Brit. J. Pharmacol. (1955), **10**, 51.

HISTAMINE RELEASE AND THE "STRESS" PHENOMENON

BY

P. A. NASMYTH

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

(RECEIVED SEPTEMBER 3, 1954)

Various authors have shown that histamine causes depletion of the rat's adrenal ascorbic acid (Sayers and Sayers, 1947; Nasmyth, 1951; and Halpern and Benos, 1952), and in 1948 Ellinger suggested that released histamine might be a mediator of the "stress" phenomenon. Attempts to test this possibility (Tepperman, Rakieten, Birnie, and Diermeier, 1951) showed that both phenoxadrine and tripelennamine reduced the effect of histamine on the rat adrenal cortex, but not that of intraperitoneal carbon tetrachloride. They considered that they had no evidence either for or against the possibility that released histamine is concerned in the stress phenomenon. More recently, Nasmyth (1954) examined the effect of morphine, which produces the stress phenomenon, on the rat's adrenal, and concluded that, though both the adrenaline and the histamine which the drug releases affected the phenomenon, they were not wholly responsible for it.

These experiments were not conclusive, and it was felt that some useful information might be gained from an investigation of the adrenal response to a more potent histamine liberator than morphine, namely, compound 48/80, using depletion of the adrenal ascorbic acid as an index of cortical activity.

METHODS

Except that only Wistar strain rats from Dr. Mandl's colony were used, and that 48/80 (0.5 mg./100 g. body wt.) replaced morphine, the methods (for the administration of drugs, the extraction of the adrenal glands, the extraction and estimation of skin histamine, the demedullation of the adrenals, and the depletion of skin histamine) were as described in a previous paper (Nasmyth, 1954).

Blood-pressure records were taken from the carotid artery of heparinized animals anaesthetized with urethane (7 ml./kg. of a 25% w/v solution), and were recorded by a Hg manometer similar to that of Condon (1951).

RESULTS

Effect of 48/80 in Normal Rats.—The subcutaneous injection of 0.5 mg./100 g. body weight of 48/80 in normal rats caused prostration, cyanosis, oedema of the paws and snout, and itching. These signs persisted for 2–3 hr. and were accompanied by copious water drinking. Subsequently they lessened, and after 5 hr. the behaviour of the test animals was hardly distinguishable from that of the controls.

The changes in the adrenal content of ascorbic acid during the experiment reflected these events. Control animals, given a subcutaneous injection of normal saline, had an average adrenal ascorbic acid content of 330 mg./100 g. gland. One and a half hr. after the injection of 48/80 the content was 182 mg./100 g., or 55% of normal. It remained low, at 184 mg./100 g., 3 hr. after the 48/80; but after 5 hr. the recovery process had begun, and the mean value was 235 mg./100 g., or 71% of normal (Table I).

A substantial amount of histamine was released from the tissues of the test animals, as indicated by the mean values for the abdominal skin histamine. The average figure for the abdominal skin histamine of saline-injected controls was 58.1 μg./g. skin.* One and a half hr. after the injection of 48/80 the value was 22.9 μg./g. Three and five hours after the dose the figures were 19.2 μg./g. and 19.3 μg./g. respectively (Table I).

Effect of 48/80 in Histamine-depleted Rats.—An attempt was made to deplete the tissue histamine with propamidine, in doses of 0.4 to 2.0 mg., using a technique similar to that described for 48/80 (Nasmyth, 1954). As these doses did not cause any significant depletion of the skin histamine, and higher doses killed the animals, the

* This high value was obtained in animals which were used just after weaning. It originally gave some concern until Hardwick (1954) showed that at weaning there is a considerable but transient rise in the rat's skin histamine.

TABLE I

THE EFFECT OF COMPOUND 48/80 (0·5 MG./100 G.) ON THE ADRENAL ASCORBIC ACID OF RATS (CONTROL ANIMALS WERE GIVEN 0·9% SALINE, 0·25 ML./100 G.)

Rats	Drug	Duration of Action (Hr.)	Ascorbic Acid Content (mg./100 g Gland ± S.E.)	Ascorbic Acid (% Normal)	No. of Glands	Abdominal Skin Histamine (μg. Base/g. Skin ± S.D.)
Normal	Saline	1 to 3	330 ± 7	100	18	58·1 ± 16
	48/80	1·5	182 ± 5	55	18	22·9 ± 7·6
	,,	3·0	184 ± 8	56	15	19·2 ± 3·9
	,,	5·0	235 ± 13	71	18	19·3 ± 4·9
Histamine-depleted	Saline	1 to 3	411 ± 9	100	18	7·5 ± 2·5
	48/80	1·5	206 ± 11	50	18	5·9 ± 3·5
	,,	3·0	251 ± 12	61	18	7·5 ± 2·7
	,,	5·0	315 ± 19	77	14	7·0 ± 1·3
Demedullated (36–56 days)	Saline	1 to 3	276 ± 13	100	8	26·4 ± 9·3
	48/80	1·5	167 ± 8	60	9	19·2 ± 7·3
	,,	3·0	160 ± 14	58	8	13·3 ± 5·6
	,,	5·0	148 ± 10	54	6	15·8 ± 17·0
Demedullated (6 months)	Saline	1 to 3	369 ± 15	100	9	21·1 ± 5·6
	48/80	1·5	244 ± 12	66	8	12·3 ± 3·3
	,,	3·0	221 ± 20	60	8	13·4 ± 3·4
	,,	5·0	261 ± 24	71	9	15·5 ± 7·0
Rats given 48/80 pretreated with adrenaline mucate (1 μg./g.).	Saline	1 to 3	279 ± 16	100	6	—
	48/80	1·5	130 ± 8	48	6	—
	,,	3·0	126 ± 12	45	4	—
	,,	5·0	194 ± 13	70	6	—

tissue histamine was depleted with 48/80. The skin histamine was never reduced to undetectable amounts, but it was diminished to about 12% of normal (Table I).

The subcutaneous injection of 0.5 mg./100 g. body weight of 48/80 in these histamine-depleted animals produced none of the signs seen in normal animals. There was no further significant reduction of the already low histamine value of the skin. The pattern, but not the magnitude, of the adrenal ascorbic acid response was different. One and a half hr. after 48/80 the adrenal ascorbic acid had fallen from 411 mg./100 g. gland to 206 mg./100 g., or 50% of normal. In contrast with the effect in normal animals, the adrenal ascorbic acid level in histamine-depleted rats began to return towards the control level 3 hr. after 48/80. The average value for the adrenal ascorbic acid at this time was 251 mg./100 g. of gland, or 61% of normal. After 5 hr. the ascorbic acid level was 315 mg./100 g., or 77% of the control value (Table I).

Effect of 48/80 in Rats with Demedullated Adrenal Glands.—The animals were taken for experiment between 36 and 56 days after demedullation. In some rats only one gland had regenerated; in a few, one of the glands was very large and the other very small. No glands under 5.0 mg. were used, as they seldom had ascorbic acid levels comparable with those of their larger partners.

In the demedullated animals the test dose of 48/80 produced great prostration, cyanosis, oedema, and itching. The symptoms of distress were more marked than in normal rats, being aggravated by the absence of the adrenal medullary secretion. Twenty per cent. of the animals died from 1½ to 4 hr. after the test dose. In normal rats the signs did not persist to the end of the experiment, but in demedullated animals they were still present 5 hr. after the test dose. The response of the adrenal ascorbic acid differed from that in normal rats, as the level fell continuously throughout the experiment (Table I).

The abdominal skin histamine was depleted, indicating a considerable release of histamine from the tissues. It should be noted, however, that the control level of 26.4 μg. histamine base/g. skin was lower than in normal animals and the depletion was proportionately less (Table I).

Effect of 48/80 in Rats Pretreated with Adrenaline.—The result of the experiment on demedullated rats suggested that the medullary secretion might possibly antagonize the effect of 48/80 on the adrenal ascorbic acid. To test this possibility, 1 μg./g. adrenaline mucate ("Hyperduric Adrenaline") was injected subcutaneously in normal animals immediately before the usual dose of compound 48/80.

As in untreated normal rats there was prostration, cyanosis, oedema, and itching, but the signs were less severe, and they subsided 1½ hr. after the 48/80. Nevertheless the effect on the adrenal ascorbic acid was not reduced by pretreatment with adrenaline. The lowest levels were reached

1½ and 3 hr. after the test dose and were 48% and 45% of the control level respectively. After 5 hr. the level had risen to 70% of the control value (Table I). The figures indicate that pretreatment with adrenaline does not antagonize the effect of 48/80 in causing adrenal cortical activity, but rather serves to reinforce it.

Rats with Demedullated Adrenal Glands and a Low Tissue Histamine.—It was not possible to deplete the tissue histamine of demedullated rats by the procedure described, as they were killed by intraperitoneal injections of 48/80. However, it has been shown that with advancing age the skin histamine of the rat is reduced (Hardwick, 1954; and Nasmyth, 1954). Accordingly, a group of rats was demedullated one week after weaning and then kept for 6 months before being taken for experiment.

In these animals the 48/80 produced only mild oedema, cyanosis, and itching, and sometimes it was difficult to distinguish the effect from that of saline injections. The response of the adrenal ascorbic acid was not significantly different from that in normal animals. One and a half hours after injection of 48/80 the ascorbic acid level had fallen from 369 mg. to 244 mg./100 g. gland, representing 66% of the resting value. After 3 hr. the level was 221 mg./100 g., or 60% of the control value; but after 5 hr. it had begun to recover and stood at 261 mg./100 g., or 71% of the control value (Table I).

In previous work (Nasmyth, 1954) the abdominal skin of 7-month-old rats had been found to contain about 12 μg. of histamine base/g. skin. In the present experiments the tissue histamine of the control animals was not as low (21.1 μg./g. skin) as might have been expected; and was reduced to 12.3 μg./g. 1½ hr. after the injection of 48/80 (Table I).

Effect of 48/80 on the Rat's Blood Pressure.—Though the values for the abdominal skin histamine gave some indication of the activity of 48/80 in releasing histamine from the tissues, it was no guide to the physiological activity of the released histamine. In an attempt to determine this, the effect of intravenous doses of the compound on the rat's blood pressure was investigated.

In three experiments with normal rats the mean blood pressure was 129 mm. Hg. The intravenous injection of 0.5 mg./kg. produced a fall in pressure in about 8 sec. This was followed immediately by a rise in pressure and then by a precipitous and persistent fall. The lowest point was always reached about 15 min. after the injection. During the following 30 min. the pressure rose a little and then gradually fell again during 1½ hr. observation. The pressure did not rise again, but it was not possible to say whether this was caused by the drug or by deterioration of the preparation. The average maximal fall in pressure in the three experiments was 82.5 mm. Hg and was reached about 15 min. after the injection of the drug. A typical tracing is shown in Fig. 1 (A, B, and C).

In 3 experiments with histamine-depleted rats the pattern of events was similar, but the fall in blood pressure was less precipitous and less extensive (Fig. 1D). The mean initial pressure was 117 mm. Hg, and the average maximal fall was 31.6 mm. Hg. One hour after the injection of 48/80 the pressure began to fall

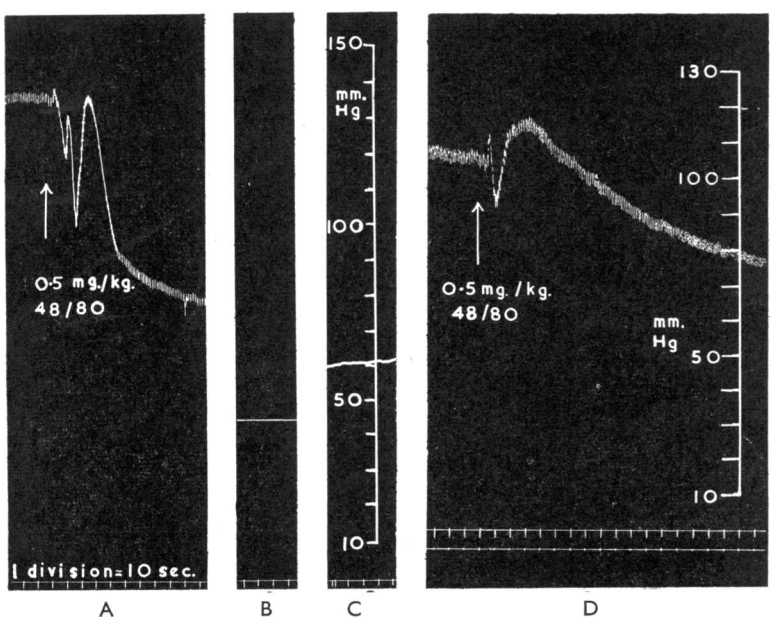

FIG. 1.—Effect of intravenous 48/80 on the arterial blood pressure of rats anaesthetized with urethane. A, B and C, normal rat; B, 15 min., and C, 47 min. after injection. D, typical response to 48/80 in histamine-depleted rat.

steadily, and, again, it was not possible to say whether it was due to the drug or to deterioration of the preparation.

In three rats which had been demedullated for 6 months the average blood pressure was 102 mm. Hg and the pulse pressure was smaller than in normal and histamine-depleted rats. After an intravenous injection of 0.5 mg./kg. of 48/80 the blood-pressure response followed a similar pattern to that seen in the other two groups, but the fall was more precipitous and death invariably occurred between 20 and 30 min. after the dose. A typical record is shown in Fig. 2.

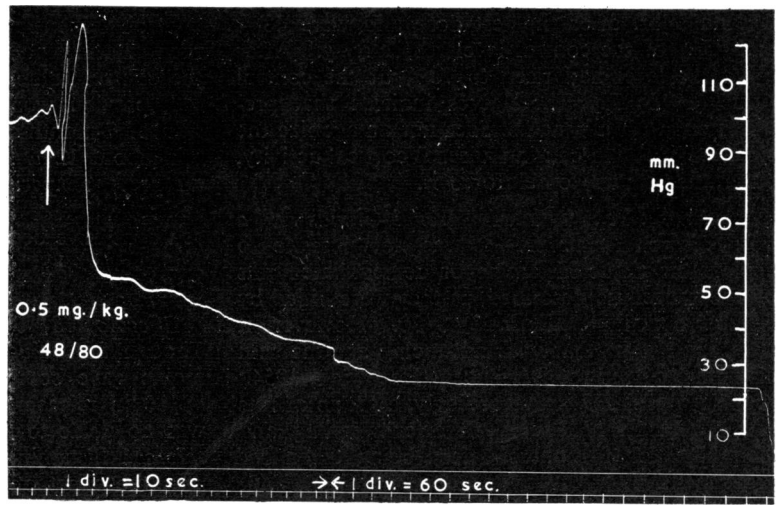

FIG. 2.—Effect of intravenous 48/80 on the arterial blood pressure of rat with adrenal glands demedullated 6 months previously. Urethane anaesthesia. The abrupt fall in pressure 25 min. after the injection (extreme right-hand end of record) indicates death.

DISCUSSION

The present experiments show that the activity of the adrenal cortex of the rat in response to an injection of the potent histamine liberator, compound 48/80, is modified when the tissue histamine has been depleted. The modification consists of a less prolonged reduction of the adrenal ascorbic acid, which could be explained on the assumption that the "substance" producing the "stress" is reduced. This assumption is based on the observation by Hodges (1953) that in rats a subcutaneous injection of 20 μg./100 g. body weight of adrenaline produced a fall in the adrenal ascorbic acid which was as great as, but less prolonged than, that caused by 40 μg. In the present instance, the dose of 48/80 was the same in normal and histamine-depleted animals, and, since the effect of the drug on the rat's blood pressure was less in histamine-depleted rats than in normal, it seems likely that the reduced effect on the adrenal ascorbic acid was due to a reduced release of histamine into the circulation. The difference in the depressor action of 48/80 in the normal and the histamine-depleted rat is certainly due to the fact that in the depleted rat less histamine is released. It is, however, by no means certain that the reduced depressor action which remains in the depleted rat is at all caused by histamine release. However, Brocklehurst (personal communication, 1954) has shown that the local area of "blueing" produced at the site of a subcutaneous injection of 48/80 when Pontamine Sky Blue is injected intravenously is less in histamine-depleted than in normal animals, but is, nevertheless, still present. It seems likely, therefore, that at least some of the remaining effect of 48/80 on the blood pressure of the depleted rat is due to released histamine. Release of histamine, therefore, may well play some part in the effect of 48/80 on the adrenal cortex in the histamine-depleted rat, but it is not possible to say how important this release is for the reduction of ascorbic acid in these animals.

The difference between the effects of 48/80 on the ascorbic acid content of the adrenal cortex of normal and histamine-depleted rats is small, probably because histamine release is not eliminated. It is therefore unlikely that it will be possible to show whether or not released histamine plays a part in "non-specific stress" until some way of completely eliminating the release of tissue histamine is available.

The results obtained in animals with demedullated adrenal glands show that a subcutaneous dose of 0.5 mg./100 g. body weight of 48/80 is more toxic to rats with adrenal glands demedullated 35–56 days previously than it is in normal animals. On the other hand, if the animals are kept for 6 months after demedullation, the response of the adrenal cortex to 48/80 does not differ significantly from that in normal animals. Since adrenaline does not appear to antagonize the effect of 48/80, it seems likely that the more toxic effect of 48/80 in the rats demedullated 36–56 days previously is a sign of inadequate regeneration of cortical tissue, although this interval has been regarded by various authors using different forms of stress (Vogt, 1952; Hodges,

1953; Nasmyth, 1951, 1954) as being adequate. The stresses employed in these previous experiments, however, were probably less severe than the stress used in the present work, and this may account for the fact that no signs of inadequate regeneration were observed. There is also the possibility that the difference in age between the two groups of animals has affected the result.

Summary

1. The subcutaneous injection of 0.5 mg./100 g. body weight of 48/80 causes a profound fall in the ascorbic acid content of rats' adrenals.

2. In histamine-depleted rats 48/80 causes a less prolonged fall in the adrenal ascorbic acid than in normal animals.

3. In rats with demedullated adrenals 48/80 causes a more prolonged effect on the adrenal ascorbic acid content when given 36–56 days after the operation. When given six months after the operation, the effect of 48/80 is not significantly different from that in normal animals. This suggests that a period of 36–56 days is insufficient for adequate regeneration of the cortical tissue in demedullated rats.

4. The effect of 48/80 on the blood pressure of normal, demedullated, and histamine-depleted rats is described.

5. It is concluded that released histamine plays some part in the effect of 48/80 on the adrenal cortex.

My sincere thanks are due to Dr. H. C. Stewart for his interest and encouragement, and to Miss B. Morris for valuable technical assistance. I am grateful to Dr. A. C. White, of the Wellcome Research Laboratories, for a generous supply of compound 48/80, and to the Sir Halley Stewart Trust for continued financial support.

References

Condon, N. E. (1951). *Brit. J. Pharmacol.*, **6**, 19.
Ellinger, F. (1948). *Radiology*, **51**, 394.
Evans, G. (1936). *Amer. J. Physiol.*, **114**, 297.
Feldberg, W., and Talesnik, J. (1953). *J. Physiol.*, **120**, 550.
Halpern, B. N., and Benos, S. A. (1952). *Bull. Acad. Suisse Sci. Med.*, **8**, 110.
Hardwick, D. C. (1954). *J. Physiol.*, **124**, 157.
Hodges, J. R. (1953). *J. Endocrinol.*, **9**, 342.
Jarrett, I. G. (1951). *Brit. J. Pharmacol.*, **6**, 294.
Nasmyth, P. A. (1951). *J. Physiol.*, **112**, 215.
—— (1954). *Brit. J. Pharmacol.*, **9**, 95.
Roe, J. H., and Kuether, C. A. (1943). *J. biol. Chem.*, **147**, 399.
Sayers, G., and Sayers, M. (1947). *Endocrinology*, **40**, 265.
Tepperman, J., Rakieten, N., Birnie, J. H., and Diermeier, H. F. (1951). *J. Pharmacol.*, **101**, 144.
Vogt, M. (1952). *J. Physiol.*, **118**, 588.