Pharmacology Biochemistry & Behavior, Vol. 17, pp. 1203–1206, 1982. Printed in the U.S.A.

Opposite Effects of CRF and ACTH on Reserpine-Induced Hypothermia

ABBA J. KASTIN,[1] LYNDA C. HONOUR,[2]
JAVIER SUEIRAS-DIAZ AND DAVID H. COY

VA Medical Center and Tulane University School of Medicine, New Orleans, LA 70146

Received 2 June 1982

KASTIN, A. J., L. C. HONOUR, J. SUEIRAS-DIAZ AND D. H. COY. *Opposite effects of CRF and ACTH on reserpine-induced hypothermia.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1203–1206, 1982.—The effects of CRF, ACTH 1–24, α-MSH, and an ACTH 4–49 analog, at doses of 0, 0.1, 1, and 10 mg/kg, were tested on temperature, ptosis, and sedation in mice pretreated 18 hr previously with reserpine. IP injection of CRF at doses of 1 and 10 mg/kg significantly potentiated the reserpine-induced hypothermia while ACTH 1–24 at the same two doses had the opposite effect of significantly reversing the hypothermia as compared to diluent. The highest dose of α-MSH exerted a similar action to that of ACTH 1–24, but none of the doses of the ACTH 4–9 analog changed body temperature. β-endorphin also failed to cause a reliable effect even though naloxone blocked the action of CRF on body temperature. The results suggest that CRF, like other hypothalamic peptides, can exert extra-pituitary actions after peripheral administration.

Temperature Peptides Rauwolfia Depression Ptosis Sedation ACTH MSH CRF
β-endorphin Naloxone Hypophysectomy

OUR concept of the multiple, independent actions of peptides in mammals has led to the description of effects of peptides, including endogenous opiates, that differed from those by which they were originally described [7,8]. Since this idea was first demonstrated with hypothalamic peptides, the terms "extra-pituitary" and "extra-endocrine" were coined for their effects on the central nervous system (CNS). It seemed appropriate, therefore, to investigate the central effects of the most recently described [16] hypothalamic hormone, corticotropin-releasing factor (CRF).

CRF is a 41 amino acid peptide that stimulates the release of corticotropin (ACTH) and β-endorphin from the pituitary gland [15,16]. Originally isolated from ovine hypothalamic extracts [16], the presence of immunoreactive CRF in hypothalamic tissue has been confirmed by radioimmunoassay [10] and immunocytochemistry [11].

ACTH and the structurally related α-melanocyte-stimulating hormone (α-MSH) are known to influence thermoregulation, particularly when body temperature has been altered by pretreatment with other compounds [4, 9, 17, 18]. Reversal of the hypothermia caused by reserpine has been used as an animal model of depression [1] and abnormalities in the adrenocortical system are frequent in mental depression [5]. Accordingly, we compared the effects of CRF with those of ACTH, α-MSH, and an ACTH analog on reserpine-induced hypothermia, sedation, and ptosis in mice and examined the possible involvement of the opiate system in these actions.

EXPERIMENT 1

Method

The same methods used by us previously [6] to study the effects of another hypothalamic peptide (MIF-1) were used for CRF, α-MSH, ACTH 1–24, and an ACTH 4–9 analog (4-Met(0_2),8 D-Lys,9-Phe-ACTH 4–9). These synthetic peptides were dissolved in diluent (0.9% NaCl acidified to 0.01 M with acetic acid) daily before injection.

Male, albino mice (20–25 g) obtained from Harlan Sprague-Dawley (Madison, WI), received a single intraperitoneal (IP) injection of diluent or one of the peptides 18 hr after reserpine (2.5 mg/kg, IP in corn oil). The doses used for each peptide were 0 (diluent), 0.1, 1.0, and 10.0 mg/kg. In this and the subsequent experiments, 7–10 mice were used in each group.

Measurements of colonic temperature, ptosis of the upper eyelid [12], and motor activity (Stoelting Activity Meter model 31407) were recorded before (time 0) and 60, 120, and 180 min after injection of the coded solutions. The changes from time 0 were analyzed by analysis of variance followed by Duncan's Multiple Range Test.

Results

Reserpine lowered body temperature from 36.9±0.1°C to 32.8±0.2°C ($p<0.01$) 18 hr later at time 0. The return toward normal body temperature over the testing period was 0.8°C

[1]Reprint requests to Dr. A. J. Kastin, VA Medical Center, 1601 Perdido Street, New Orleans, LA 70146.
[2]Present address: Department of Psychology, University of California, Riverside, CA 92502.

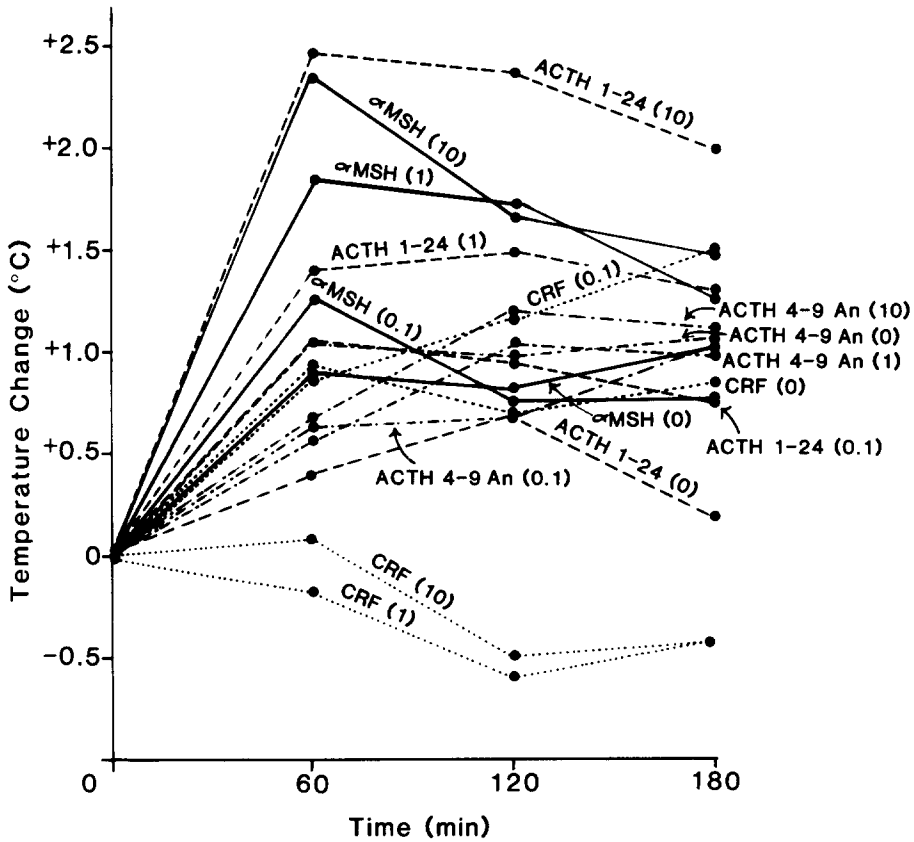

FIG. 1. Mean change in colonic temperature 1, 2, and 3 hr after peripheral injection of 4 peptides in intact mice pretreated 18 hr earlier with reserpine.

in the 4 diluent groups, which did not significantly differ from each other at any of the times examined.

Figure 1 shows the effect of each dose of the various peptides at each time period and Fig. 2 combines the time periods to show the overall effect of each dose of each peptide. These figures show that ACTH 1–24 and α-MSH accelerated the return to normal body temperature in a dose-dependent manner, but CRF, at the two higher doses, prolonged the reserpine-induced hypothermia. The ACTH 4–9 analog had no effect at any of the doses tested. These results were supported by a significant effect of peptide, $F(3,127)=6.14$, $p<0.001$, and peptide × dose interaction, $F(9,127)=3.93$, $p<0.001$.

A significant reversal of the hypothermia was found after administration of 1 and 10 mg/kg of ACTH 1–24 as compared to the 0 dose; the effect of the 10 mg/kg dose was reliably greater than that of the lower doses. The 10 mg/kg dose of α-MSH was also significantly ($p<0.05$) different from its 0 dose, but none of the doses, including 0 mg/kg, of the ACTH 4–9 analog differed from each other at any of the times tested.

By contrast, at 10 mg/kg and 1 mg/kg, administration of CRF resulted in a highly significant ($p<0.001$) persistence of the reserpine-induced hypothermia relative to the control group (0 mg/kg CRF). Statistically, the 0.1 mg/kg dose of CRF was indistinguishable from the 0 dose. Both the 1 mg/kg dose and the 10 mg/kg dose of CRF were significantly ($p<0.001$) different from these two doses of each of the other three peptides.

The various peptides also showed some differences in the

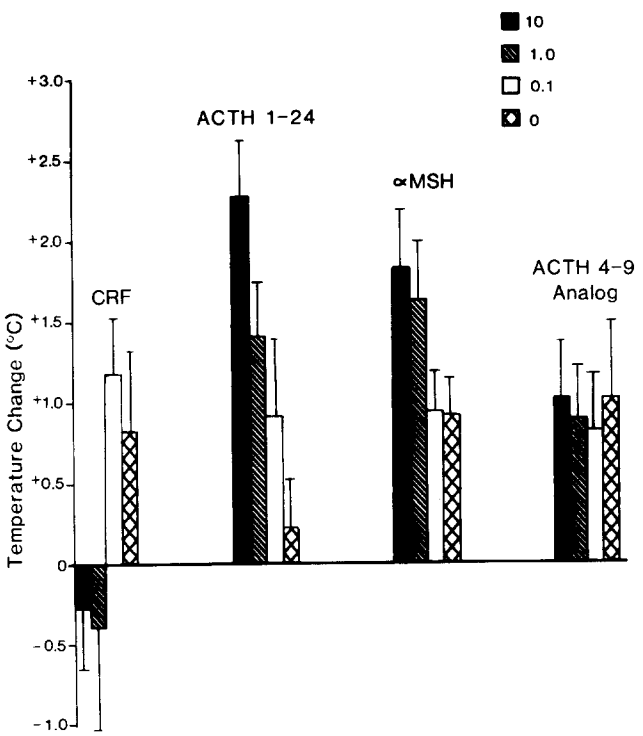

FIG. 2. Mean (±SEM) change in colonic temperature over the 3 hr test period for each dose of the 4 peptides in intact mice pretreated with reserpine.

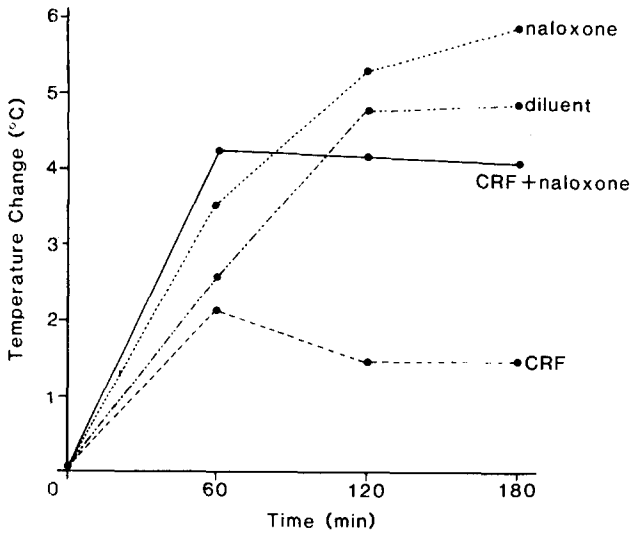

FIG. 3. Mean change in colonic temperature after CRF, naloxone, or their combination in intact mice pretreated with reserpine.

time course of their action. As shown in Fig. 1, the ACTH and α-MSH groups showed a maximal increase in temperature 60 min after injection of the peptide. The temperature of animals given the ACTH 4–9 analog, like the diluent-treated animals, increased more gradually to a peak at 120 or 180 min. The maximal decrease in temperature induced by the two higher doses of CRF was also seen at these later time periods. These results were supported by a significant peptide × time interaction, $F(2,254)=2.65$, $p<0.05$.

For ptosis, a main effect of peptide was found, $F(3,126)=2.80$, $p<0.05$, but no other main effects or interactions were seen. Although at each time interval the reversal of ptosis by the 10 mg/kg dose of ACTH 1–24 did not reach statistical significance at the 5% level, when the times were combined, the overall effect of this dose was reliable. The next greatest reversal tended to occur with the 1 mg/kg dose of ACTH 1–24 followed by all three doses of the ACTH 4–9 analog, but these were not statistically significant. Statistical analysis of the combined doses and times revealed significant differences between the effects on ptosis of both ACTH 1–24 and the ACTH 4–9 analog as compared with the relative lack of effect of either CRF or α-MSH.

EXPERIMENT 2

Method

Thirty mice received 2.5 mg/kg reserpine IP and were randomly divided into four groups. Eighteen hours after the reserpine these groups received one of the following treatments: CRF (1 mg/kg, IP), naloxone (2 mg/kg, IP), naloxone (2 mg/kg, IP)+CRF (1 mg/kg, IP), or diluent. For the group receiving both naloxone and CRF, the naloxone was injected 30 min before the CRF. Similarly, for the group receiving only CRF, an injection of diluent was given 30 min before the peptide. Observations were made before any injection of peptide or diluent (time 0) as well as 60, 120, and 180 min after the last injection.

Results

The reserpine itself lowered mean body temperature from 36.9±0.2 to 28.1±0.1°C ($p<0.01$) 18 hr later. This greater hypothermia than in Experiment 1 after the same dose of reserpine probably contributed to the greater reversal of the hypothermia with time; as shown in Fig. 3, the body temperature of the control animals increased 4.0°C over the 4 time periods. The temperatures of animals given naloxone alone or in combination with CRF were not significantly different from those of the diluent group, but CRF-treated animals maintained a reduced body temperature for at least 180 min. These results were supported by a significant treatment × time interaction, $F(6,50)=3.26$, $p<0.01$ and a significantly lower overall temperature after CRF than after diluent ($p<0.01$), naloxone alone ($p<0.0001$), or naloxone + CRF ($p<0.01$).

Although overall there was a slight reversal of ptosis by naloxone, naloxone + CRF, and CRF alone, none of the changes were greater than 1.0 on the scale of 0 to 4 nor were they statistically significant. None of the treatments reliably affected motor activity.

EXPERIMENT 3

Method

Eighteen hours after the reserpine (2.5 mg/kg IP), 30 mice were divided into four groups to receive a single injection of one of three doses of β-endorphin or diluent. The doses used were 10 mg/kg, 1.0 mg/kg, 0.1 mg/kg or 0 mg/kg. The remainder of the experimental situation was identical to that of the other experiments in this study.

Results

For temperature, ptosis, and activity, there were no significant main effects of treatment or time and no significant interaction between them. Body temperature fell from 36.7±0.2°C to 28.5±0.6°C ($p<0.01$) 18 hr after reserpine, rising 4.5°C 3 hr after injection of diluent.

DISCUSSION

Administration of CRF significantly prevented the reversal with time of the hypothermia induced by reserpine (Figs. 1–3). In contrast, ACTH 1–24, a compound with the same adrenal stimulating properties as endogenous ACTH, reliably accelerated the reversal of the hypothermia induced by reserpine. The highest dose of α-MSH also significantly reversed the reserpine-induced hypothermia, but the ACTH 4–9 analog that is reported to be 1000 times more active than α-MSH in some [3] but not all [13] experimental situations did not affect the temperature.

Unlike the hypothermic effects described for ACTH 1–24 in animals with experimentally increased body temperature [4, 9, 20], we found significant reversal of hypothermia after ACTH 1–24. The opposite direction of these results could be explained by differences in dose, pretreatment, or species of animal used, but probably not by differences in route of administration since peripheral injections were used in both experimental situations ([20], and present study). It is also possible that ACTH 1–24, and perhaps other peptides, may exert an optimizing effect on body temperature whereby abnormalities in either direction tend to be corrected.

ACTH 1–24 and α-MSH have been found to lower body temperature in untreated animals as well as in animals with fever [4, 9, 17, 20]. The ACTH 4–9 analog however, has been reported to cause a significant reversal of hypothermia induced in mice by pentobarbital [2] even though the antipyretic activities of the MSH/ACTH peptides appear to require a longer portion than the 4–9 sequences present in the substituted analog [9].

Structural requirements for reversal of reserpine-induced hypothermia, however, may be different from those for reversal of reserpine-induced ptosis. This is suggested by the similarly increased activity on ptosis of the ACTH 4–9 analog and ACTH 1–24 as compared with that of α-MSH.

The reversal of reserpine-induced hypothermia and ptosis we observed after injection of ACTH 1–24 has also been described after injection of the tricyclic antidepressants [1]. Compounds ineffective in depression, however, have also been shown to be active in this system [1], and ACTH 1–24 failed to reverse the sedation considered to be an additional part of this model of depression.

The effects of CRF on temperature have not been previously reported. They are compatible with an extra-pituitary or extra-endocrine action of this peptide because the presence of the pituitary is usually not required for the thermal effects of most peptides [18]. The opposite actions of CRF and ACTH 1–24 on reserpine-induced hypothermia more strongly support this extra-pituitary action (Figs. 1 and 2). Since CRF stimulates the release of ACTH [15,16], the effect of CRF should have been similar to that of ACTH if it were mediated only by the pituitary; the effects of these two peptides on the hypothermia, however, occurred in different directions.

Blockade by naloxone of the actions of CRF on reserpine-induced hypothermia (Fig. 3) raised the possibility that these actions were mediated by the release of β-endorphin from the pituitary. The lack of significant effect of any of the 3 doses of β-endorphin tested did not support this idea. Naloxone by itself does not affect body temperature [18], but it can block the thermal actions of amphetamine and of β-endorphin even though β-endorphin and naloxone both act in the same direction to reverse amphetamine-induced hypothermia [19]. Although mediation of the thermoregulatory effects of CRF by other opiate peptides is possible, consideration should also be given to the possibility that the interaction of CRF and naloxone may not occur at an opiate receptor.

Significant effects of some of the peptides on body temperature persisted for several hours in the present study (Figs. 1–3). This emphasizes again [8] the lack of correlation between the levels of a peptide in the blood and its biological actions.

The results also reinforce the observations made during the last two decades [3, 7, 8] that peripherally administered peptides can exert central effects. The actions of several other peptides on temperature, however, are apparently only seen with the central route of administration [2,18]. While this manuscript was in preparation, a report appeared describing altered locomotor activity of rats after CRF [14]. This effect was observed only after intracerebroventricular injection but not after subcutaneous injection. It is hoped that the lack of effect on activity after peripheral injection of this 41 amino acid peptide will not discourage future investigations of its actions by this route of administration even though such a situation almost occurred with the opiate peptides [8]. Although the mechanisms by which peptides can exert CNS effects after peripheral administration are not fully known [8], CRF is one of the largest peptides yet found to be centrally active by this route.

ACKNOWLEDGEMENTS

The authors thank Dr. James E. Zadina for review of the manuscript, Maureen R. Cancienne for its preparation, Lynn A. Fabre for technical assistance, and Endo Labs for the naloxone. The study was supported by the Veterans Administration.

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