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THE ACTION OF CHLORPROMAZINE ON DIENCEPHALIC SYMPATHETIC ACTIVITY AND ON THE RELEASE OF ADRENOCORTICOTROPHIC HORMONE

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Chlorpromazine exerts a peculiar type of sedative action in man and in animals, potentiates and prolongs the action of analgesics and hypnotics, antagonizes excitation by alcohol and nikethamide, and protects animals from shock (for reference see Courvoisier, Fournel, Ducrot, Kolsky and Koetschet, 1953). To explain some of these effects, it has been suggested that the drug has an inhibitory action on sympathetic diencephalic centres (Laborit and Huguenard, 1951 ; Delay and Deniker, 1953) and that it prevents the release of adrenocorticotrophic hormone (ACTH) in conditions of stress (Aron, Chambon, and Voisin, 1953), an action which might also be interpreted as an inhibition of hypothalamic activity. The object of this paper is to investigate these possibilities.

METHODS

Hypothalamic Activity in Cats.—Six cats were subjected to left adrenal denervation, under ether anaesthesia, with aseptic precautions ; on the left side, both splanchnic nerves were severed and the first three lumbar sympathetic ganglia extirpated. In two of the cats, the left superior cervical ganglion was removed a fortnight later. Between two and three weeks after the first operation, the drugs under investigation were injected subcutaneously ; between 4 and 5 hr. after the injection of the first drug, the cats were rapidly anaesthetized with chloroform, bled to death, and their hypothalamus and adrenal glands removed for examination.

Morphine HCl and nalorphine HBr were injected as 2% solutions in 0.9% NaCl ; chlorpromazine was given as 0.15% solution, since higher concentrations have an irritant action.

Estimation of hypothalamic noradrenaline and of the amines of the adrenal medulla was carried out by biological assay on extracts subjected to paper chromatography ; the separated amines were eluted from the paper and the eluates assayed on the rat's blood pressure (urethane anaesthesia ; pretreatment with 2 mg./kg. atropine sulphate and 16 mg./kg. hexamethonium bromide intravenously). In some experiments the results of the adrenaline estimations were checked on the rat's uterus. All details of the methods have been reported earlier (Vogt, 1954).

Blood Pressure and Pupil Size of Dogs.—The action of chlorpromazine as an antagonist of adrenaline was followed on a dog anaesthetized with chloralose. The femoral blood pressure was recorded and observations were made of the effects on pupil size produced by the injection of drugs and by faradic stimulation of the preganglionic fibres of the severed cervical sympathetic trunk.

Experiments on the Adrenal Cortex of Rats.—The release of ACTH in conditions of stress was followed in rats by adrenal ascorbic acid estimations according to the method of Roe and Kuether (1943). One set of experiments was carried out in anaesthesia (1.75 g. urethane/kg. subcutaneously), and "operative stress" used to stimulate the release of ACTH. When the rat was anaesthetized an abdominal incision was made, the intestine exposed, and the mesentery handled for a period of 2 min. The wound was sewn up, the rat replaced in a warm box and killed, while still deeply anaesthetized, by severing the neck 1 hr. later; the adrenals were then removed for ascorbic acid estimations. The effects obtained in normal rats were compared with those seen in rats injected subcutaneously with chlorpromazine 30 min. or 3 hr. before the anaesthetic.

In a second group of experiments, the fall in adrenal ascorbic acid following a subcutaneous injection of adrenaline was compared under normal conditions and 3 hr. after the subcutaneous injection of chlorpromazine. All rats used in this experiment had been accustomed to handling by being given subcutaneous injections of 0.9% NaCl for a preliminary period of one week.

RESULTS

Hypothalamic Activity in Cats.—For testing central sympathetic activity use was made of the fact that morphine, given to cats, causes stimulation of the hypothalamic sympathetic centres. This can readily be measured by denervating one adrenal and estimating the difference in amine con-

tent between the innervated and denervated (resting) gland elicited by an injection of morphine. A second index of sympathetic hypothalamic stimulation is the fall in the noradrenaline content of the tissue observed when the stimulus has been acting over a period of several hours (Vogt, 1954). If chlorpromazine was able to antagonize a central sympathetic stimulation, the effects of morphine in depleting the stores of amines in the innervated adrenal and of noradrenaline in the hypothalamus might be inhibited.

Table I illustrates the results. The first cat was used as a control and injected with chlorpromazine only. As might be expected, there was no secretion of adrenaline from the innervated adrenal, and the hypothalamic noradrenaline was not significantly different from normal. Cats 2, 3, and 4 were given 40 mg./kg. of morphine 45 min. after a first dose of 15 mg./kg. chlorpromazine; a second dose of chlorpromazine (10 mg./kg.) was injected about 2 hr. after the morphine. Hypothalamic noradrenaline was low, and there was secretion of amines from the innervated medulla; the figures are not different from those of the last row, which represent previous observations on cats given the same dose of morphine but no chlorpromazine.

Thus chlorpromazine, given in a high dose, had no antagonistic effect on the diencephalic stimulation of the sympathetic centres produced by morphine. In another experiment, this result was compared with the effect of a proved morphine antagonist, nalorphine, on the hypothalamic stimulation produced by the same dose of morphine. Cats 5 and 6 (Table I) were given an injection of morphine 12 min. after the subcutaneous administration of nalorphine. The fall in hypothalamic noradrenaline was completely prevented in both cats; a small stimulation of the innervated adrenal medulla was seen in cat 5, which had the smaller dose of nalorphine, whereas this effect was abolished in cat 6, which was given the larger dose. Suppression of the clinical signs too was more complete in cat 6, which showed no abnormality until nearly 5 hr. after the nalorphine: when it was picked up in order to be anaesthetized with chloroform, there was sudden excitation and tremor, indicating that the effect of the nalorphine was beginning to subside. Cat 5, in contrast, showed salivation and some muscular twitching throughout the experiment, though the animal was much quieter than would have been expected as a result of morphine alone.

The clinical picture after chlorpromazine followed by morphine was varied: cat 2 showed no muscular twitches and no motor excitement; instead, it was sitting still or lying down with its head drooping to one side. In cats 3 and 4 muscular twitching and tremor were not suppressed by chlorpromazine as they had been in cat 2. One short fit of convulsions was seen in cat 4.

One of the cats vomited, a fact which is of interest in view of the excellent anti-emetic action of chlorpromazine in the dog given apomorphine (Courvoisier *et al.*, 1953).

A striking sign, in view of the adrenolytic action of chlorpromazine, was, however, a dilatation of the pupils, whereas the nictitating membranes were relaxed. This was first seen in cat 2 and was obviously caused by the morphine (which is a mydriatic in cats), since the chlorpromazine control (cat 1) had narrow pupils and relaxed nictitating membranes.

In order to determine whether the mydriasis was a sign of circulating adrenaline, or was due to some other cause, the next two experiments (Nos. 3 and 4) were carried out on cats in which the left superior cervical ganglion had been re-

TABLE I

HYPOTHALAMIC NORADRENALINE, AND SECRETION FROM INNERVATED ADRENAL MEDULLA, AFTER SUBCUTANEOUS MORPHINE ALONE AND IN COMBINATION WITH CHLORPROMAZINE OR NALORPHINE

Cat No.	Chlorpromazine* (mg./kg.)	Nalorphine HBr (mg./kg.)	Morphine HCl (mg./kg.)	Hypothalamic Noradrenaline			Amines‡ in Innervated Adrenal Medulla (% of Denervated Medulla)	
				μg./g. Fresh Tissue	Mean (μg./g.)	% of Normal†	Individual Expts.	Mean
1	25	0	0	1·3		94	100	
2	25	0	40	0·8			75	
3	25	0	40	1·2	0·98	68	44	59
4	25	0	40	0·8			59	
5	0	25	30	1·4		101	78	
6	0	37·5	40	1·4		101	100	
Mean of 5	0	0	40		0·92	67	40, 57§	

* Given in two doses, interval 2½ hr. † The normal figure (a mean of 29 cats) is 1.38 μg./g. fresh tissue (Vogt, 1954). ‡ Adrenaline and noradrenaline in the adrenal medulla were estimated separately and added together. § 2 cats only.

The cats had been subjected to denervation of the left adrenal 2–3 weeks previously. Cat 1 was killed 5 hr. after the first injection of chlorpromazine; the other cats were killed 4½ hr. after the injection of morphine.

moved one week previously. The nictitating membranes of both cats remained relaxed throughout the experiment, but dilatation of both pupils—and, more strikingly, of the previously narrower, denervated pupil—occurred, particularly towards the end of the experiment. The obvious interpretation was that the pupils responded to circulating medullary amines and that the adrenolytic effect of chlorpromazine was not very powerful.

Antiadrenaline Effect.—The unexpected mydriasis in response to circulating adrenaline (and noradrenaline) in cats given chlorpromazine prompted a reassessment of the adrenolytic effect of chlorpromazine. Since the substance is credited with a ganglion-blocking action, its effect on transmission in a sympathetic ganglion was also tested. In the cats, response to circulatory medullary amines had been noticed as early as 80 and 95 min. after chlorpromazine in a dose of 15 mg./kg. An acute experiment was therefore carried out on a dog anaesthetized with chloralose, and the responses of blood presssure and pupil to injected adrenaline and noradrenaline, and to faradic stimulation of the preganglionic sympathetic fibres in the neck, were observed.

Between 5 and 15 min. after a first intravenous dose of chlorpromazine (1.5 mg./kg.), the effect of adrenaline (4 μg./kg.) on the blood pressure was reversed and that of the same dose of noradrenaline reduced; the mydriatic effects on the pupil had ceased. Preganglionic stimulation of the sympathetic chain, however, was as effective on the pupil as before. Half an hour after the administration of chlorpromazine, the effect of adrenaline began to recover, and a second, higher dose of chlorpromazine (3.0 mg./kg.) was injected. Adrenaline reversal on the blood pressure again ensued, but the threshold to electrical stimulation of the sympathetic chain remained unaltered. Two hr. 20 min. later, pressor and mydriatic effects of adrenaline were back to normal. The effect of sympathetic stimulation, hitherto unchanged, was then abolished by an injection of hexamethonium bromide (2.7 mg./kg.).

It follows from this experiment that the adrenolytic effect of chlorpromazine need not be as prolonged as is often supposed. As with other adrenaline antagonists, the effects of noradrenaline are more resistant to the drug than those of equal doses of adrenaline, and the effects of sympathetic stimulation are more resistant still. In addition, with the dosage (total of 4.5 mg./kg.) used, there was no sign of inhibition of transmission at the sympathetic ganglionic synapse. The fairly short duration of the adrenaline antagonism explains the observation on cats premedicated with chlorpromazine, in which pupillary dilatation in the sympathetically denervated eye occurred after a dose of morphine causing secretion of the adrenal medulla.

Release of ACTH in Stress.—The first part of this work dealt with possible effects of chlorpromazine on centrally initiated secretion by the adrenal medulla. The second part deals with the question whether the drug influences the stimulation of the adrenal cortex elicited by noxious or other stimuli capable of releasing ACTH.

In a first series of experiments, "operative stress" was employed in view of the fact that chlorpromazine protects animals from traumatic shock. The "stress" consisted in anaesthetizing the rats by subcutaneous injection of urethane, carrying out a laparotomy and handling the intestine. The results are shown in Table II.

In Groups 1–3 chlorpromazine was given in a dose of 10 mg./kg. and the operation was carried out 3 hr. later. The rats were killed and the adrenals removed at 4½ hr. The mean ascorbic acid content of the adrenals of Group 1, given chlorpromazine but not operated on, was 321 mg./100 g., and that of the rats operated on without pretreatment (Group 2) was 212 mg./100 g.

TABLE II

THE EFFECT OF CHLORPROMAZINE ON THE ASCORBIC ACID CONTENT (MG./100 G. FRESH ADRENAL) OF THE ADRENALS OF RATS UNDERGOING AN ABDOMINAL OPERATION

Group	No. of Rats	Treatment			Killed at	Adrenal Ascorbic Acid. Mean ± S.E. of Mean
		At Zero Hr.	At 30 min.	At 3 hr.		
1	7	Chlorpromazine, 10 mg./kg.	—	—	4½ hr.	321 ± 9·6
2	6	—	—	Urethane followed by operation	4½ ,,	212 ± 9·5
3	7	Chlorpromazine, 10 mg./kg.	—	Urethane followed by operation	4½ ,,	218 ± 12·4
4	6	0·9% NaCl	—	—	2 ,,	316 ± 12·4
5	6	Chlorpromazine, 15 mg./kg.	—	—	2 ,,	225 ± 10·0
6	6	Chlorpromazine, 15 mg./kg.	Urethane followed by operation	—	2 ,,	236 ± 6·9

Chlorpromazine was injected subcutaneously as 0·1 or 0·15% solution in saline.
The differences between the ascorbic acid values of Groups 2, 3, 5, and 6 are not significant.

When operation and chlorpromazine were combined (Group 3), the value was 218 mg./100 g.; thus no protection had been afforded the adrenals by preceding the operative stress with chlorpromazine.

In order to obviate the objection that the dose was too low or the timing inadequate, we examined the effect of increasing the dose to 15 mg./kg. and shortening the interval between injection and operation to 30 min. The mean ascorbic acid concentration of Group 5 (chlorpromazine alone) was 225 mg./100 g. Practically the same figure was obtained in Group 6, in which chlorpromazine was combined with the operation. Nearly the same value had been found in Group 2, subjected to the operation only. Saline controls (Group 4) gave a mean ascorbic acid content of 316 mg./100 g. Obviously, the higher dose of chlorpromazine itself acted as a stress of about the same severity as the operation. There is no simple additive effect of multiple stressing stimuli on the depression of adrenal ascorbic acid. Thus the fact that the combination of drug injection and operation, each of which are about equally potent in releasing ACTH, does not produce a greater fall in ascorbic acid than each procedure alone cannot be taken as proof—though it may suggest—that the injection has rendered the organism less susceptible to the effect of the operation.

Though inhibition by chlorpromazine of ACTH release by operative stress could not be demonstrated, the possibility remained that, perhaps as a result of the adrenolytic properties of the drug, release of ACTH in response to administration of adrenaline might be diminished. This possibility was examined in the next series of experiments. A special precaution was, however, necessary. It will be seen from Table II that the rats which had been given a subcutaneous injection of saline (Group 4) showed an adrenal ascorbic acid content of 316 mg./100 g., which is nearly 25% below normal. This response to the injection of saline, which is an effect of emotion, was undesirable in experiments in which conscious animals were to be subjected to several subcutaneous injections. It can be abolished by accustoming the rats to injections; this was done, in all rats used in the next experiment, by injecting 0.9% NaCl twice daily for a week before carrying out the final test with drugs. Table III shows the results. The duration of all experiments was 4½ hr. The "trained" rats, killed 4½ hr. after an injection of saline, had an adrenal ascorbic acid content of 409 mg./100 g.; when the saline was replaced by 10 mg./kg. chlorpromazine, there was a fall to 323 mg. When, 2½ hr. after the chlorpromazine, adrenaline (200 μg./kg.) was injected, there was a further significant fall in ascorbic acid; if the initial injection of chlorpromazine was omitted, the response to the same dose of adrenaline was a little but not significantly larger. It follows that a dose of chlorpromazine, large enough to cause some release of ACTH by itself, is nevertheless unable to inhibit appreciably the pituitary response to a dose of adrenaline administered 2½ hr. later, at a time when, to judge from the fall in rectal temperature, some, at least, of the actions of chlorpromazine are still present.

TABLE III

ASCORBIC ACID (MG./100 G. FRESH ADRENAL) IN THE ADRENALS OF "TRAINED" RATS INJECTED SUBCUTANEOUSLY WITH DRUGS AND KILLED AT 4½ HR.

Group	No. of Rats	Treatment	Adrenal Ascorbic Acid. Mean±S.E. of the Mean
1	11	0·9% NaCl at zero hr. ..	409±19
2	23	Chlorpromazine at zero hr. ..	323±16
3	23	Chlorpromazine at zero hr. Adrenaline 200 μg./kg. at 2½ hr.	286± 7·8
4	23	Adrenaline 200 μg./kg. at 2½ hr.	270± 7·2

Chlorpromazine (10 mg./kg.) was injected as 0.1% solution in 0.9% NaCl; the same volume of 0.9% NaCl without chlorpromazine was injected into the control rats of Group 1.

The differences between Groups 1 and 2, 2 and 3, 2 and 4 are significant ($P < 0.01$), but not that between 3 and 4 ($P < 0.1$, > 0.05).

DISCUSSION

The clinical picture of morphine poisoning in the cat is modified by premedication with chlorpromazine; actions of chlorpromazine, such as muscular weakness and relaxation of the nictitating membranes, occur side by side with a somewhat damped manifestation of the effects of morphine —salivation, vomiting, tremors, excitement, and, very occasionally, convulsions. The characteristic central sympathetic stimulation by morphine is not inhibited by chlorpromazine. In contrast, premedication with a true morphine antagonist, nalorphine, abolishes all clinical signs of morphine poisoning, and prevents the central sympathetic stimulation which results in a fall in hypothalamic noradrenaline and in a depletion of amines from the stores of the innervated adrenal gland.

The observation that a peripheral sign of circulating medullary amines, mydriasis, occurred in the denervated pupil of cats treated with chlorpromazine and morphine led to the revision of the view that the peripheral adrenolytic action of chlorpromazine need last for many hours. Confirmation was obtained by observations on the dog, in which no vestige of adrenolytic action was

found 140 min. after 4.5 mg./kg. chlorpromazine given intravenously, and partial recovery had occurred much earlier.

In the French clinical literature, chlorpromazine is sometimes classed among the ganglion-blocking agents (Laborit and Huguenard, 1951). In their experimental study, Courvoisier *et al.* (1953) demonstrated that large doses have a mild inhibitory action on transmission in the vagal ganglia of the heart. No corresponding effect in the sympathetic system was found by Reuse (1954), who compared the effects on the nictitating membrane of stimulating the preganglionic and the postganglionic fibres of the superior cervical ganglion of the cat. In complete agreement with our results on the dilatation of the dog's pupil, Reuse found that even large doses of chlorpromazine did not impair transmission in the ganglion. Reuse also showed that sympatholytic in contrast to adrenolytic action required very high doses (above 5 mg./kg.) of chlorpromazine, and the same observation was made by us in the dog.

The experiments on rats, intended to investigate whether adrenocortical response to stress was inhibited by chlorpromazine, failed to demonstrate such an action; it was, however, necessary to choose a dosage and timing that would minimize interference by the release of ACTH caused by chlorpromazine itself. If no more than 10 mg./kg. were administered and 4½ hr. allowed to elapse between injection of drug and ascorbic acid estimations, some depletion of adrenal ascorbic acid was caused by the chlorpromazine, but not enough to obscure the greater depletion due to the operative stress. This dose, given 3 hr. before the operation, did not inhibit the adrenal response to the second stress. It may be argued that larger doses would have produced such an inhibition; but, as they cause a depletion of adrenal ascorbic acid equal to that of the operation, the determination of the share to be attributed to each stressing agent when both are superimposed is impossible, and interpretation of the results would necessarily be ambiguous. Recently, Georges and Cahn (1953) have also come to the conclusion that administration to rats of a "lytic cocktail" containing approximately 11 mg./kg. chlorpromazine did not prevent the release of ACTH in operative stress, provided the body temperature was above 27° C. Contrary claims by Aron, Chambon and Voison (1953), who used doses of chlorpromazine of 10–50 mg./kg. in order to prevent the adrenal ascorbic acid fall caused by unilateral adrenalectomy, are possibly due to the use of very small numbers of rats and the lack of unoperated controls treated with the drug alone. Georges and Cahn (1953) describe a fall in eosinophils 4 hr. after an injection of 22 mg./kg. chlorpromazine, and Filk and Loeser (1954) report a loss of adrenal lipids a few hours after feeding chlorpromazine. Both phenomena indicate a release of ACTH in response to this drug.

It seemed possible that, even if chlorpromazine did not prevent operative stress from releasing ACTH, it might prevent adrenaline from doing so, at least as a result of its peripheral adrenolytic action. Here again, dosage and timing had to be chosen so that there was only a moderate depletion of ascorbic acid by the chlorpromazine alone. Under these conditions, the results were not impressive. The ascorbic acid depletion by adrenaline alone was just very slightly greater than that caused by adrenaline after premedication with chlorpromazine, but the difference was not significant ($p<1, >0.05$) in spite of the use of large groups of rats. Whatever slight protection might have been exerted may, of course, be entirely due to the inhibition of the peripheral action of the adrenaline.

The foregoing experiments have not substantiated the hope, expressed by Courvoisier *et al.* (1953), that the administration of chlorpromazine might produce a kind of "chemical adrenalectomy" by inhibiting adrenomedullary secretion, or the claim by Aron *et al.* that the drug caused the equivalent of a hypophysectomy by preventing the release of ACTH. The explanation of the shock-preventing action of this drug, rather than lying in an inhibition of the adrenal defence mechanism of the organism, must be sought elsewhere —perhaps in the metabolic effects it produces.

Summary

1. Chlorpromazine (25 mg./kg. subcutaneously) does not inhibit the stimulation of the sympathetic centres produced by morphine in the cat, as judged by the following criteria: a fall in hypothalamic noradrenaline and a depletion of the stores of medullary amines in the innervated adrenal. During certain periods of the experiment, the amount of medullary amines released into the circulation dilates the (innervated and denervated) pupil and is thus sufficient to overcome the adrenolytic action of chlorpromazine. In contrast, nalorphine inhibits all manifestations of morphine poisoning including the stimulation of the sympathetic centres.

2. In the dog, the duration of the antiadrenaline effect of chlorpromazine was followed, and its

alleged inhibitory action on ganglionic transmission was examined ; no such action was found on the superior cervical ganglion.

3. The release of ACTH in operative shock was not prevented in the rat by chlorpromazine (10 mg./kg.) administered 3 hr. before the operation ; nor was the release of ACTH by adrenaline (200 μg./kg. subcutaneously) prevented. Chlorpromazine itself caused some release of ACTH at this dose level ; if the dose was increased to 15 mg./kg., the release of ACTH was of the same magnitude as that caused by the operation, or by an injection of adrenaline ; a satisfactory investigation of a possible inhibitory effect of such doses of chlorpromazine on the ACTH releasing power of other procedures was thus not possible.

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