Brit. J. Pharmacol. (1954), 9, 481.

THE ACTION OF HISTAMINE AND PILOCARPINE ON THE SUPERIOR CERVICAL GANGLION AND THE ADRENAL GLANDS OF THE CAT

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(RECEIVED JULY 16, 1954)

Dale and Laidlaw (1912) demonstrated stimulation of the adrenal glands of the cat by pilocarpine, and Burn and Dale (1926) observed it after the injection of histamine. This action on the adrenal glands by both histamine and pilocarpine is partly responsible for the contractions of the normal and denervated nictitating membranes of the intact cat. But another factor seems to be involved in the response of the membranes, as Burn and Trendelenburg (1954) observed that sometimes the normal membrane responded more than the denervated membrane and that in some experiments the membranes responded after adrenalectomy. By perfusing the head of the cat with a Locke-dextran solution they demonstrated that both histamine and pilocarpine had no direct action on the nictitating membranes. The superior cervical ganglion of the normal side was not removed in their experiments on the whole animal and it thus seemed possible that these substances stimulated the ganglion.

Dale and Laidlaw (1912) demonstrated the stimulation of the superior cervical ganglion of the cat by pilocarpine in the whole cat, and Ambache (1949) confirmed this in the perfused preparation. Histamine, on the other hand, was found by Feldberg and Vartiainen (1935) not to stimulate the perfused superior cervical ganglion, and Konzett (1952) confirmed this for most of his experiments. He observed, however, a stimulation of the ganglion in 4 out of 22 preparations.

These mostly negative findings obtained with perfused preparations stand in contrast to the ability of histamine to stimulate the adrenal glands which are left in the normal circulation (Szczygielski, 1932). It was therefore decided to re-investigate the question of a stimulation of sympathetic ganglia by histamine and pilocarpine, using preparations in which the normal blood supply of the superior cervical ganglion was not interfered with.

METHODS

Cats of 2 to 4 kg. were used. After inducing anaesthesia with ether, 80 mg./kg. chloralose was injected intravenously. Intra-arterial injections were made into the central end of the lingual artery while occluding the external carotid artery. Thus the injected substance was diverted towards the superior cervical ganglion. With the exception of the internal carotid artery which was occasionally found and tied, no further attempts at isolation were made.

The contractions of the nictitating membrane (and in some experiments of both membranes) were recorded with an isotonic lever fitted with a frontal writing point magnifying the movements of the membrane 7.5 times. The cervical sympathetic chain was cut. For preganglionic stimulation it was placed on a pair of shielded electrodes and covered with warm liquid paraffin. The stimulus had a frequency of 17/sec. and a duration of 0.7 msec. The blood pressure was recorded from either the carotid artery of the other side or from the femoral artery.

In some experiments spinal preparations were used, set up as described by Burn and Trendelenburg (1954). As the results with these preparations did not differ from those obtained under chloralose anaesthesia, they will not be mentioned separately.

For arterial injection to adrenal glands, cats under chloralose anaesthesia were eviscerated and a cannula was tied into the central end of the coeliac artery. The aorta was tied below the kidneys. The blood pressure was recorded from the carotid artery.

The following substances were used: histamine dihydrochloride or histamine acid phosphate, dissolved in saline and neutralized with N/10 NaOH, the dose being expressed as free base; pilocarpine nitrate, the dose being expressed as free base; hexamethonium bromide, nicotine hydrogen tartrate, cocaine hydrochloride, atropine sulphate, mepyramine maleate, all expressed as salts.

RESULTS

Intravenous Injections

Stimulation of the superior cervical ganglion of a cat under chloralose anaesthesia by intravenous injection of histamine is demonstrated in Fig. 1,

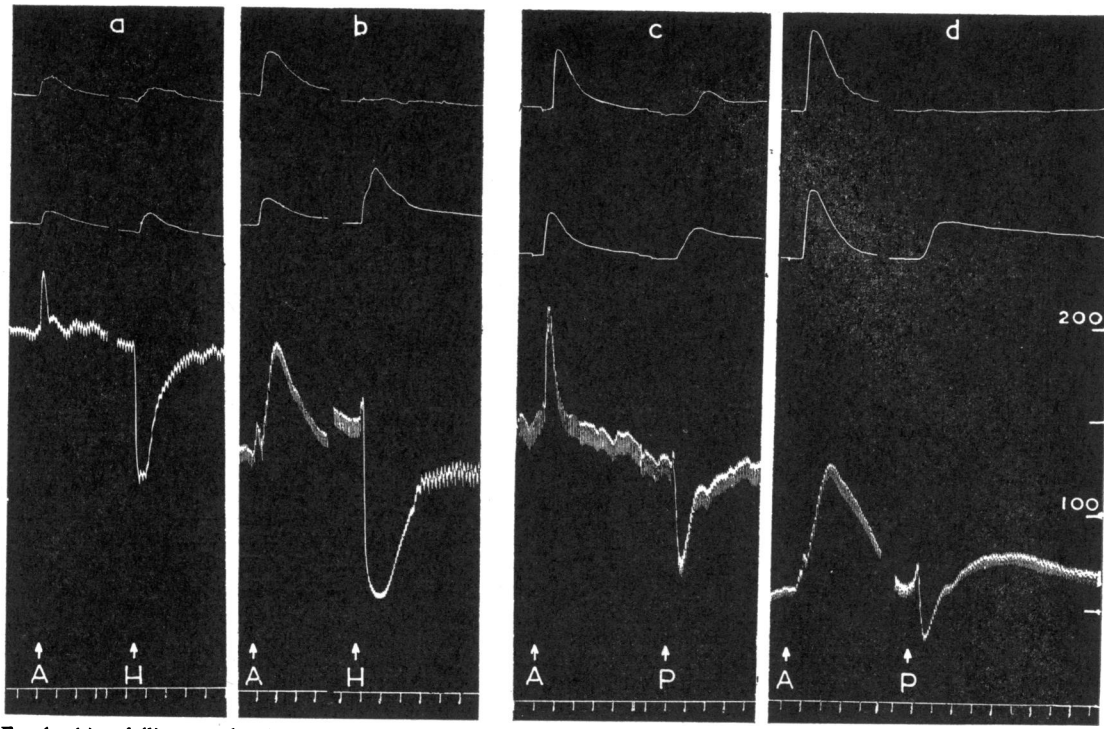

FIG. 1.—(*a*) and (*b*), cat under chloralose; (*c*) and (*d*), spinal cat. Records from above are: acutely denervated nictitating membrane; normal nictitating membrane with its preganglionic fibres cut; arterial blood pressure; time in 30 sec. Intravenous injections were: A, 5 μg. adrenaline; H, in (*a*) 65 μg. histamine; in (*b*), 130 μg. histamine; P, 150 μg. pilocarpine. Adrenalectomy and evisceration between (*a*) and (*b*) and between (*c*) and (*d*). Note stronger contraction of normal side where the ganglion was left intact.

sections (a) and (b). Throughout the experiment injections of 5 μg. adrenaline (A) were given as a control. Section (a) shows equal contractions of the acutely denervated and the normal nictitating membrane elicited by the injection of 65 μg. histamine at H ; section (b) the effect of twice that dose after adrenalectomy and evisceration: the denervated membrane no longer contracted, whereas a large contraction was elicited on the innervated side. The preganglionic fibres of the innervated side were cut in order to exclude central impulses.

Sections (c) and (d) show similar results on a spinal cat given 150 μg. pilocarpine at P intravenously, (c) before, and (d) after adrenalectomy and evisceration.

Intra-arterial Injections

To obtain stimulation of the superior cervical ganglion by intravenous injections, large amounts of histamine were required, and from these recovery of the blood pressure and of sensitivity of the ganglion was slow. The method of intra-arterial injection into the lingual artery was therefore adopted for the following experiments.

Intra-arterial injections of 2–55 μg. histamine (using histamine dihydrochloride in most of the experiments), dissolved in 0.2 to 0.3 ml. of saline and neutralized, caused a contraction of the nictitating membrane in 23 out of 28 cats. The usual dose was 15–20 μg. histamine and contractions of the membrane from 4 to 45 mm. (on the drum) were recorded. Increasing responses were observed with increasing doses. It was found that desensitization regularly occurred when injections were repeated. Care was therefore taken in each experiment to find the suitable interval between injections and to keep this interval constant. It varied from 5 to 30 min.

Intra-arterial injections of 25–40 μg. pilocarpine were found to stimulate the superior cervical ganglion in 6 out of 10 cats. The responses were very similar to those produced by histamine, but they were of longer duration (see Figs. 1 and 2) and desensitization was still more marked ; time intervals from 20–40 min. between successive injections were necessary.

As it was observed that the latent period between the intra-arterial injection and the response of the

membrane was considerably longer than that observed after intra-arterial injection of acetylcholine or nicotine, it was necessary to show that this response of the membrane was of ganglionic origin. There were three possible sources of error: (1) the stimulation of the ganglion was unspecific; (2) the contraction of the membrane was due to stimulation of the adrenal glands; and (3) the substances had an action on the membrane itself. In order to exclude these possibilities the following experiments were carried out.

(1) Control injections of saline into the lingual artery were without any effect. All solutions were carefully neutralized and the use of histamine dihydrochloride excluded a stimulation of the ganglion by the phosphate present in histamine acid phosphate.

(2) When comparing the effect of intra-arterial with intravenous injections of equal amounts of histamine or pilocarpine, it was found that up to 50 μg. of these substances injected intravenously had very little or no effect at all on the nictitating membrane (Fig. 2). Adrenalectomy did not modify the response to intra-arterial histamine or pilocarpine if the injection was made rather soon after the operation. When recording both membranes it was observed that only the membrane of the injected side contracted. Removal of the superior cervical ganglion or cutting of the post-

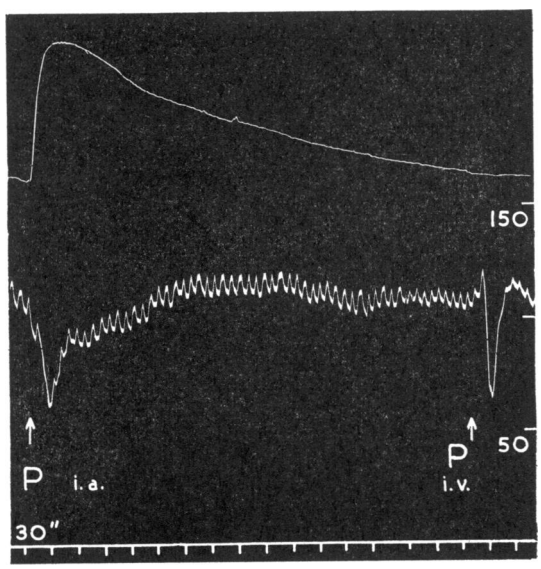

FIG. 2.—Cat under chloralose. Normal nictitating membrane and arterial blood pressure. Injection of 40 μg. pilocarpine (P) into the lingual artery during occlusion of the external carotid artery (i.a.) and intravenously (i.v.). Note duration of ganglionic response and absence of response to intravenous injection.

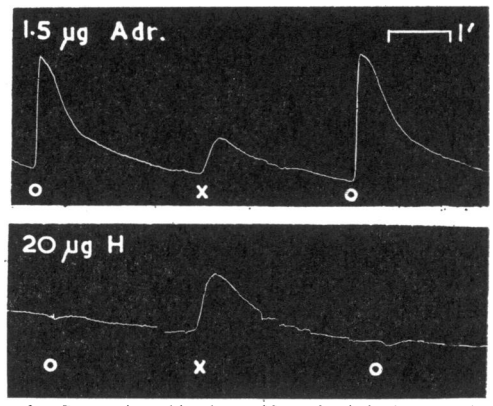

FIG. 3.—Cat under chloralose. Normal nictitating membrane. Upper record: injection of 1.5 μg. adrenaline into the lingual artery without occlusion (o) and during occlusion (×) of the external carotid artery. Lower record: injection of 20 μg. histamine into the lingual artery without occlusion (o) and during occlusion (×) of the external carotid artery. Note the direct action of adrenaline on nictitating membrane in contrast to ganglionic stimulation by histamine.

ganglionic fibres abolished the response to intra-arterial injections of histamine or pilocarpine.

(3) As no attempt was made to isolate the superior cervical ganglion, the injected histamine or pilocarpine might act directly on the nictitating membrane. Burn and Trendelenburg (1954) demonstrated that both substances were unable to contract the nictitating membrane in a perfused preparation. In order to confirm this for membranes the circulation of which was left undisturbed, the following experiment was performed. An injection of 20 μg. histamine was made into the lingual artery (a) when the external carotid artery was occluded and (b) when it remained open. The injection caused a contraction of the nictitating membrane when injected during occlusion of the external carotid artery, but not when it was unoccluded, as Fig. 3 shows. Adrenaline, on the other hand, readily elicited a contraction in as low a dose as 1.5 μg. when the external carotid artery was open, but had a much smaller action during occlusion. Pilocarpine was found to have similar actions to those of histamine, thus confirming earlier observations.

These findings indicate that the contraction observed after intra-arterial injection of a neutralized solution of histamine or pilocarpine was due to stimulation of the superior cervical ganglion.

Hexamethonium.—Intravenous injections of up to 20 mg. hexamethonium were repeatedly found to have no effect on the ganglionic response to histamine or pilocarpine, or to reduce it but slightly. One experiment, shown in Fig. 4, may illustrate this. After two control injections of

20 μg. histamine the cervical sympathetic chain was stimulated and the strength of the stimulus was chosen so as to give a contraction of approximately the same height. When 5 mg. hexamethonium was injected intravenously the effect of pre-

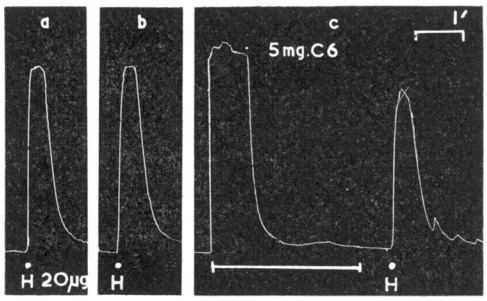

FIG. 4.—Cat under chloralose. Normal nictitating membrane Injection of 20 μg. histamine into the lingual artery during occlusion of the external carotid artery (H). 20 min. interval between (*a*) and (*b*) and (*b*) and (*c*). In (*c*) submaximal preganglionic stimulation (——) and i.v. injection of 5 mg. hexamethonium bromide. Note that response to histamine was not much affected by hexamethonium in a dose which blocked transmission.

ganglionic stimulation was abolished completely, but the response to 20 μg. histamine was only slightly depressed. Similar experiments were carried out with pilocarpine and similar results were obtained. Control experiments showed that in atropinized cats these doses of hexamethonium regularly abolished the response to intra-arterial injections of 25–100 μg. acetylcholine. Thus it was found that hexamethonium in doses which blocked both transmission and injected acetylcholine had but a very slight effect on the response to histamine and pilocarpine.

Nicotine.—In some experiments nicotine was given intravenously, paralysing ganglia by successive injections of increasing doses (0.5 to 10 mg.) and repeating the last dose until there was neither a response of the nictitating membrane nor a rise of blood pressure. Histamine or pilocarpine then injected intra-arterially into the lingual artery always failed to stimulate the superior cervical ganglion. In other experiments 6 to 8 injections of 50 μg. nicotine each were made into the lingual artery within 2–3 min. There was a response of the ganglion to the first three injections, the ganglion then being paralysed. The next injection of histamine or pilocarpine always failed to stimulate the superior cervical ganglion and partial recovery was observed 30 min. later. In some experiments it was observed that 30–60 min. after nicotine the response to histamine or pilocarpine was much larger than that to the control injections before any nicotine had been given.

Cocaine.—This substance was found to reduce the action of histamine and pilocarpine on the superior cervical ganglion, as Fig. 5 illustrates. After 2 control injections of 20 μg. histamine (sections (a) and (b)) preganglionic stimulation

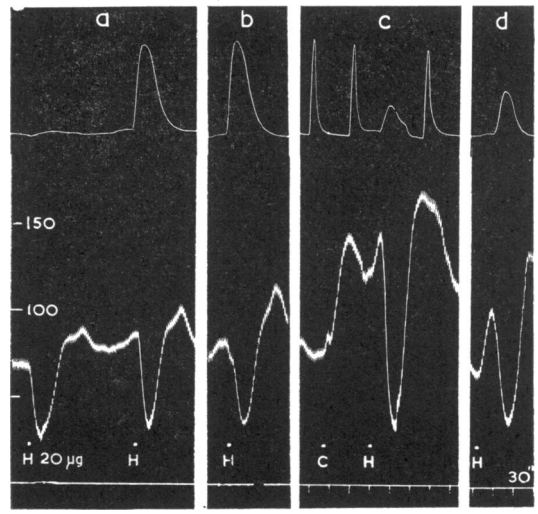

FIG. 5.—Spinal cat. Normal nictitating membrane and arterial blood pressure. (*a*) Injection of 20 μg. histamine into the lingual artery, first without and then with occlusion of external carotid artery. (*b*) As second injection in (*a*) after 20 min., (*c*) 20 min. later: 5 sec. of submaximal preganglionic stimulation, then i.v. injection of 0.5 mg. cocaine hydrochloride, followed by preganglionic stimulation, injection of 20 μg. histamine into the lingual artery and preganglionic stimulation. (*d*) 20 min. later. Note that the response to histamine was reduced by an amount of cocaine which did not affect preganglionic stimulation.

was applied for 5 sec., the strength of stimulus being chosen so as to give a submaximal contraction of similar height. After intravenous injection of 0.5 mg. cocaine the response to preganglionic stimulation was unaltered whereas the response to histamine was much reduced. Partial recovery was observed after 18 min. There was no diminution of the fall of blood pressure after histamine. In another experiment the intramuscular injection of 20 mg. cocaine abolished the response to 2 μg. histamine for more than 4 hr., 20 μg. histamine starting to elicit a response after 160 min. Furthermore it was found that the ganglionic response to pilocarpine was also reduced by cocaine in doses which did not interfere with the response to preganglionic stimulation. Control experiments showed that in atropinized cats small amounts of cocaine did not depress the response to intra-arterial injections of acetylcholine whereas 2.5 mg. cocaine or more potentiated the response.

Mepyramine.—Increasing amounts of the antihistamine substance mepyramine reduced and

finally abolished the response to 20 μg. histamine, as shown in Fig. 6. Preganglionic stimulation, recorded before and after injection of the highest dose of mepyramine, was not affected. 150 μg. mepyramine was found to block both the ganglionic and the blood-pressure responses to 20 μg. histamine. Recovery was observed after 40 min. but was not complete. Pilocarpine, on the other hand, still exerted its ganglion-stimulating action after 500 μg. mepyramine.

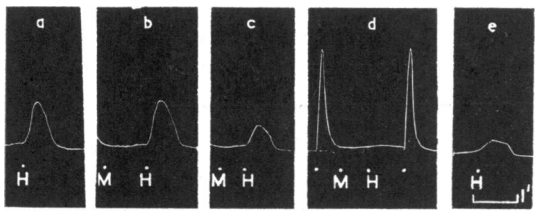

FIG. 6.—Spinal cat. Normal nictitating membrane. H, 20 μg. histamine injected into lingual artery during occlusion of external carotid artery. Time intervals between tracings 20 min. Mepyramine maleate (M) i.v.: 20 μg. in (*b*), 60 μg. in (*c*), and 150 μg. in (*d*). In (*d*) submaximal preganglionic stimulation for 5 sec. before and after injection of mepyramine. Note that an amount of mepyramine which did not affect preganglionic stimulation abolished response to histamine.

Atropine.—This substance in doses up to 2 mg. had no effect on the ganglionic response to histamine, whereas 200 μg. atropine abolished the response to pilocarpine for about 3 hr.

Stimulation of Adrenal Medulla

Injections of 0.13 to 3.5 μg. histamine into the central end of the coeliac artery led to a rise of blood pressure of 15–50 mm. Hg, due to stimulation of the adrenal glands. A constant response was obtained if a time interval of at least 10 min. was allowed between injections. Desensitization was evident with shorter time intervals, the blood-pressure response to a given dose of histamine declining the shorter the interval.

Pilocarpine had a very similar action, but greater amounts were required (7.5–40 μg.). This was not expected, as the ratio of equiactive doses of intralingual injections of histamine and pilocarpine was *ca.* 1:2. These figures were approximate and were obtained from comparisons in several experiments; it was unfortunately not possible to compare the ganglionic effects of both drugs in the same animal. As desensitization was much less pronounced after stimulation of the adrenal glands by histamine or pilocarpine, it was possible to find equiactive doses of these two substances. In 3 cats ratios of 1:57, 1:57 and 1:43 were found, thus showing that pilocarpine was considerably less active on the adrenal glands than histamine.

Hexamethonium.—Hexamethonium in intravenous doses up to 54 mg. increased the pressor response to histamine in proportion as it increased the response to adrenaline or noradrenaline.

Nicotine.—Szczygielski (1932) observed that paralysing doses of nicotine reduced or abolished the pressor rise after intra-arterial injection of histamine. The same was found to be true of pilocarpine, as Fig 7 shows. After 14 intra-arterial injections of 50 μg. nicotine each, the blood-pressure rise after 45 μg. pilocarpine was less than half of the control, full recovery being observed 15 min. later (Fig. 7 (b)).

Cocaine was injected intravenously in doses up to 10 mg., and always potentiated the responses to both histamine and pilocarpine as well as to control injections of adrenaline. There was no sign of a depression of the stimulation of the adrenal glands by histamine or by pilocarpine (Fig. 8). This was also observed in preparations the splanchnic nerves of which had been freshly cut.

Mepyramine, which was found by Emmelin and Muren (1949) to block the action of histamine on the adrenal glands in as low a dose as 10 μg., did not modify the response to pilocarpine. The amount of mepyramine injected intravenously was 300 μg. (Fig. 7 (c)).

Atropine in a dose of 400 μg. abolished the response to pilocarpine (Fig. 7 (d)) without interfering with the action of histamine.

These experiments showed that with one exception there was general agreement between the

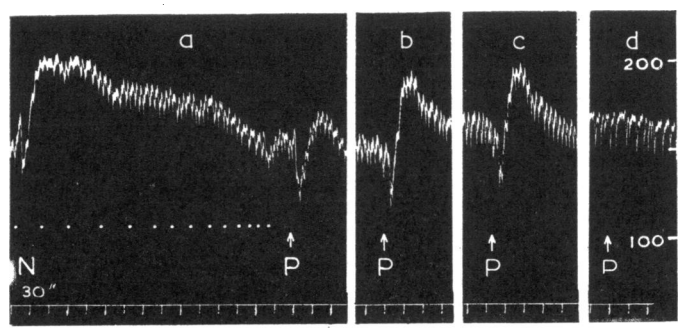

FIG. 7.—Cat under chloralose, eviscerated, and with aorta tied below kidneys. (*a*) 14 injections, each of 50 μg. nicotine tartrate into coeliac artery, followed by intra-arterial injection of 45 μg. pilocarpine (P). (*b*) 15 min. later. (*c*) 15 min. later after 300 μg. mepyramine i.v. (*d*) After 400 μg. atropine sulphate i.v. Note that nicotine reduced the response of the adrenal glands to pilocarpine; that mepyramine did not affect the response, whereas atropine abolished all actions of pilocarpine.

characteristic properties of the superior cervical ganglion and the adrenal glands, as far as the actions of histamine and pilocarpine were concerned. The only substance which exhibited a discrepancy was cocaine, blocking the superior cervical ganglion in very low doses and not modifying the liberation of pressor amines from the adrenal glands by histamine or pilocarpine even after 20-fold doses.

Discussion

The action of histamine and pilocarpine on the nictitating membrane is well known to be due in part to an action on the adrenal glands. The experiments, in which Dale and Laidlaw (1912) demonstrated that pilocarpine had an action on the superior cervical ganglion also, have escaped similar attention. Evidence has now been obtained confirming this action of pilocarpine and showing that histamine also exerts a direct stimulant action on the ganglion. The varying results seen on the nictitating membranes with both these substances (Burn and Trendelenburg, 1954) thus receive an explanation. The frequent observation that the denervated membrane contracts more than the normal membrane may be explained by the release of adrenaline and noradrenaline from the adrenal glands producing a large effect on the denervated membrane, an effect which is increased by cocaine and abolished by adrenalectomy. If, however, the superior cervical ganglion is acutely removed in the course of an experiment, histamine then has been shown to have a greater effect on the innervated side, and this effect persists after adrenalectomy and is abolished by cocaine. It is due to a stimulation of the ganglion itself, since it is seen when small quantities of histamine (or pilocarpine) are injected into the lingual artery during occlusion of the external carotid artery, and it is not seen when the external carotid remains open, leaving a direct pathway to the nictitating membrane.

The actions of histamine and of pilocarpine on the ganglion are similar in being paralysed by excess of nicotine and by cocaine, and in being unaffected by hexamethonium. They are dissimilar in that the action of histamine is blocked by mepyramine but not by atropine, whereas the reverse is true for pilocarpine.

The adrenal glands responded to low doses of histamine and to moderate doses of pilocarpine when these substances were injected into the central end of the coeliac artery after tying the aorta below the kidneys. Desensitization to repeated injections was much less prominent than in the superior cervical ganglion. The only difference between the actions of these substances was quantitative. Whereas they seemed to be roughly equiactive when injected into the lingual artery, pilocarpine was found to have only one-fiftieth of the activity of histamine on the adrenal glands.

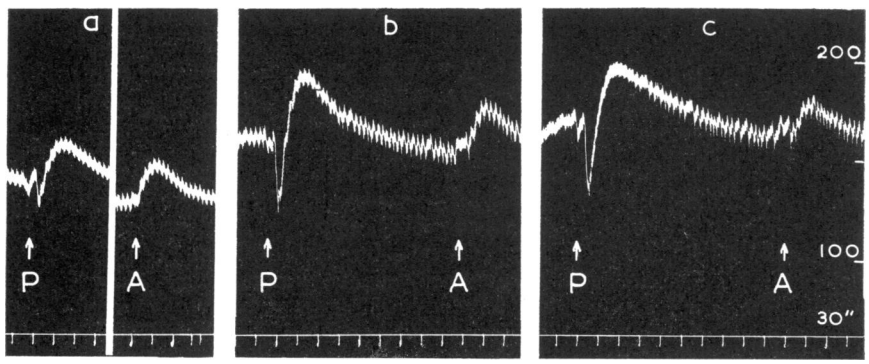

FIG. 8.—Cat under chloralose, eviscerated, and with aorta tied below kidneys. Arterial blood pressure. P, 45 μg. pilocarpine injected into central end of coeliac artery. A, 0.2 μg. adrenaline i.v., (*a*) before, (*b*) after 1 mg. cocaine i.v., and (*c*) after 5 mg. cocaine i.v. Note that ten times more cocaine than used in Fig. 5 did not reduce the response of the adrenal glands to pilocarpine.

The finding that hexamethonium on the superior cervical ganglion readily blocked both transmission and the response to injected acetylcholine without interfering with the response of the superior cervical ganglion or of the adrenals to histamine or pilocarpine indicated that these substances have a direct action on the ganglion cell, acetylcholine not being involved.

Szczygielski (1932) found that the adrenal glands were very sensitive to histamine and that nicotine abolished the response to small doses of histamine. In the present series of experiments it was found that nicotine blocked the effect of histamine and

pilocarpine on the superior cervical ganglion and that it reduced the response of the adrenal glands to intra-arterial injection of pilocarpine.

Cocaine was found to potentiate the blood-pressure rise after injection of either histamine or pilocarpine into the coeliac artery, but to block the action of these substances on the superior cervical ganglion. This blocking action of cocaine needs further investigation.

When comparing the response of the superior cervical ganglion to either histamine or pilocarpine with the stimulation of the ganglion by acetylcholine or nicotine, three observations were consistently made: the latency period between the injection of histamine or pilocarpine and the onset of contraction of the nictitating membrane was longer, and both the contraction and the desensitization were of much longer duration than those observed after approximately equiactive doses of acetylcholine or nicotine.

With our present knowledge it is impossible to answer the question why histamine usually failed to stimulate the perfused superior cervical ganglion (Feldberg and Vartiainen, 1935; Konzett, 1952) while stimulating the ganglia the circulation of which is intact. Whatever the reasons for this discrepancy, histamine must be regarded as a substance able to exert an influence on the autonomic nervous system of the cat. Various adrenaline-like effects of histamine require consideration from this point of view. It is not known whether the liberation of histamine from tissues in the body by drugs or under certain conditions such as anaphylaxis or shock may lead to a change in excitability of the autonomic nervous system, but it is of interest that Chauchard and Mazoué (1954) detected, by measurements of chronaxie, an increased excitability of the sympathetic nervous system during anaphylactic reactions.

Summary

1. By comparing the response of a normal nictitating membrane with that of an acutely denervated one to intravenous injections of histamine or pilocarpine, a stimulation of the superior cervical ganglion was demonstrated in intact and adrenalectomized cats.

2. Intra-arterial injections of histamine or pilocarpine into the lingual artery during occlusion of the external carotid artery were found to stimulate the superior cervical ganglion without interference from unspecific factors or liberation of medullary amines from the adrenal glands.

3. It was confirmed that both histamine and pilocarpine have no direct action on the nictitating membrane.

4. Ganglionic responses to histamine or pilocarpine were found to show rapid desensitization. They were not abolished by doses of hexamethonium which abolished preganglionic stimulation. They were abolished by paralysing doses of nicotine. They were abolished by cocaine in doses which did not interfere with preganglionic stimulation. Atropine specifically inhibited pilocarpine; mepyramine equally specifically inhibited histamine.

5. Injections of histamine or pilocarpine into the central end of the coeliac artery were found to stimulate the adrenal glands. The substances mentioned under 4 were tested and were found to have the same actions, except that cocaine did not interfere with stimulation of the adrenal glands.

I wish to express my thanks to Professor J. H. Burn for his advice and guidance throughout this work.

The experiments were carried out during the tenure of a British Council scholarship.

References

Ambache, N. (1949). *J. Physiol.*, **110**, 164.
Burn, J. H., and Dale, H. H. (1926). Ibid., **61**, 185.
—— and Trendelenburg, U. (1954). *Brit. J. Pharmacol.*, **9**, 202.
Chauchard, B. P., and Mazoué, H. (1954). *J. Physiol. Path. gén.*, **46**, 99.
Dale, H. H., and Laidlaw, P. P. (1912). *J. Physiol.*, **45**, 1.
Emmelin, N., and Muren, A. (1949). *Acta physiol. scand.*, **17**, 345.
Feldberg, W., and Vartiainen, A. (1935). *J. Physiol.*, **83**, 103.
Konzett, H. (1952). *J. Mt. Sinai Hosp.*, **19**, 149.
Szczygielski, J. (1932). *Arch. exp. Path. Pharmak.*, **166**, 319.