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THE ACTION OF ANTAGONISTS OF ACETYLCHOLINE ON THE VESSELS OF THE RABBIT'S EAR

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Our interest in the work to be described began with an observation made in this laboratory by H. W. Ling, that when the vessels of a dog's hind leg were perfused with defibrinated blood containing adrenaline, the injection into the cannula of atropine sulphate caused vasodilatation and increased blood flow through the leg. The observation led to an investigation of the vascular action of atropine by Bussell (1940), who found in the course of his observations that the constrictor action of adrenaline on the vessels of the rabbit's ear, perfused with Locke's solution, was abolished by atropine, though atropine did not affect the constrictor action of posterior lobe extract.

Since that work was done, fresh light has been thrown on the action of atropine by the work of Dawes (1946), who showed that it exerted a quinidine-like action on the rabbit auricles, and that its properties were shared in varying degrees by other substances, including local anaesthetics such as procaine, and the analgesic pethidine. These substances revealed themselves as antagonists of acetylcholine, and a comparison of the properties of some of them was made by de Elío (1948); a study of their effect on body temperature was also made by Dutta (1948). It remained to investigate their vascular action and to observe in particular whether like atropine they would abolish the constrictor action of adrenaline in the vessels of the rabbit's ear. The substances used have been procaine, pethidine (demerol), benadryl, quinidine, and atropine, though a few observations have also been made with antistin, anthisan (neoantergan), and *iso*conessine. Since Gowdey (1948) had observed that priscol (benzylimidazoline) reversed the action of adrenaline in the rabbit's ear, we studied the effect of this substance also.

METHODS

All observations were made on the vessels of the rabbit's ear perfused with Locke's solution. The method of Gaddum and Kwiatkowski (1938) was used first in order to stimulate the postganglionic sympathetic fibres. Later experiments were carried out by perfusing the ear severed from the head according to the method of Rischbieter (1913) (originally introduced by Bissemski). The outflow recorder described by Stephenson (1948) was found well suited to record small changes in the diameter of the vascular bed, which would not have been observed using the drop-timer.

RESULTS

The abolition of the vasoconstrictor action of adrenaline

Injections of atropine sulphate, benadryl, pethidine hydrochloride (demerol), procaine hydrochloride, and quinidine hydrochloride were found to diminish or abolish not only the vasoconstriction produced by the subsequent injection of adrenaline but also that produced by sympathetic nerve stimulation. These effects are illustrated in Figs. 1 and 2. In Fig. 1 the effect of 200 μg. pethidine was transitory, being present 2 min. after its injection but absent 8 min. later. In Fig. 2 the effect of 500 μg. quinidine was still present, though fading, 28 min. after its injection. C. S. Jang (1940) had previously observed that procaine antagonizes adrenaline and sympathetic nerve stimulation on blood vessels. The amounts of the different substances required to reduce the effect of sympathetic nerve stimulation were in general the same as those required to reduce the effect of adrenaline, but this was not true for atropine. Whereas 5 μg. atropine abolished the effect of

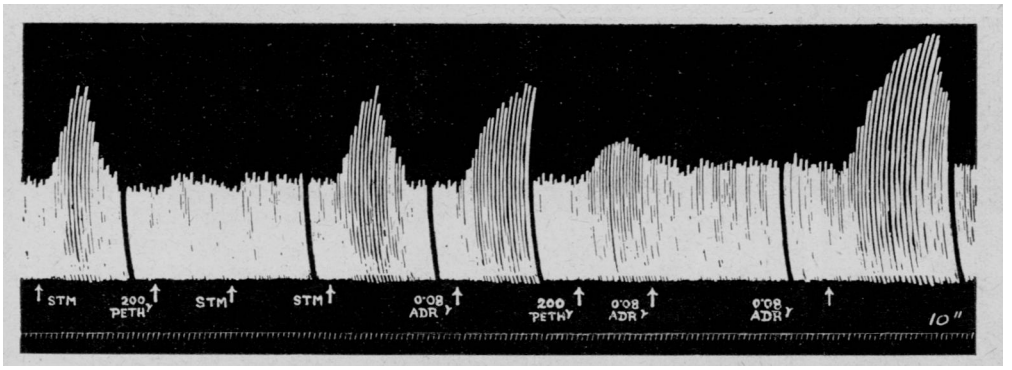
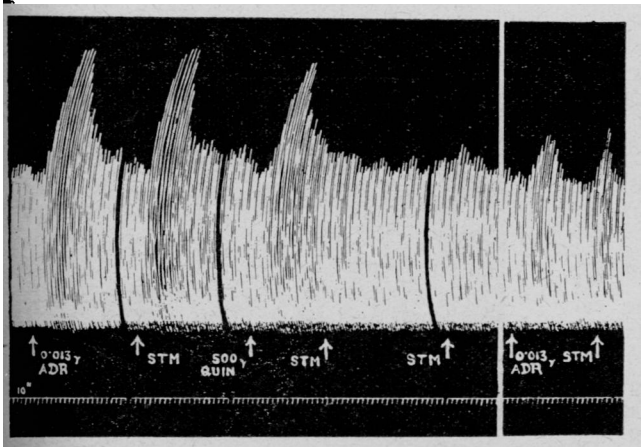


FIG. 1.—Rabbit ear perfusion using Gaddum's drop-timer. Vasoconstriction produced by sympathetic stimulation; after injection of 200 μg. pethidine, one stimulation was without effect; the next stimulation was again effective. Vasoconstriction produced by 0.08 μg. adrenaline; 200 μg. pethidine when injected caused some vasoconstriction; the next injection of 0.08 μg. adrenaline was without effect, but the succeeding injection produced augmented vasoconstriction.

TABLE I

Adrenaline hydrochloride	Histamine acid phosphate	Vasoconstriction abolished by
0.015 μg.	0.2 μg.	5 μg. pethidine hydrochloride 10 μg. „ „
0.1 μg.	0.5 μg.	0.5 mg. procaine hydrochloride 0.2 mg. „ „
0.01 μg.	0.01 μg.	4 μg. benadryl 0.02 μg. „
0.075 μg.	0.2 μg.	30 μg. quinidine hydrochloride 0.3 mg. „ „
0.015 μg.	0.2 μg.	4 μg. atropine sulphate 6 μg. „ „



2.—Record as Fig. 1. Vasoconstriction produced by 0.013 μg. adrenaline and also sympathetic stimulation. Injection of 500 μg. quinidine caused vasoconstriction, and then stimulation was twice ineffective. Later 0.013 μg. adrenaline caused much less vasoconstriction than before, and stimulation a similar reduced effect.

adrenaline, 2 mg. was required to abolish that of sympathetic nerve stimulation.

Observations were also made on the vasoconstriction produced by histamine; this was also abolished by these substances. The figures given in Table I illustrate the order of the amounts required to abolish the effects of adrenaline and of histamine. Against adrenaline, atropine, benadryl, and pethidine were strongest, quinidine next, and procaine weakest. Against histamine, benadryl was strongest, then atropine and pethidine, and finally procaine and quinidine weakest. Anthisan (neoantergan) and antistin were also observed to abolish the constrictor action of adrenaline.

The abolition of the vasodilator action of adrenaline

The reversal of the constrictor action of adrenaline to a dilator action by perfusing priscol in a concentration of 0.2 mg./ml. (Gowdey, 1948) made it possible for us to examine the effect of these compounds on the dilator action of adrenaline. For these experiments we used Stephenson's recorder. We observed that atropine, benadryl, and pethidine abolished the vasodilator action; the effect of pethidine is illustrated in Fig. 3. We were not able to observe a similar action of procaine and of quinidine because when injected in the presence of priscol they themselves caused vasodilatation, which made it difficult to determine the effect of adrenaline. When, however, a succession of small doses of quinidine was injected, it was possible to see

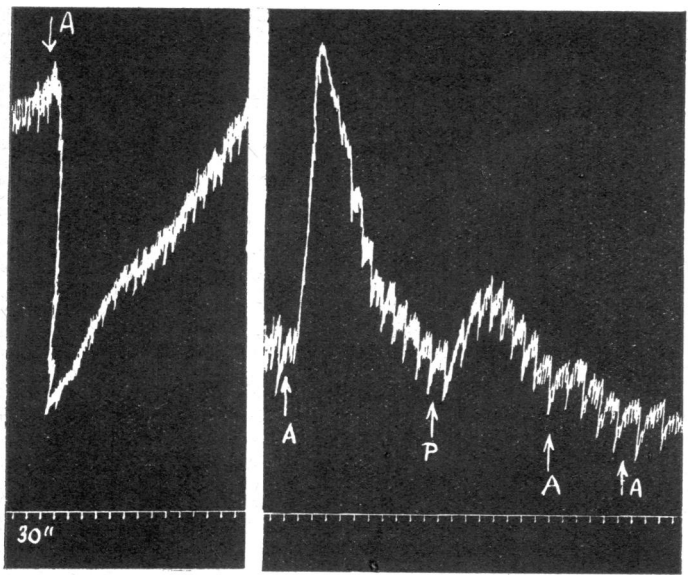


FIG. 3.—Outflow record from ear using Stephenson's recorder. Vasoconstriction due to 0.004 μg. adrenaline (A). Between t e two parts of the record priscol was added to perfusion fluid (200 μg./ml.), and adrenaline (0.008 μg.) at A then caused dilatation. At P, 10 μg. pethidine hydrochloride was injected, and 0.008 μg. adrenaline was then twice injected at A; it had no effect.

that the vasodilator action of adrenaline was reduced.

Substances causing adrenaline reversal

We found evidence in the literature that some of the compounds we were considering reverse the constrictor action of adrenaline. Thus Wehland (1924) found that, in the perfused vessels of the frog, atropine reversed the action of adrenaline. Further the observation of Murakami (1930) and Akamatsu (1933) that quinidine reversed the action of adrenaline on the blood pressure of the cat was supported by the evidence of Keogh and Shaw (1943), who found that quinine reversed the action of adrenaline but that reversal by quinidine was more difficult to produce. We therefore carried out experiments on the ear vessels perfused by Locke's solution containing quinidine hydrochloride (20 μg. per ml.) and found that the constrictor action of adrenaline was reversed, as shown in Fig. 4. A similar reversal was also observed when the perfusion fluid contained benadryl (10 μg. per ml.) or procaine hydrochloride (200 μg. per ml.). The effects of pethidine hydrochloride and of atropine sulphate were less clear, for when perfused in a concentration of 10 μg. per ml. and 30 μg. per ml. respectively, the constrictor phase was not wholly abolished, and the vasodilatation which followed was not so well marked as with the other substances. The vasodilatation was nevertheless present, as shown in Fig. 5. In one experiment we confirmed Rothlin's observation (1925) that ergotamine reversed the action of adrenaline, since we observed a reversal with ergotoxine.

Vascular action of benadryl, pethidine, etc.

In the course of these experiments we observed that the injection of benadryl or of the other substances had an effect on the vessels. In Fig. 1 the second injection of pethidine had a small vasoconstrictor effect and in Fig. 2 the injection of quinidine caused vasoconstriction. All except atropine were found to cause constriction, and we observed in addition that the constriction was reversed to dilatation when priscol was present in the perfusing fluid. During perfusion with priscol, atropine also caused dilatation. These effects are illustrated in Figs. 6 and 7. (In one experiment in a freshly prepared ear, benadryl caused slight vasodilatation in the absence of priscol. It seems to us probable that a dilator effect can be observed with these substances at an early stage, but that

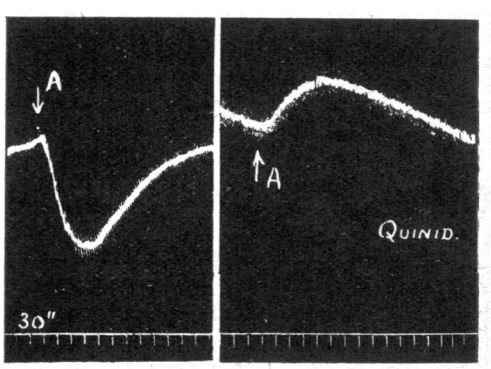


FIG. 4.—In the first part of the record, at A, 0.01 μg. adrenaline caused vasoconstriction. Between the two parts of the record, quinidine hydrochloride was added to the perfusion fluid (20 μg./ml.) and in the second part 0.05 μg. adrenaline at A caused vasodilatation.

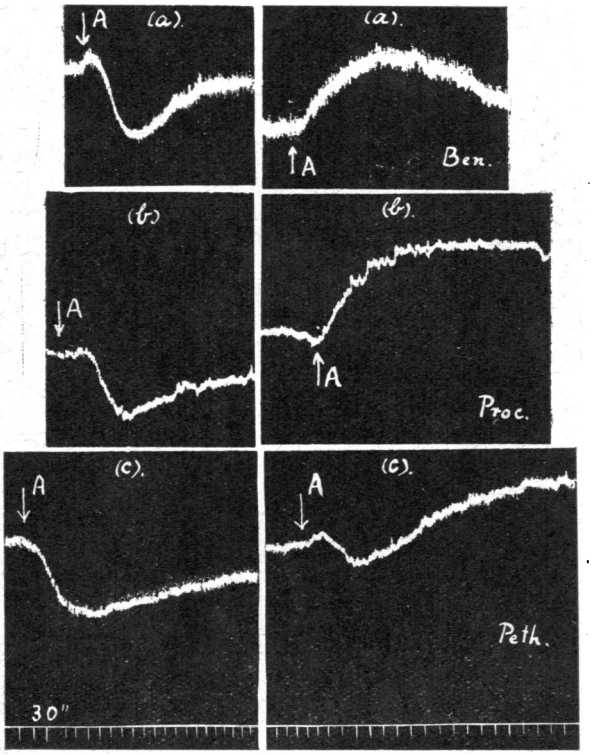


FIG. 5.—Similar to Fig. 4. Reversal of adrenaline (A). Action due to: (a) benadryl perfused in concentration 10 μg./ml., (b) procaine 200 μg./ml., and (c) pethidine 10 μg./ml. In (a) 0.005 μg. adrenaline was injected before and also during benadryl infusion. In (b) 0.01 μg. adrenaline before procaine, 0.02 μg. adrenaline during procaine infusion. In (c) 0.005 μg. adrenaline before pethidine, 0.01 μg. adrenaline during pethidine infusion.

this gives place as perfusion continues to a constrictor effect, as with acetylcholine. This requires further investigation.)

Action of acetylcholine and histamine

When the vessels of the rabbit ear are freshly prepared it is usual to observe that acetylcholine causes vasodilatation during the first 8 hours. At the end of 24 hours, this dilator response usually changes to a constrictor response. We observed that this constrictor action of acetylcholine was also reversed to a dilator action when the perfusing Locke's solution contained priscol (0.2 mg./ml.). This is shown in Fig. 8. On the other hand, the constrictor action of histamine was not reversed, though it was reduced by priscol; thus, before adding priscol to Locke's solution for the perfusion, constriction was produced by 0.03 μg. histamine acid phosphate; during priscol perfusion constriction was produced by amounts from 0.25–1.0 μg.

Exceptional actions of adrenaline and histamine

When adrenaline and histamine are injected constriction is the ordinary response in the freshly prepared organ. We have, however, observed a dilator response to adrenaline and to histamine exceptionally. In one rabbit ear the vessels were in high tone when the perfusion began, and it was necessary to raise the Marriotte bottle to a height of 75 cm. Gradually the perfusion became faster, but the resistance remained considerable and for several hours dilator responses to adrenaline and histamine were consistently recorded, as well as to acetylcholine. These responses are illustrated in Fig. 9. Repeated control observations were made with Locke's solution. On the next morning, however, the dilator responses had disappeared, and all three substances caused vasoconstriction.

In another ear, when freshly prepared, it was observed that adrenaline caused constriction followed by dilatation of rather greater extent than the constriction, and did so after several injections. Here again histamine also caused dilatation, though the effect was very small.

DISCUSSION

Adrenaline is commonly described as a vasoconstrictor substance, histamine and acetylcholine as vasodilator substances. Yet so long ago as 1918 Dale and Richards showed that, when injected into the vein of a cat anaesthetized with ether, all three substances caused the expansion of the volume of a fully denervated hind leg, the expansion being accompanied by a fall in blood pressure. Evidently, under the conditions described, all three substances acted as vasodilators. If, on the other hand, these substances are injected into the Locke's solution perfusing the vessels of the rabbit ear, the perfusion having continued for 24 hours, then each substance causes vasoconstriction. It is therefore clear that all three substances can act either as vasoconstrictors or as vasodilators, according to the conditions in which they are applied.

Some evidence suggests that the vasodilator effect of adrenaline, which has been observed only *in vivo*, may not be due to adrenaline itself but to the release of histamine by adrenaline. Thus Staub (1946) has shown that the injection of adrenaline causes a rise in the amount of histamine in the blood. It seems now reasonably certain that adrenaline has a direct vasodilator action of its own. The observation of Rothlin (1925), that in

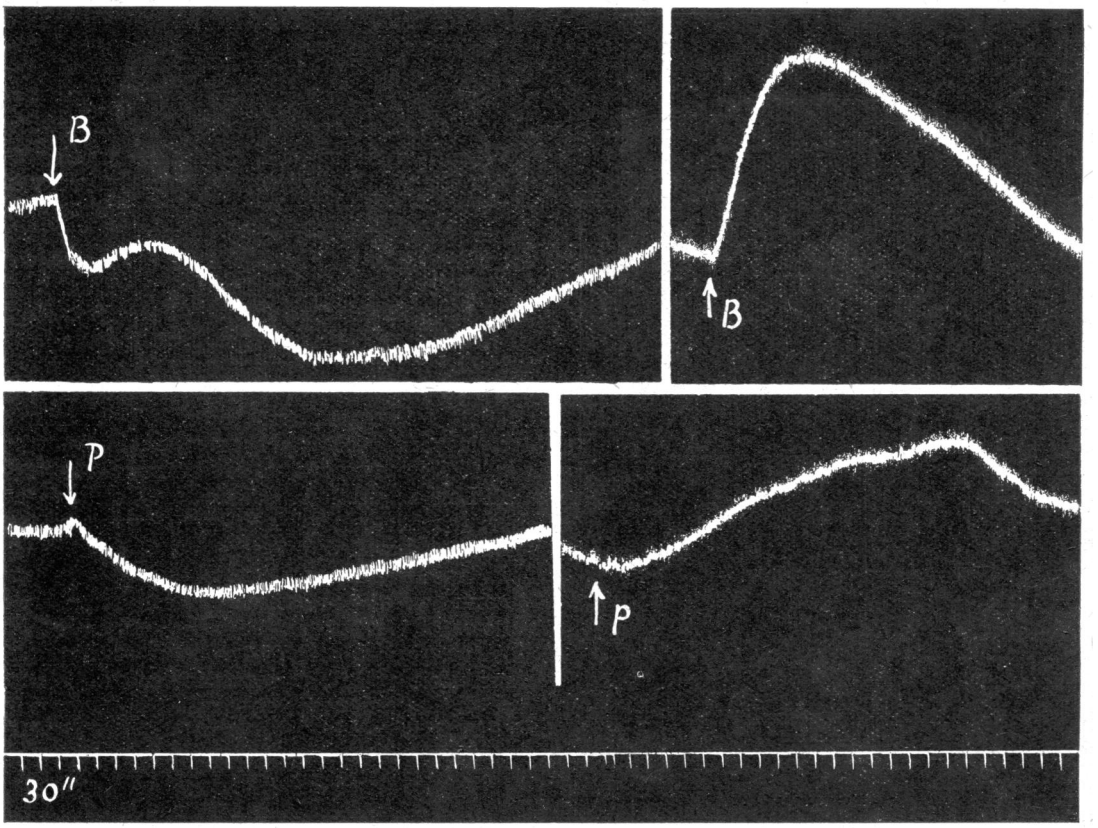


FIG. 6.—Upper tracings show action of benadryl (B): 100 μg. benadryl produced constriction; during perfusion with priscol (200 μg./ml.), benadryl (10 μg.) produced dilatation. Lower tracings show action of pethidine (P): 2 μg. pethidine caused vasoconstriction; during perfusion with priscol, pethidine (10 μg.) produced dilatation.

the presence of ergotamine adrenaline dilates the vessels of the perfused rabbit ear, has been confirmed by us using ergotoxine, and our colleague Gowdey (1948) has observed that in the presence of priscol adrenaline dilates the vessels of the rabbit ear. During priscol infusion the constrictor action of histamine itself was reduced but never reversed, so that the dilatation caused by adrenaline could not be due to histamine. More important, however, is our observation that in two fresh preparations adrenaline produced vasodilatation without the injection of any reversing agent. In one of these the vessels responded by dilatation during 8 hours' observation not only to adrenaline but to histamine and acetylcholine as well; no other substance than these three was injected at any time; the perfusion with Locke's solution was continued during the night, and on the following morning the response to the injection of adrenaline, histamine, and acetylcholine had become constrictor. In the second preparation the injection of adrenaline caused an initial constriction followed by dilatation; this was also observed several times. In the course of 20 preparations a vasodilator action of acetylcholine has almost always been observed during the early stages of perfusion; but repeated vasodilator responses to adrenaline and histamine have been seen in two preparations only.

There is, as Bülbring and Burn (1948) have found, a steady change in the response of the perfused vessels of the rabbit ear to the injection of acetylcholine; the response, which is at first dilator, becomes constrictor. There may be a similar change in the response to adrenaline, for although the initial response is almost always constrictor the vessels become much more sensitive with continued perfusion so that at a later stage a given dose produces more constriction than before. We have observed that this increased

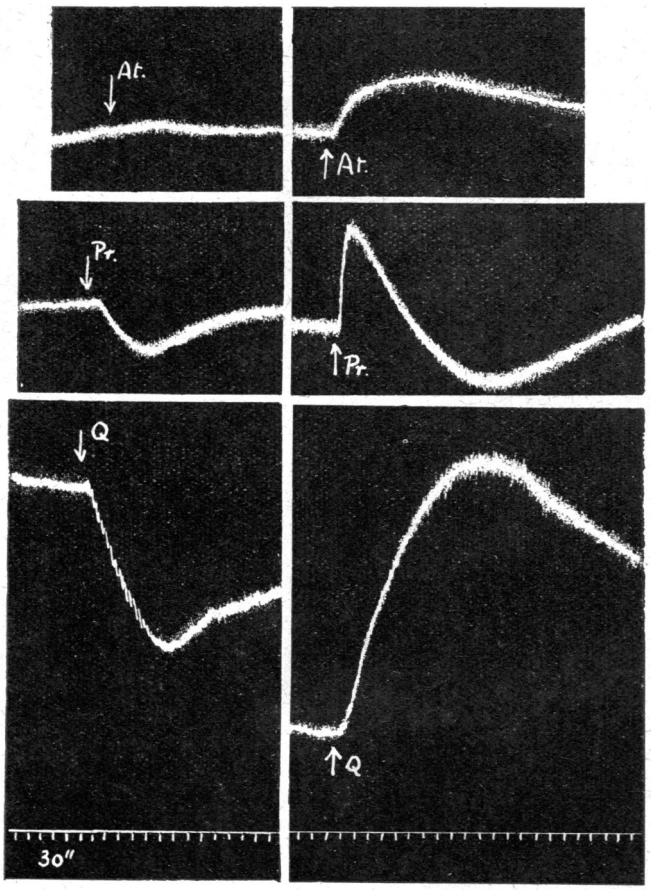


FIG. 7.—Similar to Fig. 6. Upper tracings 50 μg. atropine (At) before, and 100 μg. atropine during, priscol infusion. Middle tracings 500 μg. procaine (Pr) before and also during priscol infusion. Lower tracings 200 μg. quinidine (Q) before, and 100 μg. during, priscol infusion.

sensitiveness is reduced by perfusing a steady concentration of acetylcholine through the vessels, so that we do not think that the increased sensitiveness is due to oedema.

The view that the dilator effect of adrenaline or histamine is exerted on a different part of the vessels from that where these substances produce vasoconstriction has long been held ; such a view is less easy to hold when the system of vessels is as simple as that of the perfused rabbit ear. It seems at least equally probable that both effects are produced at the same site. Adrenaline stimulates the smooth muscle of the isolated rabbit uterus, but in the presence of ergotoxine adrenaline causes relaxation ; in this tissue it is very unlikely that there are two sites of action and much more likely that contraction and inhibition are produced at the same receptors. Both stimulation and inhibition by adrenaline were observed by McSwiney and Brown (1926) in several other tissues in which the site of action for both effects was probably the same. Recently Burn and Vane (1948) have demonstrated that acetylcholine causes both stimulation and inhibition in the isolated rabbit auricles after treatment with paludrine, and also in the isolated rabbit intestine and the isolated rat uterus. These opposed effects presumably occur at the same site in each organ, and we therefore favour the view that the two effects of adrenaline in blood vessels are also produced at the same site.

The evidence is stronger that the dilator and constrictor effects of histamine are produced at different sites, though Burn and Dale (1926) observed that histamine caused dilatation of the perfused mesenteric artery of the dog with its fine arterial branches, thus showing that dilatation is not produced by histamine in capillaries only.

Our observations also indicate the close relation which exists between the action of acetylcholine and that of adrenaline on the blood vessels. Substances such as atropine, pethidine, quinidine, benadryl, and procaine, which inhibit the constrictor action of acetylcholine, also inhibit the constrictor action of adrenaline in the rabbit ear. The close relation is also shown by the observation that the perfusion of priscol through the vessels reversed not only the constrictor action of adrenaline but that of acetylcholine as well. Vasodilatation thus produced by adrenaline was reduced or abolished by a preceding injection of

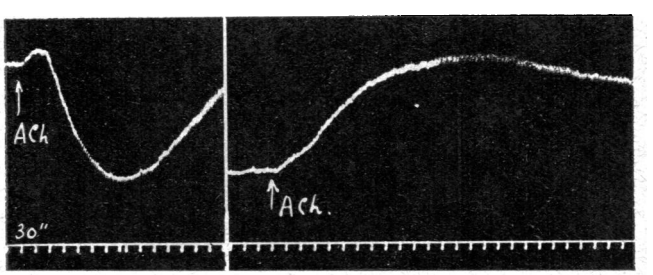


FIG. 8.—Reversal of constrictor action of acetylcholine by priscol. 10 μg. acetylcholine before, and 100 μg. acetylcholine during, priscol infusion.

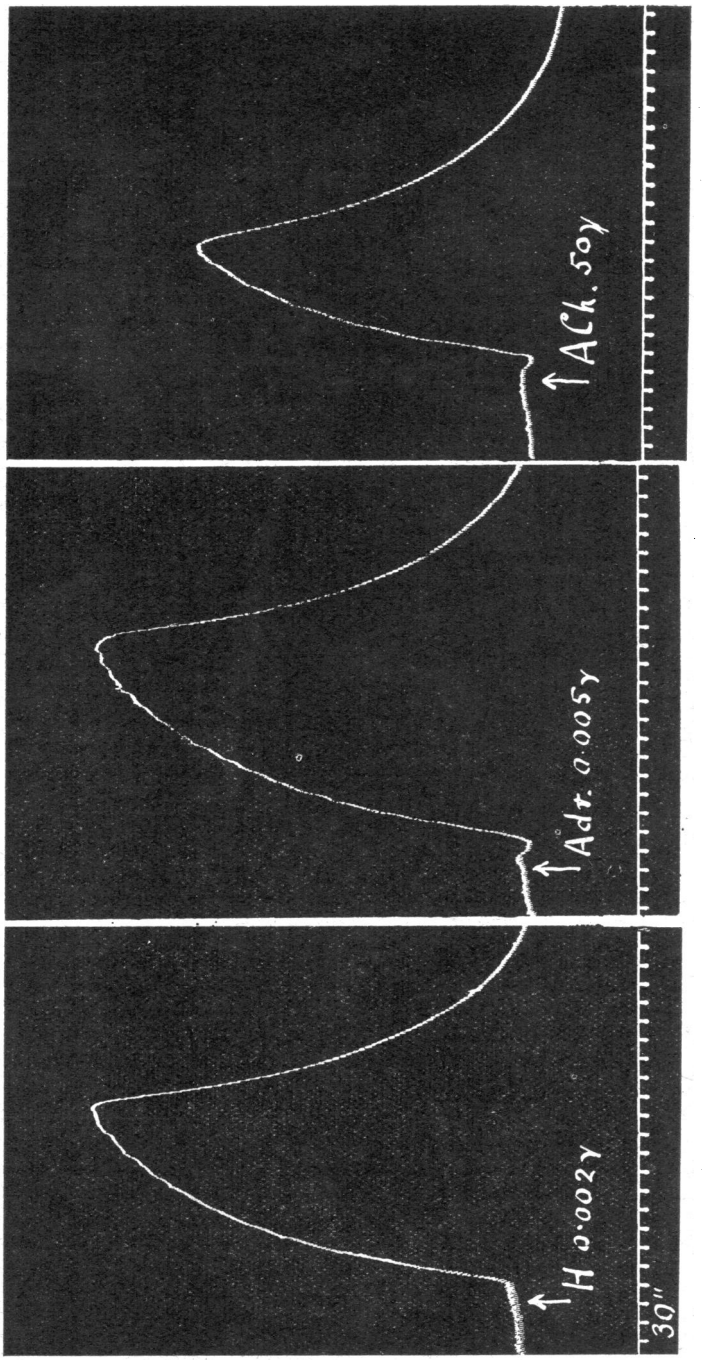


FIG. 9.—Exceptional dilator effects of histamine acid phosphate, 0.002 μg. (H), and of adrenaline hydrochloride, 0.005 μg. (Adr), in freshly prepared ear. The usual dilator effect of acetylcholine chloride, 50 μg. (Ach), is also shown. After 24 hours all three substances caused vasoconstriction in this preparation.

substances like benadryl, pethidine, and atropine.

Möller (1937) has observed that procaine constricts the vessels of the rabbit ear, and we found that large doses of benadryl, pethidine, and quinidine also cause vasoconstriction; these constrictor effects are reversed like that of adrenaline by perfusing the vessels with priscol. It was surprising to observe that quinidine reversed the vascular action of adrenaline like ergotoxine. We performed the experiment because Keogh and Shaw (1943) described an adrenaline reversal in the cat under nembutal anaesthesia following the injection of quinine. Having observed an adrenaline reversal by quinidine in the vessels of the rabbit ear, we proceeded to test the other substances in the same way, and found that when these are perfused in constant concentration through the vessels, the constrictor action of adrenaline is reversed by benadryl, procaine, and even pethidine, though the initial phase of the constrictor action is not completely abolished by pethidine. The substances which reverse the action of adrenaline have been hitherto regarded as a group apart, but our findings indicate that the properties first described for ergotoxine are properties shared by substances as dissimilar as quinidine and benadryl.

These additional observations that benadryl, procaine, pethidine, and quinidine are themselves constrictor substances which, like adrenaline or acetylcholine, are reversed in their vascular action by priscol suggests that when they inhibit the action of adrenaline or acetylcholine they do so because of their adrenaline-like or acetylcholine-like property; that is to say, the inhibition is due to competition for the same receptors.

If it is true that they compete for the same receptors, how is their ergotoxine-like property to be explained? The constrictor action of adrenaline is, of course, a motor effect, and the dilator action after ergotoxine is an inhibitor effect. Burn and Vane (1948) have suggested that with acetylcholine the transition from a motor to an inhibitor effect always occurs when excess of it is present. Such an explanation may apply to adrenaline. If the addition of benadryl or pethidine simulates the addition of a maximal amount of adrenaline, then the further addition of adrenaline would cause inhibition.

SUMMARY

1. Earlier work has shown that a group of substances which includes atropine, benadryl, pethidine (demerol), procaine, and quinidine have the common property of antagonizing the action of acetylcholine. Experiments on the perfused vessels of the rabbit's ear have now shown that these substances will also inhibit the constrictor action of adrenaline and sympathetic stimulation.

2. When the constrictor action of adrenaline is reversed by priscol, these substances, which inhibit the action of acetylcholine, also inhibit the vasodilatation caused by adrenaline.

3. These substances, when perfused through the vessels, exert an action like that of ergotoxine or priscol; they reverse the constrictor action of adrenaline.

4. These substances can themselves exert a constrictor action on the vessels, and this constrictor action, like that of acetylcholine seen in the rabbit's ear vessels after 24 hours, is reversed under the influence of priscol.

5. In occasional fresh preparations, the injection of adrenaline regularly causes vasodilatation; though after several hours' perfusion with Locke's solution, the injection causes vasoconstriction.

6. The constrictor action of histamine in the rabbit ear vessels is abolished by the substances which abolish that of adrenaline. Priscol does not reverse the action of histamine. In occasional fresh preparations, histamine causes vasodilatation.

7. The view that adrenaline has a dilator action of its own is discussed.

8. The view that adrenaline dilatation and constriction are produced at the same site is discussed.

9. The similarity in the action of adrenaline and acetylcholine is emphasized.

10. The evidence is considered to support the theory of drug action by competition, and to suggest the mechanism of adrenaline reversal.

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