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MECHANISM OF THE PREVENTION OF NICOTINE CONVULSIONS BY HEXAMETHONIUM AND BY ADRENALINE BLOCKING AGENTS

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In a previous paper (Laurence and Stacey, 1952a) we have shown that hexamethonium and pentamethonium protect mice and rats against nicotine convulsions to a marked degree, but that they afford no protection against the action of a number of other convulsants nor against insulin convulsions (Laurence and Stacey, 1952b). In the present work we have sought an explanation for this protection.

Hexamethonium does not pass freely into the cerebrospinal fluid (Paton), so that there are good reasons for investigating possible peripheral mechanisms. Evidence has already been advanced for believing that, although violent stimulation of the carotid body may, according to Schmidt and Comroe (1940), lead to convulsions, it is not by blocking afferent impulses from this structure that hexamethonium exerts its protection (Laurence and Stacey, 1952a). Attention was therefore turned to other peripheral actions of nicotine which are modified by hexamethonium. Hexamethonium blocks stimulation of the adrenal medulla by nicotine, and it was thought possible that the adrenaline liberated might play a part in precipitating nicotine convulsions, since adrenaline is known to facilitate spinal reflexes (Bülbring and Burn, 1941) and the passage of impulses across synapses (Bülbring and Burn, 1942), and to potentiate the action of various substances on the central nervous system (Friedemann and Elkeles, 1932). The convulsant action of nicotine was therefore examined under conditions in which the effects of sympathetic stimulation were increased (by simultaneous administration of adrenaline or noradrenaline) or decreased (by blocking agents or adrenalectomy).

METHODS

Mice and rats were used. In each experiment a comparison was made between the convulsion rates in two groups of animals of the same sex, from the same colony, and of similar weights and ages which had received the same dose of a convulsant (either nicotine or leptazol). One group had received previously an injection of a solution of the drug whose anticonvulsant action was being examined, while the other had received an equal volume of solvent. For dibenamine the time interval between the two injections was four hours, for all other drugs 30 minutes. *Mice* were given nicotine (0.2 mg. base/ml.) or leptazol (2 mg./ml.) into a tail vein, the injection occupying exactly 10 seconds timed by a metronome. Premedication was by intraperitoneal injection. *Rats* received nicotine (2 mg. base/ml.) subcutaneously or leptazol (25 mg./ml.) intraperitoneally and premedication by subcutaneous injection. Each experiment was repeated after one week with the groups crossed over; the results were then summed. Doses of convulsants were adjusted to give a suitable convulsion rate for the purpose of the experiment. In *mice* these were: nicotine 0.2–0.4 mg./kg., leptazol 20–40 mg./kg.; and for *rats*: nicotine 2–3 mg./kg., leptazol 50–70 mg./kg. In the experiments in which adrenaline, noradrenaline, and pitressin were used, these substances were added to the solution of the convulsant and used at once.

Adrenalectomized animals had all recovered completely from the operation before use and were not receiving cortical steroids.

Probability figures were obtained from Mainland's fourfold contingency tables (Mainland, 1948).

RESULTS

Effect of Premedication with Various Substances on the Convulsion Rate in Normal and Adrenalectomized Animals

It will be seen from Table I that all adrenaline blocking agents tested protected against nicotine convulsions but not against leptazol convulsions; tolazoline had the weakest action. Hexamethonium, artane, and diparcol also afforded protection, hexamethonium being much the most potent. All the other drugs tested (atropine,

hyoscine, ergometrine, and promethazine) gave no protection in doses up to 15 mg./kg.

TABLE I

EFFECT OF VARIOUS COMPOUNDS ON THE NICOTINE CONVULSION RATE. CORRESPONDING FIGURES FOR THE LEPTAZOL CONVULSION RATE ARE GIVEN IN BRACKETS

"Antagonist"	Dose mg./kg.	Animals	No. of Animals Convulsed out of 20		P
			Control	Exptl.	
Dibenamine HCl	5	Rats	19 (*13*)	2 (*15*)	<0·01
,, ,,	5	Mice	15	5	
Yohimbine HCl	5	Rats	16 (*14*)	0 (*16*)	
,, ,,	5	Mice	18	10	
SKF 688-A ..	5	Rats	14	1	
Ergotamine tartrate	5	,,	16	0	
DHE methane sulphon. ..	5	,,	9	0	
,, ,,	5	Mice	18	13	0·06
Tolazoline HCl	15	Rats	15	1	<0·01
,, ,,	5	,,	16	16	—
Hexamethonium I_2	5	Mice	17	3	<0·01
,, ,,	2	Rats	15 (*17*)	4 (*16*)	
Artane	15	,,	12	6	0·06
,, ...	5	,,	16	14	—
Diparcol	15	,,	14	5	<0·01
,, ...	5	,,	6	7	—
TEAB	40	,,	11	8	0·26
Atropine sulphate	5	,,	10	9	—
,, ,, ..	15	,,	5	8	—
Hyoscine HB ..	15	,,	8	13	—
Ergometrine maleate ..	5	,,	10	10	—
,,	15	Mice	11	17	—
Promethazine HCl	15	Rats	12	9	0·26

When similar experiments were performed using adrenalectomized rats (Table II) some protection was still afforded by hexamethonium and dibenamine; the figures suggest that this is less than with intact rats, while tolazoline now gave no significant protection. It was also noticed that adrenalectomized rats were slightly less sensitive to nicotine than intact rats.

TABLE II

EFFECT OF ADRENALINE BLOCKING AGENTS ON THE NICOTINE CONVULSION RATE IN ADRENALECTOMIZED RATS

"Antagonist"	Dose mg./kg.	No. of Rats Convulsed out of 20		P
		Control	Exptl.	
Hexamethonium I_2	2	14	7	<0·05
Dibenamine HCl ..	5	16	10	
Tolazoline HCl ..	15	11	9	—
,, ,, ..	5	11	12	—

Effect of Adrenaline and Noradrenaline on the Convulsant Action of Nicotine and Leptazol

The effect of mixing adrenaline and noradrenaline with the convulsants was next investigated; the results are recorded in Table III. Experiments 1–4 show that both substances potentiated the action of both nicotine and leptazol. A number of experiments were performed, of which examples only are recorded here, with the intention of bringing to light any difference between the actions of adrenaline and noradrenaline in this respect, but no constant differences could be established.

TABLE III

EFFECT OF ADRENALINE, NORADRENALINE, AND PITRESSIN ON NICOTINE AND LEPTAZOL CONVULSION RATES

Expt.	Convulsant	"Adjuvant"	No. of Mice Convulsed out of 20		P
			Control	Exptl.	
1	Nicotine	Adrenaline 40γ/kg.	3	9	0·05
	,,	,, 80γ/kg.		12	0·01
2	,,	,, 40γ/kg.	2	10	<0·01
	,,	Noradrenal. 40γ/kg.		9	
3	Leptazol	Adrenaline 40γ/kg.	2	14	<0·01
	,,	Noradrenal. 40γ/kg.		14	
4	,,	Adrenaline 20γ/kg.	1	9	<0·01
	,,	Noradrenal. 20γ/kg.		6	<0·05
5	Nicotine	Pitressin 0·4 i.u./kg	3/10	3/10	—
		,, 1·2 ,, ,,		2/10	—
		,, 4·0 ,, ,,		2/10	—
6	Leptazol	,, 2 ,, ,,	3	10	0·02
7	Nicotine preceded by C_6I_2 5 mg./kg. i.p.	,, 2 ,, ,,	5	13	0·01

Effect of Pitressin on the Convulsant Action of Nicotine and Leptazol

The results recorded above suggested that the effect of other pressor substances might be of interest. Pitressin was selected, as there is no evidence that it has any central action. When it was added to leptazol, potentiation was observed (Table III, Experiment 6), but over a wide range of dose no potentiation of nicotine was found (Experiment 5). If, however, the circulatory disturbances provoked by nicotine were inhibited by a previous injection of hexamethonium to both groups of rats, the potentiation of nicotine by pitressin became evident.

DISCUSSION

The first series of experiments shows that two groups of drugs among those investigated antagonize nicotine convulsions, namely, ganglion-blocking substances and adrenaline-blocking substances. This antagonism is well marked with hexamethonium with doses as low as 2 mg./kg., but is also easily demonstrable with artane and diparcol when doses of 15 mg./kg. are given. This dose level of diparcol is required to produce

ganglion blockade (Heymans, Estable, and Castillo de Bonneveaux, 1949). If the protection against nicotine convulsions afforded by these substances depends on their ganglion blocking action, tetraethylammonium might be expected to afford protection also. According to Paton and Zaimis (1949) it has a blocking action on sympathetic ganglia only 1/20th that of hexamethonium. This may account for the absence of definite anticonvulsant action at the maximum tolerated dose of 40 mg./kg. Of the adrenaline blocking substances tolazoline provided least protection, 15 mg./kg. being required to give protection comparable with that given by 5 mg./kg. of the other compounds. This is in accordance with its "weaker adrenergic blocking action," found by Nickerson and Smith (1949). These experiments accord with the view that sympathetic block, whether this takes place at ganglia or at adrenergic nerve endings, protects against nicotine convulsions.

When adrenalectomized animals were used, protection was still afforded by hexamethonium and dibenamine, though there was evidence that this was less complete, and tolazoline failed to protect even at the higher dose. The effects of sympathetic stimulation by nicotine in these animals would be less than in intact animals, and sympathetic block might therefore be expected to have less action.

The results of the experiments with adrenaline and noradrenaline, which agree with those of Friedemann and Elkeles (1932), who showed that adrenaline and pituitrin potentiate the action of a number of narcotics and convulsants, can be interpreted as indicating that increasing the effects of sympathetic stimulation increases the convulsant action of nicotine and leptazol.

From these experiments it is concluded that the convulsant action of nicotine is increased by the sympathetic stimulation it causes, and that substances reported here as protecting against nicotine convulsions do so by diminishing those effects of nicotine caused by sympathetic stimulation. Other convulsants, such as leptazol, in whose action sympathetic stimulation does not play an important part, are not antagonized.

When we come to consider the way in which sympathetic stimulation could potentiate the action of a convulsant, two main possibilities present themselves : (i) changes in the circulation might lead to a higher concentration of the convulsant being reached in cerebral tissue, either by an increased cerebral blood flow or by a raised blood pressure resulting in a more rapid passage of the convulsant through the blood-brain barrier, or (ii) adrenaline and noradrenaline might facilitate the development of convulsions by a direct action on the central nervous system.

Though our experiments do not give a final answer as to the relative importance of these two factors, some further deductions may be drawn. We have found that pitressin will potentiate the action of leptazol and, in certain circumstances, of nicotine, and since there is no evidence that pitressin has any direct action on the brain it presumably potentiates by a vascular mechanism. Vascular changes caused by adrenaline and noradrenaline might therefore well cause a potentiation of the order observed. This is confirmed by the observation of Friedemann and Elkeles (1932) that adrenaline and pituitrin increase the passage into the brain of those substances which normally pass the blood-brain barrier. Nicotine, adrenaline, noradrenaline, and pitressin all cause a violent rise in blood pressure, but their effect on cerebral flow in the doses used is not known. King, Sokoloff, and Weschler (1952) have shown that in man adrenaline increases while noradrenaline decreases cerebral flow, but the doses they used were much smaller than ours, and, apart from species differences, no conclusion could be safely drawn from them as to the changes in cerebral flow in our experiments.

It may be concluded that a rise in blood pressure, possibly accompanied by an increased cerebral blood flow, is probably an important factor in the potentiation of convulsants by excitation of sympathetic effector organs.

Considering now the second of the two possibilities mentioned above, our experiments have not shown what part, if any, a central action of adrenaline or noradrenaline plays in the development of nicotine convulsions. This point is being investigated further. Where the central effects of adrenaline and noradrenaline have been compared, as in their effect on the tremor of Parkinsonism (Barcroft, Peterson, and Schwab, 1952), and on the oxygen consumption of the brain (King *et al.*, 1952), adrenaline has been found to produce marked changes and noradrenaline none. In our experiments adrenaline and noradrenaline appeared to have equal effect, but our doses were so much greater that a difference in central action might have been obscured.

Finally, our experiments show that the circulatory disturbances caused by a drug may markedly modify its action on the central nervous system, so that in assessing the potency of a drug acting on the central nervous system the part played by

accompanying changes in the cerebral circulation must be assessed before any theoretical conclusions can be drawn.

Summary

1. Hexamethonium, dibenamine, SKF 688-A, yohimbine, ergotamine, and dihydroergotamine protect mice and rats against nicotine convulsions. Tolazoline, diparcol, and artane have a similar but weaker action. These substances do not protect against leptazol convulsions.

2. Adrenalectomized rats are less effectively protected than intact rats. The simultaneous administration of adrenaline and noradrenaline potentiates the convulsant action of nicotine and leptazol. Pitressin potentiates the action of leptazol; it increases that of nicotine only if hexamethonium has first been given.

3. It is concluded that the action of nicotine on the brain is intensified by the sympathetic discharge it causes. Blocking the sympathetic system reduces its convulsant action.

4. The mechanism whereby sympathetic discharge intensifies the action of a convulsant is discussed, and it is concluded that the vascular changes produced are of major importance. Hence, in comparing the potency of drugs acting on the central nervous system it is essential that the condition of the cerebral circulation should be comparable.

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