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THE EFFECTS OF HISTAMINE AND ANTIHISTAMINES ON THE ASCORBIC ACID CONTENT OF RAT'S ADRENAL GLANDS

By P. A. NASMYTH

From the Pharmacological Laboratory of the School of Pharmacy, University of London

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An observation indicating a relationship between the effect of histamine and the activity of the adrenal cortex was made by Gottesman & Gottesman in 1928. They showed that the adrenalectomized animal was twenty times more sensitive to the drug than the normal. In 1947, direct evidence for this relationship was produced by Sayers & Sayers, who demonstrated that histamine was capable of reducing the adrenal content of ascorbic acid.

In 1945, Hoskins & Long immersed rats in water at 70° C. for 5 sec. at intervals during a period of 12 hr., and observed a fall in the cholesterol content of the adrenal glands, which was less marked, if cortical extract or alcoholic saline solution had been injected earlier. This finding suggests that burning increases the activity of the adrenal cortex. Dekanski (1945) noted that immersion of mice in water at 60° C. increased the total histamine content of the body, and the same author (1947) also recorded an increase in the amount of extractable histamine in the skin of anaesthetized cats subjected to the same treatment. It is, therefore, possible that the changes in the adrenal cortex might be due to the action of histamine released from the burnt skin.

In these circumstances it seemed possible that a more detailed investigation of the effect of histamine on the adrenal cortex might prove to be profitable. This has been done by using the fluctuation in the ascorbic acid content of the gland as a guide to the activity of the cortex. The evidence in support of such a procedure has been summarized in an earlier paper (Nasmyth, 1949).

METHODS

Administration of drugs. Litter-mate rats of the Wistar strain were used from Dr K. H. Coward's colony. A few animals were of the Hooded strain, and these were very kindly presented by Dr S. K. Kon from his colony at the National Institute of Dairying at Shinfield near Reading. Since Rogoff & Stewart (1928) have shown that oestrus interferes with cortical hormone requirements, buck rats only were used. They all received the full stock diet used in Dr Coward's colony and were not deprived of food or water at any time during the experiment. Litters having at least four bucks were chosen, and two or more animals from each litter were used as controls. The test animals received a subcutaneous injection of 15 mg. of histamine acid phosphate per 100 g. body weight,

and were killed at intervals of 1½, 3 and 5 hr. respectively. The controls were similarly injected with an equal volume of normal saline and were killed at times between those chosen for the test animals.

The method of removing and extracting the adrenal glands has been described in an earlier paper (Nasmyth, 1949). The ascorbic acid was estimated by the method of Roe & Kuether (1943).

Demedullation of the adrenal glands. Litter-mate male rats of the Wistar strain were taken at weaning and the adrenal glands were demedullated using the technique described by Evans in 1936.

After operation the animals were kept in a room at 28° C. which was thermostatically controlled. Upon recovery from the anaesthetic, each animal was given a few drops of 2% glucose solution from a teat pipette. They were then maintained on the full stock diet, but received 0·2% saline in place of drinking water. Three to four weeks after operation glands consisting entirely of cortical cells had regenerated from the few cells left adhering to the capsule at the time of operation, and it was then possible to replace the saline with ordinary drinking water.

Some of the glands were taken for sectioning, after fixing in Muller's fluid. The sections were stained with Schmörl's adaptation of Giemsa's stain, described by Carleton & Leach (1938). Examination of the sections showed the glands to consist of cortical cells surrounding a central scar tissue. Very occasionally an island consisting of two or three green-staining chromaffine cells was observed. This was in accordance with the finding of Evans in 1936.

Determination of effective doses of antihistamines in the rat. There was no literature available concerning the effective doses of benadryl and mepyramine maleate (neoantergan) in the rat. An experiment was devised to determine them and the period for which the drugs would be active.

Large rats of the Wistar strain weighting 245 g. or more were anaesthetized with urethane, and the carotid artery and jugular vein cannulated. Heparin, 1 mg./100 g. body weight, was injected into the jugular vein. Carotid arterial pressure was then recorded. Doses of 0·009 mg. of histamine acid phosphate were injected into the jugular vein and the resultant falls in blood pressure recorded. Following this, the antihistamine under test was injected subcutaneously under the skin covering the chest, where it was calculated that the respiratory movements would render absorption comparable with that in conscious animals. Further intravenous injections of 0·009 mg. of histamine acid phosphate were given every 10 min. until the fall of blood pressure was abolished. Subsequently, injections of histamine were given every 30 min. in order to determine the period during which the antihistamine under test was effective.

RESULTS

Normal rats. One and a half hours after a subcutaneous injection of histamine acid phosphate (15 mg./100 g. body weight) there was a fall in the ascorbic acid content of the adrenal glands to 62·5% of the resting level. After 3 hr. the level was still low at 65%, but after 5 hr. it had risen to 82·5% of the resting value. Details of the results are given in Table 1 and they are depicted graphically in Fig. 4.

Rats pre-treated with antihistamine. Having established the fact that histamine was effective in reducing the ascorbic acid content of the adrenal glands, experiments were performed to determine whether or not the effect could be blocked with either mepyramine or benadryl.

In every experiment for determining the effective dose of the antihistamines the animal either died or the recording of the blood pressure became unreliable before the effect of the drug under test had disappeared. However, the period of effectiveness was shown to be at least long enough to cover the time range usually employed in the experiments demonstrating the effect of histamine on the ascorbic acid content of the adrenal glands.

Reference to Fig. 1 shows that a subcutaneous injection of 20 mg. of benadryl/100 g. body weight was effective in abolishing the fall of blood pressure

caused by an intravenous injection of 0·009 mg. of histamine acid phosphate in 30 min., and that the effect persisted for at least 3 hr.

TABLE 1. The effect of histamine acid phosphate on the ascorbic acid content of the adrenal glands of rats in various conditions

Condition	No. of estimations	Histamine dose/100 g. body weight (mg.)	Time interval (injection to sacrifice) (hr.)	Vit. C in mg./100 g. gland	Vit. C % of normal
Normal	12	—	—	387±93	100
	6	15	1½	243±65	62·5
	6	15	3	252±78	65·0
	6	15	5	319±26	82·5
Pre-treated with 20 mg. benadryl/100 g. body weight	8	—	—	318±142	100
	4	15	1½	234±48	73·5
	4	15	3	214±51	67·5
	2	15	5	278·5	87·5
Pre-treated with 5 mg. benadryl/100 g. body weight	6	—	—	449±20	100
	4	15	1½	240±28	53·5
	4	15	3	332±89	74·0
	2	15	4	402	89·5
Pre-treated with 5 mg. mepyramine maleate/ 100 g. body weight	3	—	—	233	100
	2	15	1½	224	96
	2	15	3	193	83
Pre-treated with 0·5 mg. mepyramine maleate/ 100 g. body weight	8	—	—	256±43	100
	4	15	1½	170±15	66·5
	4	15	3	177±17	69·0
Rats having demedullated adrenals	12	—	—	323±59	100
	7	15	1½	221±33	68·5
	6	15	3	217±25	67·5

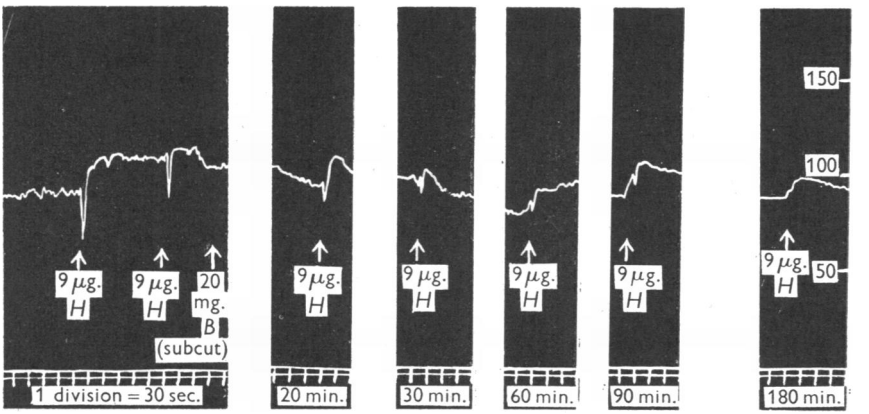

Fig. 1. The effect of a subcutaneous injection of 20 mg./100 g. body weight of benadryl in blocking the fall of blood pressure in the rat caused by an intravenous injection of 9 μg. of histamine acid phosphate. *H* = histamine acid phosphate; *B* = benadryl.

Mepyramine maleate was tried at two dose levels because the dose of 5 mg./100 g. body weight was shown to affect the ascorbic acid content of the adrenals. The results obtained with the 5 mg. dose are depicted in Fig. 2. It will

be observed that this drug was effective much more quickly than was benadryl, the fall in blood pressure due to histamine having been abolished completely in 10 min. The tracing also shows that the drug was effective for at least 3 hr. 20 min. The effects of a dose of 0·5 mg. of mepyramine maleate/100 g. body weight are seen in Fig. 3. Again the drug was effective in 10 min., but in this case the fall in blood pressure due to histamine was not converted into a small

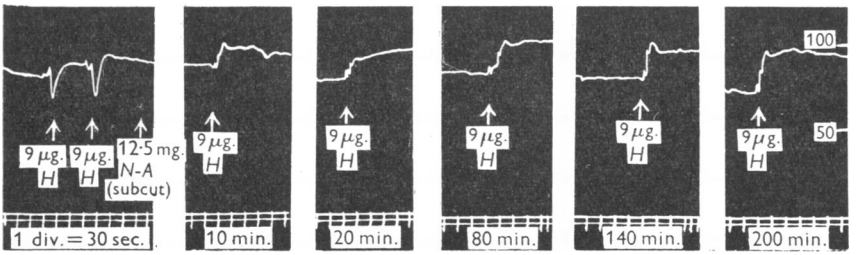

Fig. 2. The effect of a subcutaneous injection of 5 mg./100 g. body weight of mepyramine maleate in blocking the fall in blood pressure in the rat caused by an intravenous injection of 9 μg. histamine acid phosphate. *H* = histamine acid phosphate; *N–A* = neoantergan (mepyramine maleate).

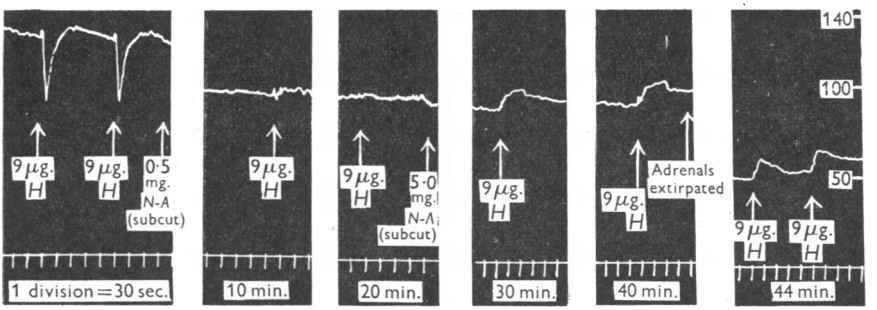

Fig. 3. The difference in the effects produced by subcutaneous injections of 0·5 and 5 mg./100 g. mepyramine maleate in blocking the fall of blood pressure caused by an intravenous injection of 9 μg. histamine acid phosphate. *H* = histamine acid phosphate. *N–A* = neoantergan (mepyramine maleate).

rise as had been the case with the larger dose of mepyramine maleate and with benadryl. Consequently, as a point of interest, the dose of mepyramine maleate was increased after 20 min. to bring it up to the level of 5 mg./100 g. body weight. Ten minutes later another intravenous injection of histamine produced a small rise in blood pressure, which was repeatable after a further 10 min. The abdomen was then opened and the adrenals excised, after clamping the blood supply to both glands with Spencer-Wells forceps. This procedure occupied about 4 min.; histamine was injected twice and a rise in blood pressure resulted in each instance.

Benadryl was the first drug to be used in the attempt to antagonize the effect of histamine on the ascorbic acid content of the adrenals. Every animal in the group, including those to be used as controls, received a subcutaneous injection of 20 mg. of benadryl/100 g. body weight. Half an hour later, the test animals were injected with histamine as before, and the controls were given a subcutaneous injection of normal saline. The test animals were killed at intervals of 1½, 3 and 5 hr. respectively after injection of histamine. At 1½ hr. the level of ascorbic acid in the glands was 73·5% of normal; at 3 hr. 67·5% and at 5 hr. 87·5%. At first the figures seemed to indicate some blocking of the histamine effect, as they were higher than those obtained in the group of animals not pre-treated with benadryl. More careful comparison of the figures showed that the control values obtained for the animals pre-treated with benadryl were lower than those obtained for control animals not so treated. Moreover, the benadryl-treated animals had exhibited marked lethargy during the experiment, and it was thought that the dose of benadryl had been too high and had caused a slight fall in the adrenal ascorbic acid of the controls.

The experiment was repeated using a dose of 5 mg. of benadryl/100 g. body weight. This time the control figures obtained were higher than those for untreated animals. The fall 1½ hr. after the injection of histamine was greater but the effect seemed to be a little more transient.

It would thus appear that benadryl exerts no significant antagonism towards this particular action of histamine.

Detailed results are given in Table 1, and they are depicted graphically in Fig. 4.

The procedure used for benadryl was repeated for mepyramine maleate. A pilot experiment was performed using a dose of 5 mg. mepyramine maleate/100 g. body weight. The control value for the ascorbic acid content of the adrenals obtained in this experiment was 233 mg./100 g. of gland which suggested that the mepyramine maleate itself had reduced it.

With a larger group of animals, an experiment was performed using a dose of 0·5 mg. mepyramine maleate/100 g. body weight. The test animals were killed 1½ and 3 hr. after the injection of histamine, and the values obtained for the adrenal content of ascorbic acid at these times were not significantly different from those obtained in animals receiving no antihistamine. Even with this dose of mepyramine maleate, the control values were low.

Details of the figures obtained appear in Table 1, and those obtained with 0·5 mg. dose of mepyramine maleate are expressed graphically in Fig. 4.

Mepyramine maleate in normal rats. This experiment was performed to determine whether mepyramine maleate alone could reduce the ascorbic acid concentration in the adrenal glands. The procedure employed was the same as that used for the effect of histamine on the adrenal ascorbic acid of normal rats except that, instead of histamine, a subcutaneous injection of 5 mg.

mepyramine maleate/100 g. body weight was given to the test animals. The test animals were killed 1½ or 3 hr. after injection and a fall in the level of ascorbic acid in the adrenal glands was observed. The effect was not as great as that

TABLE 2. The effect of mepyramine on the ascorbic acid content of the adrenal glands of normal rats

No. of estimations	Dose per 100 g. body weight (mg.)	Time interval (injection to sacrifice) (hr.)	Vit. C in mg./100 g. gland	Vit. C % of normal
8	—	—	402±66	100
4	5	1½	328±32	81·5
2	5	3	341	85

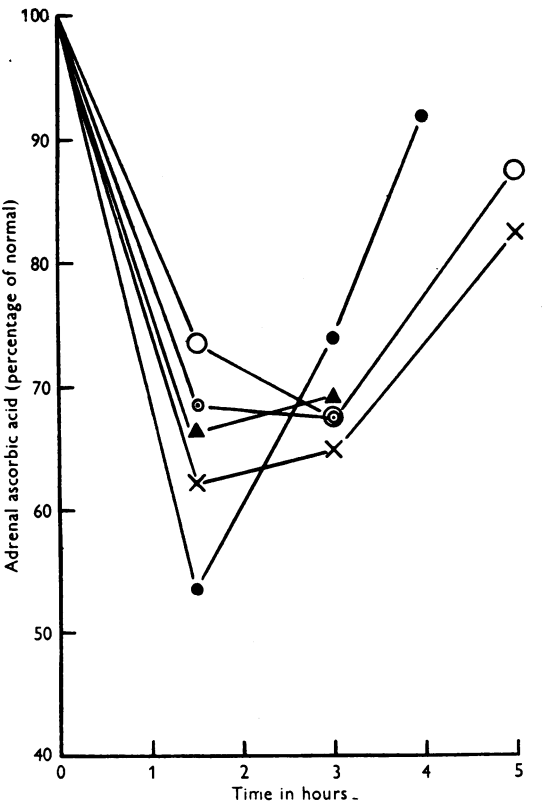

Fig. 4. The effect of a subcutaneous injection of 15 mg./100 g. body weight of histamine acid phosphate on the ascorbic acid content of the rat's adrenal glands in various conditions. ×——×, normal; ○——○, pre-treated with benadryl (20 mg./100 g.); ●——●, pre-treated with benadryl (5 mg./100 g.); ▲——▲, pre-treated with mepyramine maleate (0·5 mg./100 g.); ⊙——⊙ rats with demedullated adrenal glands.

produced by histamine, the level of ascorbic acid in the glands being 81·5% of normal 1½ hr. after injection and 85% after 3 hr. Details of the results obtained are given in Table 2.

Histamine in rats with demedullated adrenals. The procedure adopted for the determination of the effect of histamine in normal rats was repeated in rats having demedullated adrenal glands. The results indicate that the effect on the adrenal ascorbic acid content was not significantly less than it was in normal rats. Details of the results obtained are shown in Table 1 and represented graphically in Fig. 4.

DISCUSSION

During these experiments, it was noticed that rats of the Hooded strain gave higher values for the level of ascorbic acid in the adrenal glands of the controls than did rats of the Wistar strain. The control figure in the group of animals used in the determination of the effect of histamine under normal conditions was 387 ± 93 mg. of ascorbic acid/100 g. of gland. The rather high standard deviation in this case was due to the use of Wistar and Hooded strains in the same group of animals. The controls in the group of animals pre-treated with 20 mg. of benadryl/100 g. body weight showed an average ascorbic acid level in their adrenals of 318 ± 142 mg./100 g. of gland. All the animals in this group were of the Wistar strain, and the only explanation which can be suggested for this very wide standard deviation is that the benadryl had itself reduced the amount of ascorbic acid in the adrenal glands of some of the controls. Controls pre-treated with mepyramine showed consistently low values which were undoubtedly due to the activity of the antihistamine. Considering that the weight of the scar tissue in the demedullated glands must have varied quite considerably, the control values for the ascorbic acid expressed as the content of 100 g. of gland were remarkably consistent.

The work has confirmed, by a more direct method, the experiments of other workers which indicated that histamine caused hyperactivity of the adrenal cortex. It is certain that the effect produced by histamine cannot be blocked by either benadryl or mepyramine; in fact, mepyramine itself causes a reduction in the adrenal content of ascorbic acid.

The mechanism by which histamine activates the adrenal cortex remains obscure. In 1941, Binet showed that histamine causes a release of adrenaline from the adrenal medulla, confirming the suggestion of Dale (1920) and the work of Kellaway & Cowell (1922). They demonstrated that histamine caused dilatation of the pupil which was abolished by adrenalectomy, and argued that it must be due to adrenaline liberated from the adrenal medulla. This might explain the rise of blood pressure which was observed when histamine was injected into the rats pre-treated with subcutaneous injections of benadryl and mepyramine in the foregoing experiments. The failure to abolish the rise when the adrenals were excised may have been due to the serious fall in blood pressure occasioned by opening the abdomen. Since histamine may cause release of adrenaline from the medulla, and since it has been shown by Nasmyth (1949) that 0·02 mg. of adrenaline/100 g. body weight produces a greater fall in the

adrenal ascorbic acid concentration than does 15 mg. of histamine acid phosphate/100 g. body weight, it was clearly possible that the histamine effect might be mediated through the liberation of adrenaline. However, it was in fact found that demedullation did not significantly alter the response to histamine. The possibility of sympathin release at sympathetic nerve endings was not excluded, but there is no evidence that histamine can act directly to cause adrenaline liberation at these endings, and Kellaway & Cowell failed to obtain dilatation of the pupil with histamine in adrenalectomized animals, which suggested that the drug does not stimulate the sympathetic system.

Further experiments are in progress to show whether histamine is active in hypophysectomized animals.

SUMMARY

1. A subcutaneous injection of 15 mg. of histamine acid phosphate/100 g. body weight caused a marked fall in the adrenal content of ascorbic acid.

2. The doses of benadryl and mepyramine required to antagonize the hypotensive action of histamine in the rat were determined and the time course of the effectiveness of these drugs was measured.

3. Doses of benadryl and mepyramine, previously shown to be effective in preventing the hypotensive action of histamine in the rat, were ineffective in antagonizing the effect of histamine acid phosphate on the adrenal content of ascorbic acid.

4. A subcutaneous injection of 5 mg. of mepyramine maleate/100 g. body weight caused a reduction in the adrenal content of ascorbic acid.

5. Demedullation of the adrenals does not markedly affect the fall in adrenal ascorbic acid caused by histamine.

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