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Short communication

INTERACTION BETWEEN ACTH FRAGMENTS, BRAIN OPIATE RECEPTORS AND MORPHINE-INDUCED ANALGESIA

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The present study confirms that N-terminal fragments of ACTH have an affinity for rat brain opiate receptors in vitro. Such peptides, devoid of corticotrophic activity, were found to inhibit morphine-induced analgesia if they also possessed affinity for opiate receptors in vitro. The structure—activity relationship for these two parameters is comparable to that observed for the same peptides on the induction of excessive grooming.

Morphine ACTH Opiate receptor Analgesia

1. Introduction

Morphine has profound effects on pituitary-adrenal function (De Wied et al., 1974). Conversely, evidence is accumulating to suggest an interaction of ACTH with CNS effects of morphine: e.g. counteraction of morphine-reduced spinal reflex activity in vivo and in vitro (Zimmermann and Krivoy, 1973) and the inhibition by opiate antagonists of ACTH-induced excessive grooming activity in the rat (Gispen and Wiegant, 1976). Moreover, ACTH and some N-terminal fragments show affinity for CNS opiate receptors (Terenius, 1975; Terenius et al., 1975). However, the data with respect to opiate-induced analgesia are confusing (Gispen et al., 1975a), probably because of the complexity of the interaction of pituitary-adrenal hormones and morphine (De Wied et al., 1974). The present experiments were designed to compare the affinity of ACTH fragments with little or low corticotrophic activity for rat brain opiate receptors with their effectiveness in inhibiting morphine-induced analgesia.

2. Materials and methods

2.1. Receptor assay

Binding of 7,8-^{3}H-dihydromorphine (DHM, 55 Ci/mmole, NEN, Boston) to the synaptic plasma membrane (SPM) fraction of rat brain, was studied as described previously (Terenius, 1974). The standard incubation mixture contained 0.8×10^{-9} M ^{3}H-DHM and 0.4 mg protein SPM in a total volume of 0.4 ml buffer. Separation of free and bound DHM was achieved by centrifugation. Non-specific binding was determined by the addition of unlabelled DHM (10^{-6} M) and was always below 20% of the total binding observed. Logarithmic dilutions of peptides were added to the standard mixture in a volume of 25 μl. Blank values were subtracted from all experimental values and values of half-maximum inhibition were obtained graphically from semi-logarithmic plots. Each substance was tested at least 3 times at 3 or more concentrations.

2.2. Hot plate technique

Analgesia was measured using the hot plate method of Eddy and Leimbach (1953) as modified by De Wied and Gispen (1976). Female rats of an inbred Wistar strain weighing 140—160 g were used. The initial reaction time (IRT) of each rat on the hot plate was first determined. 30 min later all rats received a s.c. injection of either saline or peptide (100 μg/rat) followed 30 min later by an i.p. injection of either saline or morphine-HCl (5 mg/kg body weight). 30 min after the i.p. injection the rats were retested on the hot plate and their reaction time minus IRT was computed (ΔRT). As indicated in Results, in some experiments 1 mg instead of 100 μg peptide per rat was used. Furthermore, in one experiment rats were tested not only 30 min after the last injection but also after 60 and 90 min. Bilateral adrenalectomy or sham operation was performed 5 days prior to the hot plate tests. Adrenalectomized rats were given saline instead of water to drink. Such animals are known to be more sensitive to morphine (Gebhardt and Mitchell, 1972; Gispen et al., 1975a) and in the present study, with 5 and 10 mg/kg of morphine i.p.; the ΔRT of adrenalectomized rats 30 min after this injection was 1.25× larger than that of intact animals. Therefore an equianalgesic dose of morphine (4 instead of 5 mg/kg) was used in adrenalectomized rats.

3. Results

A number of N-terminal fragments of ACTH were tested for their affinity for the opiate receptors in the rat brain in vitro and for their ability to counteract morphine-induced analgesia.

Table 1A shows the concentration of peptide which resulted in 50% inhibition of the binding of DHM to the SPM fraction (IC_{50}). Where less than 50% inhibition was reached

TABLE 1

Affinity of ACTH-like peptides for rat brain opiate receptors in vitro (A) and counteraction of morphine-induced analgesia (B).

	(A)		(B)	
ACTH peptide	IC_{50}	% inhibition at 3×10^{-5} M	n = 10 ΔRT on hot plate in sec 6.5 — 13.0	
Saline	—	—		
1-16-NH_2	6×10^{-6}			1
5-16-NH_2	2×10^{-5}			1
5-14	3×10^{-5}			1
4-10	10^{-5}			
4-10 [1]				1
[D-Phe^7] 4-10	10^{-5}			
1-24	3×10^{-6}			1
1-24 [3]				1
11-24		<10		
11-24 [1]				1
11-16		<10		
11-19		<10		
11-17		<10		

[1] $p < 0.05$ two tailed Student's *t*-test. Significant from saline. [2] 1 mg peptide s.c. 60 min prior to hot plate test.
[3] Tested in adrenex animals and compared to adrenex controls.

at 3×10^{-5} M, the percentage inhibition at this concentration is given. The following sequences were active (1—24), (1—16), (5—16), (5—14) and (4—10). Sequences starting at amino acid residue no. 11 of ACTH were inactive. As was reported previously (Terenius et al., 1975) substitution of D-phenylalanine in position 7 of $ACTH_{4-10}$ did not influence the affinity of this sequence for the opiate receptors.

Table 1B shows the effect of N-terminal fragments of ACTH on morphine-induced analgesia as measured on the hot plate. In intact rats, morphine treatment (5 mg/kg i.p.) resulted 30 min later in an increase in reaction latency (ΔRT) of about 13 sec. Pretreatment with the sequences (1—16), (5—16), (5—14) and [D-Phe^7]-$ACTH_{4-10}$ inhibited the morphine-induced analgesia up to about 50—60%; pretreatment with the sequence (1—24) resulted in a tendency to inhibit analgesia. However, in adrenalectomized rats, similar treatment with $ACTH_{1-24}$ did in fact significantly impair morphine-induced analgesia to about 60% of the control value.

The sequences (4—10) and (11—24) were retested in a dose of 1 mg instead of 100 μg per rat s.c. 30 min prior to the morphine injection. Under these conditions (4—10) was effective whereas (11—24) remained unable to alter the morphine-induced reaction latency. In the last experiment, the effect of [D-Phe^7]-$ACTH_{4-10}$ on the morphine-induced analgesia was tested 30, 60 and 90 min after the morphine treatment. As can be seen in fig. 1, morphine (5 mg/kg i.p.) markedly increased the reaction time of rats on the hot plate, with the effect still significant 90 min after morphine treatment. Pretreatment with [D-Phe^7]-$ACTH_{4-10}$ reduced the analgesic response. Repeated testing hardly influenced the reaction time on the hot plate (saline/saline group) and peptide treatment per se did not alter the reaction latency. The latter observation was confirmed by testing $ACTH_{4-10}$ and [D-Phe^7]-$ACTH_{4-10}$ in doses up to 50 mg/kg over a period of 15—120 min after the injection and the sequence (5—16) up to 1 mg/kg without finding a detectably different reaction time from that of the controls.

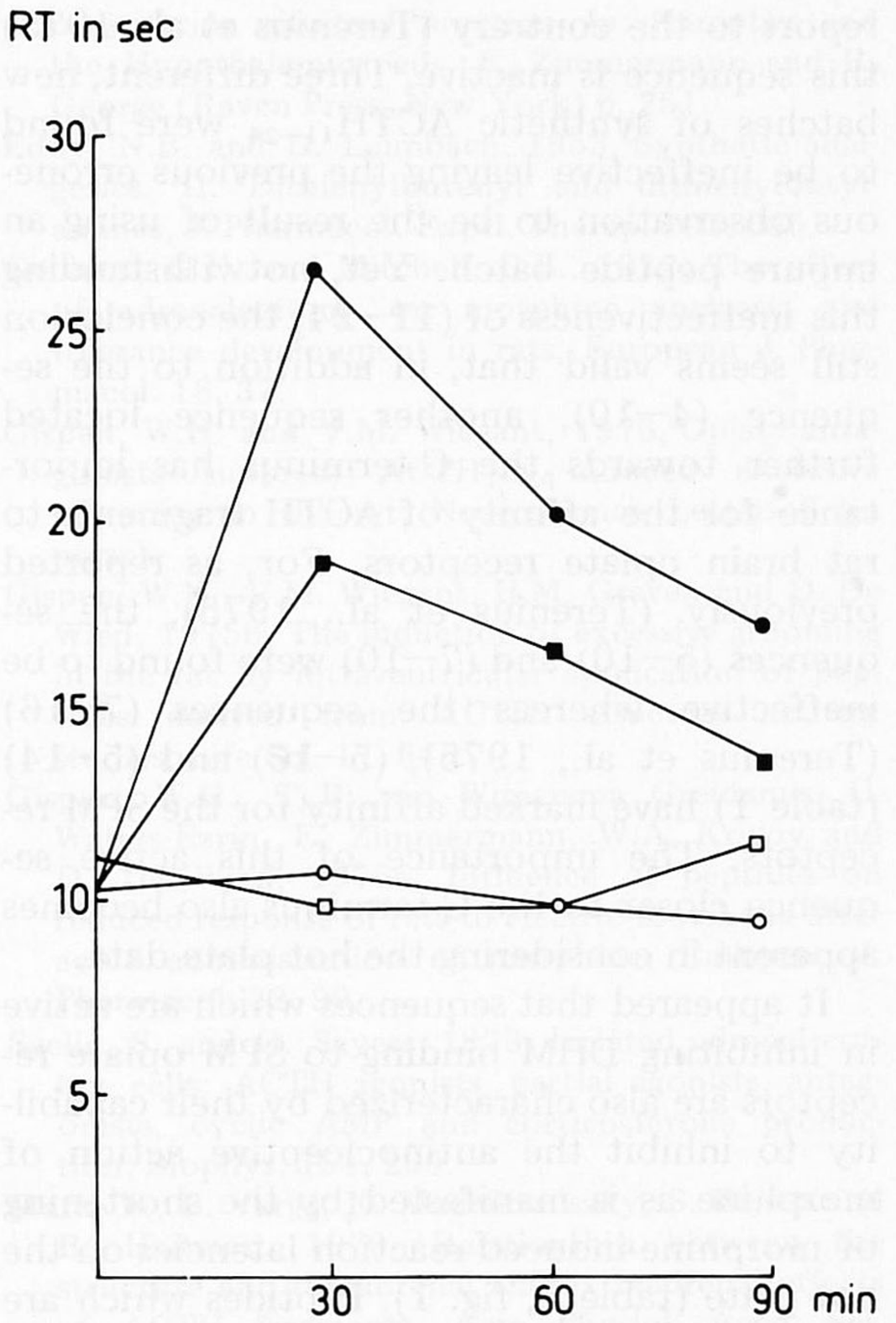

Fig. 1. The effect of pretreatment with [D-Phe^7]-$ACTH_{4-10}$ on morphine-induced analgesia, as measured on the hot plate (for experimental details see text). ●——● saline/morphine n = 10; ■——■ [D-Phe^7]-$ACTH_{4-10}$/morphine n = 10; ○——○ saline/saline n = 10; □——□ [D-Phe^7]-$ACTH_{4-10}$/saline n = 10.

4. Discussion

The present data confirm that N-terminal fragments of ACTH have an affinity for opiate receptors in the rat brain SPM fraction (Terenius, 1975; Terenius et al., 1975). However, with respect to the sequence (11—24) it should be noted that despite a previous

report to the contrary (Terenius et al., 1975) this sequence is inactive. Three different, new batches of synthetic $ACTH_{11-24}$ were found to be ineffective leaving the previous erroneous observation to be the result of using an impure peptide batch. Yet, notwithstanding this ineffectiveness of (11—24) the conclusion still seems valid that, in addition to the sequence (4—10), another sequence located further towards the C-terminus has importance for the affinity of ACTH fragments to rat brain opiate receptors. For, as reported previously (Terenius et al., 1975), the sequences (5—10) and (7—10) were found to be ineffective whereas the sequences (7—16) (Terenius et al., 1975), (5—16) and (5—14) (table 1) have marked affinity for the SPM receptors. The importance of this active sequence closer to the C-terminus also becomes apparent in considering the hot plate data.

It appeared that sequences which are active in inhibiting DHM binding to SPM opiate receptors are also characterized by their capability to inhibit the antinociceptive action of morphine as is manifested by the shortening of morphine-induced reaction latencies on the hot plate (table 1, fig. 1). Peptides which are devoid of an appreciable affinity for rat brain opiate receptors in vitro also lack the ability to interfere with morphine analgesia in vivo. The interpretation of previous in vivo studies of ACTH morphine interaction is confused by the property of ACTH to stimulate corticogenesis in the adrenal cortex. Here, however, it appeared that the fragments (1—16), (5—16) and (5—14), which have no corticotrophic activity (Seelig and Sayer, 1973; Stark et al., 1970), can inhibit DHM binding to its CNS receptors in vitro and counteract morphine-induced analgesia. In intact animals, it was difficult to demonstrate such an effect for $ACTH_{1-24}$, which has the full corticotrophic activity of ACTH. $ACTH_{1-24}$ was also tested at the same dose in adrenalectomized rats against an equianalgesic dose of morphine. In these rats, morphine antagonism was demonstrated which indicates that the (1—24) is also an antagonist. The sequence (4—10) was less active than [D-Phe^7]-$ACTH_{4-10}$ which has been shown in several behavioral and neurochemical studies to act differently or even in the opposite direction to the "all L" sequence (4—10). This emphasizes the specificity and complexity of the ACTH-CNS interaction (De Wied, 1974). The structure-activity relationship which seems to hold for the ACTH-morphine interaction in vivo ressembles that which was found for the effectiveness of ACTH fragments to induce excessive grooming in the rat (Gispen et al., 1975b). From the same study, it was also concluded that the presence of the sequence (4—10) is important but that the expression of its effect is enhanced upon elongation of the sequence towards the C-terminus.

Recently it was found that peptide-induced grooming behavior could be blocked by the administration of specific opiate antagonists like naltrexone and naloxone (Gispen and Wiegant, 1976). Terenius (1976) demonstrated that $ACTH_{1-24}$ shows little difference in affinity for agonist and antagonist sites as had also been observed for $ACTH_{4-10}$ (Terenius et al., 1975). Since this kind of receptor selectivity is characteristic of partial agonists such as nalorphine, it was suggested that ACTH acts as a partial agonist-antagonist on opiate receptors in the CNS (Terenius, 1976). This could very well underlie the somewhat confusing data obtained on the ACTH-morphine interaction in vivo. The partial *agonistic* property could explain the morphine-like activity of ACTH fragments in inducing grooming and the partial *antagonistic* property, the counteraction of morphine-induced analgesia. Depending on the test, one or the other of these properties will be the dominating one.

In a previous study in rats, it was found that $ACTH_{1-24}$ counteracted the morphine-induced (10 mg/kg) reduction of motor responding to inescapable electric footshock (EFS) (Gispen et al., 1975a). Although a direct effect of corticosteroids was difficult to demonstrate, it was suggested that $ACTH_{1-24}$ would counteract morphine-re-

duced responsiveness to EFS through a permissive action of corticosteroids. However, in the present experiments, using various ACTH sequences which lack corticotrophic activity, a more favorable peptide/morphine concentration ratio and a more sensitive technique, we could obtain clear evidence for a direct ACTH—morphine interaction at the analgesic level. This was to be expected from a variety of experiments on ACTH-morphine interactions in the CNS (Zimmermann and Krivoy, 1973; Terenius et al., 1975; Gispen and Wiegant, 1976). ACTH fragments themselves are not likely to be important endogenous opiate receptor ligands in physiological situations because of their comparatively low receptor affinity. If these fragments are present in sufficient concentrations, their partial agonistic features suggest a modulatory role. The fact that $ACTH_{4-10}$, like all hitherto characterized morphinomimetic peptides, is part of the high-molecular weight peptide hormone, β-lipotrophin, may also suggest such a role.

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