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ACTH-LIKE PEPTIDES INCREASE PAIN SENSITIVITY AND ANTAGONIZE OPIATE ANALGESIA

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The role of the pituitary and of ACTH in pain sensitivity was investigated in the rat. Pain sensitivity was assessed by measuring paw-lick and jump latencies in response to being placed on a grid at 55°C. Hypophysectomy reduced pain sensitivity, and this effect was reversed by the intracerebroventricular (ICV) injection of the opiate antagonist naloxone. Similarly, the analgesia produced by a dose of morphine was antagonized by the administration of ACTH or α-MSH. The peripheral injection of ACTH or α-MSH in normal rats did not increase pain sensitivity. However, ACTH administered ICV increased pain sensitivity within 10 min. The results indicate that the pituitary is the source of an endogenous opiate antagonist and hyperalgesic factor and that this factor is ACTH or an ACTH-like peptide. This activity resides in the N-terminal portion of the ACTH molecule since $ACTH_{4-10}$ is not active in this respect, nor does this activity require a free N-terminal serine since α-MSH appears to be almost as potent as the $ACTH_{1-24}$ peptide. It is concluded that ACTH-like peptides of pituitary origin act as endogenous hyperalgesic and opiate antagonistic factors.

Adrenocorticotropin-like peptides Opiate antagonism

Hyperalgesia Hypophysectomy

1. Introduction

Adrenocorticotrophic hormone (ACTH) and structurally related peptides exert profound influences on a wide range of behaviors (De Wied, 1977) and on brain function (Vernikos-Danellis, 1972). We have recently demonstrated that pain sensitivity could be altered significantly by physiological manipulation of endogenous levels of pituitary-adrenocortical hormones (Heybach and Vernikos-Danellis, 1978). Increased sensitivity to pain was correlated with increased circulating levels of ACTH, but was independent of any direct influence of circulating corticosteroids. Based on these findings we proposed a direct neuromodulatory role for ACTH in pain perception mechanisms. The site or sites of action of such a role for ACTH are not known; however, in in vitro brain homogenate preparations, ACTH antagonizes morphine binding to opiate receptors (Terenius et al., 1975) and is capable of displacing β-endorphin binding (Akil et al., 1980) suggesting that the likely site of action of ACTH is on opioid receptors in the central nervous system (CNS).

In the present series of experiments we investigated the hypothesis that the pituitary is the source of an endogenous opioid antagonist and that this substance is ACTH or an ACTH-like peptide. We further demonstrated that unlike naloxone the intracerebroventricular administration of $ACTH_{1-39}$ (the complete peptide sequence), $ACTH_{1-24}$, and the structurally related peptide α-melanocyte stimulating hormone (α-MSH) are capable of increasing the sensitivity to pain in normal animals by a direct action on the CNS.

2. Materials and methods

Male Sprague Dawley rats (Simonsen Laboratories, Gilroy, California) weighing 205 ±10 g, were used. They were housed five per cage in large plastic, top-loading cages with wood-chip bedding. Rats had ad libitum access to standard laboratory chow and water. They were kept on a 12-h light-dark schedule (lights on at 0700) and the temperature was maintained at 23 ±3°C. Groups of at least eight animals were used for each experimental condition.

Drugs used and their suppliers were as follows: ACTH was obtained from Armour Laboratories, Kankakee, Illinois; $ACTH_{1-24}$, $ACTH_{4-10}$, and α-MSH were obtained from Bachem Inc., Torrance, California; and morphine and naloxone were obtained from Endo Laboratories, Garden City, New York. Doses of morphine and naloxone were calculated as the free base.

Hypophysectomies were performed under ether anesthesia, using the transauricular approach with the animal in a stereotaxic instrument (Heybach and Venikos-Danellis, 1978). Animals were provided with 5% dextrose instead of drinking water and were used 1 day postsurgery.

Intraventricular cannulations were done according to the method of Severs et al. (1970), under pentobarbital anesthesia (50 mg/kg i.p.). Cannulas constructed from disposable 20-gauge hypodermic needles, filled with silastic, were implanted 1 mm caudal to the coronal suture and 1.5 mm lateral to the midsagittal suture. They were secured to the skull with acryllic cement. Substances to be injected intraventricularly were dissolved in 0.9% saline immediately before use. Animals received either intraventricular injections (2 µl) of saline, the peptides, or

naloxone in 2 µl saline. Injections were made with Hamilton microliter syringes fitted with a stop to prevent the needle from passing beyond the tip of the cannula. Animals were tested for pain sensitivity at 10, 20, and 30 min after an ivt injection. At the completion of all experiments, 5 µl of blue ink was injected through the cannula before sacrificing the animal. Brains were examined to ensure that the dye was distributed throughout the ventricular space.

The apparatus used to assess sensitivity to pain has been described previously (Heybach and Vernikos-Danellis, 1978). The procedure for estimating pain sensitivity was standardized for all experiments. All animals were initially allowed three 90-sec sessions (one session per day for 3 consecutive days) in the testing chamber with the temperature of the floor maintained at 23 ±1°C. This procedure familiarized the rats with handling and allowed for habituation to the novelty of the apparatus. To initiate testing, the rat was placed on the grid floor of the apparatus, maintained at 55 ±1°C, and a stopwatch was started. Two responses were then recorded: the latency to lift from the floor and lick one paw (i.e., paw-lick latency), and subsequently the latency to show a jump response consisting of vigorously lifting both hind paws off the grid floor simultaneously (i.e., jump latency). The paw-lick and jump latencies were used as indices of sensitivity to the painful stimulus. During testing, the experimenter was not aware of the condition of the rat being tested. If neither a paw-lick nor a jump response was made in 90 sec, the test was terminated and a latency of 90 sec was recorded for each response. All testing was carried out between 0800 and 1200 h.

Statistical analysis of the response latencies was carried out using one-way analyses of variance for relevant two-group comparisons.

3. Results

Figure 1 shows the paw-lick and jump latencies of rats that had been hypophysectomized or sham-hypophysectomized and injected intraventricularly with either saline or naloxone. Hypophysectomy increased the latency of both responses. Whereas naloxone had no significant effect on the responses of the sham-hypophysectomized animals, it antagonized the analgesic effect of hypophysectomy and restored the pain sensitivity of the hypophysectomized rats to normal.

Since opiate antagonist properties of the pituitary were implied by this and previous data, and since the changes in pain sensitivity after adrenalectomy correlated well with the changes in circulating ACTH (Heybach and Vernikos-Danellis, 1978), we studied the effect of ACTH and α-MSH on the analgesic action of morphine. Rats injected with a dose of 5 mg/kg morphine (i.p.) were given, 30 min later, a second injection of either saline, α-MSH, or ACTH (i.p.) and tested for paw-lick and jump responses. Figure 2 shows that 10 min after the injections of these peptides, this dose of morphine produced only half of its usual analgesic effect. Antagonism of morphine's analgesic action was even greater 20 or 30 min after ACTH injection. It should be noted, however, that the control injection of saline also antagonized the morphine effect to some extent; this was particularly evident in the paw-lick response.

Figure 3 shows that the peripheral administration of ACTH or α-MSH alone did not alter either the paw-lick or the jump latency of normal

rats. However, after intracerebroventricular (ICV) administration, $ACTH_{1-24}$ or α-MSH markedly reduced the paw-lick and the jump latencies at doses that were one fourth of those that were ineffective when given peripherally (fig. 4). $ACTH_{4-10}$ had no effect on pain sensitivity.

4. Discussion

We have recently obtained evidence that ACTH may be a critical pituitary factor involved in mediating the perception of pain (Heybach and Vernikos, 1978). We found that whereas sensitivity to pain was unaltered 3 days after adrenalectomy when circulating corticosterone was absent and ACTH levels were low, animals adrenalectomized for 9 or 18 days, when plasma ACTH levels were significantly increased, showed a marked increase in pain sensitivity as well. In contrast, removal of the pituitary gland led to a prompt decrease in pain sensitivity. Such a decrease has also been observed in the clinical studies of Luft and Olivecrona (1955). Based primarily on these results, we proposed that ACTH or possibly some other pituitary ACTH-like peptide that is also regulated by glucocorticoid negative feedback, may function as an endogenous opioid antagonist, modulating pain sensitivity. If this were the case, then synthetic opiate antagonists should reverse the analgesia that follows hypophysectomy, and the direct central administration of ACTH should increase pain sensitivity and antagonize the actions of opiates.

Our findings support this hypothesis. The ICV administration of naloxone restored the pain sensitivity of hypophysectomized rats to normal, though it had no apparent effect in normal animals. Similarly,

ICV-administered ACTH increased pain sensitivity. This increased sensitivity or responsiveness to pain was apparent after ICV injections of crude $ACTH_{1-39}$ (Armour), $ACTH_{1-24}$, or α-MSH. Intraperitoneal injections of ACTH or α-MSH were ineffective in this respect even at doses that were four times as great as those administered centrally. Therefore, it appears that ACTH and related peptides are capable of increasing sensitivity to a painful stimulus by a direct effect on the central nervous system and that they display some degree of structural specificity in exerting this effect. The activity seems to reside in the N-terminal portion of the molecule, whether the N-terminal serine is acetylated or not. It is conceivable that larger, peripherally administered doses of these peptides might also be able to increase pain sensitivity. However, although these peripheral doses themselves produced no change in pain sensitivity, both ACTH and α-MSH were very effective in antagonizing the analgesic action of morphine. Such an effect of ACTH in antagonizing morphine-induced analgesia has been reported previously (Gispen et al., 1976; Paroli, 1967) but this work demonstrates that ACTH and ACTH-like peptides can themselves directly increase pain sensitivity.

The finding that the peripheral injection of saline reduced the effect of morphine on pain sensitivity, but to a lesser extent and with a somewhat different time course than either ACTH or α-MSH, is consistent with the notion that the stressful nature of the injection procedure itself, via release of endogenous ACTH or related peptides from the pituitary, can lead to antagonism of morphine-induced analgesia (Vernikos et al., submitted to European J. Pharmacol., 1980).

It is particularly interesting that the synthetic peptide fragment $ACTH_{4-10}$, which has no adrenocortical stimulating activity, had also no effect on pain sensitivity when administered ICV. Similarly, it has been reported that $ACTH_{4-10}$ is ineffective in antagonizing morphine-induced analgesia in spite of its apparent affinity for rat brain opiate receptors (Wiegant et al., 1977). Thus, although the ample evidence for interactions of ACTH with opiate receptors (Terenius et al., 1975) is encouraging support for the hypothesis that ACTH exerts its hyperalgesic effects and its opiate antagonistic effects by acting on opiate receptor systems, the discrepancy between the receptor affinity and physiological action of $ACTH_{4-10}$ suggests caution in extrapolating receptor-binding data to physiological activity.

The presence of ACTH in various areas of the central nervous system has now been well documented (Orwall et al., 1979) although it is still not clear what its source and function there might be (Krieger, 1980). Nevertheless, our studies show that the pituitary is the primary source of this ACTH-like peptide that exerts hyperalgesic and opiate antagonistic effects; this is because hypophysectomy reduces pain sensitivity while increases in pituitary and circulating ACTH, as a function of time after adrenalectomy, result in a similar, time-dependent increase in the sensitivity to pain (Heybach and Vernikos-Danellis, 1978).

In contrast, although the pituitary is also a rich source of endorphins, which have been shown to be secreted concomitantly with ACTH (Rossier et al., 1980), the primary function of this endogenous opiate in the pituitary would not appear to be in pain suppression, since hypophysectomy should have been expected to result in increased pain sensitivity.

Nor is it likely that a compensatory increase in endorphin activity in the CNS would appear within 24 h of hypophysectomy. It is tempting to suggest instead that its function there might be to modulate CRF, and thereby ACTH secretion, by a short feedback-loop effect on the hypothalamus. Unlike met-enkephalin, which stimulates CRF secretion, β-endorphin alone does not affect CRF secretion, but antagonizes the stimulatory actions of morphine and enkephalin (Buckingham and Hodges, 1979). More recently, we have found that β-endorphin in concentrations as low as 10^{-5} M antagonizes in a dose-dependent manner the acetylcholine stimulated secretion of CRF from isolated hypothalami in vitro (Buckingham and Vernikos, unpublished observations).

The reversal of hypoalgesia, evident at 1 day following hypophysectomy, by the acute ICV administration of the synthetic opiate antagonist naloxone, and the direct effect of ACTH and α-MSH in increasing sensitivity to pain and in antagonizing morphine-induced analgesia, lead us to suggest that under normal conditions sensitivity to pain might be regulated by a balance of endogenous opioids of central origin and ACTH-like peptides of pituitary origin interacting at opiate receptor sites.

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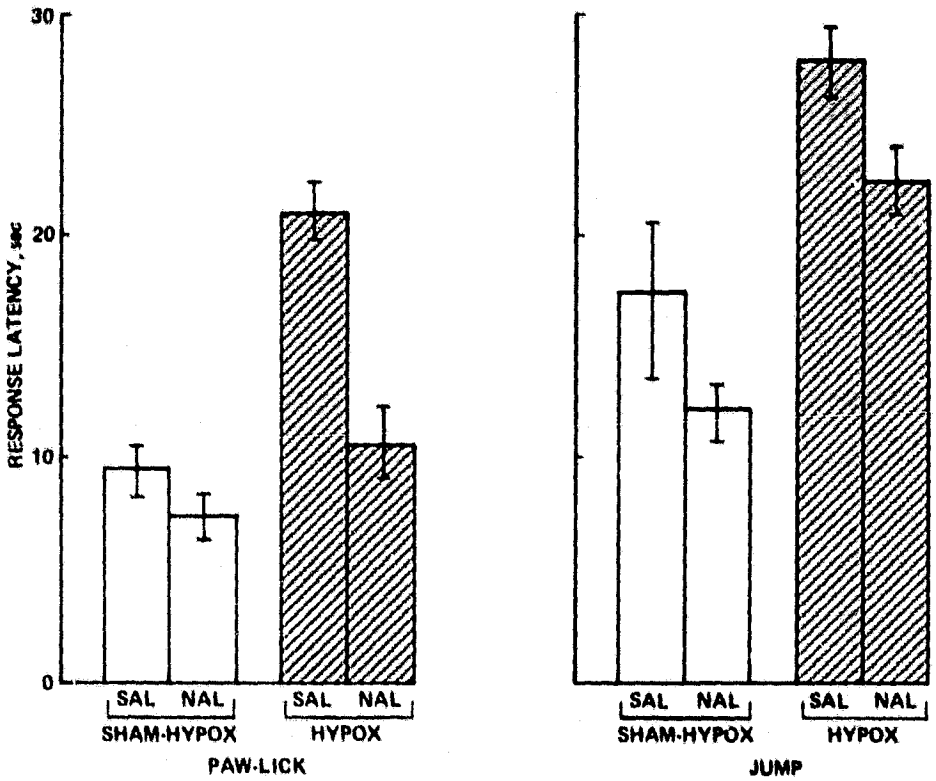
FIGURE LEGENDS

Fig. 1. Paw-lick (left) and jump (right) latencies in hypophysectomized (crosshatched bars) and sham-hypophysectomized (open bars) rats, after intracerebroventricular injection of saline (2 µl) or naloxone (20 µg). Each column represents the mean ±S.E. of the mean. (1) Sham-hypox, Sal vs Hypox, Sal: paw lick — $F(1,18) = 18.4$, $p < 0.001$; jump — $F(1,18) = 12.6$, $p < 0.005$. (2) Hypox, Sal vs Hypox, Nal: paw lick — $F(1,16) = 11.3$, $p < 0.01$; jump — $F(1,17) = 21.3$, $p < 0.001$.

Fig. 2. Percent change in latency of paw-lick and jump responses of morphine-treated rats (5 mg/kg i.p., 30 min previously) at 10, 20, and 30 min after an i.p. injection of saline, ACTH (Armour, 50 µU), or α-MSH (50 µg).

Fig. 3. Paw-lick and jump latencies in response to i.p. injections of saline, α-MSH, or ACTH.

Fig. 4. Paw-lick (left) and jump latencies (right) in normal rats after intracerebroventricular injection of saline (2 µl), $ACTH_{1-24}$ (25 or 50 µg), $ACTH_{4-10}$ (25 µg), or α-MSH (25 µg). (1) $ACTH_{1-24}$ (25 µg) vs Sal: paw lick — $F(1,23) = 19.4$, $p < 0.005$; jump — $F(1,23) = 21.1$, $p < 0.001$. (2) $ACTH_{1-24}$ (50 µg) vs Sal: paw lick — $F(1,26) = 20.3$, $p < 0.005$; jump — $F(1,26) = 32.8$, $p < 0.001$. (3) α-MSH vs Sal: paw lick — $F(1,24) = 28.3$, $p < 0.001$; jump — $F(1,24) = 17.3$, $p < 0.005$.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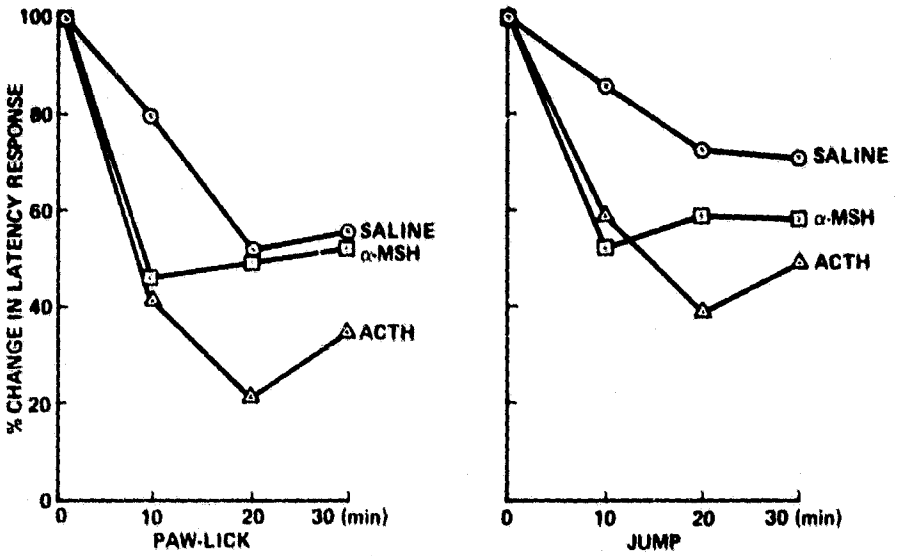
JUMP

Fig. 1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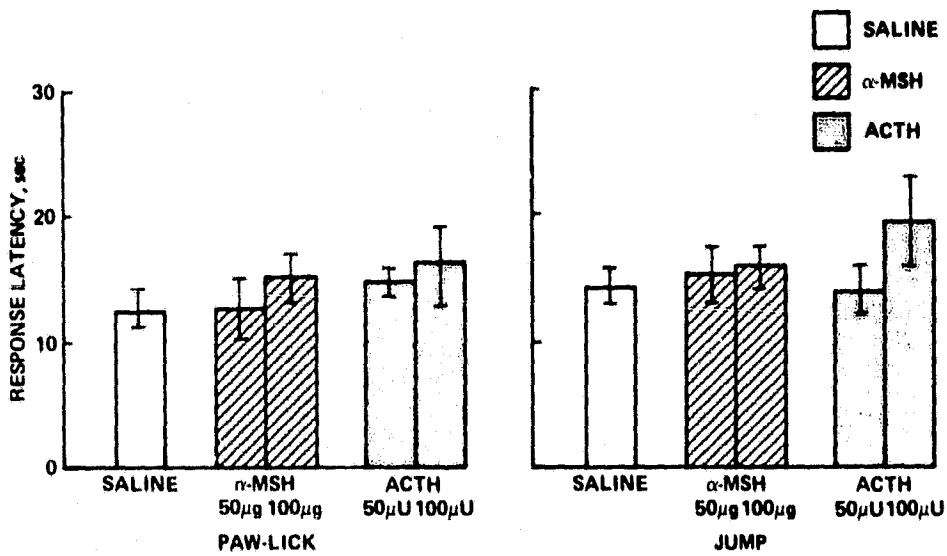
30 (min)
JUMP

Fig. 2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RESPONSE LATENCY, sec
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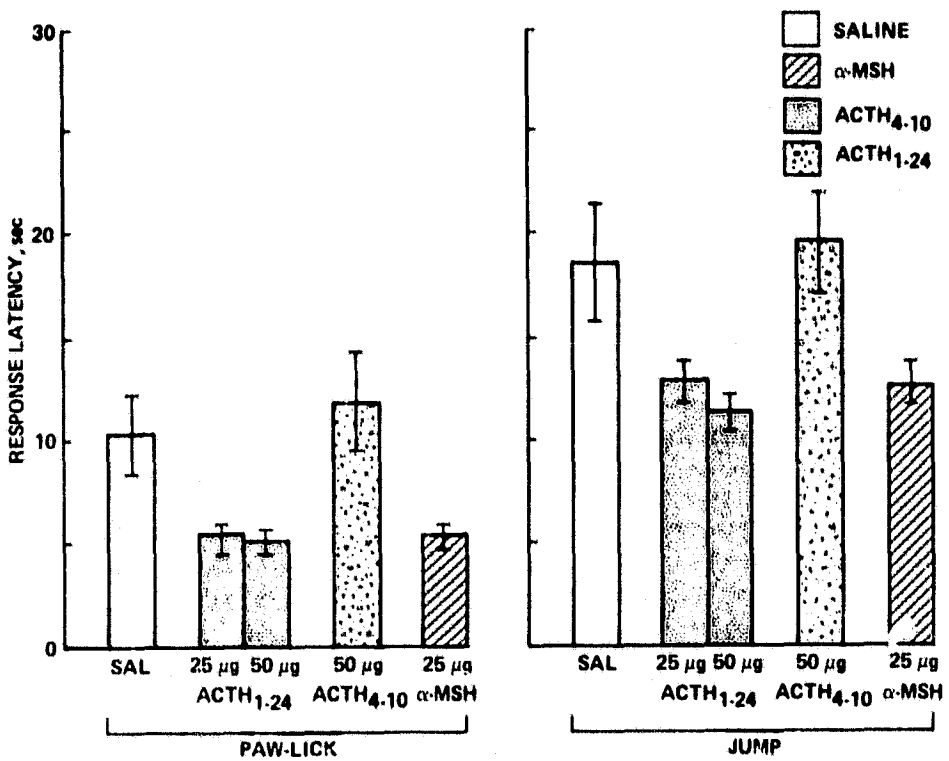
ACTH 50μU 100μU
JUMP

Fig. 3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25 µg ACTH1-24
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ACTH1-24
SAL
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50 µg ACTH1-24
50 µg ACTH4-10
25 µg α-MSH
JUMP

Fig. 4