

N O T I C E

THIS DOCUMENT HAS BEEN REPRODUCED FROM
MICROFICHE. ALTHOUGH IT IS RECOGNIZED THAT
CERTAIN PORTIONS ARE ILLEGIBLE, IT IS BEING RELEASED
IN THE INTEREST OF MAKING AVAILABLE AS MUCH
INFORMATION AS POSSIBLE

(NASA-TM-81253) NALOXONE INHIBITS AND
MORPHINE POTENTIATES THE ADRENAL
STEROIDOGENIC RESPONSE TO ACTH (NASA) 17 p
BC 102/12 201 USCI 981

181-160c

040145

03/81 25534

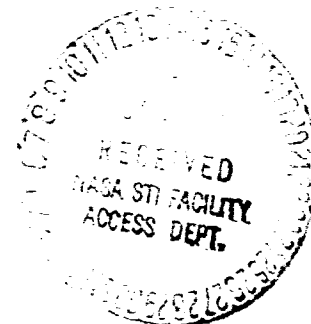
Naloxone Inhibits and Morphine Potentiates The Adrenal Steroidogenic Response to ACTH

John Heybach and Joan Vemikos

December 1980

NASA

National Aeronautics and
Space Administration



Naloxone Inhibits and Morphine Potentiates The Adrenal Steroidogenic Response to ACTH

John Heybach and
Joan Vernikos, Ames Research Center, Moffett Field, California

NASA

National Aeronautics and
Space Administration

Ames Research Center

Moffett Field, California 94035

J. P. HEYBACH and J. VERDIKOS, Naloxone inhibits and morphine potentiates the adrenal steroidogenic response to ACTH, *European J. Pharmacol.* XI(1981)XI-XI.

The administration of morphine to hypophysectomized rats potentiated the steroidogenic response of the adrenal cortex to exogenous adrenocorticotrophic hormone (ACTH) in a dose-dependent fashion. Conversely, the opiate antagonist naloxone inhibited the adrenal response to ACTH. Naloxone pretreatment also antagonized the potentiating effect of morphine on ACTH-induced steroidogenesis in a dose-dependent manner. Neither morphine nor naloxone, administered to hypophysectomized rats, had any direct effect on adrenal steroidogenesis. These adrenal actions were stereospecific since neither the (+)-stereoisomer of morphine, nor that of naloxone, had any effect on the adrenal response to ACTH. The administration of human β -endorphin to hypophysectomized rats had no effect on the adrenal corticosterone concentration nor did it alter the response of the adrenal gland to ACTH. These results indicate that morphine can potentiate the action of ACTH on the adrenal by a direct, stereospecific, dose-dependent mechanism that is prevented by naloxone pretreatment and which may involve competition for ACTH receptors on the corticosterone-secreting cells of the adrenal cortex.

1. Introduction

Administration of morphine has diverse effects on various endocrine systems believed to reflect the action of this drug on hypothalamic endocrine control mechanisms. For example, morphine administration results in inhibition of ovulation in rats (Cicero et al., 1976; Packman and Rothchild, 1976) and reduces the proestrous increase of circulating luteinizing hormone (Pang et al., 1977). Both of these effects are reversed by treatment with the opiate antagonist naloxone.

Intrahypothalamic (Lotti et al., 1969) and peripheral (Kokka and George, 1974) morphine injections stimulate the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary, as reflected by increased plasma levels of adrenal corticosterone in the rat. This stimulation is also abolished by naloxone pretreatment (Kokka and George, 1974). Morphine also stimulates the release of growth hormone, but this effect is not altered by naloxone pretreatment (Kokka and George, 1974).

It has been demonstrated that ACTH can competitively inhibit opiate binding in vitro showing mixed agonist-antagonist actions (Krivoy et al., 1977; Terenius et al., 1975; Wiegant et al., 1977). In vivo studies have shown that naloxone blocks the effect of chronic ACTH treatment in retarding the development of reproductive function in mice (Yasukawa et al., 1978). These observations suggest that compounds with an affinity for opiate receptors might interact with ACTH receptors. We investigated this hypothesis by determining in vivo the action of acute morphine, β -endorphin, and naloxone pretreatment on the steroidogenic response of the adrenal cortex to ACTH.

2. Materials and methods

Male Sprague-Dawley rats weighing 100 ± 10 g (Simonsen Laboratories, Gilroy, Calif.) were used. The rats were housed 5 per cage in large plastic cages and maintained with free access to food and water in an animal colony with controlled temperature (23°C) on a regulated photoperiod of 12L:12D (lights on at 0800 h).

Drugs used and their suppliers were: ACTH from Armour Labs, Kankakee, Illinois; Human β -endorphin from Bachem, Inc., Torrance, California; both (-)-morphine and (-)-naloxone from Endo Labs, Garden City, New York; stereoisomers (+)-morphine and (+)-naloxone supplied by Dr. Kenner C. Rice, Medicinal Chemistry Section, National Institutes of Health, Bethesda, Maryland. The β -endorphin and ACTH were dissolved in 0.9% NaCl solution containing 0.1% acetic acid. Morphine and naloxone were dissolved in 0.9% NaCl. Doses of morphine and naloxone were calculated as the free base.

All rats used in these experiments were hypophysectomized. At 18-20 h prior to drug administration, the pituitary glands were removed under ether anesthesia to eliminate the source of endogenous circulating ACTH. Pituitaries were removed by a transauricular approach in a stereotaxic instrument (Gay, 1967). Experimental procedures were carried out between 0800-1100 h on the morning following hypophysectomy.

Initially, each drug was administered to different groups of rats to determine any direct effect they might have on adrenal gland steroidogenesis. ACTH (100 μU), β -endorphin (100 μg), or vehicle solution was injected into the femoral vein of the animals under ether anesthesia. They were sacrificed 5 min later and their adrenal glands removed, cleaned, and weighed. Various doses of (-)-morphine (0, 5, 10, and 20 mg/kg) or (-)-naloxone (0, 12.5, 25, and 50 mg/kg) were injected intraperitoneally and rats were sacrificed

20 min later. All compounds were injected in a total volume of 1 ml. Adrenal glands were homogenized in 0.9% saline solution and frozen for later determination of corticosterone content by a fluorometric method (Vernikos-Daneliis et al., 1966).

To determine the time course of the effect of pretreatment with these compounds on the adrenal steroidogenic response to ACTH, (-)-morphine (10 mg/kg, i.p.) or (-)-naloxone (50 mg/kg, i.p.) were injected at various times prior to ACTH (100 μ U, i.v.). Rats were sacrificed 5 min following the injection of ACTH and the corticosterone concentration in their adrenal glands was determined. Dose-response relationships of the effect of morphine or naloxone on the steroidogenic responses to ACTH (100 μ U, i.v.) were also determined 20 min following pretreatment with various doses of (-)-morphine (0, 5, 10, and 20 mg/kg, i.p.) or (-)-naloxone (0, 12.5, 25, and 50 mg/kg, i.p.) or 5 min following pretreatment with β -endorphin (100 μ g, i.v.). Rats were sacrificed 5 min following ACTH injection for measurement of adrenal corticosterone concentrations.

Finally, the steroidogenic response to ACTH (100 μ U, i.v.) was determined 20 min following pretreatment with either the (-)- or (+)-stereoisomeric forms of morphine and naloxone (i.p.). Rats were sacrificed 5 min following the ACTH injection and adrenal glands were removed and processed as described above.

Results are expressed as μ g of corticosterone per 100 mg of adrenal gland tissue, fresh weight. The data were statistically analyzed by using overall one-way analyses of variance followed by constructing 95% confidence limits around the appropriate control group mean with the conservative post-hoc procedure of Dunnett (Keppel, 1973).

3. Results

3.1. Effect of ACTH, morphine, and naloxone on adrenal gland content of corticosterone

As shown in Table 1, ACTH (100 μ U) stimulated steroidogenesis and led to an almost threefold increase in the corticosterone content of the adrenal glands. Neither morphine, naloxone, nor β -endorphin, when administered alone, had any effect on the adrenal gland content of corticosterone at any of the doses injected.

3.2. Time course of the effect of morphine and naloxone on the steroidogenic response to ACTH

Pretreatment with (-)-morphine (10 mg/kg) potentiated the steroidogenic response of the adrenal gland to ACTH (100 μ U), [F(5,41) = 12.1, $p < 0.01$] (fig. 1A). This effect was most evident when the time interval between the morphine pretreatment and ACTH injection was greater than 10 min ($p < 0.05$ at 20, 40, and 60 min). Although adrenal gland corticosterone levels were elevated when ACTH was injected at 10 min following morphine pretreatment, this elevation was not significantly different from that produced by ACTH alone ($p < 0.05$).

Pretreatment with (-)-naloxone antagonized the steroidogenic response of the adrenal gland to ACTH [F(5,40) = 9.7, $p < 0.01$], with a time course of action similar to that seen for morphine's potentiation of the response (fig. 1B).

3.3. Effect of increasing doses of morphine and naloxone on the steroidogenic responses to ACTH

Figure 2A shows that morphine, injected 20 min before the ACTH, potentiates the steroidogenic responses to ACTH at doses up to 10 mg/kg [F(6,44) = 10.7,

$p < 0.01$]. However, at higher doses (20 mg/kg), morphine no longer potentiated ACTH and may even have caused some inhibition of this response.

There was a dose-response relationship in the antagonism of the steroidogenic response to ACTH by naloxone. This drug was particularly effective at two higher doses (25 and 50 mg/kg) [$F(6,44) = 10.1, p < 0.01$] (fig. 2B).

3.4. Stereospecificity of the effects of morphine and naloxone on the steroidogenic response to ACTH

Pretreatment with (-)-morphine (10 mg/kg) significantly ($p < 0.05$) increased the adrenal steroidogenic response to ACTH (100 μ U) injected 20 min following morphine pretreatment (fig. 3). Pretreatment with (+)-morphine (10 mg/kg) was without effect.

Similarly, (-)-naloxone (10 mg/kg) inhibited the response to ACTH ($p < 0.05$) while (+)-naloxone (10 mg/kg) was without effect. When (-)-naloxone (10 mg/kg) was injected prior to the (-)-morphine (10 mg/kg), the response of the adrenal cortex to ACTH (100 μ U) did not differ from the steroidogenic response to the injection of ACTH alone. Pretreatment with β -endorphin (100 μ g) 5 min prior to ACTH injection (100 μ U) had no effect on the steroidogenic response of the adrenal.

4. Discussion

Morphine administration has been shown to stimulate corticosterone secretion and adrenal gland function (Briggs and Munson, 1955). This response has been presumed to be mediated solely by the hypothalamic-pituitary axis or by a direct action at the pituitary (Kokka and George, 1974; Lotti et al., 1969; Zimmerman and Critchlow, 1973). The present results indicate that

morphine can also influence the steroidogenic response to ACTH by a direct action on the adrenal cortex.

At doses up to 10 mg/kg, the potentiation of ACTH-induced steroidogenesis by morphine appears to be dose-dependent and stereospecific since (+)-morphine was without effect. The opiate antagonist naloxone exerted effects on the steroidogenic response to ACTH that were also stereospecific and dose-dependent but opposite to those of morphine. In addition, naloxone prevented the potentiation by morphine of the ACTH-induced steroidogenic response. These results suggest that morphine and naloxone interact with receptors on corticosterone-secreting cells of the adrenal cortex.

It has been shown that addition of the opiate antagonists naloxone or naltrexone to dispersed rat adrenal cell preparations inhibited the steroidogenic response to ACTH, but had no effect on the steroidogenic response to the addition of dibutyryl cyclic AMP (Ginsburg et al., 1978). This observation and our results lend further support to the hypothesis that naloxone and, presumably, morphine exert effects on the adrenocortical response to ACTH by competition at ACTH adrenal receptor sites.

Alternately, mediation by an adrenal opiate receptor must also be considered. However, this is unlikely since, in the present work, the administration of β -endorphin or of morphine alone, at doses up to 20 mg/kg, did not stimulate steroidogenesis. Morphine's effect was only detectable in the presence of ACTH. Furthermore, other work has failed to demonstrate the presence of specific opiate receptor binding in adrenal-gland homogenates (Simantov et al., 1978).

The evidence that ACTH can interact with opiate receptors in the central nervous system and in the periphery (Krivoy et al., 1977; Gispén et al., 1976; Terenius et al., 1975; Wiegant et al., 1977) suggests the possibility that

opiates may also interact with ACTH receptors. The block by naloxone of the ACTH-induced delay of puberty and the reversal of ACTH-induced decreases in reproductive organ weights of mice (Uasukawa et al., 1978), as well as the antagonism by naloxone and potentiation by morphine of the steroidogenic effects of ACTH seen here, lend support to this possibility.

The present results strongly indicate that morphine and naloxone exert opposite effects on the response of the adrenal cortex to ACTH by acting at ACTH receptor sites. The results also demonstrate a pharmacological effect of morphine most probably at the adrenal gland level that is dose-dependent, stereospecific, and prevented by naloxone. The lack of effect of the endogenous opiate β -endorphin on the response of the adrenal to ACTH might then be a function of the dose or time course used here, or may indicate no significant physiological effect of this particular endogenous opiate on the adrenal cortex. On the other hand, the recent evidence that the adrenal medulla is the primary source of circulating enkephalins (Tam and Yu, 1980) introduces the possibility that this opiate, of adrenomedullary origin and in close proximity to the adrenal cortex, may serve a physiological function in regulating adrenal sensitivity to ACTH. Some evidence exists to suggest that β -endorphin and enkephalins may bind differently to the same receptor or exert their opiate-like effects by binding to different receptors but with overlapping specificity (Hazum et al., 1979).

References

- Briggs, F. N. and P. L. Munson, 1955, Studies on the mechanism of stimulation of ACTH secretion with the aid of morphine as a blocking agent, *Endocrinology* 57, 205-219.
- Cicero, T. J., E. R. Meyer, R. D. Bell and G. A. Koch, 1976, Effects of morphine and methadone on serum testosterone and luteinizing hormone levels and in the secondary sex organs of the male rat, *Endocrinology* 98, 367-372.
- Edwards, C. and V. L. Gay, 1967, A stereotaxic approach to transauricular hypophysectomy in the rat, *Endocrinology* 81, 1177-1179.
- Ginsburg, M., A. Gibson, M. Hall and S. L. Hart, 1978, Steroidogenesis in adrenal cortex: efferents of Li^+ , opioid antagonists and Na^+ dependence of ACTH response, *Proc. Int. U. Pharmac.* 7, 1256.
- Gispén, W. H., J. Buitelaar, V. M. Wiegant, L. Terenius and D. de Wied, 1976, Interaction between ACTH fragments, brain opiate receptors, and morphine induced analgesia, *Eur. J. Pharmac.* 39, 393-397.
- Hazum, E., K.-J. Change and P. Cuatrecasas, 1979, Interaction of iodinated human [D-Ala²] β -endorphin with opiate receptors, *J. Biol. Chem.* 254, 1765-1767.
- Keppel, G., 1973, *Design and Analysis* (Prentice-Hall, Inc., Englewood Cliffs, New Jersey), pp. 153-157.
- Kokka, N. and R. George, 1974, Effects of narcotic analgesics, anesthetics, and hypothalamic lesions on growth hormone and adrenocorticotrophic hormone secretion in rats, in: *Narcotics and the Hypothalamus*, eds. E. Zimmerman and R. George (Raven Press, New York), pp. 137-157.
- Krivoy, W. A., D. C. Kroeger and E. Zimmerman, 1977, Neuropeptide influence acute and chronic effects of opiates, *Psychoneuroendocrinology* 2, 43-51.

- Lotti, V. J., N. Kokka and R. George, 1969, Pituitary-adrenal activation following intrahypothalamic microinjection of morphine, *Neuroendocrinology* 4, 326-332.
- Packman, P. M. and J. A. Rothchild, 1976, Morphine inhibition of ovulation: reversal by naloxone, *Endocrinology* 99, 7-10.
- Pang, C. N., E. Zimmerman and C. H. Sawyer, 1977, Morphine inhibition of the preovulatory surges of plasma luteinizing hormone and follicle stimulating hormone in the rat, *Endocrinology* 101, 1726-1732.
- Simantov, R., S. R. Childers and S. H. Snyder, 1978, The opiate receptor binding interactions of ³H-methionine enkephalin, an opioid peptide, *Eur. J. Pharmac.* 47, 319-331.
- Tan, L. and P. H. Yu, 1980, Biosynthesis of enkephalins by chromaffin cells of bovine adrenal medulla, *Biochem. Biophys. Res. Communic.* 95, 1901-1908.
- Terenius, L., W. H. Gispen and D. de Wied, 1975, ACTH-like peptides and opiate receptors in rat brain: structure-activity studies, *Eur. J. Pharmac.* 33, 395-399.
- Vernikos-Danellis, J., E. Anderson and L. Trigg, 1966, Changes in adrenal corticosterone concentration in rats: Method of bio-assay for ACTH, *Endocrinology* 79, 624-630.
- Wiegant, W. M., W. H. Gispen, L. Terenius and D. de Wied, 1977, ACTH-like peptides and morphine. Interaction at the level of the CNS, *Psychoneuroendocrinology* 2, 63-69.
- Yasukawa, N., H. Monder, S. D. Michael and J. J. Christian, 1978, Opiate antagonist counteracts reproductive inhibition by porcine ACTH extract, *Life Sci.* 22, 1381-1390.
- Zimmerman, E. and V. Critchlow, 1973, Inhibition of morphine-induced pituitary-adrenal activation by dexamethasone in the female rat, *Proc. Soc. Exp. Biol. Med.* 143, 1224-1226.

TABLE 1

Effect of administration of ACTH, morphine, or naloxone on the adrenal gland content of corticosterone.^a

Drug	Dose	Adrenal gland corticosterone ^b
		content, $\mu\text{g}/100 \text{ mg} \pm \text{S.E.}$
	<u>μU</u>	
ACTH ^c	0	1.23 \pm 0.05
	100	3.40 \pm 0.07 ^e
	<u>μg</u>	
β -endorphin ^c	100	1.23 \pm 0.05
	<u>mg/kg</u>	
(-)-morphine ^d	0	1.30 \pm 0.05
	5	1.27 \pm 0.10
	10	1.24 \pm 0.06
	20	1.05 \pm 0.03
(-)-naloxone ^d	0	1.25 \pm 0.05
	12.5	1.30 \pm 0.08
	25	1.41 \pm 0.19
	50	1.0 \pm 0.06

^aAll rats were hypophysectomized 18-20 h prior to drug administration.

^bN = 5 rats/group.

^cAdministered intravenously.

^dAdministered intraperitoneally.

^ep < 0.01.

●—● MORPHINE
10 mg/Kg

○—○ NALOXONE
50 mg/Kg

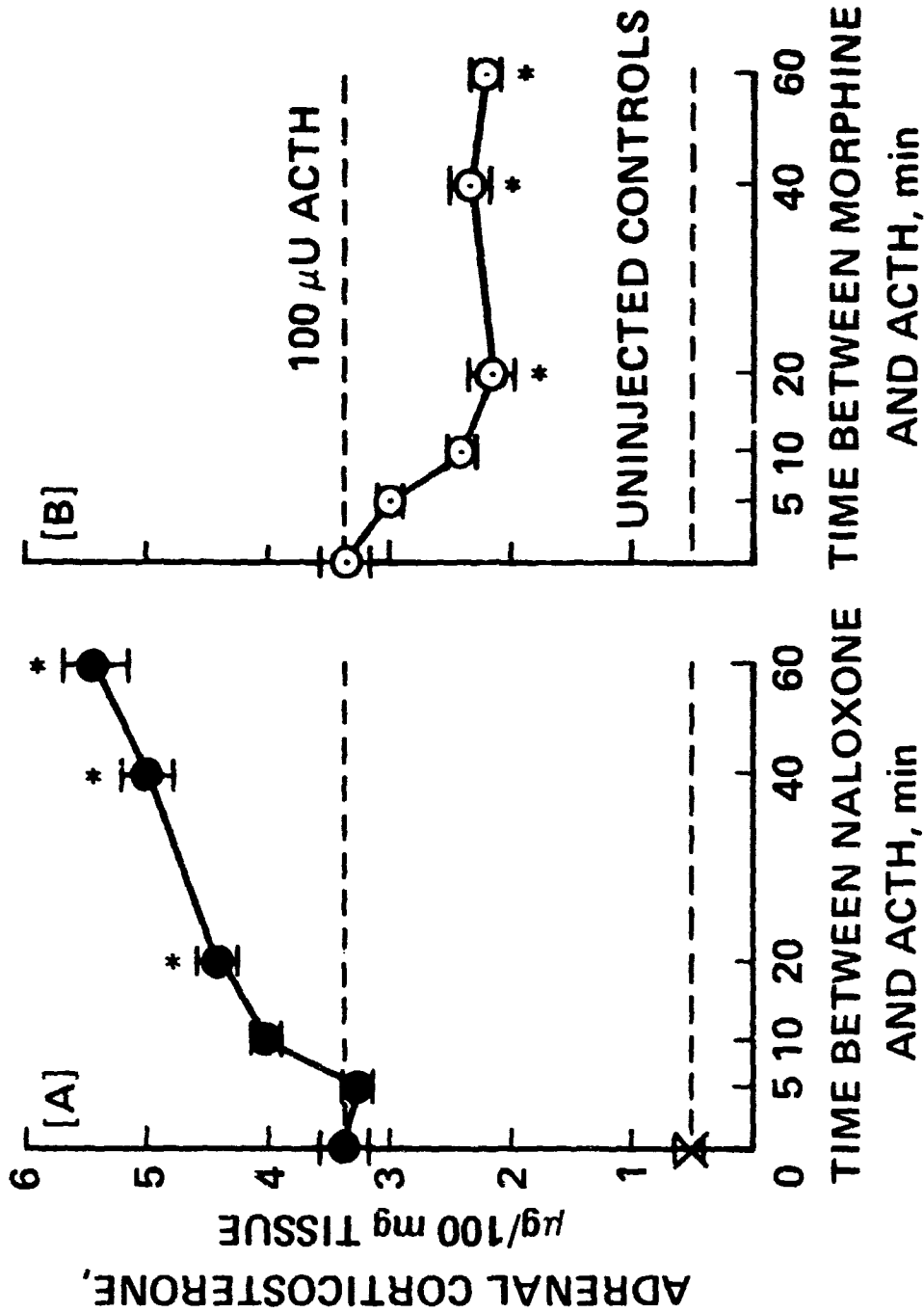


Figure 1.- Time course of the effect of (-)-morphine [A] or (-)-naloxone [B] pretreatment on the adrenal gland steroidogenic response to ACTH in hypophysectomized rats. The time zero value ($n = 10$ rats) represents the response to ACTH administered alone. $N = 5$ rats/time point. Asterisks indicate significant differences from the time zero control ($p < 0.05$).

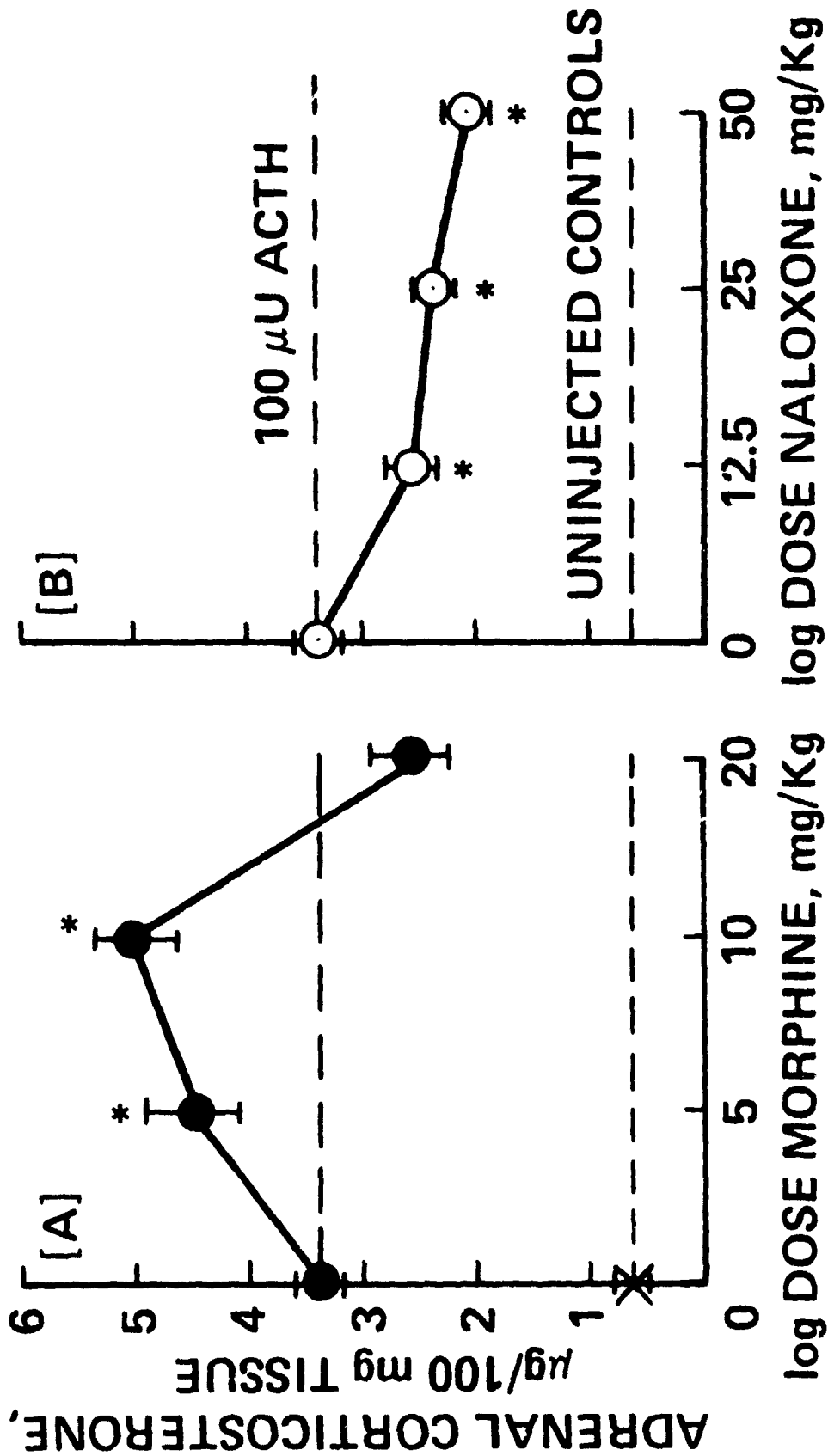


Figure 2.- The effect of pretreatment with various doses of (-)-morphine [A] or (-)-naloxone [B] on the adrenal gland steroidogenic response to ACTH in hypophysectomized rats. The time zero value represents the response to ACTH administered alone (n = 10 rats). N = 5 rats/dose. Asterisks indicate significant differences from the time zero control (p < 0.05).

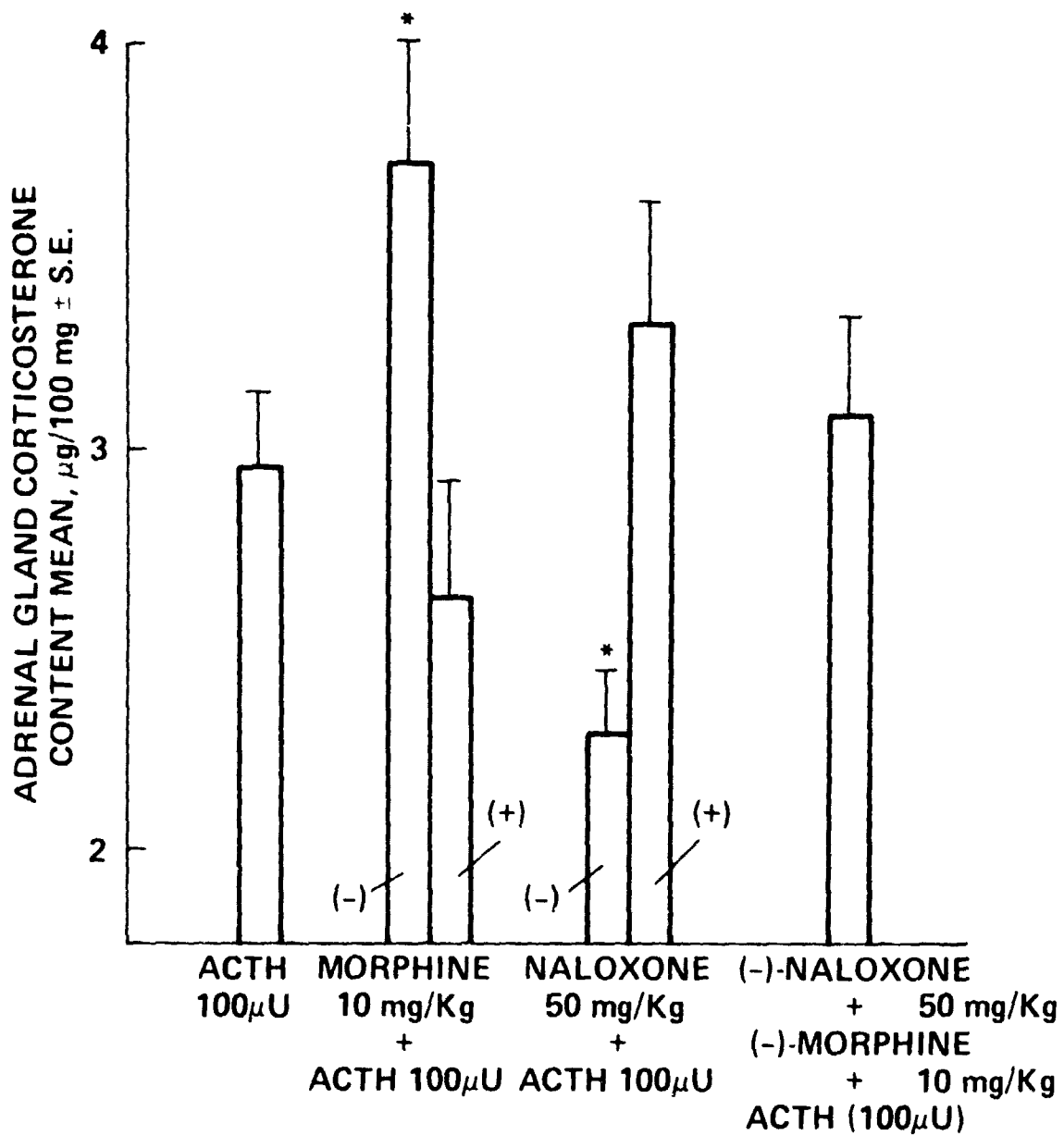


Figure 3.- The effect of pretreatment with the stereoisomeric forms of morphine or naloxone on the adrenal gland steroidogenic response to ACTH and the adrenal response to morphine following naloxone pretreatment. Asterisks indicate significant differences from the group administered ACTH alone (n = 10), at $p < 0.05$. N = 5 rats/group.