Brit. J. Pharmacol. (1954), **9**, 95.

FACTORS INFLUENCING THE EFFECT OF MORPHINE SULPHATE ON THE ASCORBIC ACID CONTENT OF RATS' ADRENAL GLANDS

BY

P. A. NASMYTH

From the Department of Pharmacology, St. Mary's Hospital Medical School, London, W.2

(RECEIVED OCTOBER 25, 1953)

In 1923 Lewis showed that morphine was 400–500 times more toxic to adrenalectomized than to normal rats. This suggests that the drug stimulates the adrenal cortex; it has since been shown (Nasmyth, 1948) that the subcutaneous injection of morphine causes a depletion of the ascorbic acid content of rats' adrenals. Morphine also affects the secretion of the adrenal medulla; Elliott, in 1912, demonstrated that the pressor amine content of the cat's adrenal was much reduced by morphine. More recently, Emmelin and Strömblad (1951) showed a similar reduction in the adrenaline and noradrenaline content of the cat's adrenal. In 1952 Evans, Nasmyth, and Stewart showed that after intravenous injection of morphine the pressor activity of blood samples from the adrenal vein of the cat was increased tenfold for more than 10 minutes. By direct measurement, Vogt (1944) has shown that the injection of adrenaline causes an increased secretion of cortical hormone in the dog, and various authors have described the depletion of the ascorbic acid content of the rat's adrenal glands when adrenaline is injected subcutaneously (Long and Fry, 1945; Nasmyth, 1950; and Jarrett, 1951).

Morphine can also liberate histamine (Feldberg and Paton, 1950 and 1951; and Nasmyth and Stewart, 1950) and a relationship between histamine and the activity of the adrenal cortex was indicated by Gottesman and Gottesman (1928), who showed adrenalectomized rats to be 20 times more sensitive to histamine than normal animals. Binet (1941) showed that histamine causes a release of adrenaline from the adrenal medulla, thus confirming the suggestion of Dale (1920) and the work of Kellaway and Cowell (1922). Histamine causes a depletion of ascorbic acid in the rat adrenal cortex (Sayers and Sayers, 1947; Nasmyth, 1951; and Halpern and Benos, 1952), and this effect is independent of its ability to release adrenaline from the adrenal medulla (Nasmyth, 1951).

Since morphine can liberate both adrenaline and histamine, it was of interest to determine how much these two substances contribute to the effect of morphine on the adrenal cortex.

METHODS

Administration of Drugs.—Litter mate, male rats of either the Hooded or Wistar strains were used. The Hooded strain was obtained from Mr. Locke's colony at Crooke's Laboratories and the Wistar strain from Dr. Mandl's colony at Birmingham University, and from the colony at Glaxo Laboratories. These strains were distributed as evenly as possible between the various groups of animals used in the following experiments. The rats were on a stock diet.

In each experiment 2 mg./100 g. body weight of morphine sulphate was injected subcutaneously in the test animals, whereas the controls, taken from the same litter, were injected with an equal volume of normal saline. The test animals were killed by a blow on the back of the head 1½, 3, and 5 hr. after the injection; the controls were killed between 1 and 3 hr. after the saline dose, as Jarrett (1951) has shown the effect of saline on the rat's adrenal ascorbic acid to be maximal between these times. Immediately after each rat was killed, the abdomen was shaved and a piece of skin weighing between 0.5 and 1 g. was taken for determination of its histamine content.

Extraction of Glands.—The adrenal glands were removed, dissected free of superfluous tissue, and weighed to 0.0002 g. This procedure occupied approximately the same time in each experiment, so that any loss in weight due to drying could be assumed to be approximately constant. Each gland was then ground in a test-tube containing 10 ml. of 6% trichloracetic acid and a little acid-washed sand, with the aid of a glass rod having an end shaped to fit the bottom of the tube. This was sufficient to extract all the ascorbic acid, which was estimated as described by Roe and Kuether (1943).

Extraction and Estimation of Skin Histamine.—The piece of abdominal skin was freed from subcutaneous tissue before weighing. Each sample was ground in a mortar containing a little acid-washed sand, with

2 ml. N-HCl and 10 ml. distilled water for each g. of tissue. When sufficiently disintegrated it was transferred to an evaporating basin, the mortar and pestle being rinsed three times with normal saline. After boiling for 1 min. the extract was filtered through cotton-wool and the basin, funnel, and cotton-wool pad were rinsed twice with a little normal saline. The extract was brought to about *p*H 7.5 with N-NaOH and the volume adjusted by adding normal saline until 1 ml. was equivalent to 20 mg. tissue. The extract was stored in a refrigerator until assayed, within 6 hr. of preparation, on the atropinized guinea-pig ileum.

Demedullation of Adrenal Glands.—The adrenal glands of litter mate, male rats were demedullated within a week or two of weaning, using the technique described by Evans (1936). Anaesthesia was by intraperitoneal pentobarbitone (1 mg.), supplemented with ether. Upon recovery, each rat was given a few drops of 2% glucose solution by mouth from a teat pipette. The operated rats were kept in a room at 28° C. and given 0.2% saline to drink for three weeks following the operation. Regeneration of cortical tissue was sufficiently advanced by this time to enable the 0.2% saline to be replaced with water.

Depletion of Skin Histamine.—This was effected by a technique similar to that of Feldberg and Talesnik (1953). Intraperitoneal injections of 48/80 in normal saline were given, one in the morning and one at night for five days. On the first day, 1 μg./g. body weight was given at each injection. On the second day a total dose of 200 μg. was injected each time. On each successive day the dose was increased by 100 μg., until, on the fifth day, each rat received two injections, each of 500 μg. Observations were made on the third day following the last dose.

Results

Effect of Morphine Sulphate on Normal Rats.—Within 20 min. of the subcutaneous injection of morphine sulphate, normal rats became inactive and remained so for 2 to 3 hr. Their behaviour was in marked contrast to that of saline-injected controls. Morphine-treated rats showed gradually increasing activity between 3 and 5 hr. after the drug. Care was taken not to disturb them until they were taken from the cage, after the elapse of the appropriate time interval.

Between 1½ and 3 hr. after the injection most rats were indifferent to handling and remained lethargic ; a few were irritable and resented handling, although they remained immobile if left alone. These observations are consistent with those on other rodents in which high doses of morphine often cause reflex irritability without motor excitement.

The sequence of depression followed by a return to normal activity fitted in well with the pattern of events in the adrenal cortex. One and a half hours after the injection of morphine sulphate the ascorbic acid content of the glands was 70% of the control value. After 3 hr. the level was still low at 73%, but after 5 hr. it had returned to 98% of the resting level (Table I).

The abdominal skin histamine of these animals was estimated to determine the normal level so as to indicate the degree of depletion obtained in rats treated with compound 48/80. It was much higher in Hooded (average 48 μg. histamine base/g. of skin) than in Wistar rats (26 μg./g.). The mean values for abdominal skin histamine given in Table I are derived from both Hooded and Wistar stains.

Effect of Morphine Sulphate on Rats with Demedullated Adrenal Glands.—After demedullation, cortical tissue regenerates from the empty capsules to which a few cortical cells remain attached. In some rats only one gland regenerated ; in a few, one of the glands was very large and the other very small. No glands under 5.0 mg. were used in the experiments, as they seldom gave values comparable with those given by their larger partners. Adequate regeneration of the glands had usually occurred 28 days after operation, but the animals were not used until at least 40 days had elapsed.

The behaviour of the demedullated animals after morphine was similar to that of normal rats, but irritability seemed to be more frequent. There was a marked fall in body temperature. The drug was evidently more toxic to demedullated than to normal rats, as it often caused wheezy respiration, and two animals died between 2 and 5 hr. after injection.

One and a half hours after the injection of morphine the adrenal ascorbic acid had fallen to 75% and after 3 hr. stood at 73% of the control value. Five hours after the injection the level had risen to 89%. The fall in the ascorbic acid both at 1½ and at 3 hr. after morphine was significant ($P<0.001$), but at neither time was it significantly different from the fall in normal animals: at 1½ hr. $P>0.2$ and at 3 hr. $P>0.6$ (Table I).

The concentration of histamine in abdominal skin was appreciably lower in the demedullated rats than in the normal animals, so it was decided to investigate this phenomenon.

Behaviour of Skin Histamine in Normal and Demedullated Rats.—The rats which had low values for the abdominal skin histamine differed from those with higher values in two respects: they had demedullated adrenal glands and they were older. The difference might therefore be due either to age or to demedullation.

TABLE I
THE EFFECT OF MORPHINE SULPHATE ON THE ADRENAL ASCORBIC ACID OF RATS

	Drug (Morphine Sulphate 2 mg. or 0·9% Saline 0·1 ml. per 100 g. Body Wt.)	Duration of Action in Hr.	Ascorbic Acid Content (mg./100 g. Gland ± S.E.)	Ascorbic Acid as % Normal	No. of Glands	Abdominal Skin Histamine (μg. Base/g. Skin)
Normal	Saline	1–3	352±13	100	17	34
	Morphine	1·5	248±10	70	12	35
	,,	3·0	257±17	73	14	32
	,,	5·0	345±19	98	6	33
Demedullated	Saline	1–3	294±17	100	13	27
	Morphine	1·5	220± 8	75	17	25
	,,	3·0	213±10	73	12	24
	,,	5·0	263±14	89	5	31
Histamine depleted ..	Saline	1–3	409±15	100	13	8
	Morphine	1·5	324± 8	79	14	9
	,,	3·0	359± 8	88	12	11
	,,	5·0	367± 5	90	12	11
Demedullated and with low skin histamine	Saline	1–3	274± 9	100	10	10
	Morphine	1·5	221±13	81	8	13
	,,	3·0	252±13	92	10	13

To test these possibilities 48 six-week-old male Wistar rats (not litter mates) were divided into two groups. The adrenal glands of one group were demedullated; the other group had sham operations. Four animals from each group were taken eight days after the operation, and four more every fourth week for six months. The abdominal skin histamine was estimated; it fell gradually in both groups during this period.

In view of this, the abdominal skin histamine of one-day-old rats was determined; it was found to be lower than that of the six-week-old rats. It was concluded, therefore, that the abdominal skin histamine rises between birth and six weeks of age, and that the subsequent fall is a function of age and not of demedullation. These results are shown in Table II.

TABLE II
EFFECT OF AGE ON ABDOMINAL SKIN HISTAMINE OF WISTAR RATS WITH DEMEDULLATED ADRENALS AND NORMAL SHAM-OPERATED CONTROLS
(The animals were operated on when 42 days old. Each figure is the average from four animals)

Age (Days)	Histamine Base (μg./g. Abdominal Skin ± S.D.)	
	Demedullated Rats	Sham-operated Rats
1	—	18·7±1·8
50	27·2±6·8	25·7±5·2
70	23·1±2·6	23·3±4·7
98	17·1±5·1	20·4±2·9
126	14·3±2·3	13·1±4·0
154	11·5±3·5	16·2±2·9
182	10·9±3·0	8·5±1·2

Since Riley and West (1952 and 1953) have demonstrated a correlation between the amount of histamine and the mast cell content of certain tissues, skin sections were prepared at each stage of the experiment and stained according to their technique. These sections never showed more than eight mast cells in one high-power field, and since no gross changes were observed as the histamine content of the skin diminished with age, no conclusions could be drawn concerning the relationship between the skin histamine and the mast cell population.

Rats Having a Depleted Skin Histamine.—The technique used to deplete the tissue histamine was similar to that described by Feldberg and Talesnik (1953), who showed that treatment with 48/80 greatly reduced the histamine content of the skin, skeletal muscle, and heart, but not that of stomach, duodenum, and liver. In the present experiments, the skin histamine level was used as a guide to the degree of tissue histamine depletion. It was never reduced to undetectable amounts, but it was diminished to levels varying between 12 and 30% of normal.

The behaviour of the histamine-depleted rats after morphine sulphate was the same as that of similarly injected normal animals. At 1½ and 3 hr. the response of the adrenal ascorbic acid appeared to be less than in the normal animals. However, statistical analysis showed that this was not so: at 1½ hr. $P>0.4$ and at 3 hr. $P>0.1$ (Table I).

Rats with Demedullated Adrenal Glands and a Low Tissue Histamine.—The first two attempts to obtain rats with demedullated adrenal glands and a low tissue histamine failed. In the first, a group of rats was demedullated and, 36 days after the operation, treatment with 48/80 was begun. The animals proved very sensitive to the drug and were nearly all killed by the early doses. In the second attempt, a group of rats was first treated with 48/80 in gradually increasing doses as previously described. On completion of this treatment,

the rats were demedullated and given daily maintenance doses of 50 μg. of 48/80. Between 40 and 50 days after the operation the animals were taken for experiment, but were found to have a nearly normal level of abdominal skin histamine.

In view of these failures it was decided to determine the effect of morphine sulphate on the adrenal ascorbic acid of demedullated rats having a low tissue histamine in consequence of their age. Accordingly, a group of rats was demedullated and kept for 21 weeks after the operation before performing the experiment. In these animals the abdominal skin histamine was reduced to levels very nearly as low as those in animals pretreated with 48/80. The response of the adrenal ascorbic acid was less than in normal animals. The level was 81% of the normal figure 1½ hr. after the morphine sulphate and had returned to 92% 3 hr. after the injection. The difference between these falls and those seen in normal animals was significant: at 1½ hr. $P<0.05$ and at 3 hr. $P<0.01$.

Discussion

The fall in the rats' adrenal ascorbic acid caused by the subcutaneous injection of 2 mg./100 g. body weight of morphine sulphate is less in demedullated and in histamine-depleted rats than in normal animals, but the significance of the difference is very small, P never being less than 0.2. In rats having a low tissue histamine and demedullated adrenals the fall in adrenal ascorbic acid caused by morphine is significantly less than in normal animals ($P<0.05$ at 1½ hr. and $P<0.01$ at 3 hr.). It seems from these results that the effect of morphine sulphate on the rat adrenal cortex is affected partly by released histamine and partly by adrenaline released from the adrenal medulla. The fact that both the elimination of the adrenal medulla and the reduction of tissue histamine to very low levels is necessary to obtain a significant effect, suggests either that the individual part played by the released substances is very small, or that the one can compensate for the absence of the other. It must be emphasized that the liberation of histamine and adrenaline is probably not responsible for the whole effect on the adrenal ascorbic acid, since there is still a significant fall in demedullated, histamine-depleted rats. Porter (1952) has shown that intravenous adrenaline causes increased electrical activity in the posterior hypothalamus of the rat, and that destruction of this area prevents the eosinopenic response to the drug. Other stress stimuli had similar effects. It is possible, therefore, that the adrenaline and histamine released by morphine, as well as the morphine itself, may stimulate the hypothalamus.

It was observed that the abdominal skin histamine of the rat rises between birth and six weeks of age and then falls. This seemed to be inconsistent with the findings of Riley and West (1953) in man, the sow, and the cat: they reported a higher level of tissue histamine in adults than in infants. Their observations were based on determinations in infants aged from 1 to 7 days, and in fully grown members of the various species. It would be possible to present a similar picture for rats by selecting two appropriate points on the curve. The fall in the level of the rats' abdominal skin histamine with advancing age is incidentally apparent in the figures of Geiringer and Hardwick (1953), though they were not concerned with this particular phenomenon.

Summary

1. The subcutaneous injection of 2 mg./100 g. body weight of morphine sulphate caused a significant fall in the ascorbic acid content of rats' adrenals.

2. The same dose of morphine sulphate in rats with demedullated adrenals, and in animals with a depleted tissue histamine, caused a reduction in adrenal ascorbic acid; this reduction was not significantly less than that observed in normal rats.

3. The fall in adrenal ascorbic acid caused by a similar dose of morphine sulphate, in rats with demedullated adrenals plus depleted tissue histamine, was significantly less than that in normal rats.

4. The histamine content of the abdominal skin in Wistar rats was shown to increase between birth and six weeks of age; during the subsequent six months it diminished to a level considerably below that at birth.

My sincere thanks are due to Dr. K. H. Coward for advice on the statistical treatment of the results, and to Dr. H. C. Stewart for his interest and encouragement. I am grateful to Dr. A. C. White of the Wellcome Research Laboratories for a generous supply of compound 48/80; to Mr. M. Wood for technical assistance; and to the Sir Halley Stewart Trust for continued financial support.

References

Binet, L. (1941). *C.R. Soc. Biol., Paris*, **135**, 1197.
Dale, H. H. (1920). *Brit. J. exp. Path.*, **1**, 103.
Elliott, T. R. (1912). *J. Physiol.*, **44**, 374.
Emmelin, N., and Strömblad, R. (1951). *Acta physiol. scand.*, **24**, 261.
Evans, G. (1936). *Amer. J. Physiol.*, **114**, 297.

Evans, A. G. J., Nasmyth, P. A., and Stewart, H. C. (1952). *Brit. J. Pharmacol.*, **7**, 542.
Feldberg, W., and Paton, W. D. M. (1950). *J. Physiol.*, **111**, 19*P*.
—— —— (1951). Ibid., **114**, 490
—— and Talesnik, J. (1953). Ibid., **120**, 550.
Geiringer, E., and Hardwick, D. C. (1953). Ibid., **119**, 410.
Gottesman, J. M., and Gottesman, J. (1928). *J. exp. Med.*, **47**, 503.
Halpern, B. N., and Benos, S. A. (1952). *Bull. Acad. suisse Sci. med.*, **8**, 110.
Jarrett, I. G. (1951). *Brit. J. Pharmacol.*, **6**, 294.
Kellaway, C. H., and Cowell, S. J. (1922). *J. Physiol.*, **56**, 20.
Lewis, J. T. (1923). *Amer. J. Physiol.*, **64**, 506.
Long, C. N. H., and Fry, E. G. (1945). *Proc. Soc. exp. exp. Biol., N.Y.*, **59**, 67.
Nasmyth, P. A. (1948). Ph.D. Thesis, University of London.
—— (1950). *J. Physiol.*, **110**, 294.
—— (1951). Ibid., **112**, 215.
—— and Stewart, H. C. (1950). Ibid., **111**, 19*P*.
Porter, R. W. (1952). *Fed. Proc.*, **11**, 124.
Riley, J. F., and West, G. B. (1952). *J. Physiol.*, **117**, 72*P*.
—— —— (1953). Ibid., **120**, 528.
Roe, J. H., and Kuether, C. A. (1943). *J. biol. Chem.*, **147**, 399.
Sayers, G., and Sayers, M. (1947). *Endocrinology*, **40**, 265.
Vogt, M. (1944). *J. Physiol.*, **103**, 317.