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THE EFFECT OF CHLORPROMAZINE ON ADRENOCORTICAL ACTIVITY IN STRESS

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Avon, Chambon, and Voisin (1953) claimed that doses of 10 to 50 mg./kg. of chlorpromazine blocked the effects of surgical stress and of formalin injections on the rat's adrenal ascorbic acid. Holzbauer and Vogt (1954), on the other hand, found that the release of ACTH caused by surgical stress or by injection of adrenaline was not blocked by doses of 10 mg./kg. of chlorpromazine. They also showed that this dose of chlorpromazine alone caused some release of ACTH. For this reason they allowed 3 hr. to elapse between injecting the chlorpromazine and applying the stress. Accordingly, it seemed to be of interest to determine whether or not smaller doses of chlorpromazine, which failed to release ACTH, would block the effects of stress on the adrenal cortex, since a shorter time between giving the drug and applying the stress could then be used without obscuring the issue.

METHODS

Animals.—Litter-mate buck rats of the Wistar strain were used. They were kept on a diet of cubes, cabbage, and water in a room thermostatically controlled at 70° F. Neither food nor water was withheld at any time during the experiments.

Drugs.—All drug solutions were freshly made in normal saline and were injected subcutaneously unless otherwise stated. The doses of histamine are in terms of the hydrochloride, and those of adrenaline in terms of the *laevo*-rotatory base.

Surgical Stress.—The animals were anaesthetized with ether, and a mock adrenalectomy was performed through a midline incision in the skin of the back. The wound was sutured and the animals were allowed to recover.

Demedullation of Adrenal Glands.—Adrenal glands were demedullated by the technique of Evans (1936). The demedullated rats were used between 75 and 90 days after operation.

Extraction of Adrenal Glands.—All animals were killed by a blow on the back of the head 1½ hr. after applying the stress. The adrenals were removed, weighed, and extracted separately as described by Nasmyth (1954). The ascorbic acid content of the extracts was estimated by the technique of Roe and Kuether (1943).

Record of Rat's Blood Pressure.—Animals were anaesthetized with 7 ml./kg. of 25% urethane solution given subcutaneously. Pressure was recorded from the carotid artery using the manometer described by Condon (1951). Heparin was introduced into the arterial cannula to prevent clotting. Doses of histamine were injected into the cannulated jugular vein; chlorpromazine and mepyramine maleate were given subcutaneously on the chest.

RESULTS

Chlorpromazine Effect.—Within a few minutes of the subcutaneous injection of 10 mg./kg. of chlorpromazine, the animals became lethargic. One and a half hours after the injection the adrenal ascorbic acid content had fallen to 68.4% of its normal level. When 2.5 mg./kg. of chlorpromazine was injected, followed by 1 ml./kg. of normal saline 30 min. later, the same signs of lethargy were seen. One and a half hours after the dose of saline the rats were killed; their ascorbic acid content was not significantly different from that of rats given saline alone. These observations (Table I) established the fact that the higher dose of chlorpromazine caused a release of ACTH, which was evident 1½ hr. after injection, whereas the lower dose did not. Accordingly, a dose of 2.5 mg./kg. of chlorpromazine was used to assess the effect of the drug on the release of ACTH caused by various forms of stress.

Adrenaline Effect.—Two doses of adrenaline were used, which were calculated to produce submaximal effects on the adrenal ascorbic acid. Injection of 50 μg./kg. of adrenaline caused a fall in adrenal ascorbic acid to 62.7% of the control after 1½ hr. The smaller dose of 25 μg./kg. caused a fall of 91% which was significant ($P<0.05$). When the animals were given chlorpromazine 30 min. before these doses of adrenaline, bigger falls in the

TABLE I

THE EFFECT OF VARIOUS STRESSES ON THE ASCORBIC ACID CONTENT OF RATS' ADRENAL GLANDS

	Chlorpromazine or Mepyramine 2·5 mg./kg. 30 min. before Stress	Stress	Duration of Stress (hr.)	Ascorbic Acid Content mg./100 g. Gland ± S.E.	Ascorbic Acid as % Normal	No. of Rats
Normal ..	Nil	1 ml./kg. 0·9% NaCl	1½	425 ± 9·3	100	18
	,,	10 mg./kg. chlorpromazine	1½	291 ± 29·0	68·4	3
	,,	10 ,, histamine	1½	276 ± 17·3	64·9	3
	,,	100 ,, ,,	1½	199 ± 3·9	46·8	4
	,,	50 μg./kg. adrenaline	1½	267 ± 6·9	62·7	3
	,,	25 ,, ,,	1½	387 ± 16·1	91·0	3
	,,	Surgical	1½	175 ± 16·4	41·2	4
	Chlorpromazine	1 ml./kg. 0·9% NaCl	1½	427 ± 8·2	100	16
	,,	10 mg./kg. histamine	1½	372 ± 24·1	87·5	6
	,,	100 ,, ,,	1½	210 ± 10·6	49·3	4
	,,	50 μg./kg. adrenaline	1½	213 ± 6·6	50·2	3
	,,	25 ,, ,,	1½	337 ± 19·7	79·3	3
	,,	Surgical	1½	168 ± 8·0	39·5	5
	Nil	2·5 mg./kg. mepyramine	1½	342 ± 8·8	80·6	3
	Mepyramine	10 ,, histamine	1½	341 ± 7·6	80·3	3
Demedullated	Nil	1 ml./kg. 0·9% NaCl	1½	298 ± 9·7	100	7
	,,	100 mg./kg. histamine	1½	152 ± 2·5	51	6
	,,	Surgical	1½	222 ± 6·3	74·5	4
	Chlorpromazine	1 ml./kg. 0·9% NaCl	1½	313 ± 7·6	100	4
	,,	100 mg./kg. histamine	1½	212 ± 5·7	67·7	5
	,,	Surgical	1½	218 ± 18·0	73·5	3

adrenal ascorbic acid were recorded (Table I). Only the difference between the falls due to the larger dose when given alone and when given after chlorpromazine was significant ($P<0.01$). These observations showed that chlorpromazine reinforced the release of ACTH caused by small doses of adrenaline.

Histamine Effect.—The subcutaneous injection of 10 mg./kg. of histamine produced no obvious signs, but 1½ hr. after the dose the adrenal ascorbic acid had fallen to 64.9% of the control. Ten times the dose of histamine caused prostration and a fall in the adrenal ascorbic acid to 46.8% of the control. When chlorpromazine was injected 30 min. before the histamine, the release of ACTH caused by the smaller dose was inhibited ($P<0.01$), but that caused by the larger dose was not significantly affected.

Since chlorpromazine did not block the release of ACTH caused by the smallest dose of adrenaline, the difference between its effects on large and small doses of histamine could be explained by supposing that the large dose, but not the small one, caused a substantial release of adrenaline from the adrenal medulla. The experiments with the large dose of histamine were therefore repeated in rats with demedullated adrenal glands.

One and a half hours after a dose of 100 mg./kg. of histamine in these animals, the ascorbic acid level of the adrenal glands had fallen to 51% of the control value. When the demedullated rats were given chlorpromazine before the dose of histamine, the fall in the adrenal ascorbic acid was inhibited. The difference between the two groups was significant ($P<0.01$). The results are given in Table I.

Surgical Stress.—Mock adrenalectomy under ether anaesthesia caused a profound fall in the adrenal ascorbic acid. One and a half hours after completion of the operation the glands contained only 41.2% of the control amount. The injection of chlorpromazine, 30 min. before performing the operation, did not alter the adrenal response (Table I).

As the operation was performed under ether, there was probably a considerable release of adrenaline from the adrenal medulla. For this reason the experiment was repeated in rats with demedullated adrenal glands. In these animals the response of the adrenal ascorbic acid was less than in normal animals, and it was unaffected by the prior injection of chlorpromazine. Without chlorpromazine the ascorbic acid level fell to 74.5% of the control 1½ hr. after the operation; with chlorpromazine it fell to 73.5%. These results (Table I) indicate that the release of medullary adrenaline caused by this procedure contributes to the release of ACTH from the pituitary gland. Furthermore, response to the stress which remains in the absence of medullary secretion is unaffected by chlorpromazine.

The Effect of Mepyramine Maleate on Histamine Stress.—It seemed possible that the ability of chlorpromazine to inhibit the release of ACTH caused by small doses of histamine in normal rats, and that caused by large doses in demedullated rats,

might be due to its antihistamine activity. The effect of a similar dose of mepyramine maleate given 30 min. before the small dose of histamine in normal rats was therefore determined. The results shown in Table I indicate clearly that mepyramine is effective in inhibiting the release of ACTH caused by small doses of histamine, though the mepyramine itself seems to have produced some fall in the adrenal ascorbic acid. The fact that 2.5 mg./kg. of mepyramine maleate, given subcutaneously, causes some fall in the adrenal ascorbic acid obscures to some extent the degree to which it is capable of inhibiting the release of ACTH by histamine, since two stresses are not simply additive.

Comparison of the Antihistaminic Activities of Chlorpromazine and Mepyramine on the Rat's Blood Pressure.—Since it was suspected that the effect of chlorpromazine was due to its activity as an antihistamine, it was of some interest to make an assessment of this property in the rat. The rat is well known to be insensitive to histamine, but the blood pressure usually gives fairly constant responses to repeated doses, and the assessment was accordingly made using this phenomenon. The procedure adopted was to find the smallest dose of histamine which would just cause a fall in blood pressure, then to re-determine the dose 30 min. after the subcutaneous injection of 2.5 mg./kg. of either mepyramine or chlorpromazine. In eight such experiments the average ratio of antihistamine activity of mepyramine and chlorpromazine was 7:1 respectively.

Discussion

It has been shown that chlorpromazine does not reduce the release of ACTH in surgical stress or in the stress caused by small doses of adrenaline, which confirms the observation of Holzbauer and Vogt (1954). Chlorpromazine did, however, inhibit the response of the adrenal ascorbic acid to small doses of histamine, but the response to large doses of histamine was only inhibited in animals with demedullated adrenal glands. The response to a large dose of histamine or to surgical stress was less in adrenal-demedullated than in normal animals, suggesting that medullary adrenaline normally contributes to the release of ACTH in both these forms of stress. The effect in adrenal-demedullated animals could be inhibited by chlorpromazine if the stressing agent was histamine, but not if it was trauma. Since the possibility of secretion from the adrenal medulla had been eliminated in these animals, it is probable that the observed response of the adrenal ascorbic acid in each type of stress was mediated by the hypothalamus. The action of chlorpromazine in inhibiting the effect of large doses of histamine in adrenal-demedullated rats could not, therefore, be attributed to any effect that it may have on the hypothalamus, since any action in that site must also have affected the response to surgical stress.

The question then arises, by what mechanism does chlorpromazine inhibit the release of ACTH by histamine ? It seems most likely to do so by blocking the peripheral effects of histamine. This view is supported by the experiments on the response of the rat's blood pressure to histamine before and after doses of chlorpromazine and mepyramine, which indicate that chlorpromazine has a considerable antihistamine effect. Furthermore, the more potent antihistamine, mepyramine maleate, was shown to produce a more complete inhibition of the release of ACTH by histamine—which is in accordance with the results of Halpern and Benos (1952).

The observation that chlorpromazine significantly increased the fall in adrenal ascorbic acid caused by 50 μg./kg. adrenaline is interesting. The mean fall caused by 25 μg./kg. of adrenaline was similarly increased, but not significantly, since the standard error of the mean was large and reflected the fact that this dose was uncertain in its effect on the pituitary. Such an increase in the effect of adrenaline on the adrenal ascorbic acid was not observed by Vogt and Holzbauer. However, they used 200 μg./kg. of adrenaline, which would produce a nearly maximal effect. The explanation of the phenomenon is not obvious, but may simply be an example of synergism.

Summary

1. The subcutaneous injection of 10 mg./kg. of chlorpromazine to rats reduced the adrenal ascorbic acid. No reduction was seen when a dose of 2.5 mg./kg. was given.

2. The injection of 2.5 mg./kg. of chlorpromazine did not block the release of ACTH caused by adrenaline, by surgical stress, or by large doses of histamine in the normal rat. The effect of a small dose of histamine was inhibited.

3. The release of ACTH by large doses of histamine and by surgical stress was reduced in demedullated rats. The remaining release of ACTH in these animals was inhibited by chlorpromazine when the stressing agent was histamine, but not when it was trauma.

4. Chlorpromazine was shown to be 7 times less active than mepyramine in blocking the effects of histamine on the rat's blood pressure.

5. The subcutaneous injection of 2.5 mg./kg. of mepyramine maleate caused a small fall in the adrenal ascorbic acid. Histamine, 10 mg./kg., injected subsequently, did not add to the fall caused by the mepyramine alone.

6. It is concluded that the role of the hypothalamus and that of medullary adrenaline in the release of ACTH is not directly affected by chlorpromazine. Chlorpromazine blocks the action of histamine by a peripheral antihistamine effect.

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