Brit. J. Pharmacol. (1955), **10**, 260.

STUDIES ON THE MECHANISM OF PITUITARY-ADRENAL ACTIVATION BY MORPHINE

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(RECEIVED MARCH 28, 1955)

That a relationship exists between the effect of morphine and the activity of the adrenal cortex was first shown by Lewis (1920), who observed that adrenalectomized rats were four to five hundred times more sensitive to morphine than were the control rats. MacKay and MacKay (1926) found that the adrenal cortex hypertrophied when a rat was treated with increasing doses of morphine. Similar results have been observed by Sung, Way, and Scott (1953) while studying rats made tolerant to morphine and methadone; however, the integrity of the adrenals does not appear to be essential in the development of chronic tolerance to the narcotic analgesics (Way, Sung, and Fujimoto, 1954). Recently, Nasmyth (1954) reported that adrenal ascorbic acid depletion occurred in rats following a single dose of morphine.

Since numerous other agents have been shown to deplete adrenal ascorbic acid, and since there appear to be gaps in the knowledge as to the manner in which such compounds exert this effect, studies were initiated in normal, adrenal demedullated and hypophysectomized rats to investigate the relative degree of specificity of the adrenal response to morphine as well as to methadone, and to assess the possible mechanisms by which these drugs stimulate the pituitary-adrenal axis.

METHODS

Administration of Drugs.—Male rats of the Sprague-Dawley strain, weighing 130–200 g., were used. They were maintained on a diet of Purina Lab Chow and tap water. The doses of morphine and of the *dextro* and *laevo* isomers of methadone were selected as fractions of the LD50. The LD50 of each drug was determined by the method of Litchfield and Wilcoxon (1949). The compounds were dissolved in distilled water and injected subcutaneously in the test animals, and the controls were injected with equal volumes of distilled water.

Estimation of Adrenal Ascorbic Acid.—All animals were killed by decapitation 90 min. after drug administration. Both adrenals of each animal were removed, dissected free of fat and weighed to 0.5 mg. The glands were then homogenized in 10 ml. of 4% trichloroacetic acid, and the ascorbic acid was determined by Geschwind, Williams, and Li's (1951) modification of the method of Roe and Kuether (1943).

Demedullation of Adrenal Glands.—The procedure was carried out according to the technique described by Evans (1936). All animals were given 0.5% saline for seven days following surgery, and were then given tap water. The groups studied in the morphine experiments were injected and sacrificed 21 days following demedullation; in the methadone studies the postoperative interval was 32–40 days. One group of animals was selected for histological examination. The adrenals of this group were fixed in formalin, sectioned serially at 7 μ, stained with haematoxylin and eosin, and examined microscopically for adrenal medullary tissue.

Hypophysectomy.—Animals were hypophysectomized by the parapharyngeal approach and were examined five days later.

RESULTS

Toxicity and Effect on Adrenal Ascorbic Acid in Normal Rats.—The acute toxicity and effect of morphine, (−)- and (+)-methadone on adrenal ascorbic acid content are summarized in Table I. All three compounds produced a significant depletion at 1/5 LD50 but not at 1/15 LD50; (−)-methadone gave the most pronounced effect and (+)-methadone the least. The results with (+)-methadone were not nearly as uniform as with morphine or (−)-methadone, and attempts to increase the response to (+)-methadone by increasing the dosage to as high as 1/2 LD50 resulted in little or no enhancement of its adrenal ascorbic acid depleting effect.

It is of interest that our toxicity data indicate that *laevo*methadone given subcutaneously is approximately 5.5 more toxic than the *dextro*-isomer, particularly since other investigators (Scott, Robbins, and Chen, 1948; Thorp, 1949) have

TABLE I

TOXICITY AND EFFECT OF MORPHINE, (−)- AND (+)-METHADONE ON THE ADRENAL ASCORBIC ACID CONTENT OF NORMAL RATS FOLLOWING SUBCUTANEOUS ADMINISTRATION

Compound	LD50 and 19/20 Confidence Limits	No. of Rats	Dose		Adrenal Ascorbic Acid		P
			Mg./kg.	Approx. Fraction of LD50	Mean mg. % ±S.E.	% of Control	
Distilled water	—	18	—	—	489±17	—	—
Morphine sulphate	108 (80·2–146·0) mg./kg.	6	22·0	1/5	324±34	66·4	0·001
		6	8·0	1/15	433±19	88·7	0·1
(−)-Methadone hydrochloride	20 (5·3–26·2) mg./kg.	6	4·0	1/5	280±15	57·4	0·001
		6	1·4	1/15	433±14	88·7	0·1
(+)- " "	112 (75·0–166·9) mg./kg.	3	60·0	1/2	357±60	73·0	0·01
		10	30·0	1/4	380±21	77·7	0·01
		6	22·0	1/5	387±29	79·3	0·01
		6	8·0	1/15	453±18	92·8	0·3

shown that the two isomers have approximately the same LD50 in mice when injected intravenously. In the latter instance the identical LD50s appear to be fortuitous since apparently the isomers kill by different mechanisms. Our unpublished observations indicate that nalorphine antagonizes the toxic effects of *laevo*methadone but not of *dextro*methadone.

TABLE II

EFFECT OF MORPHINE, (−)- AND (+)-METHADONE ON THE ADRENAL ASCORBIC ACID CONTENT OF HYPOPHYSECTOMIZED RATS FOLLOWING SUBCUTANEOUS ADMINISTRATION

Compound	No. of Rats	Dose (mg./kg.)	Adrenal Ascorbic Acid		P
			Mean mg. %±S.E.	% of Control	
Distilled water ..	8	—	433±33	—	—
Morphine sulphate	5	22·0	454±50	103·0	0·7
(−)-Methadone hydrochloride ..	5	4·0	422±26	96·0	0·8
(+)-Methadone hydrochloride ..	4	22·0	410±19	93·2	0·6

Effect on Adrenal Ascorbic Acid in Hypophysectomized Rats.—It can be seen from Table II that doses of morphine, and of the methadone isomers, which deplete adrenal ascorbate in normal rats are ineffective in hypophysectomized animals, thus indicating that integrity of the adenohypophysis is essential for this effect.

Effect on Adrenal Ascorbic Acid in Demedullated Rats.—Bilateral adrenal medullectomy did not impair the adrenal cortical response to morphine or the methadones. In fact, the surgical procedure may have enhanced the effect, since the compounds depleted adrenal ascorbate at doses much lower than those required in normal animals (Table III). With morphine a significant depletion was obtained with 1/10 of the dose that was effective in normal rats. However, Nasmyth (1954) reported that the same dose of morphine

TABLE III

EFFECT OF MORPHINE, (−)- AND (+)-METHADONE ON THE ADRENAL ASCORBIC ACID CONTENT OF DEMEDULLATED RATS FOLLOWING SUBCUTANEOUS ADMINISTRATION

Compound	No. of Rats	Mean Adrenal Weight (mg.)	Dose (mg./kg.)	Adrenal Ascorbic Acid		P
				Mean mg. %±S.E.	% of Control	
Controls for morphine (H_2O)	6	19·4	—	307±29	—	—
Morphine sulphate	6	19·5	8·0	207±9	67·5	0·01
	6	18·8	4·0	234±18	76·2	0·05
	6	19·3	2·0	234±10	76·2	0·05
Controls for methadone (H_2O)	5	26·6	—	547±24	—	—
(−)-Methadone hydrochloride	6	27·8	1·4	395±46	72·2	0·02
	6	26·5	0·7	526±26	96·0	0·3
(+)-Methadone hydrochloride	6	24·9	8·0	466±19	85·3	0·05
	6	27·6	4·0	574±26	104·5	0·5

reduced adrenal ascorbic acid in normal and demedullated rats to approximately the same degree. It cannot be stated for certain whether our findings indicate a true enhancement of the adrenal ascorbate response since the demedullated animals appeared to be more sensitive to the toxic effects of morphine and LD50 determinations were not made in this group. However, the results indicate clearly that adrenal medullary discharge of adrenaline does not play a major role in effecting adrenal ascorbic acid depletion with the above compounds.

It would appear from these data that morphine is relatively more specific than the methadones, but it is not possible to compare the effect of morphine and the methadones on the adrenal ascorbate response in medullectomized animals because experimental conditions were not identical. The groups of animals in the morphine study were used only 21 days post-operatively, and therefore were probably more sensitive than those used in the methadone studies in which the post-operative interval was 32 to 40 days. The difference in recovery period gives a plausible explanation for the disparity in the ascorbic acid content and adrenal weight of the control values in the two studies. This conclusion is supported by the report of Greep and Deane (1949) that the adrenal ascorbic acid concentration becomes constant and is present in all of the cortical cells in normal amounts about the 32nd day after enucleation.

Effect of Morphine, Aspirin, and Adrenaline on the Adrenal Ascorbic Acid of Rats Pretreated with Cortisone.—In order to elucidate the mechanism by which morphine depletes adrenal ascorbic acid, experiments were undertaken to determine whether this effect was due to an increased cellular utilization of circulating adrenal corticoids or to some other mechanism. The circulating level of cortisone was elevated by injecting animals with 5 or 10 mg./kg. of cortisone three hours before the administration of morphine. It can be seen from Table IV that neither of these doses of cortisone abolished the fall in adrenal ascorbic acid following morphine, although the response was partially blocked. Neither did cortisone pretreatment block the response to aspirin or adrenaline, though the 10 mg. dose of cortisone appeared to have some inhibitory effect. These results are not in harmony with those of Sayers and Sayers (1947), who reported that 1.0 mg./kg. of cortisone blocked the ascorbic acid depleting effect of adrenaline in unilaterally adrenalectomized animals under anaesthesia. Under our experimental conditions we were unable to demonstrate any blocking effect to morphine, aspirin, or adrenaline with a 1.0 mg./kg. dose of cortisone. Our findings are supported by those of Cronheim and Hyder (1954), who found that doses of cortisone as large as 80 mg./kg. failed to abolish the adrenal cortical response to aspirin.

TABLE IV

EFFECT OF MORPHINE, ASPIRIN, AND ADRENALINE ON THE ADRENAL ASCORBIC ACID CONTENT OF RATS PRETREATED WITH CORTISONE ACETATE

Compound	No. of Rats	Dose (mg./kg.)	Adrenal Ascorbic Acid		P
			Mean mg. %±S.E.	% of Control	
a. Cortisone (5 or 10 mg./kg.) distilled water	8		449±21	100 ±4·7	—
b. Morphine sulphate	8	30	266±8	59·4±1·8	0·001 (a–b)
c. Cortisone (5 mg./kg.)+ morphine	10	30	362±20	80·6±4·6	0·01 (a–c) 0·001 (b–c)
d. Cortisone (10 mg./kg.)+ morphine	10	30	371±17	82·7±3·8	0·01 (a–d) 0·001 (b–d)
e. Aspirin	8	150	280±16	62·4±3·6	0·001 (a–e)
f. Cortisone (5 mg./kg.)+ aspirin	6	150	285±16	63·5±3·6	0·001 (a–f) 0·9 (e–f)
g. Cortisone (10 mg./kg.)+ aspirin	6	150	354±40	78·8±8·9	0·05 (a–g) 0·07 (e–g)
h. Adrenaline hydrochloride	4	0·1	322±5	71·7±1·1	0·001 (a–h)
i. Cortisone (5 mg./kg.)+ adrenaline	10	0·1	322±5	71·7±1·1	0·001 (a–i) 1·0 (h–i)
j. Cortisone (10 mg./kg.)+ adrenaline	8	0·1	363±25	80·8±5·6	0·02 (a–j) 0·2 (h–j)

Effect of Morphine and Aspirin on Adrenal Ascorbic Acid in Rats Pretreated with Nalorphine.—All animals were treated with 5 mg./kg. nalorphine subcutaneously 5 min. before the injection of morphine, (−)-methadone, (+)-methadone, or aspirin. It can be seen from Table V that nalorphine in this dose, which does not significantly lower the adrenal ascorbic acid content, produced a pronounced inhibition of the ascorbic acid depleting effect of morphine and (−)-methadone but not of (+)-methadone and aspirin.

TABLE V

EFFECT OF MORPHINE, (−)- AND (+)-METHADONE AND ASPIRIN ON THE ADRENAL ASCORBIC ACID CONTENT OF RATS PRETREATED WITH NALORPHINE

Compound	No. of Rats	Dose (mg./kg.)	Adrenal Ascorbic Acid		P
			Mean mg. %±S.E.	% of Control	
a. Distilled water	10	—	450±21	100 ±4·7	—
b. Nalorphine hydrochloride	18	5	415±11	92·4±2·4	0·1 (a–b)
c. Morphine sulphate	8	30	266±8	59·2±1·8	0·001 (a–c)
d. Nalorphine+ morphine	9	5 30	360±13	80·0±2·9	0·001 (a–d) 0·01 (b–d) 0·001 (c–d)
e. (−)-Methadone hydrochloride	8	6	326±14	72·4±3·1	0·001 (a–e)
f. Nalorphine+ (−)-methadone	9	5 6	398±18	88·5±4·0	0·1 (a–f) 0·3 (b–f) 0·01 (e–f)
g. (+)-Methadone hydrochloride	10	30	380±21	84·3±4·7	0·05 (a–g)
h. Nalorphine+ (+)-methadone	11	5 30	368±12	81·8±2·7	0·01 (a–h) 0·01 (b–h) 0·5 (g–h)
i. Aspirin	8	150	280±16	62·2±3·6	0·001 (a–i)
j. Nalorphine+ aspirin	8	5 150	274±19	61·0±4·2	0·001 (a–j) 0·001 (b–j) 0·8 (i–j)

DISCUSSION

The data clearly indicate that morphine and *laevo*methadone deplete adrenal ascorbic acid and that the integrity of the pituitary is essential for this effect. The precise mechanism by which these compounds mediate an action on the pituitary is in need of clarification. Conceivably, morphine activates the anterior pituitary gland to release ACTH by one or more of several mechanisms which may be independent or interdependent: (*a*) a reflex secretion of endogenous adrenaline which then acts on the pituitary; (*b*) a release of tissue histamine; (*c*) a fall in the level of circulating adrenal cortical hormones as a result of increased cellular utilization; (*d*) a direct action on the anterior pituitary; and (*e*) a neural or neuro-humoral discharge in the hypothalamus.

Although our results do not completely exclude the first three possibilities, it is unlikely that morphine acts by one of these mechanisms. The results with demedullated rats show conclusively that the adrenal response to morphine and methadone occurs in the absence of the release of adrenal medullary adrenaline. Moreover, an earlier study indicated that the dose of morphine required to effect adrenal medullary discharge of adrenaline in the rat, as measured by the hyperglycaemic response, is at least double that required in the present study to deplete adrenal ascorbic acid (Miller *et al.*, 1955). These data are supported by the results of Nasmyth (1954), who reported a depletion of adrenal ascorbic acid in demedullated rats following morphine administration. In the same study Nasmyth showed that tissue release of histamine following morphine was not an important factor in depleting adrenal ascorbic acid. Finally, an increase in the level of circulating cortisone failed to block the release of ACTH following the injection of morphine.

In light of recent evidence that the adrenocortical response to stress may be controlled by the hypothalamus (DeGroot and Harris, 1950; Hume and Wittenstein, 1950; Porter, 1952; McCann, 1953; Ganong and Hume, 1954), it seems more likely that morphine acts via a neural or neurohumoral mechanism. Experiments are at present in progress to investigate this effect of morphine in rats with hypothalamic lesions. Evidence that morphine acts on the hypothalamus has been demonstrated by McCrum and Ingram (1951), who found that cats with lesions in the caudal hypothalamus failed to exhibit the characteristic symptoms of hyperthermia and hyperactivity following morphine administration. Further evidence is indicated by the antidiuretic effect of morphine in dogs (DeBodo, 1944) and rats (Giarman, Mattie, and Stephenson, 1953; Winter, Gaffney, and Flataker, 1954). DeBodo reported that morphine, while producing a marked antidiuretic effect in normal dogs, failed to inhibit water diuresis caused by pituitary stalk section or hypophysectomy; Winter *et al.* have shown that the antidiuretic effect of morphine is effectively blocked by nalorphine and the urine of morphine-treated rats contains an antidiuretic substance which is not morphine, and which is not antagonized by nalorphine. More recently, the results of McCann and Brobeck (1954) indicate that the supraopticohypophyseal tract may play a role in the regulation of ACTH secretion by release of antidiuretic hormone into the hypophyseal portal vessels. They reported that destruction of the supraopticohypophyseal tract blocks pituitary ACTH release in response to stress and that ascorbic acid depletion, which normally follows stress, failed to occur in rats with severe diabetes insipidus. These data make it attractive to speculate that the site of action of morphine, which evokes ascorbic acid depletion and antidiuresis, may be one and the same.

Inasmuch as a variety of stresses and a variety of compounds can evoke adrenal ascorbic acid depletion, the question arises, Can this action of morphine and methadone be merely a non-specific effect? It would seem apparent from our results that the adrenal ascorbic acid depleting effect of morphine—or of any other compound acting systemically—cannot arbitrarily be classified as non-specific. Not only is the specificity of the response relative in nature but the mechanisms concerned with mediating the effect may be different. Our studies clearly show that nalorphine, a specific morphine antagonist, blocks the adrenal ascorbic acid response in rats injected with morphine but fails to inhibit this effect following aspirin administration. This strongly suggests that different receptors or sites are concerned with mediating this effect for morphine and aspirin as well as for (−)-methadone and (+)-methadone. Evidence that morphine may act differently from other chemical agents is also indicated by the studies of Briggs and Munson (1954), who reported that the adrenal ascorbic acid depleting effect of morphine but not of histamine, pitressin, and adrenaline can be suppressed by previous injection of pentobarbitone sodium.

It is clear, therefore, that pharmacological agents can effect depletion of adrenal ascorbic acid by one or more of several mechanisms. Rather than characterize an agent by a mechanism which remains to be elucidated, it appears more appropriate to define specificity of action of the agent in relative terms from quantitative data obtained by standardized pharmacological techniques. Thus, until the information becomes available, the relative specificity of the response by which an agent causes depletion of adrenal ascorbic acid can better be described in terms of (*a*) its minimal effective dose, (*b*) the potency ratio of the compound in relation to its toxicity, and (*c*) whether or not other pharmacological effects are evident with a dose of the compound which evokes adrenal ascorbic acid depletion.

Summary

1. The acute toxicity and effect of morphine, and of (−)- and (+)-methadone on adrenal ascorbic acid were studied in normal rats. All three compounds produced a significant fall in the ascorbic acid content of rat adrenals at 1/5 LD50 but not at 1/15 LD50.

2. Doses of morphine and (+)- and (−)-methadone, which deplete adrenal ascorbic acid in normal rats, were ineffective in hypophysectomized animals.

3. Bilateral adrenal demedullation did not impair the adrenal cortical response to morphine and (+)- or (−)-methadone. In fact, the compounds depleted adrenal ascorbic acid at doses much lower than those required in normal animals.

4. Pretreatment with cortisone (5 or 10 mg./kg.) did not prevent a reduction in adrenal ascorbic acid following morphine, aspirin, or adrenaline, but did modify the response.

5. Pretreatment with nalorphine produced a pronounced inhibition of the ascorbic acid depleting effect of morphine and (−)-methadone but not of (+)-methadone and aspirin. This suggests that different receptors or sites are concerned with mediating this effect, possibly in the hypothalamus.

This investigation was supported by grants from the Division of Research Grants and Fellowships of the National Institutes of Health and the Institute of Analgesic and Sedative Drugs. We are indebted to Mrs. Joan Taylor and Messrs. Dirk Van Peenen and A. E. Takemori for their co-operation.

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