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SOME EFFECTS OF MORPHINE ON PITUITARY–ADRENOCORTICAL FUNCTION IN THE RAT*

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Abstract—Morphine evokes ACTH hypersecretion in rats at doses larger than 3·75 mg/kg i.p., a maximal response occurring with 30 mg/kg. The ACTH hypersecretion that follows a dose of 20 mg/kg reaches maximum within 30 min and lasts for 4–6 hr. Repeated doses (3 × 30 mg/kg) in 24 hr cause a significant lowering of pituitary ACTH content.

THE LITERATURE contains many references to the effects of morphine on the pituitary–adrenocortical system. For example, morphine was one of the agents which led Selye[1] to form the concept of the alarm reaction. Similarly, several investigators have shown that morphine evokes an increased secretion of ACTH in unanesthetized rats.[2–6] In addition, pretreatment of rats with morphine has been shown to block the pituitary–adrenocortical hyperactivity usually induced by agents such as epinephrine,[2, 7] histamine,[2] and vasopressin[2, 7] or by stressful procedures such as laparotomy or sham adrenalectomy.[2, 7] This inhibitory action of morphine may be overcome by using larger doses of histamine[8, 9] or vasopressin[9, 10] or by a more severe trauma.[11]

Previous studies have shown that Rauwolfia alkaloids such as reserpine[12] and benzoquinolizines such as Ro 4-1284,[13] as well as phenothiazine tranquillizers such as chlorpromazine, promazine, and trifluoperazine[14] evoke a marked hypersecretion of ACTH. In fact, the ACTH hypersecretion induced by a single large dose of reserpine[12] or by repeated doses of chlorpromazine[14] produces a 70 per cent depletion of pituitary stores of the hormone. This depletion is similar to that produced by exposure of rats to an environmental temperature of 4° for 24 hr.[12] The end result is an animal unable to hypersecrete ACTH in response to a subsequent stressful stimulus.

The present paper describes the hypersecretion of ACTH evoked by morphine in rats. Time-response and dose-response data are presented. Repeated doses of morphine are shown to cause a depletion of pituitary ACTH stores sufficient to reduce markedly the magnitude of response on subsequent exposure of the animals to an environmental temperature of 4°.

MATERIALS AND METHODS

Adult, male Sprague–Dawley rats (180–200 g) were used in all experiments. The

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animals were maintained in a relatively undisturbed room for 24 hr prior to use in experiments, with not more than 15 rats in a single cage of 6·25 ft². Handling during injections was minimal, and decapitation was performed within 5–10 sec after stunning. Solutions of morphine sulfate in distilled water were injected i.p. in a volume of 0·5 ml. All values in tables are given as dose of the free base. Control animals were injected with distilled water. Blood was collected into beakers containing heparin, transferred to tubes, and centrifuged immediately. Plasma, adrenals, and pituitary glands were stored at −10° until assayed.

Adrenal ascorbic acid was determined as described by Maickel,[15] plasma corticosterone by the method of Guillemin *et al.*[16] and pituitary ACTH by the procedure of Sayers *et al.*[17]

RESULTS

The duration of pituitary-adrenal hyperactivity evoked by a single dose of morphine (20 mg/kg, i.p.) is described by the data presented in Table 1. Hypersecretion of ACTH

TABLE 1. ADRENAL ASCORBIC ACID AND PLASMA CORTICOSTERONE AT VARIOUS TIMES AFTER MORPHINE ADMINISTRATION TO RATS*

Time (hr)	No. of rats	Adrenal ascorbic acid (mg/100 ml)	Plasma corticosterone (μg/ml)
0	16	398 ± 27	0·14 ± 0·02
0·5	6	261 ± 38†	0·53 ± 0·02†
1	6	272 ± 37†	0·54 ± 0·05†
2	5	264 ± 30†	0·50 ± 0·05†
4	5	266 ± 13†	0·45 ± 0·05†
6	5	270 ± 29†	0·21 ± 0·04†
8	6	263 ± 29†	0·21 ± 0·05
24	6	400 ± 33	0·16 ± 0·03

* Morphine (20 mg/kg, i.p.) or distilled water was administered at zero time. Values are reported as mean ± S.D.
† Significantly different from controls ($P < 0·05$).

lasted at least 4 hr; in 6 hr, the plasma level of corticosterone approached the normal value. The adrenal ascorbic acid remained depleted for more than 8 hr.

The pituitary–adrenal responses to various doses of morphine are presented in Table 2. From these results it may be inferred that doses of less than 3·75 mg morphine/

TABLE 2. THE EFFECT OF VARIOUS DOSES OF MORPHINE SULFATE ON ADRENAL ASCORBIC ACID AND PLASMA CORTICOSTERONE IN RATS*

Dose (mg/kg)	No. of rats	Adrenal ascorbic acid (mg/100 ml)	Plasma corticosterone (μg/ml)
0	27	422 ± 36	0·14 ± 0·03
0·75	6	391 ± 14	0·16 ± 0·04
1·50	6	385 ± 47	0·16 ± 0·04
3·75	6	364 ± 24†	0·21 ± 0·03†
7·5	5	340 ± 32†	0·27 ± 0·04†
15·0	5	336 ± 21†	0·43 ± 0·04†
22·5	6	301 ± 16†	0·48 ± 0·06†
30·0	6	291 ± 29†	0·51 ± 0·06†

* Morphine (various doses, i.p.) or distilled water was administered at zero time and all animals were killed 75 min later. Values are reported as mean ± S.D.
† Significantly different from controls ($P < 0·05$).

kg body weight were ineffective as pituitary–adrenal stimulants. A maximum response was achieved with a dose of 20–30 mg/kg. It is of interest to note that visible signs of sedation appeared at the 7·5 mg/kg dose and were maximal with the 30 mg/kg dose.

In view of previous reports demonstrating that large doses of reserpine[12] or chlorpromazine[14] deplete pituitary ACTH, a similar study was performed with morphine in various dosages. Table 3 presents the data on the ability of morphine to deplete the pituitary content of ACTH. It is evident that a single dose of morphine, either

TABLE 3. PITUITARY ACTH CONTENT AFTER VARIOUS TREATMENTS*

Group	t = 0		t = 9		t = 20		Pituitary ACTH	
	H_2O (ml)	Morphine (mg/kg)	H_2O (ml)	Morphine (mg/kg)	H_2O (ml)	Morphine (mg/kg)	(mU/mg)	(% of control)
I	0·5		0·5		0·5		59(54–64)	100
II		7·5					56(53–63)	95
III		7·5	0·5		0·5		58(53–61)	98
IV		7·5		7·5		7·5	53(50–57)	90
V		30					56(54–59)	95
VI		30	0·5		0·5		58(50–65)	98
VII		30		30	0·5		42(37–49)	72
VIII		30		30		30	21(17–26)	36

* Each value represents the mean of three experiments. In each experiment the pituitary glands from 4 rats were pooled. Figures in parentheses are the range of values obtained. The ACTH content was measured at t = 4 hr for groups II and V and t = 24 hr for all other groups.

7·5 or 30 mg/kg, i.p., had no significant effect on the pituitary ACTH content measured 4 or24 hr later. However, three doses of morphine (30 mg/kg each) during 24 hr caused a reduction of pituitary ACTH by 64 per cent, similar to that observed with agents such as reserpine or chlorpromazine.[12, 14]

This depletion of pituitary ACTH stores is confirmed by the data presented in Table 4. Groups of rats were pretreated with various doses of morphine during the 24-hr

TABLE 4. EFFECT OF MORPHINE PRETREATMENT ON PITUITARY–ADRENAL RESPONSE TO COLD EXPOSURE*

Group	No.	Pretreatment†	Cold exposure	Adrenal ascorbic acid (mg/100 ml)	Plasma corticosterone (μg/ml)
30–0	18	I	No	471 ± 34	0·14 ± 0·03
	18	I	Yes	342 ± 33‡	0·53 ± 0·08‡
30–1	6	VI	No	467 ± 27	0·15 ± 0·03
	6	VI	Yes	350 ± 29‡	0·50 ± 0·05‡
30–2	6	VII	No	489 ± 24	0·17 ± 0·03
	6	VII	Yes	398 ± 36‡	0·39 ± 0·03‡
30–3	6	VIII	No	423 ± 23	0·24 ± 0·11
	6	VIII	Yes	425 ± 36	0·30 ± 0·06

* Rats were pretreated with morphine as described in Table 3. Values are reported as mean ± S.D. Cold exposure for 2 hr at 4°.

† Pretreatment as in Table 3 for appropriate group.

‡ Significantly different from corresponding non-cold-exposed group ($P < 0·05$).

period prior to a 2-hr exposure to low environmental temperature (4°). The single dose of morphine (30 mg/kg) did not cause a depletion of pituitary ACTH and had no effect on the fall in adrenal ascorbic acid or the elevation in plasma corticosterone evoked by the subsequent cold exposure. In contrast, the triple dose of morphine (3 × 30 mg/kg), which depleted pituitary ACTH, virtually abolished the pituitary–adrenal response to the subsequent cold exposure. The intermediate dose (2 × 30 mg/kg) had only a partial effect on both pituitary ACTH content and the pituitary–adrenal response to a subsequent cold exposure.

Since Paroli and Melchiorri[18, 19] suggested that morphine treatment impaired the responsiveness of rat adrenal glands to ACTH *in vitro*, this decreased pituitary–adrenal response could also be due to a decreased effect of ACTH *in vivo*. Accordingly, the plasma corticosterone response to a dose of ACTH was measured in rats pretreated with morphine (3 × 30 mg/kg in 24 hr). The results, shown in Table 5, indicate that the morphine pretreatment had only a slight effect on the adrenocortical response to exogenous ACTH; the increase in plasma corticosterone due to ACTH was reduced from 0·36 μg/ml to 0·24 μg/ml.

TABLE 5. EFFECT OF MORPHINE PRETREATMENT ON ADRENAL RESPONSE TO ACTH *IN VIVO**

Group	Pretreatment†	Plasma corticosterone (μg/ml)
Control	I	0·12 ± 0·03
Control-ACTH	I	0·48 ± 0·04
Morphine	VIII	0·15 ± 0·03
Morphine-ACTH	VIII	0·39 ± 0·04

* Rats were pretreated as in Table 3, groups I and VIII. ACTH (Acthar gel, 25 U/rat, s.c.) was administered 1 hr prior to sacrifice. Each value is the mean ± S.D. of 6 rats.

† Pretreatment as in Table 3 for appropriate group.

DISCUSSION

The actions of morphine on the pituitary–adrenocortical system of animals are somewhat unclear despite the efforts of numerous investigators. Administration of pharmacologically active doses to rats has been shown to cause a hypersecretion of ACTH[2–6], which can be blocked by pretreatment with anesthetic doses of pentobarbital.[2, 7] This latter combination of barbiturate and morphine has been shown to block the ACTH-releasing actions of many stressful stimuli.[2, 7] In addition, Burdette *et al.*[8] have shown that this blocking action of morphine can be antagonized by doses of nalorphine which antagonize the psychic effects of morphine. Thus, morphine would appear to interfere with central nervous system pathways concerned with the control of ACTH release.

However, Paroli and Melchiorri[18, 20] have suggested that prolonged treatment of rats with morphine (20 mg/kg daily for 5–30 days) causes a decreased adrenal responsiveness to ACTH. These studies were performed with preparations of rat adrenal glands *in vitro*. Both Paroli and Melchiorri[19] and Briggs and Munson[2] reported that chronic treatment of rats with morphine reduced or abolished the normal pituitary–adrenocortical response to a subsequent stressful stimulus.

Previous reports[12, 14] had established that drugs such as reserpine and chlorpromazine could evoke ACTH hypersecretion of such severity as to cause a virtual depletion of pituitary stores of the hormone. A similar study of morphine shows that, in rats morphine exhibits a stimulating effect on ACTH hypersecretion at doses of 3·75 mg morphine sulfate/kg or greater. A maximal effect in 1·25 hr was observed with a dose of 20–30 mg/kg. The duration of action, as measured by elevated plasma corticosterone levels, is 4–8 hr; after 24 hr, the levels of plasma corticosterone and adrenal ascorbic acid have returned to normal.

Repeated dosage with morphine (3 × 7·5 mg/kg in 24 hr) has little effect on the pituitary level of ACTH. In contrast, a larger dose (3 × 30 mg/kg in 24 hr) caused a marked lowering of pituitary ACTH content to 36 per cent of control levels. Such animals exhibit almost normal levels of plasma corticosterone and adrenal ascorbic acid but do not show the typical pituitary–adrenal response when exposed to a stressful stimulus as cold exposure. The adrenals of the ACTH-depleted animals are slightly less responsive to a dose of exogenous ACTH.

The action of morphine appears to be 2-fold. When given in doses $> 3·75$ mg/kg, the compound produces a typical "stress response" with hypersecretion of ACTH. This response, as measured by increased plasma levels of cortcosterone and decreased adrenal ascorbic acid, is maximal with doses of 20–30 mg/kg. One must remember, however, that the limit of the response is the output of steroid by the adrenals, not the output of ACTH by the pituitary. Studies of plasma levels of ACTH would add considerably to our knowledge in this area. However, morphine when given in repeated doses has the ability to lower pituitary ACTH stores markedly, an activity similar to that of the phenothiazine tranquillizers.[14] Thus, the blocking action of chronic morphine treatment on stress responses may be due to a depletion of pituitary ACTH stores, making the gland refractory to a subsequent chemical or physiological stimulus.

The second action of morphine, that of blocking the ACTH hypersecretion usually evoked by other stimuli, has been explored in some detail by Briggs and Munson[2] and by Leeman.[9] These authors used pentobarbital to prevent the ACTH hypersecretion usually evoked by morphine. Thus, morphine is capable of eliciting a hypersecretion of ACTH from the pituitary and, when this reaction is blocked by pentobarbital, of preventing such hypersecretion by other stressors.[2, 9]

The action of morphine on the pituitary–adrenal axis may be part and parcel of its effects on other neural pathways. For example, morphine evokes ACTH hypersecretion only in doses which also sedate the animals, a relationship similar to that reported by Smith *et al.*[14] for chlorpromazine. In addition, George and Way[4] have shown that morphine-evoked ACTH hypersecretion is prevented by administration of nalorphine, the pharmacological antagonist of morphine. If, however, the ACTH hypersecretion evoked by morphine or chlorpromazine is related to the sedation produced by these drugs, a different mechanism must be involved in reserpine-induced ACTH hypersecretion, which has been shown to be independent of the sedating action of this drug.[21]

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