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EFFECT OF CHLORPROMAZINE, RESERPINE, BENACTYZINE AND PHENOBARBITONE ON THE RELEASE OF CORTICOTROPHIN IN THE RAT

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A single injection into the rat of chlorpromazine, reserpine, benactyzine or phenobarbitone stimulates the release of corticotrophin. This effect is not seen after the drugs have been injected daily for 5 days, nor when the rats are hypophysectomized or pretreated with hydrocortisone. The stimulant effect of ether on corticotrophin release is not modified by pretreatment with a single injection, nor to any great extent after 5 daily injections of these drugs.

It has been reported that central nervous system depressants may either depress or stimulate the pituitary-adrenal axis, depending on the conditions under which they are examined. Various authors have claimed that both chlorpromazine and reserpine when administered alone (Van Peenen & Way, 1957; Mahfouz & Ezz, 1958), and after pentobarbitone (Sevy, Ohler & Weiner, 1957; Olling & de Wied, 1956), block the release of corticotrophin following a stress, but do not themselves cause a depletion of adrenal ascorbic acid. In contrast, Holzbauer & Vogt (1954) showed that the chlorpromazine does not prevent the effects of operative stress and itself causes a depletion of adrenal ascorbic acid in the intact, non-stressed rat. Similarly reserpine (Wells, Briggs & Munson, 1956) and morphine (Briggs & Munson, 1955) induce corticotrophin release when given in single doses, the effect being lost when they are given daily for 5 days.

It is now realized (Gaunt, Chart & Renzi, 1961; Munson, 1961) that many drugs cause a secretion of corticotrophin from the pituitary gland when first injected, and that their repeated administration leads to adaptation.

We have investigated the action of chlorpromazine, reserpine, benactyzine and sodium phenobarbitone on corticotrophin release in the rat, using the depletion of adrenal ascorbic acid as an index of activity.

The effect of one injection or several daily injections of these compounds was studied in intact rats, and in order to confirm that the effect observed is mediated through the pituitary gland the drugs were also injected into hypophysectomized and into hydrocortisone-treated rats.

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METHODS

Male albino rats weighing between 120 and 170 g were maintained for at least one week prior to use in a constant environment at a temperature of 80° F. Test drugs were administered intraperitoneally in aqueous solution, with the exception of reserpine which was administered as a suspension. The volume of injection was 0.5 ml./100 g body weight. Control animals received the diluent only.

The stressing procedure consisted of putting the rats into an ether chamber for 2.5 to 3 min, and was carried out 1 hr before killing. At various times after injection, as indicated in the tables, the adrenals were rapidly removed under ether anaesthesia, trimmed free of fat, weighed, and homogenized in 2.5% metaphosphoric acid. The homogenate was centrifuged and ascorbic acid was estimated in the centrifugate by the dichlorphenol indophenol dye method. Hydrocortisone acetate was injected as a 25 mg/ml. aqueous suspension, a first injection of 6 mg/100 g body weight being given intraperitoneally at 2 p.m. on the day previous to the experiment and a second similar injection at 9 a.m. the following day. Experimental drugs were given 3 hr later.

Hypophysectomy was carried out by the parapharyngeal approach 24 hr before the test and the hypophysectomized rats were given dextrose to drink overnight. The completeness of hypophysectomy was checked visually at the end of the experiment and the results from rats with a vestige of the pituitary left were discarded.

In Tables 1 and 2 the actions of the drugs in stressed and non-stressed animals are compared with their respective controls, and a comparison is also made between the effects of the drugs themselves in stressed and non-stressed rats.

RESULTS

Intact rats given single injections only. All the compounds examined caused a depletion of adrenal ascorbic acid in the intact non-stressed rat (Table 1). The threshold dose for chlorpromazine was between 1.4 and 2.8 mg/kg.

Ether also markedly depleted adrenal ascorbic acid. The possible effects which the central nervous system depressants may have on the depleting action of ether were obscured by their own stimulant effects on corticotrophin release. However, chlorpromazine at 1.4 mg/kg over a period of 2.5 hr and at 2.8 mg/kg over a period of 6 hr before killing did not inhibit the depletion caused by ether in spite of the fact that the drug itself is non-stimulant under these conditions. In two cases, reserpine at 0.1 mg/kg and phenobarbitone at 50 mg/kg, the combination of drug and stress reduced the adrenal ascorbic acid level to a significantly greater degree than stress alone. These lower levels of ascorbic acid represent in our experience the maximum possible depletion.

Intact rats given daily injections for 5 days. The administration of chlorpromazine, reserpine, benactyzine and sodium phenobarbitone daily for 5 days resulted in the loss of the ability to deplete adrenal ascorbic acid (Table 2). Indeed, the level of ascorbic acid was significantly increased over that of the controls in the groups treated with reserpine (0.5 mg/kg) and phenobarbitone. However, the stressing action of ether was still mainly unaffected by drug treatment, although there were indications that chlorpromazine at 2.8 mg/kg, reserpine at 0.5 mg/kg and phenobarbitone at 50 mg/kg antagonized it to some extent.

Hydrocortisone-treated rats and hypophysectomized rats. The depleting effect of the experimental drugs on adrenal ascorbic acid was abolished both by pretreatment with hydrocortisone acetate and by hypophysectomy (Table 3).

TABLE 1

THE EFFECT OF SINGLE INJECTIONS OF CHLORPROMAZINE, RESERPINE, BENACTYZINE AND SODIUM PHENOBARBITONE ON THE ADRENAL ASCORBIC ACID LEVEL OF STRESSED AND NON-STRESSED RATS

Treatment	Dose mg/kg	Hr from injection to killing	No. of rats/ group	Non-stressed. Ascorbic acid mg/100 g adrenal tissue ±s.e.		P	No. of rats/ group	Stressed. Ascorbic acid mg/100 g adrenal tissue±s.e.		P	Treated groups, non-stressed compared with stressed P
				Treated group	Control group			Treated group	Control group		
Chlorpromazine	1·4	2·5	13	278·7± 9·8	310·2±16·2	N.S.	7	236·6± 8·0	253·8±16·3	N.S.	<0·01
	2·8	2·5	6	302·2±13·5	381·5±12·6	<0·01	6	231·0±13·2	247·2± 9·9	N.S.	<0·01
	2·8	2·5	8	281·4±12·6	319·5±10·6	<0·05	8	185·8± 7·4	192·6± 9·3	N.S.	<0·001
	2·8	6·0	8	340·2±12·5	339·9±11·0	N.S.	8	235·5± 8·9	223·6± 7·8	N.S.	<0·001
	28·0	3·0	7	220·0±11·9	325·4±22·4	<0·01	—	—	—	—	—
Reserpine	0·1	2·5	6	261·8±19·8	381·5±12·6	<0·001	6	205·5±. 6·4	247·2± 9·9	<0·01	<0·05
	0·5	2·5	8	265·7±12·5	319·5±10·6	<0·01	8	184·1± 9·7	192·6± 9·3	N.S.	<0·001
	0·5	6·0	4	200·2± 3·2	362·8±10·7	<0·001	—	—	—	—	—
	0·5	6·0	—	—	—	—	8	223·5±19·9	223·6± 7·8	N.S.	—
Benactyzine	15·5	2·5	6	245·0±10·3	381·5±12·6	<0·001	6	222·0±11·3	247·2± 9·9	N.S.	N.S.
	15·5	3·0	7	232·7±15·9	338·2±16·7	<0·001	—	—	—	—	—
Sodium phenobarbitone	50·0	2·5	6	270·0±18·2	381·5±12·6	<0·001	6	184·3±12·1	247·2± 9·9	<0·01	<0·01
	50·0	3·0	7	240·8±17·7	338·2±16·7	<0·01	—	—	—	—	—

TABLE 2

THE EFFECT OF 5 DAILY INJECTIONS OF CHLORPROMAZINE, RESERPINE, BENACTYZINE AND SODIUM PHENOBARBITONE ON THE ADRENAL ASCORBIC ACID LEVEL OF STRESSED AND NON-STRESSED RATS

Treatment	Dose mg/kg day	Hr from final injection to killing	No. of rats/ group	Non-stressed. Ascorbic acid mg/100 g adrenal tissue±s.e. Treated group	Non-stressed. Ascorbic acid mg/100 g adrenal tissue±s.e. Control group	P	No. of rats/ group	Stressed. Ascorbic acid mg/100 g adrenal tissue±s.e. Treated group	Stressed. Ascorbic acid mg/100 g adrenal tissue±s.e. Control group	P	Treated groups non-stressed compared with stressed P
Chlorpromazine	2·8	2·5	6	351·2±16·5	354·5±11·3	N.S.	6	239·8±18·6	234·5±14·3	N.S.	<0·001
	2·8	2·5	8	365·1±12·4	328·2±12·2	N.S.	8	250·4± 9·9	212·0± 6·1	<0·01	<0·001
Reserpine	0·1	2·5	6	346·3±14·6	354·5±11·3	N.S.	6	259·6±24·6	234·5±14·3	N.S.	<0·02
	0·5	2·5	8	389·9± 8·6	341·0±13·4	<0·01	8	275·6± 6·8	243·1± 7·1	<0·01	<0·001
Benactyzine	15·5	2·5	6	301·6±29·1	354·5±11·3	N.S.	6	231·4±31·7	234·5±14·3	N.S.	N.S.
	15·5	2·5	8	344·2±12·7	328·2±12·2	N.S.	8	226·8± 5·0	212·0± 6·1	N.S.	<0·001
Sodium phenobarbitone	50·0	2·5	6	400·2±12·1	354·5±11·3	<0·02	6	251·8±17·0	234·5±14·3	N.S.	<0·001
	50·0	2·5	8	337·6±15·5	328·2±12·2	N.S.	8	269·4±12·0	212·0± 6·1	<0·001	<0·01

TABLE 3

THE EFFECT OF CHLORPROMAZINE, RESERPINE, BENACTYZINE AND SODIUM PHENOBARBITONE ON THE ADRENAL ASCORBIC ACID LEVEL OF HYPOPHYSECTOMIZED AND HYDROCORTISONE-TREATED RATS

Treatment	Dose mg/kg	Hr from injection to killing	No. of rats/ group	Hydrocortisone-treated. Ascorbic acid mg/100 g adrenal tissue±s.e. Treated group	Hydrocortisone-treated. Ascorbic acid mg/100 g adrenal tissue±s.e. Control group	P	No. of rats/ group	Hypophysectomized. Ascorbic acid mg/100 g adrenal tissue±s.e. Treated group	Hypophysectomized. Ascorbic acid mg/100 g adrenal tissue±s.e. Control group	P
Chlorpromazine	2·8	2·5	—	—	—	—	5	468·2± 5·9	465·6±16·6	N.S.
	28	3·0	5	460·8±17·3	454·0±11·6	N.S.	—	—	—	—
Reserpine	0·5	6·0	5	428·0±24·6	462·8±13·0	N.S.	—	—	—	—
Benactyzine	15·5	3·0	7	421·3±21·6	396·4±19·6	N.S.	—	—	—	—
	15·5	2·5	—	—	—	—	7	421·1±13·2	432·8±34·8	N.S.
Sodium phenobarbitone	50·0	3·0	7	380·6±12·8	396·4±19·6	N.S.	—	—	—	—
	50·0	2·5	—	—	—	—	7	400·7±20·7	432·8±34·8	N.S.
Ether	—	1·0	16	387·1± 9·8	370·0± 7·2	N.S.	—	—	—	—

DISCUSSION

Chlorpromazine, reserpine, benactyzine and phenobarbitone caused a fall in the adrenal ascorbic acid level of intact rats. Since this action was abolished by hypophysectomy, it is reasonable to assume that the drugs stimulate the release of corticotrophin from the anterior pituitary gland and do not affect the adrenal cortex directly.

Hydrocortisone inhibits the release of corticotrophin from the pituitary gland (Sydnor, 1955) by a feedback mechanism and may act at the level of the hypothalamus since plasma from the hypothalamico-hypophyseal portal vessels of the dog will still cause a depletion of adrenal ascorbic acid when injected into the hydrocortisone-blocked rat (Porter & Jones, 1956). Since pretreatment with hydrocortisone blocked the stressing action of all the compounds which we examined, it is probable that they do not act directly on the pituitary gland but at some point earlier, possibly on the hypothalamus.

Daily administration of the drugs for 5 days led to a loss of the ability to release corticotrophin, an effect which is similar to that reported by Wells *et al.* (1956) for reserpine and by Briggs & Munson (1955) for morphine. However, repeated injections of the drugs used in this investigation did not block the stressing effect of ether, although chlorpromazine, reserpine and phenobarbitone significantly reduced it in some experiments. This finding is not in complete agreement with those of the above-mentioned workers, who showed that, after 5 days of treatment, morphine blocked the effects of histamine, and reserpine blocked the effects of ether on corticotrophin release. We also failed to show any inhibition of ether stress by the central nervous system depressant drugs after their initial injection, although this may well have been due to our choice of conditions under which each depressant agent acted as a stress in itself. However, chlorpromazine in the two instances in which it did not cause adrenal ascorbic acid depletion still failed to block ether.

Maickel, Westermann & Brodie (1961) and Saffran & Vogt (1960) showed that reserpine lowers the corticotrophin content of the pituitary gland and prevents (Maickel *et al.*, 1961) the release of corticotrophin when the rats are exposed to cold 20 hr later. Conversely, the stressing effect of reserpine on corticotrophin release is blocked by an initial exposure to cold when reserpine is given 20 hr later. It was suggested that the depletion of corticotrophin in the pituitary gland caused by the first stimulus was responsible for the failure of the second stimulus to evoke a stress reponse (Maickel *et al.*, 1961).

This suggestion cannot explain the tolerance that developed toward chlorpromazine, benactyzine, reserpine and phenobarbitone after they have been injected repeatedly, since, on the 5th day of treatment, ether still evoked a discharge of corticotrophin. Presumably the level of corticotrophin in the pituitary had risen sufficiently by this time, in spite of continued drug treatment, to cause a depletion of adrenal ascorbic acid on discharge. The fact that hydrocortisone blocked the stressing action of ether may mean that ether was also acting through the hypothalamus.

The initial stressing action of phenobarbitone is of interest in view of the fact that barbiturates are generally thought to depress corticotrophin release without first stimulating (Gaunt *et al.*, 1961).

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