Effect of peptides and amino acids on dihydromorphine binding to the opiate receptor

Drug receptors show a very high degree of ligand specificity and the opiate receptor is no exception. Thus, while all tested narcotic analgesics and antagonists have affinity for the receptor, none of the known neurotransmitters has a significant affinity (Pert & Snyder, 1973; Terenius, 1973; Wong & Horng, 1973; Snyder, Pasternak & others, 1975). Of a variety of other pharmacologic agents tested there seems to be very few which have a demonstrable affinity. Only physostigmine (Terenius, 1973; Klee & Streaty, 1974) and phenoxybenzamine (Cicero, Wilcox & Meyer, 1974) have been reported to possess some affinity. Since various peptides have been considered to be involved in pain mechanisms or to affect morphine action, a number of peptides have now been analysed for opiate receptor affinity.

The details of the experimental procedure have been described (Terenius, 1974). The receptor containing preparation was a fraction from rat brain enriched in synaptic plasma membranes (SPM). [7,8-^{3}H]Dihydromorphine (55 Ci mmol^{-1} from New England Nuclear, Boston, Mass.) was the labelled indicator drug. The following non-radioactive drugs were used: dihydromorphine, prepared by the author from morphine; $ACTH_{1-28}$, oxytocin, 8-Lys-vasopressin, Ferring Co., Malmö, Sweden, substance P, Dr. H. Sievertsson, University of Uppsala, Sweden; bradykinin, Sigma Chemical Co., St. Louis, Mo., $ACTH_{4-10}$, Dr. D. de Wied, Department of Pharmacology, University of Utrecht, The Netherlands. Other compounds were of A.R. grade from usual sources.

The standard incubation mixture contained $0{\cdot}8 \times 10^{-9}$M [^{3}H]dihydromorphine SPM membranes corresponding to 0·4 mg of protein in 0·4 ml of HEPES buffer with physiological concentrations of salts and of pH 7·4. In all experiments, samples containing an excess of unlabelled dihydromorphine (10^{-6}M) were included to obtain the non-specific binding (blank value). Following incubation for 40 min at 25°, the incubation mixtures were cooled and centrifuged at +4° in a Microfuge (Beckman) for 10 min. The SPM pellets were digested by Soluene (Packard) and the tritium content was measured by liquid scintillation counting. The blank values were subtracted from all experimental values and the effects of the various peptides were expressed in percent of the specific = saturable binding.

A number of peptides, and a few amino acids known to be present in brain in high concentrations, were tested for affinity for the opiate receptor. The compounds (M) oxytocin (10^{-5}), substance P (10^{-5}), bradykinin (10^{-5}) and GABA (10^{-5}) were inactive (giving less than 25% inhibition) or only slightly active, 8-Lys-vasopressin (10^{-5}; 35% inhibition). Glutamic acid and glycine were even inactive at 10^{-3}M. Two compounds, $ACTH_{1-28}$ and $ACTH_{4-10}$, were markedly active at 10^{-5}M and were therefore tested over a wider concentration range (Fig. 1). The figure shows that both compounds produced a concentration-dependent inhibition of the binding of dihydromorphine. When subjected to double-reciprocal analysis, there was evidence that $ACTH_{1-28}$ inhibited the binding in an apparently competitive manner.

The present results are particularly interesting in the sense that representatives of a new class of chemical substances, the peptides, have been found to have considerable affinity for the opiate receptor. This can be taken as support for the hypothesis that there may be endogenous peptides, such as the previously described factor(s) from brain extracts (Terenius & Wahlström, 1974), which are the naturally occurring ligands for the opiate receptor.

The apparent dissociation constants for the two ACTH fragments are $2{\cdot}5 \times 10^{-6}$ and 8×10^{-6}M respectively and it is not known whether micromolar concentrations

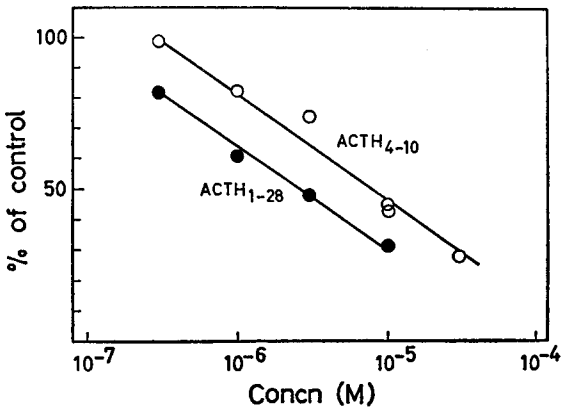

FIG. 1. Effect of ACTH fragments on the binding of dihydromorphine to the opiate receptor.

of ACTH or fragments of it are present in receptor areas under physiological conditions. On the other hand, there are reports in the literature on the direct interaction between morphine and administered ACTH fragments.

Thus, $ACTH_{1-24}$ (tetracosactin) was found to antagonize the depressant action of morphine on the spinal cord of decerebrate cats (Krivoy, Kroeger & others, 1974) or on the isolated spinal cord of frogs (Zimmermann & Krivoy, 1974). The concentrations necessary to inhibit morphine action on the frog spinal cord are of the same order as found active here.

The fragments $ACTH_{1-28}$ and $ACTH_{1-24}$ have potent corticotrophic activity while $ACTH_{4-10}$ is devoid of such activity (Schwyzer, 1963). The latter has been reported to affect behaviour, the acquisition of a conditioned avoidance response in rats (de Wied, 1969) and it is interesting that it is active here.

Since substance P, which has been reported to antagonize morphine analgesia (Stern, Ćatović & Stern, 1974) and bradykinin, which is analgesic when given intraventricularly (Ribeiro & Rocha e Silva, 1973), did not exhibit any receptor affinity, it is likely that they exert their respective activities by other mechanisms than via the opiate receptor.

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Are spinal excitatory muscarinic receptors important for cardiovascular control?

It is well known that transection of the spinal cord cranial to the spinal sympathetic outflow decreases sympathetic tone and leads to a fall in arterial blood pressure. Conceivably, this is due to an interruption of excitatory pathways descending from supraspinal nervous structures. The transmitters of the spinal descending neurons responsible for the maintenance of sympathetic tone are not known. Noradrenaline is an unlikely candidate since its amino acid precursor L-3,4-dihydroxyphenylalanine (L-dopa), does not produce a pressor response in the spinal rat (Henning, Rubenson & Trolin, 1972) pretreated with a peripheral decarboxylase inhibitor (hydrazinomethyldopa, MK 486). Neither does the 5-hydroxytryptamine (5-HT) precursor, DL-5-hydroxytryptophan under the same conditions (Henning & Trolin, unpublished) so 5-HT is excluded also. In a search for a spinal excitatory transmission mechanism acting on the sympathetic nervous system we have investigated the role of muscarinic receptors on blood pressure regulation at spinal levels.

In all experiments, male Sprague-Dawley rats (200–220 g) were spinalized at the level of the seventh cervical vertebra under pentobarbitone anaesthesia (40 mg kg^{-1}, i.p.) and venous and arterial catheters were implanted as previously described (Henning, 1969). A few experiments were made on rats with both vagi cut or which had been adrenodemedullated (Farris & Griffith, 1949) under pentobarbitone anaesthesia 7 days previously.

On the day after the spinal transection, mean arterial blood pressure was recorded in conscious animals using Statham P23 Dc pressure transducers writing on a Grass Model 7 Polygraph; heart rate was triggered by the pressure signal. Oxotremorine (2 mg kg^{-1}, i.p.) (97%, Aldrich-Europe, Beerse, Belgium), was injected 20–40 min after methylscopolamine nitrate (10 mg kg^{-1}, i.p.) or atropine sulphate (10 mg kg^{-1}, i.p.). The doses refer to the salts. A solution of 0·9% NaCl was always injected after the drugs to a final volume of 0·5 ml. Significant differences were determined with Student's *t*-test. The results are shown in Fig. 1.

The methylscopolamine-pretreated group did not differ significantly from the atropine-pretreated group in blood pressure ($P > 0{\cdot}1$) or heart rate ($P > 0{\cdot}1$) at the time for the oxotremorine injection. The blood pressure increase after oxotremorine in the methylscopolamine group (22·0 ± 3·5, 22·8 ± 2·2, 20·8 ± 2·0, 16·0 ± 2·7 and 8·5 ± 2·5 mm Hg) was significantly higher than the pressure changes after oxotremorine in the atropine group (8·6 ± 4·0, 2·6 ± 2·0, 2·0 ± 0·9, 0·2 ± 0·8 and −1·4 ± 0·6 mm Hg) at 5, 10, 15, 30 and 60 min ($P < 0{\cdot}05$, $P < 0{\cdot}001$, $P < 0{\cdot}001$, $P < 0{\cdot}001$ and $P < 0{\cdot}01$, resp.). The heart rate increase after oxotremorine in the methylscopolamine group (26·7 ± 9·5, 35·0 ± 8·9, 35·8 ± 9·0) was significantly different from the decrease in heart rate in the atropine group (−18·0 ± 7·3; −16·0 ± 4·0, −40 ± 4·0) at 5, 10 and 15 min ($P < 0{\cdot}01$, $P < 0{\cdot}005$ and $P < 0{\cdot}05$, resp.). At 30 and 60 min the difference was not significant ($P > 0{\cdot}05$ and $P > 0{\cdot}25$, resp.).

Section of the vagi or adrenal demedullation did not change the effect of oxotre-