SLEEP-WAKING STATES AND THE ENDOGENOUS OPIOID SYSTEM

(HET SLAAP-WAAK PATROON EN HET ENDOGENE OPIOIDEN SYSTEEM)

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In the general introductory part of this thesis (Chapters 1 and 2) a review of some pertinent literature related to sleep-waking states and opioid peptides is offered. A global view of the neurochemical mechanisms and theories of functions of sleep, as well as the physiological and possible clinical consequences of total or selective REM sleep deprivation is given in Chapter 1. In Chapter 2, which is concerned with the role of endogenous opioid peptides, particular attention is paid to the possible modulatory role of endogenously released opioid peptides in the regulation of some behavioural states in physiological and pathological conditions.

It is generally known that exogenously administered opiates and opioid peptides can alter sleep pattern and decrease REM sleep. A possible interaction between sleep-waking states and endogenous opioid system is suggested by the report that the episodic release of plasma humoral endorphins during sleep is associated with the REM sleep phase (Chapter 1, section 1.1.7). In addition, the concentrations of opioid peptides in some brain nuclei of the rat which are known to be important in sleep-waking regulation, are highest in the dark (active) phase, during which wakefulness is high and lowest in the light (rest) phase, when the propensity to sleep is at its highest (Chapter 1 section 1.3.2). Thus in order to clarify the effect of endogenously released opioid peptides in the regulation of sleep-waking pattern, we studied the effects of phosphoramidon, an inhibitor of enkephalinase A on sleep-waking states (Chapter 3). Several clinical studies suggest that the sleep-waking cycle may modulate the occurrence of some types of epileptic phenomena in human subjects (Chapter 1, section 1.5.2, iii). In addition, some studies indicate a similarity between enkephalin induced epileptic phenomena and petit mal epilepsy (Chapter 4, discussion). Therefore, we studied the effects of different sleep stages on enkephalin-induced epileptic phenomena using electrophysiological parameters (Chapter 4).

These initial studies (Chapters 3 and 4) suggested an interaction between sleep-waking states and endogenous opioid system. The observation that REM sleep deprivation (REMSD) reduced the pain threshold to noxious electrical stimulation (Chapter 1 section 1.5.2d i) was an indication of the importance
of REM sleep in the regulation of nociception. Therefore in Chapters 5 and 6, the experiments were designed to explore a direct effect of REMSD on the analgesic effects of morphine, an enkephalinase inhibitor phosphoramidon and cold-water-swim.

The profound antagonistic effect of REMSD on opiate/opioid peptide induced analgesia stimulated further interest to investigate the relationship between REMSD and other opiate/opioid peptide modulated behavioural phenomena. Therefore the following opioid modulated behaviours were investigated: akinetic-cataleptic syndrome, spontaneous vertical motor activity, convulsions, grooming, wet-dog-shakes and morphine withdrawal symptoms (Chapters 7-9). Additional experiments with nitrous oxide were performed (Chapter 10) since it is known that this anaesthetic agent can stimulate the release of endogenous enkephalins and induce opiate-like withdrawal symptoms (Chapter 2, section 2.5.2, iv).

Finally, the possible clinical consequences of our findings are described in the relevant chapters.
PART I: GENERAL INTRODUCTION

CHAPTER 1

NEUROBIOLOGY OF SLEEP-WAKING STATES

Sleep is a heterogeneous process organised into rhythmically occurring cycles of different stages which are characterised by specific behavioural, electrophysiological, autonomic and endocrine changes. Broadly, sleep is divided into non-rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep, also called paradoxical sleep (PS) (Aserinsky and Kleitman, 1953). REM sleep occupies a large part of neo-natal mammalian sleep (Roffwarg et al., 1966) suggesting an involvement of REM sleep in brain maturation (Corner et al., 1980). In adult mammals REM sleep appears to regulate adaptivity and plasticity of waking behaviour (McGrath and Cohen, 1978).

1.1 Sleep-wakefulness phenomenology

Sleep and wakefulness, like many life processes from heart-beating to bird migration and hibernation, follow a certain pattern of oscillations, which are regulated by a strict space-time system as well as body position. In nocturnal rodents such as rats, sleep occupies 80% of the light phase and 20% the dark period. REM sleep, which occupies 15–20% of the total sleep time in adult rats, shows a slight preponderance during the light phase (Borbely, 1982).

1.1.1 Behaviour

Pre-sleep behavioural repertory is characterised by the searching for a safe niche and preparing the body (grooming) for sleep (Parmegiani, 1980; Cooper, 1979). Behaviourally, sleep onset is characterised by the suspension of active contact with the environment (Parmegiani, 1980).

A close relationship between electrographic recordings and behaviour has been observed in most animals so far studied. However there is a growing
body of evidence that behaviour and the usual electrophysiological correlates may become dissociated (Sakai, 1985).

1.1.2 Motor functions

Sleep-wakefulness cycle is accompanied by phasic changes in motor activity. In general, quiescence of motor and postural support is a basic feature of sleep. With the onset of sleep there is a progressive decrease in electromyogram (EMG). During NREM sleep muscle tonus is present in an attenuated form, whereas when the animal enters into REM sleep, there is complete loss of the antigravity muscle tonus with consequent failure of postural support (Ursin, 1968; Timo-Iaria et al., 1970). Other important phasic motoric phenomena associated with REM sleep include, myoclonic twitching and burst of rapid eye movements.

1.1.3 Neuronal activity

The pattern and intensity of neuronal activity in some brain areas is often related to the state of vigilance. Bioelectric rhythms of the neocortex, hippocampus, lateral geniculate body, reticular formation and other brain areas have been studied extensively during sleep-wakefulness cycle (Steriade et al., 1977).

1) Neocortex

The neocortical EEG patterns of the rat during different vigilance states has been well documented (Timo-Iaria et al. 1970; Terrier and Gottesman, 1978). Based on these reports the EEG of sleep-wakefulness cycle of the rat can be differentiated into four distinct stages as follows:

a) Wakefulness in rat is characterised by desynchronised (fast) waves (30-40 Hz and 30 μV amplitude).

b) Light slow wave sleep (LSWS) in the rat consists of spindles (6-12 Hz range, 20-40 msec, 50-300 μV), K-complexes and some delta waves in frontal-parietal cortex EEG.

c) Delta sleep: This stage of NREM sleep consists of predominantly delta waves (>50%).

d) REM sleep in the rat is characterised by desynchronised (fast) waves (20 Hz, 40 μV) in the frontal-parietal cortex.

In the cat sleep-wakefulness is differentiated into five stages as
follows: active and quiet wakefulness, light slow sleep, deep slow wave sleep and REM sleep (Ursin, 1968).

ii) Hippocampus and entorhinal cortex

The electrical activity of the hippocampus and the entorhinal cortex undergo distinct oscillations which correlate well with specific vigilance states in the sleep-wakefulness continuum. During exploratory wakefulness and REM sleep, the dorsal hippocampal EEG show a regular theta wave pattern (4-6 Hz) in both rats and cats (Jouvet et al., 1959; Monmaur et al., 1979). In NREM sleep the hippocampus and the entorhinal cortex present an irregular EEG pattern consisting of high amplitude sharp waves intermingled with slow waves (4-6 Hz) in both rats and cats (Jouvet et al., 1959). In the ventral hippocampus spikes waves (100-300 µV, 50-100 msec duration) are most frequent during NREM sleep and are rare during wakefulness and REM sleep (Jouvet et al., 1959; Hartse et al., 1979).

iii) Reticular formation and lateral geniculate body

Phasic EEG spike activity during REM sleep was first demonstrated to occur in the cat pontine reticular formation by Jouvet and Michel (1959), lateral geniculate body by Mikiten and co-workers (1961) and in occipital (visual) cortex by Mouret and co-workers (1963). These spikes have been designated ponto-geniculo-occipital (PGO) waves according to the loci from which they were recorded. PGO waves have not been demonstrated in rats (Stern et al., 1974). They have not been unambiguously demonstrated in human subjects (Gaillard, 1980). The phasic PGO spike waves occur a few minutes prior and during REM episodes and are generally absent during wakefulness and NREM sleep (Hartse et al., 1979).

1.1.4 Respiration

Cyclic changes in respiration have been observed during the sleep-wakefulness cycle (Parmeggiani, 1980; McGinty and Beahm, 1984). During NREM sleep breathing is generally slower, shallower and more regular than in wakefulness. Only small changes in ventilatory response to carbon dioxide and mechanoreceptive reflex occur during NREM sleep compared with wakefulness. In both experimental animals and human subjects, the respiratory rate is irregular, highly variable and may be accompanied by brief apneas during REM sleep. Arousal thresholds in response to hypoxia and
hypercapnia are increased during REM sleep compared with NREM sleep. In addition, response to laryngeal stimulation during REM sleep could consist of apnea instead of the usual coughing.

1.1.5 Cardiovascular system

The heart rate and arterial blood pressure are decreased in both man and animals during NREM sleep compared with wakefulness (Snyder et al., 1964). The largest drop in heart rate and systemic arterial blood pressure in cat was observed during REM sleep (Guazzi and Zanchetti, 1965). However in man heart rate and blood pressure are increased during REM sleep with respect to NREM sleep (Snyder et al., 1964).

1.1.6 Thermoregulation

An ultradian rhythm of homeostasis-poikilostasis which correlate with specific vigilance state has been documented (Parmeggiani, 1980; McGinty and Beahm, 1984). During NREM sleep, the core temperature is regulated at a lower set point compared with wakefulness. The decrease in body temperature is probably due to a combination of the thermoregulatory and passive processes such as reduction in motor action. In contrast to NREM sleep, hypothalamic thermoregulatory mechanisms are inactivated during REM sleep. Physiological and behavioural symptoms such as shivering, thermal vasodilation, polypnea, sweating and thermogenesis in response to either ambient or hypothalamic temperature changes disappeared during REM sleep. The lack of shivering during REM sleep may not be related to muscle atonia, since animals which exhibited REM without atonia after pontine lesion still failed to show shivering (McGinty and Beahm, 1984).

1.1.7 Endocrine and neuropeptide secretions

Sleep-related growth hormone secretion occur during the NREM sleep phase (Takahashi et al., 1968; Mitsugi and Kimura, 1985). The episodic secretion of prolactin is triggered by sleep onset (Parker et al., 1980), while the secretion of cortisol and thyroid-stimulating-hormone are decreased during sleep compared with wakefulness (Parker et al., 1980; Mitsugi and Kimura, 1985).

Recently it was demonstrated that the episodic release of plasma humoral
endorphin is associated with REM sleep (Oksenberg et al., 1980). These data together suggest that sleep processes might play an important role in regulating the functional reactivity of hormonal and neuropeptide systems.

1.1.8 Genital response

Nocturnal penile tumescence (NPT) during sleep has been shown to occur during REM sleep in man (Karacan et al., 1966). The female counterpart of nocturnal NPT, clitoral erection and increase in vaginal pulse pressure are associated with REM sleep (Karacan et al., 1970; Rogers et al., 1985). The highly predictable occurrence of NPT during REM sleep (80-90%) has been used to distinguish organic from psychogenic impotence. For example in organic impotency associated with diabetes mellitus the REM sleep related NPT is greatly diminished, whereas in psychogenic impotency there is little or no reduction in NPT (Karacan et al., 1978).

1.1.9 Circadian sleep-wakefulness phenomena

Biological cycles with a period of approximately one lunar day (24 h), and that persist in the absence of external environmental cues ("zeitgebers"), have been termed circadian oscillations (Aschoff, 1964). In animals and human subjects isolated from external time cues, the sleep-wakefulness cycle continues to show a clearly defined rhythm whose period is only slightly different from 24 h (Groos, 1984). The sleep-wakefulness cycle is therefore a true circadian rhythm which has been demonstrated in rats (Borbely, 1982) and in man (Wever, 1979). Circadian influences on sleep-wakefulness in rats affect phase timing and not the daily amount of sleep. Lesion of the suprachiasmatic nucleus abolished the sleep-wakefulness circadian rhythm but not the daily amount of sleep (Ibuka and Kawamura, 1975). Recently the hypothalamic paraventricular nucleus was demonstrated to be involved in the maintenance of REM sleep rhythm (Piepenbrock et al., 1985).

1.2 Neural basis of sleep-wakefulness

The study of the pathology of viral encephalitis and the associated sleep disorders led von Economo (1929) to differentiate two syndromes, a)
hypersomnia related to lesions of mesencephalic tegmentum and posterior hypothalamus and b) sleeplessness in which the lesion affected basal forebrain and related striate structures. These observations led to the notion of "neural determinism of sleep-waking states".

1.2.1 Neural substrates of wakefulness

The demonstration of ocular and EEG signs of sleep after the transection of the brain stem at the level of the oculomotor nuclei "cerveau isolé" (Bremer, 1935), suggested the presence of an arousal center in the anterior third of the pons. Further lesion studies in the rat by Nauta (1946) placed the "waking center" at the mesencephalo-hypothalamic junction. Later Moruzzi and Magoun (1949) described an arousal system located in the reticular formation of the rostral segments of the pons and the midbrain.

Neural elements rostral to the brain stem reticular formation contributed to wakefulness mechanism. One such brain area was localised in the posterior hypothalamus (Moruzzi, 1964). This observation is supported by the finding that in "cerveau isolé" preparations in which the locus coeruleus and raphe nuclei were completely isolated from the forebrain of the animals, an initial period of hypersomnia was followed by behavioural and EEG patterns of wakefulness (Hanada and Kawamura, 1981).

1.2.2 Neural mechanism of non-rapid-eye-movement (NREM) sleep

The initial report of Hess (1927), that electrical stimulation of midline thalamic nuclei induce behavioural and EEG signs of sleep was the first experimental indication of the existence of an active hypnogenic center. Other workers have since demonstrated that stimulation of other brain areas can induce behavioural and electrophysiological signs of sleep (Favale et al., 1961). Three hypnogenic brain areas may be differentiated: ponto-bulbar raphe system, hypothalamic and thalamo-cortical.

1) Ponto-bulbar raphe system

The existence of medullary sleep centers was suggested by reports that medullary anesthesia, or cooling, converted sleep EEG into an activated, awake, pattern (Berlucchi et al., 1964). EEG synchronisation and behavioural sleep were elicited by low frequency stimulation of the n. tractus solitarius (Favale et al., 1961).
The lesion of raphe complex induced, initially, total insomnia followed by partial sleep recovery in cats (Jouvet, 1974). However other workers failed to implicate raphe nuclei in sleep regulation. For example midbrain raphe lesions in rats failed to alter sleep-wakefulness (Bouhuys and van den Hoofdakker, 1977). Furthermore unit discharge studies, showed that neurons in the dorsal raphe fired less during slow wave sleep compared with waking (McInty and Harper, 1976) and that electrical stimulation of the raphe nucleus resulted in arousal (Jacobs et al., 1973). Thus the exact role of the raphe nuclei in the regulation of sleep and awake vigilance states remains to be clarified.

ii) Hypothalamic system

The sleep modulating effect of the hypothalamic area was first suggested by the study of Hess (1944) in which he elicited sleep by stimulating the preoptic area. Profound insomnia was demonstrated in rats after inducing a lesion in the preoptic area (Nauta, 1946).

iii) Thalamo-cortical NREM sleep system

Medial thalamic stimulation induce behavioural and EEG signs of sleep (Akert et al., 1952). However, the complete destruction of the thalamus eliminated only sleep spindles but not slow waves (Naquet et al., 1965). Sleep has also been elicited by stimulation of the basal forebrain and orbital cortex (Sterman and Clemente, 1962)

1.2.3 Neural mechanisms of REM sleep

The pontine brain structures are essential for REM sleep generation. However a large body of evidence indicate that different features of REM sleep, such as ponto-geniculo-occipital (PGO) spikes, postural atonia and neocortical desynchronisation are regulated by different neural substrates (Sakai, 1985). The pontine-medullary brain nuclei, locus coerulus and n. reticularis magnocellularis are essential for postural atonia during REM sleep (Sakai, 1985).

PGO generators are located in the caudal mesencephalic and rostral pontine tegmental area such as brachium conjunctivum, rostral n. parabrachialis lateralis, locus coerules and n. laterodorsalis, whereas EEG activation during REM sleep involves the n. reticularis magnocellularis (Sakai, 1985).
1.3 Neurochemical basis of sleep-wakefulness

Several endogenous substances, such as the biogenic amines (noradrenaline, dopamine, serotonin and \( \beta \)-phenylethylamine), acetylcholine, gamma-amino-butyric acid and many neuropeptides (enkephalins, endorphins, delta sleep peptide) have been proposed as regulators of different vigilance states.

1.3.1 Biogenic amines, acetylcholine and GABA

i) Noradrenaline (NA)

Inhibition of tyrosine hydroxylase with \( \alpha \)-methyl-p-tyrosine (\( \alpha \)MT) or the blockade of dopamine-\( \beta \)-hydroxylase with disulfiram suppressed REM sleep, reduced waking and decreased the concentrations of NA in the brain (Jouvet, 1974). These data suggested an involvement of NA in the regulation of waking and REM sleep. In contrast \( \alpha \)-MT enhanced REM sleep in the rat (Hartmann et al., 1971). However, Kafi and co-workers (1977) have demonstrated that only high doses of \( \alpha \)-MT, which induced an almost total inhibition of NA synthesis, suppressed REM sleep while lower doses facilitated this sleep stage.

The possible involvement of NA in regulating the sleep-waking states is also suggested by the finding that the electrolytic or surgical lesions of noradrenergic neurons of the locus coeruleus which decreased brain NA levels suppressed REM sleep and reduced wakefulness (Jouvet, 1974). In contrast the presence of REM sleep was demonstrated inspite low brain NA concentrations (Jones et al., 1977).

In summary, inspite of some contradictory data it appears that transmission in NA neurons is involved the modulation of REM sleep and wakefulness.

ii) Dopamine (DA)

Destruction of the ascending DA-fibers of the ventral mesencephalic tegmentum decreased behavioural wakefulness but did not alter electroencephalographic signs of arousal (Jones et al., 1973).

In rats the administration of spiroperidol, a DA receptor antagonist, produced a dose-dependent increase in total sleep and a decrease of REM sleep (Kafi and Gaillard, 1976). Apomorphine (Apo) in doses sufficient to
stimulate post-synaptic DA receptors decreased total sleep and increased waking, whereas low doses of Ape which stimulate DA autoreceptors increased REM sleep time (Kafi and Gaillard, 1976). Similarly small doses of DA enhanced REM sleep (Hartmann et al., 1975). In addition, the possible involvement of various DA receptors in the modulation of alertness has also been suggested (Dzoljic and Godschalk, 1978). However, more recent data suggest that the activation of D-1 receptors induced arousal, while the stimulation of D-2 receptors was associated with sedation and sleep (Gessa et al., 1985).

It thus appears that the dopaminergic system is involved in the regulation of behavioural wakefulness and REM sleep.

iii) 5-Hydroxytryptamine (5-HT, serotonin)

Destruction of the raphe system, which contain the cell-bodies of 5-HT neurons induced insomnia which was associated with decreased brain 5-HT levels in cats (Jouvet, 1974). This finding indicated that 5-HT containing neurons in the rostral part of the raphe system are involved in sleep mechanisms.

The involvement of serotonin in NREM sleep induction was also supported by the finding that para-chlorophenylalanine (PCPA) which depleted brain 5HT decreased both NREM and REM sleep stages in cats and rats (Jouvet, 1974, Borbely, 1982). The sleep suppressant effect of PCPA was reversed by the administration of 5-HT precursors, tryptophan and 5-hydroxytryptophan.

The serotonin theory of sleep has been challenged by several reports. During chronic studies, sleep in raphe-lesion animals tended to return to pre-lesion baseline even though 5-HT levels remained low (Morgane and Stern, 1974). Brain lesions in rats which reduced brain 5-HT concentrations did not alter sleep (Bouhuys and van den Hoofdakker, 1977). Similarly PCPA or a chronic tryptophan-deficient diet, both of which can reduce brain 5-HT, had no effects in rats (Rechtschaffen et al., 1973; Clancy et al., 1978). In addition, serotonin neurons were most active during wakefulness compared with any sleep stage (McCinty and Harper, 1976). Similarly, the release of 5-HT was highest during wakefulness than when the animals were asleep (Puizzillout et al., 1979). In addition, electrical stimulation of 5-HT rich neurons of the dorsal raphe nucleus reduced both NREM and REM sleep stages (Jacobs et al., 1973). Although these data are inconsistent with the general postulate
that 5-HT is a hypnogenic neurotransmitter, 5-HT neurons may play some role in the PGO spikes "gating mechanisms" For example a decreased brain 5HT was associated with the release of PGO spikes into the NREM and awake states (Jouvet, 1974).

Although the role of 5-HT in the regulation of vigilance states is not clear it has been recently proposed that 5-HT, released as neurotransmitter during waking, might also act as a neurohormone in inducing the synthesis and/or the liberation of hypnogenic factor(s). These would be stored, and later influence SWS and FS (Jouvet, 1984).

iv) p-phenylethylamine (PEA)

β-Phenylethylamine is an endogenous occurring amine present in the mammalian brain. It is the substrate for monoamine oxidase B (MAO-B). The structural similarity between PEA and amphetamine (α-methylphenylethylamine) has led to the suggestion that PEA may act as an endogenous amphetamine (Sandler and Reynolds, 1976). Increasing the concentrations of PEA in the brain results in desynchronised EEG pattern and behavioural arousal in several mammalian species (Sabelli et al., 1975; Dzoljic et al., 1977). Sleep polygraphic studies also demonstrated that both NREM and REM sleep stages were suppressed by inhibitors of MAO-B (Cohen et al., 1982). PEA may therefore be considered as one of the neuromodulators of wakefulness.

v) Acetylcholine (ACh)

Several experiments in animals and human subjects have demonstrated an involvement of acetylcholine (Ach) in the induction of REM sleep. Both blockade of Ach receptors and inhibition of Ach synthesis using atropine, scopolamine or hemicholinium is generally accompanied by a decrease in REM sleep. (Domino et al., 1968; Domino and Stawisk, 1970) and reduced the frequency of PGO spikes (Henriksen et al., 1972).

The cholinesterase inhibitor, physostigmine and the muscarinic agonist arecoline, facilitated REM sleep at low doses and wakefulness at high concentrations (Sitaram and Gillin, 1986). In cats, physostigmine prolonged REM sleep periods with increase in rapid eye movements, PGO spikes and atonia (Domino et al., 1968).

vi) Gamma-aminobutyric acid (GABA)

GABA and it's metabolite gamma-hydroxybutyrate (GHB), appear to possess a sleep enhancing effect in several mammalian species. GHB stimulated REM
A sleep-like state in cats (Stock, 1982). A hypnogenic role for GABA, is also supported by the finding that GHB induced EEG synchronisation (Dzoljic et al., 1975). Recently L-cycloserine, a substance which can increase brain GABA increased NREM and REM sleep stages. However REM sleep was decreased by high doses of this substance (Scherschlicht, 1985).

1.3.2 Neuropeptides and hormones

1) Endogenous opioid peptides

Endogenous opioid peptides appear to play an important role in the regulation of sleep-wakefulness cycle in both man and animals. However, many aspects of the relationship between opioid peptides and sleep have not been elucidated yet. Many suggestions related to sleep and opioid peptides are derived from stimulation and/or blockade of opioid receptors with drugs as morphine and naloxone.

Morphine is an opium alkaloid named by Serturner in 1803 after Morpheus the Roman god of dreams. Electrophysiological studies indicate that this name is not appropriate. The intravenous (iv) administration of morphine produced a dose-dependent increase in wakefulness, and muscular tension, while delta sleep, REM sleep (dreaming period) and sleep efficiency were decreased in human subjects (Kay et al., 1969). Lewis and co-workers (1970), demonstrated that heroin also reduced REM sleep, delayed sleep onset and increased wakefulness. Thus, stimulation of opioid receptors consistently induced insomnia in human subjects.

Studies in animals are also supportive of a stimulatory role for opioid peptides. Diurnal variations in enkephalins, β-endorphin and dynorphin in brain areas important for the regulation of sleep-wakefulness correlate with the basic light (rest)-dark (active) cycle in rodents. Thus the peak of methionine-enkephalin concentrations in the anterior, medial basal hypothalamus, preoptic area and stratum occur during the dark period and decline to lowest levels during the light phase (Kumar et al., 1982; Tang et al., 1984). β-Endorphin also reached a peak concentration in the preoptic area, pons, medulla oblongata, cerebellum and anterior pituitary during the dark phase (Kerdelhue et al., 1983). Similarly immunoreactive dynorphin concentrations were highest during the dark phase in the hypothalamus and...
pituityary (Przewlocki et al., 1983). The diurnal variation in opioid peptide concentrations, with peaks during dark periods, suggest that opioid peptides might play an important role in priming animals for wakefulness as the dark period is known to suppress sleep in rats (Inoue et al., 1984; 1985).

Other animal experiments have demonstrated that stimulation of opioid receptors with opioid peptides or opiates is accompanied by an increased wakefulness. Administration of $\beta$-endorphin or morphine decreased both NREM and REM sleep stages and increased wakefulness in rats and cats (Khasan et al., 1967; Echols and Jewett, 1972; King et al. 1981; Scherschlicht et al., 1982). Microinjection of (D-Ala$^2$)-Met$^5$-enkephalinamide (DALA) and $\beta$-endorphin and des-Y-endorphin into the ventral tegmental area stimulated behavioural wakefulness manifested as increased locomotor activity (Broekkamp and Phillips, 1979; Stinus et al., 1980). The lack of a clear sleep-influencing effect after intracerebroventricular administration of met- or leu- enkephalin (Riou et al., 1982) could be explained by the fact that these peptides are rapidly metabolised by enkephalinases present in the cerebrospinal fluid (Dzoljic et al., 1985). Studies with DALA a more resistant analog of met-enkephalin, have demonstrated an increase in both behavioural and EEG wakefulness (Tortella et al., 1978; Dzoljic and Crucc, 1979). DALA was found to induce biphasic effects, an initial stupor with high voltage slow waves with eyes open and arousal with activated EEG pattern.

Collectively these data indicate that endogenous opioid system play an important role in the maintaince of wakefulness in human and animals. The mechanism of the arousal effects of opiates and opioid peptides is still not clear. It does not appear to involve 5-HT or NA, since pretreatment with 5-hydroxytryptophan or $\beta$-methyltyrosine did not alter the arousal effect of morphine (Echols and Jewett, 1972).

An additional point of interest is that diurnal physiological sleep period is preceded by intensive grooming which declines prior to diurnal arousal phase (Bolles, 1960; Cooper, 1981). Following the administration of morphine or the synthetic enkephalin RX 783030 grooming episodes were extended and sleep reduced. Naloxone however decreased grooming and increased sleep (Echols and Jewett, 1972; Cooper, 1981). In contrast, naloxone failed to alter sleep-wakefulness (King et al., 1981). However,
besides this and the fact that the mechanism (s) by which opioid/opiates suppress sleep is not fully understood, it has been proposed that the activation of endogenous opioid system impaired the mechanism responsible for switching from grooming to sleep (Cooper, 1981).

ii) Endogenous sleep-waking factors

The concept of sleep factors (hypnotoxins) was initiated by the classical experiments of Lengendre and Pieron (1910) in which the cerebrospinal fluid (CSF) or serum of sleep deprived donor dog induced sleep in non-deprived recipient dogs. Since then several sleep promoting factors have been extracted from various tissues and fluids from different animals. Two basic approaches have been utilised in the search for the endogenous sleep promoting factors: a) that sleep promoting factor should accumulate during prolonged waking and b) that sleep promoting factors should be extractable during spontaneous sleep.

1) Factor S

Sleep inducing Factor S is derived from CSF and brains of sleep deprived animals (Pappenheimer et al., 1967). The icv administration of Factor S was able to increase delta sleep in rabbits and rats (Pappenheimer et al., 1967; Fencl et al., 1971). Recently Krueger (1983) identified Factor S derived from the urine of sleep deprived subjects as a muramyl peptide. The icv administration of the synthetic analog of muramyl peptide such as muramyl dipeptide (MDP) induced sleep and fever in rat in a similar fashion to Factor S (Krueger, 1983). A slight and slow cumulative increase of SWS in rats were observed only after icv administration of MDP during the dark period (Inoue et al., 1984). MDP is present in the cell membrane of bacteria found in the urine. Whether MDP is truely the endogenous sleep factor S or derived from bacteria floral in the urinary tract remains to be clarified.

2) Sleep-promoting-substance (SPS)

The accumulation of sleep-promoting-substances (SPS) in the brain stem from 24 h sleep deprived rats has been well documented (Inoue et al., 1985). The crude brain stem extracts have been demonstrated consistently to reduce locomotor activity and increase total SWS and REM sleep in mice and rats (Inoue et al., 1985).

Four active components have been separated from the original crude extract SPS. A fraction which appears to be identical to the nucleoside
uridine, increased both SWS and REM sleep in mice (Komoda et al., 1983) and rats (Inoue et al., 1985). An interesting aspect of these studies is that SPS facilitates sleep during the dark period but not in the light phase. This is in line with the concept that an endogenous sleep substance should not induce additional sleep when the physiological demand for sleep has been satisfied (Inoue et al., 1985).

3) REM sleep factor

REM sleep deprivation appears to induce the accumulation of REM sleep factor. The icv administration of CSF from REMSD cats restored REM sleep during PCPA induced insomnia in cats (Sallanon et al., 1982) and reversed the REM sleep deficit induced by β-adrenergic blockade (Adrien and Dugovic, 1984).

4) Delta sleep-inducing peptide

Delta sleep-inducing peptide (DSIP) is derived from the brain and CSF of animal kept asleep by electrical stimulation of the intralaminar thalamus (Monnier et al., 1963). This sleep factor has been identified as nonapeptide (Schoenenberg and Monnier, 1977). Central administration of DSIP enhanced both NREM and REM sleep stages (Ursin and Larsen, 1983; Inoue et al., 1984). However, DSIP injected ip was not effective and tended to increase wakefulness (Tobler and Borbely, 1980). The hypnogenic effect of DSIP was mostly detected at low doses, while high doses were not effective (Scherschlicht et al., 1984). In clinical trials DSIP given intravenously increased REM sleep, SWS, spindle sleep, sleep efficiency and decreased the frequency of awakening in insomniacs. No sleep rebound was demonstrated after drug withdrawal (Schneider-Helmert, 1985). DSIP appear to interact with opioid receptors since it could antagonized stress or morphine-induced insomnia and the naloxone-precipitated withdrawal in dependent animals (Scherschlicht et al., 1984). It thus appear that the pharmacological action of DSIP in human and animals depends on environmental conditions and the pathophysiology of the organism.

5) Mesencephalic reticular formation perfusate

Drucker-Colin (1973) found that the perfusate collected via a push-pull cannula from the mesencephalic reticular formation (MRF) of a sleeping donor cat decreased sleep latency and increased SWS, without affecting REM sleep. Antibodies produced to MFR perfusate peptides collected during REM sleep.
decreased REM sleep in cats.

6) Sleep and waking factors from cerebrospinal fluid (CSF)

CSF collected from rats during the light period (sleep phase) inhibited dark time locomotor activity, while CSF obtained during dark period enhanced locomotor activity in the light phase (Sachs et al., 1976). These findings are of interest since opioid peptides which are possibly neuromodulators of wakefulness, reached peak concentrations during the dark phase (Kumar et al., 1982; Kerdilhue et al., 1983; Tang et al., 1984).

7) Growth hormone (GH)

GH induced a dose-related increase in REM sleep but decreased SWS in both animals and man (Drucker-Colin et al., 1975a; Mendelson et al., 1980). These data support the suggestion that SWS induced GH release is essential for REM sleep induction (Stern and Morgane, 1977).

1.4 Functions of sleep

It is generally known that one night sleep loss is followed by very annoying symptoms such as fatigue and decreased vigilance. Sleep is often assigned a restorative function. Such simplistic a concept would have been tenable if sleep was monophasic and accompanied by a near halt of all activities. However the discovery that NREM and REM sleep stages were distinct states was a tacit indication they may serve different functions. Attempts to formulate the roles of sleep in the life of an organism has given rise to many theories of the function of sleep.

1.4.1 Ontogenetic function

One important observation during ontogenesis is the preponderance of REM sleep (active sleep) in human neonates, rat pups and kittens (Roffwarg et al., 1966; Jouvet-Mounier et al., 1970). Thus in human neonates REM sleep occupies 50 % of the total time and even higher in premature infants. In rat pups, kittens and foetal guinea pigs, REM sleep occupy about 60-80 % of the total time. These reports indicate that REM sleep may provide the necessary endogenous stimuli for brain maturation and development at the critical time during which neural elements are being rapidly organised. This line of reasoning forms the basis for the "ontogenetic theory" of REM sleep function.
This hypothesis is supported by the fact that from birth to young adulthood human NREM sleep was reduced by 25% while REM sleep was reduced by 75% (Roffwarg et al., 1966).

Suppression of active sleep in rat pups with chlorimipramine not only altered neural growth and DNA content of some brain areas but also induced increased anxiety level and a deficiency in sexual activity of adult animals (Corner et al., 1980). Although several reports are supportive of the ontogenetic theory of REM sleep, the persistence of this sleep state in adult animals is still a puzzle. It has been suggested that REM sleep in adults may regulate adaptive behaviours (McGrath and Cohen, 1978).

1.4.2 Cognitive function

In adult animals and humans REM sleep appear to be essential for learning, memory and intellectual functions. A recent comprehensive review showed a consistent positive relationship between learning and an increase in REM sleep (Smith, 1985). Studies based on REM sleep deprivation also suggest a functional role in unprepared learning (McGrath and Cohen, 1978). The cognitive theory of function of REM sleep is also supported by the clinical observations that REM sleep was reduced in mentally retarded patients, (Petre-Quadens, 1966).

1.4.3 Synthetic function

REM sleep provide a condition for increased polypeptide synthesis (Drucker-Colin and Valverde-R, 1982). The peptide synthesis function of REM sleep appear to be related it’s role in learning and memory. Whilst learning increases protein synthesis, substances which can decrease protein synthesis disrupts retrieval and memory consolidation (Rogers et al., 1974; Flood et al., 1975) and suppress REM sleep (Drucker-Colin and Valverde-R 1982). Thus REM sleep in adult animals might allow the high turnover of proteins necessary for neural reprogramming.

1.4.4 Restitutive function

Hartmann (1973) and Oswald (1974) proposed a cerebral or brain restitution function for REM sleep. This view is supported by the findings that REMSD in human subjects reduced the ability to cope with stressful
events (Greenberg et al., 1972). Since REMS is associated with increased motivational behaviour, such as hypersexuality and aggressiveness, REM sleep has also been considered to reduce waking drive behaviours (Vogel, 1979).

The main theory of the function of SWS is that serves bodily restitution and musculoskeletal recovery (Hartmann, 1973; Oswald, 1974). A behaviourally active day increased SWS stages without altering sleep length and REM sleep (Horne and Minard, 1985). In addition, GH, which stimulates uptake of amino acid into tissue (Korner, 1965), is released by SWS (Takahashi et al., 1968).

### 1.5 Sleep deficiency

#### 1.5.1 Total sleep deprivation (TSD)

TSD in rats and man was followed by sleep rebound. An increase in SWS being the most pronounced effect (Borbely, 1982).

Changes in hormones, enzymes, proteins and some neurotransmitters have been observed after TSD. For example, TSD induced an increase in the release of thyroid stimulating hormone, thyroid hormones, melatonin and tyrosine hydroxylase activity (Sinha et al., 1973; Parker et al., 1976; Palmblad et al., 1979). Sterioids such as testosterone, androstenedione, dihydrotestosterone and estradiol were decreased after TSD, while the pituitary hormones prolactin, follicle stimulating hormone, luteinizing hormone and the adrenal cortex hormone, cortisol were not affected (Cortes-Gallegos et al., 1983).

In rats, TSD increased cerebral GABA levels but decreased glutamic acid, glycine, alanine and lysine (Godin and Mandel, 1965). Comparing the effects of TSD and REMS, Panov (1982) found that TSD increased the RNA contents of the locus coeruleus and n. raphe pontis, while REMS was associated with a decrease in RNA in these brain areas.

There were no changes in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels following TSD (Wesemann and Weiner, 1982), whereas there was an increase in 5-HT and 5-HIAA after 1 h sleep recovery (Borbely et al., 1980).

Profound behavioural changes have been demonstrated after TSD. Thus, there was a decrease in speed of performance, alertness, accuracy (Lubin et al., 1974; Loveland and Williams, 1963), motivation, and induced mood.
deterioration (Johnson, 1969). Prolonged TSD (60-90 h) induced depersonalization, hallucination, cognitive disorganisation and loss of thought train (Morris et al., 1960).

Sleep deprivation has also been demonstrated to increase epileptic phenomena (Pratt et al., 1968). Little is known of the biological consequences of selective SWS deprivation.

1.5.2 REM sleep deprivation (REMSD)

1.5.2a Methodological aspects of REM sleep deprivation

In human REMSD is induced by using the forced arousal technique (Dement, 1960). The same method can be used in animals. In this method animals are implanted with EEG/EMG electrodes for sleep stage monitoring and subsequent arousal with sensory stimulus any time the animal entered into REM sleep. Selective REMSD using the forced arousal technique presents very serious practical problem. Using this technique in rats Morden and co-workers (1967) found that the number of awakenings from REM sleep, rose rapidly from 135/8 h on first day to 350 on the third day. It became practically impossible to arouse animals from REM sleep without curtailing NREM sleep. An ingenious method was devised by Jouvet and co-workers (1964) which involves placing a cat on an inverted "flower pot" surrounded by a pool of water. The technique is based on differences in muscle tone during NREM and REM sleep stages. While sitting on the flower pot (=platform, island or pedestal), the animal can obtain NREM sleep since muscle tone is present, but during REM sleep muscle tone is abolished which causes the animal to wet it's nose or fall into the water. The "flower pot" technique was adapted for rats by Cohen and Dement (1965) and for mice by Fishbein (1970). REMSD by the flower pot technique has obvious advantages over forced arousal:

a) several animals can be REM sleep deprived at the same time

b) the experimenter is not required to monitor the sleep polygraph

Both of these, help to reduce the overall cost of the experiments. There are however other practical problems associated with using "flower pot" for REMSD:

a) the extent of REMSD
b) the technique introduces stress factors such as dampness and confinement

b) there is the additional problem of cleaning the tank and animals daily

Several studies have demonstrated that rats on small platforms (4-7 cm diameter) were more REM sleep deprived than animals on large platforms (11.5-14 cm diameter) (Morden et al., 1967; Mouret et al., 1969). These studies revealed that REM sleep was also reduced in the large platform animals although to less extent. However in these studies, sleep was monitored for a few hours rather than the for the whole 4-5 day period. The elaborate studies of Mendelson and co-workers (1973; 1974) in which they followed sleep polygraph of rats on platforms continuously for 4 days have clarified this problem. In these reports rat weighing 200-225 g, and platform with diameters 6.5 cm (REMSD) and 11.5 cm (stress group) were used. Under these conditions, there was no essential difference in the degree of REMSD in the small and large groups during the first 24 h. However in the fourth 24 h rats on large platforms has no REM or NREM sleep loss, whereas in the small platform group REM sleep was reduced by 50~ but NREM sleep was not different from baseline. The work of Mendelson's group sets a good standard (100 g rat body weight/14.7-15.6 cm² platform area) for using the "flower pot" technique to selectively deprive rats of REM sleep. However rats must be maintained for at least 48 h on platforms to achieve REMSD selectivity (Vogel, 1975). A corresponding stress group (large platform, 100g rat body weight/ 54-61 cm² platform area) should be added to the experimental design. The selectivity of the pedestal technique for REMSD in rats has also been recently validated electrophysiologically (Hilakivi et al., 1984). The discrepancies often encountered in studies using the "flower pot" technique are probably due to the uncritical attitude of some workers in disregarding the ratio of animal size to platform diameter or area . This problem has been fully discussed (Hicks et al., 1977).

A second confounding factor in the "flower pot" technique of REMSD is the stress (dampness and isolation) which is associated with the procedure. It has been reported that rats on small platforms lost about 10% of their initial body weight compared with control animals in home cages (Dement et al., 1967). However, Selye's indices of stress (weight loss, adrenal
hypertrophy and thymus atrophy) and stomach ulceration were not significantly different between small (REMSD) and large platform (stress) rats (Mendelson et al., 1974; Levental et al., 1975; Coenen and van Luijtelalaar, 1985). In addition there were no differences in blood counts and lymphoid tissue histology between REMSD and stressed animals (Drucker-Colin et al., 1974). Another procedure has also been used to control for stress factors associated with REMSD by "flower pot" technique. In this rats are forced to swim in water of 18-19 °C, 10 cm deep, for 1-2 h daily and thereafter allowed to have undisturbed sleep. Adrenal hypertrophy and body weight loss were not different between "swimming" rats and animals REM sleep deprived by the "flower pot method (Stern et al., 1971; Mendelson et al., 1974). The Seyle's stress indices were not essentially different in rats REM sleep deprived by the new pendulum procedure or the classical "flower pot" technique (Coenen and van Luijtelalaar, 1985).

Studies in mice (25 g body weight) also indicate that these animals placed on platforms (1-3 cm diameter) were more REM sleep deprived than animals on large platforms (8-25 cm diameter) (Fishbein, 1970; Kitahama and Valatx, 1980).

In conclusion, these data indicate that the "flower pot" technique is still a reliable procedure for REMSD in rodents. The large platform or forced swimming are adequate controls for the stress factors associated with the flower pot technique for REMSD. In order to reduce the stress due to confinement, animals in both small and large platforms should be allowed daily free locomotion in home cages.

1.5.2b Biochemical changes after REM sleep deprivation

1) Serotonergic system

It has been demonstrated that REMSD increased the rat brain serotonin (5-HT) turnover (Pujol et al., 1968). Weiss and co-workers (1968) also confirmed that REMSD increased 5-hydroxyindoleacetic acid (5-HIAA) but decreased 5-HT in the brains of rats deprived of REM sleep. Following REMSD there was also an increase in the formation of [3H]-serotonin from [7H]-tryptophan in the brainstem-mesencephalon in rats (Hery et al., 1970). In cats there were no alterations in the CSF 5-HIAA levels (Radulovacki,
after REMSD, but Livrea and co-workers (1977) demonstrated an increase in 5-HIAA in the lumbar CSF of human subjects, deprived of REM sleep. However, no changes in 5-HT\textsubscript{2} binding sites in the frontal cortex of REM sleep deprived animals have been demonstrated (Farber et al., 1983). REMSD induced changes in 5-HT levels in some brain regions. 5-HT concentrations were decreased in the locus coeruleus and A5 but increased in frontal cortex and ventral tegmentum area without alterations in 5-HIAA concentrations in these brain areas (Mattiace et al., 1983).

\textbf{ii) Dopaminergic system}

Biochemical analysis shows that REMSD for 4-10 days increased striatal dopamine concentrations in rats (Ghosh et al., 1976), without altering lumbar CSF concentrations of homovanillic acid (HVA) in human subjects (Livrea et al., 1977). Farber and co-workers (1983) found that REMSD increased the concentration of dihydroxyphenylacetic acid (DO\textsubscript{PAC}) in the striatum and frontal cortex of rats compared with controls but not with the stress group. There were no changes in the binding of pre- or post-synaptic DA receptors.

\textbf{iii) Noradrenergic system}

REMsd did not alter noradrenaline (NA) turnover brain of rats (Pujol et al., 1968b). However increases in NA turnover after REM sleep rebound was reported (Pujol et al., 1968b). Changes in NA and its metabolites, 3-Methoxy-4-hydroxyphenylglycol (MHPG), after REMSD have recently been demonstrated (Mattiace et al., 1983). It was found that REMSD induced a decrease in NA and MHPG levels in A5, and an increase in MHPG levels, without affecting NA concentrations in the striatum, ventral tegmental area and dorsal raphe. REMSD did not alter \textsuperscript{3}H]clonidine (\textalpha\textsubscript{2}) binding sites (Mogilnicka and Pilo, 1981), but decreased \textbeta receptor binding (Mogilnicka et al., 1980). However other workers found no changes in \textbeta receptor binding in the brain of REMSD animals (Abel et al., 1983).

\textbf{iv) Cholinergic system}

A decrease in Ach concentration was observed in the telencephalon of REMSD rats (Tsuchiya et al., 1989). There were no alterations in striatal concentration of Ach after 96 hr REMSD, whereas a decrease was observed after 10 days of REMSD (Ghosh et al., 1969).

\textbf{v) Amino acids}

In cats deprived of REM sleep GABA concentrations were increased in the
reticular formation, thalamus and frontal cortex, while decreases were observed in the colliculi and caudate nucleus (Micic et al., 1969; Karadzic et al., 1971). Whole brain GABA, glutamic acid, glutamine and threonine were not changed in the brain of REM sleep deprived animals (Himwich et al., 1973).

vi) Peptides
Alterations in peptides in some brain areas and the pituitary have been observed after REMSD. Thus substance P was reduced in locus coerulus (LC), central grey area (CG), medial and dorsal raphe (MR, DR), ventral tegmental (VTA), substantia nigra zona compacta (SNC), medial hypothalamus (HMC) and caudate areas, whereas somatostatin levels were reduced in DR, CG, SNC and VTA. Increases of these peptides were seen in caudate and preoptic areas after REMSD (Mattiace et al., 1981). $\beta$-Endorphin concentrations were decreased in the pituitary but increased in the hypothalamus of REM sleep deprived rats (Przewlocki, 1984).

vii) Nucleic acids and protein metabolism
The neuronal RNA content of the brain stem nuclei, including the supraoptic nucleus, was decreased in REM sleep deprived animals (Demin and Rubinskya, 1974). Similarly, Panov (1982) found a decrease in RNA concentrations in the n. raphe dorsalis, n. raphe pontis and locus coeruleus after REMSD.

Protein synthesis in some brain areas, such as the cerebellum, telencephalon, cerebrum and brainstem, was inhibited by REMSD in rats (Bobillier et al., 1974; Shapiro and Girdwood, 1981).

1.5.2c Electrophysiological changes after REM sleep deprivation

i) Neuronal excitability
Several studies have demonstrated that REMSD lowered seizure thresholds in both animals and man (Cohen and Dement, 1965; Bergonzi et al., 1973). Studies of evoked potentials also indicate that REMSD can modulate neuronal excitability. Thus REMSD facilitated recovery of cortical potentials evoked by external auditory stimuli (Dewson et al., 1967). Satinoff and co-workers (1971) have reported that REMSD increased the paleocortical excitability as assessed by evoked potentials, but decreased evoked activities in the
hindbrain sensory areas induced by stimulation of hindbrain sensory nuclei. It was then suggested that REMSD amplifies cortical responsiveness by inhibiting internally generated signals (Satinoff et al., 1971). In line with the concept that REMSD increased cortical excitability, Bowersox and Drucker-Colin (1982) demonstrated that the amplitude of entorhinal cortical evoked potentials, following prepyriform cortex stimulation, was increased by REMSD. In contrast, photic evoked potentials in the visual cortex was decreased after REMSD (van Hulzen and Coenen, 1984). In general REMSD increased neuronal excitability.

1.5.2d Behavioural changes after REM sleep deprivation

i) Nociception

REM sleep appears to be essential for the regulation of nociception. Thus it has been demonstrated that REMSD reduced the pain threshold to noxious electrical stimulation. The decrease in pain threshold was still evident 96 hr after the termination of REMSD (Hicks et al., 1978; 1979a).

ii) Waking motor and motivated behaviours

An increase in the number of cage crossings has been demonstrated during the 10-15 min after REMSD (Albert et al., 1970; van Hulzen and Coenen, 1981). REMSD reduced neophobia, increased exploration, ambulation and rearing (Hicks et al., 1979b; Moore et al., 1979; Mogilnicka et al., 1985). Shock provoked aggression was facilitated in REM sleep deprived animals (Hicks et al., 1979).

Hypersexuality, manifested as compulsive mounting behaviour was provoked by chronic REMSD in cats (Dement et al., 1967).

REMSD lowered the threshold and frequency for intracranial self stimulation (ICSS) at the medial forebrain bundle site in rat (Steiner and Ellman, 1972), whereas ICSS of the lateral hypothalamic area was not altered (Marti-Nicolovius et al., 1984).

Further support for the concept that REM sleep is involved in motivational behaviours, comes from the report that REMSD also enhanced food competition between male rats (Hicks et al., 1981).

iii) Learning and memory

Several authors have studied the effect of REMSD on learning and memory,
however most of the results are conflicting (Smith, 1985). For example Stern (1971) reported that REMSD induced a clear learning deficit in one way active and passive avoidance tests but Albert and co-workers (1970) could not confirm this observation. Similarly, retention of a condition passive avoidance response was disrupted by REMSD (Leconte and Bloch, 1970). These conflicting findings appear to be due to the lack of proper control of rat body weight to platform size, so that there may have been no great difference in REMSD between small (REMSD) and large (control) platform animals (Hicks et al., 1977). However another group using a different procedure for REMSD found no alteration in the two way shuttle avoidance response in animals deprived of REM sleep (van Hulzen and Coenen, 1979).

Contradictory effects of REMSD on memory in humans have also been reported. REMSD had no effect on memory (Chernik, 1972) whereas a decrease in memory was reported after REMSD (Pouler et al., 1975). In another study REMSD decreased creativity (divergent or flexible thinking) but improved serial memory task (Lewin and Glaubman, 1975).

Although some inconsistency exist in literature, the weight of evidence suggests that REMSD may disrupt complex and unprepared forms of learning, especially those with emotional components (McGrath and Cohen, 1978).

1.5.3 Clinical aspects of REM sleep deprivation

1) Depression

Sleep pattern is generally altered in depressive conditions. The major disturbance in depression involves shortened REM latency, an extended first REM period, increased REM density/activity and a decrease in delta sleep (Kupfer et al., 1984).

It has been demonstrated that REMSD can improve some forms of endogenous but not reactive depressions (Vogel et al., 1975; Vogel, 1980). In these elaborate clinical studies, endogenous depressives that were not improved by REMSD did not respond to imipramine. Summarizing the recent literature, Vogel (1983) outlined evidence for the therapeutic efficacy of REMSD in a subtype of endogenous depression:

a) REMSD and imipramine have similar therapeutic efficacies

b) The usefulness of antidepressant was related to their
ability to induce sustained and large REMSD
c) Drugs such as barbiturates, alcohol, diphenylhydantoin, opiates and amphetamines can induce short-lasting REM sleep reduction. In addition tolerance to REM sleep inhibiting properties of these drugs develops rapidly, usually within one week or less. These drug possess no antidepressant properties.
d) The behavioural effects of REMSD in animals, such as increased sexuality and aggressiveness, are opposite to the behavioural alterations present in human depression.

In addition, wide range of typical and atypical antidepressants, induced selective REM sleep suppression with little or no alterations in NREM sleep stage (Schereschlicht et al., 1982).

It has become generally accepted that REMSD can improve some forms of endogenous depression and may in fact be part of the mechanism by which antidepressants exert their therapeutic action (Vogel, 1983). The antidepressant action of REMSD was long lasting up to 21 days.

ii) Schizophrenia

Unlike depression, REMSD did not alter schizophrenic symptoms (Vogel and Traub, 1968; Gillin et al., 1974). It was also demonstrated that REM sleep rebound, following REMSD, was exaggerated in schizophrenics. Thus it was suggested that active schizophrenia was associated with a decrease in REM sleep need (Gillin et al., 1974).

iii) Epilepsy

Sleep disturbances and seizure phenomena often co-exist in human epileptics. For example, patients with grand mal seizure had reduced REM sleep, whereas NREM sleep stage 2 was increased (Besset, 1982). Sleep pathology in epileptics with cortical or deep temporal foci was mainly in the form of a decrease in NREM stages 3 and 4 (Montplaisir et al., 1982).

In addition to sleep disturbances associated with epilepsies, some seizure activities occur preferentially in sleep while others occur in wakefulness.

The paroxysmal discharges in petit mal epilepsies were facilitated by sleep onset and awakening. Two subgroups of petit mal seizure exist, classified according to the distribution of epileptic discharges during
sleep. In one group epileptic discharges occur during REM sleep, while in the second group paroxysmal discharges were suppressed during REM sleep (Ross et al., 1966; Billiard, 1982).

REMSD has been demonstrated consistently to lower the seizure threshold in experimental animals (see section 1.5.2c). In human epileptics, REMSD facilitated seizure activity during the night after REMSD was stopped (Bergonzi et al., 1973). In another report selective SWS deprivation, rather than REMSD, was more effective in provoking epileptic attacks in pycnoleptic children (Beck et al., 1977).
REFERENCES


Domino EF, Yamamoto K, Dren AT (1968) Role of cholinergic mechanisms in states of wakefulness and sleep, In Anticholinergic Drugs and Brain function in animal and human, Progress in Brain Research, PB Bradley, M Fink (eds), Elsevier, Amsterdam, vol 28, pp 115-133


Drucker-Colin RR, Jaques LB, Cunningham TA (1974) Anemia from sleep
deprivation with anticoagulants in rats with enhancement by PCPA.
Drucker-Colin RR, Spanis CW, Hunyadi J, Sassin JF, McGaugh JL (1975a)
Growth hormone effects on sleep and wakefulness in the rat.
Neuroendocrinol., 18:1-8
Drucker-Colin RR, Valverde-R C (1982) Endocrine and peptide functions in
the sleep-waking cycle. In Sleep: clinical and experimental aspects,
Current topics in Neuroendocrinology, D Ganen. D Pfaff (eds), pp 35-81
Dzoljic MR, Bonta IL, Godschalk M, Lagendik, Stefaako S (1975)
EEG-synchronizing effect of 3-hydroxybutyrate and
1-hydroxy-3-amino-pyrrolidone-2 (HA-966) in relation to dopaminergic
brain function, Neuropharmacol., 14: 591-599
Dzoljic MR, Bruinvels J, Bonta IL (1977) Desynchronization of electrical
activity in rats induced by deprenyl- an inhibitor of monamine
oxidase-B and relationship with selective increase of dopamine and
8-phenylethylamine, J. Neural Transm., 40: 1-12
Sleep Res., 8: 93
Dzoljic MR, Godschalk M (1978) The role of different dopamine receptors in
electrophysiological alertness, Waking Sleep, 2: 153-155
Dzoljic MR, Ukponmwan CE, Ruprecht J, Haffmans J (1985) Role of the
enkephalinergic system in sleep studied by an enkephalinase inhibitor,
In Sleep: Neurotransmitters and Neuromodulators, A Wauquier, JM
Caillard, JM Monti, M Radulovacki (eds), Raven Press, New York, pp
25-265
Echols SD, Jewett RE (1972) Effects of morphine on sleep in the cats,
Psychopharmacol., 24: 435-448
Farber J, Miller JD, Crawford KA, McMillen BA (1983) Dopamine metabolism
and receptor sensitivity in rat brain after REM sleep deprivation,
Favale E, Loeb C, Rossi GF, Sacco G (1981) EEG synchronization and
behavioral signs of sleep following low frequency stimulation of the
Fencl V, Kosti G, Pappenheimer JR (1971) Factors in cerebrospinal fluid
from goats that affect sleep and activity in rats, J. Physiol. (Lond), 216: 565-589


Gaillard JM (1980) Electrophysiological semiology of sleep, Experientia, 36: 3-6


Greenberg R, Pillard R, Pearlman C (1972) The effect of dream (stage REM) deprivation on adaptation to stress, Psychosomatic Medicine, 34: 257-262


Hartmann EL (1973) The functions of sleep, New Haven, Yale University Press
Hartmann E, Zwilling G (1975) Intraventricular norepinephrine and dopamine effects on sleep in the rat, Neurosci., 1: 248
Hartse KM, Eisenhart SF, Bergmann EM, Rechtschaffen A (1979) Ventral hippocampus spikes during sleep wakefulness and arousal in the cat, Sleep, 1: 231-246
Horne JA, Minard A (1985) Sleep and sleepiness following a behaviourally "active" day, Ergonomics, 28: 567-575
Ibuka N, Kawamura K (1975) Loss circadian rhythm in sleep-wakefulness cycle in the rat by suprachiasmatic nucleus lesions, Brain Res., 96: 76-81


Karadzic V, Micic D, Rakic L (1971) Alterations of free amino acids concentrations in cat brain induced by rapid eye movement sleep deprivation, Experientia, 27: 509-511

Kay DC, Eisenstein RB, Jasinski DR (1969) Morphine effects on human REM state, waking state and NREM sleep, Psychopharmacol.. 14: 404-416


King C, Masserano M, Codd E, Byrne WL (1981) Effects of 8-endorphin and morphine on sleep-wakefulness behavior of cats, Sleep, 4: 255-262


Loveland NT, Williams HL (1963) Adding, sleep loss and body temperature, Percept. Motor Skill, 16: 923-929


McIntyre DJ, Beahm EK (1984) Neurobiology of sleep, In Sleep and breathing NA Saunders, CE Sullivan (eds), Marcel Dekker Inc, pp 1-89


Mendelson WB, Guthrie R, Frederick G, Wyatt RJ (1973) Should flower pots be used for flower pots or rats, Sleep Res., 15: 501


Mitsugi N, Kimura F (1985) Simultaneous determination of blood levels of corticosterone and growth hormone in the male rat: relation to
sleep-wakefulness cycle, Neuroendocrinol., 41: 125-130


Moruzzi G, Magoun HW (1949) Brain stem reticular formation activation of the EEG, Electroenceph. clin. Neurophysiol., 16: 2-17


Parmeggianni PL (1980) Behavioural phenomenology of sleep (somatic and vegetative), Experientia, 36: 6-11

Parmelee A, Wenner W, Schulz H (1964) Infant sleep patterns from birth to 16 weeks of age, J. Pediat., 65: 576-582


Pujol J, Hery F, Durand M, Glowinski J (1968a) Augmentation de la synthesis de la serotoninine dans le tronc cerebral paradoxale, CR. Acad. Sci., 267: 267-372

Pujol J, Mouret J, Glowinski J (1968b) Increased turnover of cerebral norepinephrine during rebound of paradoxical sleep in the rat, Science, 159: 112-114

Radulovacki M (1973) Comparison of effects of paradoxical sleep deprivation and immobilization stress on 5-hydroxyindoleacetic acid in cerebrospinal fluid, Brain Res., 60: 255-258


Rogers LJ, Drennen HD, Mark RF (1974) Inhibition of memory information in imprinting period: irreversible action of cyclohexamide in young
Sandler M, Reynolds GP (1976) Does D-phenylethylamine cause schizophrenia. Lancet, 70-71
Schoenenberger Ga, Monnier M (1977) Characterization of
Sinha AK, Ciarnello RD, Dement WC, Bachas JD (1973) Tyrosine hydroxylase activity in rat brain following REM sleep deprivation, J. Neurochem., 20: 1289-1290
Stern WC (1971) Acquisition impairments following rapid eye movement sleep deprivation in rats, Physiol. Behav., 7: 345-352
Stern WC, Miller FP, Cox RH, Maiokel RP (1971) Brain norepinephrine and serotonin levels following REM sleep deprivation in the rat, Psychopharmacol., 22: 50-55


Tobler I, Borbely AA (1980) Effect of delta sleep inducing peptide (DSIP) and arginine vasotocin (AVT) on sleep and motor activity in the rat, Waking Sleeping, 4: 139-153


Ursin R (1968) The two stages of slow wave sleep in the cat and their relation to REM sleep, Brain Res., 11: 347-356


Weiss E, Bordwell B, Seeger M, Lee J, Dement WC, Barchas JD (1968) changes in brain serotonin (5HT) and 5-hydroxyindole-3-acetic acid (5-HIAA) in REM sleep deprived rats, Psychophysiol., 5: 209
ENDOGENOUS OPIOID PEPTIDES

2.1 Nomenclature and classification

The discovery that electrical stimulation of the periaqueductal gray area induced morphine-like analgesia (Akil et al., 1972) led to the search for an endogenous opiate ligand. Later Hughes and co-workers (1975; 1975a) isolated an endogenous compound from the brain with pharmacological properties similar to that of morphine. They termed this endogenous morphine-like compound enkephalin (from en kephalos, in the head). From the initial extract named enkephalin two related pentapeptides which differ only in the carboxyl terminal amino acid were identified (Hughes et al., 1975b), namely methionine-enkephalin (met-enkephalin, met-ENK) and leucine-enkephalin (leu-enkephalin, leu-ENK). Since the initial discovery of endogenous opioid pentapeptides by Hughes and co-workers, several other ligands with opioid activity have been isolated from various tissues in different animals (Hughes et al., 1980; Bloom, 1983).

Other peptides with opioid activity were found in the pituitary and designated $\beta$-endorphin (Li and Chung, 1976), $\alpha$- and $\gamma$-endorphin (Bradbury et al., 1976; Ling et al., 1976). Opioid peptides which are extended leu-ENK sequences have been extracted from the pituitary. Goldstein and co-workers (1979) isolated and named a pituitary opioid peptide as dynorphin$\textsubscript{1-13}$. In the same year another opioid peptide, a decapeptide described as "big" leu-ENK was named $\alpha$-neo-endorphin (Kangawa and Matsuo, 1979). Other peptides include dynorphins$\textsubscript{1-6}$, dynorphin$\textsubscript{1-17}$ and $\beta$-neo-endorphin (Minamino et al., 1980; 1981; Goldstein et al., 1981). Several other morphine-like substances have been isolated from various animal tissues for example, $\beta$-casomorphins derived from $\beta$-casein (Brantl et al., 1979) and humoral (H) endorphin (Sarne et al., 1978).
2.2 Metabolism of endogenous opioid peptides

At present three classes of endogenous opioid peptides have been described: β-endorphin, enkephalins and dynorphin. There is now sufficient evidence that these opioid peptides originate from three different precursors (Hughes et al., 1980).

2.2.1 Biosynthesis

i) β-endorphin

Biochemical evidence in the literature indicate that β-endorphin and other non-opioid peptides such as corticotropin, α- and β-melanocyte-stimulating hormone (MSH), α-lipotropin and adrenocorticotrophic hormone (ACTH) are formed from a common precursor termed pro-opiomelanocortin (POMC) (Hughes et al., 1980; Bloom, 1983). The POMC derived peptides are produced by proteolytic cleavage of the lipotropin.

ii) Enkephalins

Several studies have demonstrated that enkephalins are produced from a ribosomally synthesized protein precursor termed pro-enkephalin (Hughes et al., 1980). This pro-enkephalin contains one copy of leu-ENK and six of met-ENK (Noda et al., 1982). Several synthetic analogues of enkephalins have been produced for example, D-Ala²-met⁵-enkephalinamide (DALA) and D-Ala²-D-Leu⁵-enkephalin (DADLE).

Structures of enkephalins

Met-enkephalin: Tyr-Gly-Gly-Phe-Met
Leu-enkephalin: Tyr-Gly-Gly-Phe-Leu

iii) Dynorphin

Dynorphin and α-neo-endorphin are derived from a protein precursor termed prodynorphin (Kakidani et al., 1982).
2.2.2 Biodegradation of enkephalins

Opioid peptides are inactivated by several peptidases present in mammalian tissues.

i) Soluble aminopeptidases

Aminopeptidases degrade enkephalins by cleavage of the Tyr-Gly peptide bond. The major metabolite following aminopeptidases hydrolysis of enkephalins is tyrosine (Hambrook et al., 1976). These enzymes can be inhibited by bestatin.

ii) Enkephalinase A

Enkephalinase A is a dipeptidyl-carboxypeptidases which cleaves Gly-Phe peptide bond in enkephalins. Enkephalinase A is sensitive to inhibition by thiorphan, phosphoramidon and kelatorphan (Hudgin et al., 1981; Waksman et al., 1985).

iii) Enkephalinase B

Enkephalinase B is a dipeptidyl-aminopeptidase which inactivates enkephalins by cleavage of Gly-Gly amide bond (Gorenstein and Snyder, 1979). No selective inhibitor of this enzyme has been demonstrated.

iv) Angiotensin-converting-enzymes (ACE)

Similar to enkephalinase A, ACE hydrolyses Gly-Phe amide bond of enkephalins, but does not appear to play an essential role in vivo situations (Erdos et al., 1978). Captopril which is an inhibitor of this enzyme, does not modulate the enkephalinergic system in vivo.

Enkephalins are inactivated in vitro by all four enzymes mentioned above. However, the soluble amino peptidases and enkephalinase A appear to be involved in the biotransformation of synaptic enkephalins in vivo.

Knowledge of the biological mechanisms involved in the inactivation of $\alpha$-endorphin and dynorphin is still poor.

2.3 Opioid receptors

The existence of specific opioid receptors in the mammalian brain was demonstrated simultaneously by three groups (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973). Later studies of Martin and co-workers (1976) on the effects of opiates in spinal dogs indicated the heterogeneity of opioid receptors. The three different opiates, morphine,
ethylketocyclazocine (EtKCZ) and N-Allylnorphenazocine (SKF 10047) produced distinct syndromes. Based on this behavioural study the existence of three different opioid receptors have been suggested: \( \mu \) (mu) for morphine, \( \kappa \) (kappa) for EtKCZ and \( \sigma \) (sigma) for SKF 10047 (Martin et al., 1976).

The concept of multiple opioid receptors was also confirmed and extended in studies utilizing, guinea-pig ileum and mouse vas deferens bioassays and autoradiography (Lord et al., 1977). These authors concluded like Martin and co-workers that morphine showed preference for \( \mu \)-receptors. However the enkephalins, especially leu-ENK, interacted mainly with the opioid receptors designated \( \delta \)-receptors. \( \delta \)-Endorphin was equipotent at \( \mu \) and \( \delta \)-receptors. More recently two subtypes of \( \mu \) receptor designated \( \mu_1 \) and \( \mu_2 \) have been suggested (Pasternak et al., 1983). Enkephalin bind to \( \mu_1 \) and \( \delta \) sites, while morphine bind to both \( \mu_1 \) and \( \mu_2 \) sites. Dynorphins show preference for \( \kappa \)-receptors (Garzon et al., 1983). The relative affinities of some opiates and opioid peptides for opioid receptors are shown in table I.

**Table I: Relative affinity of ligands for opioid receptors**

*(modified after Hughes et al. 1980, Garzon et al., 1983)*

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu )</td>
</tr>
<tr>
<td>met-ENK</td>
<td>++</td>
</tr>
<tr>
<td>leu-ENK</td>
<td>+</td>
</tr>
<tr>
<td>( \alpha )-endorphin</td>
<td>+++</td>
</tr>
<tr>
<td>morphine</td>
<td>+++</td>
</tr>
<tr>
<td>dynorphin(1-17)</td>
<td>++</td>
</tr>
<tr>
<td>EtKCZ</td>
<td>+</td>
</tr>
<tr>
<td>Naloxone</td>
<td>+++</td>
</tr>
</tbody>
</table>

Key: +=low, ++=moderate, +++=high
Similarly a heterogeneity of opioid receptors has been demonstrated in the human brain (Maurer et al., 1983). The importance of multiple opioid receptors for physiological functions of endogenously released opioid peptides is not yet clear. The opioid receptor types possible involved in the pharmacological effects of opiate and opioid peptides are indicated in table III.

### 2.3.1 Opioid receptor distribution in the brain

The idea that the various opioid receptors are differentially distributed in various brain areas is supported by considerable body of evidence (Atweh and Kuhar, 1983). Recently the distribution of µ and δ-opioid receptors in the rat brain was assayed by using highly selective ligands $[^2]\text{Tyr-D-Ala-Gly-NMethyl-Phe-GL-ol}$ (DAGO) for µ-receptor and $[^2]\text{D-Thr}^2,\text{Thr}^6\text{-Leu-enkephalin}$ (DTLET) for δ-receptors (Quirion et al., 1983b). Table II show the relative preponderance of µ and δ-receptors in some brain regions.

The distribution of κ-receptors in the rat brain is similar to that of µ-receptors (Quirion et al., 1983a).

### 2.4 Physiological implications of regional distribution of opioid peptides

#### 2.4.1 Analgesia

Opioid peptides are present in high concentrations in the brain areas (periaqueductal gray area, intralaminar thalamic nuclei and raphe nuclei) and spinal cord (laminae I and II of dorsal horn) related to pain and analgesia (Hokfelt et al., 1977; Simantov et al., 1977). Electrical stimulation of the PAG and pituitary has been demonstrated to induce naloxone reversible analgesia (Akil et al., 1972; Yamagida et al., 1985) possibly by causing the release of opioid peptides. A lesion of the arcuate nucleus, which reduced $\delta$-endorphin levels in the hypothalamus, periventricular area and in the neurointermediate lobe of the pituitary was reported to decrease pain threshold (Millan et al., 1980). REMSD decreased the pain threshold to noxious electric shock (chapter 1, section 1.5.2b) and the pituitary $\delta$-endorphin concentrations (Przewlocki, 1984). The involvement of
Table II: Relative preponderance of opioid receptors in some brain areas (modified after Quirion et al. 1983b)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>n. accumbens</td>
<td>+++</td>
</tr>
<tr>
<td>frontal cortex</td>
<td>++</td>
</tr>
<tr>
<td>layers II-IV of cortex</td>
<td>+++</td>
</tr>
<tr>
<td>layers V-IV of cortex</td>
<td>+</td>
</tr>
<tr>
<td>caudate-putamen</td>
<td>+++</td>
</tr>
<tr>
<td>olfactory tubercle</td>
<td>+</td>
</tr>
<tr>
<td>septum</td>
<td>+</td>
</tr>
<tr>
<td>thalamus</td>
<td>+++</td>
</tr>
<tr>
<td>amygdala</td>
<td>+++</td>
</tr>
<tr>
<td>hypothalamus</td>
<td>+</td>
</tr>
<tr>
<td>hippocampus</td>
<td>++</td>
</tr>
<tr>
<td>habenula</td>
<td>+++</td>
</tr>
<tr>
<td>interpeduncular nucleus</td>
<td>+++</td>
</tr>
<tr>
<td>central gray</td>
<td>+</td>
</tr>
<tr>
<td>cerebellum</td>
<td>-</td>
</tr>
<tr>
<td>n. tractus solitarius</td>
<td>+++</td>
</tr>
<tr>
<td>locus coeruleus,</td>
<td>+++</td>
</tr>
<tr>
<td>dorsal horn (spinal cord)</td>
<td>+++</td>
</tr>
</tbody>
</table>

Key: -= absent, += low, ++= moderate, +++= high, ++++= very high
endogenously released opioid peptides in the regulation of nociception is also supported by other reports. The icv administration of enkephalinase A inhibitors, thiorphan and phosphoramidon which are known to potentiate the enkephalinergic system (Hugdin et al., 1981) increased the pain threshold (Carenzi et al., 1981; Rupreht et al., 1983). $\alpha$-Endorphin given centrally induced analgesia in both animals and human subjects (Loh et al., 1976; Hosobuchi and Li, 1978).

2.4.2 Locomotor activity

Opioid peptides are present in high concentrations in caudate nucleus, putamen, globus pallidus (basal ganglia), amygdala, substantia nigra and in the ventral tegmental area (Khachaturian et al., 1983). These brain areas are important in the regulation of skeletal muscle tone and locomotor activities (Chambers et al., 1971). In Parkinsonism a deficiency of ENK in the pallidum, putamen, substantia nigra and the ventral tegmental area has been demonstrated (Taquet et al., 1983; Agid and Javoy-Agid, 1985). Opioid receptors in the substantia nigra and striatum have been implicated in opiate-induced muscular rigidity and catalepsy (Turski et al., 1983).

The administration of $\alpha$-endorphin or met-enkephalin icv can stimulate morphine-like catalepsy in rats (Bloom et al., 1976; Chang et al., 1976). Similarly, the administration of an enkephalinase inhibitor, thiorphan also induced hypotonic immobility in mice (Chaillat et al., 1983). The medial preoptic area, n. accumbens, periaqueductal gray area, and anterior hypothalamus are particularly sensitive to the cataleptogenic effects of opiates and opioid peptides (Tseng et al., 1980; Winkler et al., 1982). However the administration of low doses of morphine or opioid peptides icv stimulated locomotor activity, wet-dog-shakes (WDS) and body scratching in rats (Wei et al., 1977; Brady and Holtzman, 1981). Microinjection of morphine, enkephalins and $\beta$-endorphin into ventral tegmental area or n. accumbens produced behavioural changes characterised by sniffing and grooming interrupted by bursts of locomotor activity. These stimulant actions of opiates and opioid peptides were naloxone-reversible (Pert and Sivit, 1977; Kelly et al., 1980; Stinus et al., 1980). Similarly naltrexone-sensitive WDS, grooming and body scratching were also observed following icv administration of the enkephalinase inhibitor phosphoramidon (Rupreht et al.,
2.4.3 Temperature

A role for endogenously released opioid peptides in temperature regulation is suggested by the report that administration of an enkephalinase inhibitor, thiorphan or met-enkephalinamide could induce a naloxone-reversible hypothermia. This hypothermia due to endogenously released opioid peptides involves the preoptic and the anterior hypothalamic areas (Stanton et al., 1985). Similarly central administration of β-endorphin induced hyperthermia in low concentrations and hypothermia in high doses (Tseng et al., 1980).

2.4.4 Feeding

Endogenously released opioid peptides appear to regulate feeding (Jalowiec et al., 1981). Systemic or intracerebral administration of morphine, β-endorphin or enkephalins facilitated food consumption (Grandison et al., 1977; Jalowiec et al., 1981; Tepperman and Hirst, 1983). Opioid peptides in the hypothalamic brain area are particularly important for the regulation of feeding behaviours (Przewlocki et al., 1983).

2.4.5 Respiratory and cardiovascular system

Local application of morphine and enkephalins to the dorso-rostral surface of the pons in cats selectively decreased the frequency of respiration, whilst tidal volume or the response to carbon dioxide were left unchanged or increased (Hurle et al., 1983). Morphine was less effective than enkephalin in reducing the frequency of respiration. However, naloxone can easily reverse morphine-induced respiratory depression and hypoventilation (Bowman and Rand, 1980) but not the enkephalin-induced depression of respiration (Pazos and Florez, 1983). It was suggested that both both μ and δ-receptors are involved in the respiratory depressant actions of opioid peptides and opiates.

A decrease in blood pressure after the application of met-enkephalin to the ventral surface of the brain stem in the cat has been reported (Florez and Mediavilla, 1977). The microinjection of met- or leu-enkephalin into the brain produced either hypertension or hypotension, depending on the site of
injection. It has been suggested that two types of opioid receptors exist in
the medulla. A naloxone-resistant receptor which mediate vasopressor
responses and naloxone-sensitive receptor which mediate vasodepressor
responses (Fuxe et al., 1979).

2.4.6 Sexual behaviour

The possible involvement of endogenous opioid peptides in sexual
behaviour is suggested by several authors. A decrease in sexual function in
narcotic addicts has been reported (Crowley and Simpson, 1978). Whereas
opiate withdrawal may be associated with premature ejaculation, spontaneous
erection in men and sexual arousal in women (Farr, 1976). Pretreatment of
opiate-dependent rats with enkephalinase inhibitors thiorphan or phelorphan
stimulated penile-licking during naloxone-precipitated withdrawal (Dzojlic et
al., in preparation). A similar activation of penile licking was observed
following the administration of another enkephalinase inhibitor
phosphoramidon to REMSD animals (unpublished observation). However central
administration of $\beta$-endorphin or D-Ala$^2$-met$^5$-enkephalaminamide reduced mounting
behaviour in normal, non-dependent male rats (Meyerson and Terenius, 1977;
Gessa et al., 1979). These inhibitory actions of opioid peptides on sexual
behaviour were blocked by naloxone and naltrexone. Further studies are
necessary for the clarification of the role of different opioid receptors in
sexual behaviours.

2.4.7 Neuronal excitability

Accumulating evidence indicate that both opium alkaloids and opioid
peptides are capable of exerting an inhibitory or a facilitatory action on
cerebral excitability. The administration of large doses of morphine, icv or
systemically, can induce convulsive behaviours in mice and rats (Gilbert and
Martin, 1975; Sneed and Bearden, 1982). Pretreatment with subconvulsant
doses of morphine can block the convulsant effect of morphine and enkephalins
(Uroa and Frenk, 1983; Dzoljic, 1982). Similar pretreatment with morphine,
$\beta$-endorphin and [D-Ala$^2$-D-Leu$^5$]enkephalin (DADLE) also antagonised
electroshock-induced seizures (Puglishi-Allepra et al., 1984; Berman and
Alder, 1984). This anticonvulsant action of opiates and opioid peptides was
blocked by naloxone (Berman and Alder, 1984). Recent data indicate that the
δ-opioid receptors mediate the epileptic actions of enkephalins, whilst μ-opioid receptors are involved in the anticonvulsant actions of opioid peptides (Dzoljic and vd Poel-Heisterkamp, 1982; Frenk, 1983; Haffmans and Dzoljic, 1983). The target area of this action appear to be in the limbic area (Henriksen et al., 1978), particularly in the hippocampus (French and Siggins, 1980; Haffmans et al., 1983; 1984). However in the fluorothyl seizure test both δ and μ opioid receptor agonists appear to be anticonvulsants (Tortella et al., 1985). It does appear that exogenously administered opioid peptides may have both pro- and anti-convulsant actions depending on the experimental conditions. The role of pro- and anti-convulsant opioid systems in human epilepsies is still unknown.

2.4.8 Tolerance and dependence

Similar to morphine the development of tolerance to opioid peptides has been reported (Wei and Loh, 1976; Tseng et al., 1977). Interestingly, in animals tolerant to the μ agonist sufentanyl, a δ-opioid receptor agonist DADLE was still able to induce analgesia and catatonia (Schulz et al., 1981). This might indicate the lack of cross-tolerance between μ and δ receptor agonists. It also appears that an organism can develop tolerance and dependence to endogenously released opioid peptides. Namely naloxone precipitated an opiate-like abstinence syndrome after chronic stress or enkephalinase inhibition (Christie and Chester, 1982; Bean and Vaught, 1984). Social interaction appear to activate endogenous opioid peptides resulting in an opioid-like "social dependence". Isolation or social separation provoked symptoms such as vocalisation and irritability. Symptoms induced by social isolation could be reduced by morphine and potentiated by naloxone (Panksepp, 1981).

The possible involvement of changes in endogenous opioid peptides in the process of opiate dependence is not clear. However, chronic morphine treatment increased the activity of a high affinity enkephalinase (Malfroy et al., 1978). Enkephalinase inhibition attenuated some symptoms of opiate withdrawal in morphine dependent animals (Dzoljic et al., in preparation).

An increase in protein synthesis also appears to be involved in the mechanism of tolerance to opiates. Several drugs with the common ability to inhibit protein synthesis reduce the development of tolerance and dependence
to morphine (Bowman and Rand, 1980). In addition, it has been reported that the development of opiate dependence is associated with an increase in the synthesis of secretory proteins in the pons-medulla and striatum-septum (Retz and Steele, 1983). These brain areas are functionally involved in opiate tolerance and dependence (Herz, 1978). The hippocampal CA3 area also appear to be modulate both opioid and opiate-withdrawal WDS (Isaacson and Lanthorn, 1981).

Some pharmacological actions of opiates or opioid peptides and the possible brain areas involved have been described in this section. Table III summarizes some pharmacological actions of opioid peptides/opiates and the receptor types involved.

2.5 Drugs and behavioural states affecting endogenous opioid peptides

2.5.1 Neuropeptidase inhibitors

Potentiation of enkephalinergic activities following the inhibition of neuropeptidases have been demonstrated under several experimental conditions. For example kelatorphan a potent inhibitor of enkephalinase increased met\(^5\)-enkephalin (met-ENK) levels in rat striatum and blocked the biodegradation of exogenously administered enkephalins (Waksman et al., 1985). Similarly thiorphan an enkephalinase inhibitor A (Hudgin et al. 1981), given alone, or in combination with bestatin (peptidase inhibitor), elevated in vivo striatal and midbrain met-ENK levels and induced naloxone-reversible analgesia (Zhang et al., 1982; Yaksh and Harty, 1982). Phosphoramidon, another potent inhibitor of enkephalinase A (Hudgin et al., 1981), induced naltrexone-sensitive analgesia (Ruprecht et al., 1983) and insomnia (see chapter 3). Other neuropeptidase inhibitors such as bestatin and leucinal increased brain met-ENK levels and \(\beta\)-endorphin-stimulated analgesia (Waksman et al., 1985; Davis et al., 1983). In addition, naloxone precipitated some behaviours characteristic of the opiate abstinence syndrome in rats after chronic inhibition of enkephalinase (Bean and Vaught, 1984). These reports indicate that inhibition of some neuropeptidases can induce increases in opioid peptides with concomitant alterations in behaviour. The effect of peptidase inhibitors on the brain concentrations of other opioids such as \(\beta\)-endorphin and dynorphin, still remains to be established.
Table III: Pharmacological effects of opiates and receptor subtypes involved (modified after Trends in Pharmacol. Sci., vol 6 centre'fold)

<table>
<thead>
<tr>
<th>Pharmacological actions</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$  $\delta$  $\xi$  $\mu_1$  $\sigma$</td>
</tr>
<tr>
<td>supraspinal analgesia</td>
<td>* - - ** -</td>
</tr>
<tr>
<td>stress-induced analgesia</td>
<td>* ** ** - -</td>
</tr>
<tr>
<td>euphoria</td>
<td>** ? - ? -</td>
</tr>
<tr>
<td>dysphoria</td>
<td>- ? ** ? *</td>
</tr>
<tr>
<td>sedation</td>
<td>* ? ** - -</td>
</tr>
<tr>
<td>catalepsy</td>
<td>** * * ** -</td>
</tr>
<tr>
<td>increased locomotion</td>
<td>** ? ? - **</td>
</tr>
<tr>
<td>decreased locomotion</td>
<td>* ? ** * -</td>
</tr>
<tr>
<td>tolerance</td>
<td>** ** ** ** **</td>
</tr>
<tr>
<td>withdrawal signs</td>
<td>** * * - *</td>
</tr>
<tr>
<td>ictal EEG spiking</td>
<td>** ** - ? ?</td>
</tr>
<tr>
<td>anticonvulsant</td>
<td>** ** ** - ?</td>
</tr>
<tr>
<td>hyperthermia</td>
<td>* ** - - -</td>
</tr>
<tr>
<td>hypothermia</td>
<td>* - - ** -</td>
</tr>
<tr>
<td>respiratory depression</td>
<td>** ** - - -</td>
</tr>
</tbody>
</table>

Key: ** = direct involvement, * = possible involvement, ? = not established, - = not involved
2.5.2 Centrally acting drugs

1) Narcotic analgesics

Morphine elicited a decrease in plasma \( \beta \)-endorphin but elevated the concentration of this peptide in whole brain (without the cerebellum) and in the pituitary (Bruni et al., 1985). The analgesic effect of morphine has been ascribed, partly, to the release of endogenous opioid peptides (Schlen and Bentley, 1980). However, prolonged morphine administration (>1 month) decreased enkephalin levels in the striatum and pituitary. A similar reduction in \( \beta \)-endorphin contents were demonstrated in the septum, midbrain and pituitary (Herz et al., 1980a). Plasma concentration of \( \beta \)-endorphin-like material was reduced in heroin addicts (Ho et al., 1980). In contrast, other studies failed to demonstrate changes in brain enkephalin concentrations after acute or chronic morphine treatment or naloxone-precipitated withdrawal (Fratta et al., 1977; Wesche et al., 1977). Chronic (10 days) treatment with morphine increased enkephalinase activity and \( \mu \)-opioid receptors in the striatum (Takashi et al., 1981).

ii) Neuroleptics

Chronic administration of the antipsychotic drugs haloperidol and chlorpromazine increased the formation of \( \gamma \)-endorphin and des-\( \text{Tyr}^1 \)-\( \gamma \)-endorphin in brain slice. Other central depressants such as phenobarbital and promethazine did not alter the concentrations of these opioid peptides (Davis et al., 1984). However, neuroleptic therapy has been reported to increase plasma- and CSF- \( \beta \)-endorphin activity in chronic schizophrenics (Emrich et al., 1980; Naber et al., 1984).

\( \text{Met-enkephalin} \) levels in the striatum and nucleus accumbens were elevated after chronic treatment with the antipsychotic drugs such as clozapine, haloperidol and reserpine. The non-cataleptogenic neuroleptic such as clozapine was less effective in elevating \( \text{met-ENK} \) levels (Hong et al., 1980). The clinical relevance of the neuroleptic induced alterations in opioid peptides is not yet clear.

iii) Antidepressant drugs

Antidepressant drugs such as clomipramine, desipramine, amitriptyline and iprindole provoked a selective increase in \( \text{met}^5 \)-enkephalin-like immunoreactivity in the striatum and nucleus accumbens (De Felipe et al.,...
An increase in \( \beta \)-endorphin contents in the pituitary and hypothalamus has also been reported after treatment with some antidepressants (Prewlocki, 1984).

Interestingly, antidepressant drugs decreased plasma \( \beta \)-endorphin in depressive patients in correlation with therapeutic activity (Rapisarda and Bongiorno, 1982; Genazzani et al., 1984).

**iv) Anaesthetic agents**

The analgesia induced by nitrous oxide has been attributed to enhanced endorphinergic transmission since it could be attenuated by naloxone and naltroxone (Yang et al., 1980). In addition, morphine attenuated whilst naloxone potentiated nitrous oxide withdrawal convulsions in mice (Manson et al., 1983). Recently nitrous oxide has been demonstrated to increase met-ENK levels in the CSF of rats (Quock et al., 1985). Chronic ethanol consumption decreased the release of enkephalin in the striatum inducing supersensitivity of \( \delta \)-opioid receptors, but reduced the affinity of the \( \mu \)-opioid receptors (Lucchi et al., 1984). The role of endogenous opioid peptides in the pharmacological actions of anaesthetic agents is not fully understood. There is however the idea that the analgesic and euphoric effects of nitrous oxide are due partially to release of endogenous opioid peptides.

**v) GABA and benzodiazepins**

GABA and muscimol were found to decrease potassium-evoked release of striatal met-ENK. In a parallel in vivo study, acute administration of benzodiazepines such as diazepam decreased met-ENK levels in the striatum but increased them in the hypothalamus. The drug induced enhancement of enkephalin was rapid in onset (2-5 min) (Herz et al., 1980b).

**vi) Drugs modulating 5-HT system**

The serotonin releaser, fenfluramine increased met-ENK and \( \beta \)-endorphin contents in the hypothalamus but not in the frontal cortex, hippocampus and brain stem (Harsing et al., 1982). Conversely, PCPA and 5-7-DHT which depletes 5-HT levels in the brain reduced \( \beta \)-endorphin concentrations in the hypothalamus, thalamus, and brain stem but not in the pituitary. Acute administration of these substances did not alter brain contents of this peptide (Harsing et al., 1982).

The clinical significance of changes in opioid peptides induced by drugs interfering with GABA and 5-HT system is not clear.
2.5.3 Stress

Abundant evidence suggests that endogenous opioid peptides are modulated by various stress regimens. Electroshock induced a naloxone-reversible analgesia and motor inhibition (Nabeshima et al., 1985). A parallel increase in brain enkephalin levels and the pain threshold has been demonstrated after electric shock (Madden et al., 1977). Electroconvulsive shocks, which are associated with a naloxone-reversible postictal analgesia and catalepsy (Urca et al., 1981), elevated preproenkephalin mRNA and enkephalin concentrations in the hypothalamus and limbic area (Yoshikawa et al., 1985). Painful stimuli, such as arthritis or injecting formalin into rat paws, increased enkephalin levels in the brain (Cesselin et al., 1980; Kuraishi et al., 1981). Immobilisation stress or forced swimming did not alter dynorphin concentrations in the cortex and hypothalamus, whereas tail-pinches stress enhanced dynorphin in these brain regions (Morley et al., 1982). The pituitary gland contains high concentrations of opioid peptides (Przewlocki et al., 1983; Kerdelhue et al., 1983; Tang et al., 1984), which are released during acute stress exposure (Guillemin et al., 1977), probably indicating an involvement of pituitary opioid peptides, along with ACTH, in the stress response.

2.6 Role of opioid peptides in psychopathology

Several behavioural actions of opioid peptides studied in animals suggested possible implication of endogenous opioid peptides in human psychopathology. In addition opioid peptides are present in the brain stem and limbic areas where they are well placed to modulated vigilance, motivation and emotions.

2.6.1 Opioid peptides and depressive states

In several studies an increase in plasma and CSF \( \beta \)-endorphin levels in depressive conditions have been demonstrated (Risch, 1982; Genazzani et al., 1984), while in other reports no changes in the plasma or CSF concentrations of \( \beta \)-endorphin were found (Naber et al., 1982). Patients with endogenous depression were also more tolerant to pain than normal volunteers (Davis et al., 1979). Antidepressant drugs decreased plasma \( \beta \)-endorphin in parallel
with their therapeutic activity (Rapisarda and Bongiorno, 1982; Genazzani et al., 1984). These data indicate that excess of opioid peptides may be involved in the pathogenesis of some forms of endogenous depression.

2.6.2 Opioid peptides in schizophrenia

The icv administration $\beta$-endorphin elicited rigid immobility in rats. This observation led Bloom and co-workers (1976) to propose that an excess of central opioid peptides might be involved in the pathophysiology of schizophrenia. In contrast, Jaquet and Mark (1976) proposed that $\beta$-endorphin may have a neuroleptic-like therapeutic action, since this peptide induced extrapyramidal-like rigidity. These propositions have given rise to two schools of thought i.e. i) that excess and ii) that a deficiency of opioid peptides, underlie schizophrenia. Increased $\beta$-endorphin levels in CSF of schizophrenic and manic patients were reported by some workers (Rimon et al., 1980), and naloxone appeared to reduce psychotic symptoms (Watson et al., 1978). However other authors could not confirm these findings (Naber et al., 1981). Direct biochemical evidence that excess opioid peptides are secreted in schizophrenia or that opiate antagonist helps in this disorders is at best tenous. $\beta$-Endorphin either slightly accentuated (Gerner et al., 1980) or alleviated some symptoms of schizophrenia (Kline et al., 1977). Des-Tyr$^1$-Y-endorphin (DTYE) decreased psychotic symptoms in schizophrenics (Verhoeven et al., 1979). In contrast other workers did not find any therapeutic effects following DTYE (Emrich et al., 1980). However antipsychotic drugs such as haloperidol and chlorpromazine increased the formation of $\gamma$-endorphin and des-Tyr$^1$-$\gamma$-endorphin in vitro in animal brain slice (Davis et al., 1984). Plasma and CSF $\beta$-endorphins were increased in schizophrenics, although there was no correlation between changes in plasma opioid peptides and the therapeutic effects of the antipsychotic drugs (Emrich et al., 1980; Naber et al., 1984).

Evidently the role of endogenous opioid peptides in the psychotic disorders needs further clarification.
REFERENCES


Bradbury AF, Smyth DG, Snell CR (1976) Prohormones of α-melanotropin (α-melanocyte-stimulating hormone, α-MSH) and corticotropin (adrenocorticotropic hormone, ACTH): structure and activation. In Polypeptide Hormones: Molecular and cellular aspects (CIBA foundations symp), Elsevier/Excepta Medica, North Holland, pp 61-75


Bowman WC, Rand MJ (1980) Drugs used to relieve pain, In: Textbook of


Chaillet P, Marcais-Collado H, Costentin J (1983) Catatonic or hypotonic immobility induced in mice by intracerebroventricular injection of mu or kappa opioid receptor agonists as well as enkephalins or inhibitors of their degradation, Life Sci., 33: 2105-2111


Davis GC, Buchsbaum MS, Bunney WE (1979) Analgesia to painful stimuli in affective illness, Amer. J. Psychiat., 136: 1148-1151


Dzoljic MR (1982) Opiate receptors and seizures: proconvulsant actions of δ-receptors and anticonvulsant action of μ-receptor, In: Current...
status of centrally acting peptides. Dhawan BN (ed), Pergamon press, New York, pp 107-113


opioid peptides: comparative evaluation of their receptor affinities in mouse brain, Life Sci., 33: 291-294


Cessa OL, Paglietti E, Pelligrini Quarantotti B (1979) Induction of copulatory behavior in sexually inactive rats by naloxone, Science, 204: 203-204

Gilbert PE, Marin WR (1975) Antagonism of the convulsant effects of heroin, d-propoxyphene, meperidine, normeperidine and thebain by naloxone in mice, J. Pharmac. Exp. Ther., 192: 538-541


Biochem. Psychopharmacol., 24: 223-232
Hughes J (1975) Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine, Brain Res. Osaka, 88: 295-308
Hughes J, Smith T, Morgan B, Fothergill L (1975a) Purification and properties of enkephalin, the possible ligand for the morphine receptor, Life Sci., 16: 1753-1758
Kelly AE, Stinus EL, Iversen SD (1980) Interactions between
D-Ala-met-enkephalin, A10 dopaminergic neurons and spontaneous behavior in the rat, Behav. Brain Res., 1: 3-24


morphine on convulsions in mice following withdrawal from nitrous oxide, Can Anaesth. Soc. J., 30: 28-31


Pert CB, Snyder SH (1975) Opiate receptor: Demonstration in nervous tissue, Science, 179: 1011-1014


Quirion R, Zajac JM, Morgat JL, Roques BP (1983b) Autoradiographic
distribution of mu and delta opiate receptors in the rat brain using
highly selective ligands, Life Sci., 33: 227-230
Qock RM, Kouchich FJ, Tseng L-F (1985) Does nitrous oxide induce release of
brain opioid peptides?, Pharmacol., 50: 95-99
Rapisarda V, Bongiorno G (1982) Double blind test with mianserin versus
chlorimipramine, In: Typical and atypical antidepressant clinical
practice, E Costa, G Racagni (eds), Raven press, New York, pp 141-149
Retz KC, Steele WJ (1983) Blockade of morphine dependence-related
enhancement of secretory protein synthesis in the pons-medulla and
striatum-septum by naltrexone, Neuropharmacol., 22: 183-189
Rimon R, Terenius L, Kampman R (1980) Cerebrospinal fluid endorphins in
Risch SC (1982) Beta-endorphin hypersecretion in depression: possible
cholinergic mechanism, Biol. Psychiat., 17: 1071-1079
Ruprecht J, Ukponmwan OE, Admiraal PV, Dzoljic MR (1983) Effect of
phosphoramidon- a selective enkephalinase inhibitor on nociception and
behaviour, Neurosci. Lett., 41: 331-335
substance in human CSF, Brain Res., 151: 399-403
Schlen H, Bentley GA (1980) The possibility that a component of
morphine-induced analgesia is contributed indirectly via the release of
endogenous opioids, Pains, 9: 73-84
the brain by the selective development of tolerance, Pharmacol.
Biochem. Behav., 14: 75-79
Simantov R, Kuhar MJ, Uhl GR, Snyder SH (1977) Opioid peptide enkephalin:
Acad. Sci. USA, 74: 2167-2171
Simon BJ, Hiller JM, Edelman I (1975) Stereospecific binding of the potent
Acad. Sci. USA, 70: 1947-1949
Snead OC, Bearden LJ (1982) The epileptogenic spectrum of opiate agonists,
Neuropharmacol., 21: 1137-1144
Stanton TL, Sartin NF, Beckman AL (1985) changes in body temperature and
metabolic rate following microinjection of met-enkephalinamide in the preoptic/anterior hypothalamus of rats. Regulatory Peptides, 12: 333-343


Tortella FC, Robes L, Holaday JW (1985) The anticonvulsant effects of DADLE are primarily mediated by activation of δ opioid receptors: interactions between δ and μ receptor antagonists, Life Sci., 37: 497-503


Turski L, Havenmann U, Kuschinsky K (1983) The role of the substance nigra in motility of the rat: muscular rigidity body asymmetry and catalepsy after injection of morphine into the nigra, Neuropharmacol., 22:


Yang JC, Clark WC, Ngai SH (1980) Antagonism of nitrous oxide analgesia by
naloxone in man, Anaesthesiol., 52: 414-417
Intracerebroventricular (i.c.v.) administration of the enkephalinase inhibitor phosphoramidon (25-100 μg) induced a dose-related decrease in non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) time, with a corresponding increase in wakefulness.

The local application of phosphoramidon (1-25 μg) into the locus coeruleus (LC) or periventricular gray (PVG) substance also inhibited both NREMS and REMS, and increased wakefulness.

Pretreatment with naltrexone (0.1 mg/kg, i.p. 15 min prior) significantly reduced the phosphoramidon-induced insomnia. Similarly, local application of naltrexone (10 μg/brain area) also decreased the insomnia induced by the administration of phosphoramidon into the PVG or LC.

These findings indicate that endogenous opioid peptides are important modulators of wakefulness.

INTRODUCTION

Few studies on this subject with controversial results have been performed. It has been found that β-endorphin had an arousal effect in rats (Havlicek et al., 1978). This was confirmed in cats by i.c.v. administration of 0.5 μg β-endorphin (King et al., 1981). However other authors did not find significant changes in sleep parameters after i.c.v. injection of either 1 μg β-endorphin, met-enkephalin or leu-enkephalin (Riou et al., 1982). The doses, species and way of administration of the corresponding opioid peptide might be of crucial importance in the interpretation of these results. For example, recent data indicate that
REM sleep stages using the combined EMG, ECoG and hippocampal EEG parameters (Fig 1). REM sleep episodes were identified by the appearance of low voltage ECoG fast waves, hippocampal theta pattern and EMG silence. NREM sleep was characterised by high voltage slow ECoG waves together with low EMG. Awake stage was identified by high EMG and fast low voltage ECoG.

**Drug**

Phosphoramidon (Peninsula Lab) dissolved in saline was administered i.c.v. (maximum volume 2 μl, i.c.v. or 1 μl local application). Naltrexone hydrochloride (Endo Lab) was administered intraperitoneally (i.p.) dissolved in saline.

**Statistics**

Statistical analysis was done using paired Student t-test. Statistical significance was accepted at P-value of 0.05 or less.

**RESULTS**

**Intraventricular administration of enkephalinase inhibitor**

The administration of phosphoramidon increased wakefulness whilst it decreased the NREM and REM sleep stages in a dose-dependent manner (Fig 2). The sleep suppressant effect of phosphoramidon was associated with behavioural signs of excitation such as head shakes and body scratching. The opiate antagonist naltrexone administered in a dose range which did not alter the sleep-waking pattern in the control animals (0.1-0.5 mg/kg) antagonized phosphoramidon-induced insomnia (Fig 2).

**Microinjection of enkephalinase inhibitor**

**Locus coeruleus**

Application of phosphoramidon (1-25 μg) into the locus coeruleus decreased NREM 40-50% and REM 20-27% sleep stages in a dose-related manner. Naltrexone (10 μg, 5 min prior) decreased the phosphoramidon-induced insomnia.

**Central gray substance**

Phosphoramidon (1-25 μg) injected into the periventricular gray (PVG) substance of the brain induced a significant increase in wakefulness. This arousal effect of phosphoramidon was decreased by naltrexone (10 μg, 5 min prior) (Fig 3).
Figure 2: Enkephalinase inhibition and wakefulness in rats. Each bar is mean±S.E.M for 6 rats. Note that the insomnia induced by i.c.v. administered enkephalinase inhibitor phosphoramidon, was decreased by naltrexone.
Figure 3: Enkephalinase inhibition and wakefulness in rats. Each bar is mean ± S.E.M for 3-5 rats. Note that the insomnia induced by intracerebral local administered enkephalinase inhibitor phosphoramidon, was abolished by naltrexone.
DISCUSSION

The results of this study indicate that specific enkephalinase inhibitor phosphoramidon induces both NREM and REM sleep suppression. Naltrexone antagonized the sleep suppressive action of enkephalinase inhibitor which suggests that the increased wakefulness induced by phosphoramidon is mediated by activation of opioid receptors.

The local application of phosphoramidon into specific brain regions induced naltrexone sensitive insomnia in this study. This is consistent with the fact that microinjection of opiates into these brain areas induces behavioural excitation (Jacquet and Wolf, 1981). The above data might indicate that an endogenous opioid system, particularly the enkephalinergic system plays an important role in the maintenance of wakefulness. Furthermore, a number of pathological conditions such as stress, anxiety and other psychic disturbances, in which an increase wakefulness is a common symptom, are known to be associated with increased endorphin levels. It is therefore conceivable that activation of the enkephalinergic system might constitute the common mechanism underlying various sleep-waking disturbances.

REFERENCES


King C, Masserano JM, Cody E, Byrne WL (1981) Effects of a-endorphin and morphine on the sleep-wakefulness behaviour of cats. Sleep, 4: 259-262


CHAPTER 4

STAGES OF VIGILANCE AND ENKEPHALIN-INDUCED SEIZURES

ABSTRACT

The effects of different sleep-wakefulness stages on enkephalin-induced seizure phenomena were studied in the rat by recording the electrical activities of the parieto-frontal cortex, dorsal hippocampus and submandibular/nuchal muscle.

Administration of [D-Ala^2]-Met-enkephalinamide (DALA, 10 μg/2μl. i.c.v.) induced electrographic signs of seizure during SWS, REM sleep and wakefulness. The DALA-induced epileptiform activities in ECoG, EMG and hippocampus were significantly higher during wakefulness compared with any sleep stage. REM sleep significantly inhibited DALA-induced ECoG spiking activity compared with SWS and wakefulness. Similarly SWS decreased the ECoG spiking activity compared with wakefulness.

It is suggested that an enkephalinergic system may be involved in the ethiopathogenesis of epilepsy of the "awaking type" particularly that with petit mal characteristics.

INTRODUCTION

The clinical finding that some epileptic attacks occur more frequently during the day and others at night suggests an influence of the sleep-waking cycle on the occurrence of epilepsy (Janz, 1962). Although some types of epilepsy occur mainly during sleep, their occurrence is not facilitated evenly by different sleep stages.

Recent data indicate that intracerebroventricular administration of enkephalin in rats induces electrographic and behavioural epileptic phenomena (Dzoljic et al., 1979; Urca et al., 1977), and it has been suggested that endogenous opioid peptides play a modulatory role in the pathogenesis of epilepsy (Dzoljic and Poel-Heisterkamp, 1982).

In order to understand better the relationship between stages of
vigilance, endopioids and epilepsy, we studied the effect of sleep-wakefulness cycle on enkephalin-induced seizures.

**MATERIALS AND METHODS**

Adult, male Wistar rats weighing 175-200 g were used. For the recording of the electrocorticogram, silver screw electrodes were threaded into the bone overlying the frontal and parietal cortices. The electromyogram (EMG) was recorded from the neck and/or submandibular muscles. Hippocampal electrical activities were recorded from the CA1 region by means of bilaterally implanted stainless steel electrodes. A new cannula system provided the possibility of intracerebroventricular administration of \([D-\text{Ala}^2-\text{met}]-\text{enkephalinamide} \) (DALA, 10 μg/2 μl) in unrestrained rat, in any stage of vigilance, with full external control upon the rate of flow, frequency and volume of drug injection. All rats were allowed at least a 7-day recovery period before experiments commenced. Animals were maintained under constant light/dark periods (light phase 06.00-22.00 h) and experiments were carried out between 12.00-16.00 h to avoid variations due to changes in the circadian rhythm. Results were analysed statistically using paired Student’s t-test. Statistical significance was accepted at P-value of 0.05 or less.

**RESULTS**

DALA given during wakefulness induced electrographic signs of seizure phenomena such as cortical or hippocampal spikes and myoclonic contractions in the muscles. However, the same dose of DALA given during slow wave sleep (SWS) or rapid eye movement (REM) sleep produced significantly less electrographic signs of seizure (Fig 1). The REM sleep stage proved more resistant to DALA -induced seizure compared to SWS. However, the inhibitory effects of SWS and REM sleep on hippocampal spikes and myoclonic contractions were not significantly different from each other. The most prominent DALA-induced epileptic phenomena were observed during the awake stage. Behavioural phenomena such as "wet-dog-shakes" and "fall down" were associated with the epileptic burst in the EEG and appeared only if DALA was
Figure 1: The effect of vigilance states on enkephalin (DALA, 10μg/21, intracerebroventricularly)-induced seizure phenomena during the 4 time periods after drug administration. Only spikes up to 200 and 400μV in the ENG and ECoG, respectively were counted. Each vertical bar is mean±S.E spikes for 7 rats. Note that sleep, particularly REM sleep significantly reduced the intensity of the electrographically registered epileptic phenomena in the cortex, hippocampus(hipp) and the submandibular muslces (sub.m.).
given during wakefulness and not during the SWS and REM sleep.

DISCUSSION

It is known that REM sleep deprivation increases neuronal excitability (Cohen and Dement, 1965). However, the results of this study indicate that normal REM sleep and to a lesser extent SWS decrease significantly all the electrographic signs of DALA-induced seizure phenomena in the hippocampus, cortex and submandibular/neck muscle when compared to wakefulness. The cause of the inhibitory effect of sleep on enkephalin-induced seizure is not clear. However the epileptic properties of enkephalin can be antagonized only by anti-petit mal and not anti-grand mal drugs (Snead and Bearden, 1980).

Furthermore, petit mal paroxysm occurs mainly during wakefulness or sleep-waking transition period (Janz, 1962). This clinical observation is in accordance with the inhibitory effects of sleep on enkephalin-induced seizure demonstrated in this study. Therefore, a possible involvement of an endogenous opioid system in the ethiopathogenesis of epilepsy of the "awaking type", particularly those with petit mal characteristics should be considered. In addition, this experimental model can be used as a reliable tool in future studies of the relationship between different vigilance states and drug-modulated neuronal excitability.

REFERENCES


Snead OC, Bearden LJ (1980) Anticonvulsant specific for petit mal antagonize
epileptogenic effect of leucine enkephalin, Science, 210: 1031-1033
PART III: MUTUAL INTERACTIONS BETWEEN DISTURBED SLEEP AND OPIATE/OPIOID PEPTIDES

CHAPTER 5

REM SLEEP DEPRIVATION DECREASES THE ANTINOCICEPTIVE PROPERTY OF ENKEPHALINASE-INHIBITION, MORPHINE AND COLD-WATER-SWIM

ABSTRACT

1) In this study, the effect of REM sleep deprivation (REMSD) or chronic stress was investigated on three analgesic procedures as follows: enkephalinase-inhibition (phosphoramidon), morphine and cold-water-swim induced antinociceptions.

2) REMSD (96 hr) completely abolished the analgesic effect of phosphoramidon (250 μg, i.c.v.), morphine (20 μg, i.c.v.) and 5 min cold-water-swim (5°C, cold).

3) Rats exposed to chronic stress regimen did not show any tolerance to the analgesic effect of phosphoramidon or CWS.

4) These data indicate that REMSD can decrease pain threshold, probably by altering enkephalinergic and other transmitter systems.

5) It is suggested that pharmacological manipulations and/or pathological conditions which decrease REM sleep might affect the efficacy of opiate and other analgesic procedures. Additional clinical studies are necessary to clarify the relationships of REMSD and pain threshold in human.

INTRODUCTION

The inhibitory action of opiates and opioid peptides on REM sleep is well documented (Khazan et al., 1967; King et al., 1981), while the effect of REM sleep or REM sleep deprivation (REMSD) on opiate activity is less clear. However, it is known that the episodic release of humoral endorphin is associated with REM sleep (Oksenberg et al., 1980) and REM sleep inhibits
neuronal excitation induced by exogenously administered enkephalins (Ukponmwan and Dzoljic, 1983). These data suggest that phasic changes during the sleep-waking cycle can modulate response to opiates and opioid peptides.

In order to clarify further the relationship between sleep disturbances and opiates, the effect of REMSD on the antinociceptive property of morphine and enkephalinase inhibitor phosphoramidon was investigated. Phosphoramidon potentiates enkephalinergic activity in the brain by decreasing the biotransformation of enkephalins (Hudgin et al., 1981). By using phosphoramidon and morphine, it was possible to study the effect of REMSD on analgesic effects induced by endogenous opioid peptides and exogenously administered opiates.

Since it is known that REMSD can reduce the pain threshold to noxious electrical stimulation (Hicks et al., 1979), we included in this study, the relationship between REMSD and physically induced antinociception such as cold-water-swim analgesia.

MATERIALS AND METHODS

All experiments were performed on male adult Wistar rats weighing between 150 and 175 g at implantation.

Surgery

For the intracerebroventricular (i.c.v.) administration of drugs, stainless steel, guide cannula was stereotaxically directed 1 mm above the lateral ventricle. The injection cannula protruded 1 mm below the guide cannula into the ventricle. At least 5-7 day recovery period was allowed before experiments were commenced. Throughout this study, all animals were kept in a constant environment chamber with a light-dark cycle 12 hr (light period 09.00-21.00 h) and a room temperature 22±1°C. Food and clean drinking water were available ad libitum.

Three groups of experiments were performed (i) analgesic effect of phosphoramidon, (ii) the effect of morphine on pain threshold, (iii) the cold-water-swim (CWS) analgesia. The animals in each experiment were divided into three groups as follows: REM sleep deprived, chronic stressed and non-stressed (controls) rats. Each group was submitted to CWS procedure or treated either with phosphoramidon or morphine.
REM sleep deprivation (REMSD)

REMSD was carried out using the conventional "flower pot" technique previously described (Mendelson et al., 1974). In order to avoid the problem of unequal REMSD (Hicks et al., 1977), we used platforms whose area corresponded to the rat body weights (14 cm²: 100 g). Each rat was REM sleep deprived for 96 hr continuously. In the present set up, the animals had free access to food and clean drinking water. Throughout the REMSD, the water in the tank was changed regularly (11.00-12.00 hr) once every 24 hr. During the period of cleaning the animals were allowed free locomotion in individual cages, for 1 hr during which rats were kept awake manually.

Stress

The rats in this group were forced to swim in water 17-18 °C and 7 cm for 2 hr daily (11.00-13.00 hr) for 4 days. The animals were then allowed spontaneous amount of sleep for the remaining period of the day (22 hr). Weight loss in the stress and REMSD groups were not different from each other.

Control

These animals were housed singly and allowed to have spontaneous amounts of sleep.

Cold-water-swim analgesia (CWS)

CWS was produced using the procedure described by Bodnar and Sperber (1982), with a slight modification in duration of swimming and water temperature. The rats were forced to swim for 5 min in water 5 °C. Pain threshold was determined before and then at 30, 60, 90 and 120 min after CWS. The measurement of pain threshold was commenced 30 min after CWS to allow animals to become completely dry.

Phosphoramidon or morphine analgesia

This was induced by intracerebroventricular administration of these substances.

Determination of nociception

Pain sensitivity test was carried out between 13.00 and 16.00 h according to the analgesiometric method (Randall and Seltto, 1957). The nociception was expressed in the form of analgesiometric scores (AMS) g mm⁻² pressure. The cut off value was maintained at 500 g mm⁻² to avoid damage to the paw. Response to pain in this study is measured by squeak or paw-withdrawal.
Animals which scored above 150 g mm$^{-2}$ during control testing were not used for further experimentation. In all three groups: REMSD, stress or controls baseline pain threshold was measured before saline, morphine or phosphoramidon was administered. Nociception was then followed for 2 hr after drug treatment.

**Drugs**

The following drugs were used in this study. morphine hydrochloride (Merck) and phosphoramidon (Peninsula Lab) were administered dissolved in saline. A maximum of 2μl was given i.c.v. over a period of 10 sec.

**Statistical analysis**

The significance of differences between the analgesic scores obtained after different treatments was evaluated by Student t-test, once a one way analysis of variance (ANOVA) had revealed that the samples represented different populations (Steel and Torrie, 1980). Statistical significance was accepted at P-values of 0.05 or less (two tailed).

**RESULTS**

**The effect of REMSD on the analgesia induced by enkaphalinase-inhibition**

The intracerebroventricular (i.c.v.) administration of phosphoramidon (250μg/μl) in control or stressed animals caused an increase in pain threshold during the first 30 min after drug treatment. Phosphoramidon-induced analgesia in control and stressed animals was accompanied by signs of central excitation such as wet-dog-shakes, excessive grooming, hypermotility. The antinociceptive action of phosphoramidon was completely abolished by REMSD (Fig 1).

**The effect of REMSD on morphine analgesia**

Morphine (20 μg/2μl, i.c.v.) induced profound analgesia in both control and stressed animals. The antinociceptive action of morphine was lowered in stressed group compared with control animals. The increase pain threshold induced by morphine lasted about 2 hr during which rats remained quiet with decreased motility (not evaluated). REMSD completely antagonized analgesia (Fig 2).
Figure 7: The inhibitory effect of REM sleep deprivation (REMSD) on phosphoramidon (ph) induced analgesia. Each point is mean ± S.E.M for control n=26, REMSD n=25 and stress n=19 groups. The number of animals receiving phosphoramidon in each group are as follows: control + ph=8, REMSD + ph=6 and stress + ph=6. Note that phosphoramidon given intracerebroventricularly induced an increase in pain threshold in both control and stressed animals. This analgesic effect of phosphoramidon was completely abolished by REMSD.
Figure 2: The inhibitory effect of REM sleep deprivation (REMSD) on morphine (mo) analgesia. Each point is mean ± S.E.M for control n=26, REMSD n=25 and stress n=19. The number of animals receiving morphine in each group are as follows: control + mo=10, REMSD + mo=10 and stress + mo=8. Note that morphine induced long lasting increase in pain threshold was completely abolished by REMSD.
The effect of cold-water-swim (CWS) analgesia

The pain threshold was significantly higher in rats exposed to cold-water-swim compared to control or stressed animals. This CWS analgesia was antagonized by REMSD (Fig 3).

![Graph](image-url)

*Figure 3: The antagonistic effect of REM sleep deprivation (REMSD) on cold-water-swim analgesia (CWS). Each point is mean ± S.E.M for control n=26, REMSD n=25 and stress n=19. The number of animals submitted to CWS in each group was n=6. Note that CWS analgesia in both control and stress groups was completely abolished in REM sleep deprived rats.*

**DISCUSSION**

It is of interest to note that the antinociceptive activity of enkephalinase-inhibitor (phosphoramidon) and morphine was completely abolished in REM sleep deprived rats. The phosphoramidon-induced analgesia
(Rupreht et al., 1983) is probably due to the known enkephalinase inhibition and the consequent increase in the enkephalinergic activity (Hudgin et al., 1981). In addition it has been demonstrated that the enkephalinase inhibitor thiorphan also possesses antinociceptive activity (Roques et al., 1980).

The mechanism by which REMSD antagonizes the analgesic effect of phosphoramidon is not clear. However, one possible explanation could be that animals deprived of REM sleep might have a lowered level in functional activity of the enkephalinergic system. This possibility is consistent with the known inhibitory effects of REMSD on peptide synthesis (Shapiro and Girdwood, 1981). Consequently, the reduced activity of opioid peptides in the brain may decrease pain threshold. Previous studies have demonstrated that a decrease in peptide synthesis did not alter morphine analgesia (Loh et al., 1969; Tulunay and Takemori, 1974). Hence the decreased antinociceptive action of morphine cannot be explained by a possible reduction in opioid peptides.

Therefore, another explanation such as alteration in other neurotransmitter(s) which are known to modulate nociception should be considered. For example it has been demonstrated that increase of dopaminergic activity and/or decrease serotonergic and cholinergic transmission which occur during REMSD (Farber et al., 1983; Mogilnicka et al., 1981; Tsuchiya et al., 1969) could antagonize the analgesic activity of opiates (McGilliard and Takemori, 1979; Gorlitz and Frey, 1972; Tulunay et al., 1976). These data suggest that changes in biogenic amines might be an important factor in the inhibitory action of REMSD on analgesia induced by exogenous and endogenous opioid peptides.

The physiological basis of CWS analgesia is not known, but due to the lack of cross-tolerance with morphine produced antinociception, it appears to be mediated by a non-opioid mechanism (Bodnar et al., 1978) e.g. the GABAergic system which has been shown to play a role in analgesia in response to environmental stress (Skerritt et al., 1981). Changes in the activity of GABA system were demonstrated in REMSD animals (Micic et al., 1967).

Relative to controls, no change in threshold to noxious paw pressure was observed in chronically stressed animals. Thus stress does not appear to play an important role in the slight decrease in pain threshold observed in rats deprived of REM sleep.
Finally, although the mechanism by which REMSD decreases pain threshold is not certain, some clinical consequences should be considered. Namely, it should be expected that pathological conditions and/or drug treatments accompanied with decrease REM sleep could modify the therapeutic effectiveness of opiates and other analgesic procedures. Additional clinical studies are required to clarify the significance of REM sleep in maintenance of normal nociception in human.

REFERENCES

Farber J, Miller JD, Crawford KA, McMillen BA (1983) Dopamine metabolism and receptor sensitivity in rat brain after REM sleep deprivation, Pharmacol Biochem Behav 18: 509-513
Hicks RA, Coleman DD, Ferrante F, Sahatjian M, Hawkins J (1979) Pain thresholds in rats during recovery from REM sleep deprivation, Percept Mot Skills 48: 687-690
King C, Masserano JM, Lod EM, Byrne WL (1981) Effects of β-endorphin and morphine on the sleep-wakefulness behaviour in cats, Sleep 4: 259-262
Loh HH, Shen FH, Way EL (1969) Inhibition of morphine tolerance and physical dependence development and brain serotonin synthesis by cycloheximide.
Biochem Pharmacol 18: 2711-2721


Mogilnicka E (1981) REM sleep deprivation changes behavioural response to catecholaminergic and serotonergic receptor activation in rats, Pharmacol Biochem Behav 15: 149-151

Randall OL, Selitto JJ (1957) A method for measurement of analgesic activity on inflamed tissue, Arch Int Pharmacodyn 111: 409-419


Tulunay FC, Takemori AE (1974) Further studies on the alteration of analgesic receptor-antagonist interaction induced by morphine, J Pharmacol Exp Ther 190: 401-407


CHAPTER 6

ANALGESIC EFFECT OF ENKEPHALINASE INHIBITION IS MODULATED BY MONOAMINE OXIDASE B AND REM SLEEP DEPRIVATION

ABSTRACT

Both the MAO-B inhibitor deprenyl (2.5-10 mg/kg, i.p., 60 min prior) and the MAO-B substrate \( \beta \)-phenylethylamine (PEA, 40 \( \mu g \), i.c.v.) potentiated the analgesic action of the enkephalinase inhibitor phosphoramidon (250 \( \mu g \), i.c.v.) in animals allowed normal sleep. The enhancing effect of PEA on phosphoramidon analgesia was further potentiated by deprenyl (5 mg/kg, i.p.) pretreatment. Deprenyl (5 mg/kg, i.p.) or PEA (40 \( \mu g \), i.c.v.) given alone did not induce analgesia in animals allowed undisturbed sleep.

REM sleep deprivation (REMSD) decreased the basal pain threshold and abolished the analgesic effect of phosphoramidon. The administration of deprenyl and/or PEA failed to restore the analgesic effect of phosphoramidon in REM sleep deprived animals.

The results indicate that excess PEA has a stimulatory effect on the analgesic activity of endogenously released enkephalins in rats allowed undisturbed sleep but not in REM sleep deprived animals.

It is suggested that the failure of phosphoramidon to induce analgesia after REMSD, is probably due to a functional insufficiency of an enkephalinergic system.

INTRODUCTION

Two forms of monoamine oxidase (MAO) are present in the mammalian brain, MAO-A and MAO-B. Serotonin, dopamine and noradrenaline are preferred substrates for MAO-A, while MAO-B shows selectivity for \( \beta \)-phenylethylamine (PEA) (Yang and Neff, 1974; Garrick and Murphy, 1980). Several lines of evidence suggested that inhibition of MAO activity increased the pharmacological effects/toxicity of opiates in patients (Taylor, 1962) and animals (Iwamoto and Ho, 1972; Boden et al., 1984), although this effect was
only seen when both MAO-A and MAO-B were inhibited (Jounela et al., 1977). Nevertheless some interactions between MAO-B inhibitors and opiates/opioid peptides have been reported. For example, an inhibition of MAO-B or excess of PEA (the substrate for MAO-B) potentiated the analgesia induced by exogenously administered opiates/opioid peptides (Fuentes et al., 1977; Garzon et al., 1980). In addition, some pharmacological actions of PEA can be modulated by opioid receptor blockade (Kubota et al., 1982; Dourish and Cooper, 1984) suggesting a possible interaction between PEA and opioid receptors.

This study was undertaken to clarify the relationship between MAO-B and the analgesic effect of endogenous (synaptic) enkephalins following administration of an enkephalinase inhibitor to rats allowed undisturbed sleep and animals subjected to REMSD. In these experiments REM sleep deprived rats were used because it has been shown that both MAO-B inhibitors and REMSD possess antidepressant activity (Mann and Gershon, 1980; Vogel et al., 1980) and modulate the analgesic action of opiates/opioid peptides (Garzon et al., 1980; Ukponmwan et al., 1984a).

MATERIALS AND METHODS

Adult, male Wistar rats weighing 150-175 g were used in this study. Drugs were injected intracerebroventricularly (i.c.v.), when required, via a stainless steel cannula implanted in the lateral ventricle. Correct placement of i.c.v. cannula was verified using the procedure recently described (Ukponmwan et al., 1985). A 4 day recovery period was allowed after cannula implantation before the experiments were commenced.

REM sleep deprivation

REMSD(96 h) and the corresponding stress-control, were carried out as previously described (Ukponmwan et al. 1984a) using a modification of the method of Mendelson (1974). This method is known to selectively deprive rats of REM sleep after 96 h (Mendelson et al., 1974). Throughout this study, all animals were maintained in a constant environment room with an ambient temperature of 22±1°C and automatically regulated light-dark cycle of 12 h (light period 09.00-21.00 h). Food and clean drinking water were available ad libitum.
Assessment of nociception

Pain sensitivity to noxious paw pressure was assessed between 13.00-16.00 h using the analgesiometric technique of Randall and Selitto (1957). Nociception was measured 15, 30, 60 and 120 min after drug administration and expressed as analgesiometric scores (AMS) g mm\(^{-2}\) pressure. The cut off value was measured by a squeak or paw-withdrawal. Animals scoring above 150 g mm\(^{-2}\) during control testing were not used for further experimentation.

Drugs

The following drugs were used in this study: phosphoramidon (Peninsula Laboratories, San Carlos, CA, USA), deprenyl (Chinoin, Budapest, Hungary) and \(\varepsilon\)-phenylethylamine (PEA, Sigma, St. Louis, Mo, USA). Drugs for i.c.v. or i.p. administration were dissolved in physiological saline and administered in volumes of 2μl or 500μl respectively. Since the half-life of PEA in the brain is known to be very short (Wu and Boulton, 1975), this substance was given 5 min after phosphoramidon and the pain threshold was measured 10 min later.

Statistics

The significance of differences between the analgesic scores obtained after different treatments was evaluated by Duncan's new multiple range test, once a one way analysis of variance (ANOVA) had revealed that samples represented different populations (Steel and Torrie, 1980; Saxena, 1985). Statistical significance was accepted at P-values of 0.05 or less (two tailed).

RESULTS

A significant difference in the analgesic scores across the various groups and time intervals was found after the administration of phosphoramidon (Fig 1). Similarly the effect of pretreatment with deprenyl and/or \(\varepsilon\)-phenylethylamine (PEA) on phosphoramidon induced analgesia was significant across the treatment groups (Fig 2, \(p<0.01\)).

The effects of deprenyl and/or \(\varepsilon\)-phenylethylamine on phosphoramidon-induced analgesia in animals allowed normal sleep

The administration of the enkephalinase inhibitor phosphoramidon (Hudgin et al. 1981) (250 μg, i.c.v.) significantly increased the pain threshold to
Figure 1: The effect of deprenyl on the analgesic action of the enkephalinase inhibitor phosphoramidon in rats allowed undisturbed sleep. Nociception was determined by withdrawal of hind paw from pressure stimulation (modified Randall-Selitto test). Each point is mean ± SEM at each time point. The number of animals per treatment group is indicated in parenthesis. Note that deprenyl (2.5-10mg/kg, ip, 60 min prior) potentiated the analgesic effect of phosphoramidon (250μg icv) in a dose-related manner. (*) indicate significant difference from saline pretreated group (p<0.05).
paw pressure. The most prominent analgesic effect was registered between 15-30 min after drug administration (Fig 1).

Deprenyl the MAO-B inhibitor, (2.5-10 mg/kg, i.p., 60 min prior) potentiated the analgesic effect of phosphoramidon in a dose-related manner (Fig 1). Similarly the MAO-B substrate, PEA. (40ug, i.c.v., 5 min post-phosphoramidon) also enhanced the phosphoramidon-induced analgesia (Fig 2, p < 0.05). Pretreatment with deprenyl (5 mg/kg, i.p., 60 min prior) further increased the potentiating effect of PEA (40 ug, i.c.v.) on the analgesic action of phosphoramidon (Fig 2, p<0.05). Neither deprenyl (5 mg/kg, i.p.) (Fig 2, p>0.05) nor PEA (40 ug, i.c.v., data not shown) induced analgesia in animals allowed undisturbed sleep.

The effects of deprenyl and s-phenylethylamine on phosphoramidon-induced analgesia in REM sleep deprived animals

The basal nociceptive threshold in REM sleep deprived rats was slightly, but significantly, lower than in animals allowed undisturbed sleep (Fig 2, p<0.05). Deprenyl (5 mg/kg, i.p., 60 min prior) induced a slight increase in the pain threshold of REMSD animals. A similar effect was observed during the first 10 min after PEA (40 ug, i.c.v.) administration (data not shown). However, the analgesic scores of REM sleep deprived rats treated with deprenyl (5 mg/kg, i.p., Fig 2) or PEA (40 ug, i.c.v., not shown) were not different from those of control animals (rats allowed undisturbed sleep). Phosphoramidon (250 ug, i.c.v.) had no analgesic action in animals subjected to REMSD (Fig 2). The analgesic score of REM sleep deprived rats after administration of deprenyl (5 mg/kg, i.p., 60 min prior) and/or PEA (40 ug, i.c.v., 5 min post-phosphoramidon) plus phosphoramidon (250 ug, i.c.v.), was not different from those treated with deprenyl alone (Fig 2, p >0.05).

DISCUSSION

In the animals allowed undisturbed sleep, the antinociceptive effect of the enkephalinase inhibitor phosphoramidon was potentiated by both PEA (specific substrate for MAO-B) and deprenyl (selective inhibitor of this enzyme). The analgesic effect of phosphoramidon was probably due to an increase in endogenous enkephalins and the consequent activation of opioid receptors sensitive to naloxone and naltrexone (Chaillet et al., 1983;
Figure 2: The effects of deprenyl (DEP), REM sleep deprivation (REMSD) and β-phenylethylamine (PEA) on the analgesic effect of the enkephalinase inhibitor phosphoramidon (PH). Nociception was determined by withdrawal of hind paw from pressure stimulation (modified Randall-Selitto test). The analgesic score was measured 60 min after DEP and 15 min after PH or saline (2 μl icv, SAL). Each bar is the mean analgesic score ± SEM. The number of rats per group is indicated in parenthesis. Note the following: a) DEP (5 mg/kg, ip) and/or PEA (40 μg, icv) significantly potentiated the analgesic effect of PH in animals allowed undisturbed sleep; b) REMSD decreased the basal pain threshold and PH-induced analgesia compared to rats allowed undisturbed sleep; c) DEP and/or PEA did not alter the blockade of PH-induced analgesia by REMSD. The levels of significance are given in the text.
We suggest that excess of PEA facilitates the analgesic action of enkephalins at the synaptic sites. This is in accordance with reports indicating that MAO-B inhibition and/or excess PEA potentiate the pharmacological effects of exogenously administered opiates/opioid peptides (Fuentes et al.; 1977, Garzon et al., 1980; Ukponmwan et al., 1983).

The mechanism by which MAO-B inhibition or excess PEA potentiates the analgesic effect of endogenously released enkephalins is not clear. It is possible that PEA enhances the interaction between opiates and their receptors (Fuentes et al., 1977). Thus, the MAO-B system may be an important regulator of the activity of opioids at the synaptic site in animals allowed undisturbed sleep.

The described facilitatory action of MAO-B inhibition on enkephalinergic transmission in animals allowed undisturbed sleep might be of relevance not only in the physiology of nociception, but also in human disorders in which the alterations in MAO-B have been reported. For example, it has been demonstrated that endogenous depression is associated with a decrease in brain PEA levels (Wolf and Mosnaim, 1983), whereas an increase in MAO-B activity is associated with the aging process (Benedetti and Keane, 1980). In such cases an alteration in MAO-B activity and PEA levels could modify the analgesic effects of endogenous opioid peptides.

However, the results of this study indicate that the possible alterations in the bioavailability of the MAO-B substrate, PEA, may not play an essential role in the failure of phosphoramidon to induce analgesia in REM sleep deprived rats. This statement is based on the fact that deprenyl and/or PEA did not alter the inhibitory effect of REMSD on phosphoramidon-induced analgesia.

The basal pain threshold was lowered in rats deprived of REM sleep. This is in accordance with a previous study in which, using noxious electric shock to assess pain sensitivity, it was established that REMSD decreased the pain threshold (Hicks et al., 1979). The reason for the reduction in the pain threshold in REM sleep deprived rats is not clear. It might be due to the already suggested functional insufficiency of enkephalinergic/endorphinergic system during REMSD (Ukponmwan and Dzoljic, 1984b; Ukponmwan et al., 1985) since opioid peptides play an important role in the regulation of the pain threshold.
threshold (Basbaum and Fields, 1984).

A functional insufficiency of an opioid system during REMSD (Ukponmwan et al., 1985) might partly explain why REM sleep curtailment is beneficial in treating some forms of depression (Vogel et al., 1980), since an increased opioid activity and corresponding decrease in pain sensitivity have been observed in this affective disorder (Risch, 1982; Pickar et al., 1982; Davis et al., 1979).

Further clinical experiments are necessary to clarify the roles of MAO-B and the enkephalinergic/endorphinergic systems in the regulation of the pain threshold in human diseases.

REFERENCES


Davis GC, Buchsbaum MS, Bunney WE (1979) Analgesia to painful stimuli in affective illness Amer J Psychiat 136: 1148-1151


Garrick NA, Murphy DL (1980) Species differences in the deamination of dopamine and other substrates for monoamine oxidase in the brain, Psychopharmacol 72:27-33

Hicks RA, Coleman DD, Ferrante F, Sahatjian M, Hawkins J (1979) Pain thresholds in rats during recovery from REM sleep deprivation, Percept Mot Skills 48: 687-690


Iwamoto ET, Ho IK (1972) The effects of MAO inhibition on morphine analgesia in mice, Fed Proc 31: 504


Randall OL, Selitto JJ (1957) A method for measurement of analgesic activity on inflamed tissue, Arch int Pharmacodyn 111: 409-419


Taylor DC (1962) Alarming reaction to pethidine in patients on phenelzine,

Ukponmwan OE, Rupreht J, Dzoljic MR (1984a) REM sleep deprivation decreases the antinociceptive property of enkephalinase inhibition, morphine and cold-water swim, Gen Pharmacol 15: 255-258

Ukponmwan OE, Dzoljic MR (1984b) Enkephalinase inhibition antagonizes the increased susceptibility to seizure induced by REM sleep deprivation, Psychopharmacol 83: 229-232

Ukponmwan OE, Poel-Heisterkamp AL van den, Dzoljic MR (1985) REM sleep deprivation decreases the grooming and shaking behaviour induced by enkephalinase inhibitor or opiate withdrawal, Pharmacol Biochem Behav 23: 385-389

Vogel GW, Vogel F, McAbee RS, Thurmond AJ (1980) Improvement of depression by REM sleep deprivation, Arch Gen Psychiat 37: 247-253

Wolf ME, Mosnaim AD (1983) Phenylethylamine in neuropsychiatric disorders, Gen Pharmacol 14: 385-390


REM SLEEP DEPRIVATION ANTAGONIZED THE MORPHINE-INDUCED AKINESIA AND CATALEPSY

ABSTRACT

An examination was made of the effect of REM sleep deprivation (REMSD) on some forms of altered motor activity, such as akinesia and catalepsy, induced by intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) administration of morphine in adult, male Wistar rats. Administration of morphine (25 mg/kg i.p.) induced an akinetic-cataleptic syndrome and decreased spontaneous vertical motor activity (SVMA) in animals allowed undisturbed sleep. REMSD decreased the morphine-induced akinesia and catalepsy that are known to be mediated by an inhibitory μ-opioid system. The locomotor depressant action of morphine was converted to excitation (manifested as increased SVMA and hopping behaviour) by REMSD. Similarly, decreased motor activity following i.c.v. administration of morphine (25 μg) was replaced by excitation in the form of jumping behaviour after REMSD. Naltrexone (1 mg/kg, i.p.) blocked the akinetic and cataleptic effects, but not the excitatory effects of morphine.

It is suggested that REMSD is associated with a functional insufficiency of an inhibitory μ-opioid system, thus unmasking the excitatory morphine effects. The proposed insufficiency of an endogenous opioid system might explain an increase in neuronal excitation during REMSD and the therapeutic effect of REM deficiency in some types of depression.

INTRODUCTION

Interactions between sleep and endogenous opioid systems have been documented. For example, activation of opioid receptor(s) suppresses REM sleep (1). REM sleep was associated with the episodic release of humoral endorphin (2) and can decrease the neuronal excitation induced by exogenously administered opioid peptides (3).

It has been suggested that alteration in REM sleep and endogenous opioid system are involved in some psychosomatic disturbances; for example,
increased REM sleep density and opioid activity are associated with endogenous depression (4-7). In addition, it has been shown that pharmacological and mechanical REM sleep deprivation can improve some forms of depression (8) and affect an opiate/opioid-induced analgesia (9). These data taken together suggest a functional interaction between REM sleep and opioid system in both physiological and pathological conditions.

To further clarify the role of REM sleep in the regulation of opioid activity, the effects of REM sleep deprivation (REMSD) on morphine-induced behaviours were studied. Particular attention was paid to some morphine-induced effects such as akinesia and catalepsy, which are frequently present in psychopathological conditions (10).

METHODS

Adult, male Wistar rats weighing 150-200 g were used. Animals were housed in groups of 3-5 rats per cage (40 x 20 x 15 cm) in constant environment chamber with a light-dark cycle 14:10 h (light phase, 07.00-21.00 h) and ambient room temperature 22±1°C.

REMSD

Animals were deprived of REM sleep continuously for 96 h using the classical "flower pot" technique previously described (11). In this procedure, rats were placed on platforms (14±0.5 cm²/100 g body weight) to avoid the problem of unequal REMSD (12). The platform was surrounded by water 0.5-1.0 cm below the island surface. This procedure is known to selectively deprive rats of REM sleep (11). In our experiment, the roof of the REMSD tank was designed to permit free access to food and clean drinking water. The water in the tank was changed once every 24 h, during which time the animals were subjected to 1-2 min of handling and then allowed to rest in home cages for 45-60 min and kept awake manually.

Stress control

To control for the unspecific stress factors associated with "flower pot" technique for REMSD, rats were placed on platforms large enough (57-59 cm²/100 g body weight) for them to curl up and have normal sleep without falling into the water. The large platform is known to stimulate the unspecific stress factors (isolation and dampness) associated with the
"flower pot" technique of REMSD without affecting REM sleep after 96 h (11,13). Otherwise, the stressed group of animals were treated as in REMSD group.

Control
Animals in this group were housed in home cages (40x20x15 cm) for 96 h. Like the REMSD and stressed groups, rats in the control groups were handled daily for 1-2 mins. All animals had free access to drinking water and food.

Behavioural observations
Behavioural observation and scoring were carried out in a quiet room with a temperature 22±2°C. Changes in behaviours were monitored in a transparent cylinder 18 cm in diameter and 27 cm height. The floor was covered with saw dust. REM sleep-deprived rats and stressed animals were placed in the recording cage within 5-10 min after discontinuation of these procedures. REM sleep deprived or stressed animals were first dried using absorbent towel, since they were often wet on removal from platforms. All rats were observed individually after 30 min habituation to the cage. Morphine-induced behaviours such as akinesia, catalepsy, or rearing were assessed and scored as present or absent every 10 min for the first 30 min and every 30 min thereafter for another 90 min.

Akinetic and cataleptic behaviours
Akinesia was defined as loss of spontaneous locomotor activity. A rat was scored as akinetic if it did not move for 5 min after placement at the corner of the cage.

Catalepsy was determined using the bridge test and/or loss of righting reflex (14,15). In this procedure rats were placed gently across a 10 cm wide bridge and/or on their backs. Animals that maintained this position for at least 1 min were scored as cataleptic. All cataleptic animals were akinetic, but not all akinetic rats displayed catalepsy simultaneously; both phenomena were seperately evaluated. Hopping behaviour was defined as the sudden jump along a horizontal plane.

Measurement of spontaneous vertical motor activity (SVMA) using an automated method
During preliminary experiments, it was observed that REMSD converted morphine-induced locomotor inhibition into excitation, characterised by increased rearing. This effect of REMSD was particularly evident 60-120 min
after morphine treatment. Therefore the SVMA was assessed quantitatively in both REMSD and control groups. SVMA was recorded by means of a computerized Varinex (Columbus Instrument, Columbus, Ohio, USA), in which rearing animals interrupted a magnetic field located between the floor of the cage and a plane 18 cm above.

On the experimental day, rats were transferred from home cages or platforms, weighed, and placed in transparent Plexiglas cages (40 x 20 x 15 cm) without sawdust and allowed 30 min habituation. All animals were then injected with saline (1 ml/kg, i.p.), and motor activity was recorded for an additional 30 min. Then, morphine (25 mg/kg, i.p.) was administered, and SVMA was monitored during the 60-120 min period after drug administration. The effects of morphine on SVMA were studied between 11.00-14.00 h to avoid known circadian variation in locomotion (16) and opioid receptor reactivity (17).

**Drug, dose and route of administration**

Morphine hydrochloride (Brocacef, Maarsen, The Netherlands) was administered dissolved in physiological saline. The cataleptogenic and locomotor inhibitory effects of morphine were studied at a fixed dose of 25 mg/kg, i.p., since it has been shown that 20 mg/kg i.p. induce robust catalepsy/ridigity in rats (18).

The intracerebroventricular (i.c.v.) administration of morphine (25 μg/rat) was carried out by means of chronically implanted cannulae. This dose was selected because it is known to induce the inhibitory effects of opiates (19).

**Statistical analysis**

Results expressed as percentages were analyzed using a one-tailed Fisher exact probability test. Data presented as counts were analyzed using the Kruskal Wallis one way analysis of variance (ANOVA) followed by a Mann-Whitney test. Statistical significance was accepted at P-values of 0.05 or less.
RESULTS

Intraperitoneal (i.p.) administration of morphine

Effect of REMSD on morphine-induced akinesia. Morphine (25 mg/kg) induced a significant akinetic behaviour in animals allowed undisturbed sleep (control and stress, Fig. 1, p<0.01). This akinetic effect of morphine in control and stressed animals reached maximum intensity within first 30-60 min and disappeared at 120 min after drug administration. However, there was no significant difference in the akinetic effect of morphine between control and stressed animals.

REMSD significantly decreased morphine-induced akinesia compared with the control group at 30 min and 60 min (Fig. 1, p<0.05, p<0.005, respectively, for each time period). Furthermore, REMSD converted the akinetic effect to excitation characterised by rearing, body jerks, and hopping behaviours. Saline (1 ml/kg, i.p.) did not induce akinesia in control, stressed or REMSD animals.

Effect of REMSD on morphine-induced catalepsy. The administration of morphine (25 mg/kg) induced significant catalepsy in animals allowed undisturbed sleep (control and stressed rats) 30 and 60 min after drug treatment (Fig. 2, p<0.05). The morphine-induced catalepsy was characterised by a profound state of muscular rigidity (evident in the bridge test) and loss of righting reflex, which disappeared at 120 min. The number of rats showing morphine-induced catalepsy in the control group was not significantly different from stressed groups. A close relationship was observed between akinesia and catalepsy, i.e. a significant catalepsy score was registered in the control group only when almost all animals showed akinesia.

Naltrexone (1 mg/kg, i.p.) completely blocked the morphine-induced akinetic-cataleptic syndrome in animals allowed undisturbed sleep (n=10 for control and stress groups together).

REMSD significantly decreased morphine-induced catalepsy compared with control animals allowed spontaneous amounts of sleep (Fig. 2, p<0.02, p<0.005 for 30 and 60 min, respectively).
Figure 1: Effect of REM sleep deprivation (REMSD) on morphine-induced akinesia. Number of animals per treatment group is indicated in parentheses. Note that REMSD significantly decreased the morphine-induced akinesia.
Figure 2: Effect of REM sleep deprivation (REMSD) on morphine-induced catalepsy. Number of animals per treatment group is indicated in parentheses. Note that REMSD significantly decreased the morphine-induced catalepsy 60 min after drug treatment.
Effect of REMSD on morphine-induced inhibition of SVMA. Kruskal Wallis ANOVA showed a significant difference across the treatment groups (Fig. 3, p<0.001, H=20.39, df=5).

Morphine (25 mg/kg) decreased SVMA in control animals. However, the morphine-induced inhibition of SVMA in stressed rats was not different from that in control animals (Fig. 3). In the REMSD group, morphine had the opposite effect, which was manifested as a significant increase in SVMA (Fig. 3, p<0.02). This morphine-induced increase in SVMA in the REM sleep deprived group was not altered by pretreatment with naltrexone (1 mg/kg, i.p., not shown).

Intracerebroventricular administration of morphine. In animals allowed undisturbed sleep i.c.v. administration of morphine (25 µg) induced decreased locomotion (with associated symptoms such as akinesia and catalepsy). However, the administration of morphine (25 µg) to animals deprived of REM sleep provoked excitatory behaviours such as hopping and jumping. The intensity of jumping behaviour in the REMSD group (25±9.5 jumps/2h, n=7) was significantly higher than in control (no jumps in 2 h, n=5) and stressed animals (1.2±0.8 jumps/2h, n=5, p<0.01).

DISCUSSION

The results of this study indicate that the akinetic-cataleptic syndrome and locomotor suppressant effects of morphine were antagonised by REMSD and replaced by excitatory behaviours manifested as increased SVMA: hopping and jumping.

It has been suggested that inhibitory effects of morphine such as akinesia and catalepsy are due to the activation of a naloxone/naltrexone sensitive µ-opioid receptor (20-22). This idea could be supported by this study, since a relatively low dose of µ receptor blocking substance naltrexone decreased the morphine-induced akinetic-cataleptic syndrome. Therefore, a decrease of morphine-induced akinetic-cataleptic syndrome by REMSD might suggest an insufficiency of an endogenous opioid system mediating the inhibitory effects of opiates. Similarly, REMSD antagonised the analgesic effects of opiates (9), which is supposed to be mediated by the
Figure 3: Effect of REM sleep deprivation (REMSD) on the inhibitory action of morphine on spontaneous vertical motor activity (SVMA). Number of animals per treatment group is indicated in parentheses. Note that REMSD converted the locomotor inhibitory effect of morphine to excitation manifested as increased SVMA.
same type of receptors (23,24).

Although morphine fails to induce akinetic/cataleptic syndrome in REMSD animals, changes in other transmitter/modulators are probably not involved. For example, the observed decrease in brain concentrations of acetylcholine, \( \gamma \)-aminobutyric acid (GABA), and somatostatin during REMSD (25-27) cannot account for the inhibition of morphine-stimulated akinesia and catalepsy in REM sleep deprived animals, since blockade of cholinergic and GABAergic systems or the administration of somatostatin did not alter opiate-induced akinetic-cataleptic syndrome (28,29). Similarly, increased serotonin release facilitated akinesia and catalepsy (30), while an elevated brain turnover of serotonin was observed during REMSD (31). A number of studies indicate a functional hyperactivity of dopaminergic system following REMSD (32). It is known that clinically effective neuroleptics that act as antagonists at dopamine receptors may induce catalepsy (33). However, opiates do not act as antagonists of dopamine receptors (34).

A second important finding was that REMSD converted the depressant effects of intracerebroventricularly administered morphine to excitation. The reason for the conversion of the depressant effects of morphine to excitation by REMSD is not clear. However, morphine can induce both inhibitory and excitatory behaviours, which are mediated by two groups of receptors. One is the naloxone-sensitive opioid receptor system which mediates analgesia, akinesia and catalepsy: the other is the naloxone-insensitive receptor system which mediates excitation (21,35,36). Activation or blockade of the \( \mu \)-opioid receptor can inhibit and facilitate, respectively, the excitatory effect of morphine (36). Thus, the conversion of the motor inhibitory effects of morphine to excitation during REMSD could be explained by the suggested deficiency of an inhibitory \( \mu \)-opioid system in REM sleep deprived animals and a corresponding predominance of the excitatory opioid receptor system.

Therefore, in conclusion, we suggest that functional insufficiency of a \( \mu \)-opioid system during REMSD might be a background to increased neuronal excitability in REM sleep deprived animals. Namely, it is known that endogenous opioids exert an inhibitory influence on the release of excitatory transmitters (37). This possibility can be supported by the report that blockade of opioid receptors with naloxone facilitated epileptogenesis (38).
The proposed insufficiency of an opioid system during REMSD might be an explanation for a therapeutic and diagnostic value of REM sleep curtailment in some cases of depression (8) and epilepsy (39), respectively.

REFERENCES


22. Chaillet P, Marcais-Collado H, Costentin J (1983) Catatonic or hypotonic immobility induced in mice by intracerebroventricular injection of \( \mu \) or kappa opioid receptor agonists as well as enkephalins or inhibitors of their degradation, Life Sci 33: 2105-11


CHAPTER 8

REM SLEEP DEPRIVATION DECREASES THE GROOMING AND SHAKING BEHAVIOUR INDUCED BY ENKEPHALINASE INHIBITOR OR OPIATE WITHDRAWAL

ABSTRACT

Intraventricular administration of enkephalinase inhibitor, phosphoramidon (1 x 10^-8 - 5.6 x 10^-7 moles, i.c.v.) induced a behavioural syndrome consisting of excessive grooming with body scratching as the most prominent symptom and wet-dog-shakes (WDS). The frequency of the phosphoramidon-induced WDS and body scratching were decreased by the pretreatment with the opioid receptor blocking agent, naltrexone (2.9 x 10^-6 moles/kg, i.p.). Both the phosphoramidon-induced WDS in naive rats and naloxone-precipitated withdrawal WDS were decreased in REM sleep deprived rats compared with animals allowed normal sleep (control and stress groups).

The results are discussed in light of a possible functional insufficiency of an endogenous opioid system during REMSD. It has been suggested that this insufficiency might be a background to the increased neuronal excitability during REMSD.

INTRODUCTION

Several endogenous substances including opioid peptides have been demonstrated to induce grooming and wet-dog-shakes (5,6,9,12). Recently, we demonstrated that the administration of the enkephalinase inhibitor, phosphoramidon, induced behaviours such as grooming and wet-dog-shakes (WDS) (28). These behavioural phenomena, which can be induced by various drugs in naive animals, are part of the morphine withdrawal syndrome. There are indications that both grooming and WDS may share a common neural mechanism (10).

It has been demonstrated that inhibition of protein synthesis reduced the severity of opiate withdrawal phenomena (19). In addition it is known that REM sleep deprivation (REMSD) can decrease protein synthesis (30). REMSD
also inhibited morphine induced analgesia (38). These data suggest that alterations in REM sleep can modulate both protein synthesis and pharmacological effects of opiate substances. Therefore, we analyzed the relationship between REMSD and grooming and/or shaking behaviour induced by enkephalinase inhibitor phosphoramidon in naive rats and naloxone-precipitated withdrawal in the opiate-dependent rats.

METHODS

Adult, male Wistar rats (100-125 g) housed in transparent plastic cages in a constant environment room with a light-dark cycle 14:10 (light phase 07.00-21.00 h) were used.

Intracerebroventricular (i.c.v.) administration of drug solutions

For i.c.v. administration of drugs a stainless steel guide cannula was stereotaxically directed 1 mm above the lateral ventricle. Drug solutions (maximum volume 2 µl) were injected into the lateral ventricle with gauge 30 needle, attached to a Hamilton microsyringe by polyethylene (PE) tubing. The length of the needle was made such that it protruded 1 mm into the lateral ventricle. The injection was made over 10 sec and the needle maintained in position for an additional 10 sec. Correct ventricular cannulation was verified before and after each experiment using a modification of the technique previously described by Paakkari (24). In this procedure a PE tubing is attached to the injection needle and filled with artificial cerebrospinal fluid (CSF) or saline. To test the correct placement of i.c.v. cannula during surgery, the tubing is raised above the head of the animal on the stereotaxic apparatus, and a rapid inflow of saline denotes a correct placement of cannula. The cannula was moved only in a downward direction to avoid the possible false positive effect due to an upward movement of the cannula after the first unsuccessful cannulation (14).

REM sleep deprivation (REMSD)

REMSD was carried out according to the conventional "flower pot" technique previously described (21). In this procedure, rats were placed on platforms (14 cm²/100 g rat) surrounded by water, such that the water level was 0.5-1.0 cm below the platform. Rats made morphine-dependent were placed on platform and deprived of REM sleep from day 7-11 (96 hr) of
morphinization. During this period animals received the normal doses of morphine for these days. Forty-five to 60 min after the discontinuation of REMSD the animals were injected with the enkephalinase inhibitor phosphoramidon. In this period of time the animals were kept awake manually. The behavioural changes were scored during the following 30 min.

Control for the unspecific stress factors associated with the "flower pot" technique

In order to control for the known stress factors (dampness, isolation and immobilisation) associated with the "flower pot" technique rats were placed on platforms large enough (60 cm²/100 g rat) for them to curl up and have normal sleep. The large platform can simulate the chronic stress condition associated with REMSD without affecting REM sleep level after 96 hr (20,21).

Phosphoramidon-induced behaviour

Behavioural observation and scoring were carried out on each individual rat housed singly in Plexiglas cages (40 x 20 x 15 cm) containing saw dust. Wet-dog-shakes consisting of paraxysmal shudder of the whole body along the spinal axis were registered and quantified. The body scratching (BS) episode is defined as head or body scratches followed immediately by the licking of the paw used in scratching.

Induction of morphine dependence

Animals were made dependent on morphine according to the repeated injection procedure previously described (32). In this method rats were given two intraperitoneal injections of morphine daily (at 07.30 and 15.30 hr). The dosage schedule was as follows: Days 1 and 2 (7 x 10⁻⁵ moles/kg/day); Days 3 and 4 (14 x 10⁻⁵ moles/kg/day); Days 5 and 6 (28 x 10⁻⁵ moles/kg/day) and Days 7-11 (56 x 10⁻⁵ moles/kg/day).

Precipitation of morphine withdrawal shaking behaviour

Abstinential behaviour in morphine dependent animals was provoked by injecting naloxone (3.1 x 10⁻⁶ moles/kg i.p.) three hours after the last injection of morphine. Prior to receiving naloxone treatment each rat was allowed a habituation period of 30-60 min in the observation area. Naloxone-precipitated WDS in morphine-dependent rats and phosphoramidon-induced behavioural phenomena in naive animals were studied in the following three groups of rats:— a) Control group;— these rats were housed singly in
home cages throughout the experimental procedure and allowed spontaneous amounts of sleep: b) REM sleep deprived group:– these rats were submitted to 96 hr continuous REM sleep deprivation; c) stressed group:– these animals were chronically stressed for 96 hr.

**Drugs**

The following drugs were used: morphine hydrochloride (Merck), naloxone hydrochloride (Endo Lab) and naltrexone hydrochloride (Endo Lab) were administered dissolved in physiological saline. Phosphoramidon (Peninsula Lab) was dissolved in CSF prepared fresh and administered i.c.v.

**Data Analysis**

The results were analyzed using the Kruskal-Wallis one-way ANOVA. The statistical difference between two groups of treatment were carried out using a two-tailed Mann Whitney U test, except when indicated in text.

**RESULTS**

**Effects of REMSD on behavioural syndrome induced by enkephalinase inhibition**

The intracerebroventricular administration of the enkephalinase inhibitor phosphoramidon (1 x 10^-8 - 5.6 x 10^-7 moles i.c.v.) induced a behavioural syndrome consisting of excessive grooming (as measured by body scratching) and wet-dog shakes (WDS) in all three groups of animals (control, REMSD and stressed). These symptoms appeared within 5 min after phosphoramidon administration and were still observed after 240 min. The Kruskal-Wallis one way ANOVA showed a significant difference in the phosphoramidon-induced WDS across the groups (H=106.21, NDF=3 p<0.001). The frequency of phosphoramidon-induced WDS was dose-related in both control (p<0.05) and stressed (p<0.02) groups of animals (Fig 1). However, the frequency of the phosphoramidon-induced WDS in stressed rats was not significantly different from control animals (Fig 1, p>0.05). REMSD significantly decreased the WDS induced by three doses of phosphoramidon (Fig 1, p<0.02, p<0.002, p<0.002 respectively, for increasing doses). The frequency of BS in the REM sleep deprived animals was significantly less intensive compared with control and stressed animals (Fig 3, p<0.02). There was no significant difference in the mean BS between control and stressed animals (Fig 3, p>0.10).

Naltrexone (2.9 x 10^-6 moles/kg i.p., 10 min prior) significantly
Figure 1: The phosphoramidon ($1 \times 10^{-8}$ - $5.6 \times 10^{-7}$ moles i.c.v.)- induced wet-dog-shakes (WDS). Each point is mean ± S.E.M. The number of rats per dose of phosphoramidon is stated in parentheses. Note that the phosphoramidon-induced WDS was significantly lowered in REMSD rats compared with control or stressed animals.
decreased the phosphoramidon-induced WDS in control (p<0.002), REMSD and stressed animals (p<0.05, Duncan New Multiple range test) (Fig 2). In the control rats the phosphoramidon-induced BS were significantly less frequent after pretreatment with naltrexone (92.8±23.6, n=11) compared with saline pretreated animals 201.8±29, n=12, p<0.05).

Figure 2: Effect of naltrexone (2.9 x 10^-6 moles/kg, i.p.) on phosphoramidon (5.6 x 10^-7 moles i.c.v.)-induced wet-dog-shakes (WDS). Each bar is mean ± S.E.M. The number of animals per treatment group is indicated in parentheses. Note that pretreatment with naltrexone significantly decreased phosphoramidon-induced WDS.
Figure 3: Effect of REMSD on phosphoramidon (5.6 × 10^{-7} moles, i.c.v.) induced body scratches. Each bar is mean ± S.E.M. The number of rats per treatment group is stated in parentheses. Note that the intense body scotches induced by phosphoramidon in control and stressed animals were significantly decreased by REMSD.

Effect of REMSD on opiate withdrawal WDS

Naloxone (5.1 × 10^{-6} mole/kg i.p.) precipitated WDS in morphine-dependent rats in control, REM sleep deprived and stressed groups of animals. The Kruskal-Wallis one way ANOVA showed a significant difference in the withdrawal WDS across the groups (Fig 4, Χ^2=16.12, NDF=2, p<0.001). The frequency of the precipitated WDS was significantly more pronounced in
Figure 4: Effect of REMSD on naloxone ($3.1 \times 10^{-6}$ moles/kg i.p.)-precipitated withdrawal wet-dog-shakes (WDS). Each bar is mean ± S.E.M. The number of rats per group is indicated in parentheses. Note that the total frequency of withdrawal WDS was significantly less in REMSD rats compared with control (p<0.01) and stressed (p<0.002) animals.
animals allowed to sleep normally (control and stressed groups) than in the REM sleep deprived rats (Fig 4, p<0.002). However, the intensity of such induced WDS in control or stressed animals were not significantly different (Fig 4, p>0.2). Body scratchings in naloxone-treated morphine dependent rats were few and irregular and therefore omitted from further detailed quantitative evaluation.

DISCUSSION

The results of this study showed that WDS and grooming induced by enkephalinase inhibitor, phosphoramidon, were inhibited by naltrexone, which might indicate an involvement of opioid receptor(s). This is consistent with the fact that enkephalinase inhibition can activate opioid receptors by blocking the biotransformation of endogenously released opioid peptides (16,25). In addition, the WDS-induced by i.c.v. administration of enkephalins were attenuated by opiate antagonists (2,5,6,13).

Grooming behaviour has also been observed after low doses of morphine (29). Taken together, these data might suggest that WDS and grooming induced by phosphoramidon or opioid substances share a common mechanism.

The biological significance of WDS and grooming induced by different chemical compounds is not clear. Some data suggest that WDS are indicative of arousal (10), whereas grooming might be a "de-arousing" homeostatic mechanism (17). In addition, it has been demonstrated that opioid peptides facilitate arousal (37) and in higher doses induced an electrophysiological and behavioural phenomena similar to epilepsy (7,8). Thus the excessive grooming observed in our experiment might be a response to the phosphoramidon-induced arousal, manifested as WDS.

However, the most important aspect of this report is the fact that REMSD suppressed the WDS and grooming induced by enkephalinase inhibition in naive rats. Why REMSD decreased these behaviours in rats is not clear. It could, however, be suggested that REM sleep deprived animals might have limited availability of opioid peptides and hence the WDS and grooming precipitated by phosphoramidon could be less pronounced. Although there is no direct biochemical evidence for the insufficiency of the enkephalinergic system during REMSD this possibility could be considered since it is known that
REMSD is associated with the inhibition of protein synthesis (30). The concept of a functional insufficiency in the enkephalinergic/endorphinergic system in REM sleep deprived animals receives further support from the fact that REMSD abolished the antinociceptive effects of morphine and phosphoramidon (38).

This hypothesis of a functional insufficiency in this opioid system might explain the increased neuronal excitability during REMSD (4) since it is known that opioid peptides exhibit tonic inhibitory effects on the release of excitatory transmitters (23). However, additional experiments are required to clarify whether this mechanism might be involved in REMSD-precipitated seizures and the therapeutic effect of REMSD in some types of endogenous depression.

A second important finding of this study is that REMSD inhibited abstinential WDS. The mechanism of opiate addiction/withdrawal is complex and probably involves alteration in several neurotransmitter/neuromodulator systems. However, the known changes in classical transmitters during REMSD can not account for the decrease in naloxone-precipitated withdrawal WDS in REM sleep deprived animals. For example, REMSD increased the functional activity of dopaminergic system (36), but did not alter the adrenergic system (31). However, substances which block these systems inhibited withdrawal WDS (18,34) Furthermore, the known changes in brain serotonin metabolism during REMSD (33) probably play no role in the inhibition of abstinential WDS in REM sleep deprived rats, since the alteration of the serotonergic system had no clear effect on WDS induced by morphine withdrawal (1). It is also known that drugs which stimulate central muscarinic receptors inhibited the shaking response (39), whereas REMSD decrease the acetylcholine content of the brain (3,35).

Although some high energy phosphates can antagonize the effects of morphine, there is no evidence that the concentrations of AMP, ADP and ATP are significantly altered by REMSD (11,22).

Therefore, an alternative explanation for the inhibitory effect of REMSD on morphine withdrawal WDS should be considered. Namely, it is known that during development of morphine dependence there is an increase of the synthesis of secretory proteins in the brain regions (pons-medulla and stratum -septum)(27), which are particularly rich in opioid receptors (26).
and functionally involved in opiate dependence (15). It has also been demonstrated that REMSD decreased protein synthesis in the cerebral and brain stem fractions (30) and that synthesis can decrease opiate withdrawal phenomena (19). Thus the decrease of protein synthesis during REMSD might explain the inhibitory effect of REMSD on withdrawal WDS.

REFERENCES


29. Schiørring E, Hecht A (1979) Behavioural effects of low, acute doses of morphine in non tolerant groups of rats in an open-field test, Psychopharmacol., 64: 67-71


the antinociceptive property of enkephalinase-inhibition, morphine and cold-water-swim. Gen. Pharmacol., 15: 255-258

CHAPTER 9

ENKEPHALINASE INHIBITION ANTAGONIZES THE INCREASED SUSCEPTIBILITY TO SEIZURE INDUCED BY REM SLEEP DEPRIVATION

ABSTRACT

In order to elucidate the relationship between REM sleep and the enkephalinergic system, the effects of REM sleep deprivation (REMSD), stress and the enkephalinase inhibitor phosphoramidon on handling-induced-convulsions were studied in mice. REMSD, stress and phosphoramidon (25-500 μg i.c.v.) increased the frequency of handling-induced convulsions (HIC) in normal mice. However, only in the last two groups were HIC antagonized by naloxone (1 mg/kg i.p.). In REMSD mice, phosphoramidon decreased the frequency of HIC, this effect being abolished by naloxone. The increase of neuronal excitability during REMSD is suggested to be associated with an insufficiency of an enkephalinergic system.

INTRODUCTION

Rapid-eye-movement (REM) sleep is known to modulate neuronal excitability in man and animals (Drucker-Colin et al., 1977; Passouant et al., 1965). However, in clinical reports and animal experiments, it has been demonstrated that REM sleep deprivation (REMSD) increases neuronal excitability and facilitates seizure activity (Pratt et al., 1969; Cohen and Dement, 1965). Evidently, the phasic changes in neuronal activities during sleep can influence the pathophysiology of seizures.

Recently, it has been suggested that the enkephalinergic system plays an important role in epileptogenesis (Frenk et al., 1978; Dzoljic et al., 1979). It is also known that β-endorphin exerts an inhibitory influence on REM sleep (King et al., 1981). In addition, we have demonstrated that REM sleep has an inhibitory effect on enkephalin-induced seizures (Ukponmwan and Dzoljic, 1983). These data might indicate an involvement of the endogenous opioid system in mechanisms regulating REM sleep and neuronal excitability.
In order to elucidate further the relationship between REM sleep and enkephalins, we studied the effects of REMSD, stress and the enkephalinase inhibitor, phosphoramidon on handling-induced convulsions in mice.

MATERIALS AND METHODS

Animals:
Adult, male mice of B10 A strain (25-30 g) were used (Olac Ltd, Bicester, England). Intracerebroventricular (i.c.v.) administration of drugs was by means of a stainless steel cannula stereotaxically implanted in the lateral ventricle. A minimum of 3-5 days was allowed for recovery before experiments were commenced.

Animals were then divided into three groups: Group A (control) mice housed individually in transparent cages and allowed spontaneous amount of sleep; Group B mice were subjected to 72 h REMSD according to the conventional "flower pot" technique described by Fishbein (1970). Only mice which could habituate to the platform within 3 h were used for experimentation. Group C (stress) mice were placed on platforms large enough (8.0 cm diameter, for 72 h) for the animals to curl up and exhibit REM sleep without falling into the water.

Handling-induced convulsions (HIC):
HICs in mice were assessed using the criteria previously described for alcohol withdrawal (Goldstein and Pal, 1971). In this procedure each mouse was picked up by the tail and/or spun gently through 180°. HIC was characterised by violent jerking or twirling, tonic convulsions and tightening of facial muscle (grimace). Only HICs occurring within 6-10 s of pick-up were recorded. Scores were assigned as follows: violent tonic-clonic convulsions upon pick up-4, tonic-clonic convulsions upon picking up-3, tonic convulsions upon picking up or tonic-clonic convulsions after gently spinning-2, tonic convulsions after gentle spinning-1, and facial grimace after gentle spinning-0.5. The scoring procedure is based upon the criteria described by Crabbe et al. (1981). HIC was determined at 10 min intervals form 120 min. The intensity of convulsions is indexed by total HIC during the first 30 min scoring period. The period was chosen to avoid the possible modifying influence of repeated handling. The observer
was "blind" during the evaluation of dose-response curve of the effect of phosphoramidon in all three groups.

**Drugs:**

Phosphoramidon (Peninsula Laboratories) and naloxone hydrochloride (Winthrop Laboratories) were dissolved in saline. The maximum volume of phosphoramidon administered i.c.v. over 20 s was 1.5μl. Naloxone was administered intraperitoneally (i.p.).

**Statistical analysis of data:**

The Kruskal-Wallis ANOVA was carried out on the mean convulsions score and across all data in all the experiments. Comparisons between any two treatment groups were made with the Mann-Whitney U-test (Siegel, 1956).

**RESULTS**

The Kruskal-Wallis test showed significant differences in convulsion scores across the groups ($H=174.47$, $p<0.001$, $NDF=15$). The levels of significance between two groups using the Mann-Whitney U test are indicated below.

**Control.** In this study, 16% of control non-treated mice displayed mild signs of HIC such as grimacing. In treated mice, saline injection (1μl i.c.v.) or naloxone also induced signs of HIC consisting mainly of grimacing. However, phosphoramidon (25-500 μg i.c.v.) significantly increased the intensity and degree of susceptibility to HIC in a dose-related manner ($p<0.01$). This effect was antagonized by naloxone (1 mg/kg, i.p.) (Fig 1A).

**REMSD group.** Mice subjected to 72 h REMSD demonstrated a significant increase in the incidence and intensity of HIC ($p<0.001$). This was not affected by naloxone ($p=0.1$) but significantly inhibited by the enkephalinase inhibitor phosphoramidon (25-500 μg) administered within 10 min after REMSD termination ($p<0.001$, Fig 1B). This effect of phosphoramidon was partially antagonised by naloxone (1 mg/kg i.p. 10 min prior, $p<0.001$).

**Stress group.** Stress also induced an increase in the intensity and susceptibility to HIC ($p<0.001$) but less intensely than in REMSD group. This effect was further potentiated by phosphoramidon (100 μg, $p<0.001$). Naloxone (1 mg/kg, i.p.) decreased the susceptibility to HIC in stress animals ($p<0.001$, Fig 1C).
Figure 1a-c: The effect of REM sleep deprivation (REMSD, 72 h), phosphoramidon (ph), naloxone (nal) and stress (72 h) on handling-induced-convulsions (HIC). Each bar is mean intensity ± S.E.M of HIC during the first 30 min. The number of animals per group is stated in parentheses.
DISCUSSION

Mice subjected to REMSD, stress or phosphoramidon treatment showed an increase in the susceptibility to HIC. However, only in stressed or phosphoramidon-treated animals was the convulsant activity antagonised by naloxone. This might indicate that the phosphoramidon- and stress-induced proconvulsant activity is due to activation of an endogenous opioid system. This agrees with data showing that concentrations of opioid peptides are increased after treatment with enkephalinase inhibitors (Patey et al., 1981) and that various stress regimens are associated with the release of endogenous opioid peptides (Christie and Chesher, 1982). The evidence that stress and enkephalinase inhibition increase susceptibility to epileptiform activity could be ascribed to the fact that opioid peptides in excess may induce seizure phenomena. The epileptogenic potential of various opioid peptides administered exogenously has been demonstrated frequently (Urcía et al., 1977; Dzoljic et al., 1979; Snead and Bearden, 1982).

However, the mechanism of epileptiform activity of opioid peptides is not completely understood. Recent data suggest that specific opioid receptors of the delta-subtype mediate the epileptiform effect of these substances (Dzoljic and vd Poel-Heisterkamp, 1982; Haffmans and Dzoljic, 1983; Frenk, 1983). The target area of this action seems to be the limbic system (Henriksen et al., 1980), particularly the hippocampus (French and Siggins, 1980; Haffmans et al., 1983; 1984). It has been shown that opioid peptides may excite hippocampal neurons by inhibiting adjacent interneurons (Zieglgansberger et al., 1979). Thus, it might be suggested that an increased susceptibility to HIC in animals, stressed or treated with phosphoramidon is due to the activation of a particular type of opioid receptor population in selective brain regions(s). The potentiating effect of phosphoramidon on stress-induced HIC is probably due to the protection of the released opioid peptides during the stress procedure.

A second significant aspect of this study is that the enkephalinase inhibitor phosphoramidon decreased convulsant behaviour in REMSD mice. This effect of enkephalinase inhibition might suggest that REMSD is associated with an insufficiency of endogenous opioid peptides. This possibility is supported by the fact that REMSD decreases peptide synthesis (Shapiro and
Girdwood, 1981) and affects the levels of some brain peptides (Mattiace et al., 1981). In addition, recent data indicate that nocturnal episodic secretion of humoral endorphins occurs during REM sleep (Oksenberg et al., 1980).

However, the exact mechanism by which an enkephalinase inhibitor antagonizes REMSD-induced neuronal excitation is not clear. One possible explanation that should be considered is the fact that enkephalins block GABA transport across plasma membrane (Cupello and Hyden, 1981), leaving GABA outside the neuronal membrane in contact with its receptors for a longer time. Such mechanism might explain enkephalin-induced neuronal inhibition in physiological circumstances. In the context of these findings, a proposed decrease of enkephalinergic activity during REMSD would be associated with a decrease GABA inhibitory activity and consequent increase in seizure susceptibility.

In summary, these results indicate that enkephalinase inhibition may have, depending on conditions, proconvulsant potential (in stress or control animals) or anticonvulsant (in REMSD animals) action. Similar contradictory data concerning the proconvulsant potential of morphine and endogenous opioids have been reported. Both the pro- and anticonvulsant activity of these substances have been demonstrated (Gilbert and Martin, 1975; Verdeaux and Marty, 1954; Cowan et al., 1979; Tortella et al., 1981; Dzoljic, 1982).

An insufficiency of endogenous opioid peptides in REMSD animals suggested by this study might be of importance for the worsening of seizures or improvement of depressive disorders during REMSD (Pratt et al., 1968; Vogel et al., 1980). It is of interest to note that in narcolepsy, the attacks of REM sleep have been prevented by naloxone, suggesting a possible involvement of endogenous opioid systems (Pasi et al., 1983).

REFERENCES


Cohen HB, Dement WC (1965) Sleep: Changes in the threshold to electroconvulsive shocks in rats after deprivation of "paradoxical" phase. Science, 150:


French ED, Siggins GR (1980) An iontophoretic survey of opioid peptides action in the rat limbic system in search of opiate epileptogenic mechanisms, Regulatory peptides, 1: 127-146


King C, Masserano JM, Codd E, Byrne WL (1981) Effects of $\alpha$-endorphin and morphine on sleep-wakefulness behaviour of cats, Sleep, 4: 259-262
Siegel S (1956) Nonparametric statistics for behavioural science, McGraw-Hill,
New York


Zielgansberger W, French ED, Siggins GR, Bloom FE (1979) Opioid peptide may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons, Science, 205: 415-417
CHAPTER 10

REM SLEEP DEPRIVATION INHIBIT THE NITROUS OXIDE WITHDRAWAL CONVULSIONS

ABSTRACT

The effect of REM sleep deprivation (REMSD) was investigated using withdrawal convulsions in mice following exposure to nitrous oxide ($N_2O$). REMSD for 72 h significantly decreased the severity of withdrawal convulsions following acute exposure to $N_2O$.

The results were interpreted in the light of protein synthesis inhibition during REMSD. REMSD procedure might stimulate a new approach to the treatment of withdrawal neuronal excitability.

INTRODUCTION

It is known that REM sleep deprivation (REMSD) results in a decrease in protein synthesis (Drucker-Colin and Rojas-Ramirez, 1976) and is often associated with an increase in neuronal excitability. For example, the threshold for electroconvulsive shocks (Cohen and Dement, 1965) and amygdaloid kindling (Calvo et al., 1982) is greatly reduced following REMSD.

Patients recovering from nitrous oxide anaesthesia are known to exhibit an increase in excitability (Eckenhoff et al., 1961). Similarly, mice exposed to nitrous oxide showed convulsions when picked up by the tail after removal from the anaesthetic (Harper et al., 1980). This seizure pattern is known to occur after exposure to nitrous oxide, ethylene and cyclopropane and it is considered to be a type of withdrawal convulsions (Smith et al., 1979).

It is of interest to note that some types of epileptic attacks are associated with REM sleep dysfunction (Passouant, 1976) and also with drug addiction (Herzlinger et al., 1977; Mendelson and Mello, 1978).

In this study we report on the inhibitory effect of REMSD on nitrous oxide withdrawal convulsions.
METHODS

Adult, male mice of the B10A strain weighing 32±0.2 g at the onset of experiment were used. Throughout the investigation the animals were housed under constant light/dark cycle (light phase 09.00-21.00). The room temperature was maintained at 24±°C. Food and water were available ad libitum. The number of animals used in each procedure is stated in the results.

REMSD was carried out according to the conventional water tank method previously described (Fishbein et al., 1971). This method selectively deprives mice of REM sleep and with slight effect on slow wave sleep. In this study, each mouse was placed on a platform (3 cm diameter, 1 cm above the water level) surrounded by water (3 cm deep). The water was replaced at least once in 24 h. Only mice which could adjust to this experimental condition within 2 h were used in experiments.

Stress control group for REMSD consisted of mice placed on platform (6 cm in diameter ) under the same experimental conditions as in REMSD. A second stress consisted of mice forced to swim in water 3 cm deep at 18°C for two, 1 h sessions daily. These two stress control procedures produced comparable weight loss as in REMSD (10-15%).

Nitrous oxide withdrawal convulsion was induced and assessed according to the method of Goldstein and Pal (1971). In this procedure mice were exposed to a mixture of nitrous oxide and oxygen (80:20) at 1.6 atm for 1 h. Each mouse was picked up by the tail and spun gently through 180°. Mice showing violent jerking or twirling and/or grimace were quantified as positive. Mice were tested for the presence or absence of handling-induced convulsions (HIC) every 10 min after the removal from N2O, until there were no convulsions in two successive tests. Throughout this study, all testing of HIC was carried out between 10.00-14.00 h to avoid circadian alterations in seizure threshold.

Statistical analysis: The number of animals convulsing after N2O withdrawal in control, stressed and REMSD groups were compared using the Kx2 method (de Jong, 1963). Statistical significance was accepted at P-values of 0.05 or less.
RESULTS

**Nitrous oxide withdrawal convulsions (n=20)**

Mice exposed to $\text{N}_2\text{O}$ showed withdrawal convulsions lasting about 2 h with highest incidence within 20 min after removal from $\text{N}_2\text{O}$. The mice convulsed when picked up by the tail following $\text{N}_2\text{O}$ anaesthesia. These handling-induced convulsions (HIC) consisted of violent jerking and twirling (clonic components) and arching of the back and contractions of head muscle ("grimace", tonic components).

**REM sleep deprivation (n=20)**

Mice subjected to REMSD for 24-120 h showed increased susceptibility to HIC. However, mice subjected to REMSD for 72 h showed a decrease in the degree of susceptibility to $\text{N}_2\text{O}$ withdrawal convulsions (Fig A1). Stress did not inhibit $\text{N}_2\text{O}$ withdrawal convulsions (n=10), but instead exacerbated it. Combined REMSD and an enkephalinase inhibitor phosphoramidon (Hudgin et al., 1981) also decreased the frequency of HIC but not to a degree significantly different from REMSD or phosphoramidon given alone (Fig A.1).

DISCUSSION

Mice exposed to $\text{N}_2\text{O}$ develop very quickly a drug dependence which is characterized by withdrawal convulsions. This is demonstrated when mice are picked up by the tail following $\text{N}_2\text{O}$ anaesthesia. This type of convulsions was counteracted by REM sleep deprivation (REMSD), in spite of the fact that REMSD could induce neuronal excitation in the normal animals.

The neurobiological basis of acute $\text{N}_2\text{O}$ tolerance and withdrawal convulsions is still unclear, it is does not appear to involve changes in synaptic membrane fatty acid, phospholipid and cholesterol (Koblin et al., 1979). However the possible involvement of endogenous opioid peptides in $\text{N}_2\text{O}$ tolerance and withdrawal excitation has been demonstrated. Acute exposure to $\text{N}_2\text{O}$ induced an increase in the cerebrospinal fluid met-enkephalin levels (Qock et al., 1985), whereas the administration of morphine or naloxone can inhibit and facilitate, respectively, the $\text{N}_2\text{O}$ withdrawal convulsions (Manson et al., 1983). This suggests that $\text{N}_2\text{O}$ stimulates an opioid-like dependence.
Figure A.1: The effect of REM sleep deprivation (REMSD) on nitrous oxide withdrawal convulsions. Control consists of mice not subjected to any treatment. (*) denotes statistical significance at $p < 0.05$ level compared to control or stress. Note that 1) REMSD significantly reduced the frequency and duration of nitrous oxide withdrawal convulsions; 2) phosphoramidon did not alter the inhibitory action of REMSD on nitrous oxide withdrawal convulsions. The effect of the enkephalinase inhibitor phosphoramidon was not considered in this chapter.
It is known that several drugs with the common ability to inhibit protein synthesis reduced the development of dependence to opiates (Feinberg and Cochin, 1972; Bowman and Rand, 1980) and decrease REM sleep (Drucker-Colin and Rojas-Ramirez, 1976). In addition REMSD has also been reported to decrease protein synthesis in some nuclei of the rat brain stem (Bobillier et al., 1974; Panov, 1982). The antagonistic effect of REMSD on N\textsubscript{2}O withdrawal convulsions might be due to decrease in protein synthesis, which in turn blocked the development of tolerance and physical dependence.

Stressed animals showed enhanced susceptibility to N\textsubscript{2}O withdrawal convulsions proving that the inhibitory action of REMSD on N\textsubscript{2}O withdrawal convulsions is not related to the unspecific effects (dampness, isolation and restriction) of the experimental procedure.

Apart from the uncertainty of the interactions between REMSD and N\textsubscript{2}O withdrawal syndrome, the results of this study indicate that REMSD might prove useful as new approach to the management of withdrawal neuronal hyperexcitability.

REFERENCES


Feinberg MP, Cochin J (1972) Inhibition of development of tolerance to morphine
by cycloheximide, Biochem. Pharmacol., 21: 3083-3085


Jonge H de (1963) Inleiding tot medische statistiek. Wolters-Noordhoff, Groningen


In chapter 1 a general review of the literature on sleep-waking mechanisms, functions of sleep, and the biological and therapeutical effects of sleep deprivation was provided. Chapter 2 gave a brief review of the role of the endogenous opioid system in physiological regulation.

In this thesis the role of endogenous opioid peptides in the regulation of sleep-waking states and the effects of REM sleep on reactivity to endogenously released and exogenously administered opioid peptides, or opiates, are reported. We explored the possibility that REM sleep is involved in regulating the functioning of opioid peptidergic neurons in the central nervous system by studying the effects of REM sleep deprivation (REMSD) on behavioural responses to endogenously released opioid peptides and exogenously administered opiates and opioid peptides. The following opioid/opiate-induced behavioural responses were examined in this thesis: analgesia, akinesia-catalepsy, spontaneous vertical motor activity (SVMA), convulsions, grooming and nitrous oxide and morphine withdrawal symptoms.

Endogenous opioid peptides and waking mechanism(s)

The possible involvement of endogenously released opioid peptides in waking mechanism(s) is suggested by the diurnal oscillations in enkephalin and &-endorphin concentrations in brain nuclei, involved in vigilance state regulation (chapter 1, sections 1.2, 1.3.2i). Thus, concentrations of &-endorphin, enkephalins and dynorphin in the rat brain are highest in the dark, during which wakefulness is high, and lowest in the light phase, when propensity to sleep is at its highest.

In our study we demonstrated that the inhibition of enkephalinase, with phosphoramidon, induced an increase in wakefulness. Both NREM and REM sleep stages were suppressed. The increased wakefulness induced by enkephalinase inhibition was accompanied by excitatory symptoms, such as head shakes, scratching (chapter 3) and excessive grooming, which normally precede sleep in rats (chapters 2, section 1.3.2i and 6). The insomnic action of phosphoramidon was decreased by naltrexone. These data suggest that opioid receptors and endogenous enkephalins play an important role in sleep-waking.
mechanism (chapter 3). This idea might be of relevance in some clinical situations. For example, an increase in opioid peptide levels has been demonstrated during stress, anxiety and psychic disturbances associated with insomnia. It is also conceivable that this new class of drugs, the enkephalinase inhibitors, might be of potential use in the treatment of sleep disorders characterised by excessive somnolence.

Sleep-waking states and enkephalin induced neuronal excitation

The intracerebroventricular (icv) administration of enkephalin was found to induce epileptiform activity in the hippocampal and cortical EEG and in the EMG derived from submandibular muscle in freely moving rats (chapters 4). Sleep, in particular REM sleep, decreased the enkephalin-induced epileptiform discharges.

These observations indicate that phasic changes during sleep-waking states can modulate the neuronal excitability regulated by opioid peptides. This might be of importance for some forms of human epilepsies affected by the sleep-waking cycle.

REM sleep deprivation and nociception

REMSD decreased the pain threshold and abolished the analgesic effects of morphine, enkephalinase inhibition and cold-water-swim (CWS) (chapters 5 and 6). The pain threshold to noxious electric shock was similarly reduced by REMSD (chapter 1, section 1.5.2d, vii). All these data suggest that REM sleep is an important factor in the physiological regulation of nociception. The finding that REMSD can reduce the pain threshold might be of relevance for those individuals who suffer from disturbed sleep e.g. insomnia or people working in shifts. In general it should be expected that pathological conditions, and/or drug treatment accompanied with REMSD, could modify the therapeutic effectiveness of opiates and other analgesic procedures.

REM sleep deprivation and monoamine-opioid interactions

There are abundant data showing that opioid peptides interact with many other physiologically active substances. In our study we paid particular attention to β-phenylethylamine (PEA), a substrate for the MAO-B enzyme,
since it is known that inhibition of MAO-B modulates some effects of opioid peptides (chapter 6, introduction). In our study, the inhibition of MAO-B (the enzyme which biodegradates PEA) and excess of PEA (a substrate for MAO-B) had a stimulatory effect on the analgesic action of endogenously released opioid peptides in rats allowed undisturbed sleep, but not in REMSD animals (chapter 6).

The described facilitatory action of MAO-B inhibition on enkephalinergic transmission might be of relevance not only in the physiology of nociception, but also in conditions associated with the alterations of MAO-B enzyme and/or REM sleep (endogenous depression, ageing etc).

*REM sleep deprivation induces a functional deficiency of μ opioid receptor system*

The finding that opiate-induced analgesia, which is due to a preferential activation of μ-opioid receptors, can be blocked by REMSD suggested a functional insufficiency of a μ-receptor system (chapters 5, 6). In order to test this concept, we studied the effects of REMSD on opiate induced motor inhibition. In this study we demonstrated that the morphine induced akinesia/catalepsy syndrome, which is characterised by rigidity and mediated mainly by the μ-opioid receptors, was abolished by REMSD and replaced by motor excitation, seen as an increase in spontaneous vertical motor activity (SVMA). Naltrexone blocked the morphine induced akinesia/catalepsy in rats allowed undisturbed sleep, but not the opiate induced increase in SVMA in REMSD animals (chapter 7). In addition to the fact that REMSD blocked opioid/opiate-induced analgesia and akinetic-cataleptic syndrome, the naltrexone sensitive wet-dog-shakes and grooming behaviours stimulated by an enkephalinase inhibitor were attenuated in REMSD animals (chapter 8).

These data collectively indicate that the blockade of akinesia/catalepsy syndrome and the reduced effects of enkephalinase inhibition in REMSD animals are due to a functional insufficiency of the μ-opioid receptor system, allowing for an increased expression of the naltrexone resistant excitatory opioid receptors responsible for the increased SVMA. It is known for example that blockade of μ-opioid receptors facilitates the excitatory effects of morphine (chapter 7, discussion). The idea that REMSD can induce a functional deficit of an opioid system derives further support from the fact
that enkephalinase inhibition blocked the proconvulsant action of REMSD (chapter 9). This proposition can be supported by the known inhibitory influence of opioid peptides on the release of excitatory transmitters and that blockade of $\mu$-opioid receptors with naloxone facilitates epileptogenesis (chapter 7, discussion). The suggested functional deficiency of an opioid system might explain the increase in neuronal excitability in REMSD animals.

Some recent biochemical studies, provide evidence for the concept of derangement of endogenous opioid system during REMSD. For example REMSD decreased the concentrations of $\beta$-endorphin in the pituitary but increased it in the hypothalamus (chapter 1, section 1.5.2b, vi). We have also observed that REMSD reduced the concentrations of leu-enkephalin in some brain areas (Haffmans, Ukponmwan, Dzoljic in preparation).

The proposed insufficiency of an opioid system during REMSD could account for the therapeutic and diagnostic value of REM sleep curtailment in some cases of depression and epilepsy respectively (chapter 7, discussion).

**REM sleep deprivation and opiate dependence**

Acute exposure to nitrous oxide can lead to the release of met-enkephalin in the CSF and opiate-like dependence phenomena (chapter 2, section 2.5.2, iv). The withdrawal convulsions in mice following exposure to nitrous oxide were reduced by REMSD (chapter 10). Similarly, naloxone-precipitated wet-dog-shakes in morphine-dependent rats were attenuated by REMSD (chapter 8). It has also been demonstrated that REMSD can attenuate naloxone-precipitated jumping and myoclonic contractions in acute morphine dependent rats (Dzoljic et al. in preparation). Although the neurochemical basis of drug dependence is not fully understood, it has been established that several drugs which can inhibit protein synthesis reduced the severity of opiate dependence and withdrawal phenomena (chapter 2, section 2.4.8). REMSD has also been demonstrated to decrease protein synthesis in the rat brain (chapter 1, section 1.5.2b, vii). It is therefore suggested that the antagonistic effect of REMSD on opiate or nitrous oxide withdrawal phenomena might be due to a decrease in protein synthesis in animals deprived of REM sleep.

The finding that REMSD can attenuate abstinential symptoms might suggest the existence of a common link between REM sleep deficiency and drug...
dependence. However, the mechanism by which REMSD inhibits abstinential syndromes and whether it modulates drug dependence in humans remain to be clarified.

The following general conclusions can be made from this thesis:—

1a) Activation of endogenous opioid peptides increased wakefulness

1b) Sleep, particularly the REM sleep stage, decreased the epileptic effects of enkephalin when compared with the waking state. Statements 1a and 1b indicate that there is an interaction between vigilance and the endogenous opioid system.

2) Inhibition of monoamine oxidase B and/or excess of β-phenylethylamine facilitated enkephalinergic transmission in rats allowed undisturbed sleep but not in REMSD animals. This finding might be of relevance in some clinical situations associated with the alterations of MAO-B and/or REM sleep (endogenous depression, ageing, etc).

3) Stimulation of the endogenous opioid system, with enkephalinase inhibition, attenuated the proconvulsant action of REMSD, suggesting an involvement of opioid peptides in the regulation of neuronal excitability.

4) The analgesic effects of endogenously released enkephalins and exogenously administered opiates, which are mediated mainly by μ-opioid receptor, were abolished by REMSD. This suggests that normal REM sleep is an important factor for proper regulation of the pain threshold.

5) The akinetic/cataleptic effect of morphine, also mediated by μ-opioid receptors, was replaced by excitatory motor activity in REMSD rats.

6) Conclusions 4 and 5 suggest that REM sleep deprivation may be associated with a functional insufficiency of μ-opioid system.

7) REM sleep deprivation decreased abstinential phenomena, suggesting that sleep deprivation could be an attractive tool in the investigation of drug dependence.
SAMENVATTING EN CONCLUSIES

In het eerste hoofdstuk werd een algemeen literatuuroverzicht gegeven over slaap-waak mechanismen, de functies van slaap en de biologische en therapeutische effecten van slaapdeprivatie. In hoofdstuk 2 werden de fysiologische regulaties hierin toegespitst op de rol van het endogene opioid systeem in de vorm van een korte samenvatting van de literatuur. Dit proefschrift beschrijft een onderzoek naar de rol van endogene opioid peptiden in de regulatie van slaap-waak stadia en de invloed van REM slaap op de effecten van endogeen geseëcretteerde- en exogeen toegediende opioid peptiden en opiaten. Met name is de mogelijke betrokkenheid van de REM slaap bij de functionele regulatie van opioid peptiderge neuronen in het centrale zenuwstelsel van de rat onderzocht, via het bestuderen van de effecten van REM slaap deprivatie (REMSD) bij bepaalde gedragsresponsies op endogeen geseëcretteerde opioid peptiden en exogeen toegediende opiaten en opioid peptiden. In dit onderzoek werden de volgende door opiaat/opioid geïnduceerde gedragsresponsies bestudeerd: analgesie, akinesie-catalepsie, spontane verticale motorische activiteit (SVMA), convulsies, poetsgedrag en opiaat onthoudingssymptomen opgewekt door lachgas en morfine.

Endogene opioid peptiden en waak mechanisme(n)

De mogelijke betrokkenheid van endogene opioid peptiden bij waak mechanisme(n) zou afgeleid kunnen worden uit de dagluchtuaties in enkefaline en \( \beta \)-endorfine concentraties in bepaalde hersenkernen die een rol spelen bij alertheideregulering (hoofdstuk 1, sectie 1.2, 1.3.21). Dienovereenkomstig zijn de concentraties van \( \beta \)-endorfine, de enkefalines en dynorfin in rattehersenen het hoogste in de nacht of in het donker, wanneer de waakzaamheid hoog is, en zijn ze het laagst gedurende de dag of in het licht, wanneer de neiging tot slapen het grootst is. De remming van het enzym enkefalinase door toediening van het farmacon fosforamidon bleek een verhoging van de waakzaamheid te induceren. Zowel de NREM als de REM slaap periodes werden onderdrukt. Deze toegenomen waakzaamheid als gevolg van de remming van enkefalinase werd vergezeld door symptomen van geprikkeldheid zoals schokbevagingen van de kop, krabben (hoofdstuk 3) en overmatig poetsgedrag, dat normaal bij ratten aan het slapen vooraf gaat (hoofdstuk 2, sectie 1.3.21 en hoofdstuk 8). Het bleek dat
De opwekkende werking van fosforamidon werd verminderd door de opiat antagonist naltrexon. Deze gegevens suggereren dat opioid receptoren en endogene enkefalines een belangrijke rol spelen in het slaap-waak mechanisme (hoofdstuk 3). Dit mogelijke verband zou in bepaalde situaties klinische implicaties kunnen hebben. Er is bij voorbeeld bij de mens een toename in endogene opioid concentraties aangetoond gedurende stress, angsttoestanden en psychische storingen die samengaan met slapeloosheid. Het is ook voorstelbaar dat een nieuw type van farmaca, de enkefalinase remmers, potentiële van nut zou kunnen zijn bij de behandeling van patienten die lijden aan excessieve slaperigheid.

*Slaap-waak stadia en inductie van neuronale excitatie door enkefaline*

De intracerebroventriculaire (i.c.v.) toediening van enkefaline induceerde epileptiforme activiteit in de hippocampus en de cortex volgens het EEG en was ook zichtbaar aan het EMG van de submandibulaire spier bij vrij bewegende ratten (hoofdstuk 4). Slaap, en in het bijzonder de REM slaap, kon de door enkefaline geïnduceerde epileptiforme ontlasting doen verminderen.

Deze observaties geven aan dat fasische verschuivingen in slaap-waak stadia het effect van opioid peptiden op de neuronale prikkelbaarheid, kunnen moduleren. Dit zou bij de mens betekenis kunnen hebben voor bepaalde vormen van epilepsie, waarop de slaap-waak cyclus een invloed heeft.

*REM slaap deprivatie en pijnprikkeling*

REMSD verminderde de pijndrempel en antagoniseerde het analgetische effect van resp. morfine, enkefalinase remming, en in-koud-water-zwemmen (CWS)(hoofdstukken 5 en 6). De pijndrempel voor een elektrische schok kan eveneens verminderd worden door REMSD (hoofdstuk 1, sectie 1.5.2.d.vii). Al deze gegevens duiden erop dat REM slaap een belangrijke factor is in de fysiologische regulatie van pijnprikkeling.

De waarneming dat REMSD de pijndrempel kan verlagen, zou betekenis kunnen hebben voor die personen die aan een gestoorde slaap lijden zoals bijvoorbeeld slapeloosheid bij mensen die in ploegendienst werken. In het algemeen zou men kunnen verwachten dat pathologische omstandigheden en/of een bepaalde behandeling met farmaca die vergezeld gaan met REMSD, de therapeutische effectiviteit van opiaten en andere analgetische behandelingen kunnen
REM slaap deprivatie en monoamine-opioïd interakties

Er is een veelheid aan informatie in de literatuur die laat zien dat de effecten van opioid-peptiden interfereren met die van vele andere fysiologisch actieve stoffen. In dit proefschrift is in dit verband bijzondere aandacht besteed aan β-fenylethylamine (PEA), een substraat voor het MAO-B enzym, omdat bekend is dat remming van MAO-B bepaalde effecten van opioid-peptiden kan beinvloeden (hoofdstuk 6: "introduction"). Remming van MAO-B (het enzym dat PEA afbreekt) en een overmaat aan PEA (het substraat voor MAO-B) hadden een stimulerend effect op de pijnstillende werking van endogeen gecodeerde opioid peptiden bij ratten die ongestoord konden slapen, maar niet bij ratten waarbij de REM slaap werd onthouden (REMSD)(hoofdstuk 6).

Het effect van MAO-B remming op enkele motorische transmissies, die zoals beschreven neurotransmissie bevorderend is, kan niet alleen van betekenis zijn voor het begrip van de fysiologie van pijnlijkheid bij de mens, maar ook voor die van bepaalde pathologische condities die verband houden met veranderingen in de activiteit van het MAO-B enzym of van de hoeveelheid REM slaap zoals bij endogene depressies of bij het verouderen.

REM slaap deprivatie en de inductie van een functionele deficiëntie in het μ-opioïd receptor systeem

De bevinding dat de analgesie die door een opiat wordt geinduceerd en die het gevolg is van een stimulering van voornamelijk de μ-type opioid receptoren, geblokkeerd kan worden door REMSD, werd geïnterpreteerd als een functionele insufficiëntie in het μ-type receptor systeem (hoofdstukken 5 en 6). Om deze opvatting te kunnen toetsen, is vervolgens het effect van REMSD op de door opiaten veroorzaakte motorische activiteitsvermindering bestudeerd. Uit deze studie kwam naar voren dat het door morfine geinduceerde akinesie/katalepsie syndroom, dat geïn dred wordt door een lichaamsstarheid als gevolg van een preferentiele μ-receptor stimulering, werd tegengegaan door REMSD en zelfs werd vervangen door motorische activiteitsvermeerdering in de vorm van een toegenomen spontane vertikale motorische activiteit (SVMA). De opioid receptor antagonistenaltrexon blokkeerde de door morfine geïn ducteerd akinesie/katalepsie in ratten die ongestoord konden slapen, maar niet de door morfine geïn ducteerd
toename in SVMA in dieren met REMSD (hoofdstuk 7). Naast de hierboven beschreven waarnemingen dat REMSD de door opioiden en opiaten geïnduceerde analgesie en het akinesie/katalepsie syndroom kan blokkeren, bleek dat in dieren met REMSD het opioid geïnduceerde gedrag, zoals de naltrexon-sensitieve "wet-dog-shakes" en het door een enkefalinaas remmer geïnduceerde poetsgedrag, eveneens verzwakt werd (hoofdstuk 8).

Uit het geheel van deze waarnemingen zou men kunnen concluderen dat de blokkering van het akinesie/katalepsie syndroom en de verminderde effecten van de enkefalinaas remming in REMSD dieren worden veroorzaakt door een functionele insufficiëntie van het $\mu$-opioid receptor systeem. De toegenomen SVMA wordt hierbij toegeschreven aan een zichtbaar geworden expressie (demaskering) van opwekkende opiaat-effecten, middels een ander en naltrexon-resistent opioid receptor type. Het is bijvoorbeeld bekend dat blokkering van $\mu$-opioid receptoren de expressie van opwekkende effecten van morfine kan vergemakkelijken (hoofdstuk 7: discussie). Het denkbeeld dat REMSD een functioneel tekort in een opioid systeem kan induceren, wordt verder ondersteund door het feit dat remming van het enzym enkefalinaas het proconvulsieve effect van REMSD kan blokkeren (hoofdstuk 9). Deze bewering wordt ondersteund door het bekende feit van de remmende werking van opioid peptiden op de afgifte van stimulerende neurotransmitters, waarbij een blokkering van $\mu$-opioid receptoren met naloxon het ontstaan van epileptische verschijnselen vergemakkelijkt (hoofdstuk 7: discussie). Het veronderstelde functionele tekort in een opioid systeem zou aldus de toename in normale prikkelbaarheid in REMSD dieren kunnen verklaren.

Enkele recente biochemische studies ondersteunen het concept van een versterking van endogene opioid systemen als gevolg van depravatie van REM slaap. REMSD vermindere de bijvoorbeeld de concentratie van $\delta$-endorfïne in de hypothalamus, maar in de hypothalamus nam deze concentratie toe (hoofdstuk 1, sectie 1.5.2b. vi). In overeenstemming hiermee vinden wij in bepaalde hersengebieden ook verminderde concentraties van leu-enkefaline als gevolg van REMSD (Haffmans, Ukponmwan, Dzoljic; in voorbereiding).

De veronderstelde functionele insufficiëntie van een opioid systeem als gevolg van REMSD zou verantwoordelijk kunnen zijn voor het gewenste therapeutische effect en de diagnostische waarde van het beperken van REM slaap bij respectieel bepaalde types depressie en epilepsie (hoofdstuk 7: discussie).
**REM slaap deprivatie en lichamelijke afhankelijkheid van opiaten**

Een acute inhalatie van lachgas kan de afgifte van Met-enkefaline in de hersenvloeistof verhogen en aldus opiaat-achtige lichamelijke afhankelijkheidsfenomenen oproepen. De convulsies als element van de onthoudingsverschijnselen bij muizen na lachgas inhalatie waren verminderd in REMS dieren (hoofdstuk 10). Hiermee vergelijkbaar was het door naloxon opgeroepen "wet-dog-shakes" gedrag in ratten die tevoren morfine afhankelijk gemaakt waren, minder uitgesproken in REMS dieren. Ook is aangetoond dat REMS het door naloxon opgeroepen spring gedrag en de myoclonische contracties bij ratten die acuut morfine-afhankelijk gemaakt zijn, kan verzwakken (Dzoljic et al., in voorbereiding). Hoewel de neurochemische basis van afhankelijkheid van verdovende middelen en andere substanties niet bekend is, is wel vastgesteld dat verschillende stoffen die de eiwitsynthese remmen de mate van opiaat afhankelijkheid en van onthoudingsverschijnselen doen verminderen (hoofdstuk 2. sectie 2.4.8). Het is aangetoond, dat REMS de eiwitsynthese in ratte hersenen vermindert (hoofdstuk 1. sectie 1.5.2b, vii). Om deze reden wordt voorgesteld, dat het antagonistische effect van REMS op opiaat en lachgas onthoudingsverschijnselen veroorzaakt zou kunnen worden door een afname van de eiwitsynthese bij dieren met REM slaap onthouding.

De waarneming dat REMS opiaat onthoudingsverschijnselen kan verzwakken, veronderstelt het bestaan van een verband tussen REM slaap tekort en lichamelijke afhankelijkheid van opiaten. Het mechanisme waardoor REMS onthoudingsverschijnselen kan verzwakken en of REMS invloed kan uitoefenen op lichamelijke afhankelijkheid van opiaten bij de mens moet echter nader onderzocht worden.

Uit de resultaten van dit proefschrift kunnen de volgende algemene conclusies getrokken worden:

1a) Activering van het endogene opioid systeem doet de waakzaamheid toenemen.

1b) Slaap, in het bijzonder het REM slaap stadium, vermindert de epileptische effecten van enkefaline, vergeleken met het waak-stadium. De stellingen 1a en 1b geven aan dat er een interactie is tussen de mate van waakzaamheid en het endogene opioid systeem.

2) Remming van monoamine oxidase B en/of een overmaat aan \( \beta \)-fenylethylamine vergemakkelijkt enkefalinerge transmissie in ratten die ongestoord kunnen
slapen, maar niet in ratten met REM slaap deprivatie. Deze conclusie zou voor de mens betekenis kunnen hebben in bepaalde klinische situaties, waarin veranderingen in MAO-B enzym activiteit en/of REM slaap een rol spelen (endogene depressie, veroudering, enz.).

3) Stimulering van het endogene opioid systeem via remming van het enzym enkefalinas verzwakt de proconvulsieve werking van REMSD, hetgeen een betrokkenheid van opioid peptiden in de regulatie van neuronale prikkelaarheid gesuggereert.

4) De analgetische werking van endogeen gesecreteerde enkefalines en van de exogeen toegepaste opioiden, die voornamelijk het µ-opioid receptor type stimuleren, wordt tegengegaan door REMSD. Hieruit kan men concluderen dat een normale REM slaap een belangrijke factor is voor een adequate instelling van de pijn Drempel.

5) Het akinetische/kataleptische effect van morfine, dat ook via de stimulatie van µ-opioid receptors werkt, wordt in REMSD ratten vervangen door een verhoogde motorische activiteit.

6) Uit de conclusies 4 en 5 zou men kunnen afleiden dat REM slaap deprivatie geassocieerd is met een functionele insufficientie van het µ-opioid systeem.

7) REM slaap deprivatie vermindert opiat onthoudingsverschijnselen, hetgeen doet vermoeden dat slaap deprivatie een aantrekkelijk hulpmiddel zou kunnen zijn bij verslavingsonderzoek.
List of Publications


Dzoljic MR, Haffmans J, Ukponmwan OE. Endogenous opioid peptides (endorphins) and neuronal excitation, Acta Yugoslav Pharmacol (in press).


Ukponmwan OE, Dzoljic MR. (1981) The modulatory effect of morphine and
monoamine oxidase B (MAO-B) system on enkephalin-induced seizures. 
In: Advances in Endogenous and Exogenous Opioids, Takagi H and BJ Simon (eds) 
Kodanska Ltd, Tokyo, pp 226-228


**Ukponmwan OE, Rupreht J, Dzoljic MR (1984) REM sleep deprivation decreases the antinociceptive property of enkephalinase inhibition, morphine and cold-water-swim, Gen Pharmacol 15: 255-258

**Ukponmwan OE, Dzoljic MR (1984) Enkephalinase inhibition antagonizes the increased susceptibility to seizure induced by REM sleep deprivation, Psychopharmacol 83: 229-232

**Ukponmwan OE, Poel-Heisterkamp ALvd, Dzoljic MR (1985) REM sleep deprivation decreases the grooming and shaking behaviour induced by enkephalinase inhibitor or opiate withdrawal, Pharmacol Biochem Behav 23: 385-389

**Ukponmwan OE, Rupreht J, Dzoljic MR (1986) An analgesic effect of enkephalinase inhibition is modulated by monoamine oxidase B and REM sleep deprivation, Naunyn-Schmied Arch Pharmacol (in press)

**Ukponmwan OE, Poel-Heisterkamp ALvd, Dzoljic MR (1986) REM sleep deprivation antagonized the morphine-induced akinesia and catalepsy, Sleep (in press)

(*) Articles partly incorporated in this thesis
(**) Articles fully incorporated in this thesis
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