ACTH-induced hyperalgesia in rats

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Summary. The injection of ACTH 1-24 into the cerebral ventricles in rats markedly reduces the reaction time in the hot-plate test and the nociception threshold in the tail-stimulation test. Morphine antagonizes and naloxone potentiates this hyperalgesic effect of ACTH. It is proposed that ACTH peptides play a physiological role in nociception.

There is mounting evidence that opiate peptides and ACTH peptides overlap in many instances. Except for leu-enkephalin, all the endogenous ligands of the opiate receptors are fragments of the C-terminal moiety of the pituitary hormone β-lipotropin[1]: met-enkephalin is the fragment 61-65; α-, β- and γ-endorphin are the fragments 61-76, 61-91 and 61-77 respectively.

On the other hand, β-lipotropin contains the complete amino acid sequence of β-MSH (= LPH 41-58) and a heptapeptide (Met-Glu-His-Phe-Arg-Trp-Gly) which is common to ACTH (= ACTH 4-10), α- and β-MSH and which is essential for the adrenocorticotropic, melanophore-stimulating and behavioral activities of these peptides[2].

In the β-LPH molecule, the aminoacid sequence of β-MSH is separated from that of β-endorphin by only 2 basic amino acids, lysine and arginine, in the positions 59 and 60; such dipeptides often border amino acid sequences which are enzymatically released from prohormones. A basic dipeptide is also located at the N-terminus of the sequence of β-MSH. ACTH, β-LPH and β-endorphin have been found in the same storage granules in corticotrophs[3] and in the same hypothalamic neurons[4] and have a common precursor of mol. wt 31,000, as demonstrated in cultures of the AtT-20/D-16v mouse pituitary tumor cell line[5].

Like enkephalins[6] and endorphins[7], ACTH and α-MSH are widely distributed in several brain regions[8-10], where their concentration is usually unaffected by hypophysectomy, suggesting that they are synthesized in these brain areas, where they may function as neuromodulators[9,11]. This view is supported by the recent immunocytochemical demonstration of the existence of 2 peptide neuronal systems, one being an enkephalin system and the other a β-LPH/β-endorphin/ACTH-positive system[4]. Met-enkephalin[12] and β-endorphin[7,13] have behavioral effects, and ACTH peptides have many different, pronounced and well-known behavioral effects also[14]. Reciprocally, enkephalins and endorphins have hormone-releasing activities[15]. Exogenous opiates and endorphins inhibit adenylate cyclase activity[16]; conversely, it is well-known that ACTH stimulates adenylate cyclase activity in target cells[17]. Intraventricular β-endorphin induces a dose-dependent decrease of the turnover rate of acetylcholine in several nuclei of the rat brain, particularly in the hippocampus[18]; α-MSH and ACTH 1-24 have an opposite effect[19].

Exogenous opiates and enkephalins reduce the neuronal firing[20]; ACTH increases it[21]. ACTH-like peptides antagonize morphine inhibition of spinal reflex activity, both in vivo, in the anaesthetized cat[22], and in vitro, using the isolated spinalcord of the frog[23].

Finally, several fragments of ACTH have marked affinity for brain opiate receptors and inhibit morphine- and endorphin-induced analgesia without having any analgesic activity per se: the heptapeptide sequence ACTH 4-10 seems to be crucial with respect to affinity, which is however highest in the case of ACTH 1-24[24]. This last finding seemed to us very important in view of the fact that the blockage of the opiate receptors should cause hyperalgesia, as has been demonstrated for naloxone, which is a powerful and selective opiate-antagonist[25]. Thus, owing to its presence in the same brain areas, or actually in the same neurons which contain endogenous opiates, ACTH might play a physiological role in nociception. Here we show that

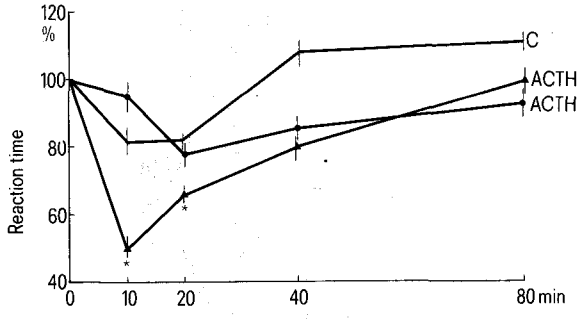

Fig. 1. Effect of the intracerebroventricular injection of ACTH 1-24 on the reaction time to a thermal stimulus (hot plate test, 45 ± 0.2 °C), in rats. Values are expressed as % ± SEM (vertical bars) of the respective basal reaction times. ACTH 20 = ACTH 1-24, 20 µg/rat, into a brain lateral ventricle; ACTH 50 = ACTH 1-24, 50 µg/rat, by the same route; C = the same volume (10 µl) of the solvent, by the same route. Injections were made at time 0. * Different from controls, $p < 0.02$ (Student's t-test).

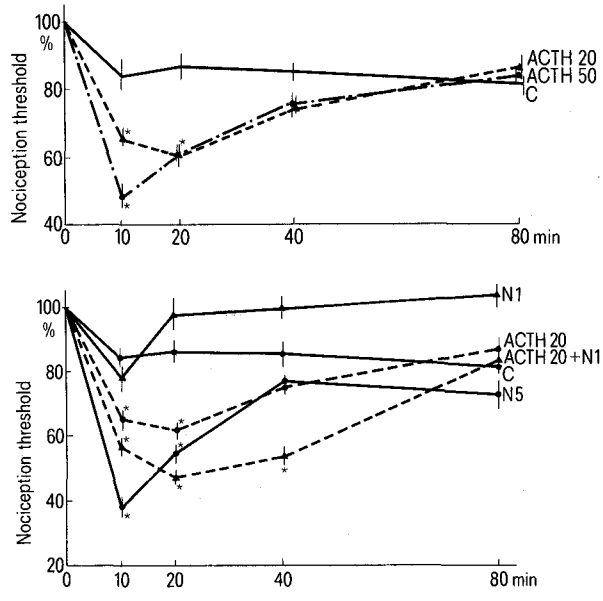

Fig. 2. Effect of ACTH 1-24 and of naloxone on the nociception threshold to an electrical stimulus (5 imp./sec through electrodes placed in 2 adjacent intervertebral spaces of the tail), in rats. Values are expressed as % ± SEM (vertical bars) of the respective basal nociception thresholds. ACTH 20 = ACTH 1-24, 20 µg/rat, into a brain lateral ventricle (i.c.v.); N 1 = naloxone, 1 mg/kg i.p. and the same volume of the solvent of ACTH (10 µl) i.c.v.; N 5 = naloxone, 5 mg/kg, i.p. and 10 µl of solvent i.c.v.; ACTH 20 + N 1 = naloxone, 1 mg/kg, i.p. and ACTH 1-24, 20 µg/rat, i.c.v.; C = the same volume of the solvent of ACTH (10 µl) i.c.v. and the solvent of naloxone, i.p. Injections were made at time 0. * Different from controls, $p < 0.001$ (Student's t-test).

the intraventricular injection of ACTH 1-24 causes hyperalgesia.

Materials and methods. Groups of 30 adult Wistar rats, kept under standard conditions, were used. ACTH 1-24 (Synacthen Ciba-Geigy) was injected into a lateral brain ventricle through a stereotaxically implanted permanent cannula, at doses of 20 and 50 µg/rat, in a volume of 10 µl; control rats received the same volume of the solvent by the same route.

In a set of experiments naloxone and morphine HCl were also administered: the doses of naloxone were 1 and 5 mg/kg i.p., that of morphine was 5 mg/kg s.c. 2 different algesimetric methods were used: the hot plate test[26] and the electric stimulation of the tail[27].

In the hot plate test the copper plate was maintained at the temperature of 45 ± 0.2 °C and in the tail-stimulation test the frequency of impulses was reduced (5/sec), in order better to detect any state of hyperalgesia. The reaction time (hot plate) and the nociception-threshold (electric stimulation of the tail) were recorded immediately before treatment and 10, 20, 40 and 80 min later.

Results and discussion. In the hot plate test, 10 min after the intracerebroventricular injection of the highest dose of ACTH (50 µg), the reaction time was reduced roughly by 50% and the reduction was still significant at 20 min; 80 min after the injection, the reaction time to the thermal stimulus returned to normal. Morphine (5 mg/kg, s.c. immediately before the intracerebroventricular injection of ACTH 1-24) completely counteracted the effect of ACTH.

The hyperalgesic effect of ACTH was shown even more clearly with the electrical stimulation of the tail: the dose of 20 µg caused a highly significant reduction of the threshold voltage, 10 and 20 min after the injection: the effect was still significant at 40 min and was comparable to that produced by the i.p. injection of 5 mg/kg of naloxone. Moreover, the combination of ACTH (20 µg/rat) with a subactive dose of naloxone (1 mg/kg) resulted in an increased hyperalgesia. These last data, together with the antagonist effect of morphine, may indicate that ACTH causes hyperalgesia by binding to brain opiate receptors.

On the whole, bearing in mind the other behavioral effects, the brain distribution, the origin, the presence in the same neurons which contain β-endorphin, the affinity for opiate receptors and the present results, it seems to us that ACTH (and probably α-MSH and other ACTH-fragments) may play a physiological role in the perception of noxious stimuli and can be considered an endogenous antagonist of opiate ligands. Recently we found that the s.c. injection of naloxone to normal adult rats induces the same behavioral syndrome observed after the intraventricular injection of ACTH peptides[28], and Grevert, Baizman and Goldstein reported that the hyperalgesic effect of naloxone is prevented by hypophysectomy, and have suggested that the pituitary contains a hyperalgesic factor and that naloxone stimulates release of this factor[29]. These findings, particularly those of Grevert et al., give further strong support to our proposal to regard ACTH peptides as endogenous antiendorphines.

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