $GABA_B$ receptors and $5\text{-}HT_1$ receptors respectively, the effect of baclofen and 8-OH-DPAT on extinction was observed during and after two weeks of fluvoxamine administration. The reported upregulation of $GABA_B$ receptor sites seems to be supported by the present experiments while, in analogy with the acute experiments, the effect of 8-OH-DPAT on extinction was not affected by fluvoxamine. The implications of these data were discussed.

The present data suggest an important role of an impairment of the 5-HT system, especially reduced activation of the postsynaptic $5\text{-}HT_1$ receptor, in the baclofen-induced extinction of conflict behaviour. It is tentative to suggest that this mechanism also plays an important role in aspects of the anxiolytic action of this drug in panic disorder. In the appendix of chapter VI, an effort was made to make a synthesis of the present results and existing theories on the pathofysiology of anxiety in panic disorder. A speculative hypothesis is described of the neurochemical mechanism behind the effects of baclofen, anti-depressants and alprazolam on panic attacks, anticipatory anxiety and avoidant behaviour.

SAMENVATTING

De paniekstoornis is een ernstige angststoornis die niet (genoeg) op de gebruikelijke angstdempende medicatie, de benzodiazepines, reageert. Deze angststoornis is vaak opgebouwd uit drie componenten: de paniekaanvallen zelf, de anticipatoire angst (angst voor toekomstige paniekaanvallen samengaand met een algemeen verhoogd angstniveau) en het vermijdingsgedrag (agorafobie, angst om in een situatie te komen waar geen adequate hulp voorhanden is ingeval er een paniekaanval start). Aangezien de benzodiazepines in eerste instantie hun werking uitoefenen via de neurotransmitter GABA, leek de redenatie van een tekort aan GABA bij deze patiënten logisch. Vanuit die redenering is er in 1978 door Pepplinkhuizen en Bruinvels een klinisch onderzoek gestart met baclofen (Lioresal), een GABA-analoog. Baclofen bleek op alle componenten van de paniekstoornis een gunstige uitwerking te hebben.

Deze klinische observatie leidde tot dit promotieonderzoek naar het mechanisme achter het anxiolytische effect van baclofen. Om dit onderzoek met proefdieren (ratten) te verrichten moest een diermodel voor angst ontworpen worden waarin ook baclofen een effect zou hebben. Het is gebleken dat baclofen geen effect vertoont in een klassiek diermodel voor angst, de Geller-Seifter test. Deze test meet gedrag bij dorstige ratten die in conflict zijn tussen hun dorst enerzijds en de angst om een lichte electrische schok te ontvangen bij het daadwerkelijke drinken anderzijds. Aangezien de rat in conflict is tussen zijn dorst en zijn angst voor de schok, noemt men het gedrag tijdens de test "conflictgedrag". Omdat baclofen niet alleen een effect heeft op de paniekaanvallen maar ook op de andere twee componenten van de paniekstoornis, werd de Geller-Seifter test gewijzigd, en wel in die zin dat de rat de electrische schok wel verwacht doch zonder dat die schok in de testsituatie aanwezig is. Indien dit een langere tijd het geval is, ebt het conflictgedrag langzaam weg, dit wordt "extinctie van conflictgedrag" genoemd. Hoewel het niet is toegestaan om in antropomorfische termen te denken bij diermodellen, is het verleidelijk om de analogie aan te duiden tussen deze "angst voor de te verwachten schok" van de rat en de anticipatoire angst van de patiënt, en tussen "het achterwege laten van het gedrag dat leidt tot een electrische schok" van de rat en de agorafobie van de mens. Extinctie van conflictgedrag wordt door baclofen versneld, en is verder als model gebruikt om het anxiolytische effect van baclofen te bestuderen.

In hoofdstuk I wordt de literatuur over de met angst gerelateerde neurotransmittoren doorgenomen. De nadruk wordt gelegd op GABA, serotonine en noradrenaline. Aandacht wordt geschonken aan anti-depressiva (welke ook een gunstig effect hebben op de paniekstoornis), de verschillende diermodellen voor angst, en de paniekstoornis zelf. Aangezien de literatuurgegevens daar het meeste aanleiding toe gaven, werd besloten om het onderzoek met name te richten op eventuele effecten van baclofen op het $GABA_A$/benzodiazepine receptor complex en het serotonerg systeem.

Hoofdstuk II legt de basis voor de rechtvaardiging van het gebruik van extinctie van conflictgedrag als diermodel voor angst. Het bleek dat baclofen, benzodiazepines en haloperidol extinctie versterken. Er werden in vitro en ex-vivo receptor binding experimentem uitgevoerd, welke aantoonden dat het angstdempende effect van baclofen niet via het $GABA_A$/benzodiazepine systeem

wordt uitgeoefend.

In het derde hoofdstuk blijkt dat cyproheptadine, een serotonine$_2$ en muscarine receptor blokker, ook extinctie van conflictgedrag versnelt en dat dit via zijn muscarine blokkade bewerkstelligd wordt. Niet uitgesloten kan worden dat het hier om een gemengd anxiolytische en geheugenverstorende werking van deze stof gaat.

Hoofdstuk IV beschrijft het onderzoek naar middelen welke de functie van het serotonerg systeem verminderen. Uit het onderzoek bleek dat vermindering van serotonine biosynthese, vermindering van serotonine afgifte, en blokkade van postsynaptische serotonine$_1$ receptoren een versnelde extinctie veroorzaakt. Blokkade van serotonine$_3$ receptoren heeft geen effect. Tevens bleek, dat bij een verminderde serotonine biosynthese geen extra effect meer op extinctie verkregen werd na baclofen of een serotonine afgifte remmer. Deze resultaten suggereren een effect van baclofen op extinctie via een verminderde functie van het serotonerge systeem. Met name de verminderde postsynaptische activatie van serotonine$_1$ receptoren zou ten grondslag kunnen liggen aan het effect van baclofen.

In het vijfde hoofdstuk wordt nu ook in vivo aangetoond dat baclofen niet via activatie van het GABA$_A$/benzodiazepine systeem kan werken, aangezien de GABA$_A$ blokker bicuculline het effect van baclofen niet tegengaat, terwijl bicuculline het effect van oxazepam, een benzodiazepine, wel tegengaat. De relatie tussen baclofen en het serotonerge systeem werd verder uitgediept door te kijken naar het effect dat de selectieve serotonine heropnameremmer fluvoxamine uitoefent op het effect van baclofen, de serotonine afgifteremmer 8-OH-DPAT en oxazepam. Het effect van fluvoxamine is zowel na acute toediening als na chronische toediening bestudeerd. Fluvoxamine heeft klinisch een anxiolytische werking na verloop van enkele weken. Het blijkt dat fluvoxamine het effect van baclofen en oxazepam op extinctie tegengaat, hetgeen de suggestie van een relatie tussen baclofen en oxazepam enerzijds en het serotonine systeem anderzijds steunt. Na chronische toediening van fluvoxamine heeft fluvoxamine zelf een kortdurend effect op extinctie. Bovendien is het effect van baclofen op extinctie licht versterkt, een effect dat al door andere groepen met behulp van andere diermodellen werd aangetoond. Een ander beschreven effect na een wat langere toediening van fluvoxamine, namelijk een vermindering van effect van 8-OH-DPAT, bleek niet aanwezig te zijn. De betekenis hiervan wordt nader besproken in de discussie van het hoofdstuk.

De verkregen resultaten suggereren een belangrijke rol van een verminderde functie van het serotonerg systeem, in het bijzonder een verminderde activatie van de postsynaptische serotonine$_1$ receptor, bij het effect van baclofen op extinctie van conflictgedrag. Het is verleidelijk om een analogie te trekken met het effect van baclofen op de paniekstoornis bij de mens, zeker omdat er reeds theorieën en bewijsmateriaal bestaan over de betrokkenheid van het serotonerge systeem bij paniekstoornis. Met behulp van bestaande theorieën wordt de implicatie van de verkregen resultaten nader besproken in de algemene discussie en appendix.

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CURRICULUM VITAE

De schrijver van dit proefschrift is geboren te Arnhem, op 26 maart 1958. In 1976 behaalde hij het Atheneum B diploma aan het St Paulus Lyceum te Tilburg. Het artsexamen werd in 1983 afgesloten aan de Erasmus Universiteit te Rotterdam. Tijdens de studie is het keuzepracticum van een half jaar in Kingston, Canada, doorgebracht waar de schrijver de diagnostiek van vernauwingen in de halsslagaders met behulp van ultrageluid heeft bestudeerd. Daarna is nog enig onderzoek in het Academisch Ziekenhuis te Rotterdam verricht naar ultrageluid-echopulsaties in de hersenen. Van juni 1984 tot juli 1988 is bij de afdeling farmacologie onder supervisie van Prof. Dr. J. Bruinvels het in dit proefschrift beschreven promotieonderzoek tot stand gekomen. In de zomer van 1988 was hij 4 maanden arts-assistent neurologie in het St Clara Ziekenhuis te Rotterdam. Vanaf 1 november 1988 is hij deels als arts-assistent, deels als staflid/onderzoeker werkzaam in de polikliniek kinder- en jeugdpsychiatrie van het Academisch Ziekenhuis te Groningen. Op 1 september 1990 wordt een aanvang genomen met de opleiding tot psychiater in het Academisch Ziekenhuis te Groningen.