RESEARCH PAPERS

THE EFFECT OF VARIOUS CHOLINE ESTERS ON THE ADRENAL GLAND OF THE CAT

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Received December 23, 1957

A comparison has been made between the abilities of various choline esters to cause a release of catechol amines from the adrenal gland of the atropinised cat, anaesthetised with chloralose. It was found that repeated intravenous doses of acetylcholine, carbamylcholine or benzoylcholine, but not acetyl-β-methylcholine, caused the liberation of similar proportions of adrenaline and of noradrenaline. This was irrespective of the overall degree of depletion of the glands, which ranged from 7·1 per cent to 86·5 per cent. Acetylcholine and benzoylcholine were more effective than carbamylcholine in causing a release of amines.

It is well known that the administration of large doses of acetylcholine to an atropinised animal causes the release of catechol amines from the adrenal medulla. A number of workers[1–4] have shown that other choline esters also cause a release. However few have studied the relative proportions of adrenaline and noradrenaline liberated by such substances. Outschoorn[5] has found that acetylcholine causes the liberation, in the adrenal venous effluent, of similar percentages of adrenaline and noradrenaline. Other substances, notably insulin[6] and nicotine[7] cause the preferential release of adrenaline and noradrenaline respectively. Thus it seemed of interest to study the effects of different choline esters, and in this paper a comparison is made of the ability of acetylcholine, carbamylcholine, benzoylcholine and acetyl-β-methylcholine to deplete the adrenal glands of cats of their catechol amines. Also the modification of the acetylcholine response by physostigmine is studied.

Method

Healthy, adult cats of both sexes were used. The animal was given 6 mg./kg. of atropine sulphate, intraperitoneally and anaesthetised 10 minutes later with ether, following by 60 mg./kg. of chloralose, intravenously. Doses of choline ester, as the chloride, were given by femoral vein and the blood pressure recorded from the carotid artery, the usual dose range being 0·2–3·0 mg./kg. Having obtained one pressor response of suitable magnitude, one adrenal gland was removed as the control gland and an extract prepared (see later). Subsequent to the unilateral adrenalectomy, repeated doses of the choline ester were given, the dose being sufficiently large to obtain a definite pressor response. In order to obtain a range of depletion, the number of doses of choline ester was

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varied in each experiment. The minimum number of doses given in any one experiment was 10 and the maximum was 166. In those experiments in which physostigmine was given, subsequent to the removal of one gland, a constant submaximal response to acetylcholine was obtained and then 0·2 mg./kg. of physostigmine salicylate was given intravenously. The main depletion of the gland was then produced by giving further doses of acetylcholine. In certain experiments where carbamylcholine was used as the depleting agent, one definite pressor response to acetylcholine

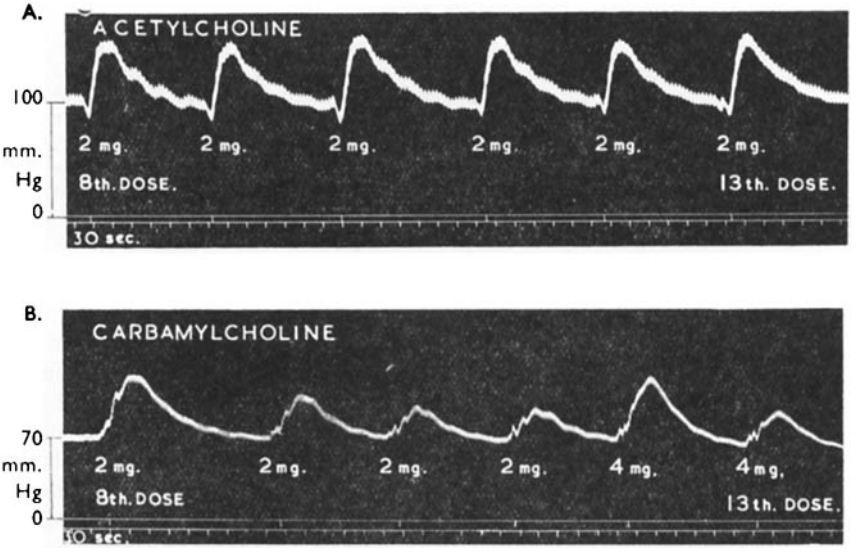

FIG. 1. A. Cat 2·1 kg. Atropine sulphate 6 mg./kg., followed by ether, and chloralose 60 mg./kg. The right adrenal gland was removed. Blood pressure tracing to show the similarity of successive responses to intravenous doses of acetylcholine. B. Cat 3·4 kg. Atropine and chloralose as above. The right adrenal gland was removed. Blood pressure tracing to show the rapid diminution in the response to consecutive intravenous doses of carbamylcholine.

and to electrical stimulation of the splanchnic nerve was obtained after the removal of one gland and before giving repeated doses of carbamylcholine. When the gland was no longer sensitive to carbamylcholine an attempt was made to obtain a rise in blood pressure by the injection of acetylcholine and by stimulation of the nerve. In all other experiments only one choline ester was employed throughout the experiment. After the depletion, the second gland was removed and an extract prepared as for the first gland.

Preparation of the extracts of the adrenal glands. Immediately after excision, the gland was dissected free from connective tissue and weighed on a micro-torsion balance. A 100 mg./ml. extract was prepared by grinding the gland in 0·1N hydrochloric acid with 300 mg. of acid-washed sand per 100 mg. of gland. After centrifugation at 5000 r.p.m. for 2 minutes the supernatant fluid was withdrawn and stored in an airtight container at 4° until assay.

Methods of assay. All the adrenal gland extracts were assayed chromatographically and some were also assayed biologically.

The chromatographic method was similar to that employed by Shepherd and West[8], using *n*-butanol—acetic acid—water as the solvent and potassium iodate to locate the catechol amines. The standard solution used was a mixture of 50 μg. (−)-adrenaline and 50 μg. (−)-noradrenaline, as base, per ml. The standard solution and each extract of

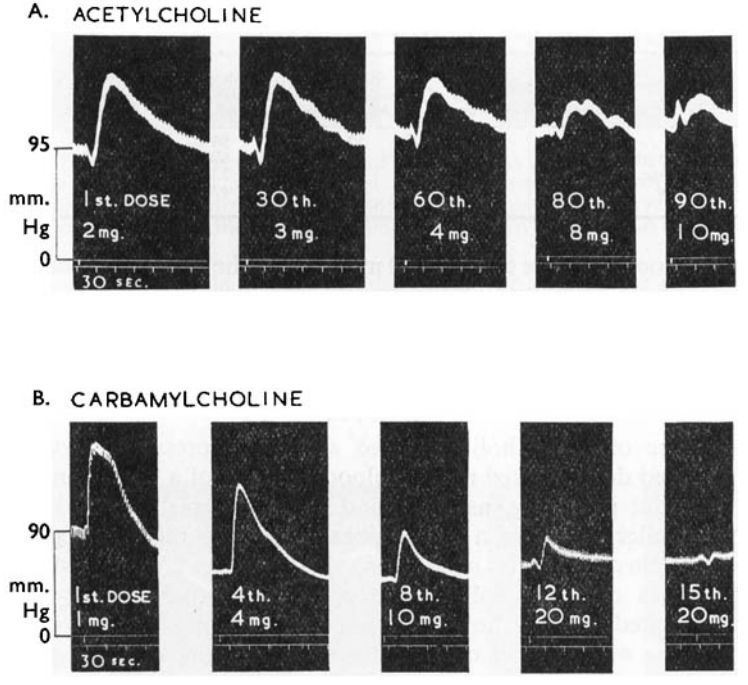

FIG. 2. A. Cat 3·6 kg. B. Cat 3·1 kg. Atropine, ether and chloralose as in Fig. 1. In both cats the left adrenal gland was removed. Fig. 2A shows the slow diminution in the response to acetylcholine. In comparison, Fig. 2B shows the rapid diminution in the response to carbamylcholine.

the glands were applied to the paper from Agla micrometer syringes. A range of spots was applied to each paper. The minimum volume used was 0·0025 ml. The maximum volume was 0·05 ml., applied in replicate drops of 0·01 ml., each spot being allowed to dry before the next spot was applied. When all the material had been applied, the papers were suspended in a glass tank and the solvent allowed to ascend for at least 15 hours. The papers were dried at 30°–40° for 10–15 minutes and then sprayed with 1 per cent (w/v) potassium iodate solution. The spots were located by placing the papers in an oven at 100°–110° for 2 minutes. Adrenaline and noradrenaline were rendered visible as pink and violet spots, having R_F values of 0·36 and 0·28 respectively. A direct comparison between the standard spots and those of the extract was made. At

least two separate assays of each gland were performed. The minimum amount of adrenaline and noradrenaline detectable varied slightly from experiment to experiment, ranging from 0·25 μg. to 1·0 μg.

The biological assays were made firstly, on the acutely denervated nictitating membrane of the cat and secondly, on the blood pressure of the same animal given hexamethonium bromide in a dose sufficient to

TABLE I

THE DEGREE OF DEPLETION OF CAT ADRENAL GLANDS, PRODUCED BY VARIOUS CHOLINE ESTERS

Choline ester	Number of experiments	Mean per cent loss		Range per cent loss	
		Adrenaline	Noradrenaline	Minimum	Maximum
Acetylcholine	15	50·4	50·1	7·1	86·5
Acetylcholine + physostigmine	12	60·1	62·8	35·7	83·5
Carbamylcholine	6	25·5	23·7	1·6	50·0
Benzoylcholine	5	46·7	47·2	26·1	67·6

lower the blood pressure to about 60 mm. Hg. The results were calculated by the formula of Bülbring[9] and were expressed, in terms of the laevo isomers of the base, as μg./gland, since Butterworth and Mann[10] have shown that this is the better method of calculation for cat adrenal glands.

RESULTS

Each dose of acetylcholine caused a marked pressor response. At first, repeated doses caused rises in blood pressure of a similar magnitude (Fig. 1A) but gradually, as the gland became depleted, the responses became smaller. If then a larger dose of acetylcholine was given the response returned to its initial magnitude (Fig. 2A). Eventually if enough doses of acetylcholine were given no further pressor response could be elicited. From the 15 experiments performed the mean depletion of adrenaline was 50·4 per cent and of noradrenaline was 50·1 per cent; there being no significant difference ($P > 0·9$) between the adrenaline depletion values and those of the noradrenaline. The range of depletion was wide; the minimum depletion obtained was 7·1 per cent and the maximum 86·5 per cent (Table I). The administration of physostigmine increased the sensitivity to acetylcholine 3 to 5 fold but again there was no significant difference ($0·7 > P > 0·6$) between the adrenaline and the noradrenaline depletion values (Table I).

Carbamylcholine, like acetylcholine, caused a pressor response. At the beginning of most experiments carbamylcholine was as active as acetylcholine in causing a release from the adrenal gland (Fig. 1B), but whereas repeated doses of acetylcholine gave rises in blood pressure of a similar magnitude, those to carbamylcholine rapidly diminished (Fig. 2B). Again there was no significant difference ($0·9 > P > 0·8$) between the adrenaline and the noradrenaline depletion values. However, due to the rapid decrease in the response to carbamylcholine, both the minimum and the maximum values were smaller than those for acetylcholine (Table I). Because of this rapid diminution in the carbamylcholine responses very

large doses (up to 1 g.) were eventually given, in some experiments, in an attempt to obtain a pressor response, but without success. This is in marked contrast to acetylcholine where there was never more than a 10-fold difference between the initial and the final doses. When carbamylcholine was no longer able to cause a pressor response, acetylcholine was

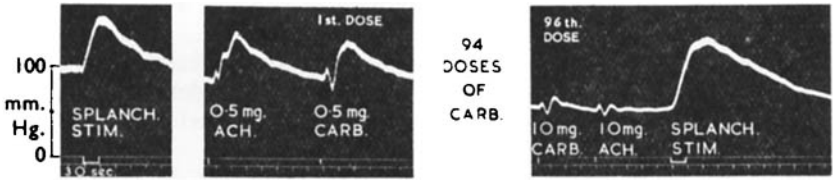

FIG. 3. Cat 3·4 kg. Atropine, ether and chloralose as in Fig. 1. The left adrenal gland was removed. A comparison of the vasopressor effects of acetylcholine (ACH.) and electrical stimulation of the greater splanchnic nerve (SPLANCH. STIM.) before and after the loss of response to carbamylcholine (CARB.).

ineffective also, but electrical stimulation of the splanchnic nerve caused as great a rise in blood pressure as it did initially (Fig. 3).

Benzoylcholine also caused a pressor response. Again there was no significant difference ($0{\cdot}6 > P > 0{\cdot}5$) between the adrenaline and the noradrenaline depletion values. The mean degree of depletion was similar to that for acetylcholine (Table I).

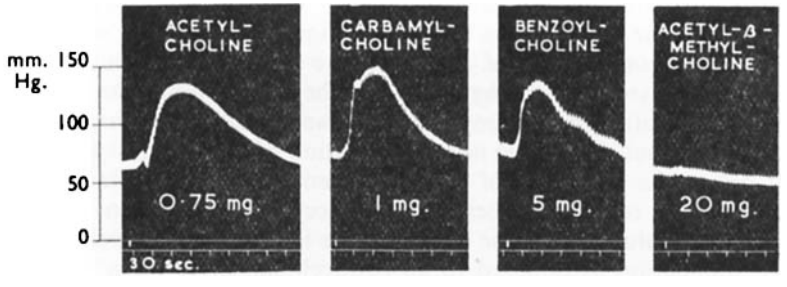

FIG. 4. Typical vasopressor responses to various choline esters given intravenously, at the beginning of each experiment, to atropinised, unilaterally adrenalectomised cats.

The fact that acetyl-β-methylcholine is incapable of producing a rise in blood pressure[11] was confirmed. Doses of up to 20 mg. had no effect. Figure 4 shows a comparison of the 4 choline esters studied.

DISCUSSION

In all experiments there was a similar depletion of adrenaline and noradrenaline. Thus, neither acetylcholine, carbamylcholine nor benzoylcholine caused a preferential loss of either amine. This similar percentage loss of amines was irrespective of the overall degree of depletion and of the actual amount (per cent) of noradrenaline present in the glands.

Although the chromatographic method of estimating the concentration of adrenaline and noradrenaline was suitable for this work, it proved to have certain disadvantages. The main disadvantage is that it is not possible to determine the accuracy of the result. Thus in some of the work biological assay methods were employed. An attempt to increase the clarity of the spots was made by previously soaking the chromatographic paper either in a 1 mg./ml. solution of ascorbic acid or in 0·01N hydrochloric acid. But little improvement was obtained. One advantage of the chromatographic method is that it is possible to detect the presence of other catechol amines; but in no experiment were any detected. Assays performed biologically gave results similar to those performed chromatographically.

There was no precise relation between the number of doses of choline ester and the degree of depletion obtained. Obviously there cannot be, since the amount of amine liberated by each dose of choline ester varied from cat to cat and also as the experiment proceeded. As might be expected, the rises in blood pressure produced by the choline esters became smaller as the doses were repeated. This was due to the liberation of less amine from the gland by each successive dose and not to a reduction in the sensitivity to adrenaline or noradrenaline. With acetylcholine and benzoylcholine this effect developed very gradually and the initial size of the response could be maintained if the dose was increased. With carbamylcholine the decrease in the response was much more rapid. This rapid reduction in the response made it impossible to deplete the glands to the same extent as with acetylcholine. This decrease in response to carbamylcholine was not due to deterioration of the drug in solution, to incomplete atropinisation of the cat or to tachyphylaxis but was due presumably to some "blocking" action of the drug. When the gland was no longer sensitive to carbamylcholine it was found to be insensitive to acetylcholine but splanchnic nerve stimulation caused as great a response as it did at the beginning of the experiment. Possible explanations of this difference could be either that the electrical stimulation is a more powerful stimulus or that the acetylcholine is liberated intracellularly on nerve stimulation as opposed to an extracellular effect when it is given intravenously. It is of interest that Eade[12] has found carbamylcholine to be ineffective as a releaser of catechol amines from chromaffin granules. He suggests that the action of choline esters on the chromaffin cell is an action on the cell membrane; possibly causing an increase in the permeability of the membrane. This would allow the amines present in the cytoplasm to leave the cell. He found no direct effect of the choline esters on the storage granules. It is hoped that further work will explain this difference between carbamylcholine and the other choline esters.

Acknowledgements. We wish to thank Professor G. A. H. Buttle for helpful criticism of the work and Mr. J. Conway for skilled technical assistance.

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