ENDOGENOUS OPIOID PEPTIDES AND EPILEPSY

Endogene opioid peptiden en epilepsie

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INTRODUCTION

In recent years a large number of peptides, many of which were originally characterized in non-neural tissues, have been reported to be present in the central nervous system (CNS). The detection of these peptides within the CNS has raised many questions regarding their source and mechanism of action.

In view of the accumulating information, it seems that the function of the classical neurotransmitters in the CNS would be better clarified by elucidating the role of the brain neuropeptides.

The classification of the major categories of the main peptides, as listed below, is a some what arbitrary one as it is based on the first localization of a given peptide, while the opioid peptide family is given separately. For many of the peptides described in brain, their major functional role is still unknown. However, even before the major discoveries in the past decade, the opiates were known to possess selective and unique pharmacological properties. It was well known that opiates were effective in the treatment of pain and were useful as cough suppressants. They are also known to depress respiration and blood pressure and to exert an effect on behaviour, like euphoria, sedation and depression. The diversity/complexity of their properties suggests that like the catecholamines, endogenous opioids may have a basic, multisystem regulation essential to the maintenance of homeostasis and to the survival of the organism.

In the last years we have tried to reveal one of these peptidergic secrets and focussed our attention on the opioid peptides, more specifically in relation to the excitatory phenomena which they might induce after systemic or intraventricular administration.

Categories of mammalian brain peptides

"Hypothalamic-releasing hormones"
- Thyrotropin-releasing hormones
- Gonadotropin-releasing hormones
- Somatostatin
- Corticotropin-releasing hormones
- Growth hormone-releasing hormone

Neurohypophyseal hormones
- Vasopressin
- Oxytocin
- Neurophysin

Pituitary peptides
- Adrenocorticotropic hormone
- α-Melanocyt-stimulating hormone
- Prolactin
- Luteinizing hormone
- Growth hormone
- Thyrotropin

Invertebrate peptides
- Phenylalanyl methionyl argininyl phenylalanylamide (FMRF amide)
- Hydra head activator

Gastrointestinal peptides
- Vasoactive intestinal polypeptide
- Cholecystokinin
- Gastrin
- Substance P
- Neurotensin
- Insulin
- Glucagon
- Bombesin
- Secretin
- Somatostatin
- Motilin

Others
- Angiotensin II
- Bradykinin
- Carnosine
- Sleep peptide(s)
- Calcitocin
- Neuropeptide Yy

Opioid peptide family
- Leu-enkephalin
- Met-enkephalin
- α-endorphin
- β-endorphin
- γ-endorphin
- Dynorphin
- Peptide E
- α-neoendorphin
- β-neoendorphin

CHAPTER I.

OPIOID PEPTIDES

1. NOMENCLATURE

The term "opioid peptides" has usually been applied to "naturally occuring peptides with opiate-like biological properties" (Morley 1983). The various members of the family of the opioid peptides (for structural formulae of some agents, see Table 1) can be divided into five main groups:

1.1. *The enkephalins*

They usually include two naturally occurring pentapeptides, methionine-enkephalin and leucine-enkephalin (Hughes et al., 1975). In this manuscript the term "enkephalin" refers to these compounds but will also embrace synthetic enkephalin analogues like: (D-ala^2,D-leu^5)enkephalin (DADL), (D-ala^2,L-met^5)enkephalin (DALA) and (D-ser^2,L-Leu^5)enkephalyl-thr (DSTLE).

1.2. *The endorphins*

These refer to β-endorphin (Bradbury et al., 1976; Li and Chung, 1976) and the related α-, γ- and δ-endorphins (Ling et al., 1976; Verhoef et al., 1980). It should be noted that the term "endorphin" (endogenous morphine-like substances) and "opioid peptide" have often been used synonymously. To avoid confusion, a more restricted definition of endorphin, i.e. those opioid peptides that arise from β-lipotropin, has lately been recommended (Morley, 1983). In this manuscript the terms "endorphin" and "endorphinergic", unless stated otherwise, are used as a general reference to all opioid peptides.

1.3. *Peptides that arise or are presumed to arise, from enkephalin-precursors.*

This category includes three peptides arising from adrenal proenkephalin (Rossier et al., 1980), peptide E (Kilpatrick et al., 1981), dynorphin (Goldstein et al., 1981) and α- and β-neoendorphin (Kangawa et al., 1981; Minamino et al., 1981).

1.4. *Pronase resistant peptides in body fluids.*

β-casomorphin-5 and -7 in bovine milk (Henschen et al., 1979) and

anodynin in blood (Pert et al., 1976) are included in this category.

1.5. *Miscellaneous peptides whose opiate-like properties do not arise from a direct interaction with opiate or opiate-like receptors.*

An example is kyotorphin , which seems to act via release of enkephalins and inhibition of enkephalin degradation (Takagi et al., 1979).

In this manuscript we will deal mainly with the pharmacological/ electrophysiological properties of the first two groups, i.e. the enkephalins and endorphins.

Table 1. STRUCTURES OF OPIOID PEPTIDES

(Leu)enkephalin	Tyr-Gly-Gly-Phe-Leu
(Met)enkephalin	Tyr-Gly-Gly-Phe-Met
(Met)enkephalinyl-Arg-Phe	Tyr-Gly-Gly-Phe-Met-Arg-Phe
(Met)enkephalin-8	Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu
Peptide E	Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-Gly-Arg-Pro- 1 5 10 Glu-Trp-Trp-Met-Asp-Tyr-Gln-Lys-Arg-Tyr-Gly- 15 20 Gly-Phe-Leu
Dynorphin A (1-17)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys- 1 5 10 Leu-Lys-Trp-Asp-Asn-Gln 15 17
Dynorphin A (1-8)	1-8 sequence
Dynorphin B (1-13)	1-13 sequence
α-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys
β-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro
PH-8P	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile
β-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln- 1 5 10 Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile- 15 20 Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu 25 30 31
α-Endorphin	1-16 sequence
γ-Endorphin	1-17 sequence
δ-Endorphin	1-27 sequence
β-Casomorphin-7	Tyr-Pro-Phe-Pro-Gly-Pro-Ile
β-Casomorphin-5	Tyr-Pro-Phe-Pro-Gly
Kyotorphin	Tyr-Arg

2. BIOGENESIS AND DEGRADATION OF THE OPIOID PEPTIDES

The different classes of the opioid peptides - the enkephalins, the endorphins and the dynorphins - may function as neurotransmitters, neuromodulators or hormones. The receptor-mediated actions of these opioids show a considerable overlap, not surprisingly in view of their close structural relationships (Table 1). Thus in order to understand the roles of these peptides in physiological or pathological events, it is necessary to elucidate the mechanisms underlying and controlling their biogenesis, inactivation and release.

2.1. *Biosynthesis*

Peptide hormones are derived from the enzymatic cleavage of larger, and generally inactive prohormones which are synthesized under the direction of mRNA on membrane-bound polyribosomes. The nascent protein characteristically contains a 18-25 residue hydrophobic amino acid sequence at its N-terminus, termed the "signal sequence". The cleavage of this pre-prohormone in its entry into the Golgi apparatus yields the prohormone. Sequential cleavage of the prohormone then results in one or more biologically active peptides that are available for secretion (Marx, 1983).

The use of modern techniques, including DNA cloning, has revealed the total sequences of the following prohormones:

1. Proopiomelanocortin (POMC) (Smyth, 1983); 2. Proenkephalin (Noda et al., 1982); 3. Prodynorphin (Kakidani et al., 1982). These proteins provide the basis for three separate opioid peptide families.

2.1.1. Proopiomelanocortin (POMC)

POMC was the first of the precursors to be identified. The sequence of amino acids in the complete POMC molecule results after cleavage in at least seven active peptides including β-lipotropin (β-LPH), β-endorphin, adrenocorticotropic hormone (ACTH), and α-, β-, and γ-melanocyte stimulating hormones (α-, β-, and γ-MSH) (Fig. 1a) (Marx, 1983). The individual peptides in the precursor are bound on both ends by pairs of amino acid residues, usually containing one lysine and one arginine residue. These pairs are the sites,

recognized and cut by the protein-splitting enzymes (this chapter), that release the active opioids.

Although Met-enkephalin is the 61-65 sequence of β-endorphin, it is very unlikely that Met-enkephalin is formed in the brain by degradation of β-endorphin (Watson et al., 1977).

The major places of synthesis of POMC are the intermediate and anterior lobes of the pituitary, the hypothalamus as well as some peripheral tissues including the placenta, gastrointestinal tract and lungs (Watson et al., 1977).

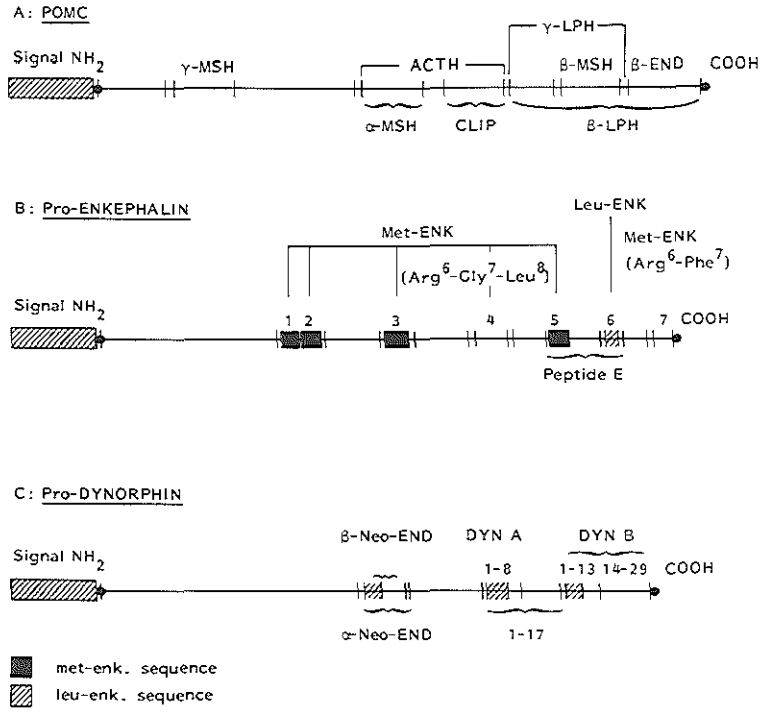

Fig.1. Schematic representation of the precursor structures of the three opioid peptide families (modified after Akil et al., 1984).

2.1.2. Proenkephalin A

Different groups of investigators (Noda et al., 1982; Gubler et al., 1982) cloned the gene coding for the polypeptide now called proenkephalin A. The proenkephalin A and not POMC is the precursor of Met- and Leu-enkephalin and also of some larger enkephalin-containing peptides, some of them more active than the enkephalins themselves (Gubler et al., 1982).

Proenkephalin A contains six copies of the Met-enkephalin sequence (Tyr-Gly-Gly-Phe-Met) and one of Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) (Fig. 1b).

2.1.3. Prodynorphin

Kakidani et al. (1982) have identified a sequence from a DNA clone of porcine hypothalamic RNA which encodes the complete sequences of dynorphin and α-neoendorphin but which contains no Met-enkephalin sequence (Fig. 1c). Analysis of the DNA structure indicates that the precursor protein consists of 256 amino acids including a signal sequence.

2.2. *Metabolism*

The final products produced and stored within a given neuron depend not only on the genetic code for the precursor, but also on the program that directs the enzymes to process the precursor in certain ways. The action of cutting specific peptides out of the precursor protein, and modifying these products by acetylation, amidation, phosphorylation, methylation, glycosylation or further cleavage is part of the biological program of a given cell. These events are capable of determining the exact mix of peptides in a given neuron. They appear to vary from one tissue to the next, in spite of the existence of a common gene for the precursor. Since these changes result in peptides of widely differing potencies, pharmacological profiles and receptor selectivities, they are critical in determining function and may constitute a critical step in the regulation and homeostasis of a given opioid system in a particular region in the CNS.

Detailed studies on the biosynthesis of these peptides are lacking,

although in the last years some progress has been made in the nature of "post-translational" proteolytic enzymes (Docherty and Steiner, 1982; Lindberg et al., 1982; Loh and Gainer, 1982). The variable processing of these multivalent prohormones would be a powerful adaptive mechanism.

2.3. *Release of enkephalins and endorphins.*

2.3.1. Stress- and pain associated release.

There is a clear association between stressful conditions and the release of opioid peptides. Injury stress causes the parallel release via hypothalamic corticotropin releasing factor (CRF) of β-endorphin and corticotropin from the anterior pituitary (Guillemin et al., 1977). Various forms of conditioning and stress are associated with analgesic states, which are either partially or fully reversed by naloxone, an opiate antagonist (Madden et al., 1977; Watkins et al., 1982).

Interestingly, electroconvulsive therapy, which can cause postictal analgesia and catalepsy (Urca et al., 1981), also results in increased enkephalin levels in hypothalamus and limbic brain areas (Hong et al., 1979). No such changes were seen in β-endorphin levels. Selective brain stimulation also leads to analgesia and increased opioid peptide release into the cerebral spinal fluid (CSF) (Akil et al., 1978a,b).

Electroacupuncture and noxious stimuli result in increased opioid peptide levels, as well (Sjölund et al., 1977; Cesselin et al., 1980).

The mechanisms underlying the release of opioid peptides during stressful or painful conditions still remain to be resolved. Opioid release may be diffuse and involve several different specific neuronal pathways.

2.3.2. Steroid modulated release.

Steroids are the best known endogenous regulators of peptide hormone synthesis and release. The release and synthesis of adrenocorticotropic hormone/endorphin peptides is subject to corticosteroid feedback inhibition in the anterior pituitary but not

in the intermediate lobe (Bloom et al., 1977). Corticosterone acts to suppress both mRNA synthesis and CRF-modulated release.

Factors controlling gonadotropin release might possibly be involved in opioid peptide release as well. Oestrogen treatment reduces the β-endorphin levels in the hypothalamus, thalamus and mid-brain, while an increased β-endorphin level was demonstrated during pregnancy (Wardlaw et al., 1982).

2.3.3. Opioid peptide releasing agents.

Some drug effects have been interpreted in terms of an induction of opioid peptide release. For example, the analgesic properties of nitrous oxide (Berkowitz et al., 1977) and of intracerebrally injected substance P (Stewart et al., 1976; Naranjo et al., 1982) are blocked or partially reversed by naloxone. It has also been suggested that the analgesic action of morphine may be due to endogenous opioid peptide release (Fu and Dewey, 1979; Schlen and Bentley, 1980).

Finally, certain inhibitors of the enkephalin breakdown will enhance enkephalin release and content. These effects are discussed below.

2.4. *Drug modulated concentration of enkephalins and endorphins.*

Reports of the effects on opiates have been contradictory. Acute morphine administration has been claimed to increase (Gros et al., 1978), or not to affect brain Met-enkephalin content (Wesche et al., 1977). Chronic morphine treatment apparently has no effect on brain enkephalin levels (Wesche et al., 1977) but decreases β-endorphin content and synthesis in the anterior pituitary (Przewlocki et al., 1979; Höllt et al., 1981). An opposite effect is seen with dynorphin. The pituitary level of this peptide is increased by chronic morphine treatment (Herz et al., 1982). These effects of morphine may be mediated via stimulation of corticosteroid release (Hughes, 1983).

2.5. *Inactivation of the opioid peptides.*

Since the activity of the peptides depends on the rate of degradation, it has been of interest to determine what happens to them after they

have been released. Most research in this field has concerned enkephalins, which are extremely rapidly degraded by enzymes in various tissue homogenates or in blood (Hambrook et al., 1976; Dupont et al., 1977). β-endorphin is considerably more stable (Miller et al., 1977). This serves to explain, at least partly, the greatly increased antinociceptive potency of β-endorphin over Met-enkephalin following intraventricular (ivt) injection (Belluzzi et al., 1976; Chang et al., 1976).

A number of proteolytic enzymes are thought to be involved in the inactivation of enkephalin. This breakdown can occur at several sites of the peptide molecule as follows:

1. The soluble *aminopeptidases* degrade enkephalins at the Tyr^1-Gly^2 peptide bond. The hydrolysis product is tyrosine (Hambrook et al., 1976; Meek et al., 1977; Vogel and Altstein, 1980). The best inhibitor presently available is bestatin.

2. *Dipeptidyl-aminopeptidase* ("enkephalinase B") hydrolyses the Gly^2-Gly^3 amide bond of enkephalin. No selective inhibitor for this enzyme is available as yet (Gorenstein and Snyder, 1979).

3. *Angiotensin-converting enzyme* (ACE) hydrolyses the enkephalin at the level of the Gly^3-Phe^4 bond (Erdos et al., 1978). This enzyme can be inhibited by captopril.

4. *Dipeptidyl-carboxypeptidase* termed "enkephalinase" (Malfroy et al., 1978) or "enkephalinase A" (Gorenstein and Snyder, 1979), which cleaves the enkephalin also at the Gly^3-Phe^4 bond, can be inhibited by phosphoramidon or thiorphan.

Recently, kelatorphan has been described as the first complete inhibitor of enkephalin metabolism. This substance inhibits the activity of "enkephalinase A", dipeptidylaminopeptidase and

aminopeptidase (Fournie-Zaluski et al., 1984).

Peptidases generally display a limited chemical specificity. A given peptide can be hydrolyzed by different enzymes and a given peptidase can act on several opioid peptides. This raises the question as to what extent these peptidases are involved in the physiological degradation of the endogenous enkephalins.

In homogenates of the guinea pig ileum (GPI) or in the brain, enkephalin appears to be degraded mainly by removal of the N-terminal tyrosine residue, although other minor modes of degradation might also exist (Hambrook et al., 1976). It is therefore not surprising that enkephalin analogues, containing a D-amino acid in the second position instead of a glycine are considerably more stable than the naturally occuring enkephalins (Hambrook et al., 1976).

The specificity or selectivity of the enzyme(s) remain(s) to be determined, particularly since specificity may be due to substrate specificity or anatomical location. The anatomical distribution, perhaps associated with limited substrate specificity, may well constitute a "functional" specificity.

Attempts to find inhibitors of the different enzymes, trying to understand the degradation of the enkephalins, resulted in the synthesis of the different enkephalinase inhibitors. All enkephalinase or aminopeptidase inhibitors tested so far enhance the analgesic potency of opioid peptides (Roques et al., 1980; Chipkin et al., 1982; Chaillet et al., 1983). The enkephalinase inhibitors thiorphan and phosphoramidon not only enhance the analgesic action of the opioids but have also analgesic properties when injected alone (Roques et al., 1980; Rupreht et al., 1983). These data indicate that enkephalinase inhibitors can potentiate an endogenous ongoing enkephalinergic activity in the CNS, which results in the analgesic effect.

Little is known about the endorphin breakdown. β-endorphin can be degraded by an enzyme in synaptosomal membranes (Austen et al., 1977). The products formed depend on the pH at which the experiment is performed and also whether or not bacitracin, a breakdown inhibitor (Miller et al., 1977) is present. Depending on the conditions

α-endorphin, γ-endorphin or Met-enkephalin can be formed. However, the nature of the various enzymes involved in these cleavages still has to be determined.

Little is known about the metabolism or inactivation of dynorphin related peptides.

It seems likely that the main method of termination of action of opioid peptides in vivo will prove to be by degradation, as with acetylcholine, rather than by presynaptic reuptake, as with the biogenic amines. At least, peptidase and enkephalinase A are essential for the biodegradation of enkephalins, in vivo.

3. CENTRAL DISTRIBUTION OF THE OPIOID PEPTIDES

Several neurochemical and immunochemical techniques, i.e. radioimmunoassay (r.i.a.) and immunohistochemistry, have been applied for the study of the distribution and cellular localization of opioids. Most studies tend to agree on the differential distribution of the opioids.

The major site of production of the peptides, derived from POMC, is the pituitary (Bloom et al., 1977; Pelletier et al., 1977). A small percentage of the anterior lobe cells produce ACTH, β-LPH and β-endorphin. In most species, the pituitary contains an intermediate lobe. All cells of this lobe produce β-endorphin/ACTH related peptides, the main end points being β-endorphin and α-MSH. The main cell group, producing β-endorphin/ACTH related peptides, is found in the arcuate nucleus of the medial basal hypothalamus and projects its fibers very widely, including many areas of the limbic system and brain stem (Bloom et al., 1978; Watson and Akil, 1979,1980).

The second major opioid neuronal pathway (proenkephalin A) is widespread and includes endocrine and CNS distributions (Elde et al., 1976; Hökfelt et al., 1977; Sar et al., 1978; Watson, 1982). The concentration of Met-enkephalin is invariably found to be several fold greater than that of Leu-enkephalin (Yang et al., 1978; Goldstein and Ghazarossian, 1980). Possible explanations can be found in the fact that the precursor molecule for the enkephalins contains both peptides in a given proportion (see this

chapter). In brain, peptides formed from pro-enkephalin are found at every level of the neuraxis, including cells in the cortex all the way to cells in the spinal cord.

Finally, the prodynorphin is found in the posterior pituitary and brain (Khachaturian et al., 1982; Watson, 1982, 1983). It has been found in several hypothalamic cell groups and brain stem, as well. Some pathways are known (supraoptic nucleus to posterior pituitary) (Akil et al., 1984) whereas others are still to be studied.

These three opioid neural pathways, will now be described in more detail. Since the majority of studies have dealt with the regional distribution of enkephalins, they will be considered first.

3.1. *Enkephalin-immunoreactive neurons.*

Most observations have been made in the CNS of experimental animals. However, a very similar distribution of enkephalin-immunoreactive neurons has been demonstrated by Cuello (1978) in human CNS. A notable observation is that enkephalin-immunoreactive neurons are located in high concentrations in areas related to pain and analgesia in the mammalian nervous system (Hökfelt et al., 1977; Simantov et al., 1977). Although minor discrepancies are found between the results of several authors the following pattern of enkephalinergic distribution emerges (Fig. 2):

Telencephalon. Numerous cell bodies occur in the n.caudate putamen, n.accumbens and n.interstitialis of the stria terminalis. Occasional cell bodies can also be found in the olfactory- and frontal cortex. Furthermore, enkephalin-immunoreactivity was found in the limbic system, with significant amounts of the enkephalins in the hippocampus, more specifically in the pyramidal neurons (Gall et al., 1981) and fibers in the CA_2 (Finley et al., 1981). Both groups found enkephalin-immunoreactivity in the granule cells of the dentate gyrus. The most important enkephalinergic fiber system of the entire brain is found in the globus pallidus, which seems to receive an enormously heavy input of enkephalinergic fibers, while enkephalin-immunoreactive cell bodies are restricted to the neighbouring caudate putamen (Cuello, 1983). Cell bodies and fibers

can also be detected in the n.amygdaloides medialis and centralis. These neurons appear to be the site of origin of some of the enkephalinergic fibers terminating in the n.interstitialis of the stria terminalis (Uhl et al., 1978).

Diencephalon. The hypothalamus seems to display a large number of enkephalinergic cell bodies. Intens enkephalin-immunoreactivity was found in the perifornical region. Cell bodies directly associated with endocrine functions (n.arcuatus, supraopticus and paraventricularis) display enkephalin-immunoreactivity (Sar et al., 1978).

Brain stem and cerebellum. Relatively few enkephalin-immunoreactive cell bodies and enkephalinergic fibers are found in the substantia grisea, while high concentrations are found in the parabrachial nuclei and dorsal tegmental nucleus. Very low concentration of enkephalin can be detected in the cerebellum.

Spinal cord. The highest concentration of enkephalin-immunoreactive fibers is present in the most superficial layers of the dorsal horn. An intense fiber network is also observed in the dorsolateral funiculus. Scattered fibers are seen all over the substantia grisea and are particularly distinctive around the motorneurons (Cuello, 1983).

3.2. *β-endorphin immunoreactive neurons.*

The first clear evidence for distinctive and separate populations of β-endorphin- and enkephalin-containing neurons comes from the work of Bloom et al. (1978). β-endorphin-containing elements (Fig.2) are highly concentrated in the hypothalamus. According to Bloom et al. (1978) the cell bodies of these neurons are clustered in the arcuate nucleus and tuberal region. The fiber system originating from these neurons is distributed rostrally in the region of the anterior commisure extending into the lateral septum and n.accumbens. The loose fiber system of the periventricular regions of the hypothalamus appears to continue dorsally and terminate in a number of brain stem structures prominently in the periaqueductal grey matter and the locus coeruleus. Bloom et al. (1978) also found immunoreactive fibers in the olfactory cortex and hippocampus.

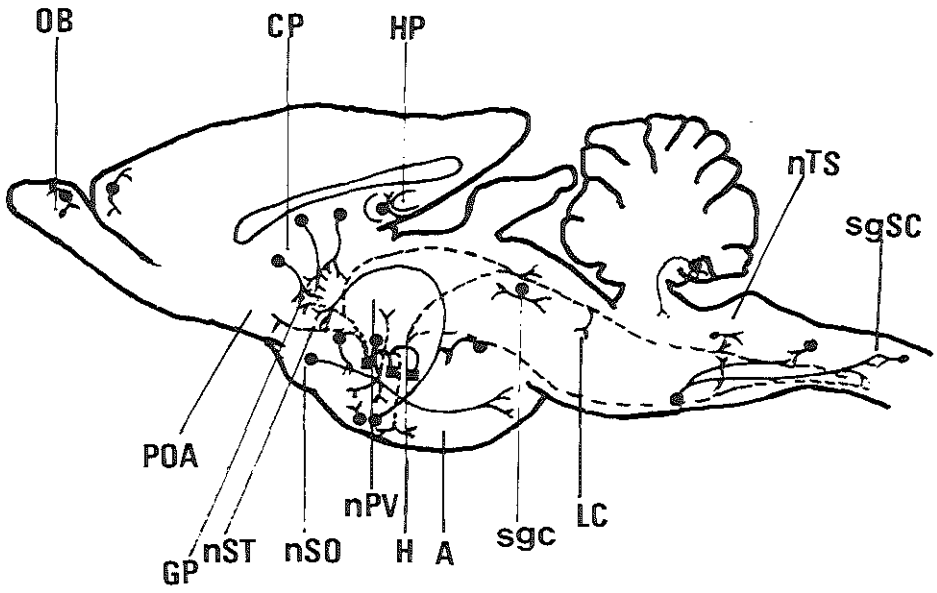

Fig.2. Saggital view of the rat brain representing the distribution of the major neuronal systems containing enkephalin (● ——) and endorphin (■ ---) immunoreactive neurones. OB: olfactory bulb; CP: caudatus putamen; HP: hippocampus; POA: preoptic area; GP: Globus pallidus; nST: nucleus interstitialis of the stria terminalis; nSO: nucleus supraopticus; nPV: nucleus paraventricularis; H: hypothalamus; A: amygdala; sgc: substantia grisea centralis of the mid-brain; LC: locus coeruleus; nTS: nucleus tractus solitarius; sgSC: substantia gelatinosa of the spinal cord;(modified after Cuello, 1983).

3.3. *Dynorphin-immunoreactive neurons.*

It has been pointed out that dynorphin-immunoreactive neurons belong to a subset of opioid-containing neurons (Watson et al., 1981). In fact some of the immunoreactivity attributed to the enkephalins could be due to the presence of dynorphin, as many antibodies against the enkephalins can potentially cross-react with dynorphin-immunoreactive material, mainly at the high concentrations used in

immunohistochemistry. This seems to be true for the enkephalin-immunoreactivity present in some neurosecretory neurons of the hypothalamus (Watson et al., 1982). A clear distinction between these opioid immunoreactive neurons has still to be made.

4. OPIOID PEPTIDES AND THE BLOOD BRAIN BARRIER.

An insignificant permeability through the blood-brain barrier after systemic administration of opioids would appear consistent with the view that the blood-brain barrier, which is composed of cerebrovascular endothelial cells that are connected by tight junctions, is essentially impermeable to water-soluble non-electrolytes, electrolytes, peptides and proteins (Rapoport, 1976).

Because of their limited cerebrovascular permeability, it has been suggested that circulating peptides enter the brain via the chorioid plexus and cerebrospinal fluid (Cornford et al., 1978), or, if produced in the pituitary, through routes such as the pituitary portal system (Klee, 1977). In contrast, some investigators have reported that peptides are significantly permeable at the blood-brain barrier (Kastin et al., 1976; Voightlander and Lewis, 1978), and that lipid solubility may play a role in the peptide transfer through the blood-brain barrier (Rapoport et al., 1980).

These investigators also demonstrated that some synthetic analogues of natural opioid peptides have a moderate cerebrovascular permeability, at least sufficient to produce significant brain uptake. There may be little uptake after a bolus injection if the peptide disappears rapidly from plasma, or if binding to plasma protein is marked. However, the fact that opiates, administered systemically, exert central effects (Yamashiro and Tseng, 1977) suggests a significant cerebrovascular permeability (Rapoport et al., 1980). This permeability indicates, furthermore, that feedback may operate between circulating peptides that have potential central effects, and brain sites that regulate their release into circulation (DeWied, 1977; Klee, 1977).

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CHAPTER II.

OPIATE RECEPTORS

1. DEMONSTRATION AND PROPERTIES OF THE OPIATE RECEPTORS.

The direct demonstration of specific saturable binding of opiates to nervous tissue was reported simultaneously by Pert and Snyder (1973), Simon et al. (1973) and Terenius (1973). The ability to assay specific opiate binding sites directly with labelled opiates and opiate antagonists of high specific activity has permitted us to learn a great deal about these sites and to provide evidence that they are the pharmacologically relevant opiate receptors.

Receptors are defined as specific sites to which a drug must bind in order to trigger biochemical or biophysical steps that result in the observed pharmacological response (Simon, 1982).

Opiate binding sites, present in the CNS and in some innervations of peripheral organs, are tightly bound to cell membranes in the vicinity of synapses. They have been found in all vertebrates so far examined and in some invertebrates as well (Stefano et al., 1980).

The opiate binding is saturable, reversible and stereospecific. Only the active enantiomer of an opiate binds with high affinity (Simon, 1982). The most convincing evidence for pharmacologically relevant opiate receptors, comes from the excellent correlation between binding affinities and pharmacological activity of opiates that vary in potency (Stahl et al., 1977; Creese and Snyder, 1975). Opiate binding is very sensitive to a variety of proteolytic enzymes and reagents that react with functional groups and amino acids of the protein. In particular, sulfhydryl reagents have been studied in detail and all such reagents have been found to inhibit specific opiate binding. Evidently, one or more proteins containing a SH group participate in the binding process. The fact that opiate receptors are highly sensitive to some phospholipases, as well as to low concentrations of detergents suggest that phospholipids also play a role, possibly holding the receptor site in the active conformation (Pasternak and Snyder, 1973).

A very specific effect on opiate binding is exerted by sodium ions and, to a lesser extent, by lithium ions. All other cations, including the closely related alkali metals, tend to inhibit the binding of all ligands to the opiate receptor in a dose-related manner (Pert et al., 1973; Simon et al., 1975). Accumulating evidence suggests that Na^+ acts as an allosteric effector, shifting the equilibrium towards a conformer of the receptor that binds antagonists with greater affinity than agonists (Simon et al., 1975; Simon and Groth, 1975).

2. MULTIPLE OPIATE RECEPTORS.

On present evidence it appears that there are in the central nervous system three independent peptidergic systems (Chapter I).
The question arises whether or not these different opioid peptides interact with one and the same receptor or whether there are several receptors subserving different physiological functions. The latter is specially important since the synthesis of highly specific ligands for one receptor class would then become possible and could provide drugs of clinical importance, such as analgesics with low addiction potential. The existence of several endogenous opiate receptor ligands and the finding that multiple receptors exist for classical neurotransmitters such as acetylcholine and catecholamines stimulated the examination of the possible existence of two or more opiate receptors.
Pharmacological differences between morphine and some synthetic analogues observed by Martin et al. (1976) in chronic spinal dog preparations suggested the existence of three types of receptors, which they named μ for morphine, κ for ketocyclazocine and σ for SKF 10047 (N-allyl norcyclazocine). After the discovery of the enkephalins Kosterlitz's group found a paradox in the potency of opiates and enkephalins in two bioassay systems. They concluded that two different receptor types predominate in the two tissues: A receptor in the guinea pig ileum that prefers morphine and its congeners, which they called the μ receptor, according to Martin and coworkers, and one that prefers enkephalins in the mouse vas deferens, which they called the δ opiate receptor (Lord et al., 1977). Similar or identical receptor heterogenity has been found in rodent and human brain

(Chang and Cuatrecasas, 1979; Bonnet et al., 1981).
The exact physiological significance of these multiple receptors is not yet fully understood but Wolozin and Pasternak (1981) suggest that the μ- and δ-receptors may correspond to high- and low-affinity receptors, respectively, as determined by (^{3}H)naloxone binding. Even more, differential tolerance of the mouse vas deferens to μ- and δ-specific drugs strongly supports the pharmacological relevance of the two receptors (Schulz et al., 1980).
However, it is still not clear if we are dealing with two distinct receptors or with an opioid receptor complex, in which the enkephalin and morphine receptor are allosterically coupled (Rothman and Westfall, 1982).
Many opiate drugs interact at multiple receptor sites. Thus the constellation of neuropharmacological actions of a particular opioid ligand may reflect its various potencies at a combination of μ, δ, κ and σ receptors. However, some peripheral and pharmacological effects have been ascribed to the different opiate receptor subtypes. For example, interactions with the μ receptor produce analgesia (Urca et al., 1977) miosis, bradycardia and hypothermia (Martin et al., 1976), whereas the δ opiate receptor can be held responsible for epileptic (Urca et al., 1977), sedative (Martin et al., 1976) and behavioural (Urca et al., 1977; Stein and Belluzzi, 1979) effects. Ketocyclazocine-like opiates produce ataxic and sedative effects and pupillary constriction via interaction with κ-receptors (Martin et al., 1976), while SKF 10047 and related opiates produce stimulant and psychomimetic effects after activation of σ-receptors (Holltzman, 1979).

3. INTERACTION WITH ENDOGENOUS LIGANDS.

In Chapter I we described three different opioid systems. It is important to consider the activity at the different binding sites of the possible fragments of these precorsors.

3.1. *POMC-derived.*

β-endorphin is equipotent in displacing the binding of tritiated μ- or δ-ligands (Kosterlitz et al., 1982). Furthermore, it does not interact with the κ-receptor, the only opiate receptors present in the

rabbit vas deferens (Oka et al., 1981), even after the inhibition of peptidase activity (McKnight et al., 1982).

3.2. *Pro-enkephalin-derived*

As already mentioned (Met^5)enkephalin and (Leu^5)enkephalin are present in this precursor. Both, and particularly (Leu^5)enkephalin, have a higher affinity for the δ- than for the μ-binding site and are almost inactive at the κ-binding site.

In binding assays, (Met^5)enkephalyl-Arg^6-Phe^7 (Stern et al., 1979) and (Met^5)enkephalyl-Arg^6-Phe^7-Leu^8 (Kilpatrick et al., 1981) do not distinguish between μ- and δ-binding sites, however, the latter one is five times more potent. Both peptides have low potencies at the κ-binding site (Magnan et al., 1982).

3.3. *Prodynorphin-derived.*

The heptadecapeptide dynorphin, dynorphin$_{1-13}$ and dynorphin$_{1-13}$-amide interact preferentially with the κ-binding site (Chavkin et al., 1982; Corbett et al., 1982).

Analysis of the binding and pharmacological profiles of the C-terminus extensions of (Leu^5)enkephalin, derived from prodynorphin, has shown that the shorter peptides exhibit different patterns of selectivity (Corbett et al., 1982). In binding assays, any extension of (Leu^5)enkephalin results in a loss of δ-selectivity, which is characteristic for the pentapeptide (Paterson et al., 1983) and a progressive increase in agonist potency at the κ-binding site (Corbett et al., 1982).

The fact that the antagonist Mr 2266, which interacts with μ- and κ-opiate receptors, is more potent than the μ antagonist, naloxone, against α- and β-neoendorphin, supports the view that these peptides interact with the κ-receptor as well (Oka et al., 1982).

4. DISTRIBUTION OF OPIOID RECEPTORS IN THE BRAIN.

4.1. *Regional distribution of opioid receptor binding.*

Advances in technology of receptor autoradiography allowed detailed histochemical mapping of opioid receptors in rodent and primate brain (Atweh and Kuhar, 1977 a,b,c; Pearson et al., 1980; Wamsley et al.,

1982). Except for minor differences, the distribution of receptors is very similar in the different species, studied.

Although opiate receptors are widely distributed in the brain, they can be observed most often in association with three major systems: 1. Sensory system; 2. Limbic system; 3. Neuroendocrine system.

4.1.1. Opiate receptors associated with sensory systems.

A high density of opioid receptors is found in the spinal cord and the brain-stem in association with afferent systems. In the spinal cord, generally higher receptor densities are found in the grey matter compared to the white matter. High receptor density can be observed in the dorsal horn and specifically in the marginal cell zone and in the dorsal layer of the substantia gelatinosa (s.g.). In the upper cervical cord and in the lower medulla, these receptors seem to be continuous within the s.g. of the descending spinal nucleus of the trigeminal nerve (Atweh and Kuhar, 1977a).

The s.g. of the spinal cord and the trigeminal system is an important structure for the transmission of sensory information, such as temperature and pain, to the CNS. Opiate receptors in the s.g. are strategically located to modulate this transmission of nociceptive sensory information. The importance of those receptors in explaining the analgesic properties of opiates, is supported by many physiological and clinical experiments (McClane and Martin, 1971; Krivoy et al., 1973; Calvillo et al., 1974; Yaksh and Rudy, 1976).

The other mechanism by which opiates exert their analgesic properties involves the higher centres in the mid-brain and thalamus. The periaqueductal grey matter in the mid-brain is probably an important area for the control of pain (Mayer and Liebeskind, 1974; Richardson and Akil, 1977) and is found to be highly enriched with opiate receptors (Kuhar et al., 1973; Atweh and Kuhar 1977a).

Certain sensory nuclei of the thalamus are also found to be enriched with opioid receptors (Atweh and Kuhar, 1977b), specifically the periventricular nuclei, that are known to be important in the

processing of nociceptive signals (Pert and Yaksh, 1974). Opioid receptors are found as well in the afferent fibers of the vagus nerve and their terminals within the n.tractus solitarius and n.commissuralis (Atweh and Kuhar, 1977a; Wamsley et al., 1982). Those receptors may underlie the mechanisms by which opiates affect certain autonomic functions such as blood pressure control (Fennessy and Rattray, 1974), respiration (Morin-Surun et al., 1984a,b) and gastric motility (Yamaguchi, 1974).

4.1.2. Opioid receptors associated with the limbic system .

In the forebrain the highest concentration of opiate receptors is associated with the amygdala and basal ganglia. The n.amygdaloides corticalis, medialis and basalis, show the highest receptor concentration. This is most pronounced in the cortical layer (Atweh and Kuhar, 1977c). The basal ganglia also show a widespread distribution of opioid receptors, specially in the striatum, where they are located in clusters of high density and in a streak localized to the superior and lateral boundaries of the striatum below the corpus callosum (subcollosal streak). There is only moderate and diffuse enrichment of opiate receptors in the globus pallidus. Patches of receptors are also seen in the n.accumbens, but their density is not as high as those seen in the caudate (Atweh and Kuhar, 1977c; Wamsley et al., 1982). Since these structures play an important role in behaviour and mood control, the interconnections, specially in the striatum, between the enkepalinergic system and the dopaminergic system, has received a lot of attention . It is suggested that some of the opioid receptors are located on the dopaminergic terminals, presynaptically, within the striatum (Pollard et al., 1978; Murrin et al., 1980). Indeed, pharmacological studies demonstrate that opiates do inhibit dopamine release in the striatum (Loh et al., 1976).

Electrical stimulation of the caudate (Lineberry and Vierck, 1975) and direct injection of morphine in the rat striatum (Jurna and Heinz, 1979) have been shown to have antinociceptive effects. The striatal opiate receptors may contribute to these analgesic effects.

Other areas in the forebrain that possess opiate receptors are the cortex and the hippocampus, specially the presubicular area (Atweh and Kuhar, 1977c). In vitro labelling of opioid receptors in tissue sections of rat and monkey brain, followed by autoradiography, however, revealed variable receptor densities in the hippocampus and cortex (Goodman et al., 1980; Duka et al., 1981).

It is generally assumed that the phylogenetically older parts of the cortex, such as the hippocampus and the cingulate gyrus, show higher densities of opiate receptors (Meibach and Maayani, 1980; Wamsley et al., 1982).

4.1.3. Opioid receptors associated with neuroendocrine systems.

It is well known that opiates exert some effect on both posterior and anterior pituitary function (Chapter I). Their effect on the posterior pituitary is probably mediated by those receptors present in the posterior lobe (Wamsley et al., 1982). Very high receptor density is seen in the infundibulum of the hypothalamus. These receptors seem to be particularly interesting, since many of the hypothalamic releasing factors that control the secretory functions of the anterior pituitary are released from neurosecretory nerve endings into the capillary bed of the infundibulum. There is some evidence that opiates do inhibit the release of hypothalamic releasing or inhibitory factors, such as thyrotropin-releasing hormone, somatostatin (which regulates the release of growth hormone) and dopamine (which inhibits the release of prolactin) (Meites, 1980). Hypothalamic nuclei are also enriched with opiate receptors (Kuhar et al., 1973; Atweh and Kuhar, 1977b). In addition to the neuroendocrine systems, other vegetative functions of the hypothalamus, such as temperature regulation and feeding behaviour, may also be affected by opiates (Chapter III).

High density of opiate receptors is found in the rat mid-brain in association with the visual system (Atweh and Kuhar, 1977b).

4.2. *Differentiation of μ and δ opiate receptor distribution.*

The localization of μ and δ opiate receptors in the cerebral cortex is strikingly different. μ receptors are localized to discrete clusters

and a subcollosal streak (Young and Kuhar, 1979; Goodman et al., 1980) in the caudate putamen, whereas δ receptors are more diffusely distributed.

The most striking difference in localization between the two receptor types occurs in the thalamus and hypothalamus. The dorsomedial and ventral thalamus, as well as the hypothalamus, contain a very high concentration of μ receptors and extremely few δ receptors (Goodman et al., 1980). The paraventricular nuclei of the hypothalamus are enriched with δ receptors (Duka et al., 1981).

δ receptors are distributed diffusely in the hippocampus, while μ receptors are highly localized in the pyramidal cell layer.

The olfactory tubercle, n.accumbens and amygdala have high densities of δ opiate receptors and relatively few μ sites. The septum contains δ opiate receptors as well (Duka et al., 1981). Areas with the highest concentration of μ receptors include the brain stem, periaqueductal grey and n.interpeduncular (Goodman et al., 1980; Duka et al., 1981). δ opiate receptors are only found in the pontine nuclei. Similar high densities of the two receptor types occur in the medulla oblongata, spinal cord, n.tractus solitarius, grey matter areas and the substantia gelatinosa.

A variety of opiates show different rank order of potency for the μ and δ opiate receptors (Chang and Cuatrecasas, 1979; Chang et al., 1979). Larsson and his coworkers (1979) showed that (Met)- and (Leu)-enkephalin are contained, at least in part, in separate neuronal populations. Snyder and Goodman (1980) suggested that the μ and δ opiate receptors are the physiological receptors for (Met)- and (Leu)-enkephalin neurons, respectively, since for example the amygdala, which possesses more δ than μ receptors, contains more (Leu)- than (Met)-enkephalin neurons, as well.

μ-selective enkephalin analogues are more potent analgesics than δ-selective peptides, which agrees with the μ receptor localization in brain regions subserving pain perception (thalamus, spinal cord, periaqueductal grey). δ-specific enkephalins are more effective in eliciting limbic seizures (Urca et al., 1977; Dzoljic, 1980) and in facilitating reward behavior (Urca et al., 1977; Stein and

Belluzzi, 1979). Indeed, some limbic structures, such as the amygdala, n.accumbens and some hippocampal areas are enriched with δ opiate receptors (Meibach and Maayani, 1980).

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CHAPTER III.

NEUROBIOLOGICAL PROPERTIES OF THE OPIOID PEPTIDES

1. PRO- AND ANTI-CONVULSIVE ACTION OF THE OPIOID PEPTIDES.

To get a better understanding of these properties, most experiments are performed using morphine, since this opiate-like substance is well known for its analgesic action and presumed to be a μ-opiate agonist. Relatively high doses of morphine or related alkaloids, administered systemically or intracerebroventricularly (icv), induced electrographic epileptiform activity and behavioral convulsions in various species (Gilbert and Martin, 1975; Urca et al., 1977; Aloisi et al., 1980). However, electrographic epileptiform phenomena, observed after icv administration of enkephalins or β-endorphin are never associated with behavioral convulsions (Urca et al., 1977; Frenk et al., 1978b; Henriksen et al., 1978; Aloisi et al., 1980) with exception of wet dog shakes (Frenk et al., 1978b; Chapter VII) and myoclonic contractions of the submandibular muscles (Dzoljic and vd Poel-Heisterkamp, 1982). Subconvulsive doses of morphine may enhance the action of different convulsant manipulations (Le Gal la Salle et al., 1977; Fuller and Olney, 1979; Urca and Frenk, 1980). However, anticonvulsive potentials of morphine (Adler et al., 1976), enkephalin analogues and β-endorphin (Tortella et al., 1981; Berman and Adler, 1984) have been demonstrated as well. Cowan et al.(1979) reported that opiates could be classified on the basis of the change these compounds produce in the seizure threshold, and most of the opioids do increase this threshold (anticonvulsive).

Furthermore, it has been shown that opiates possess both pro- and anti-convulsant properties, depending on the experimental conditions (Urca and Frenk, 1980). Similarly, pro- and anti-convulsive effects of naloxone, the opiate antagonist, have been observed (Snyder et al., 1981).

For an explanation, we must first examine the mechanisms, site(s) and receptor(s), mediating this dual effect.

1.1. *The convulsant system*

The doses of systemic or icv administered morphine, needed to produce electrographic and/or behavioral seizures are very high compared to the doses which produce analgesia (Frenk et al., 1978b) This precludes activation of specific opiate receptors.

On the contrary, β-endorphin and Leu- and Met-enkephalin produce EEG epileptic spikes at very low doses, while high doses are needed to produce analgesia (Frenk et al., 1978b; Henriksen et al., 1978). These epileptic phenomena are probably mediated by specific opiate receptors, which are different from the μ-receptors, presumed to mediate analgesia (Urca et al., 1977). The high doses of naloxone, a preferential μ antagonist, needed to reverse enkephalin-induced seizures suggests that the δ opiate receptors are involved in the opioid-induced epileptic phenomena (Frenk et al., 1978a; Dzoljic and vd Poel-Heisterkamp, 1982).

Summarizing, it appears that morphine and the opioid peptides, depending on the conditions of administration, activate two convulsant systems: 1. A non-specific system and 2. A δ receptor-mediated system.

1.1.1. Non-specific convulsant system.

In literature, a role for the GABA-ergic system in the non-specific proconvulsant system is suggested (Dingledine et al., 1978; Dzoljic and vd Poel-Heisterkamp, 1981; Werz and McDonald, 1982), although evidence for glycine involvement also exists (Werz and McDonald, 1982).

Enkephalin markedly attenuates a variety of GABAergic inhibitory pathways in the CNS, but does not affect the action of GABA (Nicoll et al., 1980). Since morphine, but not (D-ala^2,D-met^5)enkephalin, has an antagonistic effect on GABA (Nicoll et al., 1980; Werz and McDonald, 1982), the convulsant action is apparently not a factor of the putative δ system. It is suggested that the site of action of this non-specific convulsant system may be located on those sites, where morphine causes epileptiform activity and the enkephalins do not (Frenk, 1983). Such sites exist in the cerebral cortex (Sprick et al., 1981) and spinal cord (Frenk, 1983).

1.1.2. The δ-opiate receptor mediated proconvulsant system.

Since iontophoretic application of opioid peptides typically inhibits neuronal firing in most brain areas, but excites the hippocampal pyramidal cells (Nicoll et al., 1977; French and Siggins, 1980; Chapter IV), the hippocampus is proposed as the origin of the opioid-induced seizures (Henriksen et al., 1978). This hippocampal neuronal excitation is reversible by naloxone in most studies (Nicoll et al., 1977; Zieglgänsberger et al., 1979; Chapter IV). However, microinjections of opioid peptides produce activatory and epileptiform effects on the local electrical activity not only in the hippocampus (French and Siggins, 1980; Elazar et al., 1982) but in other brain regions as well such as the thalamus (Frenk et al., 1978a), n.accumbens, parietal cortex, amygdala (Elazar et al., 1982) and caudate nucleus (Dzoljic and vd Poel Heisterkamp, 1980). A non-susceptive area seems to be the periaqueductal grey (Yeung et al., 1978).

In contrast to the suggestion that δ opiate receptors mediate the opioid-induced excitatory phenomena (Frenk et al., 1978b; Dzoljic, 1982; Dzoljic and vd Poel-Heisterkamp, 1982), Gähwiler (1981) demonstrated that μ receptors are involved in the opiate-induced hippocampal firing. However, this might be a result of using (D-ala^2,D-leu^5)enkephalin, which has a low discrimination ratio between δ and μ opiate receptors (Kosterlitz and Paterson, 1980), while the experiments were performed on cultured hippocampal cells and the results in vivo might be different by a recurrent inhibitory mechanism.

1.2. *The anticonvulsant system.*

The anticonvulsive effects of morphine and the opioid peptides can be reversed by naloxone and only low doses of these substances are needed to produce the anticonvulsant action (Cowan et al., 1979; Urca and Frenk, 1980; Tortella et al., 1981). Furthermore, since morphine decreases spontaneous and enkephalin-induced electrographic epileptiform spike activity, it is suggested that activation of μ opiate receptors may result in an anticonvulsive effect (Dzoljic, 1982), which includes the existence of a tonically active

anticonvulsant system modulated by the endogenous opioid peptides.

1.3. *Interaction between the pro- and anticonvulsant systems.*

The presence of a pro- and anti-convulsant system, both activated by the same substances and mediated by possibly pharmacologically different opiate receptors, raises the question whether it is possible to activate one, but not the other.

Morphine derivates with diminished opiate receptor potency are demonstrated to possess an enhanced epileptogenic potency (LaBella et al., 1979).

Urca and Frenk (1982; 1983) have shown that the pro- and anticonvulsant effects of morphine, β-endorphin and Leu-enkephalin, may be elicited probably by employing different routes of administration and that the opiate anticonvulsant μ system was able to inhibit the proconvulsant δ system (Chapter VI).

The existence of the two systems might explain the dual effects of morphine, the opioid peptides and naloxone. Morphine as a μ agonist, in low doses, can be considered as an anticonvulsant agent (Adler et al., 1976; Urca and Frenk, 1980). However, in high doses it might interact with δ receptors as well (Wüster et al., 1980) and facilitate seizure phenomena induced by other convulsant agents (Puller et al., 1979). This is partially in contrast to the hypothesis of Frenk (Frenk et al., 1982; Frenk, 1983), who proposed along with the non-specific and δ-receptor mediated convulsant systems, a μ receptor mediated proconvulsant system. His hypothesis is mainly based on the criterion of naloxone reversibility. However, naloxone, which possesses a significantly higher blocking affinity for the μ than for the δ opiate receptors (Chang et al., 1980) can also antagonize the effects of GABA in high concentrations (Dingledine et al., 1978), leading to excitatory phenomena, independent of opioid-mediation. Furthermore, pharmacologically, pro- and anti-convulsive effects mediated by the same receptor subtype, is not comprehensible.

Similarly, naloxone may facilitate seizure activity (Gilbert and Martin, 1975; Schreiber, 1979; Snyder et al., 1980, 1981), or in high doses may block δ receptors as well, resulting in an

anticonvulsive effect (Dzoljic, 1982).

The recently described opioid antagonism of electroshock- and kindling-induced seizures (Berman and Adler, 1984; Puglisi-Allegra et al., 1984), might depend on the route of administration. The convulsions induced in these experiments are probably the result of the increased endogenous levels of endorphins and enkephalins (Hong et al., 1979; Vindrola et al., 1981). In this case, it should be interesting to examine the effects of enkephalinase inhibitors, like phosphoramidon and thiorphan, which potentiate the endogenous enkephalinergic system, on electroshock- and/or kindling-induced seizures, since it has been demonstrated that phosphoramidon can induce epileptic phenomena, when administered alone (Ukponmwan and Dzoljic, 1984).

Thus, depending on the experimental conditions (primarly the dose of the opiates) and the functional activity of the endogenous endorphinergic system, both pro- and anti- convulsant effects of morphine and the opioid peptides may be expected.

2. OTHER NEUROBIOLOGICAL PROPERTIES OF THE OPIOID PEPTIDES.

The most common pharmacological use of opiate substances is for the relief of pain. Indeed, β-endorphin and enkephalins, administered icv or systemically, induced analgesia (Wei et al., 1977; Foley et al., 1979; Kastin et al., 1979) while opioid antagonists enhanced nociceptive reactions (Jacob and Ramabadran, 1978). Furthermore, several investigators reported that acupuncture involved an activation of endorphin-mediated analgesia (Pomeranz and Chiu, 1976; Mayer et al., 1977). The results led to the conclusion that endogenous opioid peptides may be endogenous analgesics (Kosterlitz, 1979; Terenius, 1982).

Opiates are known to have mood-altering properties, such as dreamlike euphoria and reality escape, for which they presumably are self-administered in many forms and preparations. Many drugs that are abused by man, are self-administered by animals as well, and this includes the opiates like morphine and heroin (Werner et al., 1976) as well as

Leu-enkephalin (Belluzzi and Stein, 1977), which might serve as an endogenous euphorigen. Although many experiments have been performed to elucidate the underlying mechanisms of opiate addiction/tolerance (Wei, 1981; Zukin and Zukin, 1981; Zukin et al., 1982), the diversity in natural opioid agonists and in antagonist ligand binding sites prevented the determination of involvement and/or mechanisms of the endogenous opioid system(s).

Systemic injection of opioids results in locomotor activity, which is jerky and undirected, interrupted by grooming and staring (Koob and Bloom, 1983) and wet-dog shakes (Chapter VI). In high doses, it might result in extreme generalized muscular rigidity (Wand et al., 1973). An opioid-dopamine (DA) interaction is proposed (Stinus et al., 1980), which might be of importance for the putative role of the mesolimbic DA system in the pathophysiology of schizophrenia (Bloom et al., 1976; Jaquet and Marks, 1976) and affective disorders (Judd et al., 1981).

Opioid peptides block the release of catecholamines (Loh et al., 1976; Taube et al., 1976) and some other neurotransmitters (Beaumont and Hughes, 1979). This is attributed to a presynaptic inhibitory action (Snyder and Childers, 1979). In addition, they inhibit both spontaneous, and acetylcholine- or glutamate-induced firing of most neurons, with the exception of hippocampal pyramidal cells and spinal cord Renshaw cells (Zieglgänsberger and Bayerl, 1976; Snyder and Childers, 1979). This inhibition of firing suggests an influence on post-synaptic receptors. Whether pre- or post-synaptic, the physiological significance may be in the fact that enkephalin- and endorphin-containing fibers usually run in the vicinity of catecholaminergic fibers (Terenius, 1982).

Intravenous or icv administration of opioid peptides stimulates pituitary release of prolactin and growth hormone (Grandison and Guidotti, 1977; Rivier et al., 1977). It is not known whether this effect is caused by a direct action upon the hypophysis, or by way of inhibition of the catecholaminergic fibers, which normally control pituitary secretion. The latter explanation appears to be more likely (Beaumont and Hughes, 1979).

Morphine, heroin and opioid peptides stimulate feeding and drinking (McKay et al., 1981; Morley et al., 1983), while opiate antagonists possess anorexic properties (Holltzman, 1979). Stress-induced eating is shown to

be mediated by endogenous opiates, as well (Morley and Levine, 1980). Although an overwhelming body of evidence favors a role for the endogenous opioid peptides in the regulation of ingestive behavior, the exact mechanism of action is not clear (Morley et al., 1983).

An involvement of the different opiate receptor subtypes, and as such of the different opiate agonists and antagonists on respiration, is reported by several authors (Florez et al., 1980; Meldrum and Isom, 1981; Hassen et al., 1982). Where the μ agonist does not change the respiratory frequency, the δ opiate agonist results in a respiratory depression (Morin-Surun, 1984).

Although the heart is devoid of opiate receptors (Simantov et al., 1978), opioid peptides do alter cardiovascular functions as well (Bolme et al., 1978; Chapter VIII), generally resulting in hypotension and bradycardia. These effects are believed to be, in part, a consequence of opiate action upon the brain stem autonomic centers involved in the regulation of cardiovascular homeostasis. Pituitary endorphins released during stress or shock, probably act on opiate receptors in the brain to depress cardiovascular function (Holaday and Loh, 1981).

Despite the long history of opiate thermoregulatory investigations, the effects of opiates on body temperature are still the subject of scientific controversy. In general, opiates may produce either hyperthermia (Martin and Morrison, 1978; French, 1979) or hypothermia (Lin and Su, 1979; Tseng et al., 1979), depending on species, doses, ambient temperature, route of injection, degree of tolerance and endocrine status. Generally, it is concluded that endorphins may be activated by any extreme temperature (Thornhill et al., 1980) and that the pituitary endorphins are more functionally involved in heat adaptation in an associated hypothermic role (Holaday and Loh, 1981).

Last, but not least, opioid peptides play a role in memory and learning processes, where they are probably the mediators of an endogenous amnesic mechanism (Izquierdo et al., 1980).

In general, it seems that the endorphinergic system does not play an explicite role during homeostatic conditions within an organism. However, environmental or physiological alterations, which may be considered as stressful, appear to activate this system.

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CHAPTER IV.

OPIATES AND HIPPOCAMPUS

1. STRUCTURE AND NOMENCLATURE OF THE HIPPOCAMPUS

The hippocampal region is the part of the cerebral cortex that forms a relatively long, horn-shaped body along the curvature of the lateral ventricles. This region can be subdivided in the hippocampus or Ammon's horn; the fascia dentata (gyrus dentatus); and the subicular region (prosubiculum, subiculum, presubiculum and parasubiculum), which is contiguous to the entorhinal cortex (Fig.1.).

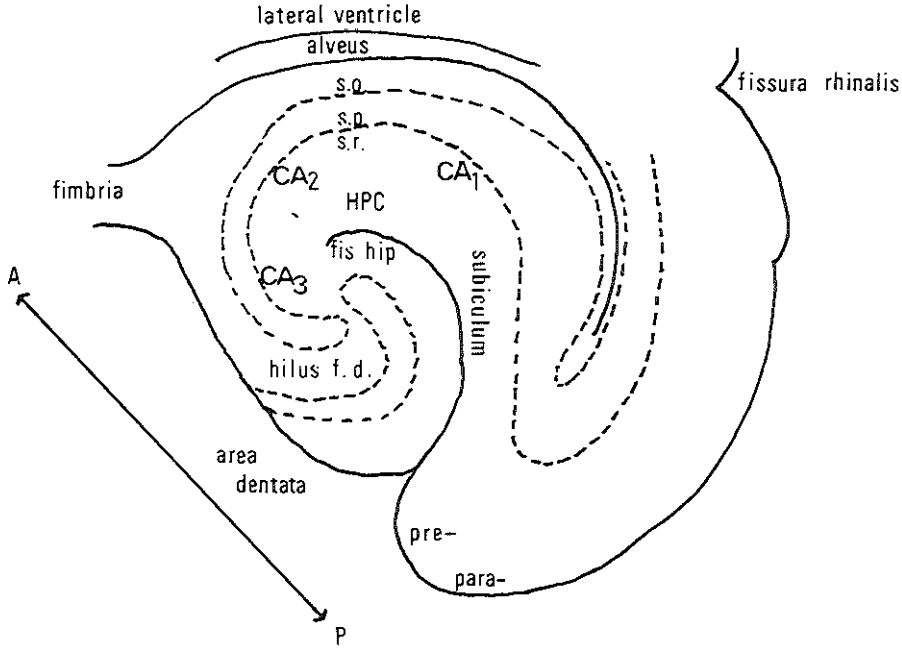

Fig.1. Diagram of a horizontal section of the hippocampal region. The following hippocampal layers are shown: alveus; stratum oriens (s.o.); stratum pyramidale (pyramidal cell bodies, s.p.); stratum radiatum (s.r.); In the dentate area: stratum granulosum (granular cell bodies, s.g.) and the hilus of the fascia dentata (hil.f.d.). The following related structures are indicated: subiculum; presubiculum (pre-); parasubiculum (para-); A: anterior; P: posterior.

The Ammon's horn (Cornus Ammonis or CA) has been divided into 4 fields: CA1, CA2, CA3 and CA4 (Lorente de Nó, 1934). The CA2 and CA3 areas are the regions with giant pyramidal cells. The pyramidal cell layer of the CA3 area enters the hilus of the fascia dentata (CA4). According to Blackstad (1956) these pyramidal cells form a deeper cortical layer which he called the "dentate area".

Since iontophoretically applied opiates and opioid peptides are known to excite hippocampal pyramidal cells (Nicoll et al., 1977), a simplified diagram of the neuronal circuitry in the CA1 region is given in Fig.2., illustrating excitatory and inhibitory synaptic connections. The pyramidal cell body layer (stratum pyramidale) and the basal dendritic layer also contain non-pyramidal cells, being used to describe basketcells and other inhibitory interneurons. Specific interneurons forming synapses exclusively with the axon initial segments of pyramidal cells, have been identified by Somogyi et al. (1983).

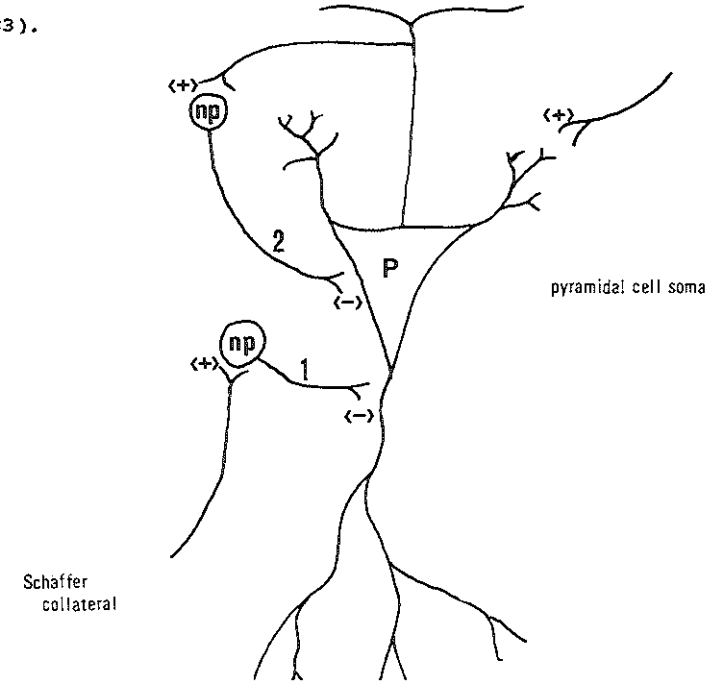

Fig.2. Schematic representation of the neuronal circuitry in the CA1 pyramidal cell region of the hippocampus. P:pyramidal cell; NP:non-pyramidal cells; +:excitatory synapse; -:inhibitory synapse; 1.:feedforward inhibition; 2.:feedback inhibition.

2. OPIOID MEDIATED HIPPOCAMPAL EXCITATIONS

Clinical and preclinical investigations of the endorphins have suggested a correlation between certain neuropsychiatric conditions, limbic system and the central function of these peptides (Bloom and McGinty, 1981; Vereby, 1982). Despite the uniform naloxone-reversible depressant action of opiates and opioid peptides in most brain regions studied, iontophoretic applications of these substances excite most hippocampal pyramidal neurons (Nicoll et al., 1977) while the neighbouring granule cells are depressed (Tielen et al., 1981). The excitatory cholinergic (ACh) septal hippocampal pathway, can not be involved in this excitation, since scopolamine and atropine, ACh blocking agents, as well as lesions of the septal nuclei, do not reduce the opioid-induced excitations (French and Siggins, 1980). These findings point to an autonomous role for the hippocampus in the epileptiform response and may underlie epileptic episodes induced by enkephalins (French and Siggins, 1980; Henderson, 1983).
Three major hypotheses have been proposed to explain these excitatory phenomena in the HPC:

2.1. *Excitation by disinhibition.*

There are two, probably GABA-ergic pathways in the HPC, which both utilize non-pyramidal interneurons, and illustrated in Fig.2. as the feedforward and feedbackward inhibitory pathways. Zieglgänsberger and coworkers (1979) postulated that excitation of pyramidal cells may activate inhibitory interneurons via recurrent collaterals of their axons. This inhibitory circuit may be interrupted by the application of opiates, which exert their effect at the level of the cell body of the inhibitory interneurone, suppressing cell discharge (Nicoll et al., 1980). The resulting disinhibition of pyramidal cells becomes evident as a strong excitation. This hypothesis was supported by several other investigators (Dunwiddie et al., 1980; Lee et al., 1980; Robinson and Deadwyler, 1981).
If disinhibition is the important mechanism in opioid action on pyramidal cells, then intracellular records should show diminished

inhibitory postsynaptic potentials (i.p.s.p.). However, opioids did not reduce the i.p.s.p. in pyramidal cells (Haas and Ryall, 1980; Dingledine, 1981).

2.2. *Facilitation*

Haas and Ryall (1980) proposed that opioid peptides enhance transmitter release from excitatory nerve terminals. Indeed, several groups of investigators did find increased excitatory postsynaptic potentials (e.p.s.p.) after opioid application (Haas and Ryall, 1980; Nicoll et al., 1980). However, others failed to observe this increase (Corrigal and Linseman, 1980; Dunwiddie et al., 1980; Dingledine, 1981; Lynch et al., 1981).

2.3. *Increased efficiency of coupling*

Opioids might act by facilitating the passage of the e.p.s.p. from the dendrites to the cell soma and thus potentiate the soma population spike activity (Dingledine, 1981; Lynch et al., 1981; Robinson and Deadwyler, 1981). However, the excitation evoked by application of excitatory amino acids is not potentiated by opioids, as would be expected (Haas and Ryall, 1980; Dingledine, 1981).

Although no final conclusion can be drawn from these results, the bulk of evidence favours disinhibition as responsible for the excitation of hippocampal pyramidal cells by opioid peptides. The question of the functional meaning of the opiate-evoked excitations in the HPC remains to be resolved.

3. OPIOID SYSTEMS IN THE HIPPOCAMPUS

By evaluating immunohistochemical studies (Hökfelt et al., 1977; Goldstein and Ghazarossian, 1980; Wamsley et al., 1980; Gall et al., 1981; Hong and Schmid, 1981), at least two possible opioid systems, identified in the HPC have been suggested. Potentially, the most significant pathway is described by the dentate-granule cells-mossy fiber system (Corrigal, 1983), where dynorphin has been identified (Henriksen et al., 1982), while dynorphin-containing cells are also found scattered throughout CA1 and CA3-4 cellular fields, possibly representing a subclass of interneurons (Henriksen et al., 1982). The second peptide system

derives from the lateral entorhinal/perirhinal cortex and appears to synapse on dentate granule cells. This latter pathway contains enkephalin-immunoreactivity, but is devoid of dynorphin (Henriksen et al., 1982). Enkephalin-like immunoreactivity was found within the somata of three types of hippocampal neurons: 1. granule cells of the dentate gyrus, 2. occasional pyramidal shaped cells of field CA1 stratum pyramidale, and 3. varied scattered interneurons. Of this last group, two types of interneurons were consistently seen. The first occupy the border between stratum radiatum and stratum pyramidale, whereas the second lie within the stratum radiatum of field CA1. Cells containing enkephalin-like immunoreactivity were also observed in the subiculum (Gall et al., 1981). Thus, opioid peptide immunoreactivity in two major opioid peptidergic pathways of the hippocampal formation may represent the presence of two entirely different prohormonal systems. Furthermore, dynorphin, in comparison to Leu^5-enkephalin, induced a longer lasting excitatory spike activity in the EEG (Henriksen et al., 1982). Although questions of the pharmacological action of exogenously applied dynorphin and its release by stimulation of the mossy fibers remain to be answered, it might be that the dynorphin family plays an important role in the opioid-induced excitatory phenomena as well.

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CHAPTER V.

CORRELATION BETWEEN THE DISTRIBUTION OF ^{3}H-LABELLED ENKEPHALIN IN RAT BRAIN AND THE ANATOMICAL REGIONS INVOLVED IN ENKEPHALIN-INDUCED SEIZURES

SUMMARY

The correlation between the distribution of ivt administered δ agonist [^{3}H](D-ala^{2},D-leu^{5})-enkephalin ([^{3}H]DADL) and the anatomical regions involved in the enkephalin-induced seizures has been studied in rat by using an autoradiographic method and recording of the electromyogram (EMG) and the electroencephalogram (EEG).

The results indicate that within 10 min, the radioactivity of the ivt administered drug reached all parts of the ventricular system, including the central canal of the spinal cord. However, within 2.5 min after ivt [^{3}H]DADL, which corresponds to the onset of DADL-induced seizures, the substance appeared mainly in the left lateral ventricle and occasionally in the third ventricle. During the first 2.5 min the substance penetrated regularly into the surrounding periventricular tissue of the striatum, septum and hippocampus to a depth of about 100 µm. The most intensive and long lasting epileptic discharges, exceeding 30 min were observed in the hippocampus, in contrast to the mild and short-lasting electrophysiological responses of the septum and corpus striatum. The experiments suggest that the short onset of enkephalin-induced excitatory phenomena is due to the rapid distribution and penetration of the substance in the surrounding periventricular tissue. According to these data, it is proposed that activation of opiate receptors localized within the first 100 µm of the periventricular tissue, mainly in the hippocampus, is essential for the triggering of endorphin-induced seizure activity.

1. INTRODUCTION

There are indications that some synthetic analogues of natural opioids and β-endorphin have a moderate cerebrovascular permeability and can exert central effects in conscious animals when administered systemically (Tseng et al., 1976; Rapoport et al., 1980). However, the variation in the amount of the opioid peptides penetrating the blood-brain barrier and the high costs of the compounds are the main reasons that most studies with endogenous opioid peptides have followed the intraventricular (ivt) route of administration.

Unfortunately, many aspects of the ivt administration which might be of relevance for the interpretation of the results with endorphins, such as distribution of peptides in the ventricular system or the volume of injection in relation to its clearance from brain tissue, are not sufficiently well known. Although the distribution of morphine and morphine-like substances have been studied by Herz and Teschemacher (1971), no data are available about the spread of various endopioids (=endorphins; Adler 1980) after ivt administration.

There is evidence from previous studies (Urca et al., 1977; Frenk et al., 1978; Dzoljic et al., 1979) that endorphins may play a role in epileptogenesis. One of the most potent seizure-inducing opioid peptides is (D-ala^2,D-leu^5)enkephalin (DADL) (Dzoljic and vd Poel-Heisterkamp, 1982). This peptide, which is also one of the most potent agonists at δ opiate receptors known at the time these experiments were performed (Chang et al., 1980), induced epileptic phenomena within a few minutes after ivt administration and the effect lasted for 30-60 min (Dzoljic and vd Poel-Heisterkamp, 1981).

The short onset of excitability indicates an involvement of brain structures in the close vicinity of the lateral ventricles. Lack of knowledge about the spread of the exogenously (ivt) applied opioid peptides has prevented the identification of those brain regions involved in the initiation of endorphin-induced seizure phenomena.

The aim of this study is to identify the anatomical regions associated with the enkephalion-induced excitatory phenomena and to correlate the extent of drug diffusion into these regions with the manifestation of pharmacological activity. An autoradiographic method and electrophysiological recording is used to detect the onset and characteristics of enkephalin-induced seizure activity in the periventricular regions and cortex. The distribution of (^{3}H)DADL (ivt) within the liquor system and its speed of diffusion into the periventricular tissue, is compared.

2. MATERIALS AND METHODS

2.1. *Animal experiments*

2.1.1. Autoradiography.

Male rats (Wistar strain, 175–250 g) were anaesthetized with urethane (1.2 g/kg, i.p.). A steel cannula for ivt injections was implanted by means of a stereotaxic instrument (coordinates: AP +6.1 mm, L −1.3 mm, H −4.0 mm from dura with lambda 0).

12.5 µCi/1µl (^{3}H)DADL (29 Ci/mmol) was administered by use of a 5 µl Hamilton syringe. In each period of 1, 2.5, 5, 10, 30 and 60 min after the injection, 4 rats (i.e. in total 24 animals) were killed by immersion in a mixture of acetone and carbon dioxide (−70/−80 °C). After a minimum of 10 min the corpse was taken out and immediately transferred to a cold room (−20 °C).

The method used was according to Ullberg (1954). Frontal brain sections 30 µ in thickness, were prepared in a cryostat (−20 °C) with a Jung model tetrander microtome using scotch tape (Minnesota Mining 810) as a support. The sections were freeze-dried and then placed in contact with LKB Ultrofilm ^{3}H. For illustration purposes, prints were made of the autoradiograms (enlargement x 8.3) showing light and dark areas which are regions of maximum and minimum radioactivity, respectively.

A hematoxyline–eosine colouring method was used for staining the sections to achieve a better contrast between the different areas. Anatomical interpretation of the distribution of the radioactivity is based on the atlas of König and Klippel (1963). To determine the extent of diffusion into the brain tissue, the penetration distance was measured from the wall of the lateral ventricle into the hippocampal, striatal and septal areas.

Statistical evaluation of the data was performed by the Student's t-test.

2.1.2. Electrophysiological recording.

Six male rats (Wistar strain, 175–200 g) were anaesthetized with urethane (1.2 g/kg,i.p.). A tracheal cannula was inserted and electrodes were implanted in the submandiblar muscles for the

registration of the electromyogram (EMG) and myoclonic contractions (MC) (Dzoljic and vd Poel-Heisterkamp, 1982). Spontaneous and drug-induced myoclonic contractions of the submandibular muscles were recorded by the method of Bieger et al.(1972). A steel cannula for ivt injections (left side) and electrodes in the left side of the striatum (AP +1.9 mm, L +3.5 mm, H -5.1 mm), hippocampus (AP -4.9 mm, L +4.0 mm, H -3.0 mm) and septum (AP +2.4 mm, L +0.5 mm, H -4.3 mm, with H measured from dura and bregma 0) (de Groot, 1972) were implanted stereotaxically. In addition, the electrocorticogram (ECoG) between the frontal and parietal electrodes was recorded by means of a polygraph Grass model 7. Rectal temperature was maintained between 36.5 and 37.5 °C.

2.2. *Drugs*

D-ala^2-[tyrosyl-1-3,5-^{3}H]enkephalin (5-D-leucine) (New England Nuclear), is supplied in 0.02M sodium dihydrogen phosphate buffer, pH 2.1. Before use the labelled compound was dried under nitrogen and diluted with an appropriate solution of artifical cerebrospinal fluid (CSP) (1 mCi/80µl) and adjusted to pH 7. Specific activity of [^{3}H]DADL was 29 Ci/mmol.

(D-ala^2,D-leu^5)-enkephalin (DADL) (Peninsula Lab.) was diluted in CSF (5 µg/2µl).

3. RESULTS

The conclusions are based on a large number of autoradiograms of which, only a small selection is shown here.

3.1. *Distribution of [^{3}H]DADL in the ventricular system*

At 1 min after administration of labelled DADL (12.5 µCi/1µl), radioactivity was observed in all 4 rats only in the left lateral ventricle, where it was injected (Fig.1). After 2.5 min, the radioactivity penetrated to the frontal parts of the third ventricle, the *recessus pinealis* (in 3 of 4 animals) (Fig.2). However, within 5 min the labelled substance reached both lateral ventricles and all parts of the third ventricle in all experimental animals (Fig.3).

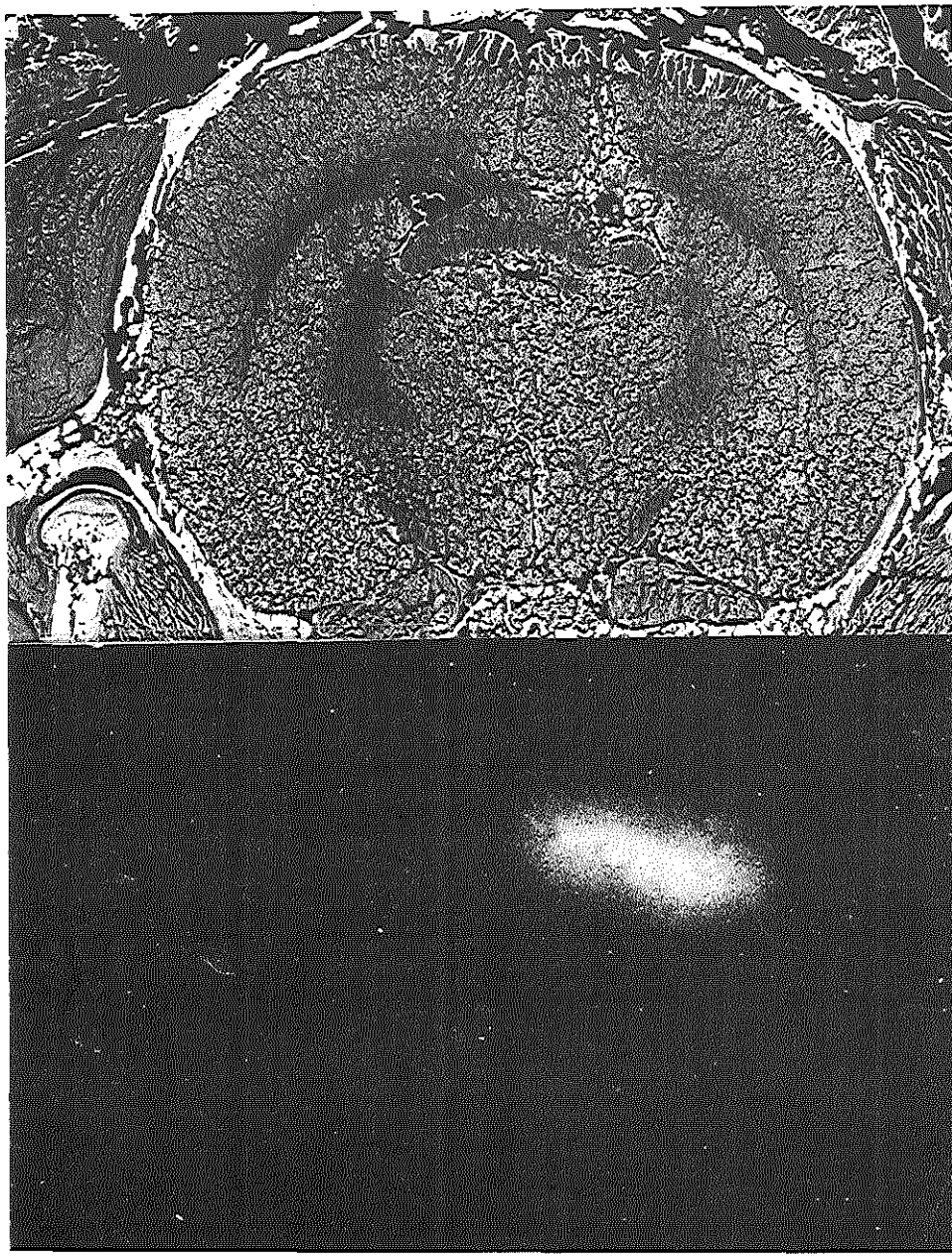

Fig.1. Brain section (A 5780 µ) and corresponding autoradiogram, 1 min after intraventricularly administered labelled (D-ala^2,D-leu^5)-enkephalin ([^{3}H]DADL, 12.5 µCi/µl) in the rat brain. Note that [^{3}H]DADL was only detected in the ventricle where it was injected.

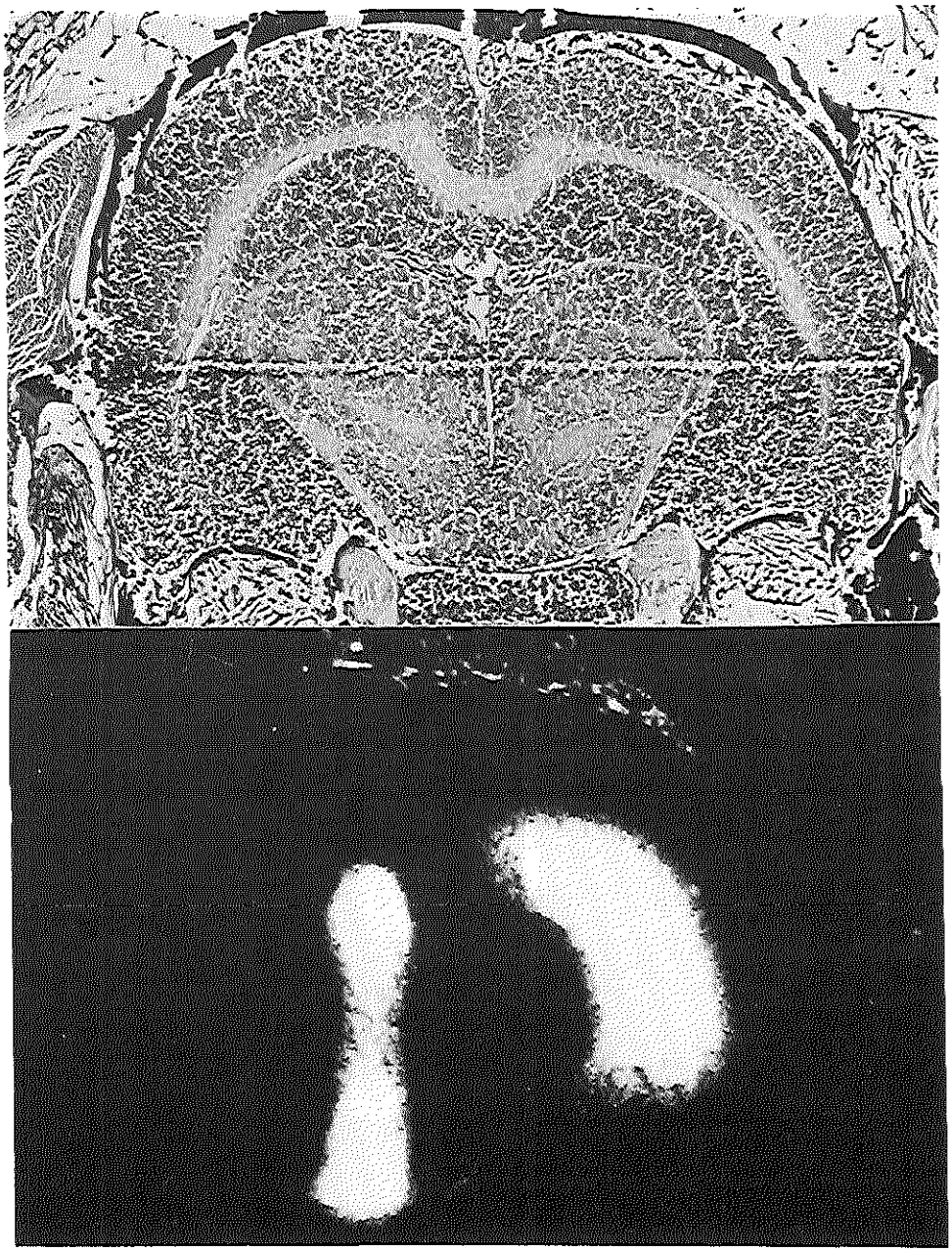

Fig.2. Brain section (A 3750 µ) and corresponding autoradiogram, 2.5 min after intraventricular administration of labelled (D-ala^2,D-leu^5)-enkephalin (12.5 µCi/µl) in the rat brain. Note that the radioactive substance penetrated into the third ventricle.

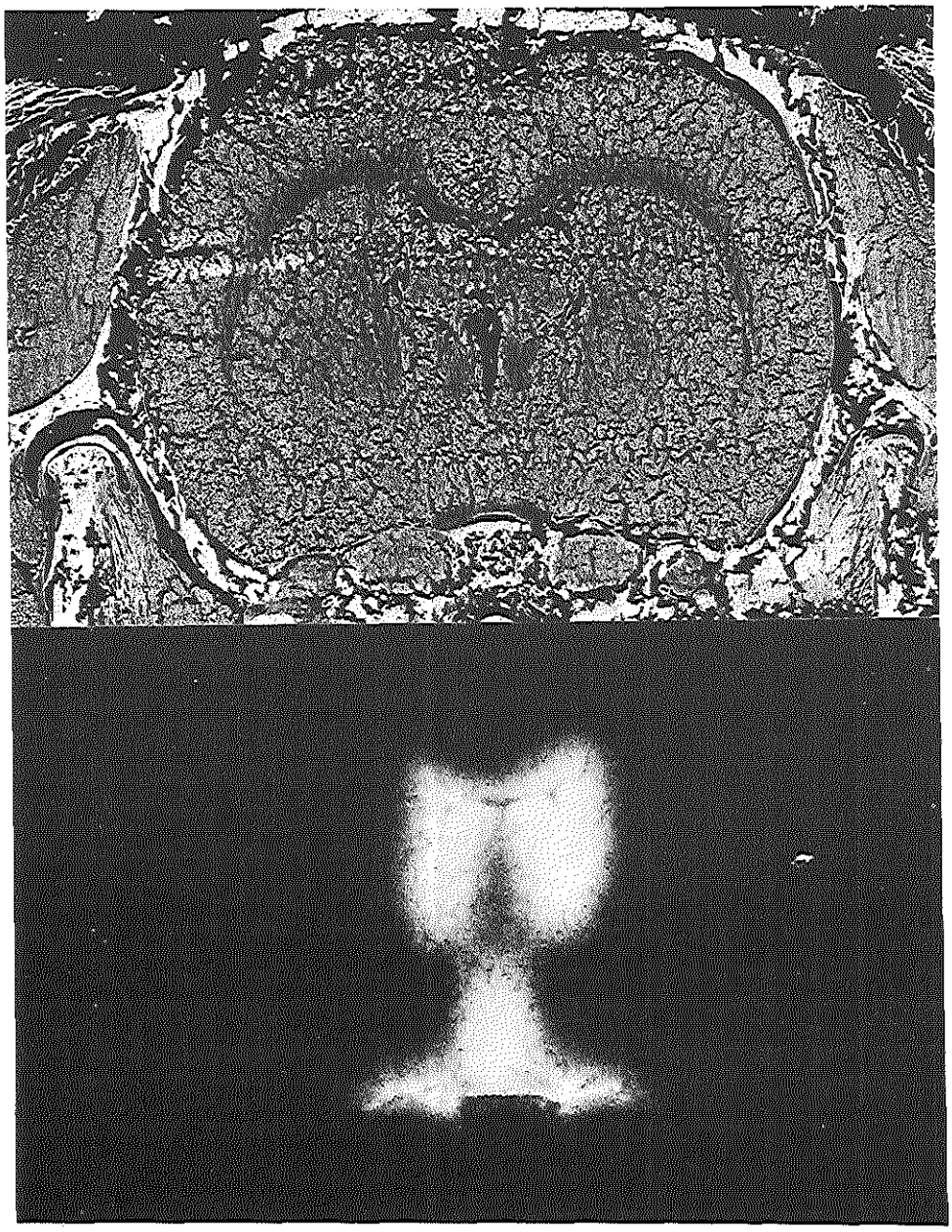

Fig.3. Brain section and corresponding autoradiogram, 5 min after intraventricular administration of labelled (D-ala^2,D-leu^5)-enkephalin (12.5 µCi/µl) in the rat brain. Note the penetration of the radioactive substance into the corresponding lateral ventricle and third ventricle.

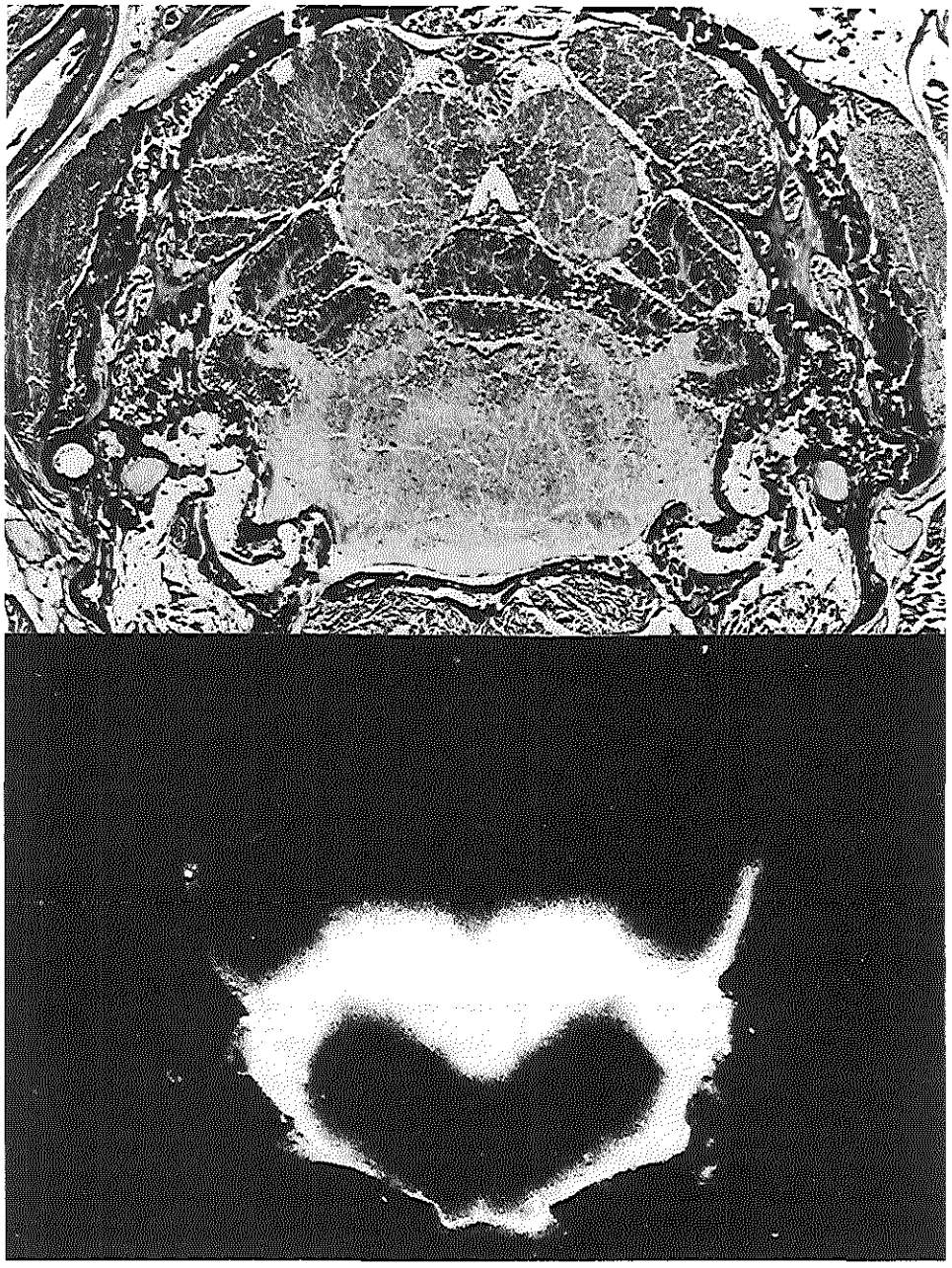

Fig.5. Brain section (A 160 µ) and corresponding autoradiogram, 10 min after intraventricularly administered labelled (D-ala^2,D-leu^5)-enkephalin (12.5 µCi/µl) in rat brain. Note that the labelled substance also reached the extracerebral space of the ventricular system.

Within 10 min, the whole ventricular system showed radioactivity, including the central canal of the spinal cord (Fig.4). During this period of time a significant amount of labelled substance could be detected in the whole extracerebral ventricular system (Fig.5).

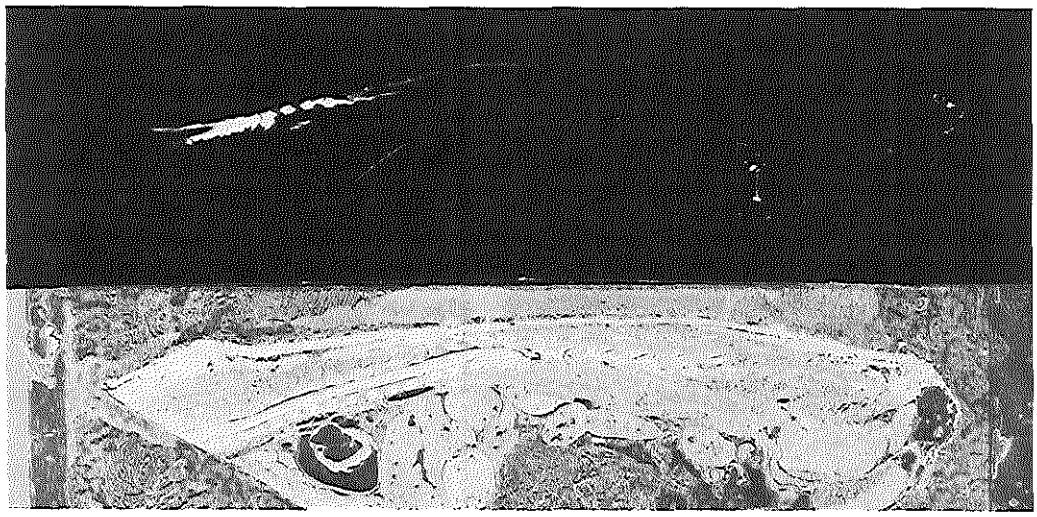

Fig.4. Sagittal section of the body and corresponding autoradiogram, 10 min after intraventricular administration of labelled D-ala^2,D-leu^5)enkephalin (12.5 µCi/µl). Note that the labelled substance penetrated into the central canal of the spinal cord.

3.2. *Distribution in the periventricular anatomical regions.*

After 1 min, radioactivity was observed in the wall of the left lateral ventricle, consisting of the septum, striatum and hippocampus (Fig.1). After 2.5 min the radioactive substance penetrated into the hippocampus and striatum as well as into the wall of the third ventricle (Fig.2). Within 10 min, [^{3}H]DADL penetrated from the ventricles into the whole septum, parts of the corpus striatum and hippocampus and small parts of the corpus callosum and thalamus. Radioactivity was pronounced in the chorioidal plexus.

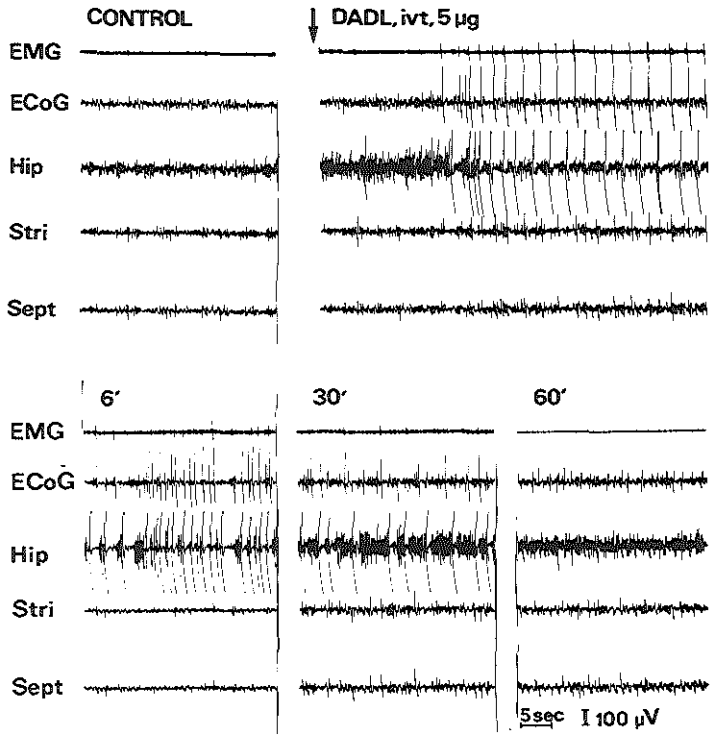

Fig.6. Electromyogram (EMG), electrocorticogram (ECoG) and electrophysiological responses of the hippocampus (hip), striatum (str) and septum (sept) before and after intraventricular (ivt) administration of ($D\text{-}ala^2, D\text{-}leu^5$) enkephalin (DADL, 5µg/2 µl). Note the short onset of epileptic spike activity and the long-lasting and intensive effect of DADL in the hippocampus.

3.3. *Electrophysiological recording*

The electrophysiological responses of the hippocampus, striatum and septum as well as the ECoG and EMG show epileptic discharges and myoclonic contractions, within the time range of 0.5–2 min after ivt administration of DADL. The latency times of the epileptiform muscular and EEG phenomena for each animal are equal. EEG or ECoG epileptic spikes are immediately followed by a MC.

The epileptic spike activity in the septum and striatum are less intensive and short-lasting. The most intensive and long-lasting spiking, exceeding 30 min, is observed in the cortex and particularly

in the hippocampus (Fig.6). No differences are observed between the electrophysiological responses induced by ivt administration of equimolar doses of labelled and unlabelled DADL.

3.4. *Diffusion of radioactivity into the brain tissue.*

Since the EMG and EEG recordings indicate that the epileptic phenomena induced by DADL (5 µg,ivt) appeared within 1-2 min, the depth of penetration of the radioactive substance into the brain tissue was measured over this period of time.

The results indicate that the mean depth of penetration into the hippocampus, striatum and septum was about 100 µm (Fig.7). After 30 and 60 min the distribution of [^{3}H]DADL was found to be diffuse and difficult to evaluate.

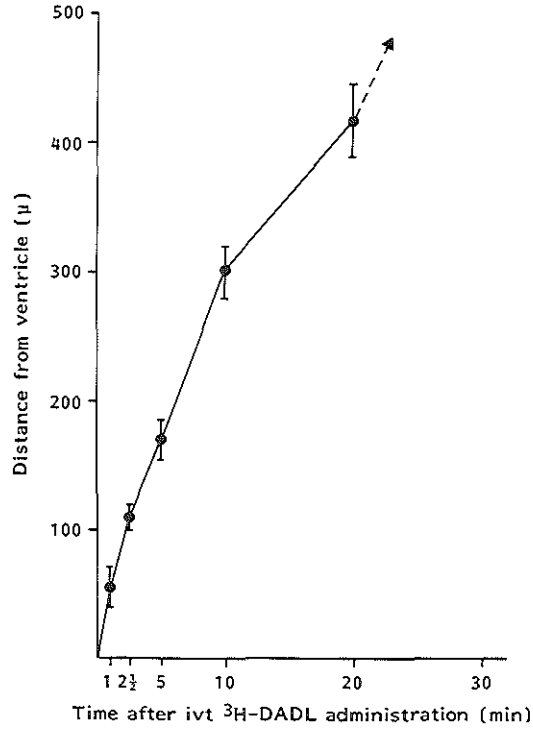

Fig.7. Speed of diffusion of radioactive (D-ala^2,D-leu^5)-enkephalin ([^{3}H]DADL, 12.5 µCi/µl) in the surrounding periventricular tissue after intraventricular (ivt) administration. Note that within 2.5 min (the onset period for DADL induced seizures) [^{3}H]DADL penetrated to a depth of about 100 µm. The vertical bars indicate SEM (n=4).

4. DISCUSSION

Presumably, the radioactivity involves intact [^{3}H]DADL, since the long-lasting effect clearly suggests that the drug was neither metabolized nor rapidly cleared (Yaksh et al., 1982).

Tritium labelled DADL reached all parts of the ventricular system, including the extracerebral liquor space, within 10 min after ivt administration. The cerebrospinal fluid leaves the ventricular system in the vicinity of the fourth ventricle through apertures in the posterior medullary velum (Zeman and Innes, 1963), which might explain why the extracerebral radioactivity is more pronounced in the posterior sections. The time course of distribution of [^{3}H]DADL within the ventricular system is consistent with the stream pattern of cerebrospinal fluid within the ventricles. The ^{3}H-labelled DADL also penetrated rapidly into the periventricular tissue. The brain regions bordering the lateral ventricles are the striatum, septum and hippocampus. Penetration of the radioactive substance into these anatomical regions occured to a depth of about 50-100 µm during the first 2.5 min after ivt administration. This period of time coincides with the appearance of epileptic discharges in the EEG and the EMG. The shortest onset period and the most pronounced and long-lasting epileptic discharges in the EEG were observed in the hippocampus and cortex. Therefore, it can be suggested that the hippocampus is the anatomical regions associated with the initiation of the seizure phenomena, after ivt injection of enkephalins.

In literature it is suggested that endorphins and particularly DADL, exert their convulsant actions through activation of δ opiate receptors (Dzoljic and vd Poel-Heisterkamp, 1982). The striatum, septum and some parts of the hippocampus contain high densities of δ receptors (Goodman et al., 1980; Duka et al., 1981). Furthermore, the hippocampus and the striatum do have the lowest threshold for excitation (Cowan et al., 1979).

It is suggested that the short onset of epileptic phenomena, after ivt administration of enkephalins or enkephalin analogs, is likely to be due to the activation of δ opiate receptors, localized within the first 100 µm of the periventricular tissue of the hippocampus.

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CHAPTER VI.

THE EFFECTS OF SELECTIVE μ and δ OPIATE AGONISTS AND ANTAGONISTS ON EPILEPTOGENIC POTENTIALS IN ANAESTHETIZED AND FREE-MOVING RATS

SUMMARY

By using electroencephalographic (EEG) and electromyographic (EMG) recordings in anaesthetized and free-moving rats, the epileptogenic properties of two opioid peptides, which selectively stimulate μ and δ opiate receptors, respectively morphiceptin and DSTLE, were investigated. In addition, we also examined the effects of two recently synthesized selective antagonists of the δ-opioid receptor (ICI 154,129 and ICI 174,864).

The δ receptor peptide (DSTLE, 4.6-18.6 nmol, ivt) produced a dose-related increase of myoclonic contractions (MC) with epileptic discharges in anaesthetized rats and severe wet dog shakes, with occasionally falling down, in free-moving animals. Morphiceptin, a specific μ opiate agonist, used in equimolar doses and under the same experimental conditions, had a significantly less pronounced effect on the number of MC and epileptiform EEG phenomena but inhibited the DSTLE-induced MC in a dose-related manner. DSTLE (18.6 nmol) injected in the CA2 area of the hippocampus, a region with a nearly equal distribution of μ and δ opiate receptors, induced epileptic discharges in anaesthetized and free-moving rats, while an equimolar dose of morphiceptin had no significant effect. Experiments with ICI 154,129 and ICI 174,864 revealed that they antagonized the epileptogenic effects of DSTLE. For this purpose ICI 154,129 was needed in a high concentration, which indicated the low potency of this substance.

It is suggested that the epileptiform activity of opioid peptides is mainly due to an activation of δ opiate receptors in the central nervous system.

1. INTRODUCTION

The concept of multiple opiate receptors (Lord et al., 1977; Martin, 1981) might explain the various pharmacological effects of opiate alkaloids and opiate peptides. In literature, it is suggested that the analgesic action of opiates may be mediated by μ receptors (Urca et al., 1977), while the epileptic effects of opiates are proposed to emerge from the δ opiate receptor stimulation (Frenk et al., 1978). However, other authors suggest that opioid-induced excitatory responses are predominantly mediated by μ receptors (Gähwiler and Maurer, 1981).

In previous studies Dzoljic (1982) demonstrated that myoclonic seizure phenomena and epileptic spikes in the electroencephalogram (EEG), induced by (D-ala^2,D-leu^5)enkephalin and other endopioids after intraventricular (ivt) administration are probably mediated by δ opiate receptors in the rat brain. The target area of this action seems to be the limbic system (Henriksen et al., 1978), specifically the hippocampus (French and Siggins, 1980; Chapter V). The increase of neuronal excitability caused by opiates in the hippocampus is of particular interest, since opiates are found to depress neurons in other brain regions (Illes, 1982).

Therefore, the aim of this study is to elucidate further the role of the μ and δ opiate receptors in the endorphin-induced neuronal activity, particularly in the hippocampus. For the experiments we used specific ligands, such as morphiceptin, which shows high specificity for the μ opiate receptor (Chang et al., 1981; Zhang et al., 1981; Chang et al., 1982) and δ receptor peptide (DSTLE), one of the most specific δ agonists presently known (Gacel et al., 1980). The last substance shows no cross-reactivity with the μ receptor sites (David et al., 1982). Both substances were injected into the hippocampus and, for comparison, into the lateral ventricle. Studies with opiate antagonists, such as naloxone and naltrexone, have revealed that these drugs exhibit selectivity for the μ-receptor over both the δ- and κ-subtypes (Magnan et al., 1982). However, the recently- described enkephalin analogues ICI 154,129 (Shaw et al., 1982; Gormley et al., 1982) and ICI 174,864 (Cotton et al., 1984), which are supposed to be selective δ opiate antagonists, were also used in these experiments. Since there is evidence for opiate-anaesthesia interactions (Urca and Liebeskind, 1979; Linseman, 1980), the experiments were performed in anaesthetized and free-moving animals.

2. MATERIALS AND METHODS

2.1. *Anaesthetized rats*

2.1.1. Intraventricular administration (ivt)

The method used in these experiments has been described previously (Dzoljic et al., 1979). In brief, male Wistar rats (200-275 g) were anaesthetized by urethane (1.2 g/kg, i.p.). A steel cannula for ivt injections (coordinates: AP -0.1 mm, L +1.5 mm, H -2.0 mm from dura, with bregma 0) was implanted by means of a stereotaxic instrument. Intraventricular injections were made by a 5 µl Hamilton syringe. The electrocorticogram (ECoG) between the frontal and parietal electrodes was recorded by means of a polygraph (Grass model 7). The electromyogram (EMG) of spontaneous and drug-induced myoclonic contractions (MC) of the submandibular muscles was recorded by the method of Bieger et al.(1963).

The term "spontaneous myoclonic contractions" corresponds to the irregular muscle twitches with low frequency (1-3 twitches/min) and low amplitude (mostly lower then 50 µV), which are due to mastication or other oral activities persisting even during complete anaesthesia. The number of MC, correlating with ECoG epileptic spikes (Dzoljic et al., 1980), were counted within the first 15 min after ivt administration of the drug. The most intensive spiking was observed during this period of time.

2.1.2. Intrahippocampal administration (ihp)

A steel cannula for ihp injections and an electrode for EEG recording, were implanted stereotaxically in the CA2 area of the hippocampus (coordinates: AP +2.6 mm, L -2.6 mm, H -2.9 mm from dura, with bregma 0) (de Groot, 1972). The ECoG, the electroencephalographic responses (EEG) of the hippocampal area and the MC were recorded. The number of EEG spikes in the hippocampus, the ECoG spikes and the MC were counted within the first 15 min after drug administration.

Only those animals, which after injections of marker dye at the end of the experiment revealed dye in the ventricle/hippocampus, were taken

into consideration for statistical analysis.

Rectal temperature was maintained between 36.5 and 37.5 °C.

2.2. *Free-moving rats*

2.2.1. **Intraventricular administration**

Male Wistar rats (200-275 g) were prepared for EEG, ECoG and EMG recording under Hypnorm (Duphar) anaesthesia (0.4 ml/100g, s.c.). Silver screw electrodes were fixed into the bone overlying the frontal and parietal cortices. The EMG was recorded from the neck- and submandibular muscles. A steel cannula for ivt injections (same coordinates as in anaesthetized rats) was implanted stereotaxically.

2.2.2. **Intrahippocampal administration**

For recording of the EEG responses of the hippocampus, an electrode was implanted in the CA2 area. A steel cannula for ihp injections (same coordinates as in anaesthetized rats) was implanted by means of a stereotaxic instrument. ECoG, EEG and MC of the submandibular- and neck muscles were recorded.

All rats were allowed at least a 7-day recovery period before the experiments started. A new hydraulic system provides the possibility of administration of drugs in unrestrained rats, with full external control upon the rate of flow, frequency and volume of drug injection. This system consists of a microsyringe (max volume 8 µl) and a connector base. The cap of the microsyringe is divided by a rubber diaphragm into two parts. The upper part is attached to a polyethylene tube filled with hydraulic oil. This tubing is used to connect the injection assembly with the injection machine microdriver. The microinjection system can be placed in the cavity of the connector base, which is cemented to the skull. In addition, the connector base is also used as a plug for maximal 7 electrode leads. Via the connector base, the rat was attached to the cable connector, for recording electrographic responses. Microinjections of the drug solution into the lateral ventricle or hippocampus was achieved by turning the microdrive.

2.3. *Statistics*

Statistical evaluation of drug-induced MC, exceeding 100 μV and EEG spikes, exceeding 400μV, was performed by the Mann-Whitney U test.

2.4. *Drugs*

The synthetic peptide, *morphiceptin* (tyr-pro-phe-pro-NH_2, Peninsula Lab.), which is an amide derivate of β-casomorphine-4 and the hexapeptide, *δ receptor activating peptide* (D-tyr-ser-gly-phe-leu-thr, DSTLE, Peninsula Lab.), were dissolved in artifical cerebrospinal fluid (CSF). The substances and the control injections were in a volume of 1-2 μl (dose-range 4.65-74.2 nmol), administered ivt over 5-10 sec. For ihp administration a dose of 18.6 nmol/0.5 μl of the drugs was given.

ICI 154,129, N,N-Bisallyl-tyr-gly-gly-ψ-(CH_2S)-phe-leu-OH, was dissolved in CSF and injected ivt in a dose range of 18.6-74.4 nmol.

ICI 174,864, N,N-diallyl-tyr-aib-aib-phe-leu-OH (aib: α-aminoisobutyric acid) was dissolved in CSF and injected ivt in a dose range of 2.2-37.2 nmol.

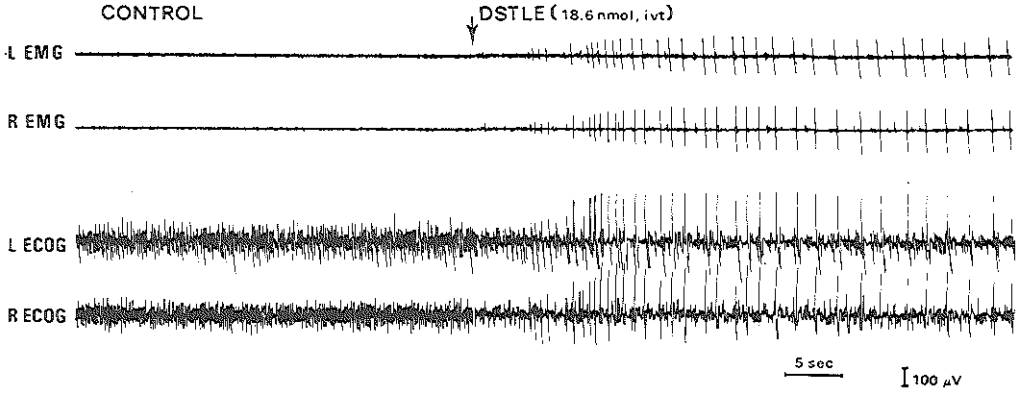

Fig.1. Left (L) and right (R) electromyogram (EMG of the submandibular muscles) and electrocorticogram (ECoG) of an anaesthetized rat, before and after intraventricular (ivt) administration of δ receptor peptide (DSTLE, 18.6 nmol/1 μl). Note the short onset of epileptic phenomena after DSTLE injection.

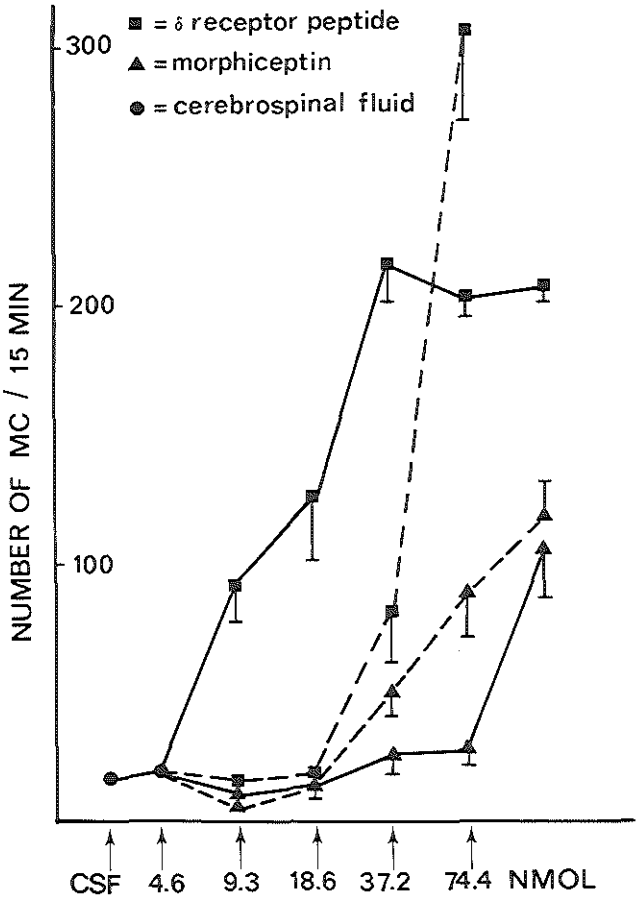

Fig.2. Myoclonic contractions (MC) recorded electromyographically in anaesthetized (___) and free-moving (---) rats, after intraventricular (ivt) administration of cerebrospinal fluid (CSF, 2 µl,) followed by δ receptor peptide (■) or morphiceptin (▲). Vertical line indicates the number of MC, obtained during the first 15 min after ivt drug administration. The horizontal line represents the different doses of δ receptor peptide or morphiceptin in nmol. Each point indicates the mean value of 8 experiments. Vertical bars denote SEM. Note the significant increase of δ receptor peptide-induced MC, compared to the effects induced by morphiceptin.

3. RESULTS

3.1. *Intraventricular administration*

In anaesthetized rats, DSTLE (4.65–18.6 nmol) induced within 0.5–1 min, an intensive and dose–related increase of MC of the submandibular muscles, associated with epileptic discharges in the ECoG (Fig.1). Further increase of the dose (37.2 and 74.4 nmol) was not followed by an increase of response (Fig.2). The most potent dose of DSTLE (18.6 nmol) in inducing the MC, was chosen for further experiments. Similar administration of DSTLE (4.65–37.2 nmol, ivt) in free–moving animals induced a dose–related increase of ECoG epileptic spikes and MC (Fig.2). A high dose of DSTLE (37.2 nmol) induced additional severe wet dog shakes (WDS), associated with epileptic bursts in the ECoG and "falling down" of the rat (Fig.3). The epileptic burst was followed by postictal ECoG depression for about 0.5–1 min. Further increase of the dose (74.4 nmol) appeared to be lethal in 3 out of 5 animals and was therefore not included in Fig.2.

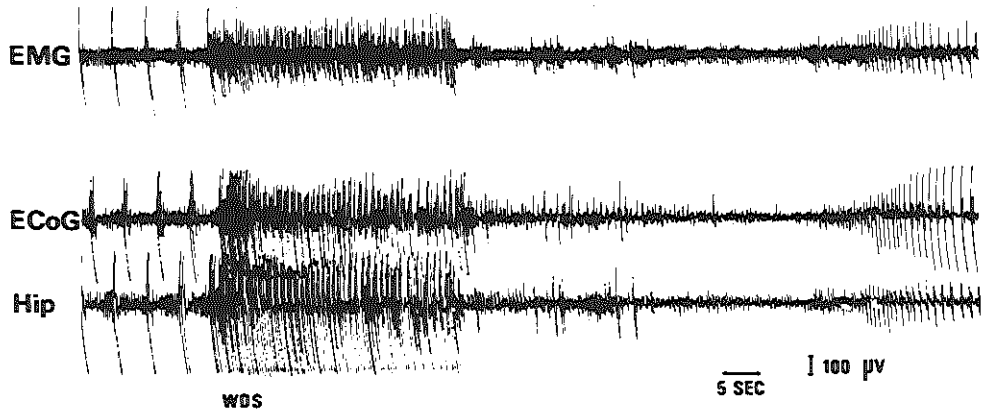

Fig.3. EMG, ECoG and hippocampal EEG (hip) 3 min after administration of δ receptor peptide (DSTLE, 20 µg, ivt). The epileptic burst was correlated with wet dog shakes (WDS). Note the subsequent postictal depression of the excitatory activity.

Lower doses of morphiceptin (4.65-18.6 nmol) injected in anaesthetized or free-moving rats, induced neither MC nor ECoG epileptic spikes, while higher doses (37.2-74.4 nmol) resulted in a slight increase of MC and ECoG discharges (Fig.2). Urethane facilitated the DSTLE-induced MC, within a certain dose-range (Fig.2). However, no significant differences could be observed after administration of different doses of morphiceptin in anaesthetized and free-moving animals

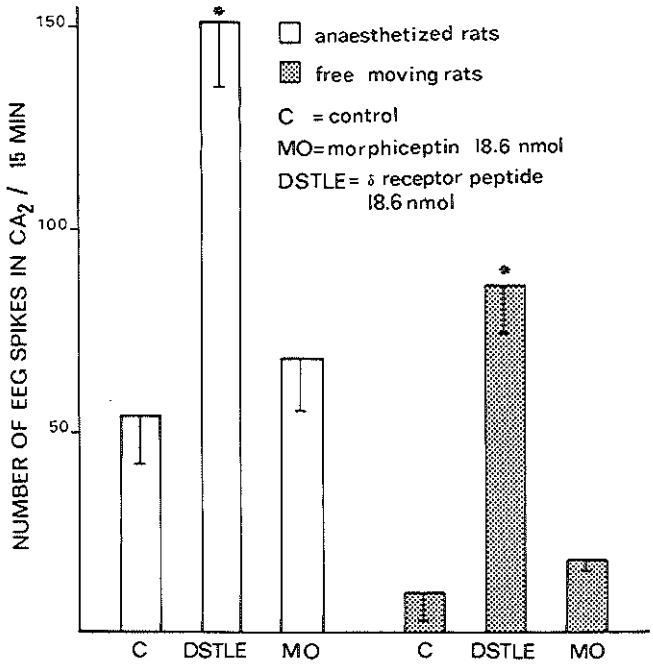

*Fig.4. Effects of intrahippocampal (CA2) injection of δ receptor peptide (DSTLE) and morphiceptin (MO) in anaesthetized and free-moving rats. The vertical line indicates the number of epileptic discharges in the CA2 hippocampal area during the first 15 min after administration of cerebrospinal fluid (C, 2 μl), δ receptor peptide (DSTLE, 18.6 nmol) and morphiceptin (18.6 nmol). Vertical bars denote SEM (n=6). * means significant difference with respect to control (p<0.01). Note the higher number of spontaneous epileptic spikes in the CA2 area of the hippocampus in urethane-anaesthetized rats, compared to the free-moving animals and the significant differences in inducing EEG epileptiform phenomena between the equimolar doses of the μ and δ opiate receptor agonists, compared to the control.*

3.2. *Intrahippocampal administration*

DSTLE (18.6 nmol) administered in anaesthetized and free-moving rats, showed a pronounced increase of epileptic discharges in the hippocampal EEG (CA2 area), ECoG and submandibular MC, while an equimolar dose of morphiceptin had no significant effect on these parameters. The difference between DSTLE and morphiceptin on hippocampal spiking is demonstrated in Fig.4. The number of DSTLE-induced neuronal discharges (hippocampal or cortical spikes) were immediately followed by a same number of MC. All these three excitatory phenomena gradually declined and disappeared within 30-60 min. In free-moving animals, DSTLE induced significantly less wet dog shake phenomena in comparison to ivt administered DSTLE. The hippocampal epileptic discharges induced by DSTLE were also facilitated by urethane anaesthesia (Fig.4).

3.3. *Pretreatment with morphiceptin*

Morphiceptin (9.3 and 18.6 nmol), administered ivt in anaesthetized rats or ihp in anaesthetized and free-moving animals, 15 min prior, significantly inhibited the number of MC and epileptiform discharges induced by DSTLE (18.6 nmol). The inhibitory effect of morphiceptin on DSTLE-induced MC is demonstrated in Fig.5. Pretreatment with morphiceptin (37.2 nmol) injected ivt in free-moving rats, 15 min prior, blocked the MC and wet dog shakes, induced by an equimolar dose of DSTLE.

3.4. *Pretreatment with δ opiate antagonists*

ICI 154,129 injected 10 min prior, was effective in inhibiting the DSTLE (18.6 nmol)-induced epileptic phenomena only in high doses (46.5-74.4 nmol) (Table 1). The antagonist caused initial spike activity in the ECoG, but no MC, in the first 30 sec after application.

Low doses (2.2-4.5 nmol) of ICI 174,864, injected 10 min prior, inhibited the intensity and duration of the MC and the correlated epileptic discharges in the ECoG, induced by DSTLE (Table 1.). Administration of ICI 174,864 (9.3-18.6 nmol) resulted in a depression of amplitude of the ECoG activity for over 2 hours. In this period of time DSTLE (18.6 nmol) did not induced any MC or epileptic discharges

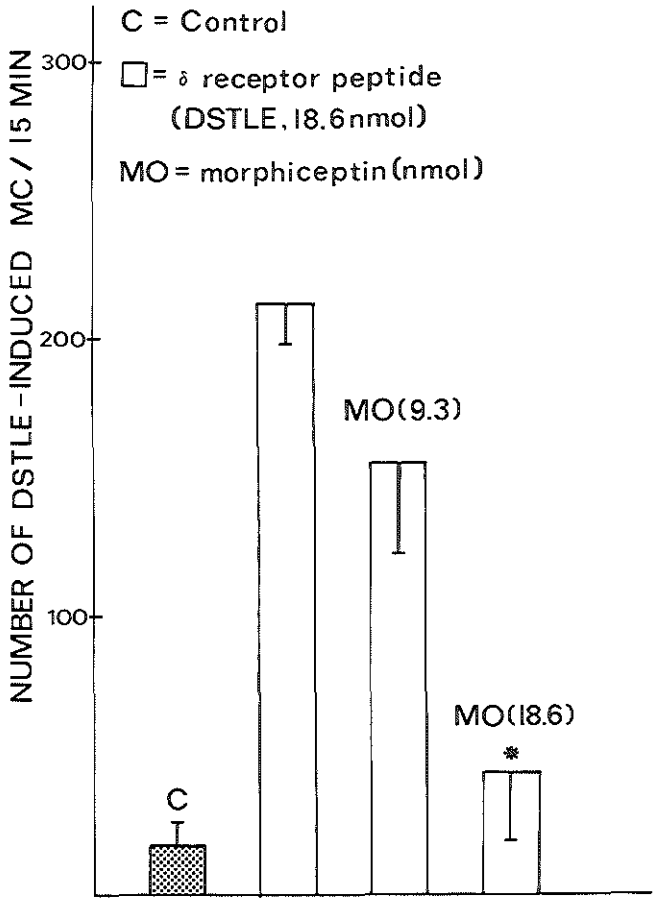

*Fig.5. Interactions between morphiceptin and δ receptor peptide in urethane-anaesthetized rats. The vertical line indicates the number of electromyographically recorded myoclonic contractions (MC) obtained during the first 15 min after intraventricular (ivt) administration of cerebrospinal fluid (2 µl, C) and DSTLE (18.6 nmol). Morphiceptin (9.3 or 18.6 nmol) was administered ivt 15 min before DSTLE. Vertical bars denote SEM (n=8). * means significant difference from DSTLE (p<0.01). Note the dose-related inhibition of DSTLE-induced MC by morphiceptin.*

(Fig. 6). A high dose of this antagonist (37.2 nmol) inhibited ECoG activity for at least 15 min and ECoG was practically without wave of spike activity ("ECoG silence") and very weak for the next hour, while breathing was normal.

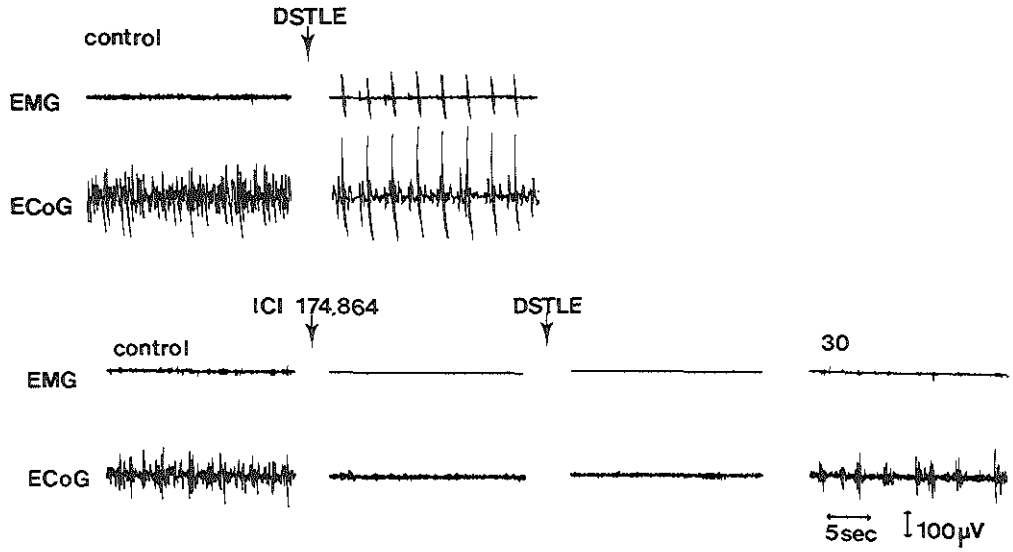

Fig.6. EMG of the submandibular muscles and the ECoG before and after intraventricular administration of DSTLE (18.6 nmol) and ICI 174,864 (9.3 nmol). Note the depression of amplitude of the ECoG induced by ICI 174,864.

Table 1. Myoclonic contractions correlated with ECoG epileptic spikes (mean ± SEM) induced by intraventricular (ivt) administration of the δ opiate agonist, DSTLE (18.6 nmol) and after pretreatment with different doses of the δ opiate antagonists, ICI 154,129 and ICI 174,864. The antagonists were injected ivt 10 min prior to DSTLE.

	$\bar{X}$ ± SEM
DSTLE (18.6 nmol, ivt)	193 ± 13
Pretreatment	
ICI 154,129 (ivt)	
18.6 nmol	183 ± 25
37.2 nmol	181 ± 9
46.5 nmol	117 ± 8*
74.4 nmol	49 ± 7*
ICI 174,864 (ivt)	
2.2 nmol	15 ± 6*
4.5 nmol	23 ± 9*
9.3 nmol	no DSTLE-induced MC
18.6 nmol	no DSTLE-induced MC
37.2 nmol	ECoG silence

*: significant difference compared to DSTLE-induced MC ($P<0.001$, n=6)

4. DISCUSSION

The specific δ opiate receptor agonist, DSTLE, administered ivt, induced a dose-related increase of MC and EEG epileptiform discharges in anaesthetized and free-moving rats.

This is in contrast to the specific μ agonist, morphiceptin, which was significantly less potent in inducing MC, unless high doses, compared to the effective doses of DSTLE, were administered. This indicates that μ receptors are probably not involved in the seizure phenomena. Furthermore, low doses, in comparison with the effective dose of DSTLE, of the specific δ opiate receptor antagonist ICI 174,864 inhibited the epileptic phenomena induced by DSTLE. This is consistent with the suggestion that δ opiate receptors are involved in the endorphin-induced seizures (Frenk et al., 1978; Dzoljic, 1982; Dzoljic and vd Poel-Heisterkamp, 1982). From autoradiographic studies and EEG recordings, we suggested that the hippocampus can be held responsible for the triggering of endorphin-induced

seizure phenomena (Chapter V). This is favoured by the demonstration of a relatively high density of opiate receptors in the hippocampus, specifically in the pyramidal cell layer (Meibach and Maayani, 1980) and enkephalin-containing fiber-systems in this brain region (HÖkfelt et al., 1977; Rossier and Bloom, 1980). Furthermore, it is known that opioids excite the hippocampal pyramidal neurons (Zieglgänsberger et al., 1981), which is in contrast to the opioid-induced depression of neurons in other brain regions (Illes, 1982). Therefore, we decided to administer the selective opiate receptor agonists in the hippocampus as well. The CA2 area of the hippocampus was selected because of the highest density of opiate binding sites (Meibach and Maayani, 1980) and the relatively equal distribution of μ and δ opiate receptors, in comparison to other hippocampal regions (Duka et al., 1981).

Local administration of DSTLE induced a significant increase of epileptic discharges in the CA2 area, while an equimolar dose of morphiceptin had no pronounced effect. This fact supports the idea of the involvement of δ opiate receptors in the generation of epileptiform phenomena. Pretreatment with morphiceptin, inhibited the stimulatory δ receptor mediated effect of DSTLE in anaesthetized and free-moving rats. This might be due to the fact that morphiceptin acts as a partial agonist for the δ receptor system (Day et al., 1981; Chang et al., 1982). Increase of MC, in this study, following administration of high doses of morphiceptin, is probably also a result of the δ opiate receptor stimulation. However, stimulation of the μ opiate receptors by morphiceptin, had a pronounced anticonvulsive effect, which supports the hypothesis about the proconvulsant δ receptors and anticonvulsant μ opiate receptors (Dzoljic, 1982).

Pretreatment with high doses of ICI 154,129 resulted in an inhibition of the MC and spike activity induced by the δ agonist. These findings are consistent with those of Tortella and coworkers (1984), who demonstrated that ICI 154,129 raises the seizure threshold in rats but at high doses exhibit μ agonist properties. The low potency of this substance at the δ receptor (Cotton et al., 1984) has limited its value as a pharmacological tool and the high doses needed to inhibit the DSTLE-induced epileptic phenomena might as well be a result of an activation of the anticonvulsive μ opiate receptors, instead of a specific antagonism at the δ receptor

site. In contrary, low doses of ICI 174,864 were effective in blocking the epileptic phenomena, while high doses resulted in a complete depression of ECoG activity. These results indicate that this substance is pharmacologically a more potent δ antagonist compared to ICI 154,129.
Intraventricular administration of DSTLE in free-moving animals, resulted in severe wet dog shakes, falling down and an excited behaviour. In contrary, a local injection of the δ agonist in the CA2 area of the hippocampus, induced less or no wet dog shakes. This difference in activity might be due to the fact that after ivt administration of DSTLE, a higher number of δ opiate receptors are activated.
Related to the interactions with anaesthesia, it was observed that urethane facilitated the DSTLE-induced MC. The reason for this stimulatory effect of urethane on enkephalin effects is not clear, but a possible additional release of endorphins during anaesthesia (Berkowitz et al., 1976) might be one of the explanations. Other data indicate that the effects of morphine can either be inhibited or stimulated in anaesthetized rats in comparison to free-moving animals (Urca and Liebeskind, 1979; Linseman, 1980). This study demonstrates that the effects of opioid peptides can also be significantly modulated by urethane anaesthesia, which indicates the necessity of parallel study in free-moving animals and which has to be taken into account in clinical circumstances, when administering opiate-like substances to post-operative patients.
In conclusion, this study with relatively specific opiate agonists and antagonists suggests that the epileptiform activity of opioid peptides is mainly due to an activation of δ opiate receptors.

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CHAPTER VII.

METABOLIC RATE IN DIFFERENT RAT BRAIN AREAS DURING SEIZURES INDUCED BY A SPECIFIC DELTA OPIATE RECEPTOR AGONIST

SUMMARY

The glucose utilization during specific δ opiate agonist-induced epileptiform phenomena, determined by the [^{14}C]2-deoxyglucose technique (2-DG) was examined in various rat brain areas at different time intervals. The peak in EEG spiking response and the most intensive 2-DG uptake occured 5 min after ivt administration of the δ opiate receptor agonist. The most pronounced 2-DG uptake at this time interval could be observed in the subiculum, including the CA1 hippocampal area, frontal cortex and central amygdala. A general decrease of glucose consumption, compared to control values, was observed after 10 min, in all regions with exception of the subiculum. Since functional activity and 2-DG uptake were correlated, we suggest that the subiculum and/or CA1 area, are probably the brain regions most involved in the enkephalin-induced epileptiform phenomena.

1. INTRODUCTION

There is evidence that endorphins may play a role in epileptogenesis (Urca et al.1977; Dzoljic and vd Poel-Heisterkamp, 1982). The target area of this action seems to be the limbic system (Henriksen et al., 1982; Illes, 1982), and specifically the hippocampus (French and Siggins, 1980, Chapters V and VI). The increase of neuronal excitation caused by opiates in the hippocampal area is of particular interest since opiates have been found to depress neurons in other brain regions (Illes, 1982).

Because functional activity and energy metabolism appear to be closely related in the nervous system (Sokoloff, 1977), local alterations in glucose utilization accompany and reflect local changes in neuronal activity in the rat brain (Kennedy et al., 1975). Studies using the [^{14}C]2-deoxyglucose histochemistry with intracerebroventricular (ivt) β-endorphin injection, show the most dramatically enhanced metabolic activity in the ventral hippocampus and the entorhinal cortex, thus

favouring these structures as the possible sites of origin for the drug-induced epileptiform activity (Henriksen et al., 1982).

Using a modification of Sokoloff's technique (Meibach et al., 1980) and a method for isolated removal of brain tissue (Palkovits, 1973), we selected some rat brain areas, which from literature and our previous studies appeared to be involved in the neuronal excitability after ivt administration of enkephalins (Frenk et al., 1978; French and Siggins, 1980; Chapters V and VI). In the present study we utilized these methods to ascertain what changes occur in the metabolic rates of different regions in the CNS, during opioid-induced seizures. Therefore, we compared the glucose uptake in some rat brain areas, in function of time, during a state of normal neuronal excitability and during δ receptor peptide (Gacel et al., 1980) (DSTLE)-induced epilepsy. DSTLE, as an δ opiate receptor agonist is selected since the epileptiform phenomena are proposed to emerge from δ opiate receptor stimulation (Chapter VI) and probably not from the activation of μ receptors (Frenk et al., 1978; Snead and Bearden, 1980).

2. MATERIALS AND METHODS

2.1. *2-Deoxyglucose uptake.*

24 Male Wistar rats (175-200 g) were anaesthetized by Hypnorm (fluanison/fentanyl base, Duphar, 0.4 ml/100 g, s.c.). A steel cannula for ivt injections (coordinates: AP -0.1 mm, L -1.5 mm, H -2.0 mm from dura, with bregma 0) (König and Klippel, 1963) was implanted stereotaxically. Each canulla implantation was checked with a positive passage of artifical cerebrospinal fluid (CSF) into the ventricle. All rats were allowed a recovery period of at least 7 days. Intraventricular injections were made by a 5 μl Hamilton syringe.

The 2-deoxyglucose (2-DG) experiments were performed according to the method of Meibach et al. (1980).

The experimental paradigm consisted of ivt injections of DSTLE (10 μg/2μl), 0, 2.5, 5 and 10 min preceding the intravenous administration of 2-D (10 μCi/100g). Control animals received CSF (2 μl, ivt)

instead of DSTLE.

30 min after the 2-DG injection, the cannula was withdrawn and the animal decapitated. The skull was opened, the brain carefully removed, frozen in dry ice and stored at −70 °C before sectioning.

The brain was cut in 300 µm serial sections in a cryostat, maintained at −20 °C. Brain nuclei were punched with hollow needles, according to Palkovits (1973). Nine individual brain regions were taken from each rat: frontal cortex (FC), parietal cortex (PC), subiculum, including CA1 (S), dentate gyrus (GD), CA3 hippocampal area, central amygdaloid nucleus (ac), cortical amygdaloid nucleus (aco), lateral septal nucleus (SL) and the nucleus parafascicularis thalami (pf).

Tissue pellets were homogenized in 100 µl of distilled water. An aliquot of 10 µl was taken in duplicate for the measurements of proteins (Lowry et al., 1951).

Radioactivity was determined of two aliquots of 40 µl from the homogenate by liquid scintillation counting. Results are given in pmol/100 µg protein.

Statistical evaluation was performed by the Mann-Whitney U-test.

2.2. *Electroencephalographic recording*

Six male rats (Wistar strain, 175-200 g) were anaesthetized by urethane (1.2 g/kg, i.p.). A tracheal canulla was inserted. A steel lOcannula for ivt injections and electrodes into the subiculum (AP +3.6 mm, L +0.4 mm, H −3.1 mm from dura), lateral septum (AP +1.1 mm, L +1.2 mm, H −4.6 mm from dura) and central amygdala (AP +0.3 mm, L +4.1 mm, H −7.1 mm from dura with bregma 0) (König and Klippel, 1963) were implanted stereotaxically. In addition the electrocorticogram of the frontal and parietal cortices was recorded by means of a polygraph Grass model 7. The regions were selected on basis of the maximal and minimal metabolic rate changes during DSTLE-induced epilepsy. Rectal temperature was maintained between 36.5 and 37.5 °C with a warm light. After the experiment, the placement of the electrodes was checked histologically.

2.3. *Drugs*

2-[1-^{14}C]Deoxy-D-glucose (2-DG; New England Nuclear).

2-DG, 51.1 mCi/mmol, suspended in ethanol-water (9:1) was placed in a

vial and the ethanol medium slowly evaporated with a gentle stream of gaseous nitrogen. The isotope was diluted in sterile 0.9% saline (250 µCi/2.5 ml). The solution was injected intravenously.

δ receptor peptide.

(D-tyr-ser-gly-phe-leu-thr, DSTLE, Peninsula Lab.) was dissolved in CSF (10 µg/2 µl) and administered intraventricularly.

3. RESULTS

3.1. *2-DG uptake.*

Simultaneous injection of DSTLE (10 µl/2 µg, ivt) and 2-DG (10 µCi/100 g, i.v.) resulted in an increase of 2-DG uptake in the frontal cortex, subiculum/CA1 area and the cortical amygdala, compared to the controls, which received CSF, 2 µl (Table 1, "0 min"). In the other selected brain regions, such as the central amygdala, parietal cortex, lateral septal nucleus, gyrus dentatus, CA3 area and the n. parafascicularis thalami, no differences in the glucose uptake could be observed. DSTLE administration, 2.5 min prior to the 2-DG pulse resulted in a general increase of the uptake in all regions, most pronounced in the frontal cortex, subiculum/CA1 and the cortical amygdala. Less pronounced uptake of 2-DG occured in the parietal cortex, dentate gyrus and CA3. No significant increase could be detected in the lateral septum, central amygdala and the parafascicular nucleus. DSTLE injection, preceding by 5 min, the 2-DG administration, induced a further significant increase of glucose utilization in the frontal cortex, subiculum and central amygdala. However, a tendency for a decrease in 2-DG uptake could already be observed in all other areas with exception of CA3 (Table 1).

10 min after the DSTLE injection, glucose utilization decreased to control values in all brain regions with exception of the subiculum.

In the n.parafascicularis and the lateral septal nucleus, the 2-DG uptake was not significantly affected by DSTLE. Because of this general decrease after 10 min, no further experiments, with longer time intervals between the DSTLE and the 2-DG injection, were carried out.

Table 1.Mean values +S.E.M. of 2-DG uptake.
[^{14}C]2-deoxyglucose (2-DG, 10 µCi/100 g, i.v.) uptake in various rat brain areas of control animals (cerebrospinal fluid, CSF, 2 µl, ivt) and at different time intervals after δ receptor peptide (DSTLE, 10 µg/2 µl, ivt) administration. Note the significant increase of the 2-DG uptake after DSTLE administration in the frontal cortex (FC), subiculum, including CA1 (S) and central and cortical amygdala (ac, aco). A less pronounced increase of the 2-DG uptake can be observed in the dentate gyrus (GD), CA3 hippocampal region and parietal cortex (PC). The 2-DG uptake in the lateral septal nucleus (SL) and n.parafascicularis thalami (pf) was not significantly changed by DSTLE injection. 10 min after DSTLE application a general decrease of the 2-DG uptake occurs. Significant difference compared to controls: ** p<0.001 and * p<0.05.

Brain area	*CSF Controls*	*Time intervals between DSTLE and 2-DG injection*			
		0 min	*2.5 min*	*5 min*	*10 min*
FC	22.7 ± 2.5	30.0 ± 1.2**	34.0 ± 4.6**	37.4 ± 2.9**	25.9 ± 2.3
PC	25.3 ± 4.6	24.1 ± 3.6	32.2 ± 3.8*	24.5 ± 2.8	24.9 ± 2.9
S	17.0 ± 1.7	22.5 ± 1.5*	29.6 ± 2.1**	46.8 ± 2.2**	26.1 ± 1.6**
GD	17.3 ± 0.9	16.7 ± 1.3	24.5 ± 2.2*	20.3 ± 2.8	19.9 ± 0.3
CA3	18.4 ± 3.9	20.4 ± 2.0	25.5 ± 3.0*	26.9 ± 6.7	20.7 ± 0.7
ac	16.6 ± 2.6	16.2 ± 1.5	20.3 ± 1.4	30.8 ± 2.6**	14.2 ± 1.6
aco	23.7 ± 4.6	31.4 ± 1.1**	31.7 ± 4.3**	29.2 ± 3.2	20.1 ± 1.8
SL	18.6 ± 2.7	16.6 ± 1.7	22.1 ± 2.9	14.9 ± 9.6	15.9 ± 3.5
pf	20.5 ± 1.8	21.8 ± 0.8	26.1 ± 3.9	20.0 ± 7.9	21.2 ± 2.9

3.2. *Electroencephalographic (EEG) recording.*

During the EEG recordings in the frontal and parietal cortices, the subiculum, the central amygdala and the lateral septum, an onset period for the DSTLE-induced epileptic discharges of about 30 s was observed in the subiculum (Fig.1). These excitatory phenomena appeared a few seconds later in the central amygdala and frontal/parietal cortex.

In all these regions, with exception of the lateral septum, the intensity of the spiking exceeded 400 µV within 1 min after DSTLE administration. However, the most intensive and long-lasting spiking was observed in the subiculum and central amygdala.

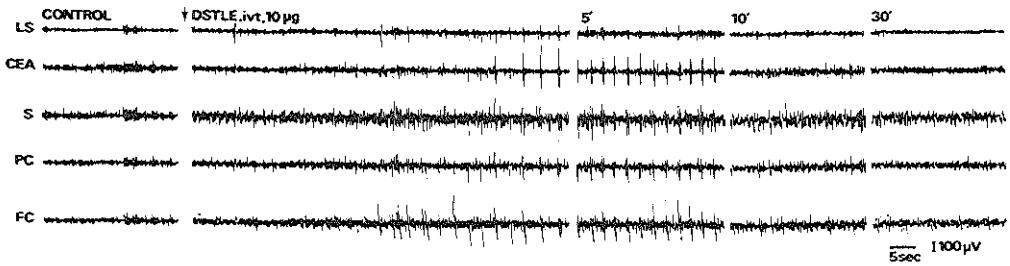

Fig.1. Electroencephalographic responses of the lateral septum (LS), central amygdala (CEA), subiculum (S), parietal cortex (PC) and frontal cortex (FC), before and after intraventricular (ivt) administration of δ receptor peptide (DSTLE). Note the unequal spiking activity in the various brain regions at the different time intervals.

4. DISCUSSION.

The EEG responses and the results of the 2-DG uptake in this study, indicate that the frontal cortex, central amygdala and the subiculum/CA1 region, are the main areas involved in the DSTLE-induced epileptic phenomena. These results are generally in accordance with the [^{14}C]2-DG autoradiographic study of Henriksen et al. (1982), who demonstrated a marked increase of 2-DG uptake in the amygdalo-hippocampal area after ivt administration of β-endorphin. Of particular interest is the subiculum, which in this study, included the pyramidal cell layer of the CA1. In this area the intensity and duration of the increased 2-DG uptake was most pronounced. The electrophysiological responses of this hippocampal region showed the shortest onset of DSTLE-induced epileptiform discharges as well as long-lasting EEG spike activity.

Based on electrophysiological studies, it is suggested that opiate evoked epileptiform activity in the limbic system arises from pyramidal cell

activity in the hippocampal formation (French and Siggins, 1980; Zieglgänsberger et al., 1979). Thus, the hippocampus and particularly the subiculum and/or CA1 area are the probable trigger-zones for seizures induced by an ivt administration of enkephalins. It is possible that these regions play an important role in the opiate-modulated neuronal excitability also in physiological and/or pathological conditions in humans. Other hippocampal areas, like CA3 and the dentate gyrus, showed only a moderate and gradually increase of the 2-DG uptake after DSTLE injection. Although the uptake was significant, it was less intensive compared to the glucose utilization in the subicular region during the epileptic phenomena.

In the lateral septum, we did not find an increase of 2-DG uptake after DSTLE application, which is in contrast to the data of Henriksen et al. (1982). This controversy might be due to the different activities of β-endorphin and the enkephalin-analogue, the δ opiate receptor agonist, DSTLE. However, of particular importance is the fact that in this study the lower level of energy metabolism correlated with the short-lasting and less intensive epileptic discharges in the EEG, compared to the responses of other brain areas.

Increase of glucose utilization in the cortical and central nucleus of the amygdala might be due to an activation of the central nucleus following stimulation of different parts of the subiculum. Pathways connecting these structures have been demonstrated by Watson et al. (1983). Furthermore, the central nucleus of the amygdala is rich in enkephalin-containing fiber systems and opiate receptors (Meibach and Maayani, 1980). The peak of the 2-DG uptake in the cortical amygdaloid nucleus occured at about 2.5 min after DSTLE administration. However, for the central nucleus the peak appeared 2.5 min later. Possibly, the activation pathway runs from the cortical to the central nucleus of the amygdala.

The increase of glucose uptake in the frontal cortex after DSTLE application is probably a reflexion of the excitation of the hippocampal area. Namely, using the 2-DG technique, pronounced labelling of the frontal cortex following elicitation of seizures in the hippocampus has been demonstrated (Watson et al., 1983).

Based on these results, we suggest that the hippocampal formation but

particularly the subiculum and/or CA1 are the probable trigger zones for seizures induced by ivt administration of endorphins. Furthermore, the results indicate a strong relationship between the electrophysiological recordings, the functional activities and glucose utilization/energy metabolism in different rat brain areas, which also supports our earlier observations concerning the brain regions involved in the endopioid-induced epilepsy (Chapters V, VI).

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CHAPTER VIII.

REGIONAL CEREBRAL BLOOD FLOW DURING ENKEPHALIN-INDUCED SEIZURES IN THE RAT

Summary
Blood flow determined by the radioactive microsphere technique during epileptiform seizures induced by D-tyr-ser-gly-phe-leu-thr (DSTLE), a specific δ opiate agonist, was examined in different rat brain areas at various time intervals.
An increase in blood flow to the hippocampus and the brain stem was observed 2.5 min after the administration of DSTLE into the left lateral ventricle. An additional flow increase occured in the striatum and cerebellum 2.5 min later (5 min after the injection), at which time both the neural and vascular effects of the drug were most marked. Ten min after the drug administration, cerebral blood flow in all regions, except the hippocampus, returned to the respective baseline values. Since the time course and the magnitude of functional activity and blood flow in the hippocampus show a good correlation, it is suggested that this brain region may play an essential role in triggering and maintaining the enkephalin-induced epileptic phenomena.

1. INTRODUCTION.

Accumulating evidence suggests that the epileptiform activity of the opioid peptides is mainly due to an activation of the δ opiate receptors (Chapter VI). Several studies have demonstrated that while the opioid peptides inhibit neuronal firing in most brain areas (Illes, 1982) they excite the hippocampal pyramidal cells (Zieglgänsberger et al., 1979). Indeed, using the 2-deoxyglucose method (Sokoloff, 1977), we showed that intraventricular (ivt) injections of D-tyr-ser-gly-phe-thr (DSTLE, Chapter VII), a peptide with specific δ opiate receptor agonist activity (Gacel et al., 1980), and of β-endorphin (Henriksen et al., 1982), significantly increase the metabolic activity of the hippocampal area, thus favouring this structure as the possible site of origin of the epileptiform activity (Henriksen et al., 1982; Chapter VII).
Changes in neuronal activity are also reflected by alterations in local cerebral blood flow (LCBF).

In patients with focal cortical epilepsy a marked increase in blood flow occurs during seizures in areas presumed to participate in the seizure activity (Meyer et al., 1966; Hougaard et al., 1976). Increases in cerebral metabolism concomitant with blood flow changes, have also been frequently demonstrated in experimental animals, during epileptic seizures induced by agents, such as pentylenetetrazol and by electroconvulsive stimulation (Plum et al., 1968; Siesjö et al., 1980).

Therefore, in this study, we measured the blood flow in different areas of the rat brain before and during epileptic seizures induced by δ receptor peptide (DSTLE). The measurements have been made at such time intervals at which enhanced neuronal excitability and metabolism was demonstrated (Chapter VII).

2. MATERIALS AND METHODS.

2.1. *General.*

Male albino rats of Wistar strain, weighing 300-350 g, were obtained from TNO Central Breeding Institute, Zeist, The Netherlands.

The animals were anaesthetized with urethane (1.2 g/kg, i.p.). The trachea was cannulated but the animals were allowed to breath spontaneously. A steel cannula was implanted stereotaxically into the left lateral ventricle (coordinates: AP −0.1 mm, L +1.5 mm, H −1.7 mm from dura with bregma 0) (König and Klippel, 1963) for intraventricular (ivt) injections. The position of the cannula was checked with an unrestricted passage of artifical cerebrospinal fluid (CSF) into the ventricle. A 5 µl Hamilton syringe was used for the ivt injections.

Subsequently, the left femoral artery was cannulated with an appropriate polyvinyl catheter to record arterial blood pressure with a Statham P 23AC transducer on a model 7 Grass polygraph. The same catheter was used for withdrawing arterial blood samples. Blood gases were measured with an ABL-2 (Radiometer, Copenhagen).

2.2. *Regional cerebral blood flow measurements.*

The radioactive microsphere technique was used for the measurements of regional cerebral blood flows in rats (McDevitt and Nies, 1976). The left ventricle of the heart was cannulated via the right common carotid artery with a thin polyvinyl catheter. A suspension of about 200.000 spheres (15 ±2[SD] µm; NEN, Dreieich; West-Germany), labelled with either ^{141}Ce, ^{103}Ru, ^{113}Sn or ^{95}Nb, in 0.2 ml of 0.9% saline containing a drop of Tween 80, was slowly injected via the cannula into the heart.

Starting 5 sec before the injection of the microspheres, arterial blood was withdrawn (rate: 0.5 ml/min) from the left femoral artery over a period of 90 sec for determining the radioactivity.

At the end of each experiment, the animal was killed by decapitation. The skull was opened and the brain carefully removed and dissected into the following areas: the cerebral cortex, striatum, hippocampus and thalamus of both the left and right sides, the septo-hypothalamic area, cerebellum and brain stem. The various tissue samples were weighed and subsequently placed in plastic vials. The radioactivity in the tissues and arterial blood samples was counted for 10 min in a γ-scintillation counter (Packard, model 5986) equipped with a multichannel analyzer using suitable windows for discriminating the different isotopes used (Saxena et al., 1980).

2.3. *Calculations*

The microsphere and other data were processed by a PDP-11/70 computer using a set of specially developed programmes (Saxena et al., 1980)

Tissue blood flow ($\dot{Q}_{tis}$) was calculated by:

$$\dot{Q}_{tis}(ml.min^{-1}) = (I_{tis}/I_{art}) \times \dot{Q}_{art}$$

where I_{art} and $\dot{Q}_{art}$ represent arterial blood radioactivity ($c.min^{-1}$) and withdrawal rate ($ml.min^{-1}$), respectively, and I_{tis} is the radioactivity (c.p.m.) in a particular tissue sample (Johnston and Saxena, 1978). All blood flow values were normalized for 100 g tissue weight.

2.4. *Experimental protocol*

The first batch of microspheres was injected about 15 min after the completion of the surgery to measure the tissue blood flow at the baseline. Five minutes later each rat was injected with either 10 µg of DSTLE (dissolved in 2 µl artifical CSF) or the same volume of the vehicle into the left lateral ventricle. The rats were then divided into 6 groups (n=6 each) and a second batch of microspheres was given after either 2.5, 5 or 10 minutes following administration of DSTLE or CSF.

Just before the injection of microspheres, values of heart rate and arterial blood pressure were collected and arterial blood was withdrawn for the determination of blood gases.

2.5. *Neuronal activity*

In order to confirm the seizure activity of DSTLE, five rats belonging to the "10 min" series (see above) had been implanted at least 7 days before the actual experiment with suitable electrodes to record ECoG, hippocampal EEG and the electrical activity of the submandibular muscles (Chapter VI).

2.6. *Data presentation and statistical evaluation.*

All data have been expressed as mean ± S.E. of the mean. The values obtained at different time intervals after CSF administration have been placed together in the Tables. However, statistical comparison was always made between the values obtained at 2.5, 5 or 10 min after DSTLE administration and those obtained at the corresponding time intervals after injection of CSF, thus eliminating the influence of the microsphere injection. The changes in the haemodynamic variables from the baseline values were calculated in each experiment and the significance of these changes was determined by using the Wilcoxon matched-paired signed-rank test (Siegel, 1956). Furthermore, since corresponding experiments with CSF were conducted, the changes obtained at different time intervals after DSTLE administration were compared to those after CSF administraion using the Mann-Whitney U test (Siegel, 1956). Statistical significance was accepted at p-values of 0.05 or less (two-tailed).

Table 1. Blood gases and haemodynamic variables.
Mean values ± SEM of the heart rate (HR), mean arterial blood pressure (MBP) and arterial blood gases in rats before (baseline) and 2.5, 5 or 10 min after intracerebroventricular (ivt) injection of δ receptor peptide (DSTLE, 10 μg) or artifical cerebrospinal fluid (CSF, 2 μl).

			min after DSTLE 10 μg, ivt		
	baseline	CSF	2.5	5	10
pH	7.27	7.29	7.27	7.29	7.26
PCO2 (Hg mm)	43±2	43±2	41±1	41±1	41±3
PO2 (Hg mm)	112±3	117±43	115±2	109±3	117±5
S_{O_2} (%)	96±0.6	97±0.6	97±0.2	96±0.4	97±0.4
HR (beats/min)	347±7	340±20	317±24	330±19	333±21
MBP (Hg mm)	102±3	107±9	87±9*	75±7*	83±9*

S_{O_2}, haemoglobin saturation

* , change was significantly different when compared with the corresponding change in control (CSF) animals as well as from baseline values.

3. RESULTS

3.1. *Blood gases*

No changes were noticed in the arterial blood gases at different time intervals after CSF or DSTLE (Table 1).

3.2. *Blood pressure and heart rate.*

Baseline values of the arterial blood pressure and heart rate and the changes produced by DSTLE or CSF are given in Table 1. When compared with CSF, the DSTLE injection caused a significant decrease in mean arterial blood pressure, while there was only a slight initial decrease in the heart rate (statistically not significant). Compared to the baseline values, no differences of these haemodynamic variables occured after CSF administration.

Effect of intraventricular injection of CSF or DSTLE

As shown in Table 2, the distribution of the blood flow to the different brain structures was little affected by ivt administration of CSF (2 µl). However, the administration of DSTLE was associated with a significant increase of the blood flow to both left and right hippocampus within 2.5 min after the drug injection. This effect, which was more marked at the side (left) of drug administration, reached its peak at 5 min and was still noticeable 10 min after DSTLE injection. At the time of peak effect in the hippocampus, blood flow also increased in the striatum, cerebellum and brain stem.

Cerebral cortical blood flow was not significantly modified but, 5 min after injection, it was more in the drug-treated animals than in the animals given CSF. No changes were noticed in the lateral septum, hypothalamus and thalamus.

Table 2. Regional blood flow.
Mean values ± SEM of the blood flow ($ml.min^{-1}.100\ g^{-1}$) to various brain regions in the rat, before (baseline) and 2.5, 5 or 10 min after intracerebroventricular (ivt) administration of δ receptor peptide (DSTLE, 10 µg) or artifical cerebrospinal fluid (CSF, 2 µl).
Note the significant increase of the flow to the striatum, hippocampus cerebellum and brain stem ($p<0.05$, $n=6$ for each time period).

			min after DSTLE, 10 µg, ivt		
Regions	baseline	CSF	2.5	5	10
left cerebral cortex	50±2	48±3	47±3	55±2**	49±5
right cerebral cortex	46±4	48±7	41±6	49±6	49±4
left striatum	49±3	45±3	43±4	61±2*	47±6
right striatum	44±4	40±4	38±6	53±3*	46±4
lateral septum + hypothalamus	25±3	28±2	24±5	29±3	26±2
left hippocampus	40±4	46±2	77±9*	131±28*	61±9*
right hippocampus	33±4	31±1	49±6*	75±14*	46±6*
left thalamus	60±8	65±7	59±9	63±2	55±5
right thalamus	51±6	60±8	54±4	61±9	56±9
cerebellum	65±4	53±7	65±5	93±13*	63±2
brain stem	59±1	54±4	71±5*	78±9*	58±7
total brain	51±4	47±4	51±5	67±4*	55±5

* ,change was significantly different when compared with the corresponding change in control (CSF) animals as well as from baseline values.

**,significantly different only from control (CSF) values.

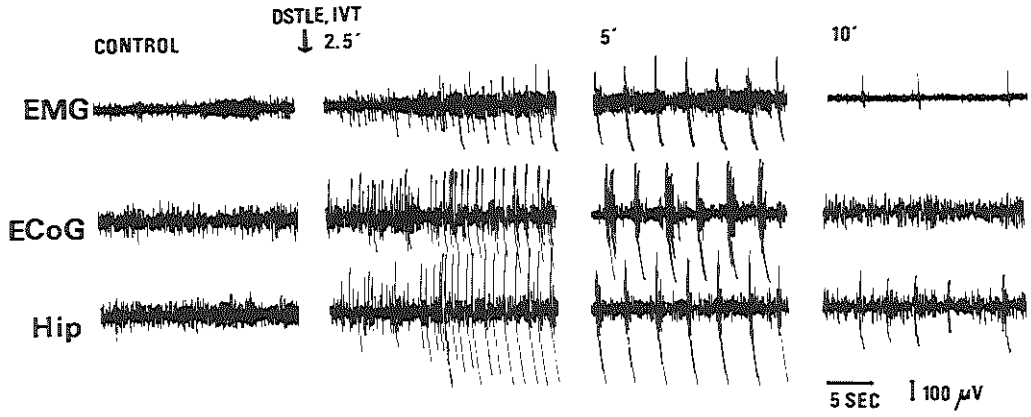

Fig.1. Electromyogram (EMG of the submandibular muscles), electrocorticogram (ECoG) and hippocampal EEG (Hip), before and after intraventricular (ivt) administration of δ receptor peptide (DSTLE 10 μg) in rat.

3.3. *Regional cerebral blood flow.*

Baseline values

Baseline values of the distribution of the carotid artery blood flow to the various brain structures are given in Table 2. Amongst the different regions of the brain, the blood flow to the cerebellum was the highest and that to the lateral septum and hypothalamus, the lowest. Furthermore, the left side of the brain areas received slightly, but consistently, higher blood flows than the corresonding areas of the right side. This variation may be due to mechanical irritation caused by the introduction of the cannula into the left lateral ventricle.

3.4. *Effect of DSTLE on neuronal activity*

Figure 1 shows the records from one of the five animals (10 min series) where neuronal activity was recorded. As has been reported earlier (Chapter VI), ivt administration of DSTLE increased the submandibular muscle activity (EMG) and caused epileptic discharges in the ECoG and hippocampal EEG. The effect was noticeable within 2.5 min and reached its peak spike activity at about 5 min. Though still noticeable, the above effects were reduced after 10 min. The hippocampal blood flow in this animal was 42 and 55 $ml.min^{-1}.100\ g^{-1}$, respectively at the baseline and 10 min after the administration of DSTLE.

4. DISCUSSION.

The administration of DSTLE significantly decreased the mean arterial blood pressure. This is in accordance with previous findings (Bolme et al., 1978) demonstrating a similar reduction of the arterial pressure after the administration of another putative δ opiate agonist, ($D\text{-}ala^2$, $D\text{-}leu^5$) enkephalin. Though the physiological role of the δ opiate receptors in the control of blood pressure is not well understood, it may be noted that ICI 154 129, a selective δ opiate antagonist (Shaw et al., 1982), can cause a transient increase in arterial blood pressure (Clark and Reid, 1984) .
Regional cerebral blood flow was not substantially altered by the administration of CSF but the increase of the flow to the different brain areas after DSTLE injection was clearly demonstrated and was more pronounced at the side where the drug was injected. The enhancement of cerebral blood flow after the administration of the δ opiate agonist occured within 2.5 min and reached its peak at about 5 min after the injection. This time course corresponds with that of the EEG epileptic spiking (Figure 1) and the increased deoxyglucose uptake (Chapter VII) after ivt administration of DSTLE. Amongst the brain regions, the blood flow to the hippocampus increased most intensively and for the longest period. This is in accordance with our earlier observations that ivt administered enkephalin reaches the hippocampus within 2.5 min (Chapter V), has the shortest onset of epileptiform discharges and a long lasting EEG

spike activity (Chapter V). Furthermore, the 2-deoxyglucose uptake, as a parameter for energy metabolism (Sokoloff, 1977), was significantly increased in the hippocampus after endorphin administration (Henriksen et al., 1982), specifically in the subicular/CA1 region (Chapter VII). The most intensive and long-lasting increase of the hippocampal blood flow after enkephalin application, as observed in this study, which apparently reflects an increase of the neuronal activity, suggests that this brain region plays an essential role in the opioid peptide-induced seizure phenomena.

The increase of the blood flow to the striatum 5 min after the enkephalin injection might be due to its periventricular position and the presence of δ opiate receptors (Duka et al., 1981). Furthermore, it is known that parts of the striatum are activated following electrical stimulation of the hippocampal formation (Watson et al., 1983). The brain stem also shows and increased blood flow after DSTLE administration. This brain area is rich of opiate receptors (Atweh and Kuhar, 1977) and the enkephalins are quickly distributed in the liquor space after an ivt injection (Chapter V), although an excitatory input to this area can not be excluded.

An increase of the blood flow to the rat cerebellum after enkephalin administration is an unexpected phenomenon, since this region has a very low density of opiate receptors (Atweh and Kuhar, 1977) and only the perikarya of the Golgi cells stain positively for enkephalin (Sar et al., 1978). A possible excitatory input via the mossy- and climbing fibers, originating from the perikarya located in the brain stem and spinal cord, to the cerebellum, might be a reason for the increased neuronal cerebellar activity associated with a rise in blood flow to this region. Unfortunately, the cerebellum was not included in our previous experiments where we studied the 2-deoxyglucose uptake during enkephalin-induced epilepsy (Chapter VII). This precludes a comparison between the observed flow increase after DSTLE administration and the possible rise of the metabolic rate in the cerebellum. Evidently, additional experiments are needed to elucidate the role of the enkephalins in the cerebellum, particularly in the light of various clinical evidences suggesting a strong relationship between the cerebellum and some types of epilepsy (Cooper, 1973; Wood et al., 1977).

Lastly, though we demonstrated a strong relationship between the electrophysiological recordings and energy metabolism in the cortical region (Chapter VII), in this study we did not observe a marked increase in blood flow to this region after administration of DSTLE. This discrepancy may be due to the nature of cortical tissues examined in the two studies. While we isolated the cortical areas for the measurements of deoxyglucose uptake (Chapter VII), the relatively poorer resolution of the radioactive microsphere technique for blood flow determination (Saxena et al., 1980), required us to examine large cortical areas which included a substantial amount of white matter. It is therefore quite likely that an increase in blood flow to the cortical nuclei may have been masked by the "over-all" cortical blood flow measured by us.

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SUMMARY AND CONCLUDING REMARKS

Part 1.

Before the major discoveries in the past decade, opiates were known to possess selective and unique pharmacological properties. To elucidate their role in relation to excitatory phenomena which they might induce, it is important to have a better understanding of the pharmacological and pharmacokinetic properties and the distribution of the opioids. Chapter 1 reviews the chemical relationships, biogenesis and degradation of the opioid peptides (enkephalins, endorphins and dynorphins). Of particular interest are the enzymes which are involved in their inactivation, since inhibitors of these enzymes can be considered as useful tools in pharmacological research. Enkephalin-like peptides are found at every level of the neuraxis, including cells in the cortex all the way to cells in the spinal cord. β-endorphin/ACTH related peptides are found in the medial basal hypothalamus and projects its fibers very widely, including many areas of the limbic system and brain stem. Dynorphins are found in the posterior pituitary and several other brain regions. These three opioid neural pathways are described in more detail in Chapter 1.

Opioid peptides exert their effects through binding with opiate receptors (Chapter II). This binding is stereospecific, saturable and reversible. The question arises whether or not the different opioid peptides interact with one and the same receptor or whether there are several receptors subserving different physiological functions. This is of particular importance since the synthesis of highly specific ligands for one receptor class would then become possible and could provide drugs of clinical importance such as analgesics with low addiction potential. Indeed, different opiate receptor subtypes have been found of which the most important ones: A receptor that prefers morphine, which was called the μ receptor; A receptor that prefers enkephalins, called the δ opiate receptor and a dynorphin binding site, which was called the κ receptor.

Many opiate drugs interact at multiple receptor sites. However, some peripheral and pharmacological effects have been ascribed to the different

opiate receptor subtypes. For example, interactions with μ receptors produce analgesia, bradycardia and hypothermia, whereas δ receptors can be held responsible for convulsive, sedative and behavioural effects (Chapter III).

Recent studies have demonstrated an important role for endogenous opioid peptides in the limbic system particularly in the hippocampus (Chapter IV). Although the predominant action of opioids in the nervous system is a naloxone-reversible depression, they excite hippocampal pyramidal cells, which may underlie epileptic episodes induced by enkephalins. Three major hypotheses have been proposed to explain these excitatory phenomena in the hippocampus: 1. Excitation by disinhibition; 2. Facilitation; 3. Increased efficiency of coupling. A bulk of evidence favours disinhibition as responsible for excitation of hippocampal pyramidal cells by opioid peptides.

Part 2.

Most neuropeptides are known to occur both in the central nervous system and in blood, and although central effects occur after peripheral administration of the peptides, the relationship between these two compartments has hardly been studied. For many peptides, such as the enkephalins, poor penetration into the brain via the blood brain barrier has been demonstrated by Meisenberg and Simmons (1983). The diffusion of the most peptides across the brain vascular endothelium seems to be severely restricted and forms the main obstacle for the development of peptides, as useful therapeutic agents.

A second problem which faces opioid peptide research is the biotransformation of these substances in the cerebrospinal liquor space. In this respect, the presence of enkephalin degrading enzymes in the cerebrospinal fluid (CSF) is of particular importance. These factors, together with the high costs of the compounds, urged investigators in opioid research to use the intracerebroventricular (icv) route of administration. However, many aspects which affect ivt administration of enkephalins such as the distribution, elimination and biotransformation of these substances, were not clear and made interpretation of the results

rather difficult.

By using a physiologically stable enkephalin analogue, like (D-ala^2, D-leu^5) enkephalin (DADL), at least one of the problems was circumvented (Chapter V). Tritium labelled DADL reached all parts of the ventricular system, including the central canal of the spinal cord and the extracerebral ventricular space, within 10 min after icv administration. However, electrographic epileptic phenomena occured within 1-2 min after the application which suggests the presence of an opioid proconvulsant system in the vicinity of the lateral ventricles. The brain regions bordering the lateral ventricles are the striatum, septum and hippocampus, in which penetration of the labelled enkephalin occured upto a depth of about 100 μm during the first 2 min. Comparison of the electrophysiological responses of these three regions after icv enkephalin, made us suggest that the hippocampus plays an essential role in the opioid-induced seizures. In this region spike activity was very intense and long-lasting and it is known that the hippocampus has a low seizure threshold (Cowan et al., 1979).

Since we used DADL, which has a low discrimination ratio between δ and μ opiate receptors (Kosterlitz and Paterson, 1981), the characteristics of the convulsant system involved remained unresolved. It is suggested that the analgesic action of opiates is mediated by μ opiate receptors (Urca et al., 1977; Chaillet et al., 1984) while the excitatory phenomena induced by opioid peptides emerge from δ opiate receptor stimulation (Dzoljic and vd Poel-Heisterkamp, 1982), although Gähwiler demonstrated μ receptor involvement in these phenomena (1981). To elucidate the role of these two opioid systems in the opiate-induced epileptiform seizures, the highly specific δ opiate agonist, D-tyr-ser-gly-phe-leu-thr (DSTLE) (Gacel et al., 1980) and morphiceptin, which shows a high specificity for the μ opiate receptors (Chang et al., 1981; Zhang et al., 1981; Chang et al., 1982) were used (Chapter VI). Indeed, icv DSTLE caused a dose-related increase of the myoclonic contractions (MC) of the submandibular muscles. The use of the number of MC, as a parameter for epileptiform activity, is justified by the fact that they are correlated with the ECoG and EEG epileptic spikes. Morphiceptin had no significant effect on the EMG or ECoG but, interestingly, inhibited the DSTLE-induced seizures in a dose-related

manner. These results support the terms "proconvulsant δ opiate receptors" and "anticonvulsant μ opiate receptors" as proposed by Dzoljic (1982) and Frenk (1983). Furthermore, the DSTLE-induced MC were inhibited by low doses of the specific δ opiate antagonist, ICI 174,864.

The convulsions induced by electroshock or kindling are probably mediated by increased endogenous levels of endorphins and enkephalins (Hong et al., 1979; Vindrola et al., 1981). The recently described antagonism of exogenously applied opioids, on these seizures (Berman and Adler, 1984; Puglisi-Allegra et al., 1984) may depend on the route of administration (Frenk, 1983) or, as stated above, upon stimulation of the different opiate receptor subtypes. In this case it should be interesting to examine the effects of enkephalinase inhibitors, which potentiate the endogenous enkephalinergic system, on these convulsant manipulations, while they induce epileptic phenomena when injected alone (Ukpommwan and Dzoljic, 1984). Whether endogenous (leu)enkephalin, the putative δ agonist, is involved remains to be established.

Increased levels of endorphins are observed during anaesthesia (Berkowitz et al., 1976). This might explain the increased electrographic excitation during anaesthesia, as well as the facilitatory effect of urethane on the DSTLE-induced epileptiform phenomena. Apparently, the effects of opioid peptides can be significantly modulated by the level of anaesthesia. Other data indicate that the effects of morphine, which acts on the same opioid peptidergic system, can either be inhibited or facilitated by urethane anaesthesia (Urca and Liebeskind, 1979; Linseman, 1980). This indicates the necessity of parallel studies with opioids in free-moving animals. Furthermore, these findings might be of clinical importance, specifically in the treatment of post-operative pain.

DSTLE injected icv in free-moving animals resulted in severe wet dog shakes and "falling down". These wet dog shakes were associated with a very intense electrographic epileptic spike activity. Wet dog shakes can also be observed during naloxone-precipitated withdrawal in morphine tolerant/dependent rats and mice, and are associated with a considerable increase in the plasma endorphin immunoreactivity. These findings may reflect the stress associated effects in these experiments, since stressors are potent stimuli to endorphin release (Guillemin et al., 1977). Much

like the shivering and "chills" which accompany withdrawal symptoms in humans, it seems possible that the thermogenic effects of wet dog shakes with an associated heat conserving behaviour, could represent an anlogous situation in rats. These behavioral epileptic phenomena (MC and wet dog shakes) are probably the result of activation of the limbic system, which is known to control motor behavior.

Increased neuronal activity, as observed during DSTLE-induced epileptic phenomena, should be correlated with an increased energy metabolism and as such with a rise of the blood flow to the regions involved in these phenomena. By using the "punching" technique we were able to isolate the different brain nuclei, while Sokoloff's (1977) 2-deoxyglucose technique was used to determine the energy metabolism in these regions during enkephalin-induced neuronal activity (Chapter VII). Generally, the limbic system showed a higher glucose uptake compared to the other brain areas, which corresponded with the more intensive and longer-lasting spike activity, as recorded after icv DSTLE. Since our attention was focused on the hippocampal area, we isolated the different structures/nuclei of this region. Compared to the CA2, CA3 and dentate gyrus, the CA1 and subiculum showed a significant increase of the energy metabolism during epileptic phenomena. The onset period of the CA1 spike activity after DSTLE administration was very short (within 1 min), which suggests an opioid mediated excitatory mechanism/pathway in the close vicinity of the lateral ventricle, where indeed the pyramidal cells of the CA1 are located. In this short period of time, the enkephalin penetrated to a depth of about 50-100 μm. Activation by opiates of the pyramidal cells might, via interneurons, result in a decreased release of GABA, which may lead to excitation (Zieglgänsberger et al., 1979). Indeed, GABA antagonists potentiate the opiate-induced epileptic activity (Dzoljic et al., 1978) while GABA inhibits these phenomena (Illes, 1982).

Similar conclusions can be drawn from the measurements of the blood flow to the different regions after administration of the enkephalin analogue (Chapter VIII). The increase of the blood flow to the striatum and brain stem after the application of DSTLE, might be a result of the high densities of opiate receptors in those regions (Duka et al., 1981). However, an excitatory input, probably originating in the limbic system,

can not be excluded.

An unexpected finding was that the opioid peptide also enhanced cerebellar blood flow. It is known that this region has a very low density of opiate receptors (Atweh and Kuhar, 1977); only the perikarya of the Golgi cells stain positively for enkephalin (Sar et al., 1978) and its location is far from the lateral ventricles. Thus, it is not likely that the compound can easily reach these structures during the first minutes, when the blood flow increase occurs. Although, if so, a similar mechanism as is proposed for the opiate-induced hippocampal excitations by disinhibition (Zieglgänsberger et al., 1979), might occur in the cerebellum as well, since this brain region has a relatively high concentration of GABA-ergic neurones/pathways (Curtis et al., 1970). However, an excitatory input via the mossy- and climbing fibers, originating from the perikarya in the brain stem and spinal cord, to the cerebellum, might be an additional reason for the increased neuronal cerebellar activity associated with the rise of the blood flow to this region. The results suggest the necessity of further experiments concerning the relationship between the enkephalins and the cerebellum, particularly because clinical evidence suggests a strong relationship between the cerebellum and some types of epilepsy, which are associated with a decrease of the CSF GABA levels (Cooper, 1973; Wood et al., 1977).

Noteworthy is the strong correlation between the EEG recordings, which reflect the neuronal activities, the energy metabolism and the blood flow of the different areas.

In summary, the results presented in this dissertation, permit the following general conclusions:

1. *Enkephalins, administered icv, reach all parts of the ventricular system including the central canal of the spinal cord and the extracerebral spaces within 10 min.*
2. *The substance penetrates into the periventricular tissue to a depth of about 100 µm within 2.5 min.*
3. *Activation of δ opiate receptors is essential for the triggering of opioid-induced epileptiform phenomena, while activation of µ opiate receptors has a more anticonvulsive effect.*

4. *The short onset of electrographic spike activity after icv administration of enkephalins, suggests an opioid peptidergic convulsant system in the vicinity of the lateral ventricles.*
5. *This convulsant system is probably located in the hippocampus, in which the CA1 or subicular area can be held responsible for the triggering and maintaining of the opiate-evoked seizures. From this area, excitatory pathways may run to other brain regions, like the brain stem and cerebellum.*
6. *In the cerebellum, the enkephalins may play an important role in some types of epilepsy.*
7. *Noteworthy is the strong correlation between the neuronal activity, energy metabolism and blood flow in the different brain regions.*

It should be emphasized that the pharmacological effects (epileptic discharges and excitatory behaviour) of the opioid peptides, as described above, have been demonstrated with exogenous administration of opioids. However, the spike activity and excitatory behaviour can also be observed after administration of enkephalinase inhibitors (Ukponmwan and Dzoljic, 1984), which indicates that endogenous opioid peptides may have similar actions. Consequently, the endorphins/enkephalins seem to be important physiological modulators of neuronal excitability in the CNS.

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SAMENVATTING

Deel 1.

Hoewel opiaten reeds eeuwen toegepast worden op grond van hun unieke eigenschappen, is pas in de laatste jaren een basis gelegd voor enig begrip omtrent hun werking. Om een inzicht te verkrijgen in de rol van de opioid peptiden met betrekking tot de excitatie verschijnselen, welke zij kunnen induceren na een systemische of intraventriculaire toediening, is het belangrijk eerst meer te weten over de eigenschappen en de verspreiding van deze stoffen en de mechanismen, waarin ze betrokken zijn.

Hoodfstuk I geeft een overzicht van de chemische structuren, de biosynthese en de afbraak van de opioid peptiden (enkefalinen, endorfinen en dynorfinen). Belangrijk hierbij zijn de enzymen, die betrokken zijn bij de inactivatie van de opioid peptiden, omdat remmers van deze enzymen als belangrijk hulpmateriaal kunnen beschouwd worden bij farmacologisch onderzoek. Enkefalinen worden overal in het centrale zenuwstelsel gevonden, van hersenschors tot ruggemerg. Endorfinen worden aangetroffen in de mediale basale hypothalamus, waar vanuit zenuwbanen naar het limbische systeem en de hersenstam lopen. Dynorfinen worden gevonden in de neurohypofyse en in verschillende andere hersengebieden. Deze drie door opioiden gemedieerde neuronale systemen worden meer gedetailleerd beschreven in Hoofdstuk I.

De werking van opioid peptiden komt tot stand door binding aan specifieke opiaat receptoren (Hoofdstuk II). Opiaat receptorkinetiek wordt gekarakteriseerd door stereospecificiteit, verzadigbaarheid en omkeerbaarheid. Aangezien er verschillende opioid peptiden bestaan, rijst de vraag of deze aan dezelfde receptor binden of dat er voor de verschillende fysiologische eigenschappen ook verschillende receptoren bestaan. Indien dit laatste het geval is, zou het van klinisch belang kunnen zijn omdat dan de mogelijkheid ontstaat om receptor-specifieke geneesmiddelen te ontwikkelen zoals analgetica zonder verslavende eigenschappen.

Tot op heden zijn er inderdaad verschillende opiaat receptoren

gevonden, met name, de μ opiaat receptoren, welke bij voorkeur morfine binden, de δ receptoren met een voorkeur voor enkefalinen, de κ- of dynorfine-receptoren en de σ en ϵ receptoren, hetgeen echter niet uitsluit dat de verschillende opiaatachtige stoffen aan meerdere receptoren kunnen binden.

Wel worden verschillende effecten van opiaten toegeschreven aan een activatie van specifieke receptoren. Analgesie, bradycardie, en hypothermie komen bijvoorbeeld tot stand via activatie van de μ receptoren terwijl δ receptoren verantwoordelijk zouden zijn voor convulsies, sedatie en veranderingen in gedrag (Hoodfstuk III).

Recente studies hebben aangetoond dat endogene opioiden een belangrijke rol spelen in het limbische systeem, voornamelijk in de hippocampus (Hoodfstuk IV). In tegenstelling tot de werking van opioiden in de rest van het centrale zenuwstelsel, waar zij de activiteit van neuronen remmen, leidt toediening van enkefalinen in de hippocampus tot een verhoogde activiteit van de hippocampale pyramidale cellen. Dit zou een onderliggend mechanisme kunnen zijn van de epileptogene werking van enkefalinen. Voor deze exciterende werking bestaan drie mogelijke verklaringen: 1. Excitatie als gevolg van disinhibitie; 2. Facilitatie; 3. Verhoogde efficientie van de impulsoverdracht.
Op grond van resultaten van meerdere onderzoeken, is de eerste hypothese de meest waarschijnlijke te noemen.

Deel 2.

Terwijl de meeste neuropeptiden zowel in het centrale zenuwstelsel als in het bloed voorkomen en hoewel effecten op het centrale zenuwstelsel onstaan na intraveneuse toediening van opioid peptiden, is een relatie tussen deze twee compartimenten nauwelijks bestudeerd. Meisenberg en Simmons (1983) hebben aangetoond dat veel peptiden, b.v. enkefalinen, de bloed-hersen barriere slecht kunnen passeren. Diffusie van de meeste peptiden door het endotheel van bloedvaten in de hersenen lijkt nauwelijks plaats te vinden, hetgeen een groot obstakel is voor de ontwikkeling van therapeutisch toepasbare peptiden. Biotransformatie

van opioiden in de liquor cerebrospinalis door daar voorkomende enzymen, is een tweede probleem waarmee onderzoekers op het gebied van peptiden, worden geconfronteerd. Mede gedwongen door de hoge kosten verbonden aan het gebruik van opioiden, noopten deze factoren onderzoekers tot het gebruiken van de intraventriculaire manier van toedienen. Echter, veel aspecten die van invloed zijn op de intraventriculaire toediening (ivt) van enkefalinen, zoals verspreiding, eliminatie en biotransformatie, waren onbekend en maakten de interpretatie van resultaten moeilijk.

Door gebruik te maken van een stabiel enkefaline, zoals (D-ala^2,D-leu^5) enkefaline (DADL), kon tenminste een van deze problemen omzeild worden (Hoofdstuk V). In een studie, waarbij gebruik werd gemaakt van tritium-gelabeld DADL, bleek deze stof binnen 10 min na ivt toediening, alle delen van het ventriculair systeem waaronder het centraal kanaal en de extracerebraleruimte bereikt te hebben. Echter tijdens de eertse 2.5 minuten, na toediening, verschijnen epileptische pieken in het EEG. Deze tijdsperiode komt overeen met penetratie van het enkefaline in de periventriculaire weefsels (hippocampus, striatum, septum) tot op een diepte van 100 µm. Dit wil zeggen dat er een opiaat receptor-gemedieerd epileptogeen systeem in de onmidddelijke nabijheid van het laterale ventrikel moet liggen. EEG's van de verschillende hersengebieden tonen aan dat na een ivt toediening van enkefaline, de eerste epileptische ontladingen ontstaan in de hippocampus.

Omdat we in ons onderzoek gebruik maakten van het niet-specifieke DADL bleven verdere karakteristieken van het convulsief systeem onopgelost. Echter, ivt toediening van de specifieke µ en δ opiaat agonisten, respectivelijk morphiceptin en δ receptor peptide (DSTLE), toonden aan dat deze excitatie- en epileptische verschijnselen het gevolg zijn van een activatie van de δ opiaat receptoren, terwijl activatie van µ receptoren resulteerde in een anticonvusief effect (Hoofdstuk VI). Voorafgaande toediening van zeer lage doses van de specifieke δ antagonist, ICI 174,864, blokkeerde de DSTLE-geinduceerde epileptische ontladingen. Deze resultaten ondersteunen de termen "proconvulsieve δ receptoren" en "anticonvulsieve µ opiaat receptoren". Uit deze experimenten bleek ook dat narcose een stimulerende invloed

uitoefende op de DSTLE-geinduceerde excitaties. Een verklaring hiervoor moet waarschijnlijk gezocht worden in een verhoging van de endogene opioid concentratie, tijdens narcose. Hieruit blijkt de noodzaak voor gelijklopende onderzoeken in wakkere dieren. Bovendien, zouden deze bevindingen van klinisch belang kunnen zijn vooral in verband met het behandelen van post-operatieve pijn.
DSTLE, ivt toegediend in vrij-lopende ratten, resulteerde in ernstige "wet dog shakes". Deze "wet dog shakes" zijn geassocieerd met intense piek activiteit in het EEG. Gelijkaardige "wet dog shakes" kunnen ook ontstaan tijdens de onthoudingverschijnselen in morfine verslaafde dieren en zijn hier geassocieerd met een aanzienlijke toename van de plasma enkefaline concentratie. Deze resultaten zouden een weerspiegeling kunnen zijn van stress factoren tijdens de experimenten, omdat stressors sterke stimuli zijn van endorfine afgifte. Net zoals het rillen bij mensen tijdens het onthoudingssyndroom, zouden "wet dog shakes" een analoge situatie kunnen zijn bij ratten. Deze epileptische verschijnselen in het gedrag zijn waarschijnlijk het resultaat van een activatie van het limbische systeem, dat een controlerende invloed uitoefent op de motoriek.

Verhoogde neuronale activiteit, geinduceerd door DSTLE, zou logischerwijs gepaard moeten gaan met een verhoging van het energieverbruik in de betrokken hersen gebieden en als zodanig met een toename van de bloeddoorstroming. Het toepassen van de "punching" techniek stelde ons in staat verschillende hersen nuclei te isoleren, terwijl Sokoloff's 2-deoxyglucose techniek werd gebruikt om het energieverbruik in deze gebieden, tijdens DSTLE-geinduceerde epilepsie, te meten (Hoofdstuk VII). Over het algemeen, vertoonde het limbisch systeem een hogere glucose opname in vergelijking met andere hersen gebieden, wat overeen komt met de sterkere en langer durende piek activiteit in het EEG. Vooral het CA1 gebied van de hipppocampus en het subiculum vertoonden een sterke toename van de glucose opname. De latentietijd van DSTLE-geinduceerde pieken was erg kort (< 1 min) in het EEG van het CA1/subiculum. Dit suggereert een opioid gemedieerd convulsief systeem in de onmiddelijke nabijheid van het laterale ventrikel, waar inderdaad de pyramidale cellen van het CA1 gelegen

zijn. Activatie door opioid peptiden van de pyramidale cellen kan, via interneuronen, resulteren in een verminderde GABA afgifte, wat op zijn beurt kan leiden tot een excitatie. GABA antagonisten potentieren deze enkefaline-geinduceerde excitatieverschijnselen, terwijl GABA zelf deze actvatie remt.

Gelijkaardige conclusies kunnen worden getrokken uit de experimenten waarbij de doorbloeding van de verschillende hersengebieden, tijdens DSTLE-geinduceerde epileptische fenomenen, werd gemeten (Hoofdstuk VIII). De toename van de bloeddoorstroming in het striatum en de hersenstam kan het gevolg zijn van de talrijke opiaat receptoren, die in deze gebieden voorkomen. Echter, een excitatoire input, waarschijnlijk vanuit het limbisch systeem, mag niet worden uitgesloten. Het is mogelijk dat activatie van het subiculum via de enkefalinerge banen, secundaire epileptische foci kan induceren. Een onverwacht effect was de toename in de doorbloeding van het cerebellum. Dit gebied bezit weinig opiaat receptoren en alleen de perikarya van de Golgi cellen kunnen "enkefalin-positief" worden genoemd. Bovendien ligt het cerebellum ver van de laterale ventrikels en is het dus niet waarschijnlijk dat het enkefaline, enkele minuten na toediening het cerebellum al heeft bereikt. Echter, indien dit het geval zou zijn, dan kan een gelijkaardig mechanisme zoals is geopperd voor de hippocampus, nl. door disinhibitie, ook hier van toepassing zijn aangezien het cerebellum een relatief hoge concentratie van GABA-erge neuronen en zenuwbanen heeft. Daarentegen kan ook een excitatoire input vanuit de hersenstam en het ruggemerg de reden zijn van een verhoogde activiteit in het cerebellum. De resultaten wijzen echter op de noodzaak van verder onderzoek naar de relaties tussen enkefalinen en het cerebellum, vooral ook omdat uit klinisch onderzoek is gebleken dat er een sterke relatie bestaat tussen het cerebellum en bepaalde vormen van epilepsie, welke geassocieerd zijn met afname van de GABA concentratie in de cerebrospinale liquor.

Samenvattend leiden de beschreven resultaten tot de volgende conclusies:

1. *Enkefalinen, ivt toegediend, bereiken alle delen van het ventriculair systeem, met inbegrip van het centraal kanaal van het ruggemerg en de extracerebrale ruimte, binnen 10 minuten.*

2. *Activatie van δ opiaat receptoren is essentieel voor het ontstaan van opioid-geinduceerde epileptische verschijnselen, terwijl activatie van μ opiaat receptoren een anticonvulsief effect heeft.*

3. *De korte latentietijd van electrografische piekactiviteit, na ivt toediening van enkefalinen, suggereert het bestaan van een opioid convulsief systeem in de directe nabijheid van de laterale ventrikels.*

4. *Dit convulsief systeem ligt waarschijnlijk in de hippocampus, meer specifiek in het subiculum of in de pyramidale cellaag van het CA1 gebied. Vanuit dit gebied kunnen excitatoire banen lopen naar andere hersengebieden zoals de hersenstam en het cerebellum.*

5. *Er bestaat een sterke correlatie tussen neuronale activiteit, energieverbruik en doorbloeding van de verschillende hersengebieden.*

6. *Bepaalde narcotiserende stoffen kunnen een stimulerende invloed uitoefenen op opiaat-geinduceerde excitatieverschijnselen.*

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