SLEEP-WAKING STATES AND THE ENDOGENOUS OPIOID SYSTEM

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

Proefschrift

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PROMOTIECOMMISSIE

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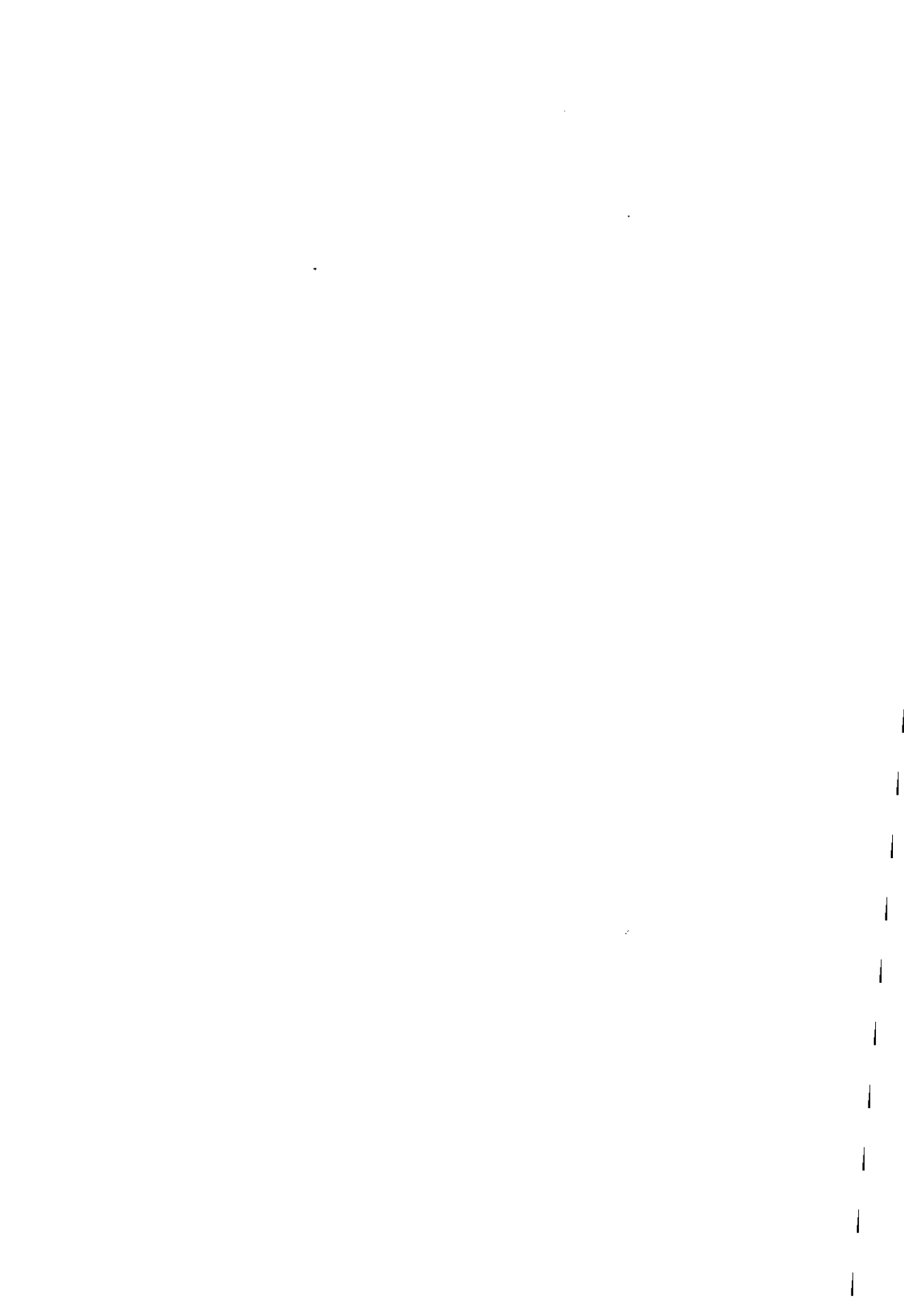CO-PROMOTOR: DR M.R. DZOLJIC

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CONTENTS

SCOPE OF THIS THESIS

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 occurence 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 phosphoramdion 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 heterogenous 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 prepondrance 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 (Parmeggianni, 1980; Cooper, 1979). Behaviourally, sleep onset is characterised by the suspension of active contact with the environment (Parmeggianni, 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, quiescience 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).

i) 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 (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 episode 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, shallow and more regular than in wakefulness. Only small changes in ventilatory response to carbon dioxide and mechanoceptive 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 occurence 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 diminshed, 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 maintaince 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 tegementum 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 occulomotor 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 coerulus 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.

i) 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 solitarus (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 fire less during slow wave sleep compared with waking (McGinty 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 coeruleus 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 ß-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 α-methyl-p-tyrosine (αMT) or the
blockade   of   dopamine-ß-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 α-MT enhanced REM sleep in the rat (Hartmann et
al., 1971). However, Kafi and co-workers (1977) have demonstrated that only
high doses of α-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 coerulus 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 Apo 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 (McGinty 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 PS (Jouvet, 1984).

iv) β-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, 1980). 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

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

i) 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 stratium 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

pituitary (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 β-endorphin or morphine decreased both NREM and REM sleep stages and increased wakefulness in rats and cats (Khazan 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 β-endorphin and des-γ-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 Crucq, 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-hydroxytyptophan or α-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 (Scherschlict 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; Kerdelhue 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 teneable 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 retrival 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 REMSD 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; Palmbald et al., 1979). Steriods 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 REMSD, Panov (1982) found that TSD increased the RNA contents of the locus coerulus and n. raphe pontis, while REMSD 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 a was 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 over all 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 continously 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 cm^2 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 cm^2 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 Luijtelaar, 1985). In addition there were no differences in blood counts and lympoid 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 ^{O}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 Luijtelaar, 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

i) Serotoninergic 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 $[^{3}H]$-serotonin from $[^{3}H]$-tryptophan in the brainstem-mesencephalon in rats (Hery et al., 1970). In cats there were no alterations in the CSF 5-HIAA levels (Radulovacki,

1973) 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 $5-HT_2$ 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 coerulus 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).

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 (DOPAC) 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.

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 it´s metabolite, 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 $[^3H]$clonidine (α_2) binding sites (Mogilnicka and Pilc, 1981), but decreased β receptor binding (Mogilnicka et al., 1980). However other workers found no changes in β receptor binding in the brain of REMSD animals (Abel et al., 1983).

iv) Cholinergic system

A decrease in Ach concentration was observed in the telencephalon of REMSD rats (Tsuchiya et al., 1969). 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).

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). β-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 coerulus 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 (Fowler et al., 1973). 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 disrupts complex and unprepared forms of learning, especially those with emotional components (McGrath and Cohen, 1978).

1.5.3 Clinical aspects of REM sleep deprivation

i) 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 substained 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 (Scherschlicht 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

Abel MS, Villegas F, Abreu J, Gimino F, Steiner S, Beer B, Meyerson LR (1983) The effect of rapid eye movement sleep deprivation on cortical β-adrenergic receptors, Brain Res. Bull., 11: 729-734

Adrien J, Dugovic C (1984) Presence of paradoxical sleep (PS) factor in the cerebrospinal fluid of PS-deprived rats, Eur. J. Pharmacol., 100: 223-226

Akert K, Koella WP, Hess R (1952) Sleep produced by electrical stimulation of the thalamus, Amer. J. Physiol., 168: 260-267

Albert I, Circala GS, Siegel J (1970) The behavioral effects of REM sleep deprivation in rats, Psychophysiol., 6: 550-560

Aschoff J (1964) Survival value of duirnal rhythms, Symp. Zool. Soc. Lond., 13: 78-98

Aserinsky E, Kleitman N (1953) Regularly occurring periods of eye motility and concurrent phenomena during sleep, Science, 118: 273-274

Baldy-Moulinier M (1982) Temporal lobe epilepsy and sleep organization, In Sleep and Epilepsy, MB Sterman, MN Shouse, P Passouant (eds), Academic Press, New York, pp 411-419

Beck U, Wenzel D, Sauer M (1977) Selective deprivation of sleep in pycnoleptic children: Effects of deprivation of slow wave sleep and REM sleep on the frequency and duration of petit mal attacks, Arch. Gen. Psychiat. Neurol. Sci., 223: 107-115

Bergonzi P, Chirulla C, Tempesta E (1973) Selective deprivation of sleep stages in epileptics, In Sleep 1972, L Levin, WP Koella (eds), Karger, Basel, pp 534-538

Berlucchi G, Maffei L, Moruzzi G, Strata P (1964) EEG and behavioral effects by cooling of medulla and pons, Arch. Ital., Biol., 102: 372-392

Besset A (1982) Influence of generalised seizures on sleep organization, In Sleep and Epilepsy, MB Sterman, MB Shouse, P Passouant (eds), Academic Press, New York, pp 411-419

Billiard M (1982) Epilepsies and the sleep-wake cycle, In Sleep and Epilepsy, MN Sterman, MB Shouse, P Passouant (eds), Academic Press, New York, pp 269-286

Bobillier P, Sakai T, Seguin S, Jouvet M (1974) The effect of sleep deprivation upon *in vivo* and *in vitro* incorporation of tritiated amino acids into brain proteins in the rat at three different age levels, J. Neurochem., 22: 23-31

Bolles RC (1960) Grooming behavior in the rat, J. Comp. Physiol. Psychol., 53: 306-310

Borbely AA (1982) Sleep regulation: circadian rhythm and homeostasis, In Sleep: clinical and experimental aspects , Current topics in neuroendocrinology, D Ganten, D Pfaff (eds), Springer Verlag, Berlin-Heidelberg, pp 83-100

Borbely AA, Steigrad P, Tobler I (1980) Effect of sleep deprivation on brain serotonin in the rat, Behav. Brain Res., 1: 205-210

Bouhuys AL, Hoofdakker vd RH (1977) Effects of midbrain raphe destruction on sleep and locomotor activity in rats, Physiol. Behav., 19: 535-541

Bowersox SS, Drucker-Colin RR (1982) Seizure modification by sleep deprivation: a possible protein synthesis mechanism, In Sleep and Epilepsy, MB Sterman, MN Shouse, P Passouant (eds), Academic Press, New York, pp 91-104

Bremer F (1935) Cerveau "isole" et physiologie du sommeil, CR Soc. Biol. (Paris), 118: 1235-1241

Broekkamp CL, Phillips AG (1979) Stiumlant effects of enkephalin microinjection into the dopaminergic A10 area, Nature, 278: 560-562

Chernik DA (1972) Effects of REM sleep deprivation on learning and recall by humans, Percept. Motor Skills, 34: 283-294

Clancy JL, Caldwell DF, Oberleas D, Sangiah S (1978) Effect of a chronic tryptophan dietary deficiency on the rats sleep-wake cycle, Brain Res. Bull., 3: 83-87

Coenen AML, Luijtelaar ELJM (1985) Stress induced by three procedures of deprivation of paradoxical sleep, Physiol. Behav., 35: 501-504

Cohen HB, Dement WC (1965) Sleep: changes in threshold to electroconvulsive shock in rats after deprivation of "paradoxical" phase, Science, 150: 1318-1319

Cohen RM, Pickar D, Garnett D, Lipper S, Gillin JC, Murphy DL (1982) REM sleep suppression induced by selective monoamine oxidase inhibitors, Psychopharmacol., 78: 137-140

Colasanti B, Kirchman A, Khazan N (1975) Changes in the electroencephalogram and REM sleep time during morphine abstinence in pellet-implanted rats, Res. Comm. Chem. Path. Pharmacol., 12: 163-172

Cooper SJ (1981) Endogenous opioid peptides and behaviour switching, In Theory in Psychopharmacology, SJ Cooper (ed), Academic Press, vol 1, pp 304-322

Corner MA, Mirmiran M, Bour HLMG, Boer GJ, Poll vd NE, Oyen vd HG, Uylings HBM (1980) Does rapid-eye-movement sleep play a role in brain development, In: Adaptive capabilities of the nervous system, Progress in Brain Research, vol 53, PS McConnell, B Ronijn, Poll vd NE, MA Corner (eds), Elservier/North-Holland, pp 347-356

Cortes-Gallegos V, Castaneda G, Alonso R, Sojo I, Carranco A, Cervantes C, Parra A (1983) Sleep deprivation reduces circulating androgens in healthy men, Arch. Androl., 10: 33-37

Dement WC (1960) The effect of dream deprivation, Science 131: 1703-1707

Dement WC, Henry P, Cohen H, Ferguson J (1967) Studies on the effect of REM sleep deprivation in humans and animals, In Sleep and altered states of conscious, S Kety, E Evarts, H Williams (eds), William and Wilkin, Baltimore, pp 456-468

Demin NN, Rubinskya NL (1974) Neuronal and neurological proteins and RNA content of supraoptic nucleus after deprivation of the paradoxical phase of sleep for 24 hrs, Dokl. Akad. Nauk. USSR Biol., 214: 949-942

Dewson JH, Dement WC, Wagner TE, Nobel K (1967) Photically evoked potentials in the visual cortex following paradoxical sleep deprivation in rats, Physiol. Behav., 32: 557-563

Domino EF, Stawiski M (1970) Effect of cholinergic antisynthesis agent HC-3 on the awake-sleep cycle of the rat, Psychopharmacol., 7: 315-316

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 113-133

Drucker-Colin RR (1973) Cross perfusion of a sleep inducing brain tissue substance in conscious cats, Brain Res., 56: 123-134

Drucker-Colin RR, Jaques LB, Cunningham TA (1974) Anemia from sleep

deprivation with anticoagulants in rats with enhancement by PCPA, Pharmacol. Biochem. Behav., 2: 817-826

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 Ganten, D Pfaff (eds), pp 35-81

Dzoljic MR, Bonta IL, Godschalk M, Lagendik, Stefanko S (1975) EEG-synchronizing effect of γ-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 monoamine oxidase-B and relationship with selective increase of dopamine and β-phenylethylamine, J. Neural Transm., 40: 1-12

Dzoljic MR, Crucq JB (1979) Enkephalin-induced hippocampal theta activity, Sleep Res., 8: 93

Dzlojic MR, Godschalk M (1978) The role of different dopamine receptors in electrophysiological alertness, Waking Sleep, 2: 153-155

Dzoljic MR, Ukponmwan OE, Rupreht J, Haffmans J (1985) Role of the enkephalinergic system in sleep studied by an enkephalinase inhibitor, In Sleep: Neurotransmitters and Neuromodulators, A Wauquier, JM Gailliard, JM Monti, M Radulovacki (eds), Raven Press, New York, pp 25-265

von Economo C (1929) Schlaftheorie, Ergeb. Physiol., 28: 312-339

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, Pharmacol. Biochem. Behav., 18: 509-513

Favale E, Loeb C, Rossi GF, Sacco G (1961) EEG synchronization and behavioral signs of sleep following low frequency stimulation of the brain stem reticular formation, Arch. Ital. Biol., 99: 1-22

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

Fishbein W (1970) Interference with conversion of memory from short-term to
long-term stage by partial sleep deprivation, Comm. Behav. Bio., 5:
171-175

Flood JF, Bennet EL, Orme AE, Rosenzweig (1975) Effects of protein
synthesis inhibiton for active avoidance training, Physiol. Behav., 14:
177-184

Fowler MJ, Suillivan MJ, Ekstrand BR (1973) Sleep and memory, Science, 179:
302-304

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

Gessa GL, Porceddu ML, Collu M, Mereu G, Serra M, Ongini E, Biggia G (1985)
Sedation and sleep induced by high doses of apomorphine after blockade
of D-1 receptors by SCH-23390, Eur. J. Pharmacol., 109: 269-274

Ghosh PK, Hrdina PD, Ling GM (1976) Effects of REMS deprivation on striatal
dopamine and acetylcholine in rats, Pharmacol. Biochem. Behav., 4:
401-405

Gillin JC, Buchsbaum MS, Jacobs LS et al.(1974) Partial REM sleep
deprivation, schizophrenia and field articulation, Arch. Gen.
Psychiat., 30: 653-662

Godin Y, Mandel P (1965) Distribution of free amino acids in the central
nervous system of rats during sleep and in protracted wakefulness, J.
Neurochem., 12: 455-460

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

Groos G (1984) The physiological organization of the circadian sleep-wake
cycle, Exp. Brain Res. suppl., 8: 241-257

Guazzi M, Zanchetti A (1965) Blood pressure and heart rate during natural
sleep of the cat and their regulation by carotid sinus and aortic
reflexes, Arch. Ital. Biol., 103: 789-817

Hanada Y, Kawamura H (1981) Sleep-waking electrocorticographic rhythms in
chronic cerveau isole' rats, Physiol. Behav., 26: 725-728

Hartmann EL (1973) The functions of sleep, New Haven, Yale University Press

USA

Hartmann E, Bridwell TJ, Schildkraut JJ (1971) Alpha-methylparatyrosine and sleep in the rat, Psychopharmacol., 21: 157-164

Hartmann E, Zwilling G (1975) Intraventricular norepinephrine and dopamine effects on sleep in the rat, Neurosci., 1: 248

Hartse KM, Eisenhart SF, Bergmann BM, Rechtschaffen A (1979) Ventral hippocampus spikes during sleep wakefulness and arousal in the cat, Sleep, 1: 231-246

Henriksen SJ, Jacobs BL, Dement WC (1972) Dependence of REM sleep PGO waves on cholinergic mechanisms, Brain Res., 48: 412-416

Hery F, Pujol JF, Lopez M, Macon J, Glowsinski J (1968) Increased synthesis and utilisation of serotonin in the central nervous system of the rat during paradoxical sleep deprivation, Brain Res., 21: 391-403

Hess WR (1927) Stammganglien Rezversuche, Ber. Ges. Physiolog., 42: 554-555

Hess WR (1944) Das Schafsyndrom als Folge diencephaler Reizung, Helv. Physiol. Pharmacolog. Acta., 2: 305-344

Hicks RA, Coleman DD, Ferrante F, Sahatjian M, Hawkins J (1979a) Pain threshold in rats during recovery from REM sleep deprivation, Perecept. Motor Skills, 48: 687-690

Hicks RA, Hirshfield C, Humphrey V, Lauber A, Giampashi J, Hawkins J (1981) REM sleep deprivation and food competition in male rats, Physiol. Behav., 26: 245-247

Hicks RA, Moore JD, Findley P, Hirshfield C, Humphrey V (1978) REM sleep deprivation and pain thresholds in rats, Percept. Motor Skills, 47: 848-850

Hicks RA, Moore JD, Hayes C, Phillips N, Hawkins J (1979b) REM sleep deprivation increases aggressiveness in male rats, Physiol. Behav., 22: 1097-110

Hicks RA, Okuda A, Thomsen D (1977) Depriving rats of REM sleep: the identification of a methological problem, Amer. J. Psychol., 90: 95-102

Hilakivi I, Peder M, Elomaa E, Johansson G (1984) Validation of the cuff pedestal technique for rapid eye movement sleep (REMS) deprivation by electrophysiological recordings, Physiol. Behav., 32: 945-947

Himwich WA, Davis JM, Stout M (1973) Cerebral amino acids of rats deprived
of paradoxical sleep, Biol. Psychiat., 6: 37-44

Horne JA, Minard A (1985) Sleep and sleepiness following a behaviourally
"active" day, Ergonomics, 28: 567-575

van Hulzen ZJM, Coenen AML (1979) Selective deprivation of paradoxical
sleep considation of shuttle box avoidance, Physiol. Behav., 28:
821-826

van Hulzen ZJM, Coenen AML (1981) Paradoxical sleep deprivation and
locomotor activity in rats, Physiol. Behav., 27: 741-744

van Hulzen ZJM, Coenen AML (1984) Photically evoked potentials in the
visual cortex following paradoxical sleep deprivation in rats, Physiol.
Behav., 32: 557-563

Ibuka N, Kawamura H (1975) Loss circadian rhythm in sleep-wakefulness cycle
in the rat by suprachiasmatic nucleus lesions, Brain Res., 96: 76-81

Inoue S, Honda K, Komoda Y (1985) Sleep promoting substances, In Sleep:
Neurotransmitters and Neuromodulators, A Wauquier, JM Giallard, JM Monti
, M Radulovacki (eds), Raven Press, New York, pp 305-318

Inoue S, Honda K, Komoda Y, Uchizono K, Ueno R, Hayaishi O (1984)
Differential sleep substance: its effects of five sleep substances
nocturnally infused in unrestrained rats, Proc. Natl. Acad. Sci.
USA, 81: 6240-6244

Jacobs BL, Ascher R, Dement WC (1973) Electrophysiological and behavioural
effects of electrical stimulation of the raphe nuclei on sleep and
locomotor activity in rats, Physiol. Behav., 19: 535-541

Johnson LC (1969) Psychological and physiological changes following total
sleep deprivation, In Sleep, physiology and pathology, AA Kales (ed)
Lippincott, Philadelphia, pp 206-220

Jones BE, Harper ST, Halaris AE (1977) Effects of locus coerulus lesions
upon cerebral monoamine content, sleep-wakefulness states and the
response to ampehtamine in the cat, Brain Res., 124: 473-496

Jouvet M (1974) The regulation of the sleep/waking cycle by monoaminergic
neurons in the cat, In: Brain and Sleep, HM van Praag, H Meinardi
(eds), De Erven Bohn Bv, Amsterdam, pp 22-38

Jouvet M (1984) Indolamines and sleep-inducing factors, Exp. Brain Res.
suppl., 8: 81-94

Jouvet M, Michel F (1959) Correlations electromyographiques du sommeil chez la chat de´cortique et mesencephalic chronique, CR Soc. Biol., 153: 422-425

Jouvet-Mounier D, Astic L, Lacote D (1970) Ontogenesis of the states of sleep in rat, cat and guinea pig during the first post-natal month, Develop. Psychobiol., 2: 216-239

Jouvet D, Vimont P, Delmorme F, Jouvet M (1964) Etude de la privation selective de la phase paradoxale de sommeil chez le chat, CR Soc. Biol., 158; 756-759

Kafi S, Bouras C, Constantinidis J, Gaillard JM (1972) Parodixical sleep and brain catecholamines in the rat after single and repeated administration of alpha-methyl-paratryosine, Brain Res., 135: 123-134

Kafi S, Gaillard JM (1976) Brain dopamine receptors and sleep in the rats; effects of stimulation and blockade, Eur. J. Pharmacol., 38: 357-363

Karacan J, Googenough DR, Shapiro A, Straker S (1966) Erection cycle during sleep in relation to dream anxiety, Arch. Gen. Psychiat., 15: 183-189

Karacan I, Rosenbloom A, Hoon E (1970) The clitoral erection cycle during sleep, Psychophysiol., 7: 338

Karacan I, Sallis PJ, Ware JC, Jervent B, Williams RL, Scott FB, Attia Sl, Beutler LE (1978) Nocturnal penile tumescence and diagnosis in diabetic impotence, Am. J. Psychiat., 135: 191-197

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, Jasinki DR (1969) Morphine effects on human REM state, waking state and NREM sleep, Psychopharmacol., 14: 404-416

Kerdelhue B, Karteszi M, Pasqualini C, Reinberg A, Merey E, Palkovits M (1983) Circadian variations in β-endorphin concentration in pituitary and some brain nuclei of the adult male rat, Brain Res., 261: 243-248

Khazan N, Weeks JR, Schroeder LA (1967) Electroencephalographic, electromyographic and behavioral correlates during a cycle of self-maintained morphine addiction in the rat, J. Pharmacol. Exp. Ther., 155: 521-531

King C, Masserano M, Codd E, Byrne WL (1981) Effects of β-endorphin and morphine on sleep-wakefulness behavior of cats, Sleep, 4 : 259-262

Kitahama K, Valatx J-L (1980) Instrumental and pharmacological paradoxical sleep deprivation in mice strain differences, Neuropharmacol., 19: 529-535

Komoda Y, Iskikawa M, Nagasaki H, Iriki M, Honda K, Inoue S, Higashi A, Uchizono K (1983) Uridine, a sleep-promoting substance from brain stem of sleep-deprived rats, Biomed. Res. suppl., 4: 223-227

Korner A (1965) Growth hormone control of biosynthesis of protein and ribonuclei acid, Recent Prog. Horm. Res., 21: 205-236

Krueger JM (1983) Sleep-promoting factor S: characterization and analogs, In Sleep 1982, WP Koella (ed), Karger, Basel, pp 107-109

Kumar MSA, Chen CL, Sharp DC, Liu JM, Kalra JM, Kalra SP (1982) Diurnal fluctuations in methionine-enkephalin levels in the hypothalamus and preoptic area of the male rats: effects of pinealectomy, Neuroendocrinol., 35: 28-31

Kupfer DJ, Ulrich RF, Coble PA, Jarrett DB, Grochocinski V, Doman J, Matthews G, Borbely AA (1984) Application of automated REM and slow wave sleep, Psychiat. Res., 13: 335-343

Leconte P, Bloch V (1970) Effet de la privation de sommeil paradoxal sur l'acquisition et al retention d'un conditionnement chez le rat, J. Physiol. (Paris), 62: 290

Legendre R, Pieron H (1910) Le probleme des facteurs du sommeil. Resultats d'injection vasculaires et intracerebrales de liquides insomnique, CR Soc. Biol. (Paris), 68: 1077-1078

Levental M, Susic V, Rusic M, Rakic L (1975) Rapid eye movement (REM) sleep deprivation: effect on acid mucopolysaccharides in rat brain, Arch. Int. Physiol. Biochim., 83: 221-232

Lewin I, Glaubman H (1975) The effect of REM deprivation; Is it detrimental or neutral, Psychophysiol., 12: 349-353

Lewis SA, Oswald J, Evants JI, Akindele MO, Tompsett SL (1970) Heroin and human sleep, Electroencephal. clin. Neurophysiol., 28: 374-381

Lubin A, Moses M, Johnson LC, Naitoh P (1974) The recuperative effects of REM sleep and stage 4 sleep on human performance after complete sleep loss, Psychophysiol., 11: 133-146

Livrea P, Di Reda L, Puca FM, Genco S, Specchio LM, Papagno G (1977) Homovanillic acid and 5-hydroxyindoleacetic acid in lumbar cerebrospinal

fluid total and REM sleep deprivation in humans, Eur. Neurol., 16: 280-285

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

Marti-Nicolovius M, Portell-Cortes I, Morgado-Bernal I (1984) Intracranial self-stimulation after paradoxical sleep deprivation induced by the platform method in rats, Physiol. Behav., 33: 165-167

Mattiace LA, Johnston C, Negro-Vilar A, Farber J (1983) Changes in NE and 5-HT metabolism in discrete brain after REM sleep deprivation in rats, 4th Int Congr. Sleep Res. (APSS), Bologna Abstr., 120

Mattiace LA, Negro-Vilar A, Roffwarg HP, Farber J (1981) The effect of REM sleep deprivation on substance P and somatostatin levels in discrete areas of the rat brain, Sleep Res., 10: 68

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

McGinty DJ, Harper RM (1976) Dorsal raphe neurons: depression of firing during sleep in cats, Brain Res., 101: 569-575

McGrath MJ, Cohen DB (1978) REM sleep facilitation of adaptive waking behaviour: a review of the literature, Psychol. Bull., 85: 24-57

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

Mendelson WB, Guthrie R, Frederick G, Wyatt RJ (1974) The flower pot technique of rapid eye movement (REM) sleep deprivation, Pharmacol. Biochem. Behav., 2: 553-556

Mendelson WB, Slater S, Gold P, Gillin JC (1980) The effect of growth hormone administration on human sleep, a dose response response study, Biol. Psychiat., 15: 613-618

Micic D, Karadzic V, Rakic L (1969) Behavior of free amino acid pool in various brain structures of cats deprived of paradoxical sleep, Elecrtoenceph. clin. Neurophysiol., 27: 554

Mikiten T, Niebyl P, Hendley C (1961) EEG desynchronization during behavioural sleep associated with spike discharges from the thalamus of the cat, Fed. Proc., 20: 327

Mitsugi N, Kimura F (1985) Simltaneous determination of blood levels of corticosterone and growth hormone in the male rat : relation to

sleep-wakefulness cycle, Neuroendocrinol., 41: 125-130

Mogilnicka E, Arbilla S, Depoortere H, Langer SZ (1980) Rapid eye movement sleep deprivation decreases the density of ^{3}H-dihydroalprenolol and ^{3}-imipramine binding sites in the rat cerebral cortex, Eur. J. Pharmacol., 65: 289-292

Mogilnicka E, Boissard CG, Hunn C, Delini-Stula A (1985) Suppressant effect of REM sleep deprivation on neophobia in normal rats and rats with selective DSP-4 induced damage of locus coerulus neurons, Pharmacol. Biochem. Behav., 23: 93-97

Mogilnicka E, Pilc A (1981) Rapid-eye-movement deprivation inhibits clonidine-induced sedation in rats, Eur. J. Pharmacol., 71: 123-126

Monmaur P, Houcine O, Delacour J (1979) Experimental dissociation between wakefulness and paradoxical sleep hippocampal theta, Physiol. Behav., 23: 471-479

Monnier M, Keller Th, Graber S (1963) Humoral influences of induced sleep and arousal electrical brain activity of animals with cross circulation, Exp. Neurol., 8: 264-277

Montplaisir J, Laverdiere M, Saint-Hilaire JM (1982) Sleep and focal epilepsy, In Sleep and Epilepsy, MB Sterman, MN Shouse, P Passouant (eds), Academic Press, New York, pp 301-314

Moore JD, Hayes C, Hicks RA (1979) REM sleep deprivation increases preference for novelty in rats, Physiol. Behav., 23: 975-976

Morden B, Mitchell G, Dement W (1967) Selective REM sleep deprivation and compensation phenomena in the rat, Brain Res., 5: 339-349

Morgane PJ, Stern WC (1974) Chemical anatomy of brain circuits in relation to sleep and wakefulness, Adv. Sleep Res., 1: 1-131

Morris GO, Williams HL, Subin A (1960) Misperception and disorientation during sleep deprivation, Arch. Gen. Psychiat., 2: 247-254

Moruzzi G (1964) Reticular influences on EEG, Electroenceph. clin. Neurophysiol., 16: 2-17

Moruzzi G, Magoun HW (1949) Brain stem reticular formation activation of the EEG, Electroenceph. clin. Neurophysiol., 1: 455-473

Mouret J, Jeannerod M, Jouvet M (1963) L´activite electrique du systeme visuel au cours de la phase paradoxale du sommeil chez le chat, J. Physiol. (Paris), 55: 305-306

Mouret J, Pujol JF, Kiyono S (1969) Paradoxical sleep rebound in rat: effects of physical procedures involved in intracisternal injection. Brain Res., 15: 501-506

Naquet R, Denavit M, Lanoir J, Albe-Fessard D (1965) Alterations transitoires ou definitives des zones diencepaliques chez le chat. Leurs effets sur l'activite corticale de le sommeil, In Neurophysiologie des etats de sommoeil, M Jouvet (ed) Centre Nationale de Recherche Scientifique Paris VII, pp 107-131

Nauta WJH (1946) Hypothalamic regulation of sleep in rats. An experimental study, J. Neurophysiol., 9: 285-316

Oksenberg A, Sarne Y, Weissman AD, Luboshitzky R, Lavie P (1980) Plasma humoral endorphin profiles during human sleep, Sleep Res., 9: 94

Oswald I (1974) Sleep, Peguin Books, Harmondsworth, Middlesex, p 215

Palmbald J, Akerstedt T, Froberg J, Melandes A, von Schenck H (1979a) Thyroid and adrenomedullary reactions during sleep deprivation, Acta. Endocrinol., 90: 233-239

Panov A (1982) RNA and protein synthesis and contents of brain stem cells after sleep deprivation, Rev. Biol., 75: 95-99

Pappenheimer JR, Miller TB, Goodrich CA (1967) sleep promoting effects of cerebrospinal fluid from sleep-deprived goats, Proc. Natl. Acad. Sci. USA, 58: 513-517

Parker DC, Rossman LG, Hershman JM, Gibson W, Davis D, Wilson K, Pekary AE (1980) Endocrine rhythms across sleep wake cycles in normal young men under basal state condition, In Physiology in sleep, J orem, C Barnes, Academic Press, New York, pp 145-179

Parker DC, Pekary AE, Hershman JM (1976) Effect of normal and reversed sleep-wake cycles upon nychtohemeral rhythmicity of plasma thyrotropin evidence suggestive of an inhibitory influence in sleep, J. Clin. Endocrinol. Metab., 43: 318-329

Parmeggianni PL (1980) Behavioural phenomenology of sleep (somatic and vegative), 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

Petre-Quadens O, Jouvet J (1966) Paradoxical sleep and dreaming in the mentally retarded, Jap. J. EEG. EMG., 4: 192-198

Piepenbrock N, Valatx JL, Malquarti V, Jouvet M (1985) Effects of hypothalamic paraventricular lesions on sleep in rats, Neurosci. Lett., 62: 151-156

Pratt KL, Mattson RH, Welker NJ, Williams R (1968) EEG activation of epileptics following sleep deprivation: a prospective study of 114 cases, Electroenceph. clin. Neurophysiol., 24: 11-15

Przewlocki R (1984) Some aspects of physiology and pharmacology of endogenous opioid peptides, Pol. J. Pharmacol. Pharm., 36: 137-153

Przewlocki R, Lason W (1983) The opioid peptide dynorphin, circadian rhythms and starvation, Science, 219: 71-73

Puizzillout JJ, Gaudin-Chazal G, Daszuta A, Seyfritz N, Ternaux JP (1979) Release of endogenous serotonin from encephale isole cats 2, Correlation with raphe neuronal activity and sleep and wakefulness, J. Physiol. (Paris), 75: 531

Pujol J, Hery F, Durand M, Glowinski J (1968a) Augmentation de la synthesis de la serotonine 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

Rechtschaffen A, Lovell RA, Freedman DW, Whitehead PK, Aldrich M (1973) Effects of parachlorophenylalanine on sleep in the rat: some implication for the serotonin-sleep hypothesis, IN Serotonin and behaviour, J Barchas, E Usdin (eds), Academic Press, New York, pp 401-424

Roffwarg HP, Muzio JN, Dement WC (1966) Ontogenetic development of the human sleep-dream cycle, Science, 52: 604-619

Rogers GS, Castle vd RL, Evans WS, Critelli JW (1985) Vaginal pulse amplitude response patterns during erotic conditions and sleep, Arch. Sexual Behav., 15: 327-342

Rogers LJ, Drennen HD, Mark RF (1974) Inhibition of memory information in imprinting period: irreversible action of cyclohexamide in young

chickens, Brain Res., 79: 213-233

Ross JJ, Johnson LC, Walter RD (1966) Spike and wave discharges during stages of sleep, Arch. Neurol., 14: 399-407

Sabelli HC, Vazquez AJ, Flavin D (1975) Behavioural and electrophysiological effects of phenylethylamine, a putative neurotransmitter, Psychopharmacol., 42: 117-125

Sachs J, Ungar J, Waser PG, Borbely AA (1976) Factors in cerebrospinal fluid affecting motor activity in the rat, Neurosci. Lett., 2: 83-86

Sakai K (1985) Neurons responsible for paradoxical sleep, In Sleep: Neurotransmitter and Neuromodulators, A Wauquier, JM Gaillard, JM Monti, M Radulovacki (eds) Raven Press, New York, pp 29-42

Sallanon M, Buda C, Janin M, Jouvet M (1982) Restoration of paradoxical sleep by cerebrospinal fluid transfer to PCPA-treated insomniac rats, Brain Res., 251: 137-147

Sandler M, Reynolds GP (1976) Does β-phenylethylamine cause schizophrenia, Lancet, 70-71

Satinoff E, Drucker-Colin RR Hernandez-Peon R (1971) Paleocortical excitability and sensory filtering during REM sleep deprivation, Physiol. Behav., 7: 103-106

Scherschlicht R (1985) Role for GABA in the control of the sleep-wakefulness cycle, In: Sleep: Neurotransmitter and Neuromodulators, A Wauquier, JM Gaillard, JM Monti, M Radulovacki (eds), Raven Press, New York, pp 197-209

Scherschlicht R, Aeppli L, Polc P, Haefely W (1984) Some pharmacological effects of delta-sleep-inducing peptide (DSIP), Eur. J. Neurol., 23: 346-352

Scherschlicht R, Polc P, Schnoenberger J, Steiner M, Haefely W (1982) Selective suppression of rapid eye movement sleep (REMS) in cats by typical and atypical antidepressants, In: Typical and atypical antidepressants molecular mechanism, E Costa, G Racagni, Raven Press New York, pp 359-364

Schneider-Helmert D (1985) Clinical evaluation of DSIP, In: Sleep: Neurotransmitters and Neuromodulators, A Wauquier, JM Gaillard, JM Monti, M Radulovacki (eds), Raven Press, New York, pp 279-289

Schoenenberger Ga, Monnier M (1977) Characterization of

delta-electroencephalogram (sleep)-inducing peptide, Proc. Natl. Acad. Sci. USA, 74: 1282-1286

Shapiro C, Girdwood P (1981) Protein synthesis in rat brain during sleep, Neuropharmacol., 20: 457-460

Sinha AK, Ciarnello RD, Dement WC, Bachas JD (1973) Tyrosine hydroxylase activity in rat brain following REM sleep deprivation, J. Neurochem., 20: 1289-1290

Sitaram N, Gillin JC (1980) Development and use of pharmacological probes of the CNS in man: evidence of cholinergic abnormality in primary affective illness, Biol. Psychiat., 15: 925-955

Smith C (1985) Sleep states and learning, Neurosci. Biobehav. Rev., 9: 157-168

Snyder F, Hobson AJ, Morrison DF, Goldfrank F (1964) Changes in respiration, heart rate and systolic blood pressure in human sleep, J. Appl. Physiol., 19: 417-422

Steiner SS, Ellman SJ (1972) Relation between REM sleep and intracranial self-stimulation, Science, 177: 1122-1224

Steriade M, Hobson JA (1977) Neuronal acitivity during the sleep waking cycle, In: Progress in Neurophysiology, GA Kerbut, JW Phillips (eds), Pergamon Press, pp 155-376

Stern WC (1971) Acquisition impairments following rapid eye movement sleep deprivation in rats, Physiol. Behav., 7: 345-352

Stern WC, Forbes WB, Morgane PJ (1974) Abstent of ponto-geniculo-occipital (PGO) spikes in rats, Physiol. Behav., 12: 293-295

Stern WC, Miller FP, Cox RH, Maickel RP (1971) Brain norepinephrine and serotonin levels following REM sleep deprivation in the rat, Psychopharmacol., 22: 50-55

Stern WC, Morgane PJ (1977) Sleep and memory: effects of growth hormone on sleep brain biochemistry and behaviour, In: Neurobilogy of Sleep and Memory, RR Drucker-Colin JL MacGaugh (eds), Academic Press, New York, pp 373-410

Stinus L, Koob GF, Ling N, Bloom FE, Le Moal M (1980) Locomotor activation induced by infusion of endorphin into the ventral tegmental area: evidence for opiate-dopamine interactions, Proc. Natl. Acad. Sci. USA, 77: 2323-2327

Stock G (1982) Neurobiology of REM sleep. In Sleep: clinical and experimental aspects, Current topics in Neuroendocrinology, D Ganten, D Pfaff (eds), Spinger-Verlag, Berlin-Heidelberk, pp 1-36

Takahashi Y, Kipnis DM, Daughaday WH (1968) Growth hormone secretion during sleep, J. clin. Invest., 47: 2079-2090

Tang F, Tang J, Chou J, Costa E (1984) Age-related and diurnal changes in met^5-enk-arg^6-phe^7- and met-^{5}-enkephalin contents of the pituitary and some brain nuclei of the adult male rat, Brain Res., 261: 243-248

Terrier G, Gottesmann CL (1978) Study of cortical spindles during sleep in the rat, Brain Res. Bull., 3: 701-706

Timo-Iaria C, Negrao N, Schmidek WR, Hoshino K, Lobato de Menezes CE, Leme de Rocha T (1970) Phases and states of sleep in the rat, Physiol. Behav., 5: 1057-1062

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

Tortella FC, Moreton JE, Khazan N (1978) Electroencephalographic and behavioral effects of D-Ala^2-methionine-enkephalinamide and morphine in the rat, J. Pharmacol. exp. Therap., 206: 636-643

Tsuchiya K, Toru M, Kobashi T (1969) Sleep deprivation and brain acetylcholine, Science, 153: 1416-1417

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

Ursin R (197) Differential effect of sleep deprivation on the two slow wave sleep stages in cat in the cat, Acta. Physiol. Scan., 83: 352-361

Ursin R, Larsen M (1983) Increased sleep following intracerebroventricular injection of the delta sleep inducing peptide in rats, Neurosci. Lett., 13: 169-172

Vogel GW (1975) A review of REM sleep deprivation, Arch. Gen. Psychiat., 32: 749-961

Vogel GW (1979) A motivational function of REM sleep, In: The functions of sleep, RR Drucker-Colin, M Mhkurovich, MB Sterman, Academic Press, New York, pp 233-250

Vogel GW (1983) Evidence for REM sleep deprivation as the mechanism of action of antidepressant drugs, Prog. Neuro-Psychopharmacol. Biol.

Psychiat., 7: 343-349

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

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

Wesemann W, Weiner N (1982) Regulation of cerebral serotonin binding and metabolism in sleep-deprived rats, Behav. Brain Res., 6: 79-84

Wever RA (1979) The circadian system of man, Springer, Berlin Hedeberg, New York

CHAPTER 2

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 β-endorphin (Li and Chung, 1976), α, and γ-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$_{1-13}$. In the same year another opioid peptide, a decapeptide described as "big" leu-ENK was named α-neo-endorphin (Kangawa and Matsuo, 1979).   Other   peptides   include   dynorphins$_{1-8}$, dynorphin$_{1-17}$ and β-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, β-casomorphins derived from β-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), β-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-Ala^2-met^5-enkephalinamide (DALA) and D-Ala^2-D-Leu^5-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 degradate 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 Synder, 1979). No selective inhibitor of this enzyme has been demonstrated.

iv) Angiotensin-converting-enzyme (ACE)

Similar to enkephalinase A, ACE hydrolyses Gly-Phe amide bond of enkephalins, but does not appear to play an essential role in *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 biotranformation of synaptic enkephalins *in vivo*.

Knowledge of the biological mechanisms involved in the inactivation of β-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: μ (mu) for morphine, χ (kappa) for EtKCZ and σ (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 μ-receptors. However the enkephalins, especially leu-ENK, interacted mainly with the opioid receptors designated δ-receptors. β-Endorphin was equipotent at μ and δ-receptors. More recently two subtypes of μ receptor designated μ_1 and μ_2 have been suggested (Pasternak et al., 1983). Enkephalin bind to μ_1 and δ sites, while morphine bind to both μ_1 and μ_2 sites. Dynorphins show preference for χ-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)

Ligands	Receptors		
	μ	δ	χ
met-ENK	++	+++	+
leu-ENK	+	+++	+
β-endorphin	+++	+++	+
morphine	+++	+	+
dynorphin(1-17)	++	+	+++
EtKCZ	+	+	+++
Naloxone	+++	+	+

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 $[^2]$-Tyr-D-Ala-Gly-NMet-Phe-Gl-ol (DAGO) for μ-receptor and $[^3]$D-Thr^2,Thr^6-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 χ-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, intralaminal 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; Yanagida et al., 1985) possibly by causing the release of opioid peptides. A lesion of the arcuate nucleus, which reduced β-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 β-endorphin concentrations (Przewlocki, 1984). The involvement of

Table II: Relative preponderance of opioid receptors in some brain areas (modified after Quirion et al. 1983b)

Brain region	Receptors	
	μ	δ
n. accumbens	++++	++
frontal cortex	++	++
layers II-IV of cortex	+++	-
layers V-IV of cortex	+	+++
caudate-putamen	++++	+++
olfactory tubercle	+	+++
septum	+	-
thalamus	+++	-
amygdala	+++	-
hypothalamus	+	-
hippocampus	++	+
habenula	++++	+
interpeduncular nucleus	+++	+
central gray	++	+
cerebellum	-	-
n. tractus solitarius	+++	+
locus coerulus	+++	-
dorsal horn (spinal cord)	+++	+

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). β-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 β-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 (Chaillet 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 β-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.,

1983).

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 mediates 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 (Parr, 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 β-endorphin or D-Ala^2-met^5-enkephalinamide 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 excitabiltiy. The administration of large doses of morphine, icv or systemically, can induce convulsive behaviours in mice and rats (Gilbert and Martin, 1975; Snead and Bearden, 1982). Pretreatment with subconvulsant doses of morphine can block the convulsant effect of morphine and enkephalins (Urca and Frenk, 1983; Dzoljic, 1982). Similar pretreatment with morphine, β-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 Dzlojic, 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 inhibiton 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 summarises 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 (Rupreht et al., 1983) and insomnia (see chapter 3). Other neuropeptidase inhibitors such as bestatin and leucinal increased brain met-ENK levels and β-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 β-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 centrefold)

Pharmacological actions	Receptors				
	μ	δ	χ	μ_1	σ
supraspinal analgesia	*	–	–	**	–
stress-induced analgesia	*	**	**	–	–
euphoria	**	?	–	?	–
dysphoria	–	?	**	?	*
sedation	*	?	**	–	–
catalepsy	**	*	*	**	–
increased locomotion	**	?	?	–	**
decreased locomotion	*	?	**	*	–
tolerance	**	**	**	**	**
withdrawal signs	**	*	*	–	*
ictal EEG spiking	**	**	–	?	?
anticonvulsant	**	**	**	–	?
hyperthermia	*	**	–	–	–
hypothermia	*	–	–	**	–
respiratory depression	**	**	–	–	–

Key: **=direct involvement, *=possible involvement, ?=not established   –=not involved

2.5.2 Centrally acting drugs

i) Narcotic analgesics

Morphine elicited a decrease in plasma β-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 (>1month) decreased enkephalin levels in the striatum and pituitary. A similar reduction in β-endorphin contents were demonstrated in the septum, midbrain and pituitary (Herz et al., 1980a). Plasma concentration of β-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 μ-opioid receptors in the striatum (Takashi et al., 1981).

ii) Neuroleptics

Chronic administration of the antipsychotic drugs haloperidol and chlorpromazine increased the formation of γ-endorphin and des-Tyr[1]-γ-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- endorphin activity in chronic schizophrenics (Emrich et al., 1980; Naber et al., 1984).

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 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 cloimipramine, desimipramine, amitriptyline and iprindole provoked a selective increase in met[5]-enkephalin-like immunoreactivity in the striatum and nucleus accumbens (De Felipe et al.,

1985). An increase in β-endorphin contents in the pituitary and hypothalamus has also been reported after treatment with some antidepressants (Pzewlocki, 1984).

Interestingly, antidepressant drugs decreased plasma β-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 naltrexone (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 δ-opioid receptors, but reduced the affinity of the μ-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 eurphoric 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 β-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 β-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 inhibiton (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-pinch 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 β-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 β-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 β-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 pathogensis of some forms of endogenous depression.

2.6.2   Opioid peptides in schizophrenia

The icv administration β-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 β-endorphin may have a neuroleptic-like therapeutic action, since this peptide induced extrapyramidal-like ridigity. These propositions have given rise to two schools of thought ie i) that excess and ii) that a deficiency of opioid peptides, underlie schizophrenia. Increased β-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. β-Endorphin either slightly accentuated (Gerner et al., 1980) or alleviated some symptoms of schizophrenia (Kline et al. 1977). Des-Tyr[1]-γ-endorphin (DTγE) decreased psychotic symptoms in schizophrenics (Verhoeven et al., 1979). In contrast other workers did not find any therapeutic effects following DTγE (Emrich et al., 1980). However antipsychotic drugs such as haloperidol and chlorpromazine increased the formation of γ-endorphin and des-Tyr[1]-γ-endorphin *in vitro* in animal brain slice (Davis et al., 1984). Plasma and CSF β-endorphins were inceased 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

Agid Y, Javoy F (1985) Peptides and parkinson's disease, Trends in Neurosci., 8: 30-35

Akil H, Mayer DJ, Liebeskind JC (1972) Comparaison chez le rat entre l'analgesie induite par stimulation de la substance grise periaqueductale et l'analgesie morphinique, CR hebd. Seance Acad. Sci. (Paris) 274: 3603-3605

Atweh SF, Kuhar MJ (1983) Distribution and physiological significance of opioid receptors in the brain, Brit. Med. Bull., 39: 47-52

Bean AJ, Vaught JL (1984) Physical dependence produced by chronic intracerebroventricular infusion of [D-Arg]kyotophan or thiorphan to rats, Eur. J. Pharmacol. 105: 333-337

Berman EF, Adler MW (1984) The anticonvulsant effect of opioids and opioid peptides against maximal electroshock seizures in rats, Neuropharmacol., 23: 367-371

Bloom FE (1983) The endorphins a growing family of pharmacologically pertinent peptides, Ann. Rev. Pharmacol. Toxicol., 23: 151-170

Bloom F, Segal D, Ling N, Guillemen R (1976) Endorphins profound behavioral effects in rats suggest new etiological factors in mental illness, Science, 194: 630-632

Bradbury AF, Smyth DG, Snell CR (1976) Prohormones of β-melanotropin (β-melanocyte-stimulating hormone, β-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

Brady LS, Holtzman SG (1981) Locomotor activity in morphine-dependent and post-dependent rats, Pharmacol. Biochem. Behav., 14: 361-370

Brantl V, Teschemacher H, Henschen A, Lottspeich F (1979) Novel opioid peptides derived from casein (β-casomorphins) I, Hoppe-Seyler's Z Physiol. Chem., 360: 1211-1216

Bruni G, Dal Pra P, Fiaschi AI, Segre G (1985) Effect of morphine and naloxone on the levels of ACTH and beta-endorphin in plasma, brain and pituitary of rats, Pharmacol. Res. Commun., 17: 659-669

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

pharmacology, Blackwell Scientific Publications, pp 16.1-16.17

Carenzi A, Frigeni V, Della Bella D (1981) Strong analgesic activity of leu-enkephalin after inhibition of brain aminopetidase: a pharmacological study, In: Advances in Endogenous and Exogenous opioids, H Takagi, EJ Simon (eds) Kodansha Ltd, Tokyo, pp 267-270

Cesselin F, Montastruc JL, Gros C, Bourgoin S, Hamon M (1980), Met-enkephalin levels and receptors in the spinal cord of chronic suffering rats, Brain Res., 191: 289-293

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

Chambers WF, Culver CM, Rech RH (1971) Essentials of neuroanatomy and neurophysiology, In: An introduction to psychopharmacology, RH Rech, KE Moore (eds), Raven press, New York, pp 41-78

Chang J-K, Fong BTW, Pert A, Pert CB (1976) Opiate receptor affinities and behavioral effects of enkephalin: structure-activity relationship of ten synthetic peptide analgues, Life Sci., 18: 1473-1482

Christie MJ, Chesher GB (1982) Physical dependence on physiologically released endorgenous opiates, Life Sci., 30: 1173-1177

Crowley TJ, Simpson R (1978) Methadone dose and human sexual behavior, Int. J. Addict., 13: 285-295

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

Davis KR, Hernandez DE, Wolfenden R (1983) Leucinal inhibits brain aminopeptidase activity and potentiates analgesia induced by leu-enkephalin, Pharmacol. Biochem. Behav., 19: 791-794

Davis TP, Schoemaker H, Culling AJ (1984) Centrally acting drugs alter *in vitro* β-endorphin processing in the rat, Eur. J. Pharmacol., 100: 249-251

De Felipe MC, De Ceballos ML, Gil C, Fuentes JA (1985) Chronic antidepressant treatment increases enkephalin levels in n. accumbens and striatum of the rat, Eur. J. Pharmacol., 112: 119-122

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

Dzoljic MR, Poel-Heisterkamp AL vd (1982) Delta opiate receptors are involved in the endopioid-induced myoclonic contractions, Brain Res. Bull., 8: 1-6

Dzoljic MR, Radermaker B, Poel-Heisterkamp AL vd, Ukponmwan OE, Haffmans J, Enkephalinase inhibition suppresses naloxone-induced jumping in morphine dependent mice (in preparation)

Emrich HM, Hollt V, Bergman M (1980) Plasma levels of β-endorphin under chronic neuroleptic treatment in schizophrenic patients: Failure of naloxone to counteract curative effects of neuroleptic drugs, In: Neural peptides and neuronal communications, Costa E, Trabucchi M (eds), Raven press, New York, pp 489-502

Emrich HM, Zaudig M, Kissling W, Dirlich G, Zerssen Dv, Herz A (1980) Des-Tyrosyl-γ-Endorphin in schizophrenia: A double blind trial in 13 patients, Pharmacopsychiat., 13: 290-298

Erdos EG, Johnson AR, Boyden NT (1978) Hydrolysis of enkephalin by cultured human endothelial cells and by purified peptidyl dipeptidase, Biochem. Pharmacol., 27: 843-848

Florez J, Mediavilla A (1977) Respiratory and cardiovascular effects of met-enkephalin applied to the ventral surface of the brain-stem, Brain Res., 138: 585-590

Fratta W, Yang HY, Hong J, Costa E (1977) Stability of met-enkephalin content in brain structures of morphine-dependent or foot shock-stressed rats, Nature, 268: 452-453

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

Frenk H (1983) Pro- and anti-convulsant actions of morphine and endogenous opioids: Involvement annd interactions of multiple opiate and non-opiate systems, Brain Res., 6: 197-210

Fuxe K, Bolme P, Jonsson G, Aganati LF, Goldstein M, Hokfelt T, Schwarcz R, Engel J (1979), In: Nervous system and hypertension, Meyer P, Schmitt H (eds) Wiley-Flammarian, Paris, PP 1

Garzon J, Sanchez-Blazquez P, Hollt V, Lee NM, Loh HH (1983) Endogenous

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

Genazzani AR, Petraglia F, Fachinetti F, Monittola C, Scarone S, Brambilla F (1984) Opioid plasma levels in primary affective disorders, Neuropsychobiol., 12: 78-85

Gerner RH, Catlin DH, Gorelick DA, Hui KK, Li CH (1980) β-endorphin: Intravenous infusion causes behavioral change in psychaitric inpatients, Arch. Gen. Psychiat., 37: 642-647

Gessa GL, 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

Goldstein A, Fischli W, Lowney LI, Hunkapiller M, Hood L (1981) Porcine pituitary dynorphin: complete amino acid sequence of the biologically active heptadecapeptide, Proc. Natl. Acad. Sci. USA, 78: 7219-7223

Goldstein A, Tachibana S, Lowney L, Hunkapiller M, Hood L (1979) Dynorphin-(1-13), an extraordinarily potent opioid peptide, Proc. Natl. Acad. USA 76: 6666-6671

Gorenstein C, Snyder SH (1979) Two distinct enkphalinases: solubilzation, partial purification and separation from angiotensin converting enzyme, Life Sci., 25: 2065-2070

Grandison L, Guidotti A (1977) Stimulation of food intake by muscimol and beta-endorphin, Neuropharmacol., 16: 533-536

Guillemin R, Vargo T, Rossier J, Minck S, Ling N, Rivier C, Vale W, Bloom F (1977) β-endorphin and adrenocorticotropin are secreted concomittantly by the pituitary gland, Science, 197: 1367-1369

Haffmans J, Blankwater YJ, Ukponmwan OE, Zijlstra FJ, Vincent JE, Hespe W, Dzoljic MR (1983) Correlation between the distribution of ^{3}H-labelled enkephalin in rat brain and the anatomical regions involved in enkephalin-induced seizures, Neuropharmacol., 22: 1021-1028

Haffmans J, De Kloet R, Dzoljic MR (1984) Metabolic changes in different rat brain areas during seizure induced by a specific delta opiate receptor agonist, Brain Res., 302: 111-115

Haffmans J, Dzoljic MR (1983) Differential epileptogenic potentials of selective μ and δ opiate receptor agonists, J. Neural. Transm., 57: 1-11

Hambrook JM, Morgan BA, Rance MJ, Smith CFC (1976) Mode of deactivation of the enkephalins by rat and human plasma and rat brain homogenates, Nature, 262: 782-783

Harsing LG, Yang H-YT, Govoni S, Costa E (1982) Elevation of met[5]-enkephalin and beta-endorphin hypothalamic content in rats receiving anorectic drugs: differences between D-fenfluramine and amphetamine, Neuropharmacol., 21: 141-146

Henriksen SJ, Bloom FE, McCoy F, Ling N, Guillemen R (1978) β-endorphin induces non-convulsive limbic seizures, Proc. Natl. Sci. USA, 75: 5221-5225

Herz A (1978) Sites of opiate action in the central nervous system, In: Development of opiate research, Modern Pharmacology-Toxicology, Herz A (ed), M-Dekker, New York, vol 14, pp 153-191

Herz A, Duka T, Gramsch C, Hollt V, Osborne H, Przewlocki R, Schulz R, Wuster M (1980b) Pharmacological manipulation of brain and pituitary endorphin content and release, In: Neural peptides and neuronal communication, E Costa, M Trabucchi (eds), Raven press, New York, pp 323-333

Herz A, Hollt V, Przewlocki R (1980a) Endogenous opioids and addiction, In: Brain and pituitary peptides, Wuttke W, Weindl A, Voight HK 9eds), S Karger AG, Basel, pp 183-189

Ho WKK, Wen HL, Ling N (1980) Beta-endorphin-like immunoreactivity in plasma of heroin addicts and normal subjects, Neuropharmacol., 19: 117-120

Hokfelt T, Elde R, Johansson O, Terenius L, Stein L (1977) The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system, Neurosci. Lett., 5: 25-31

Hollt V, Bergmann M (1982) Effects of acute and chronic haloperidol treatment on the concentrations of immunoreactive beta-endorphin in plasma, pituitary and brain rats, Neuropharmacol., 21: 147-154

Hong JS, Yang H-YT, Gillin JC, Costa E (1980) Effects of long-term administration of antipsychotic drugs on enkephalinergic neurons, Adv.

Biochem. Psychopharmacol., 24: 223-232

Hosobuchi Y, Li CH (1978) The anlagesic activity of human beta-endorphin in man, Commun. Psychopharmacol. 2: 33-37

Hudgin RL, Charlson SE, Zimmerman M, Mumford R, Wood PL (1981) Enkephalinase: selective peptide inhibitors, Life Sci., 29: 2593-2601

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, Beaumont A, Fuentes JA, Malfroy B, Unsworth C (1980) Opioid peptides: aspects of their origin release and metabolism, J. Exp. Biol., 89: 239-255

Hughes J, Smith T, Kosterltz HW, Fothergill L (1975b) Identification of two related pentapeptides from the brain with potent opiate agonist activity, Nature, 258: 577-579

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

Hurle MA, Mediavilla A, Florez J (1983) Participation of pontine structures in the respiratory actions of opioids, Life Sci., 33: 571-574

Isaacson RL, Lanthorn TH (1981) Hippocampal involvement in the pharmacologic induction of withdrawal-like behaviors, Fed Proc., 40: 1508-1512

Jacquet YF, Marks N (1976) The C-fragment of β-lipotropin: an endogenous neuroleptic or antipsychotogen?, Science, 194: 632-634

Jalowiec JE, Panksepp J, Zolovick AJ, Najam N, Herman BH (1981) Opioid modulation of ingestive behavior, Pharmacol. Biochem. Behav., 15: 477-484

Kakidani H, Furutani Y, Takahashi H, Noda M, Morimoto Y, Hirose T, Asai M, Inayama S, Nakanishi S, Numa S (1982) Cloning and sequence analysis of cDNA for porcine β-neo-endorphin/ dynorphin precursor, Nature, 298: 245-249

Kangawa K, Matsuo H (1979) α-neo-endorphin a "big" leu-enkephalin with potent opiate activity from porcine hypothalamus, Biochem. Biophys. Res. Commun., 86: 153-160

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

Kerdelhue B, Karteszi M, Pasqualini C, Reinberg A, Mezey E, Palkovits M (1983) Circadian variations in β-endorphin concentrations in the pituitary and in some brain nuclei of the adult male rat, Brain Res., 261: 243-248

Khachaturian H, Lewis ME, Watson SJ (1983) Enkephalin systems in diencephalon and brainstem of the rat, J. Comp. Neurol., 220: 310-320

Kline NS, Li CH, Lehman HE, Lathja A, Laski E, Cooper T (1977) Beta-enodrphin induced changes in schizophrenic and depressed patients, Arch. Gen. Psychiat., 34: 1111-1113

Kuraishi Y, Sugimoto M, Takagi H (1981) Noxious stimulus-induced release of met-enkephalin from the nucleus reticularis gigantocellularis of the rat, In: Advances in Endogenous and Exogenous Opioids, Takagi H, Simon EJ (eds), Kodansha Ltd, Tokyo, pp 182-184

Li CH, Chung D (1976) Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands, Proc. Natl. Acad. Sci. USA, 73: 1145-1148

Ling N, Burgus R, Guillemin R (1976) Isolation primary structure and synthesis of α and γ, endorphins two peptides of hypothalamus-hypophysial origin with morphinomimetic activity, Proc. Natl. Acad. Sci. USA, 73: 3942-3946

Loh HH, Tseng LF, Wei ET, Li Ch (1976) β-endorphin is potent analgesic agent, Proc. Natl. Acad. Sci. USA, 73: 2896-2898

Lord JAH, Waterfield AA, Hughes J, Kosterlitz HW (1977) Endogenous opioid peptides: multiple agonists and receptors, Nature, 267: 495-499

Lucchi L, Rius RA, Uzumaki H, Govoni S, Trabuchi M (1984) Chronic ethanol changes receptor function in rat striatum, Brain Res., 293: 368-371

Madden J, Akil H, Patrick RL, Barchas JD (1977) Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat, Nature, 265: 358-360

Malfroy B, Swerts JP, Guyon A, Roques BP, Schwartz JC (1978) High-affinity enkephalin-degrading peptidase in brain is increased after morphine, Nature, 276: 523-526

Manson HJ, Dyke G, Melling J, Gough M (1983) The effect of naloxone and

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

Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE (1976) The effects of morphine and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog, J. Pharmacol. Exp. Ther., 197: 517-532

Maurer R, Cortes R, Probst A, Palacios JM (1983) Multiple opiate receptor in human brain; an autoradiographic investigation, Life Sci., 33: 231-234

Meyerson BJ, Terenius L (1977) β-Endorphin and male sexual behavior, Eur. J. Pharmacol. 42: 191-192

Millan MJ, Gramsch C, Przewlocki R, Hollt V, Herz A (1980) Lesions of the hypothalamus arcuate nucleus produce a temporary hyperalgesia and attenuated stress-evoked analgesia, Life Sci 27: 1513-1523

Minamino N, Kangawa K, Chino N, Sakakibara S, Matsuo H (1981) β-endorphin, a new hypothalamic "big" leu-enkephalin of porcine orgin: its purification and complete amino acid sequence, Biochem. Biophys. Res. Commun., 99: 864-870

Minamino N, Kangawa K, Fukuda A, Matsuo H, Iagarashi M (1980) A new octapeptide related to dynorphin from porcine hypothalamus, Biochem. Biophys. Res. Commun., 95: 1475-1481

Morley JE, Elson MK, Levine AS, Shafer RB (1982) The effects of stress on central nervous system concentrations of the opioid peptide dynorphin, Peptides, 3: 901-906

Naber D, Nedopil N, Eben E (1984) No correlation between neuroleptics-induced increase of β-endorphin serum level and therapeutic efficacy in schizophrenia, Brit. J. Psychiat., 114: 651-653

Naber D, Pickar D, Post RM, van Kammen D, Ballinger J, Goodin FK, Bunney WE (1982) Endogenous opioid activity and beta-endorphin immunoreactivity in CSF of psychaitric patients and volunteers, Amer. J. Psychiat. 138: 11

Naber D, Pickar D, Post RM, van Kammen DP, Waters RN, Ballinger JC, Goodwin FK, Bunney WE (1981) Endogenous opiate receptor ligands and β-endorphin-immunoreactivity in CSF of psychiatric patients and in normal volunteers, Amer. J. Psychiat., 138: 1457-1462

Nabeshima T, Matsuno K, Kameyama T (1985) Inovlvement of different opioid receptor subtypes in electric shock-induced analgesia and motor suppression in the rat, Eur. J. Pharmacol., 114: 197-207

Noda M, Furutani Y, Takhashi S, Toyosato M, Hirose T, Inayama S, Nakanishi S, Numa S (1982) Cloning and sequence analysis of cDNA for bovine adrenal pre-proenkephalin, Nature, 295: 202-206

Panksepp J (1981) Brain opioids: a neurochemical substrate for narcotic and social dependence, In: Theory in psychopharmacology, SJ Cooper (ed), Academic press, New York, pp 147-175

Parr D (1976) Sexual aspects of drug abuse in narcotic addicts, Brit J Addict 71: 261-268

Pasternak GW, Gintzler AR, Houghten RA, Ling GSF, Goodman RR, Spielgel K, Nishimura S, Johnson N, Recht LD (1983) Biochemcal and pharmacological evidence for opioid receptor multiplicity in the central nervous system, Life Sci., 33: 167-173

Pazos A, Florez J (1983) Interactions of naloxone with μ and δ-endorphin agonists on the respiration of rats, Eur. J. Pharmacol., 87: 309-314

Petho B, Graf L, Karczag I, Borvendeg J, Bitter I, Barna I, Herman I, Tolna J, Baraczka K (1982) β-endorphin administration to acute schizophrenia patients : a doble blind study, Ann. NY. Acad. Sci., 398: 460-469

Pert A, Sivit C (1977) Neuroanatomical focus for morphine and enkephalin-induced hypermotility, Nature 265: 645-647

Pert CB, Snyder SH (1973) Opiate receptor: Demonstration in nervous tissue, Science, 179: 1011-1014

Przewlocki R (1984) Some aspects of physiology and pahrmacology of endogenous opioid peptides, Pol. J. Pharmacol. Pharm., 36: 137-158

Przewlocki R, Lason W, Konecka AM, Gramsch C, Herz A, Reid LD (1983) The opioid peptide dynorphin, circadian rhythms and starvation, Science, 219: 71-73

Puglisi-Allegra S, Castellano C, Csanyl V, Doka A, Oliverio A (1984) Opioid antagonism of electroshock-induced seizures, Pharmacol. Biochem. Behav., 20: 767-769

Quirion R, Weiss AS, Pert CB (1983a) Comparative pharmacological properties and autoradiographic distribution of [^{3}H]-ethylketocyclazocine binding sites in the rats and guinea pig brain, Life Sci., 33: 183-186

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., 30: 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 schizophrenia, Acta. Psychiat. Scan., 61: 395-403

Risch SC (1982) Beta-endorphin hypersecretion in depression: possible cholinergic mechanism, Biol. Psychiat., 17: 1071-1079

Rupreht J, Ukponmwan OE, Admiraal PV, Dzoljic MR (1983) Effect of phosphoramidon- a selective enkephalinase inhibitor on nociception and behaviour, Neurosci. Lett., 41: 331-335

Sarne Y, Azov R, Weissman BA (1978) A stable enekphalin-like immunoreactive 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

Schulz R, Wuster M, Herz A (1981) Differentiation of opiate receptors in 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 enekphalin: Immunohistochemical mapping in rat central nervous system, Proc. Natl. Acad. Sci. USA, 74: 2167-2171

Simon EJ, Hiller JM, Edelman I (1973) Stereospecific binding of the potent narcotic analgesic $[^3H]$-etorphine to rat brain homogenate, Proc. Natl. 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

Stinus L, Koob GF, Ling N, Akert N (1980) Locomotor activation induced by infusion of endorphins into the ventral tegmental area: Evidence for opiate interactions, Proc. Natl. Acad. Sci. USA, 77: 2323-2327

Takahashi M, Izumi R, Kaneto H, Koida M (1981) Effects of psychotropic drugs on the levels of enkephalins, their receptors and enkephalinase in rat brain, In: Advances in Endogenous and Exogenous Opioids, H Takagi, EJ Simon (eds), Kidansha Ltd, Tokyo, pp 235-237

Tang F, Tang J, Chou J, Costa E (1984) Age-related and diurnal changes in met[5]-enk-arg[6]-Phe[7] and met[5]-enkephalin contents of pituitary and rat brain structures, Life Sci., 35: 1005-1014

Taquet H, Javoy-Agid F, Hamon M, Legrand JC, Agid Y, Cesselin F (1983) Parkinson's disease affects differently met[5]- and leu[5]-enkephalin in the human brain, Brain Res., 280: 379-382

Tepperman FS, Hirst M (1983) Effect of intrahypothalamic injection of [D-Ala[2],D-Leu[5]]enkephalin on feeding and temperature in the rat, Eur. J. Pharmacol., 96: 243-249

Terenius L (1973) Stereospecific interactions between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex, Acta. Pharmac. Toxicol., 32: 317-384

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

Tseng L-F, Loh H, Li CH (1977) Human β-endorphin: development of tolerance and behavioural activity in rats, Biochem. Biophys. Res. Commun., 74: 390-396

Tseng L-F, Wei ET, Loh HH, Li CH (1980) β-Endorphin: central sites of analgesia, catalepsy and body temperature changes in rats, J. Pharmacol. Exp. Ther., 214: 328-332

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:

1039-1048

Urca G, Frenk H (1983) Intracerebral opiates block the epileptic effect of intracerebroventricular (icv) leucine-enkephalin, Brain Res., 259: 103-110

Urca G, Yitzhaky J, Frenk H (1981) Different opioid systems may participate in post-electroconvulsive shock (ECS) analgesia and catalepsy, Brain Res., 219: 385-396

Verhoeven WMA, van Praag HM, van Ree JM, de Wied D (1979) Improvement of schizophrenic patients treated with [Des-Tyr[1]]-γ-endorphin (DTγE) Gen. Psychiat., 36: 294-298

Waksman G, Bouboutou R, Devin J, Bourgoin S, Cesselin F, Hamon M, Fournie-Zaluski M-C, Roques BP (1985) *In vitro* and *in vivo* effects of kelatorphan on enkephalin metabolism in rodent brain, Eur. J. Pharmacol., 117; 233-243

Watson SJ, Berger PA, Akil H, Mills MJ, Barchas JD (1978) Effect of naloxone on schizophrenia. Reduction in hallucinations in a subpopulation of subjects, Science, 201: 73-76

Wei E, Loh H (1976) Physical dependence and opiate-like peptides, Science, 193: 1262-1263

Wei ET, Tseng LF, Loh H, Li CH (1977) Comparison of the behavioral effects of β-endorphin and enkephalin analogues, Life Sci., 21: 321-328

Wesche D, Hollt V, Herz A (1977) Radioimmunoassay of enkephalins. Regional distribution in rat brain after morphine treatment and hypophysectomy, Naunyn-Schmied. Arch. Pharmacol., 301;79-82

Winkler M, Havenmann U, Kuschinsky K (1982) Unilateral injections of morphine into the nucleus accumbens induces akinesia and catalepsy, but no spontaneous muscular rigidity in rats, Naunyn-Schmied Arch. Pharmacol., 318: 143-147

Yaksh TL, Harty GJ (1982) Effects of thiorphan on the antinociceptive action of intrathecal [D-Ala[2],met[5]]-enkephalin, Eur. J. Pharmacol., 79: 293-300

Yanagida H, Corssen G, Trouwborst A, Erdmann W (1985) The pituitary inhibitory system: its role in pain perception, Brain Res., 345: 356-361

Yang JC, Clark WC, Ngai SH (1980) Antagonism of nitrous oxide analgesia by

naloxone in man, Anaesthesiol., 52: 414-417

Yoshikawa K, Hong J-K, Sabol SL (1985) Electroconvulsive shock increases preproenkephalin messenger RNA abundance in the rat hypothalamus, Proc. Natl. Acad. Sci. USA, 82: 589-593

Zhang Az, Yang HYT, Costa E (1982) Nociception, enkephalin content and dipeptidylcarboxypeptidase inhibitors, Neuropharmacol., 21: 625-630

PART II: MUTUAL INTERACTIONS BETWEEN NORMAL SLEEP AND ENDOGENOUS OPIOID SYSTEM

CHAPTER 3

ENDOGENOUS OPIOID PEPTIDES AND SLEEP

ABSTRACT

Intracerebroventricular (i.c.v.) administration of the enkephalinase inhibitor phosphoramidon (25-100 μg) induced a dose-related decrease in non-rapid eye moevement 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 coerulus (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 controversal 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 coerulus

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).
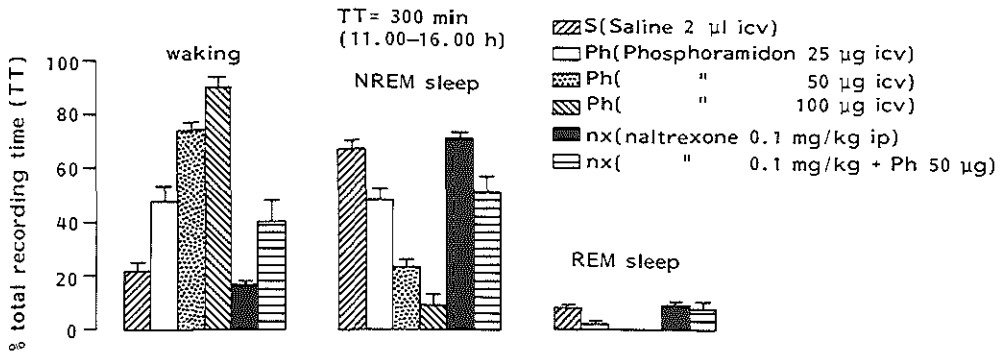
Figure 2: Enkephalinase inhibition and wakefulness in rats. Each bar is mean±S.E.M for 6 rats. Note that the insomnia induced by i.c.v. administered enkephalinase inhibitor phosphoramidon, was decreased by naltrexone.
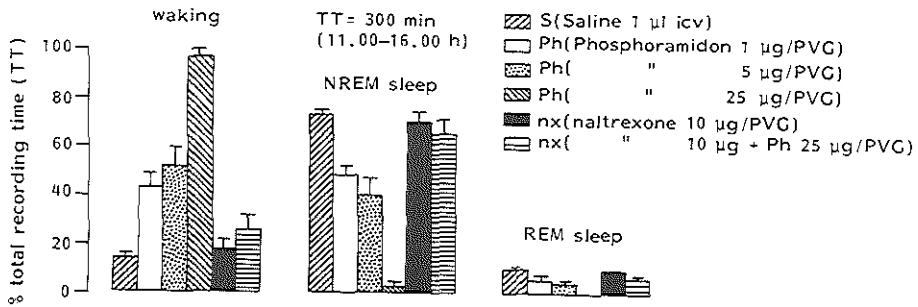
Figure 3: Enkephalinase inhibition and wakefulness in rats. Each bar is mean ±S.E.M for 3-5 rats. Note that the insomnia induced by intracerebral local administered enkephalinase inhibitor phosphoramidon, was abolished by naltrexone.

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 maintaince 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

Havlicek V, Labella F, Pinsky C, Childiaeva R, Friesen H (1978) In: Characteristics and functions of opioids, JM van Ree and L Terenius (eds) Elsevier, Amsterdam pp 423-448

Hazato T, Shimamura M, Katayama T, Kasama A, Nishioka S, Kaya K (1983) Enkephalin degrading enzymes in cerebrospinal fluid, Life Sci., 33: 443-448

Hudgin RL, Charleson SE, Zimmerman M, Mumford R, Wood PL (1981) Enkephalinase: selective peptide inhibitors, Life Sci., 29: 2593-2601

Jacquet YF, Wolf G (1981) Morphine and ACTH-24: correlative behavioral excitations following microinjections in rat periaqueductal gray, Brain Res., 219: 214-218

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

Riou F, Cespuglio R, Jouvet M (1982) Endogenous peptides and sleep in the rat II: peptides without significant effect on the sleep-waking cycle, Neuropeptides, 2: 255-264

Suda H, Aoyagi T, Takeuchi T, Umezawa H (1973) A thermolysin inhibitor produced by actinomycetes: phosphoramidon, J. Antibiotics, 31: 621-623

Umezawa S, Tatsuta K, Izawa O, Tsuchiya T, Umezawa H (1972) A new microbial metabolite phosphoramidon, Tetrahedron Letters, 1: 97-100

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 $[D\text{-}Ala^2]$-Met-enkephalinamide (DALA, 10 $\mu g/2\mu l$, 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 occurence of epilepsy (Janz, 1962). Although some types of epilepsy occur mainly during sleep, their occurence 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 pathogensis 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-Ala^2-met]-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
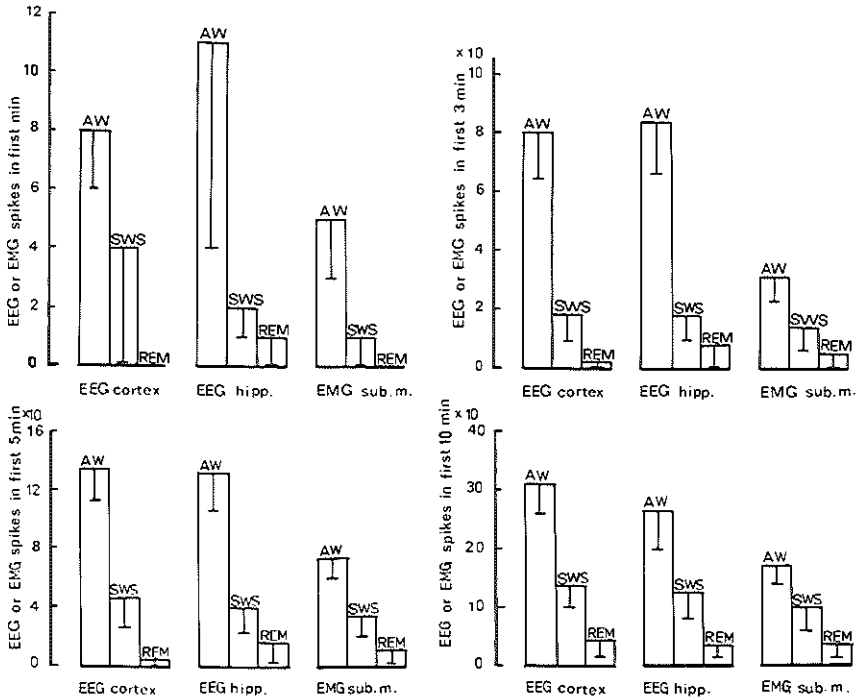
Figure 1: The effect of vigilance states on enkephalin (DALA, 10μg/2l, intracerebrovenrticularly)-induced seizure phenomena during the 4 time periods after drug administration. Only spikes up to 200 and 400μV in the EMG and ECoG, respectively were counted. Each vertical bar is mean±S.E spikes for 7 rats. 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

Cohen HB, Dement WC (1965) Sleep: changes in threshold to electroconvulsive shock in rats after deprivation of "paradoxical" phase, Science., 150: 1318-1319

Dzoljic MR, Lely AJvd, Mourik JBA (1979) Enkephalin-induced myoclonic twitches blocked by ergometrine and potentiated by haloperidol, Psychopharmacol., 66: 111-116

Dzoljic MR, Poel-Heisterkamp ALvd (1982) Delta opiate receptors are involved in the endopioid-induced myoclonic contractions, Brain Res. Bull., 8: 1-6

Janz D (1962) The grand mal epilepsies and the sleep-waking cycle, Epilepsia, 3: 69-109

Snead OC, Bearden LJ (1980) Anticonvulsant specific for petit mal antagonize

epileptogenic effect of leucine enkephalin, Science, 210: 1031-1033

Urca G, Frenk H, Liebeskind JC, Taylor A (1977) Morphine and enkephalin: analgesic and morphine properties, Science, 197: 83-86

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 pepides 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 $22\pm1^{o}C$. 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 cm^2: 100 g). Each rat was REM sleep deprived for 96 hr continously. In the present set up, the animals had free assess 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 ^{o}C and 7 cm for 2 hr daily (11.00-13.00hr) 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 ^{o}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 enkephalinase-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).
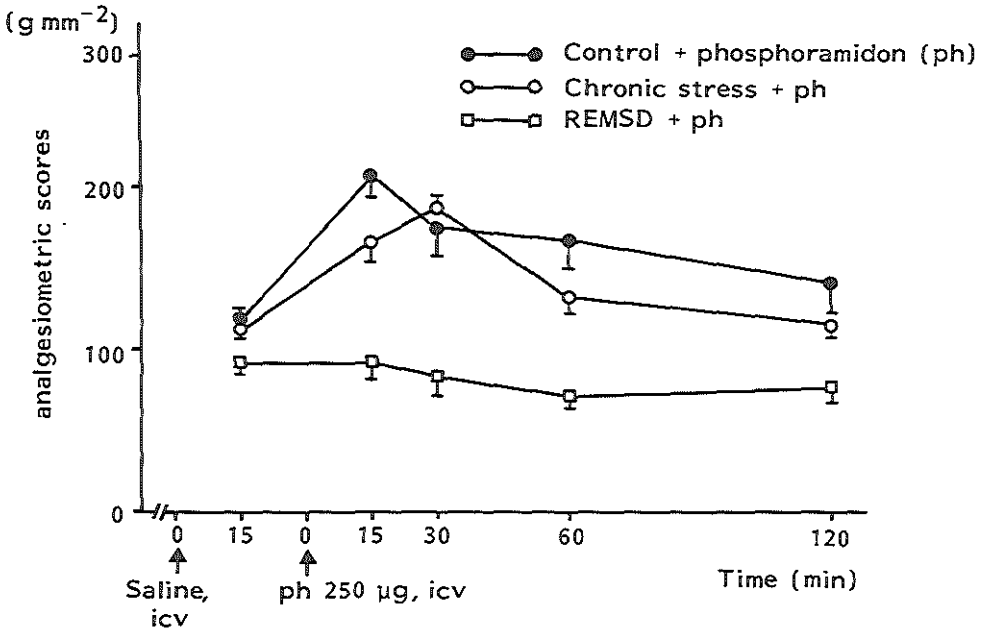
Figure 1: The inhibitory effect of REM sleep deprivation (REMSD) on phosphoramidon (ph) induced analgesia. Each point is mean ± S.E.M for control n=26, REMSD n=25 and stress n=19 groups. The number of animals receiving phosphoramidon in each group are as follows:- control + ph=8, REMSD + ph=6 and stress + 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.
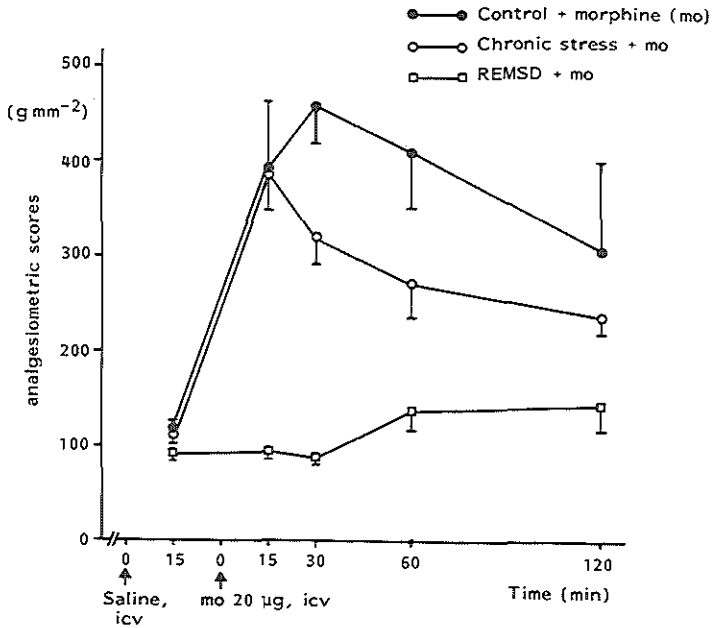
Figure 2: The inhibitory effect of REM sleep deprivation (REMSD) on morphine (mo) analgesia. Each point is mean ± S.E.M for control n=26, REMSD n=25 and stress n=19. The number of animals receiving morphine in each group are as follows: control + mo=10, REMSD + mo=10 and stress + mo=8. Note that morphine 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).
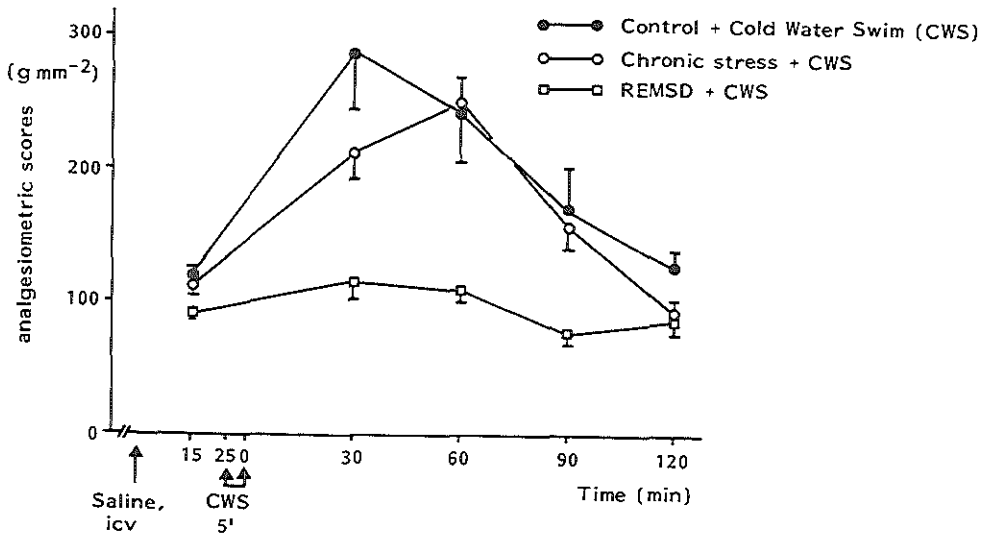
Figure 3: The antagonistic effect of REM sleep deprivation (REMSD) on cold-water-swim analgesia (CWS). Each point is mean ± S.E.M for control n=26, REMSD n=25 and stress n=19. The number of animals submitted to CWS in each group was n=6. Note that CWS analgesia in both control and stress groups was completely 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 serotoninergic 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 maintaince of normal nociception in human.

REFERENCES

Bodnar RJ, Sperber ES (1982) Cold-water-swim analgesia following pharmacological manipulation of GABA, Behav Neural Biol 36: 311-314

Bodnar RJ, Kelly DD, Steiner SS, Glusman M (1978) Stress-produced analgesia and morphine-produced analgesia: Lack of cross, Pharmacol Biochem Behav 8: 661-666

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

Gorlitz BD, Frey HH (1972) Central monoamines and antinociceptive drug action, Eur J Pharamcol 20: 171-180

Hicks RA, Okuda A, Thomsen D (1977) Depriving rats of REM sleep: the identification of a methological problem, Amer J Physiol 90: 95-102

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

Hudgin RL, Charleson SE, Zimmerman M, Mumford R, Wood PL(1981) Enkephalinase: selective peptide inhibitors, Life Sci 29: 2593-2601

Khazan N, Weeks JR, Schroeder LA (1967) Electroencephalographic, electromyographic and behavioural correlates during a cycle of self-maintained morphine addiction in the rat, J Pharmacol Exp Ther 155: 521-531

King C, Masserano JM, Codd E, 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 Pharmac 18: 2711-2721

McGillard KL, Takemori AE (1979) The effect of dopaminergic modifiers on morphine-induced analgesia and respiratory depression, Eur J Pharmacol 54: 61-68

Mendelson WB, Guthrie RD, Frederick G, Wyatt RJ (1974) The flower pot technique of rapid eye movement (REM) sleep deprivation, Pharmacol Biochem Behav 2: 553-556

Micic D, Karadzic V, Rakic LM (1967) Changes of gamma-aminobutyric acid, glutamic acid and aspartic acid in various brain structures of cats deprived of paradoxical sleep, Nature 215: 169-170

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

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

Roques BP, Fournie-Zaluski MC, Soroca E, Lecomte JM, Maleroy B, Llorens C, Schwartz JC (1980) The enkephalinase-inhibitor thiorphan shows antinocieptive activity in mice, Nature 288: 286-288

Rupreht J, Ukponmwan OE, Admiraal PV, Dzoljic MR (1983) Effect of phosphoramidon a selective enkephalinase-inhibitor on nociception and behaviour, Neurosci Letts 41: 331-335

Shapiro C, Girdwood P (1981) Protein synthesis in rat brain during sleep, Neuropharmacol 20: 457-460

Skerritt JH, Trisdikoon P, Johnston GAR (1981) Increased GABA binding in mouse brain following acute swim stress, Brain Res 215: 398-403

Steel RGD, Torrie JH (1980) Principles and procedures of statistical, biometrical approach, McGraw-Hill Kogakusha Ltd, Tokyo

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

Tulunay FC, Yano I, Takemori AE (1976) The effect of biogenic amines modifiers on morphine analgesia and its antagonism by naloxone, Eur J Pharmacol 35: 285-292

Tsuchiya K, Toru M, Kobayashi T (1969) Sleep deprivation: changes of monoamines and acetylcholine in rat brain, Life Sci 8: 867-873

Ukponmwan OE, Dzoljic MR (1983) Stages of vigilance and enkephalin-induced seizures, In: Sleep 82, pp 250-252, Karger, Basel.

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 β-phenylethylamine (PEA, 40 μg, i.c.v.) potentiated the analgesic action of the enkephalinase inhibitor phosphoramidon (250 μg, 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 (5mg/kg, i.p.) or PEA (40 μg, 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 β-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 inihibited (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 $22\pm1^{\circ}C$ 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 to 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 β-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 β-phenylethylamine (PEA) on phosphoramidon induced analgesia was significant across the treatment groups (Fig 2, p<0.01).

The effects of deprenyl and/or β-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
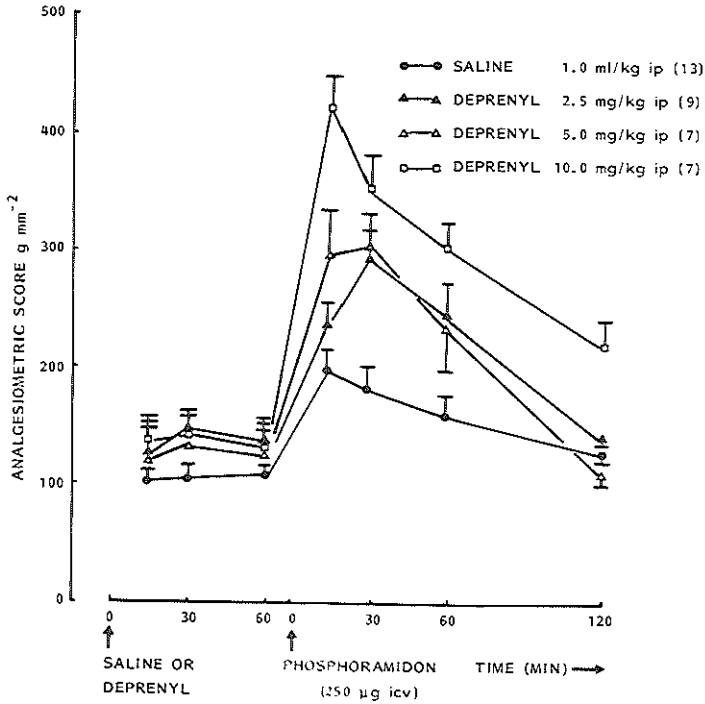
Figure 1: The effect of deprenyl on the analgesic action of the enkephalinase inhibitor phosphoramidon in rats allowed undisturbed sleep. Nociception was determined by withdrawal of hind paw from pressure stimulation (modified Randall-Selitto test). Each point is mean ± SEM at each time point. The number 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, (40μg, 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 μg, 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 μg, i.c.v., data not shown) induced analgesia in animals allowed undisturbed sleep.

The effects of deprenyl and β-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 μg. 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 μg, i.c.v., not shown) were not different from those of control animals (rats allowed undisturbed sleep). Phosphoramidon (250 μg, 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 μg, i.c.v., 5 min post-phosphoramidon) plus phosphoramidon (250 μg, 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 inihibitor phosphoramidon was potentiated by both PEA (specific substrate for MAO-B) and deprenyl (selective inhibitor of this enyzme). 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;
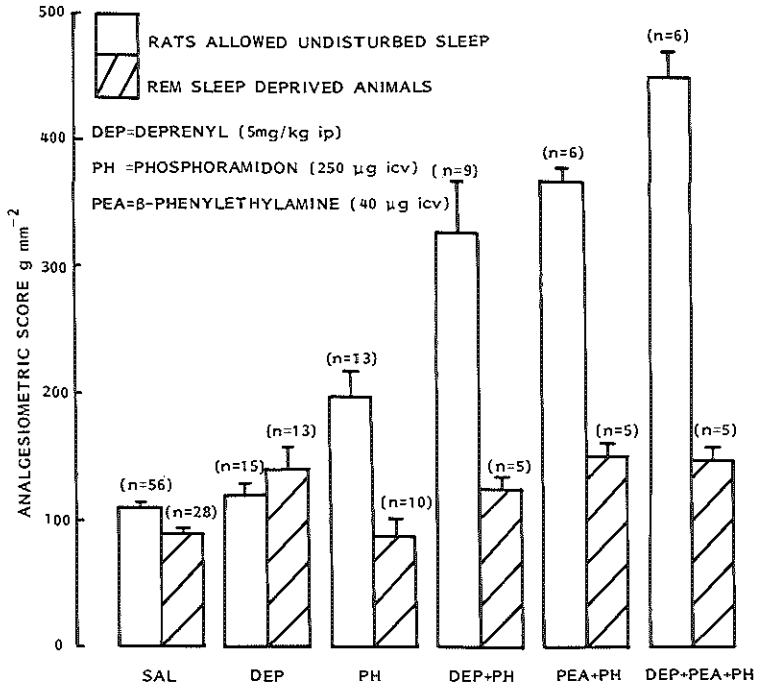
Figure 2: *The effects of deprenyl (DEP), REM sleep deprivation (REMSD) and β-phenylethylamine (PEA) on the analgesic effect of the enkephalinase inhibitor phosphoramidon (PH). Nociception was determined by withdrawal of hind paw from pressure stimulation (modified Randall–Selitto test). The analgesic 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.*

Rupreht et al., 1983). 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 (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

Basbaum AI, Fields HL (1984) Endogenous pain control systems: brain spinal pathways and endorphin circuitry, Ann Rev Neurosci 7: 309-338

Benedetti MS, Keane PE (1980) Differential changes in monoamine oxidase A and B activity in the aging rat brain, J Neurochem 35: 1026-1032

Boden R, Botting R, Coulson P, Spanswick G (1984) Effect of nonselective and selective inhibitors of monoamine oxidases A and B on pethidine toxicity in mice, Br J Pharmac 82: 151-154

Chaillet P, Coulaud A, Fournie-Zaluski MC, Gacel G, Roques BP, Costentin J (1983) Pain control by endogenous enkephalins is mediated by mu opioid receptors, Life Sci 33: 685-688

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

Dourish CT, Cooper SJ (1984) Potentiation of total horizontal activity and ambulation in rats treated with combinations of β-phenylethylamine and naloxone, Neuropharmacol 23: 1059-1064

Fuentes JA, Garzon J, Del Rio J (1977) Potentiation of morphine analgesia in mice after inhibition of brain type-B monoamine oxidase, Neuropharmacol 16: 857-862

Garzon J, Moratalla R, Del Rio J (1980) Potentiation of the analgesia induced in rats by morphine or [D-Ala^2]-met-enekephalinamide after inhibition of brain type B monoamine oxidase:the role of phenylethylamine, Neuropharmacol 19: 723-729

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

Hudgin RL, Charleson SE, Zimmerman M, Mumford R, Wood PL (1981) Enkephalinase: selective peptide inhibitors, Life Sci 29: 2593-2601

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

Jounela AJ, Mattila, MJ, Knoll J (1977) Interaction of selective inhibitors of monoamine oxidase with pethidine in rabbits, Biochem Pharmacol 26: 806-808

Kubota K, Matsuoka Y, Sakuma M, Satoh S, Uruno T, Sunagane N (1982) Characteristic of analgesia induced by noncatacholic phenylethylamines in mice, Life Sci 31: 1221-1224

Mann J, Gershon S (1980) L-Deprenyl, a selective monoamine oxidase type -B inhibitor in endogenous depression, Life Sci 26: 877-882

Mendelson WB, Guthrie RD, Frederick G, Wyatt RJ (1974) The flower pot technique of rapid eye movement (REM) sleep deprivaton, Pharmacol Biochem Behav 2: 553-556

Pickar D, Cohen MR, Naber D, Cohen RM (1982) Clinical studies of the the endogenous opioid system, Biol Psychiat 17: 1243-1276

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

Risch S (1982) β-Endorphin hypersecretion in depression:possible cholinergic mechanisms, Biol Psychiat 17: 1071-1079

Rupreht J, Ukponmwan OE, Admiraal PV, Dzoljic MR (1983) Effect of phosphoramidon - a selective enkephalinase inhibitor- on nociception and behaviour, Neurosci Letts 41: 331-335

Saxena PR (1985) An interactive computer programme for data management and parametric and non-parametric statistical analysis, Proc Br Pharmacol Soc Edinburgh Abstr D3

Steel RGD, Torrie JH (1980) Principles and procedures of statistics, biometrical approach, McGraw-Hill Kogakusha Ltd, Tokyo

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

Lancet ii 401-402

Ukponmwan OE, Poel-Heisterkamp AL van den, Haffmans J, Dzoljic MR (1983) MAO-B inhibitor deprenyl and β-phenylethylamine potentiate [D-Ala^2]-met -enkephalinamide-induced seizures, Naunyn-Schmied Arch Pharmacol 322: 38-41

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

Wu PH, Boulton AA (1975) Metabolism, distribution and disappearance of injected β-phenylethylamine in the rat, Can J Biochem 53: 42-50

Yang H-YT, Neff NH (1974) The monoamine oxidases of brain: selective inhibition with drugs and the consequences for the metabolism of the biogenic amines, J Pharmac Exp Ther 189: 733-740

CHAPTER 7

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 $22\pm1^{o}C$.

REMSD

Animals were deprived of REM sleep continously for 96 h using the classical "flower pot" technique previously described (11). In this procedure, rats were placed on platforms (14 ± 0.5 cm^2/100 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 assess 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 cm^2/100 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 assess 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 Varimex (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 ridigity (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).
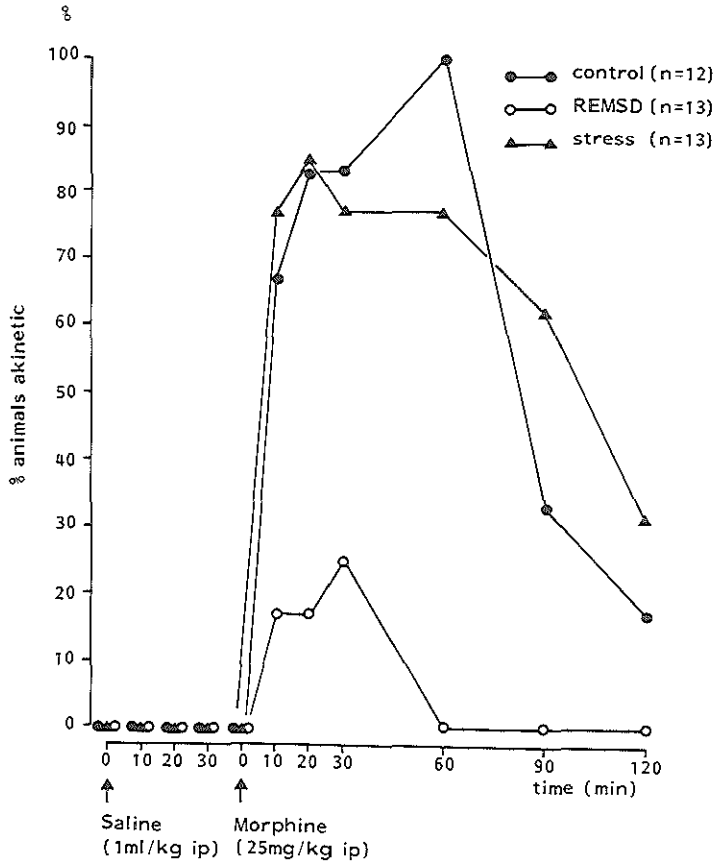
Figure 1: Effect of REM sleep deprivation (REMSD) on morphine-induced akinesia. Number of animals per treatment group is indicated in parentheses. Note that REMSD significantly decreased the morphine-induced akinesia.
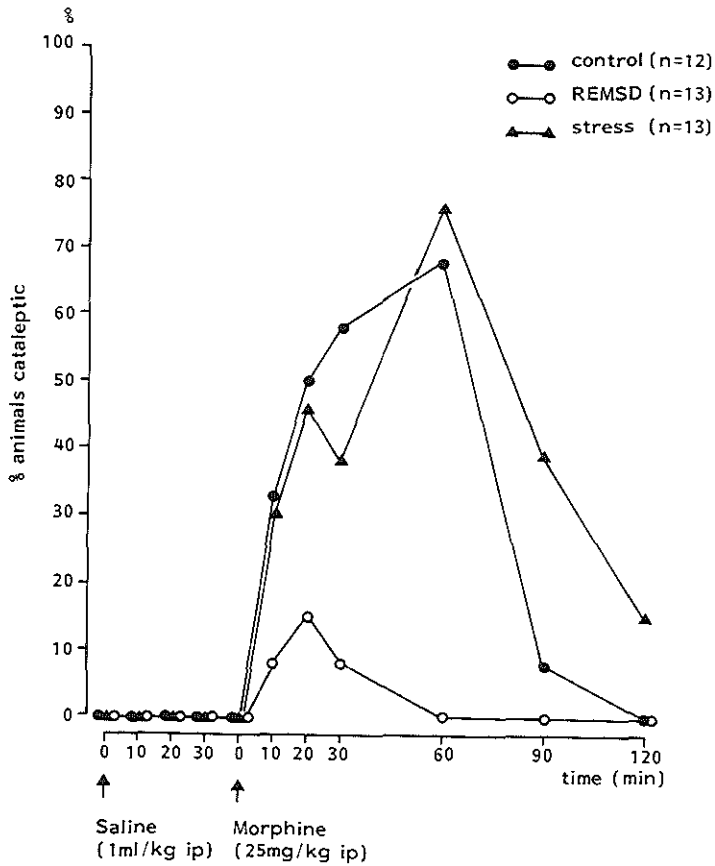
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Figure 2: Effect of REM sleep deprivation (REMSD) on morphine-induced catalepsy. Number of animals per treatment group is indicated in parentheses. Note that REMSD significantly decreased the morphine-induced catalepsy 60 min after drug treatment.

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 exctitatory 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
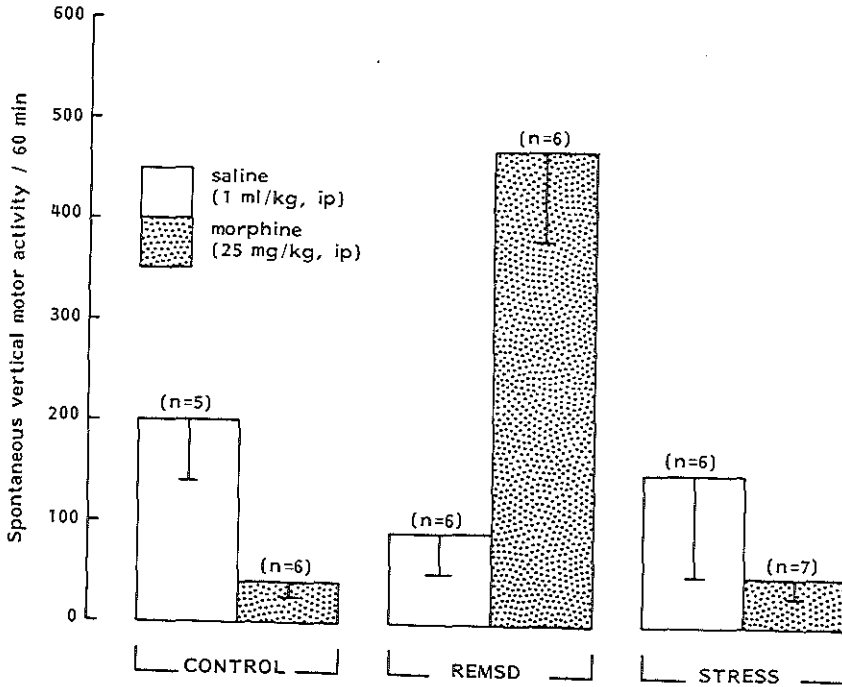
Figure 3: *Effect of REM sleep deprivation (REMSD) on the inhibitory action of morphine on spontaneous vertical motor activity (SVMA). Number of animals per treatment group is indicated in parentheses. Note that REMSD converted the locomotor inhibitory effect of morphine to excitation manifested as increased 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, γ-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 μ-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 μ-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 μ-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 possiblity 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

1. King C, Masserano JM, Codd E, Byrne WL (1981) Effects of ß-endorphin and morphine on sleep-wakefulness of cat, Sleep, 4: 259-62

2. Oksenberg A, Sarne Y, Weissman AB, Luboshitzky R, Lavie P (1980) Plasma humoral endorphin profiles during human sleep, Sleep Res, 9:94

3. Ukponmwan OE, Dzoljic MR (1983) Stages of vigilance and enkephalin-induced seizures. In:Koella W.P. ed. Sleep 1982, Basel: Karger, pp 250-252

4. Coble P, Foster G, Kupfer DJ (1976) Electroencephalographic sleep diagnosis of primary depression, Arch Gen Psychiatry, 33: 1124-27

5. Ansseau M, Kufer DJ, Reynolds CF, Coble PA (1985) "Paradoxical" shortening of REM latency on the first recording night in major depressive disorder: clinical and polysomnographic correlates, Biol Psychiatry, 20: 135-45

6. Pickar D, Cohen MR, Naber D, Cohen RM (1982) Clinical studies of endogenous opioid system, Biol Psychiatry, 17: 1243-76

7. Risch S (1982) ß-Endorphin hypersecretion in depression: possible cholinergic mechanisms, Biol Psychiatry, 17: 1071-9

8. Vogel GW (1983) Evidence for REM deprivation as the mechanism of action of antidepresant drugs, Prog Neuro-Psychopharmacol Biol Psychiatry, 7: 343-9

9. 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: 255-8

10. Wolff EA III, Putnam FW, Post RM (1985) Motor activity and affective illness, Arch Gen Psychiatry, 42: 288-94

11. Mendelson WB, Guthrie RD, Frederick G, Wyatt RJ (1975) The flower pot technique of rapid eye movement (REM) sleep deprivation, Pharmacol Biochem Behav, 2: 553-6

12. Hicks RA, Okuda A, Thomsen D (1977) Depriving rats of REM sleep: the identification of a methodological problem, A J Psychol, 90: 95-102

13. McGrath MJ, Cohen DB (1978) REM sleep facilitation of adaptive waking behaviour, Psychol Bull 85: 24-57

14. Itoh S, Katsuura G (1981) Suppressive effect of cholecystokinin and its related peptides on β-endorphin-induced catalepsy in rats Eur J Pharmacol 74: 381-4

15. Holaday JW, Pasternak GW, Faden AI (1983) Naloxazone pretreatment modifies cardiorespiratory, temperature, and behavioural effects of morphine. Neurosci Lett 37: 199-204

16. Elomaa E, Johansson GG (1980) Daily rhythm of locomotor activity is abolished during rapid eye movement sleep deprivation in the rat. Physiol Behav, 24: 327-30

17. Kafka MS, Wirz-Justice A, Naber D, Moore RY, Benedito MA (1983) Circadian rhythms in rat brain neurotransmitter receptors, Fed Proc 42: 2796-801

18. Brown DR, Robertson MJ, Goldberg LI (1983) Reversal of morphine-induced catalepsy in the rat by narcotic antagonists and their quaternary derivatives, Neuropharmacol 22: 317-21

19. Browne RG, Derrington DC, Segal DS (1979) Comparison of opiate- and opioid-peptide-induced immobility, Life Sci 24: 933-47

20. Havemann U, Kuschinsky K (1981) Further characterization of opioid receptors in the stratum mediating muscular rigidity in rats, Naunyn-Schmiedebergs Arch Pharmacol 317: 321-5

21. Ling GSF, Pasternak GW (1982) Morphine catalepsy in the rat: Involvement of μ_1 (high affinity) opioid binding sites, Neurosci Lett 32: 193-6

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

23. Jacquet YF, Klee WA, Rice KC, Iijima I, Minamikawa J (1977) Stereospecific and nonstereospecific effects of (+)-and (-)-morphine:Evidence for a new class of receptors, Science 198: 842-5

24. Chaillet P, Coulaud A, Zajac J-M, Fournie-Zaluski M-C, Costentin J, Rooques BP (1984) The μ rather than δ subtype of opioid receptors appears to be involved in enkephalin-induced analgesia, Eur J Pharmacol 101: 83-90

25. Tsuchiya K, Toru M, Koboyashi T (1969) Sleep deprivation: changes of monoamine and acetylcholine in the rat brain, Life Sci 8: 867-73

26. Karadzic V, Micic D, Rakic LJ (1971) Alterations of free amino acids concentrations in cat brain induced by rapid eye movement sleep deprivation.

Experientia 27: 509-11

27. Mattiace LA, Negro-Vilar A, Poffwarg HP, Farber J (1981) The effect of REM sleep deprivation on substance P and somatostatin levels in discrete areas of the rat brain, Sleep Res 10: 68

28. Moleman P, Versluis DJ, Bruinvels J (1978) Is morphine-induced catalepsy related to activation of dopaminergic neurons. Psychopharmacol 60: 35-9

29. Kasson BG, George R (1983) Endocrine influences on the actions of morphine, Neuroendocrinol 37:416-20

30. Broekkamp CL, LePichon M, LLoyd KG. Akinesia after locally applied morphine near the nucleus raphe pontis of the rat, Neurosci Lett 50: 313-8

31. Hery F, Pujol J-F, Lopez M, Macon J, Glowinsky J (1970) Increased synthesis and utilization of serotonin in the central nervous system of the rat during paradoxical sleep deprivation, Brain Res 21: 391-403

32. Tufik S (1981) Changes of response to dopaminergic drugs in rats submitted to REM-sleep deprivation, Psychopharmacol 72:257-60

33. De Ryck M, Teitelbaum P(1983) Morphine versus haloperidol catalepsy in the rat: An electromyographic analysis of postural support mechanism. Exp Neurol 79: 54-76

34. Carlson KR, Seeger TF (1982) Interaction of opiates with dopamine receptors: receptor binding and behavioural assays Pharmacol Biochem Behav 16: 119-24

35. Yacquet YF (1982) Dual actions of morphine on the central nervous system:parallel actions of β-endorphin and ACTH. Ann NY Acad Sci 398: 272-90.

36. LaBella FS, Pinsky C, Christy ME, Colton CD (1982) Hyperactivity versus explosive motor behaviour induced by opioids in the rat. Mechanisms elucidated with enkephalin-tyrosine-o-sulfate and morphine congeners, Life Sci 30: 1565-71.

37. North RA, Williams JT (1983) How do opiates inhibit neurotransmitter release? Trends Neurosci 6: 337-9

38. Hardy C, Panksepp J, Rossi J, Zolovick AJ (1980) Naloxone facilitates amygdaloid kindling in rats, Brain Res 194: 293-7

39. Bergonzi P, Chiurulla C, Cianchetti C, Quattrini A (1972) Effetti della "REM-deprivation" negli epilettici (attivata patological EEG, fasi e cicli del sonno), Riv Pat Nerv Ment 93: 49-59

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 enekphalinase 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 guage 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 sugery, 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 cm^2/100 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 mannually. 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 cm^2/100 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 nalxone (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 continous 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 inhibiton

The intracerebroventricular administration of the enkephalinase inhibitor phosphoramidon (1×10^{-8} - 5.6×10^{-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 phosphoramdion administration and were still observed after 240 min. The Kruskal-Wallis one way ANOVA showed a significant difference in the phosphoramdion-induced WDS across the groups ($H = 106.21$, NDF=8 $p < 0.001$). 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, $p > 0.10$).

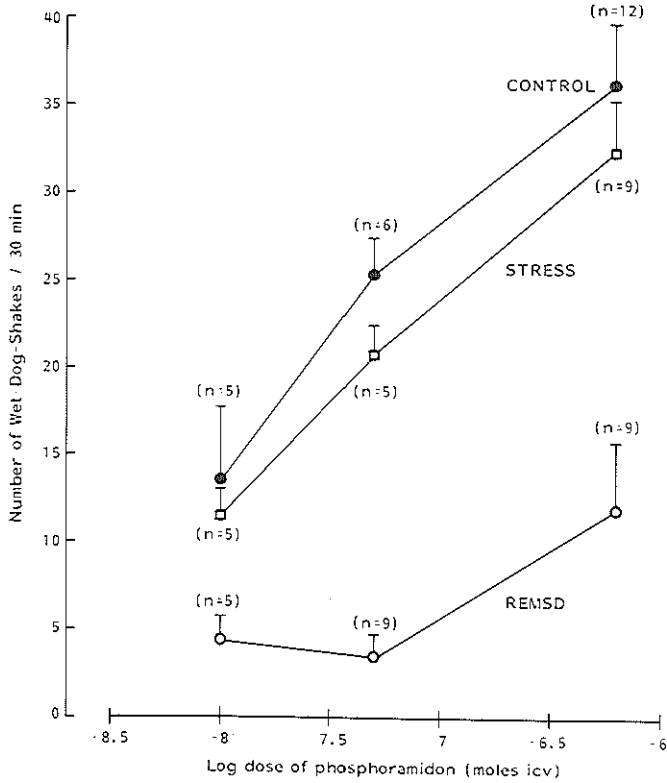Naltrexone (2.9×10^{-6} moles/kg i.p., 10 min prior) significantly

Figure 1: The phosphoramidon (1×10^{-8} – 5.6×10^{-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 phosphoramdion-induced WDS in control (p<0.002), REMSD and
stressed animals (p<0.05, Ducan 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).
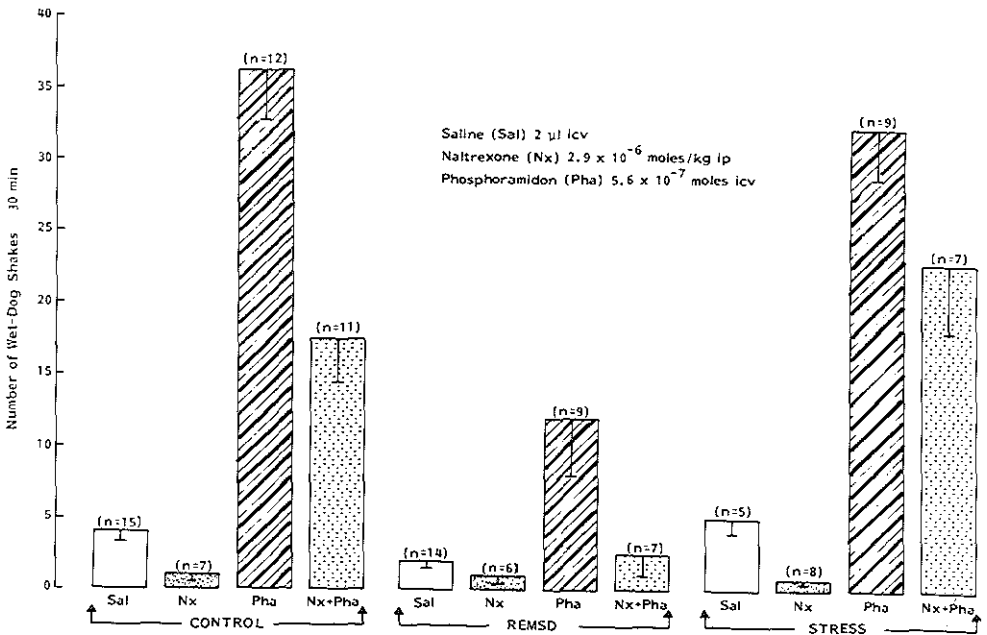
Figure 2: *Effect of naltrexone (2.9×10^{-6} moles/kg, i.p.) on phosphoramidon
(5.6×10^{-7} moles i.c.v.)-induced wet-dog-shakes (WDS). Each bar is/mean ±
S.E.M. The number of animals per treatment group is indicated in
parentheses. Note that pretreatment with naltrexone significantly decreased
phosphoramidon-induced WDS.*
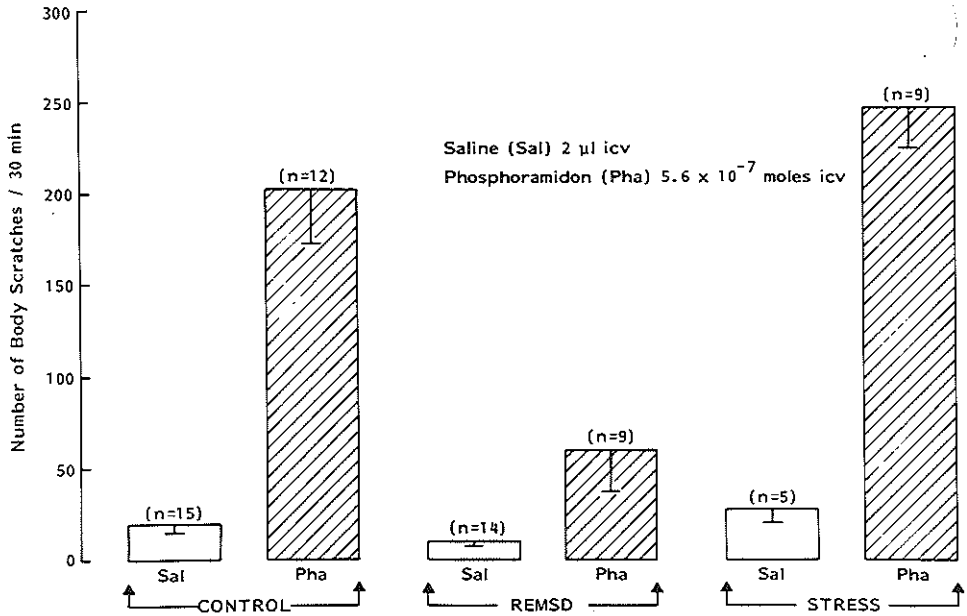
Figure 3: Effect of REMSD on phosphoramidon (5.6 x 10^{-7} moles, i.c.v.) induced body scratches. Each bar is mean ± S.E.M. The number of rats per treatment group is stated in parentheses. Note that the intense body scatches induced by phosphoramidon in control and stressed animals were significantly decreased by REMSD.

Effect of REMSD on opiate withdrawal WDS

Naloxone (3.1 x 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, H=16.12, NDF=2, p<0.001). The frequency of the precipitated WDS was significantly more pronounced in

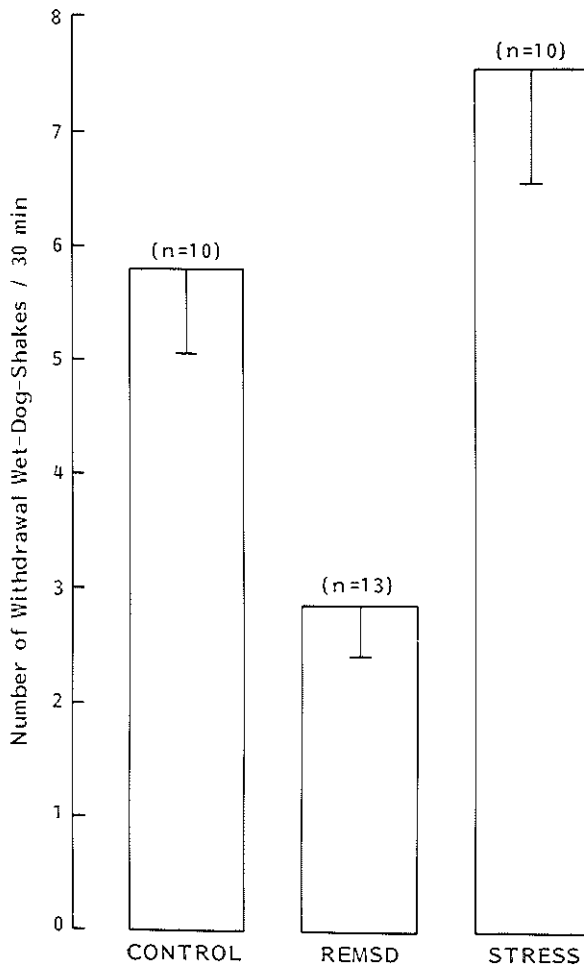Figure 4: Effect of REMSD on naloxone (3.1 x 10^{-6} moles/kg i.p.) -precipitated withdrawal wet-dog-shakes (WDS). Each bar is mean ± S.E.M. The number of rats per group is indicated in parentheses. Note that the total frequency of withdrawal WDS was significantly less in REMSD rats compared with control (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 inhibiton of abstinential WDS in REM sleep deprived rats, since the alteration of the serotoninergic 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

1. Algeri S, Costa E (1971) Physical dependence on morphine fails to increase serotonin turnover rate in rat brain, Biochem. Pharmacol., 20: 877-884

2. Aloisi F, De Carolis AS, Longo V (1980) EEG and behavioural effects of morphine, enkephalins and derivatives administered into the lateral cerebral ventricles of rats and rabbits, Pharmacol. Res. Commun., 12: 467-477

3. Bowers MB, Hartman EL, Freedman DX (1966) Sleep deprivation and acetylcholine, Science, 153: 1416-1417

4. Bowersox SS, Drucker-Colin RR (1982) Seizure modification by sleep deprivation: A possible protein synthesis mechanism. In: Sleep and Epilepsy, MB Sterman, MN Shouse, P Passouant, Academic Press, New York, pp 91-104

5. Cowan A, Tortella FC (1982) A quantitative analysis of the shaking behaviour induced in rats by β-endorphin and D-Ala^2-met^5-enkephalinamide, Life Sci., 30: 171-176

6. Drust EG, Sloviter RS, Connor JD (1981) Methionine enkephalin-induced shaking behaviour in rats: Dissociation from brain serotonin mechanisms, Neuropharmacol., 20: 473-475

7. Dzoljic MR (1982) Opiate receptors and seizures: procovulsant actions of δ-receptors and anticonvulsant action of μ-receptors. In : Current Status of Centrally Acting Peptides, BN Dhawan (ed), Pergamon Press, New York, pp 107-113

8. Frenk H, Urca G, Liebeskind JC (1978) Epileptic properties of leucine- and methionine-enkephalin: comparison with morphine and reversiblity by naloxone, Brain Res., 147: 327-337

9. Gispen WH, Wiegant VM, Greven HM, de Wied D (1975) The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure-activity studies, Life Sci., 17: 645-652

10. Gmerek DE, Cowan A (1982) A study of the shaking and grooming induced by

RX-336-M in rats, Pharmacol. Biochem. Behav., 16: 929-932

11. Gourley DRH, Beckner SK (1973) Antagonism of morphine analgesia by adenine and adenosine and adenine nucleotides, Proc. Soc. Exp. Biol. Med., 144: 774-779

12. Goujet MA, Simon P, Chermat R, Boissier JR (1975) Profil de la TRH en psychopharmacologie experimental, Psychopharmacol., 45: 87-92

13. Harston CT, Spirtes MA, Dunlap WP, Coy DH (1980) Naloxone-reversible effects of d-Ala^2-met^5-enkephalinamide-induced behavioural activity in rats, Behav., Neural Biol., 30: 1-19

14. Herman BH, Berger S, Holtzman SG (1983) Comparison of electrical resistance, bubble withdrawal and stereotaxic method for cannulation of cerebral ventricles, J. Pharmacol. Methods, 10: 143-155

15. Herz A (1978) Sites of opiates in the central nervous system. In: Developments of Opiate Research. Modern Pharmacology-Toxicology, vol 14, A Herz (ed), New York, Dekker, pp 153-191

16. Hudgin RL, Charleson SE, Zimmerman M, Mumford R, Wood PL (1981) Enkephalinase: selective peptide inhibitors, Life Sci., 29: 2593-2601

17. Jolles J, Rompa-Barendregt J, Gispen WH (1979) ACTH-induced excessive grooming in the rat: the influence of environmental and motivational factors, Horm. Behav., 12: 60-72

18. Lal H, Numan R (1975) Blockade of morphine-withdrawal body shakes by haloperidol, Life Sci., 18: 163-168

19. Loh HH, Shen F-H, Way EL (1969) Inhibition of morphine tolerance and physical dependence, development and brain serotonin synthesis by cycloheximide, Biochem. Pharmacol., 18: 2711-2721

20. McGrath MJ, Cohen DB (1978) REM sleep facilitation of adaptive waking behavior: A review of the literature, Psychol. Bull., 85: 24-57

21. Mendelson WB, Guthrie RD, Frederick G, Wyatt RJ (1974) The flower pot technique of rapid eye movement (REM) sleep deprivation, Pharmacol. Biochem. Behav., 2: 553-556

22. Mendelson W, Guthrie RD, Guynn R, Harris RL, Wyatt RJ (1974) Rapid eye movement (REM) sleep deprivation, stress and intermediary metabolism, J. Neurochem., 22: 1157-1159

23. North RA, Williams JT (1983) How do opiates inhibit neurotransmitter release? Trends Neurosci., 6: 337-339

24. Paakkari I (1980) A simple method for the verification of a successful cannulation of the rat cerebral ventricles, Experientia 36: 887-889

25. Patey G, De La Baume S, Schwartz J-C, Gros C, Roques M-C, Fournie-Zaluski E, Soroca-Lucas E (1981) Selective protection of methionine enkephalin released from brain slices by enekphalinase inhibition, Science, 212: 1153-1155

26. Pert CB, Kuhar MJ, Snyder SH (1976) Opiate receptor: autoradiographic localisation in rat brain, Proc. Natl. Acad. Sci. USA, 73: 3729-3733

27. 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

28. Rupreht J, Ukponmwan OE, Admiraal PV, Dzoljic MR (1983) Effect of phosphoramidon- a selective enkephalinase inhibitor-on nociception and behaviour, Neurosci. Lett., 41: 331-335

29. Schiorring 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

30. Shapiro C, Girdwood P (1981) Protein synthesis in the rat brain during sleep, Neuropharmacol., 20: 457-460

31. Stern WC, Miller FP, Cox RH, Maickel RP (1971) Brain norepinephrine and serotonin levels following REM sleep deprivation in the rat, Psychopharmacol., 22: 50-55

32. Tornos ME, Sacristan A, Ortiz JA (1983) Effect of oral CDP-choline on experimental withdrawal syndrome, Drug Res., 33: 1018-1021

33. Toru M, Mitsushio H, Mataga N, Takashima M, Arito H (1984) Increased brain metabolism during rebound sleep in sleep-deprived rats, Pharmacol. Biochem. Behav., 20: 757-761

34. Tseng L-F, Loh HH, Wei ET (1975) Effects of clonidine on morphine withdrawal signs in the rat, Eur J. Pharmacol., 30: 93-99

35. Tsuchiya K, Toru M, Kobayashi T (1969) Sleep deprivation: changes of monoamines and acetylcholine in rat brain, Life Sci., 8: 867-873

36. Tufik S (1981) Changes of response to dopaminergic drugs in rats submitted to REM sleep deprivation, Psychopharmacol., 72: 257-260

37. Ukponmwan OE, Dzoljic MR (1983) Stages of vigilance and enkephalin-induced seizures, In: Sleep 1982, WP Koella (ed), Basal, Karger, pp 250-252

38. 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

39. Wei ET (1981) Pharmacological aspects of shaking behaviour produced by TRH, AG-3-5, and morphine, Fed. Proc., 40: 1491-1496

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 eludicate 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 occuring 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 $1.5\mu l$. 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 ($1\mu l$ 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 ($p=o.1$) 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).
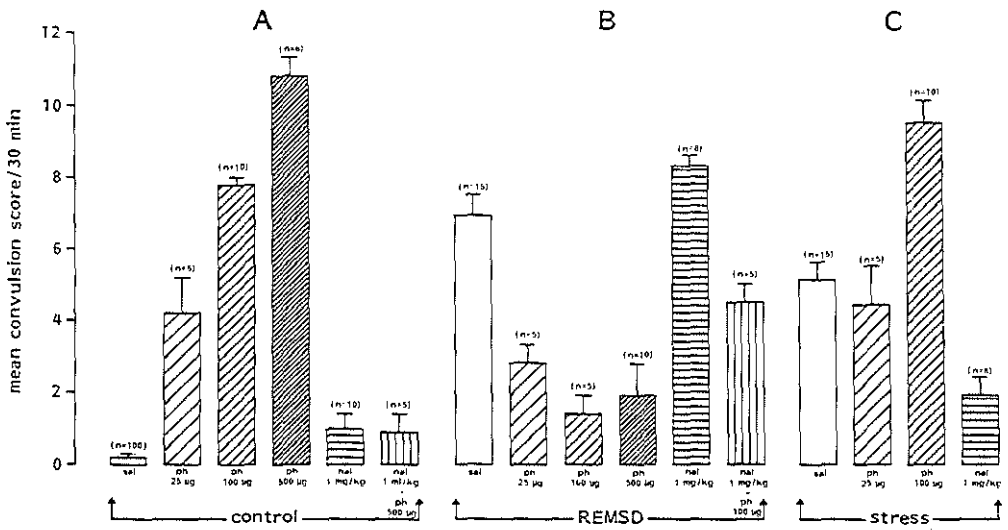
Figure 1a-c: The   effect   of   REM   sleep   deprivation   (REMSD,   72   h),
phosphoramidon (ph), naloxone (nal) and stress (72 h) on handling-induced-
convulsions (HIC). Each bar is mean intensity ± S.E.M of HIC during the
first 30 min. The number of animals per group is stated in parentheses.

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 (Urca 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 humural 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 consquent increase in seizure susceptibilty.

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 nacolepsy, the attacks of REM sleep have been prevented by naloxone, suggesting a possible involvement of endogenous opioid systems (Pasi et al., 1983).

REFERENCES

Christie MJ, Chesher GB (1982) Physical dependence on physiologically released endogenous opiates, Life Sci., 30: 1173-1177

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

1318-1319

Cowan A, Geller EB, Adler MW (1979) Classification of opioids on basis of change in seizure thresholds, Science, 206: 465-467

Crabbe JC, Young ER, Janowsky J, Rigter H (1981) Pyrazole exacerbates handling-induced convulsions in mice, Neuropharmacol., 26: 605-609

Cupello A, Hyden H (1981) On the presence of met 5-enkephalin receptors on plasma membrane of Deiters' neurons and their modulation of GABA transport, J. Neurosci. Res., 6: 579-583

Drucker-Colin RR, Spanis CW, Rojas-Ramirez JA (1977) Investigation of the role proteins in the REM sleep. In: Drucker-Colin RR, McGaugh JL (eds) Neurobiology of sleep and memory. Academic Press, New York, pp 303-319

Dzoljic MR, Lely AJ vd, Mourik JBA v (1979) Enkephalin-induced myoclonic twitches blocked by ergometrine and potentiated by haloperidol, Psychopharmacol., 66: 111-116

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

Dzoljic MR, Poel-Heisterkamp AL vd (1982) Delta opiate receptors are involved in the endopioid-induced myoclonic contractions, Brain Res. Bull., 8: 1-6

Fishbein W (1970) Interference with conversion of memory from short-term to longterm storage by partial sleep deprivation, Commun. Behav. Biol., 5: 171-175

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

Frenk H, Urca G, Liebeskind JC (1978) Epileptic properties of leucine- and methionine-enkephalin: Comparison with morphine and reversibility by naloxone, Brain Res., 147: 327-337

Frenk H (1983) Pro- and anticonvlsant actions of morphine and endogenous opioids: Involvement and interactions of multiple opiate and non-opiate systems, Brain Res. Rev., 6: 197-210

Gilbert PE, Martin WR (1975) Antagonism of the convulsant effects of heroin, D-proproxyphene, meperidine, normeperidine and thebaine by naloxone in mice, J. Pharmacol. Exp. Ther., 197: 538-541

Goldstein DB, Pal N (1971) Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction, Science, 172: 288-290

Haffmans J, Blankwater YJ, Ukponmwan OE, Zijlstra FJ, Vincent JE, Hespe W, Dzoljic MR (1983) Correlation between the distribution of ^{3}H-labelled enkephalin in rat brain and the anatomical regions involved in enkephalin-induced seizures, Neuropharmacol., 22: 1021-1028

Haffmans J, Dzoljic MR (1983) Differential epileptogenic potentials of selective μ and δ opiate receptor agonists, J. Neural. Transm., 57: 1-11

Haffmans J, De Kloet R, Dzoljic MR, (1984) Metabolic rate in different rat brain areas during seizures induced by a specific delta opiate receptor agonist, Brain Res., 302: 111-115

Henriksen SJ, Bloom FE, McCoy F, Ling N, Guillemin R (1978) β-Endorphin induces non convulsive limbic seizures, Proc. Natl. Sci., USA 75: 5221-5225

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

Mattiace LA, Negro-Vilar A, Poffwarg HP, Farber J (1981) The effect of REM sleep deprivation on substance P and somatostatin levels in discrete areas of the rat brain, Sleep Res., 10: 68

Oksenberg A, Sarne Y, Weissman AB, Luboshitzky R, Lavie P (1980) Plasma humoral endorphin profiles during human sleep, Sleep Res., 9: 94

Pasi A, Wettstein A, Foletta D, Gramsch C (1983) Naloxone effects and β-endorphin-like immunoreactivity plasma levels in idiopathic narcolepsy, In: Sleep 1982, WP Koella (ed), Karger, Basel, pp 246-249

Passouant P, Cadilhac J, Delange M (1965) Indications apportees par l'etude du sommeil de nuit sur physiopathologie des epilepsies, Int. J. Neurol., 5: 207-216

Patey G, Dela Baume S, Schwartz J-C, Gros C, Roque M-C, Fournie-Zaluski E, Soroca-Lucas E (1981) Selective protection of methionine enkephalin release from brain slices by enkephalinase inhibition, Science, 212: 1153-1155

Pratt KL, Mattson RH, Weikers NJ, Williams R (1968) EEG activation of epileptics following deprivation: A prospective study of 114 cases, Electroenceph. Clin. Neurophysiol., 24: 11-15

Shapiro C and Girdwood P (1981) Protein synthesis in the rats brain during sleep, Neuropharmacol., 20: 457-460

Siegel S (1956) Nonparametric statistics for behavioural science, McGraw-Hill,

New York

Snead OC, Bearden LJ (1982) The epileptogenic spectrum of opiate agonists, Neuropharmacol., 21: 1137-1144

Tortella FC, Cowan A, Adler MW (1981) Comparison of the anticonvulsant effects of opioid peptides and etorphine in rats after icv administration, Life Sci., 10: 1039-1045

Ukponmwan OE, Dzoljic MR (1983) Stages of vigilance and enkephalin-induced seizures, In: Sleep 1982, WP Koella (ed), Karger, Basel, pp 250-252

Urca G, Frenk H, Liebeskind JC, Taylor AN (1977) Morphine and enkephalin: analgesic and epileptic properties, Science, 197: 83-86

Verdeaux G, Marty R (1954) Action sur l'electroncephalogramme de substances pharmacodynamique d'interet clinique, Rev. Neurol., 91: 405-417

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

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 addicition (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 N_2O, 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 N_2O withdrawal in control, stressed and REMSD groups were compared using the Kx2 method (de Jong, 1963). Statistical signifcance was accepted at P-values of 0.05 or less.

RESULTS

Nitrous oxide withdrawal convulsions (n=20)

Mice exposed to N_2O showed withdrawal convulsions lasting about 2 h with highest incidence within 20 min after removal from N_2O. The mice convulsed when picked up by the tail following N_2O anaesthesia. These handling-induced convuslions (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 N_2O withdrawal convulsions (Fig A1). Stress did not inhibit N_2O withdrawal convuslions (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 N_2O develop very quickly a drug dependence which is characterized by withdrawal convulsions. This is demonstrated when mice are picked up by the tail following N_2O anaesthesia. This type of convulsions was conteracted 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 N_2O 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 N_2O tolerance and withdrawal excitation has been demonstrated. Acute exposure to N_2O 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 N_2O withdrawal convulsions (Manson et al., 1983). This suggests that N_2O stimulates an opioid-like dependence.
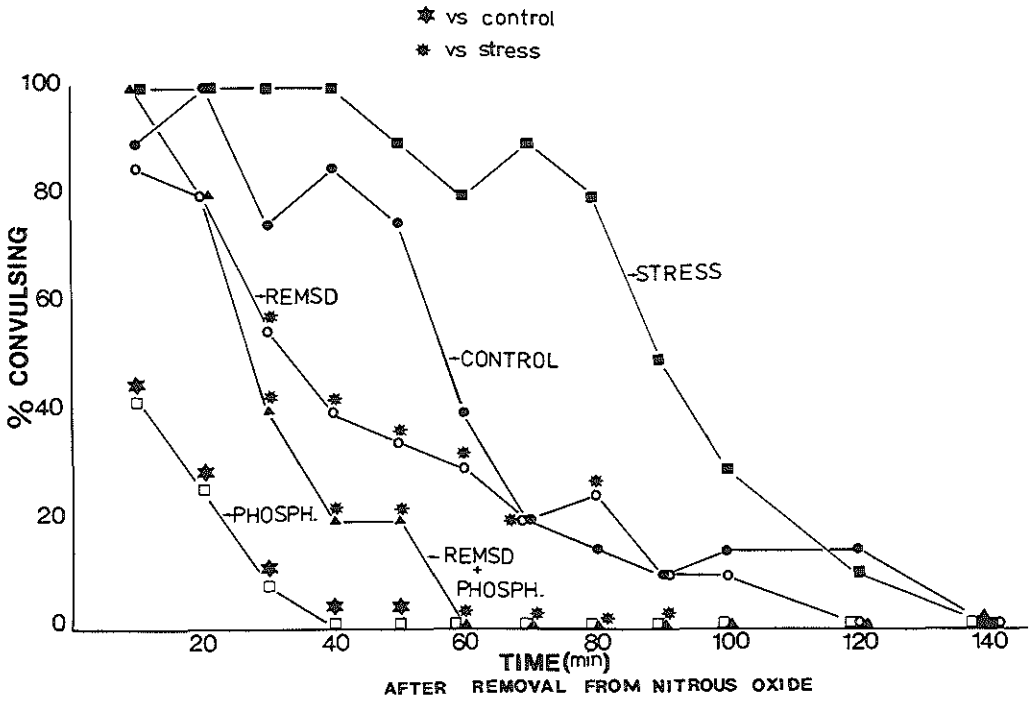
Figure A.1: The effect of REM sleep deprivation (REMSD) on nitrous oxide
withdrawal convulsions. Control consists of mice not subjected to any
treatment. (*) denotes statistical significance at p < 0.05 level compared
to control or stress. Note that 1) REMSD significantly reduced the frequency
and duration of nitrous oxide withdrawal convulsions; 2) phosphoramidon did
not alter the inhibitory action of REMSD on nitrous oxide withdrawal
convuslions. The effect of the enkephalinase inhibitor phosphramidon 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_2O 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_2O withdrawal convulsions proving that the inhibitory action of REMSD on N_2O 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_2O withdrawal syndrome, the results of this study indicate that REMSD might prove useful as new approach to the management of withdrawal neuronal hyperexcitability.

REFERENCES

Bobillier P, sakai T, Seguin S, Jouvet M (1974) The effect of sleep deprivation upon *in vivo* and *in vitro* incorporation of tritiated amino acids in brain proteins in the rat at three different age levels, J Neurochem., 22: 23-31

Bowman WC, Rand MJ (1980) Drugs used to relieve pain, In: Textbook of Pharmacology, Blackwell Scientific Publications, pp 16.1-16.17

Calvo JM, Alvarado R, Briones R, Paz C, Fernandez-Guardiola A (1982) Amygdaloid kindling during rapid eye movement (REMSD) sleep in cats, Neurosci. Lett., 29: 255-259

Cohen HB, Dement WC (1965) Sleep: changes in threshold to electroconvulsive shock in rats after deprivation of "paradoxical phase", Science, 150: 1318-1319

Drucker-Colin RR, Rojas-Ramirez JA (1976) New approaches to the study of the neurochemical basis of sleep and wakefulness. In: Advances in Psychobiology, Riesen AH, Thompson RF (eds), John Wiley, New York, pp. 1-34

Eckenhoff JE, Kneale DH, Dripps RD (1961) The incidence and etiology of post anaesthetic excitement (a clinical survey), Anaesthiol., 22: 667-673

Feinberg MP, Cochin J (1972) Inhibition of development of tolerance to morphine

by cycloheximide, Biochem. Pharmacol., 21: 3083-3085

Fishbein W, McGraugh JL, Swarz JR (1971) Retrograde amnesia: Electroconvulsive shock effect after termination of rapid eye movement sleep deprivation, Science, 172: 80-82

Goldstein DB, Pal N (1971) Alcohol dependence produced in mice by inhalation of ethanol grading the withdrawal reaction. Science, 172: 288-290

Harper MH, Winter PM, Johnson BH, Koblin DD, Eger EJ (1980) Withdrawal convulsions in mice following nitrous oxide, Anaesth. Analg., 59: 19-21

Herzlinger RA, Kandall SA, Vaughan HG (1977) Neonatal seizures with narcotic withdrawal, J. Pediatr., 91: 638-641

Hudgin RL, Charleson SE, Zimmerman M, Mumford R, Wood PL (1981) Enkephalinase: selective peptide inhibitors, Life Sci., 29: 2593-2601

Koblin DD, Dong DE, Eger EI (1979) Tolerance of mice to nitrous oxide, J Pharmacol. Exp. Ther., 211: 317-325

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

Manson HJ, Dyke G, Melling J, Gough M (1983) the effect of naloxone and morphine on convulsions in mice following withdrawal from nitrous oxide, Can. Anaesth. Soc. J., 30: 28-31

Mendelson JH, Mello NK (1978) Recent developments in chemotherapy of narcotic addiction. In: Basic Mechanisms underlying physical dependence upon alcohol, Ann. NY. Acad. Sci., New York, Kissin B (ed) 311:69-79

Passouant P (1977) Influence des etats de vigilance sur les epilepsies In: Sleep 1976, WP Koella, P Levin (eds), Karger, Basel, pp. 57-65

Panov A (1982) RNA and protein synthesis and contents of brain stem cells after sleep deprivation, Rev biol., 75: 95-99

Qock RM, Kouchich FJ, Tseng L-F (1985) Does nitrous oxide induce release of brain opioid peptides ?, Pharmacol., 30: 95-99

Smiths RA, Winter PN, Smith M, Eger EI II (1979) Tolerance to and dependence on inhalational anaesthetics, Anaesthesiol., 50: 505-509

Snead OE, Bearden LJ (1980) Anticonvulsants specific for petit mal, antagonize epileptogenic effect of leucine enkephalin, Science, 210: 1031-1033

CONCLUDING REMARKS AND SUMMARY

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 β-endorphin concentrations in brain nuclei, involved in vigilance state regulation (chapter 1, sections 1.2, 1.3.2i). Thus, concentrations of β-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 8). 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 μ-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 β-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 expose 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 gesecreteerde- 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 gesecreteerde opioid peptiden en exogeen toegediende opiaten en opioid peptiden. In dit onderzoek werden de volgende door opiaat/opioid geinduceerde 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 dagfluctuaties in enkefaline en β-endorfine concentraties in bepaalde hersenkernen die een rol spelen bij alertheidsregulering (hoofdstuk 1, sectie 1.2, 1.3.2i). Dienovereenkomstig zijn de concentraties van β-endorfine, de enkefalines en dynorfine 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 schokbewegingen van de kop, krabben (hoofdstuk 3) en overmatig poetsgedrag, dat normaal bij ratten aan het slapen vooraf gaat (hoofdstuk 2, sectie 1.3.2i en hoofdstuk 8). Het bleek dat

de opwekkende werking van fosforamidon werd verminderd door de opiaat 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, potentieel 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 spieren bij vrij bewegende ratten (hoofdstuk 4). Slaap, en in het bijzonder de REM slaap, kon de door enkefaline geinduceerde epileptiforme ontladingen 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 electrische schok kan eveneens verminderd worden door REMSD (hoofdstuk 1, sectie 1.5.2d.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 effektiviteit van opiaten en andere analgetische behandelingen kunnen

beinvloeden.

REM slaap deprivatie en monoamine-opioid 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 gesecreteerde 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 enkefalinerge transmissie, die zoals beschreven neurotransmissie bevorderend is, kan niet alleen van betekenis zijn voor het begrip van de fysiologie van pijnprikkeling 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 funktionele deficientie in het μ-opioid receptor system

De bevinding dat de analgesie die door een opiaat wordt geinduceerd en die het gevolg is van een stimulering van voornamelijk de μ-type opioid receptoren, geblokkeerd kan worden door REMSD, werd geinterpreteerd als een functionele insufficientie 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 gekarakteriseerd 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 antagonist naltrexon blokkeerde de door morfine geinduceerde akinesie/katalepsie in ratten die ongestoord konden slapen, maar niet de door morfine geinduceerde

toename in SVMA in dieren met REMSD (hoofdstuk 7). Naast de hierboven beschreven waarnemingen dat REMSD de door opioiden en opiaten geinduceerde analgesie en het akinesie/katalepsie syndroom kan blokkeren, bleek dat in dieren met REMSD het opioid geinduceerde gedrag, zoals de naltrexon-sensitieve "wet-dog-shakes" en het door een enkefalinase remmer geinduceerde 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 enkefalinase remming in REMSD dieren worden veroorzaakt door een functionele insufficientie van het μ-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 μ-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 enkefalinase 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 μ-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 verstoring van endogene opioid systemen als gevolg van deprivatie van REM slaap. REMSD verminderde bijvoorbeeld de concentratie van β-endorfine in de hypofyse, 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 insufficientie 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 respectivelijk 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 afhankelijkheids fenomenen oproepen. De convulsies als element van de onthoudingsverschijnselen bij muizen na lachgas inhalatie waren verminderd in REMSD 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 REMSD dieren. Ook is aangetoond dat REMSD 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 wél 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 REMSD de eiwitsynthese in ratte hersenen vermindert (hoofdstuk 1, sectie 1.5.2b, vii). Om deze reden wordt voorgesteld, dat het antagonistische effect van REMSD op opiaat en lachgas onthoudingsverschijnselen veroorzaakt zou kunnen worden door een afname van de eiwitsynthese bij dieren met REM slaap onthouding.

De waarneming dat REMSD opiaat onthoudingsverschijnselen kan verzwakken, veronderstelt het bestaan van een verband tussen REM slaap tekort en lichamelijke afhankelijkheid van opiaten. Het mechanisme waardoor REMSD onthoudingsverschijnselen kan verzwakken en of REMSD 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 opioïd 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 opioïd systeem.*

2) *Remming van monoamine oxidase B en/of een overmaat aan β-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 enkefalinase verzwakt de proconvulsieve werking van REMSD, hetgeen een betrokkenheid van opioid peptiden in de regulatie van neuronale prikkelbaarheid gesuggereert.*

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

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 opiaat onthoudingsverschijnselen, hetgeen doet vermoeden dat slaap deprivatie een aantrekkelijk hulpmiddel zou kunnen zijn bij verslavingsonderzoek.*

List of Publications

*Dzoljic MR, Ukponmwan OE, Baas AAG, vd Berg DT, Rupreht J. (1984) Enkephalinase inhibition and REM sleep deprivation inhibit the nitrous oxide withdrawal convulsions, In:Epilepsy, Sleep and Sleep Deprivation , Degen R and E Neidermeyer (eds) Elsevier, pp 305-310

*Dzoljic MR, Ukponmwan OE, Rupreht J, Haffmans J. (1985) The role of enkephalinergic system in sleep studied by an enkephalinase inhibitor, In: Sleep, Neurotransmitter and Neuromodulators, Mauquier et al (eds), New York, pp 251-263

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

Dzoljic MR, Haffmans J, Rupreht J, Ukponmwan OE (1985) Endogene opioid peptiden en pijn, IKR-Bulletin, 9: 12-14

Dzoljic MR, Ukponmwan OE (1985) The inhibitory effect of REM sleep deprivation (REMSD) on opiate withdrawal and quasi-morphine withdrawal syndrome (QMWS), In: Sleep 1984, Koella WP, Ruther E, Schultz H (eds), Gustav Fisher Verglag, Stuggart, New York, pp 397-399

Dzoljic MR, Radermaker B, Poel-Heisterkamp ALvd, Ukponmwan OE, Haffmans J, Enkephalinase inhibition supresses naloxone-induced jumping in morphine dependent mice, Arch Int Pharmacodyn (in press)

Grillo SA, Ukponmwan OE (1981) The effect of amphetamine and chlorpromazine on the electrolyte contents of the cerebrospinal fluid of the cat, Nig Journ Pharmac 12: 300-303

Haffmans J, Blankwater YJ, Ukponmwan OE, Zijlstra FJ, Vincent JE, Hespe W and Dzoljic MR. (1983) Correlation between the distribution of ^{3}-H-labelled enkephalin in rat brain and anatomical regions involved in enkephalin-induced seizures, Neuropharmacol 22: 1021-1028

Rupreht J, Ukponmwan OE, Admiraal PV and Dzoljic MR. (1983) Effect of phosphoramidon- a selective enkephalinase inhibitor on nociception and behaviour, Neurosci Lett 41: 331-335

Rupreht J, Ukponmwan OE, Dworacek B, Admiraal PV, Dzoljic MR. (1984) Enkephalinase inhibition prevented tolerance to nitrous oxide analgesia in rats, Acta Anaesthesiol Scand 28: 617-620

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 EJ Simon (eds) Kodanska LtD, Tokyo, pp 226-228

Ukponmwan OE, Poel-Heisterkamp ALvd, Haffmans J, Dzoljic MR. (1983) MAO-B inhibitor deprenyl and β-phenylethylamine potentiate D-Ala^2-Met-enkephalinamide-induced seizures, Naunyn-Schmied Arch Pharmacol 322: 38-41

**Ukponmwan OE, Dzoljic MR.(1983) Stages of vigilance and enkephalin-induced seizures, In:Sleep 1982, Koella WP (ed), Karger, Basel pp 250-252

**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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1) Faculty of Health Sciences, University of Ife, Nigeria

Courses: Neurobiology, Individual Behaviour and Psychophysiology, Neuromuscular Pharmacology.

M.Sc (Human Biol, 1978).

2) Division of Biocybernetics, Institute for Medical and Dental Engineering, Tokyo Medical and Dental University. Tokyo (1979/1980)(Sponsored by Japan International Co-operation Agency JICA): Sleep studies (supervision by Prof S. Inoue).

Certificate in Neurophysiology

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