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A STUDY OF THE EFFECT OF AGE AND HORMONES ON BRAIN AND PITUITARY ENDORPHINS

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A STUDY OF THE EFFECT OF AGE AND HORMONES ON BRAIN AND PITUITARY ENDORPHINS

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ABSTRACT

Optimal methods were established to obtain reproducible estimates of endorphin concentrations in rat pituitary and brain extracts. Subsequently the influence of age, sex, endocrine changes and pharmacological treatments on regional brain endorphin concentrations was determined. The highest concentrations of β-endorphin were found in the pituitary, followed by the hypothalamus, hindbrain and midbrain. The principal immunoreactive species in brain extracts was β-endorphin. After hypophysectomy, a major reduction of β-endorphin concentration in the brain was observed. After adrenalectomy, the concentration of β-endorphin was increased significantly in the pituitary gland (359 µg/g wet wt. vs 540 µg/g), hindbrain (0.7 µg/g vs 1.5 µg/g), hypothalamus (3.9 µg/g vs 8.2 µg/g), and midbrain (0.53 µg/g vs 0.86 µg/g). After thyroidectomy, the concentration of β-endorphin was significantly increased in the hypothalamus (5 µg/g vs 11 µg/g). In rats treated with thyroxine (T_4), significant increases in β-endorphin were found in the midbrain (0.35 µg/g vs 0.60 µg/g), hypothalamus (4.8 µg/g vs 7.6 µg/g), and pituitary (187 µg/g vs 290 µg/g). In orchiectomized rats, a significant decrease of β-endorphin was found in rat pituitary (359 µg/g vs 189 µg/g). No differences in

β-endorphin in rat brain regions occurred after ovariectomy. Naloxone administration to rats increased β-endorphin in hypothalamus (4.7 μg/g vs 10.5 μg/g), while pentobarbital injection caused an increase in the pituitary (390 μg/g vs 607 μg/g). No differences in β-endorphin in rat brain were found between day 8 and day 24 old rats, but very significant increases were found between day 24 and day 60 old rats in most brain regions. The midbrain was the only brain region in which a significant sex difference in β-endorphin was found.

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NOMENCLATURE

ACTH	adrenocorticotropic hormone
BSA	bovine serum albumin
CRF	corticotropin releasing factor
CSF	cerebrospinal fluid
GH	growth hormone
LH	luteinizing hormone
LPH	lipotropic hormone
MLF	morphine-like factor
MSH	melanotropin stimulating hormone
RIA	radioimmunoassay
RRA	radioreceptorassay
PRL	prolactin
T_4	thyroxine
TRH	thyrotropin releasing hormone
TSH	thyrotropin stimulating hormone
V_o	void volume

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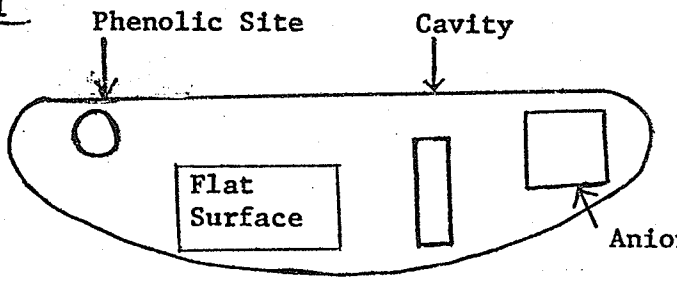
INTRODUCTION AND LITERATURE REVIEW

Opium, a word which means "poppy juice" has been used as a drug at least since the classical Greek period. It is an agent which kills pain and gives rise to euphoria. The drug is present in the milky exudate obtained by incising the unripe seedpod of the poppy "papaver somniferum". Derosine in 1803 and Serturner in 1805 both isolated crystalline material from crude opium, which Serturner called morphine and demonstrated that this material has opium like effects in dogs. The toxicity and addictiveness of morphine were recognized after the clinical utility of opium was established as a pain killer, anti-diarrheal agent and hypnotic. The search for nonaddictive synthetic opiates led to the discovery of numerous opiate agonists and antagonists. A landmark in this field occurred with the recent discovery of opiate receptors in the central nervous system of animals and man which shortly thereafter culminated in the identification and isolation of endogenous opiate-like ligands.

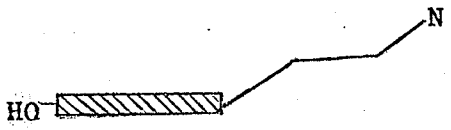
EVIDENCE FOR OPIATE RECEPTORS

Over the past 25 years (Beckett et al. 1954; Portoghese 1965; Jacobson 1972), much evidence supported the view that opiates act via specific receptors. Firstly, there was a general structural similarity among opiate agonists as shown in Fig. 1. The minimal structural requirements for opiate activity are the presence of an aromatic ring structure and a nitrogen atom, usually as a tertiary amine, that is located at a distance of 2 saturated carbon atoms from the aromatic ring (Eddy, et al. 1973). Secondly, only the naturally occurring levorotatory isomer is active, while the dextrorotatory isomer has little or no analgesic or addiction-producing activity. Thirdly, morphine cogeners such as etorphine have been synthesized that are 500-1000 times as potent as morphine, and differ in structure in only minor ways. Fourthly, Pohl (1975) found that morphine derivatives with small changes in structure may act as antagonists to opiates, for example : substitution of the N-methyl group by a large alkyl group such as allyl or cyclopropylmethyl, converts a potent analgesic to a drug that antagonizes morphine and related narcotic analgesics. Because of the steric and structural specificity of the opiates, pharmacologists assumed that specific opiate receptors must exist in brain and possibly in other tissues.

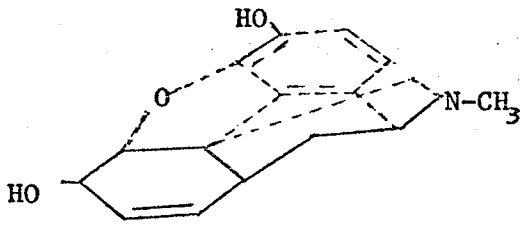
Fig. 1



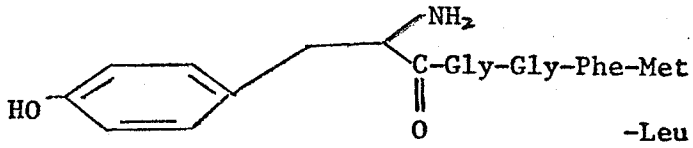
Postulated Opiate Receptor



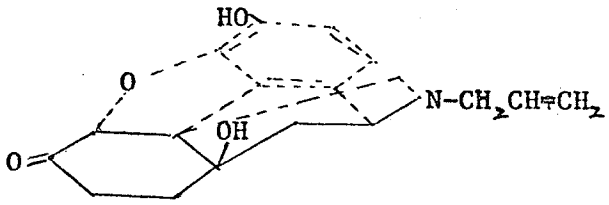
General Structural Similarity



Morphine



-Leu Enkephalins



Naloxone

Reference : Beckett et al. 1954; Hughes et al. 1975a, 1975b, 1975c.

DEVELOPMENT OF OPIATE RECEPTOR ASSAY

The search for putative opiate receptors was complicated by the difficulty of distinguishing nonspecific binding to various tissue components from specific binding to receptors and by the unavailability of isotopically labeled opiates with sufficiently high specific activity. Several early attempts (Simon et al. 1966; Ingoglia et al. 1970) were unsuccessful. In 1971, Goldstein et al. first established a set of criteria for stereospecificity of binding of opiates to their receptors. Mouse brain homogenates were incubated with ^{3}H-levorphanol in the presence of a large excess of unlabeled levorphanol and its inactive enantiomer dextrorphan. Stereospecific binding was defined as that portion of the binding of the labeled drug which is prevented by levorphanol but not by dextrorphan. Because he used a preparation of ^{3}H-levorphanol of very low specific activity (0.8 μCi/nM), he found only about 2% of the total binding to be stereospecific. Also he reported this binding to be localized in the nuclear portion of subcellular fractions and fairly evenly distributed throughout the brain. This result was discouraging, since it implied that it would be very difficult to distinguish the small amount of specific binding to the postulated receptor from the very large amount

of nonspecific binding. Other laboratories could not reproduce Goldstein's result. However, three laboratories (Pert & Snyder 1973a,1973b; Terenius 1973a,1973b; Simon et al. 1973a) using modifications of Goldstein's procedure, independently and almost simultaneously reported stereospecific opiate binding in rat brain homogenates. The modification involved the use of a very low concentration of labeled ligand of high specific activity ^{3}H-Naloxone (6.1 μCi/nM, Snyder), ^{3}H-Etorphine (Simon), and the washing of homogenate after incubation with cold buffer to remove contaminating unbound and loosely bound radioactivity. Their results differed in several respects from Goldstein's. They found that the binding was mainly in the synaptosomal fraction of brain homogenates and that there were marked regional variations throughout the brain. However stereospecific binding does not ensure that one is dealing with the opiate receptor, since various brain lipids e.g. cerebrosides (Loh et al. 1974; Abood et al. 1975) and even certain filters (Snyder et al. 1975) display "stereospecific binding" of opiates. When identifying receptors, it is desirable to examine (Cuatrecasas, 1976):(1) the binding of analogs and antagonists; (2) the capacity and affinity of binding sites; (3) the reversibility of binding; (4) the tissue distribution of specific binding sites; and (5) the simultaneous correlation of the binding data with

the biological dose-response curves in identical tissue preparations. The pharmacological relevance of the opiate receptor has been demonstrated by comparing binding and pharmacological activities in the same tissue - the guinea-pig ileum. (Creese & Snyder, 1975a; Kosterlitz & Waterfield 1975a). Their studies showed that the ability of the opiates to inhibit electrically induced contractions of the guinea-pig ileum parallels their analgesic potencies. Affinities of numerous opiate agonists and antagonists for binding sites in the guinea-pig ileum correlates remarkably well with their effects on electrically induced contractions in the same tissue, suggesting that this stereospecific binding sites was indeed a real receptor. Stahl et al.(1977) found very good correlations between the binding affinities of the drugs to sites in the brain and the ability of these opiates to inhibit contractions of the intestine, providing evidence for the similarity of opiate binding sites in the brain and the myenteric plexus of the intestine.

DISTRIBUTION OF OPIATE RECEPTORS

The stereospecific binding sites are found in the central

nervous system and in the myenteric plexus of the guinea-pig ileum. Apart from Abood's report (Abood et al. 1976) of stereospecific opiate binding in human erythrocyte membrane, opiate receptors have not been observed in non-nervous tissue. Receptor binding was detected in the brain of all vertebrates (Pert et al. 1974), but not in invertebrates. In the most primitive vertebrates such as the hagfish and dogfish shark brain, opiate receptors were as numerous as in monkey and human brain. Different species of mammals differ in their behavioral response to opiates. In many species, including man, monkey and dog, a depressive response is observed after opiate administration, while in others such as the cat, cow, sheep, horse and pig, excitatory features dominate (Jaffe, 1970). A survey of stereospecific binding of ^{3}H-Etorphine in selected area of the brains of several species - cow, cat, sheep, dog, monkey, and man (Kuhar et al. 1973; Hiller et al. 1973) revealed that except for the amygdala and frontal cortex, there was reasonably good reproducibility of binding sites in comparable anatomical regions in all six species. For species that exhibit depression after opiates at least a two fold greater ratio of opiate receptor levels in the amygdala and frontal cortex to that in the caudate nucleus was observed than for species that show an excitatory response to opiates. Opiate receptors are found throughout the brain but are concentrated in areas associated with the limbic system and the periaque-

ductal gray area is one of the few regions where microinjection of morphine elicits analgesia and where direct electrical stimulation causes analgesia that is blocked by naloxone.

Autoradiographic techniques (Jacquet et al. 1974; Pert et al. 1976a) have permitted discrete microscopic localization of receptors. Within the spinal cord, opiate receptors are localized in the substantia gelatinosa, which is the first region in the central nervous system for the intergration of sensory information. Within the brain stem, opiate receptors are particularly prominent in the solitary nuclei, which receive visceral fibers from the vagus and glossopharyngeal cranial nerves. Opiate receptors in the solitary nuclei may explain how opiates depress the cough reflex, elicit orthostatic hypotension and reduce gastric juice secretion. Also within the brain stem opiate receptors are concentrated in the area postrema, which contains the chemoreceptor trigger zone, the site at which opiates apparently induce nausea and vomiting. The greatest abundance of opiate receptors in the brain occurs in the amygdala. It is possible that receptors here are associated with influences of opiates on emotional behavior.
Duller, more chronic and less localized pain is quite effectively relieved by opiates and appears to be conveyed by a pathway that evolved earlier, called the paleospinothalamic system. This pathway ascends along the midline of the brain, its way

stations include the central gray matter of the brain stem and the central part of the thalamus. The map of the distribution of the opiate receptors strikingly parallels the paleospinothalamic pain pathway. In the subcellular studies, opiate receptors are highly associated with membrane fractions of tissue homogenates and have been reported to be most concentrated in the synaptosomal cell fraction of brain and guinea-pig ileum homogenates (Hitzeman et al. 1974; Pert et al. 1974b; Terenius et al. 1973c), suggesting a location in the vicinity of synapses. Whether the receptors are situated pre-or post-synaptically has not yet been established. Lamotte et al. (1976) found that after dorsal root section in the monkey, opiate receptor binding declined by 50% in the dorsal horn of the monkey spinal cord. Zieglgansberger et al.(1976a) found that opiates diminish spontaneous firing and glutamate induced firing of cells in the cerebral cortex and corpus striatum through a postsynaptic actions. A cell line in culture derived as somatic hybrids of a neuroblastoma clonal cell line and glioma cell line, NG108-15, is rich in opiate receptors (Klee et al. 1976). Both parent lines contain few if any opiate receptor binding sites.

FACTORS AFFECTING OPIATE RECEPTOR BINDING (Pert & Snyder, 1973b,

1974c; Soloman et al. 1973a, 1973b; Simon et al. 1973b, 1975a; Pasternak et al. 1973)

(1) Temperature: Stereospecific binding is temperature dependent with maximal binding at 35°C. At 4°C binding was reduced to 25% of values at 35°C. Heating for 10 minutes at temperatures higher than 50°C decreases specific naloxone binding by 90% or more.

(2) pH: Binding of both agonists and antagonists has a broad pH optimum between 6.5 and 8.

(3) Ions: Low physiologic concentrations of manganese and magnesium selectively increase the binding of opiate agonists by reducing receptor sensitivity to sodium. Calcium fails to enhance opiate agonist binding. Treating the brain membrane with EDTA decreases the binding of opiate agonist, while EGTA, which chelates calcium but not manganese and magnesium, has no influence on receptor binding. The most important feature is the role of sodium on binding of opiates. Inhibition of agonist binding by Na^+ was observed by Simon et al.(1973b) whereas binding of antagonists was not affected according to Pert and Snyder (1973b). Subsequent studies (Pert and Snyder, 1974c; Simon et al. 1975a) showed that Na^+ (and to some extent Li^+) specifically increases the binding affinity of antagonists and decreases that of opiate agonists. The enhan-

cement by sodium was remarkable requiring only 1 mM sodium. Anions e.g. F^-, Cl^-, Br^-, I^-, $SO_4^=$, SCN^- also favor binding of antagonists.

(4) Protein-modifying agents: The opiate receptor is highly sensitive to inactivation by various sulfhydryl regents such as N-ethylmaleimide (NEM), iodoacetamide, p-hydroxymercuribenzoate, Ellamn's regent etc.

(5) Enzymes and Detergents: Opiate receptor is extremely sensitive to digestion by proteolytic enzymes e.g. trypsin, chymotrypsin and detergents such as Triton X-100, sodium dodecylsulfate and deoxycholate. Low concentration of proteolytic enzymes selectively reduce opiate agonist binding with negligible effects on antagonist binding. Phospholipase A from Russell's Viper or bee venom block the binding while the enzyme obtained from rattle snake venom is ineffective. Phospholipase C is slightly inhibitory, while RNAase, DNAase and neuraminidase are without effect.

(6) Others: Implantation of morphine pellets or administration of opiate agonists or antagonists in vivo increases receptor binding of both agonists and antagonists to brain homogenates.

CONFORMATION OF THE OPIATE RECEPTOR

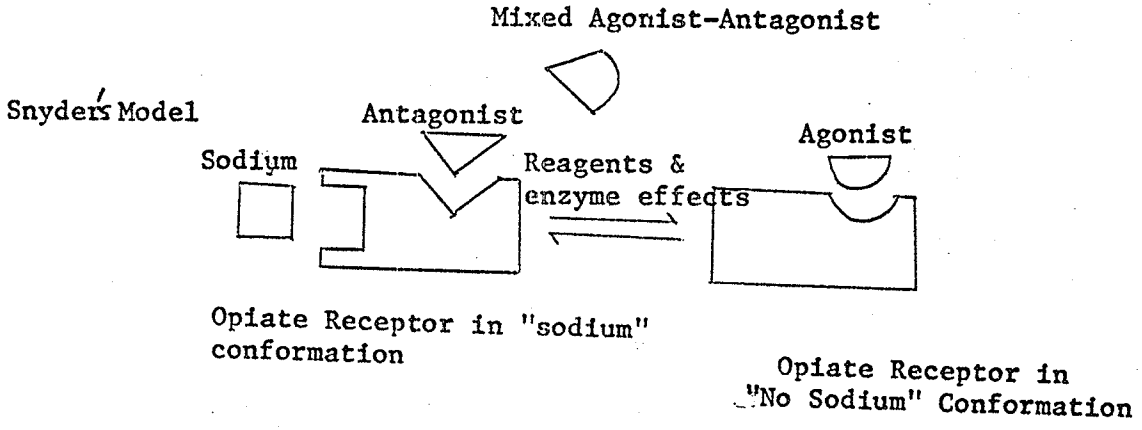
Due to the selective action of Na^+ in enhancing the opiate antagonist binding, two laboratories (Pert & Snyder, 1974c, 1975; Simon 1974, 1975c) have independently proposed similar allosteric models of the receptor. They hypothesize that the opiate receptor can exist in two states as shown in Fig. 2.

The essence of the models is that the sodium ion acts as an allosteric effector, the binding of which to an allosteric site on the receptor molecule results in a conformational change in the opiate binding site. The new conformer (Na^+- dependent) exhibits a higher affinity for antagonists and a lower affinity for agonists than the conformer that exists in Na^+- free media. Simon also found that conformational change in the receptor was produced by sodium at 37°C but that the change was amplified at 0°C. Similar results were obtained by Creese et al. (1975). The physiological significance of this conformational change in the receptor in the presence of sodium is still not clear.

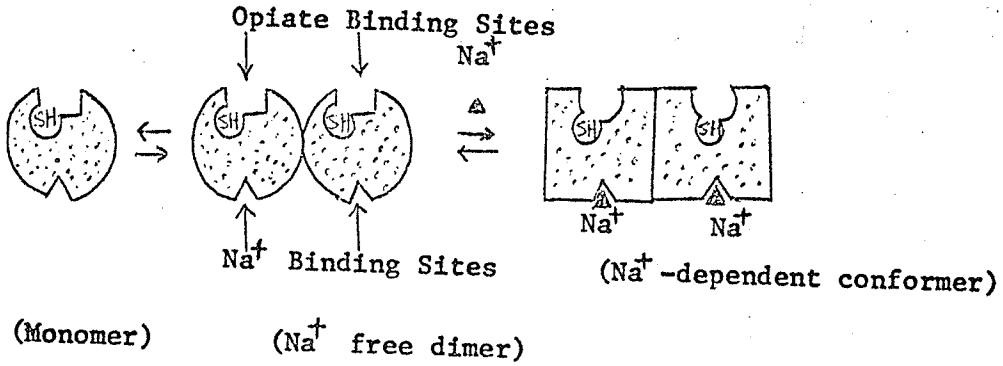
FURTHER CHARACTERIZATION OF OPIATE RECEPTOR

The solubilization and purification of the opiate receptor has proved to be difficult. The reason is that

Fig. 2



Simon's Model



Reference: Pert & Snyder 1974c, 1975; Simon 1974, 1975c.