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REVERSAL OF THE VASCULAR RESPONSE TO ACETYLCHOLINE AND ADRENALINE

BY

J. H. BURN AND JUDITH ROBINSON

From the Department of Pharmacology, Oxford University

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The phenomenon of the reversal of the vasomotor action of adrenaline by extracts of ergot was first described by Dale (1906). It has been studied by many workers since it was originally described, and it has been produced by many substances other than those found in ergot. Little light, however, has been shed on the process involved, partly because most observations have been made by recording the blood-pressure in the anaesthetized or spinal animal. So many different facts modify the blood pressure response that it is difficult to be certain what parts of the cardiovascular system are responsible for the reversal of the effect.

METHOD

We have therefore carried out observations on the vessels of the rabbit ear perfused at a constant head of pressure with Locke's solution at room temperature using the outflow recorder devised by Stephenson (1948) in this laboratory. This apparatus is simple to make from glass tubing, and gives an extremely sensitive record of changes in the size of the vascular channels in the ear.

A fine glass cannula was tied into the central artery at the base of the ear and fluid passed to this cannula by way of the small rubber-capped chamber described by Gaddum and Kwiatkowski (1938). Injections were made into this chamber through the cap, the fluid injected being Locke's solution containing the active agent. The final dilution of the agent in Locke's solution was made just before injection. When the effect observed was dilatation, we always gave control injections to ensure that this was not due to the volume of the injected fluid. When the ear vessels are perfused with a solution containing histamine, for example, they become very sensitive to the mechanical effect of injecting fluid.

Responses to acetylcholine

Spontaneous change during perfusion.—When acetylcholine (ACh) is injected into the cannula during the first few hours of perfusion the vessels nearly always respond by dilatation. The amounts sufficient to produce the effect vary from 1 to 20 μg., though dilatation may be caused by 100 μg. As perfusion continues, the dilatation in response to a given dose declines and may disappear; when a larger dose is injected constriction is then seen, and from this point onwards all injections of acetylcholine cause constriction. An example of the change is given in Fig. 1. The time which elapses between the beginning of the perfusion and the appearance of the constrictor response varies in different ears. In our experience the dilator response usually persists during the first 6 hr.; the constrictor response is seen when injections are made the

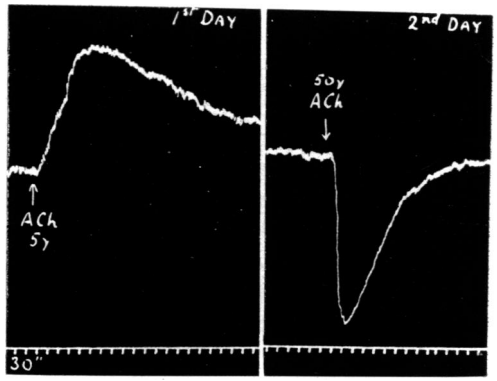

FIG. 1.—Record of outflow from rabbit ear vessels, showing increase caused by the injection of 5 μg. acetylcholine (ACh) during perfusion on 1st day, and on the right the decrease caused by the injection of 50 μg. acetylcholine on 2nd day.

next day, the perfusion having continued overnight. Sometimes, however, the dilator response persists during the second day, and then gives place to a constrictor response on the third day. Sometimes the dilator response disappears during the first day, and occasionally constriction is the response which is seen at the beginning of the perfusion. The change in the response is always in the direction of dilatation to constriction. We have never observed a spontaneous change in the reverse direction. In nineteen consecutive experiments we observed a dilator response on the first day in sixteen. The change in a succession of eleven experiments is recorded in Table I. During the first day the response to acetylcholine was dilatation in ten experiments; in the remaining experiment acetylcholine caused constriction. The perfusion was continued on the second day, and acetylcholine then caused constriction in nine experiments; in the remaining two experiments acetylcholine caused dilatation, as on the first day. In these two experiments acetylcholine caused constriction on the third day. All third-day responses were constriction.

Effect of dose.—When the response to acetylcholine is dilator, it is usually obtained with small doses; when, on the other hand, the response is constrictor it is usually obtained with larger doses. This difference, which is illustrated in Fig. 1, suggests

TABLE I

EFFECT OF ACETYLCHOLINE ON EAR VESSELS AT DIFFERENT STAGES OF PERFUSION

Exp.	Response and dose: D = dilatation, C = constriction		
	1st day	2nd day	3rd day
1	D 100 μg.	C 100 μg.	
2	D 10	C 20	
3	C 50	C 200	
4	D 20	C 20	
5	D 20	C 20	C 25 μg.
6	D 10	C 10	
7	D 1	D 2.5	C 50
8	D 5	C 10	
9	D 2.5	C 50	
10	D 4	D 4	C 10
11	D 10	C 20	

that the response is determined by the size of the dose. In some experiments dilatation has been produced during the first day's perfusion by a large dose, 0.1 mg., and constriction has been produced by the same dose during the second day's perfusion. In Exps. 1, 4, 5, and 6 of Table I constriction was produced on the second day by the same dose which produced dilatation on the first day. This indicates that the response does not depend on the dose. In other experiments, however, in which a dilator response was recorded to a certain dose, a greater dose caused a transitory constriction preceding the dilatation.

Restoration of a dilator response.—When the response to acetylcholine has become constrictor, the addition of adrenaline or of *nor*adrenaline to the Locke's solution perfusing the ear restores the dilator action of acetylcholine. This effect is illustrated in Fig. 2, which records responses obtained on the second day of perfusion. In the upper part of Fig. 2, 10 μg., 50 μg., and 100 μg. acetylcholine were injected in succession, with increasing constrictor action. *Nor*adrenaline was then added to the perfusing fluid in a concentration of 10^{-7}. The response to the injection of acetylcholine then became dilator, as shown in the lower part of Fig. 2. The dilatation was produced by the injection of 1 μg. The injection of 10 μg. produced a much larger dilatation.

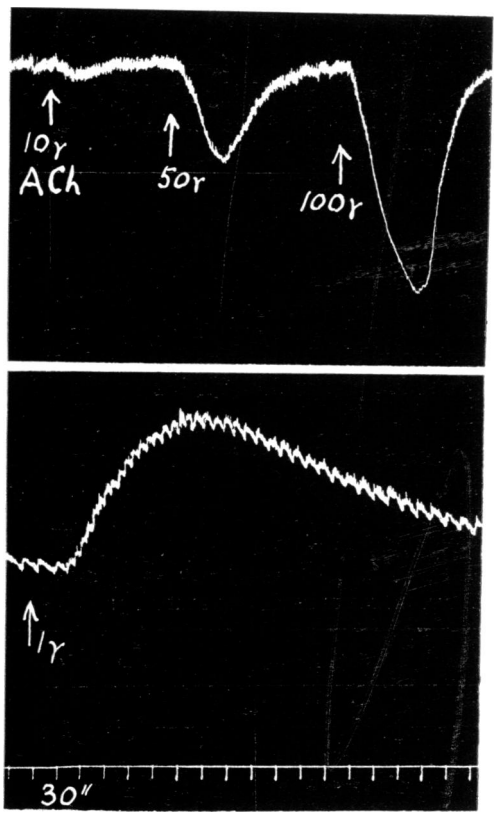

FIG. 2.—Records during perfusion on 2nd day. Decrease in outflow caused by 10 μg., 50 μg., and 100 μg. acetylcholine. *Nor*adrenaline was then added to the Locke's solution, the concentration being 10^{-7}. During perfusion with this solution, dilatation was produced by injecting 1 μg. acetylcholine.

Restoration by histamine.—Experiments were also carried out to determine the effect of histamine, since it is known from the observations of Schmiterlöw (1948) that histamine is present in the arterial walls of oxen. Fig. 3 shows observations made on the second day of perfusion ; in (*a*) the injection of 0.15 mg. acetylcholine produced vasoconstriction; histamine acid phosphate was then added to the perfusion fluid in a concentration of 10^{-5} (histamine base). This addition had a slight effect in constricting the vessels, but very little. The response to injections of 0.15 mg. acetylcholine was steadily modified, however. At first, as shown in Fig. 3 (*b*) and (*c*), the constrictor action of acetylcholine was progressively augmented. As the perfusion continued, the constriction became shorter in duration and was

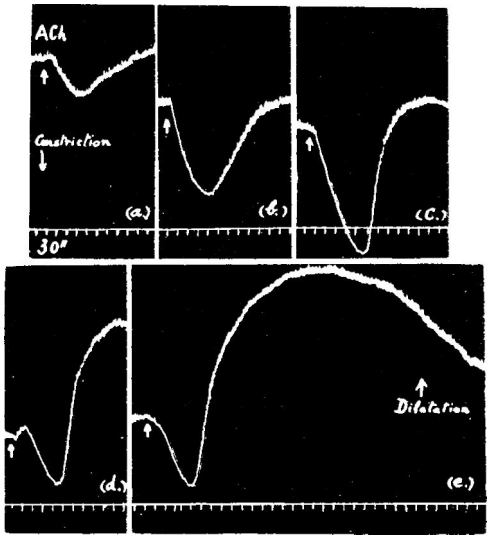

FIG. 3.—Records during perfusion on 2nd day. In (*a*) the decrease in outflow caused by injecting 150 μg. acetylcholine is recorded. Histamine acid phosphate was then added to the Locke's solution, the concentration being 10^{-5} (histamine base). In (*b*) 150 μg. acetylcholine caused a greater constriction, and in (*c*) it caused a still greater one. In (*d*) the injection caused constriction followed by dilatation, and in (*e*) the main effect of the same injection was dilatation.

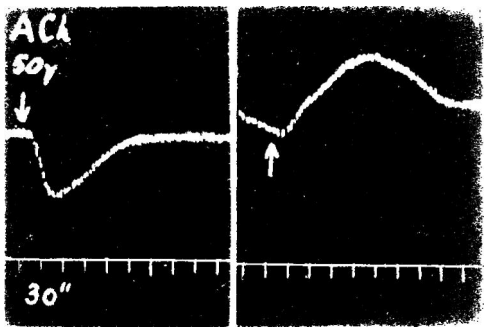

FIG. 4.—Records on 2nd day. Constrictor effect of 50 μg. acetylcholine. During perfusion with Locke's solution containing histamine 10^{-5}, the injection of 50 μg. acetylcholine caused dilatation.

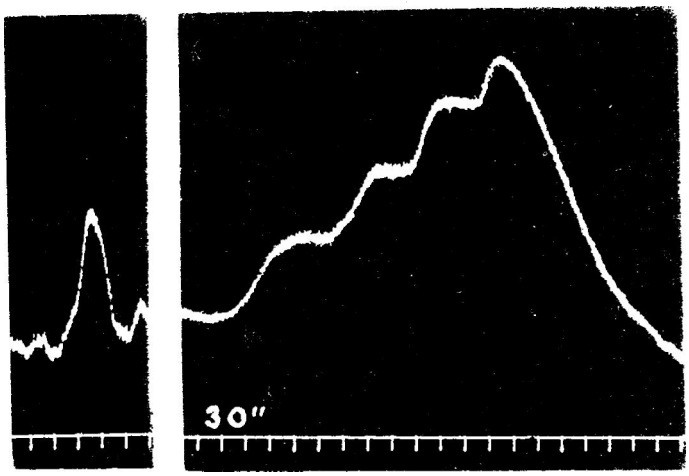

FIG. 5.—Record showing two examples of spontaneous changes in outflow during perfusion on the 1st day, when the perfusing fluid contained 10^{-5} histamine.

followed by dilatation. In Fig. 3 (*e*), the principal effect of the injection of 0.15 mg. acetylcholine was dilatation. When 0.05 mg. acetylcholine was next injected, dilatation only was recorded.

The same sequence of changes was observed in several experiments. In some, only the first phase of potentiation of the constrictor action of acetylcholine was obtained. In others simple reversal was recorded as in Fig. 4.

In all these experiments the effect of adding histamine to the Locke's solution was determined during perfusion on the second day. When histamine was added to the perfusion fluid on the first day, it led to the occurrence of spontaneous changes in the vessels (see Fig. 5), and a great sensitivity to the mechanical effect of injections of Locke's solution, the injection of 0.05–0.1 ml. causing a large dilator response.

Response to adrenaline

Initial dilator response.—In a previous paper Burn and Dutta (1948) described the unusual results they obtained in one experiment on the perfused rabbit's ear, in which they repeatedly observed dilator responses to adrenaline and histamine as well as to acetylcholine during the first day's perfusion. On the second day the responses to all three substances were constrictor. We have now observed several more examples of an initial dilator response to adrenaline at the beginning of a perfusion. This dilator response has not persisted throughout the first day's perfusion, but has rapidly diminished and then given place to the usual constrictor response. Examples of this change are given in Figs. 6 and 7. In Fig. 6 when 0.01 μg. adrenaline was injected it produced first of all the dilatation shown at the top of the figure. A control injection of fluid was given; it had no effect. Later injections of 0.01 μg. adrenaline caused

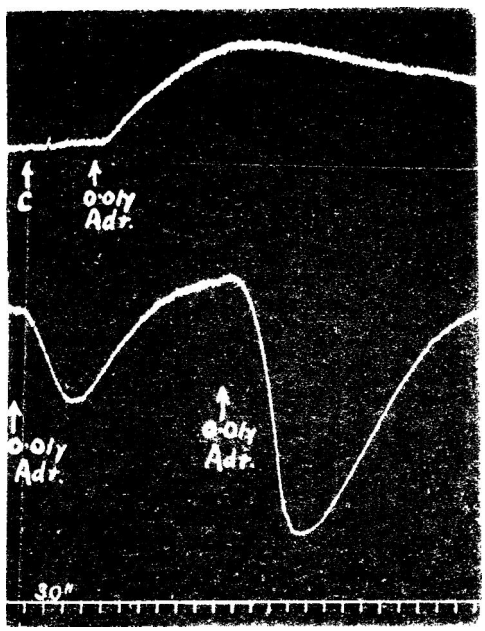

FIG. 6.—Record at beginning of perfusion showing the dilator effect of 0.01 μg. adrenaline injected in 0.1 ml. At C, 0.1 ml. perfusion fluid was injected. The lower tracings show the effects of injecting 0.01 μg. adrenaline a little later. The dilator response changed spontaneously to a constrictor response.

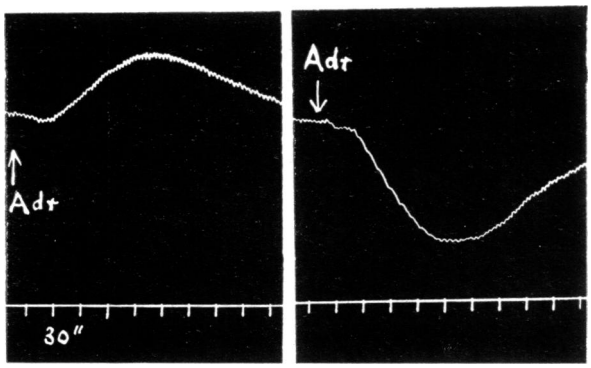

FIG. 7.—Record shows a similar change to that in Fig. 6. 0.005 μg. adrenaline produced dilatation at first, but after 30 min. it caused constriction.

in succession the small and then the larger constriction seen in the lower part of the figure. In Fig. 7 an injection of 0.005 μg. adrenaline caused dilatation, whereas the same dose injected 32 min. later caused the usual constrictor response. In this experiment the same change in response was observed when *nor*adrenaline was injected in a dose of 0.01 μg. Thus, when a long series of experiments is carried out in which rabbit ears are perfused, it is found that in a small proportion of these the initial effect of adrenaline, like the effect of acetylcholine, is dilator.

Reversal by ephedrine.—It was shown by Gaddum and Kwiatkowski (1938) that, when ephedrine is added to the fluid perfusing the vessels of the rabbit ear, the constrictor action of both adrenaline and of sympathetic stimulation is potentiated. We have recently carried out many experiments (Burn and Robinson, 1951) in which we have determined the effect of adding ephedrine to the perfusing Locke's solution, and in one of these we obtained an unusual result. The vessels of the ear in this experiment were found to be very constricted when the perfusion began, and, even when the Marriotte bottle containing Locke's solution was raised about 1.3 metres

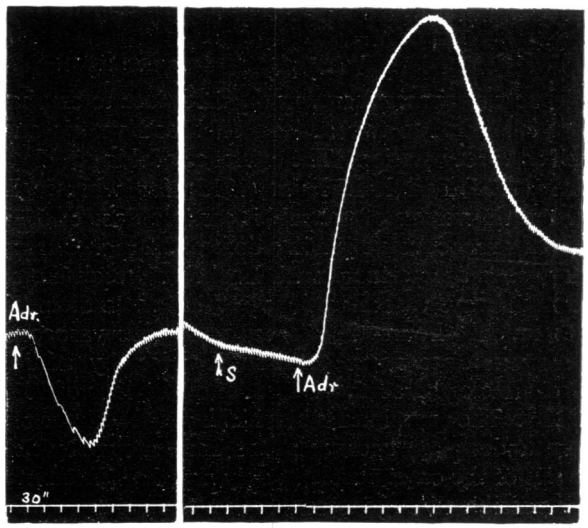

FIG. 8.—Ear perfusion on 1st day. On the left is shown the constrictor action of 0.003 μg. adrenaline. Ephedrine hydrochloride was then added to the Locke's solution in a concentration of 10^{-5}; during perfusion with this solution 0.1 ml. perfusing fluid was injected at S, and then 0.002 μg. adrenaline. This last injection caused vasodilatation.

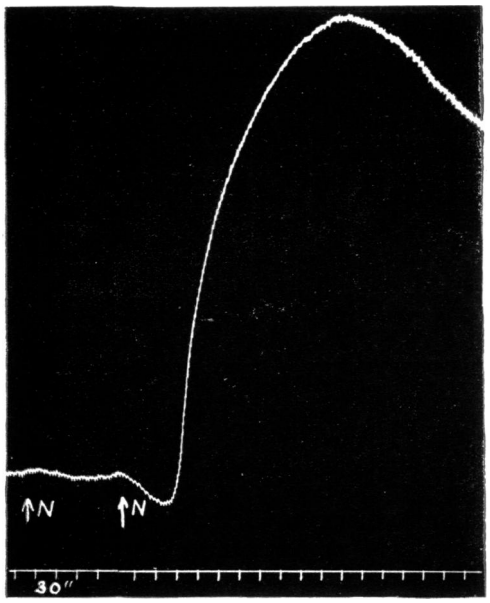

FIG. 9.—In the same experiment as shown in Fig. 8, during perfusion with Locke containing ephedrine, 0.002 μg. *nor*adrenaline and then 0.008 μg. *nor*adrenaline were injected. The last injection caused vasodilatation.

above the ear, little fluid passed through the vessels. The preparation was left for some time under this high pressure, and after 1 hr. fluid began to flow out of the veins. The pressure was then reduced. When injections of adrenaline were made, the usual constriction was produced as shown in Fig. 8. Ephedrine hydrochloride was then added to the Locke's solution in a concentration 10^{-5}, and the perfusion was continued with this solution. The injection of the same amount of adrenaline as before now produced dilatation as shown in Fig. 8. Thus in the presence of ephedrine the constrictor action of adrenaline was reversed to dilatation. A similar reversal took place of the action of Corbasil, a substance resembling adrenaline with the addition of a $-CH_3$ group to the α carbon atom. When perfusion began without the addition of ephedrine to the Locke's solution, 0.025 μg. Corbasil caused vasoconstriction similar in magnitude to that caused by 0.003 μg. adrenaline; after the addition of ephedrine, the same dose of Corbasil produced vasodilatation like that caused by 0.002 μg. adrenaline. *Nor*adrenaline was not injected before the addition of ephedrine to the perfusion fluid; during perfusion with ephedrine it caused vasodilatation as shown in Fig. 9.

DISCUSSION

The experiments serve in the first place to emphasize what is not well known, that acetylcholine has two actions on blood vessels and can cause vasoconstriction as well as vasodilatation. The existence of this double effect caused controversy between Dale (1914) and Reid Hunt (1915), for, whereas Dale observed that acetylcholine dilated the vessels of the rabbit ear in a dose of 5 μg., Reid Hunt observed either no effect or vasoconstriction in a dose of 0.2 mg. Our experiments show that when rabbit ear vessels are perfused with Locke's solution the early response to acetylcholine is almost always vasodilatation, but that when the perfusion has continued for a period measured in hours the response becomes vasoconstriction. The response can be converted once more to vasodilatation by adding to the perfusing fluid either adrenaline or *nor*adrenaline in concentrations of 10^{-7}, or histamine in a concentration of 10^{-5}. The experiments in which the vasodilator response was restored by histamine were undertaken because it has been shown by Schmiterlöw (1948) that the blood vessels of the ox contain not only *nor*adrenaline but also acetylcholine and histamine. It is possible that the same three substances are present in the vessels of the rabbit ear;

if this is so it is also possible that when the ear is continuously perfused the amount of any or all of these substances declines, and that the change in the reaction of the vessels to acetylcholine is due to this loss.

However this may be, when the perfusion of an ear has continued long enough for the reaction to acetylcholine to become constrictor, the addition of histamine to the perfusion fluid changes the reaction back to dilatation. Histamine in a concentration of 10^{-5} has little constrictor action of its own, and the reversal of the action of acetylcholine is not due to increased tone, because the initial effect of histamine is to augment the constrictor action of acetylcholine. This finding was confirmed many times, and in some experiments was the only effect which the addition of histamine to the perfusion fluid was observed to produce.

In these experiments histamine was added to the perfusing Locke's solution on the second day of the perfusion. When histamine was added on the first day it had a peculiar effect in producing spontaneous changes in the vessels. The outflow from the veins increased and declined rhythmically, and if a small injection of the perfusing fluid was made the vessels responded by a transient dilatation to such an extent that it became impossible to study the effect of other substances. This action of histamine seems to be of some interest.

The observation was previously made by Burn and Dutta (1948) that various substances, among others quinidine, pethidine, and benadryl, when injected into the arterial cannula during a perfusion of the vessels of the rabbit ear with Locke's solution caused vasoconstriction. When a known reversing agent, benzylimidazoline, was added to the perfusing fluid, in a concentration which reversed the constrictor action of adrenaline, the constrictor action of these different substances was also reversed. Thus it was shown that quinidine, pethidine, and benadryl possess a vasoconstrictor action which must closely resemble that of adrenaline, since when the action of adrenaline is reversed their action is reversed also.

Burn and Dutta (1948) found in addition that quinidine, pethidine, and benadryl were able to act as reversing agents, for when any one of them was added to the fluid perfusing the rabbit's ear in a concentration of 10^{-5}, the constriction caused by the injection of adrenaline into the arterial cannula was replaced by dilatation. Their experiments demonstrated that adrenaline-like substances were themselves reversing agents, and they therefore suggested that adrenaline reversal was essentially due to competition by the reversing agent for the receptor to which adrenaline attaches itself. The evidence now obtained gives considerable support to this view, for an experiment is described in which ephedrine added to the perfusing fluid was found to reverse the action of adrenaline, of Corbasil, and also of *nor*adrenaline. Thus a substance generally accepted as exerting many of its effects by competition with adrenaline has been shown to reverse the action of adrenaline from constriction to dilatation.

The question then arises how a competing substance can produce reversal. Current theory, which has recently been discussed (Burn, 1950), assumes that there are two kinds of receptors, the one responsible for constriction and the other for dilatation. There is, however, no evidence of two kinds of receptors for acetylcholine, which has both effects in the rabbit ear vessels. In other tissues also acetylcholine has been shown to have two effects; thus Bülbring and Burn (1949) have shown that when the isolated auricles of the rabbit heart cease to contract after beating for 25–30 hr. the normal

inhibitory action of ACh is reversed to a stimulant action; when a certain degree of activity has been re-established, the normal inhibitory action reappears. Similarly, Burn and Vane (1949) have shown that in the isolated intestine of the rabbit or the isolated uterus of the rat the stimulant action of smaller concentrations is replaced by an inhibitory action of higher concentrations. In these tissues the difference in the effect of low and high concentrations of acetylcholine can be more easily explained by calling to mind the effect of acetylcholine concentration on the rate of its hydrolysis by " true " or " specific " cholinesterase. As the concentration of acetylcholine is increased, at first the rate of hydrolysis rises; but after reaching a peak the rate of hydrolysis falls gradually to zero. In order that hydrolysis may take place each molecule of acetylcholine is supposed to be attached to the molecule of enzyme at two points A and B. When excess of acetylcholine is present, two molecules of it may attach themselves, one by point A and the other by point B. Hydrolysis cannot then occur, and the enzyme is inhibited.

In a simple vascular preparation, like the system in the rabbit ear, dilatation may be an inhibition of this kind. When the relative number of free receptors and of molecules of acetylcholine is such that there is an excess of acetylcholine, attachment of the molecules may take place in such a way that the acetylcholine cannot be hydrolysed, and then the receptors will be blocked. Dilatation will follow. On the other hand, when there is no excess of acetylcholine, the attachment of the molecules to the receptors will cause stimulation attended by splitting of the ester.

The reversal of the constrictor action of adrenaline by ephedrine is capable of interpretation on these lines. Usually ephedrine does not reverse the constrictor action, but rather increases it. The potentiation was first explained by Gaddum and Kwiatkowski (1938) as due to inhibition of amine oxidase, and support for this view has recently been obtained (Burn and Robinson, 1951). Thus the potentiation occurs because a greater number of adrenaline molecules are free to combine with the receptors.

We can imagine that in some experiments perfusion of the vessels with ephedrine would have another effect. If the number of enzyme molecules was small, ephedrine might combine with all of them, and then combine with some of the receptors in addition. The effect of this would be to reduce the number of free receptors and so to reduce the sensitivity of the vessels to adrenaline. In the course of 55 perfusions with ephedrine we observed that the amount of adrenaline required to produce a given constriction was increased in 16 perfusions, and in some of these the decrease in sensitivity was sixfold.

In the experiment in which ephedrine caused reversal we may suppose that the perfusion with ephedrine led to combination of ephedrine with all enzyme molecules and also with so many of the receptors that an amount of adrenaline which previously produced constriction was in excess for these receptors. Therefore the molecules of adrenaline became attached to them in such a way that they could not exert a stimulant action, and served only to block the receptors, with resulting inhibition or dilatation.

Experiments in which the first few injections of adrenaline into the perfused ear vessels cause vasodilatation are rare, and are easily missed unless a larger series of experiments is performed. We suppose that in these exceptional experiments very few receptors are free, and that small amounts of adrenaline cause inhibition of the vascular tone. After perfusion has continued for a time, which may be short, the

usual constrictor action of adrenaline is seen, probably because the substances which blocked the receptors are washed away.

These changes in the response to adrenaline resemble the changes in the response to acetylcholine. We see that both substances begin by causing dilatation and later cause vasoconstriction; however, the dilator response to acetylcholine is much more persistent than that to adrenaline, while the constrictor response to adrenaline is much more powerful than that to acetylcholine. With both substances the reversal from dilatation to constriction is spontaneous, not requiring the intervention of a third substance, and therefore we think it reasonable to suppose that the reversal takes place because of an increase in the number of free receptors as perfusion goes on, so that there is a rise in the ratio of free receptors to molecules of adrenaline or acetylcholine. This conception fits with the observation that, when a competing substance such as ephedrine is introduced which diminishes the number of free receptors, then constriction is reversed once more to dilatation.

SUMMARY

1. When the vessels of the rabbit ear are perfused with Locke's solution, the injection of acetylcholine at first causes dilatation, but after some hours the injection causes constriction. Larger amounts are usually needed to cause constriction than were needed to cause dilatation.

2. The constrictor response can be reversed once more to dilatation by adding to the Locke's solution either adrenaline or *nor*adrenaline in a concentration of 10^{-7} or histamine in a concentration of 10^{-5}.

3. The first effect of adding histamine to the Locke's solution is to potentiate the constrictor action of acetylcholine; the later effect is to restore the vasodilator response to acetylcholine.

4. When a large series of ears is perfused, occasional preparations respond to adrenaline initially by vasodilatation. After perfusion has continued for an hour or more, the response changes spontaneously to vasoconstriction.

5. In one experiment the addition of ephedrine to the Locke's solution reversed the constrictor action of adrenaline, of Corbasil, and of *nor*adrenaline.

6. The evidence is taken to indicate that when a substance reverses the action of adrenaline from constriction to dilatation it does so by competing with adrenaline for the receptors with which it combines. When most of the receptors are occupied by the competing substance, the molecules of adrenaline, being now in excess in relation to the number of remaining receptors, combine with these receptors in an abnormal manner and block them. Inhibition or dilatation then follows.

REFERENCES

Bülbring, E., and Burn, J. H. (1949). *J. Physiol.*, **108**, 508.
Burn, J. H. (1950). *Physiol. Rev.*, **30**, 77.
Burn, J. H., and Dutta, N. K. (1948). *Brit. J. Pharmacol.*, **3**, 354.
Burn, J. H., and Robinson, J. (1951). *Brit. J. Pharmacol.*, **6**, 00.
Burn, J. H., and Vane, J. R. (1949). *J. Physiol.*, **108**, 104.
Dale, H. H. (1906). *J. Physiol.*, **34**, 163.
Dale, H. H. (1914). *J. Pharmacol.*, **6**, 147.
Gaddum, J. H., and Kwiatkowski, H. (1938). *J. Physiol.*, **94**, 87.
Hunt, Reid (1915). *J. Pharmacol.*, **6**, 477.
Schmiterlöw, C. G. (1948). *Acta physiol. scand.*, **16**, Suppl. 56.
Stephenson, R. P. (1948). *J. Physiol.*, **107**, 162.