Pharmacology Biochemistry & Behavior, Vol 20, pp 879–882, 1984 © Ankho International Inc Printed in the U S A 0091-3057/84 $3 00 + 00

Loss of Sensitivity to Morphine Induced by Prolonged ACTH Treatment

MÁRTON I. K. FEKETE,[1] BÉLA KANYICSKA,* TIBOR SZENTENDREI AND ERVIN STARK

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
**Research Centre of Animal Nutrition and Breeding, Gödöllő, Hungary*

Received 31 August 1983

FEKETE, M I K , B KANYICSKA, T. SZENTENDREI AND E STARK *Loss of sensitivity to morphine induced by prolonged ACTH treatment* PHARMACOL BIOCHEM BEHAV **20**(6) 879–882, 1984 —The effect of long term ACTH treatment on some actions of morphine were studied. The effect of ACTH administration was compared to that induced by acute dexamethasone injection. ACTH caused a delayed inhibition of the morphine induced increase in growth hormone secretion demonstrable 24 hr after the last hormone injection The morphine induced increase of striatal DOPAC (3,4-dihydroxyphenylacetic acid) content was also inhibited by ACTH treatment, however, neither the analgesia, nor the hypermotility caused by morphine were affected. Dexamethasone did not alter significantly the responsiveness to morphine. It is concluded that the prolonged exposure to ACTH presumably causes a corticosterone-mediated loss of responsiveness of functionally restricted opiate sensitive mechanisms in the central nervous system

ACTH Morphine Growth hormone Striatal DOPAC Motility Analgesia Glucocorticoids Rats

PROLONGED treatment of rats with ACTH inhibits not only the stress-induced increase of corticosterone secretion [24,25], but also the release of prolactin induced by various noxious stimuli [9]. The effect of ACTH is demonstrable 24 hr following the last hormone injection, when the plasma hormone levels (corticosterone, ACTH, prolactin) of the hormone injected rats are not different from the controls. In a further study it was shown that chornic ACTH treatment also inhibits the decrease of dopamine metabolism in the tuberoinfundibular region and the increase of prolactin secretion produced by morphine [15]. These results have led us to suggest that ACTH, possibly by way of an effect of the central nervous system, induces a decrease of opiate sensitivity.

Morphine is known to induce several well established CNS effects: analgesia, increase of spontaneous motility in rodents [2], augmented striatal dopamine metabolism [12], and increase in corticosterone and growth hormone levels in the peripheral plasma [4, 23, 29]. Interestingly, ACTH has been reported to compete for opiate receptor binding [28] and to influence the morphine-induced changes of prolactin secretion [11] and nociception [13]. The results reported in this study are aimed to clarify how general is the inhibition by ACTH of the effects of morphine.

Since the effects of prolonged ACTH treatment on stimulation-evoked release of ACTH-corticosterone are dependent on the presence of adrenals and are therefore presumably corticosterone mediated [26], we had to differentiate the effects of ACTH from that of an acute glucocorticoid feedback action. For that purpose we have looked at the possible interactions between morphine and dexamethasone. This glucocorticoid is known to exert an immediate and relatively strong feedback inhibition of the hypothalamo-pituitary-adrenal system [1].

METHOD

Male CFY random bred rats of 180–220 g body weight were used in this study. The animals, five in each cage, were housed in an air conditioned room (22°C, 60% relative humidity) with regulated lighting (14 hr light, 10 hr dark).

The following experimental groups were employed: (1) Fourteen days treatment with ACTH (IM 3 U/rat, equivalent to 30 μg synthetic ACTH). The measurements were done 24 hr following the last ACTH injection; (2) Two injections of dexamethasone, 400 μg/kg 18 hr and 200 μg/kg 2 hr preceding the measurements. The animals were handled daily for 6 days preceding the experiments; (3) saline treated controls. The controls for ACTH treatment were injected for 14 consecutive days with saline and the measurements were done 24 hr following the last injection. For controls of dexamethasone treatment, handled animals received saline 18 and 2 hr before the measurements.

Twenty four hours before the measurements random groups were formed and pairs of the rats receiving the same treatment were housed in plastic cages. A group of each rats treated as described above received saline at the time of measurements and served as intact control of the given pretreatment. The others received morphine SC.

The animals were killed by decapitation and the trunk

[1]Requests for reprints should be addressed to Dr M I K Fekete, P.O.B. 67, Budapest, 9, Hungary H-1450

blood was collected in heparainized tubes for hormone determinations. The plasma samples were stored at −20°C until assayed with radioimmunoassays NIAMDD material was used for the growth hormone assay, using GH-RP-1 as reference preparation. The limit of detection was 6–12 ng/ml plasma. The antibody against corticosterone was raised in rabbits in our Institute. The antibody was specific to corticosterone, there was no cross reaction with naturally occurring steroids (aldosterone, gonadal steroids) in the range of physiological concentrations. The limit of detection was 30 nM/l plasma corticosterone.

The striatal 3,4-dihydroxyphenylacetic acid (DOPAC) level was measured radioenzymatically [8]. Brains were quickly removed following the decapitation and the striata were dissected and put on dry ice within 1 min. The tissue samples were homogenized in 0.1 N perchloric acid in a volume of 2 ml/one striatum. The protein content of the homogenates was measured by Lowry's method, 10 μl of the supernatant was used for the measurement of DOPAC

The spontaneous motility of individual rats was measured by a Varimex apparatus (Columbs Instruments, OH, USA)

The analgesic effect of morphine was measured in rats by tail flick method [6]. The ED_{50} values were determined nonparametrically [19]; two times increase of the latency time was considered as positive analgesic response.

The following drugs were employed: ACTH (Cortrophine-Z®), dexamethasone (Oradexon®, Organon, Oss, The Netherlands), morphine sulphate (Alkaloida, Tiszavasvari, Hungary).

One or two way analysis of variance for randomized blocks and mutliple comparison procedures [7] were used for statistical evaluation. Logarithms of hormone and amine values were used for evaluation.

RESULTS

Effects on Corticosterone and Growth Hormone Secretion

Morphine in doses of 2.5–20 mg/kg induced a dose related increase in corticosterone secretion (Fig. 1). ACTH treatment inhibited this response without altering the basal plasma corticosterone levels. In contrast, dexamethasone depressed the basal rate of corticosterone release, the corticosterone levels in all dexamethasone treated groups were less than the determination limit of the radioimmunoassay employed (30 nM/l plasma).

The growth hormone secretion (Fig. 1) exhibited a bell shaped dose-response curve to the increasing doses of morphine The maximal increase was obtained at the dose of 5 mg/kg. Both phases of this curve were depressed by ACTH pretreatment. Dexamethasone, although it caused a significant ($p<0.01$; $F(1,80)=5.32$) decrease in basal growth hormone release, did not interfere significantly $(3,80)=2.14$) with the effect of morphine.

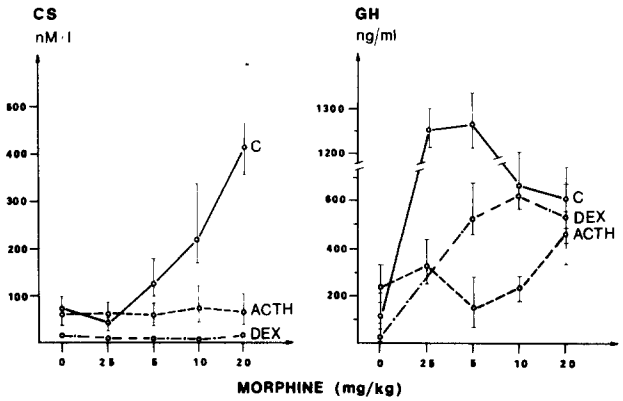

FIG 1 The effects of increasing doses of morphine (abscissae) on the plasma levels of corticosterone (CS) and growth hormone (GH) in control saline treated (C), dexamethasone (DEX), or ACTH treated rats Dexamethasone was injected in doses of 200 and 400 μg/kg SC 2 and 18 hr preceding the measurements ACTH was injected in a dose of 3 U/rat/day IM daily for 14 days The controls received the corresponding volume of saline ACTH and dexamethasone inhibited the effect of morphine on corticosterone at a limit of significance $p<0$ 01, the effect of morphine on GH secretion was inhibited only by ACTH at a level of significance ($p<0.01$) Means±S E (vertical bars), n=10–12 at each point.

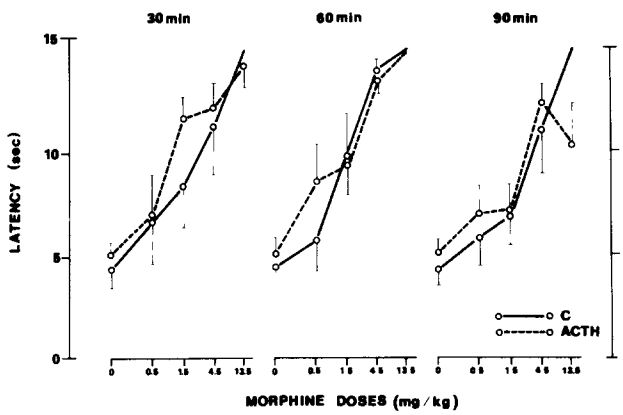

FIG. 2 Tail flick latency as affected by increasing doses of morphine at various times following the treatment, located at the top of the curves, in control (C) and ACTH treated rats Means±S.E., n=6 ACTH was given in a dose of 3 U/rat IM for 14 days, the controls received saline daily

Morphine Induced Analgesia

Morphine increased the tail flick latency between 30 and 90 min following its administration (Fig. 2). ACTH pretreatment had no effect on morphine analgesia. At 60 min the ED_{50} value in the controls and ACTH treated rats was 0.82 mg/kg and 0.62 mg/kg, respectively (the difference is not significant $p>0.10$, [19]). The ED_{50} value for dexamethasone treated animals was 0.76 mg/kg at the same time (not shown in the figure).

Spontaneous Motility

The motility counts for control saline treated rats measured for one hour (beginning of the measurements 20 min after the injection) was 104±27. In animals treated with morphine 5 mg/kg SC, the same value was 194±38 There was no change in this effect of morphine in 14 days. ACTH treated animals (124±24 versus 217±29 motility counts, n=5)

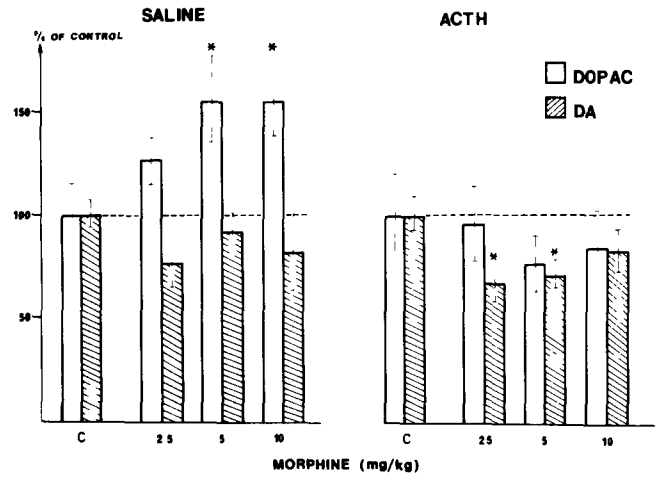

FIG. 3 The changes of striatal dihydroxyphenylacetic acid (DOPAC) and dopamine (DA) levels under the effect of increasing doses (indicated at the bottom of the columns) of morphine in saline and ACTH treated rats ACTH was given in dose of 3 U/rat daily for 14 days, the controls received the corresponding volume of saline (50 μl) The asterisks indicate significant ($p<0.01$) changes compared to the respective control taken as 100% value. The control values were for DOPAC 13 2±1 05 and 15.3±1 32; for dopamine 65 7±5 78 and 73 4±8.24 in saline and ACTH treated group, respectively (ng/mg protein values, n=8)

Changes in Striatal DOPAC Levels

Morphine in doses of 5 and 10 mg/kg significantly increased the concentration of DOPAC in the striatum. This effect was not demonstrable in ACTH pretreated rats (Fig. 3). Dexamethasone did not alter the effect of morphine augmenting the DOPAC concentration in this brain region.

DISCUSSION

Prolonged ACTH treatment inhibited not only the morphine induced increase in prolactin release [15], but also the morphine induced increase of growth hormone and corticosterone secretion. Moreover, long lasting ACTH administration diminished the morphine-responsiveness of the nigrostriatal dopaminergic system in parallel with the inhibition of the decrease of dopamine turnover induced by morphine in the tuberoinfundibular dopaminergic neurons [15].

Surprisingly, neither the morphine induced increase in motility, nor the analgesia were affected by ACTH. One possible explanation of our results could be the increased elimination of morphine by the effect of adrenal steroids on hepatic drug metabolism [27], also suggested by several recent reports [16] As certain effects of morphine are uninfluenced, while other effects are inhibited by the same hormonal pretreatment, this possibility seems improbable. Similarly, the naloxone like effect of ACTH first suggested by Gispen *et al.* [13] may be excluded at the time of measurements (24 hr following the last injection): As far as the plasma corticosterone levels are concerned, at that time no effect of ACTH injection could be detected Plasma corticosterone level is very sensitive indicator in ACTH treated rats, since the hypertrophic adrenals are very sensitive to minute amounts of ACTH.

Both the prolonged ACTH treatment [9] and the acute dexamethasone administration [14] inhibit corticosterone and prolactin secretion induced by various stimuli. Glucocorticoids are reported to inhibit growth hormone release [21]. The inhibitory effect of ACTH on corticosterone release is dependent on th presence of adrenals, and therefore is presumably corticosterone mediated [26]. Similarly the ACTH induced inhibition of morphine's action is not demonstrable in adrenalectomized rats (in preparation). Moreover the effect of ACTH could be mimicked by treatment with a long acting hydrocortisone preparation [10]. In spite of these facts the prolonged ACTH induced inhibition is not similar to steroid feedback action induced by the acute treatment with dexamethasone. The effect of ACTH is demonstrable 24 hr after the last hormone injection, under conditions, when the basal release of hormones (corticosterone, prolactin, ACTH and growth hormone) is apparently unaffected. Dexamethasone, on the other hand, induces a depression of corticosterone, prolactin and growth hormone secretion and does not affect the morphine induced release of prolactin [15,22] and growth hormone (present results).

Morphine may be affecting prolactin release at least partly via the inhibition of tuberoinfundibular dopamine release. The prolonged ACTH treatment caused inhibition of morphine induced prolactin release is possibly mediated by the change in opiate reactivity of dopaminergic neurons [15]. On the other hand, morphine may be acting on growth hormone release possibly by way of an activation of noradrenergic synapses [18]. Our preliminary data suggest that ACTH treatment decreases noradrenaline metabolism in brain areas We suggest that this effect may be responsible for the action of ACTH on growth hormone secretion.

The inhibitory effect of ACTH pretreatment seems to be restricted to certain actions of morphine. One might suggest that the effects of this trophic hormone are linked to specific opiate receptor subtypes. The receptor subtypes for opiates are well known in the literature [20]. Employing opiate agonists specific to pharmacologically defined opiate receptor subtypes, evidence was presented for the involvement of separate opiate receptors (sigma and delta) for the changes of motility [3], and striatal dopamine metabolism [5]. Our results obtained so far disprove the possibility of a possible receptor specific action of ACTH.

Glucocorticoid treatment is known to inhibit ACTH and β-endorphine release and synthesis in the pituitary [17]. It is possible that the prolonged ACTH treatment affects the synthesis and release of endogenous opioids at least partly by way of prolonged steroid action. Indeed preliminary data (Simonyi *et al.* to be published) show that the prolonged ACTH treatment increases the trypsin-like enzyme activity in the adrenal medulla.

The loss of responsiveness to repeated noxious stimuli is a well known phenomenon. A repeated stressful influence may evoke similar changes to that caused by repeated ACTH injection. Therefore, we propose the hypothesis that one step in the mechanism of adaptation process may be a hormone-induced loss of opiate sensitivity of functionally restricted CNS mechanisms.

REFERENCES

1 Arimura, A , C Y Bowers, A V Schally, M. Saito and M C Miller. Effect of corticotropin releasing factor, dexamethasone and actinomycin D on the release of ACTH from rat pituitaries in vivo and in vitro *Endocrinology* **85:** 300–311, 1969

2 Babbini, M , M Gaiardi and M. Bartoletti Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment *Br J Pharmacol Chemother* **46:** 213–224, 1972

3 Brady, L S and S G Holtzman Effects of intracerebroventricular morphine and enkephaline on locomotor activity in nondependent, morphine dependent and post-dependent rats *J Pharmacol Exp Ther* **218:** 613–620, 1981

4 Briggs, F N and P L Munson Studies on the mechanism of stimulation of ACTH secretion with the aid of morphine as a blocking agent *Endocrinology* **35:** 205–219, 1955

5 Chesselet, M. F , A Cheramy, T D Reisine and J Glowinski. Morphine and opiate agonists locally stimulate in vivo dopamine release in cat caudate nucleus *Nature* **291:** 320–322, 1981

6. D'Amour, P. E. and D L Smith A method for determining loss of pain sensation *J Pharmacol Exp Ther* **72:** 74–79, 1941

7 Dunn, O J. Multiple comparison among means *J Am Stat Assoc* **56:** 62–64, 1976.

8 Fekete, M. I K., B. Kanyicska and J P Herman Simultaneous radioenzymatic assay of catecholamines and dihydroxyphenylacetic acid (DOPAC), comparison of the effects of drugs on the tuberoinfundibular and striatal dopamine metabolism and plasma prolactin level. *Life Sci* **23:** 1549–1556, 1978

9 Fekete, M I. K , E Stark, B Kanyicska, J. P Herman and M Palkovits Inhibition of stress induced hormonal changes by chronic ACTH treatment, possible relationships with changes of monoaminergic transmission in the central nervous system In *Catecholamines and Stress Recent Advances*, edited by E Usdin, R Kvetnansky and I J Kopin New York. Elsevier, 1980, pp. 149–154

10 Fekete, M I K , B Kanyicska, T Szentendrei and E Stark Decrease of morphine induced prolactin release by a prolonged stress situation *J Endocrinol*, in press, 1984.

11 Ferri, S , D. Cocchi, V Locatelli, S Sampinato and E Muller $ACTH_{1-24}$ counteracts the prolactin releasing effect of an opioid. *Eur J Pharmacol* **77:** 143–146, 1982

12 Fukui, K and H Takagi Effect of morphine on the cerebral contents of metabolites of dopamine in normal and tolerant mice its possible relation to analgesic action *Br J Pharmacol Chemother* **44:** 45–51, 1972

13 Gispen, W H , J Buitelaar, V M Weigant, L Terenius and D De Wied Interaction between ACTH fragments, brain opiate receptors and morphine-induced analgesia *Eur J Pharmacol* **39:** 393–397, 1976

14 Harms, P G., P Langlier and S M McCann. Modification of stress induced prolactin release by dexamethasone or adrenalectomy *Endocrinology* **96:** 475–478, 1975

15 Kanyicska, B , E Stark, Gy Horváth, A Simonyi and M I K Fekete Long term ACTH induced diminished responsiveness of prolactin secretion to morphine *Life Sci* **33:** 55–69, 1983

16 Kasson, B G and R George Endocrine influences on the actions of morphine I Alteration of target gland hormones *J Pharmacol Exp Ther* **234:** 273–281, 1983

17 Kenessey, A , P Páldi-Haris, G B Makara and L Gráf Tryspsin-like activity of anterior pituitary in relation to secretory activity *Life Sci* **25:** 437–444, 1979

18 Koenig, J , M A Mayfield, R J Coopings, S M McCann and I Krulich Role of central nervous system neurotransmitters in mediating the effects of morphine on growth hormone and prolactin secretion in the rat *Brain Res* **197:** 453–468, 1980

19 Litchfield, J T and F Wilcoxon A simplified method of evaluating dose-effect experiments *J Pharmacol Exp Ther* **96:** 97–113, 1949

20 Martin, W R Multiple opioid receptors A little about their history and some implications related to evolution *Life Sci* **28:** 1547–1554, 1981

21 Pecile, A and E Muller Suppressive action of corticosteroids on the secretion of growth hormone *J Endocrinol* **36:** 401–408, 1966

22 Rossier, J , E French, C. Rivier, T Shibasaki, R Guillemin and F E Bloom Stress induced release of prolactin Blockade by dexamethasone and naloxone may indicate β-endorphine mediation *Proc Natl Acad Sci USA* **77:** 666–669, 1980

23. Simon, M , R George and J Garcia Acute morphine effects on regional brain amines growth hormone and corticosterone *Eur J Pharmacol* **34:** 21–26, 1975

24 Stark, E , J Fachet and K Mihály Pituitary and adrenal responsiveness in rats after prolonged treatment with ACTH *Can J Biochem Physiol* **41:** 1772–1777, 1963

25 Stark, E , Zs Ács, G B Makara and K Mihály. The hypophyseal-adrenocortical response to various different stressing procedures in ACTH-treated rats *Can J Physiol Pharmacol* **46:** 567–576, 1968

26 Stark, E , M Kárteszi, Gy Rappay and G. B Makara Effects of treatment with adrenocorticotrophin on the hypothalamo-pituitary-adrenal system *J Endocrinol* **88:** 131–139, 1981

27 Szabó, S , H Selye, P Kourounakis and Y Tache Comparative studies on the effect of adrenocorticotrophic hormone (ACTH) and pregnenolone-16-alpha-carbonitrile (PCN) upon drug response distribution in rats *Biochem Pharmacol* **23:** 2083–2094, 1974

28 Terenius, L , W H Gispen and D De Wied ACTH like peptides and opiate receptors in the rat brain structure activity studies *Eur J Pharmacol* **33:** 395–399, 1975

29 Van Vugt, D. A and J Meites Influence of endogenous opiates on anterior pituitary function *Fed Proc* **39:** 2533–2538, 1980