Effects of (D-Ala², Met⁵)-Enkephalinamide and Naloxone on ACTH and Corticosterone Secretion

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Abstract

An intravenous administration of (D-ala², met⁵)-enkephalinamide (DALA) caused a significant elevation of plasma ACTH and corticosterone at 10 to 20 min after injection in unanesthetized freely moving rats. An intraperitoneal administration of cyproheptadine tended to reduce plasma ACTH and corticosterone levels at 60 min after injection, but it did not attenuate the DALA-induced ACTH and corticosterone elevation. A large dose of naloxone (1-10 mg/kg body weight) caused a significant elevation in plasma corticosterone, but naloxone at 10 mg/kg body weight reduced the basal ACTH level and DALA-induced ACTH elevation. When both DALA and naloxone were injected, the steroidogenic effect was attenuated. Neither DALA nor naloxone affected the basal ACTH release and CRF-induced ACTH stimulation in rat anterior pituitary cell cultures. These results suggest that DALA acts at the extra-hypophyseal level to stimulate ACTH and corticosterone and that the naloxone stimulatory effect on steroidogenesis acts on the adrenal gland or is mediated by stimulating corticosterone stimulating factors other than ACTH.

Since the discovery of endogenous opioid peptides in the brain and pituitary, evidence has been accumulating that they play an important role in regulating pituitary-adrenal function. However, the controversy continues on the role of the opioid peptides in hypothalamo-pituitary-adrenocortical activity. Some investigators reported a stimulatory role of endogenous opioid peptides in ACTH secretion (George & Way, 1979; Gibson et al., 1979; Buckingham & Cooper, 1984), while others reported that opioid peptides tonically inhibited ACTH secretion (Stubbs et al., 1978; Del Pozo et al., 1980; Gaillard et al., 1981). Intravenous administrations of enkephalin analogues were reported to inhibit ACTH and cortisol secretion in man, while it stimulated plasma ACTH and corticosterone levels in rats (Ferri et al., 1980; De Souza & Van Loon, 1982).

There is also controversy about the effects of naloxone, an opiate antagonist, on ACTH and corticosteroid secretion. A high dose of naloxone elevated circulating ACTH (Volavka et al., 1979; Morley et al., 1980), whereas low doses were ineffective (Spiler & Molith, 1980). On the other hand, Lymangrover et al. (1981) reported that a high dose of naloxone produced a decline in basal steroidogenesis, while lower doses

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of naloxone resulted in a rise in the corticosterone level.

We previously studied the dual effects of the methionine-enkephalin analogue, (Dala², met⁵)-enkephalinamide (DALA) on CRF-ACTH secretion. When DALA was injected into the third ventricle, it potentiated the mild stress-induced elevation of ACTH and corticosterone, but it inhibited CRF release from hypothalamic tissue in an in vitro infusion system (in press). In the present paper we report on the effects of the peripheral administration of DALA and naloxone on ACTH and corticosterone secretion in rats.

### Materials and Methods

**Animals**

Male Wistar rats, weighing 250–300 g, were kept at under controlled temperature (23–26°C) and lighting (on from 700 to 1900 hr) conditions in an animal room.

**In vivo experiments**

Catheters (Silastic tubing, Dow Corning Co., USA) were inserted into the right external jugular vein one day before the experiments, according to the method of Harmes and Ojeda (1974). In the morning of the experiments, guide catheters were connected with the inserted catheters to collect blood samples. The experiments were carried out from 1200 to 1500 hr to avoid diurnal variations in plasma hormones.

To examine the effect of DALA on ACTH and corticosterone secretion, heparinized blood samples (0.7 ml) were withdrawn from the jugular catheters at minus 60 min, immediately before intravenous injection of DALA (500 µg/kg body weight) and at 10 and 20 min after injection. Cyproheptadine (1 mg/kg body weight), which is a serotonergic antagonist, was injected intraperitoneally 60 min before DALA injection to examine the involvement of the serotonergic mechanism in the DALA-induced plasma ACTH changes. To examine the effect of naloxone on ACTH and corticosterone secretion, blood samples were withdrawn before intravenous injection of naloxone (0.2, 1.0 and 10 mg/kg body weight) and at 10, 20 and 40 min after injection. In one experiment, naloxone at 5 mg/kg body weight was injected 10 min before DALA injection to examine the effect of naloxone on the DALA-induced ACTH secretion.

**In vitro experiments**

The effects of DALA and naloxone on ACTH secretion were examined in monolayer cultured rat anterior pituitary cells. Cultured anterior pituitary cells were prepared by a previously reported method (Hashimoto et al., 1979). After 4 days of culture, the cells were washed twice with fresh DMEM, and then the DMEM containing DALA (total volume, 2 ml) or rat CRF or both were added for 3 hr of incubation. After incubation the medium was separated and stored at −20°C until assay.

**ACTH and corticosterone radioimmunoassay**

Plasma ACTH levels were measured in 100 µl samples of each with the RIA kit (CEA-IRESORIN, France). Plasma corticosterone was measured with in a 50 µl sample with a cortisol RIA kit (Daiichi Radio-isotope Labs, Japan). The validity of this kit for rat plasma corticosterone assay was discussed previously (Suemaru et al., 1985). The correlation between the values obtained by this method and by fluorescence spectrometry (Guillemin et al., 1959) was high (r = 0.855, P < 0.001).

The amount of ACTH in the incubation medium of pituitary cells was quantified by radioimmunoassay, using anti-ACTH serum developed in our laboratory (Hashimoto et al., 1979).

**Statistics**

Statistical analyses were conducted using Student’s t-test.

**Results**

**Effect of DALA on ACTH and corticosterone secretion.**

DALA caused a significant rise in ACTH and corticosterone at 10 and 20 min after intravenous administration, while saline injection caused neither statistically significant ACTH nor corticosterone changes (Fig. 1).
Fig. 1. Effect of intravenous (D-ala², met⁵)-enkephalinamide (DALA) (600 μg/kg body weight) on plasma ACTH and corticosterone levels and effect of intraperitoneal cyproheptadine (1.0 mg/kg body weight) on the basal and DALA-induced ACTH and corticosterone levels in unanesthetized freely moving rats. *P < 0.05, **P < 0.01 vs saline-saline injected group or cyproheptadine-saline injected group. +P < 0.05, ++ < 0.01 vs zero time sample.

Fig. 2. Effect of intravenous naloxone (0.2–10 mg/kg body weight) administration on plasma ACTH and corticosterone levels in unanesthetized freely moving rats. *P < 0.05, **P < 0.01 vs saline injected group. +P < 0.05, ++P < 0.01 vs zero time sample.
Plasma ACTH and corticosterone tended to decrease at 60 min after intraperitoneal cyproheptadine administration, but it did not attenuate the DALA-induced ACTH and corticosterone rises.

**Effect of naloxone on ACTH and corticosterone secretion**

Naloxone at 0.2 and 1.0 mg/kg body weight did not cause a statistically significant change in plasma ACTH, whereas naloxone at 5 or 10 mg/kg body weight lowered the plasma ACTH level at 40 min (Fig. 2) and 50 min (Fig. 3) after injection. Plasma corticosterone showed a significant rise after naloxone (1.0 and 10 mg/kg body weight) administration (Fig. 2). Naloxone at 5 mg/kg body weight completely blocked the DALA-induced ACTH rise (Fig. 3). In rats that received both naloxone and DALA, the plasma corticosterone level at 20 min after naloxone injection was lower than in

**Fig. 3.** Effect of naloxone (5 mg/kg body weight) on DALA (500 µg/kg body weight)-induced plasma ACTH and corticosterone elevation in unanesthetized freely moving rats. *P<0.05, **P<0.01 vs naloxone (NALOX)-DALA injected group. +P<0.05, +++P<0.01 vs zero time sample.

**Fig. 4.** Effect of DALA on the basal and CRF-induced ACTH release in rat anterior pituitary cell cultures. The number in the bars represents the number of dishes used. The line above the bar is +SEM.
only naloxone or DALA injected rats (Fig. 3).

**Effect of DALA and naloxone on ACTH release in pituitary cell cultures**

Neither DALA at 1–1,000 ng/ml nor naloxone at $10^{-7}$ M–$10^{-4}$ M affected the basal ACTH release or the CRF-induced ACTH release in pituitary cell cultures (Fig. 4, Fig. 5).

**Discussion**

We had already observed that an intrathird ventricular administration of DALA potentiated a mild stress-induced ACTH and corticosterone rise. The present investigation showed that an intravenous DALA administration caused a rise in ACTH and corticosterone, but it did not stimulate an ACTH release from pituitary cells in *in vitro* experiments. These results indicate that DALA acts at the extra-hypophyseal level to stimulate ACTH secretion. There is, however, substantial evidence to indicate that a number of opioid precursors alter basal or stimulated adrenal steroidogenesis directly *in vitro* (Pedersen & Brownie, 1980; Racz et al., 1980; Matsuoka et al., 1980). Therefore, peripherally administered DALA may act at the extra-hypophyseal level to stimulate corticosterone secretion.

On the other hand, methionine-enkephalin analogue (Damme-FK 38–824) caused a suppression of ACTH and cortisol in man (Stubbs et al., 1978; Del Pozo et al., 1980; Grossman & Besser, 1982). Although the discrepancy between man and laboratory animals in the methionine-enkephalin analogue effect on the pituitary-adrenal system is hard to explain, it may be ascribed to a species difference.

Several investigators have shown that not only opioids but also naloxone, the specific antagonist of morphine receptors, stimulates adrenal steroidogenesis. Some investigators (Volavka et al., 1979; Gibson et al., 1979; Siegal et al., 1982; Eisenberg, 1984) suggested that naloxone acts directly on the central nervous system regulating ACTH secretion, but others reported that naloxone acts directly on the adrenal cortex (Lyman-grover et al., 1981; Jezova, 1985). Our present results have shown that naloxone stimulates corticosterone secretion without
elevating ACTH. The results suggest that naloxone has a direct stimulatory effect on corticosterone secretion or it stimulates corticosterone-stimulating factors other than ACTH.

The mechanism by which naloxone stimulates corticosterone secretion is not clear. Lymangrover et al. (1981) reported the direct effect of both methionine-enkephalin and naloxone on adrenal steroid secretion. The most prominent effect was the potentiation of the steroidogenic action of ACTH by interaction with one or more relatively nonspecific membrane receptors which are distinctly different from the ACTH receptor. They also suggested a mechanism which involves a change in membrane permeability to one or more ions, resulting in an optimal intracellular ion concentration. Although both DALA and naloxone showed a steroidogenic effect, the response was attenuated when both were given to rats. The results indicate that they act antagonistically on adrenal cortex cells.

The present investigation showed that a large dose of naloxone rather lowered the plasma ACTH level. An elevated level of plasma corticosterone may partly explain the reduction in plasma ACTH. However, naloxone at 1 mg/kg body weight did not lower the plasma ACTH level although it elevated the plasma corticosterone level. Our results showed that naloxone had no direct effect on the release of ACTH from pituitary cells. Therefore, it is also possible that naloxone lowered the plasma ACTH level by blocking the endogenous opioid effects at the hypothalamic level. The discrepancy between the effects of naloxone on plasma ACTH level in our study and others (Siegel et al., 1982; Jezova 1985) may be ascribed to experimental conditions. In our experiments, blood was sampled with a jugular cannula in unanesthetized freely moving rats, without additional stress. Siegel et al., (1982) injected naloxone intraperitoneally and collected blood samples after decapitation. Jezova (1985) also injected naloxone intraperitoneally although he used indwelling catheters.

Opioid peptides stimulate prolactin and GH secretion via a serotonergic mechanism (Spampinato et al., 1979). A serotonergic mechanism has been reported to stimulate ACTH secretion (Jones et al., 1976; Meyer et al., 1978; Hashimoto et al., 1982). Our results here show that a serotonergic mechanism may be involved in sustaining the basal plasma level but it was not involved in the DALA-induced ACTH stimulation.

In summary, the present investigation suggests that the stimulatory effect of DALA on the hypothalamo-pituitary-adrenal system is mediated by stimulating the secretion of CRF or other ACTH secretagogues and that the stimulatory effect of naloxone on steroidogenesis acts at the adrenal gland or is mediated by stimulating other corticosterone-stimulating factors.

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References


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