Brit. J. Pharmacol. (1962), **19**, 513–526.

SYMPATHETIC VASODILATATION IN THE RABBIT EAR

BY

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(*Received August* 24, 1962)

Changes in the blood content of a 1 cm^2 portion of the intact rabbit's ear were studied with transillumination and a photocell. Stimulation of the post-ganglionic sympathetic nerves produced a decrease in blood content, attributable to vasoconstriction, followed by an increased blood content, attributable to vasodilatation. The vasodilatation was enhanced by eserine and decreased by atropine. Guanethidine abolished the vasoconstriction but not the vasodilatation. After the ganglion had been decentralized by degeneration of the pre-ganglionic sympathetic nerves the vessels had an increased sensitivity to acetylcholine and the vasodilatation in response to sympathetic stimulation was enhanced. It is concluded that sympathetic stimulation results in the liberation of acetylcholine which causes vasodilatation.

There is a large body of evidence that post-ganglionic sympathetic nerves in many parts of the body contain cholinergic fibres. This evidence was reviewed by Burn & Rand (1962), who put forward the hypothesis that the post-ganglionic neurone was primarily cholinergic, the release of acetylcholine leading to a secondary release of noradrenaline. As far as the rabbit's ear is concerned this hypothesis is based on the following findings: (1) Acetylcholine, after atropine, causes vasoconstriction in the isolated perfused ear (Burn & Dutta, 1948 ; Kottegoda, 1953) and this constriction is abolished by the antiadrenaline drug tolazoline (Burn & Dutta, 1948), by bretylium and xylocholine, which prevent the release of noradrenaline (Huković, 1960), or by depleting the noradrenaline stores from the ear with reserpine (Burn & Rand, 1958). (2) The vasoconstrictor response to sympathetic stimulation is enhanced by eserine (Burn & Rand, 1960) and blocked by excessive amounts of acetylcholine (Burn & Rand, 1960) and by hemicholinium (Chang & Rand, 1960). These procedures would be expected to affect a cholinergic but not a purely adrenergic mechanism. (3) The blood vessels normally contain acetylcholine, but its concentration falls after sympathetic denervation (Armin, Grant, Thompson & Tickner, 1953). (4) After sympathetic stimulation the perfusate from the rabbit's ear contains a substance which causes contractions of the eserinized leech and is probably acetylcholine (Burn & Rand, 1960).

Thus there is considerable evidence in favour of cholinergic neurones in the sympathetic supply to the rabbit's ear, and it would be expected that the acetylcholine released from these neurones would cause vasodilatation under suitable

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conditions, but hitherto evidence for the existence of dilator sympathetic fibres to the ear is lacking. It is well known that vasodilator responses are obtained more readily when the circulation is intact than in a perfused preparation. Therefore, we have looked for sympathetic vasodilatation in the intact rabbit's ear using the photocell method of Holton & Perry (1951). Our results show that sympathetic vasodilatation occurs and that it is, at least in part, due to acetylcholine.

METHODS

Albino rabbits of either sex weighing 1.9 to 3.0 kg were anaesthetized with urethane (approximately 6 ml./kg of a 25% w/v solution given intravenously). The trachea was cannulated and the larynx and the remainder of the trachea and the oesophagus were removed from the neck. The right cervical sympathetic nerve and the superior cervical ganglion were dissected free and the ganglion was placed on the anode of bipolar platinum electrodes with the cathode on the post-ganglionic nerve. In most experiments the ganglion was covered with liquid paraffin. During the dissection the internal carotid artery was tied and the jugular foramen was cleaned in order to avoid stimulating branches of the vagus (including Arnold's nerve), hypoglossal or glossopharyngeal nerves. The greater and lesser auricular nerves were cut. The right external ear was thus completely denervated except for the sympathetic supply from the superior cervical ganglion. The left vagus was cut in the neck.

In the course of these experiments observations were made on the anatomy of the sympathetic supply to the ear vessels. In most animals the nerves did not run in the post-ganglionic trunk but emerged laterally from the superior cervical ganglion, passed through the carotid plexus, and left the carotid artery laterally dorsal to the digastric muscle and hypoglossal nerve.

The ganglion was stimulated by square pulses from a Palmer stimulator via an isolation transformer or from an Ead stimulator (Cinetronics) via a radio-frequency coupling unit. Details of the stimulus parameters are described in "Results"; the output voltage was measured with 10,000 Ω across the terminals. Blood pressure was recorded from a femoral artery with a mercury manometer. Intravenous injections were given via a polythene cannula inserted into a vein in the leg. The flow of blood through the ear was measured after intravenous administration of 1,000 units/kg of heparin by cannulating the central vein at the base of the ear, passing the blood through a Palmer photo-electric drop counter, and returning the blood to the rabbit via a cannula in the femoral vein.

Section of pre-ganglionic sympathetic nerve. Rabbits with decentralized ganglia were prepared by removal of 3 to 4 cm of cervical sympathetic trunk from 5 to 17 days before the experiment. In some experiments the stellate ganglion was also removed and in others the greater and lesser auricular nerves were also sectioned, but these additional procedures made no difference to the results. During the experiments the cervical sympathetic was examined for macroscopic signs of regeneration, but none were found. The operations were performed with strict aseptic precautions under pentobarbitone sodium (about 30 mg/kg intravenously) and ether anaesthesia after premedication with 10 mg atropine/kg intravenously.

Reserpine treatment. A solution of reserpine 10 mg/ml. was made in 20% (w/v) ascorbic acid and 0.5 ml./kg of this solution was injected intraperitoneally approximately 48 hr and 24 hr before the experiment.

Measurement of vasoconstriction and vasodilatation. The photocell method (Holton & Perry, 1951) was used to detect changes in the amount of blood in part of the ear. The circular field of observation (area 1 cm^2) was chosen from the distal third of the ear, avoiding large blood vessels. The photocell was used in conjunction with an amplifier and recording milliammeter as described by Holton & Jones (1960), and the amount of light was adjusted so that the backing off voltage was 27 V. Thus the sensitivity was approximately comparable in the different experiments.

The apparatus was arranged so that an upward deflection on the record corresponded to increased light transmission.

Calibration. The amplitude of the deflection accompanying changes in the calibre of blood vessels is related to the change in the volume of blood between the photocell and the light. For a given volume of blood the change in light transmission is greater for a thin film over a large area than for an uneven distribution. It is therefore impossible to calibrate the apparatus exactly. However, the relationship between volume of blood and deflection was determined with an even film of blood, and gives some indication of the size of the changes observed. The vertical line on the records is the deflection produced in the middle range of the recorder by the addition of 1 μl. of rabbit's blood to 1 ml. 0.9% sodium chloride solution in the 1 cm^2 field of observation. The relationship is not linear and the same size of deflection was produced by 0.7 μl. in the top half of the record and 1.3 μl. in the bottom half.

RESULTS

The usual response of the ear vessels to sympathetic stimulation was an increase in light transmission followed by a decrease as illustrated in Fig. 1. This is interpreted as a vasoconstriction followed by a vasodilatation. There was a short latency between the beginning of stimulation and the beginning of the response; the vasoconstriction reached a peak in 10 to 12 sec and was succeeded by vasodilatation immediately after the end of the 15 sec period of stimulation. The response to sympathetic nerve stimulation was not accompanied by any change in the arterial blood pressure. It is therefore justifiable to conclude that changes in the amount of blood in the transilluminated portion of ear were due to vascular conditions in the ear alone. In an experiment in which the outflow from the ear was measured there was a decreased outflow when the light transmission increased, thus indicating vasoconstriction in the ear. During the second phase of the response when the light transmission decreased the venous outflow returned to, but did not exceed, its initial level. This experiment shows that the increased amount of blood in the ear cannot be attributed to venous constriction but is due to an active vasodilatation, and that, under these conditions, this vasodilatation does not involve the resistance vessels to any significant extent (see Hilton & Holton, 1954).

The ear vessels constricted in response to noradrenaline (Fig. 1) and dilated in response to acetylcholine (Fig. 1) or histamine (5 μg) injected intravenously.

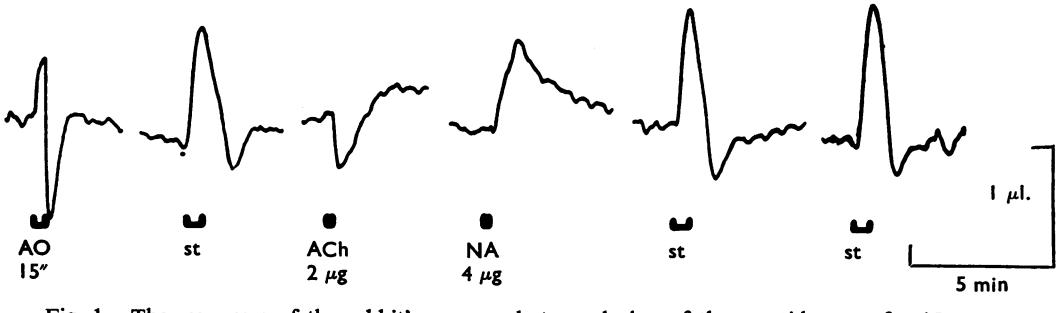

Fig. 1. The responses of the rabbit's ear vessels to occlusion of the carotid artery for 15 sec at *AO*, stimulation of the sympathetic nerves with 2 msec pulses at 20/sec for 15 sec at *st*, intravenous injection of 2 μg acetylcholine at *ACh* and 4 μg noradrenaline at *NA*. In this and later figures an upward movement of the record corresponds to increased light transmission indicating vasoconstriction. For explanation of the 1 μl. calibration, see " Methods."

Occlusion of the arterial blood supply was followed by a reactive hyperaemia (AO in Fig. 1). The responses to injections of noradrenaline, acetylcholine and histamine can be assumed to be due to changes in the diameter of blood vessels, since they were in the opposite direction from any passive effects likely to arise from changes in the blood pressure. The apparent vasoconstriction during arterial occlusion was, of course, due to preventing the inflow of blood ; the subsequent decrease in light transmission was due to vasodilatation (reactive hyperaemia), since it occurred without an increase of blood pressure.

The effect of varying the parameters of stimulation

Frequency. The effect of changing the frequency of stimulation is shown in the experiment illustrated in Fig. 2. Increasing the frequency, when the voltage, pulse

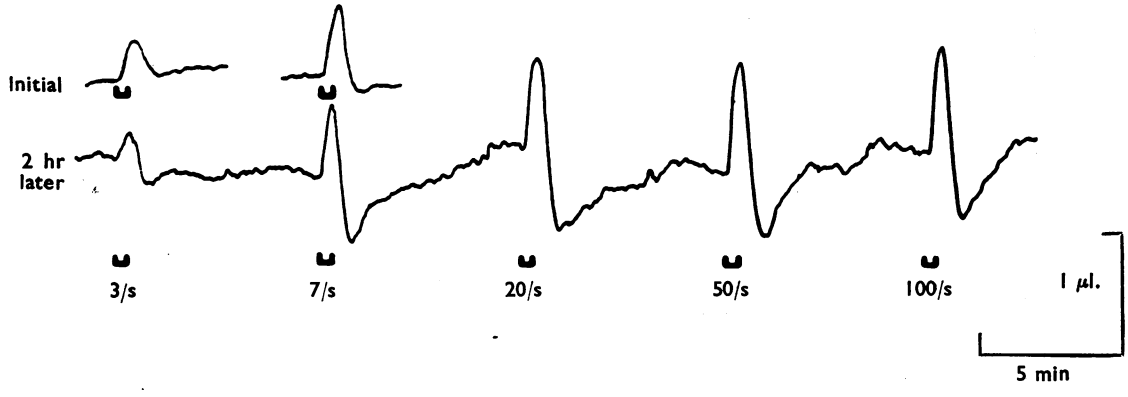

Fig. 2. The effect of increasing the frequency of stimulation with 2 msec pulses applied for 15 sec. The maximal response was obtained with a frequency of 20/sec. At the beginning of the experiment the responses were constrictor only; 2 hr later the constriction was followed by a secondary vasodilatation.

width and duration of stimulation were held constant, increased both the initial vasoconstriction and the secondary vasodilator phase of the response. This biphasic response was maximal at a frequency of 20/sec. Thus the nerve fibres involved had similar characteristics to sympathetic fibres innervating other effectors.

Voltage. At the beginning of an experiment a suitable response was obtained with pulses of 4 to 10 V across the electrodes. Increasing the voltage did not alter the nature of the response of the ear vessels, but sometimes caused a fall of blood pressure due to spread of stimulus to sensory nerves ; stimulation of sensory nerves causes a reflex fall of blood pressure in the rabbit. For this reason, in most experiments the voltage used was not large enough to produce a maximal response ; nevertheless the submaximal responses were reproducible as is shown in the Figures. In a few rabbits it was possible to increase the voltage to produce a maximal biphasic response without affecting the blood pressure.

Pulse width. The effect of altering the pulse width was investigated in order to determine whether the vasoconstrictor and vasodilator components of the response

were produced by neurones of the same diameter. A decentralized (preganglionically denervated) preparation was used for these experiments, since the vasodilator phase of the response was particularly well marked in these preparations, as described later. It was found that pulses of 0.5 msec duration had no effect; 1 msec pulses produced a small biphasic response; 2 msec pulses gave a qualitatively similar biphasic response of greater magnitude.

Duration of stimulation. A greater duration of stimulation than the 15 sec illustrated in Figs. 1 and 2 prolonged both the vasoconstrictor and vasodilator phases of the response, but did not increase their magnitude.

Alteration of stimulus parameters affected both phases of the response together. Thus it was impossible to distinguish between the nerve fibres responsible for the vasoconstrictor and the vasodilator phases by these means. It may be concluded from this that the nerve fibres involved were either identical or of similar diameter and excitability. In the remainder of the experiments we used pulses of 2 msec width of a suitable voltage at a frequency of 20/sec applied for 15 sec. Repetition of stimulation at 4 min intervals gave constant responses.

The effect of drugs on the vascular response to sympathetic nerve stimulation

Eserine. In six experiments eserine (0.4 to 0.7 mg/kg) was given without previous administration of atropine, and on every occasion the vasodilator phase of the response to sympathetic stimulation was enhanced, as was the vasodilatation produced by acetylcholine, as shown in Fig. 3. The effect of eserine on the vasoconstrictor phase was inconstant (compare A and B in Fig. 3). Recently, Burn, Rand

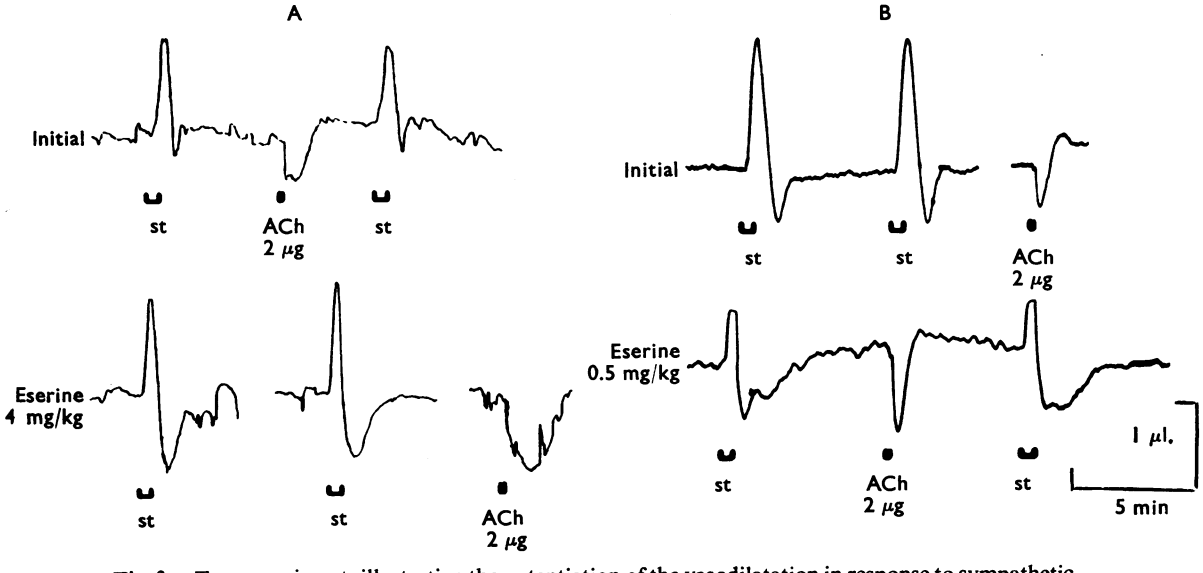

Fig. 3. Two experiments illustrating the potentiation of the vasodilatation in response to sympathetic stimulation after eserine. The dilator responses to acetylcholine were also potentiated. In *A* the vasoconstrictor phase was slightly increased, and in *B* it was decreased. Stimulation with 2 msec pulses at 20/sec for 15 sec at *st*; 2 μg acetylcholine injected intravenously at *ACh*.

& Wien (1962) found that the responses of the cat nictitating membrane and the guinea-pig vas deferens to sympathetic nerve stimulation were enhanced by eserine after atropine or hyoscine had been given to block the muscarinic effect of released acetylcholine. Therefore, in four experiments eserine was given after the vasodilator effect of acetylcholine had been antagonized by atropine, so that the vasoconstrictor response alone could be observed. In these experiments atropine decreased the vasoconstriction and eserine partly restored it, but there was no enhancement of the vasoconstrictor response above the control level.

Atropine. The effect of eserine suggests that the vasodilator component of the response of the ear vessels to sympathetic nerve stimulation is due to acetylcholine and might therefore be sensitive to atropine. The results with atropine, however, were less clear. Atropine was used in a total of eleven experiments, in four of which the superior cervical ganglion was chronically denervated.

In five of the seven unoperated rabbits atropine abolished sympathetic vasodilatation, as shown in Fig. 4. However, in the other two of these rabbits the vasodilatation was diminished but not abolished in spite of the injection of large doses of atropine (10 to 20 mg/kg) which completely blocked the response to injected acetylcholine.

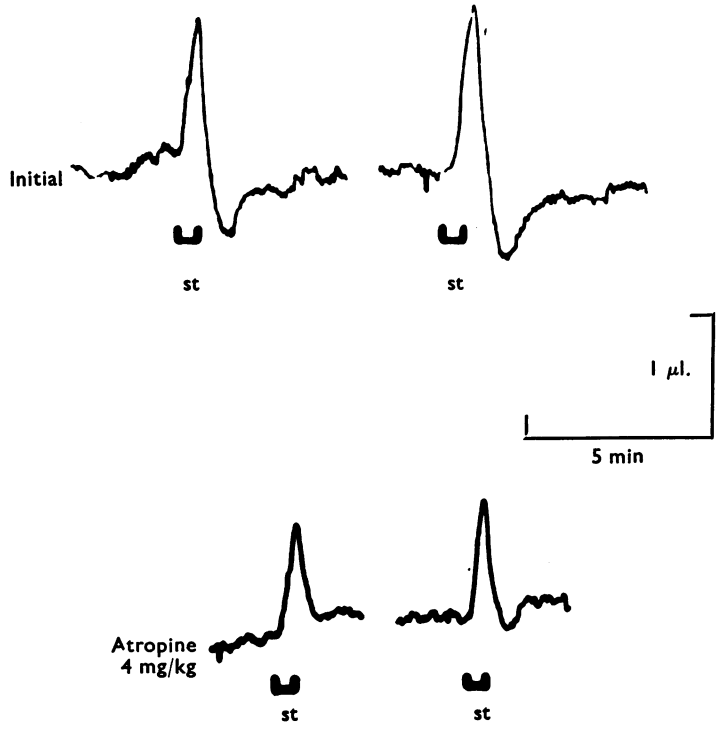

Fig. 4. Effect of atropine on the response of the ear vessels to sympathetic nerve stimulation with 2 msec pulses at 20/sec for 15 sec at *st*. After atropine the dilator phase of the response was absent.

In the four rabbits with the sympathetic supply to the ear decentralized the vessels were hypersensitive to acetylcholine and relatively refractory to the blocking action of atropine. In one of these experiments sympathetic vasodilatation was abolished by atropine, while in the others it was decreased but not to the same extent as was the response to injected acetylcholine.

In experiments in which some vasodilatation remained after atropine it always followed vasoconstriction and could have been a reactive hyperaemia. When the vasoconstriction had been abolished by the procedures described below the vasodilator response was sensitive to atropine.

Atropine was used in these experiments as a tool to indicate the muscarinic actions of acetylcholine, but, in the rabbit, it is not as selective as is desirable. The rabbit is known to be rather insensitive to the anti-muscarinic actions of atropine, and in addition some rabbits are able to hydrolyse atropine so that its effects are short-lived (see Ambache, 1955). These factors were apparent in our experiments. We found that the effect of atropine in blocking the response to injected acetylcholine or the vasodilator phase of the response to sympathetic stimulation wore off in the course of 0.5 to 2 hr. It was also necessary to give high doses (4 to 20 mg/kg) of atropine to block the vasodilatation caused by acetylcholine. In these doses atropine often diminished the constrictor phase of the response to sympathetic stimulation (for example, in Fig. 4). This finding may be related to those of Bussell (1940) that high concentrations of atropine impair the vasoconstrictor responses of the perfused rabbit's ear to adrenaline and to sympathetic stimulation.

Guanethidine. After the injection of 0.5 to 1 mg/kg of guanethidine the constrictor response to sympathetic nerve stimulation was greatly reduced or abolished. The dilator component of the response was always present after guanethidine.

The effect of guanethidine on the responses of the ear vessels, without previous injection of atropine or eserine, is shown in Fig. 5. In this experiment the initial response to sympathetic stimulation was vasoconstriction followed by vasodilatation. After guanethidine there was a small constrictor component and dilatation began during the period of sympathetic stimulation. Eserine was then given and the dilator responses to sympathetic stimulation and to 2 μg acetylcholine were prolonged to an approximately equal extent. Then after atropine both these dilator responses were virtually abolished.

Reserpine. There were only small constrictor responses to sympathetic stimulation in reserpine-treated rabbits. However, the ear vessels gave the usual response to acetylcholine and were hypersensitive to noradrenaline. No sign of sympathetic vasodilatation was visible initially, but after eserine a dilator response appeared. Atropine then abolished the dilatation, leaving only a small constriction. These results are illustrated in Fig. 6.

An infusion of noradrenaline into the reserpinized rabbit had little effect on the responses to sympathetic stimulation, but an infusion of dopamine led to an increased vasoconstrictor response for a period of about 1 hr after the end of the infusion. This provides evidence that the poor response to sympathetic stimulation was in fact a result of the pretreatment with reserpine and not due to faulty dissection.

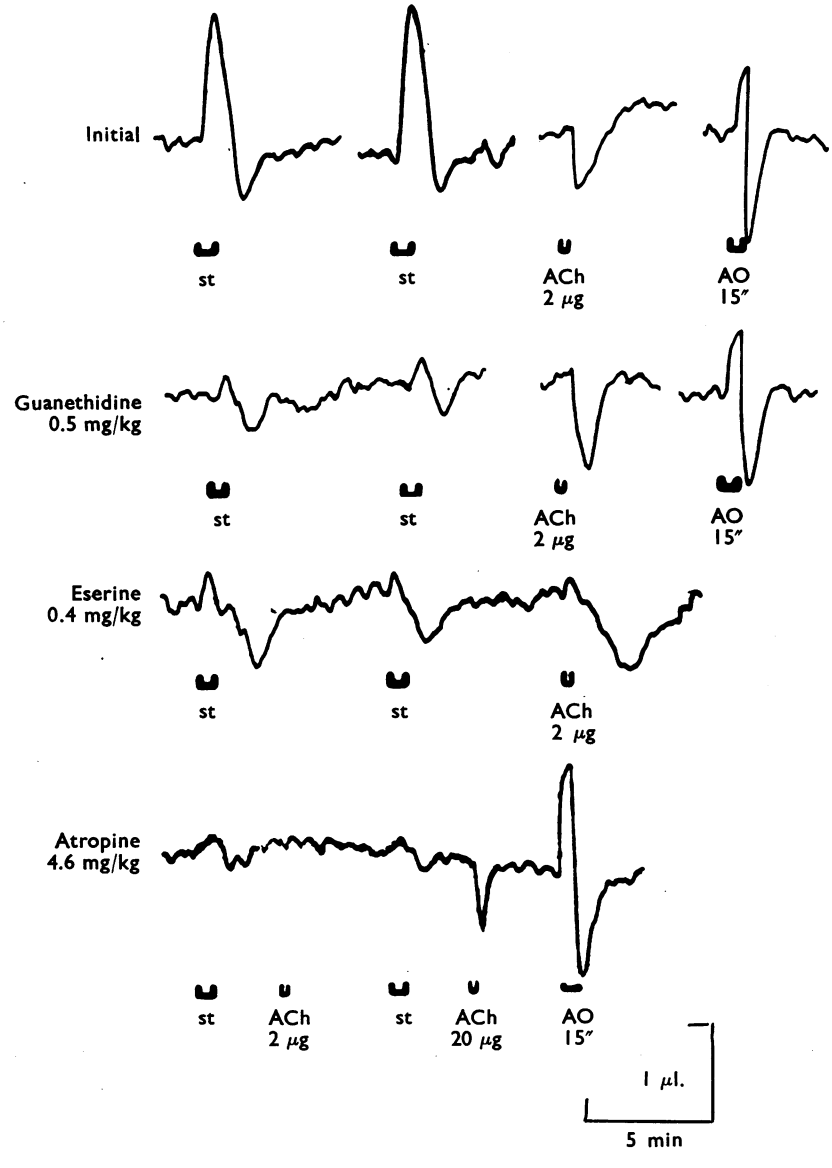

Fig. 5. Effect of guanethidine on the responses of the ear vessels to sympathetic stimulation with 2 msec pulses at 20/sec for 15 sec at *st*. Intravenous injections of acetylcholine were given at *ACh* in the doses indicated. The carotid artery was occluded for 15 sec at *AO*. Guanethidine blocked the constrictor but not the dilator phases of the response to sympathetic stimulation; the dilatation was enhanced after eserine and blocked after atropine.

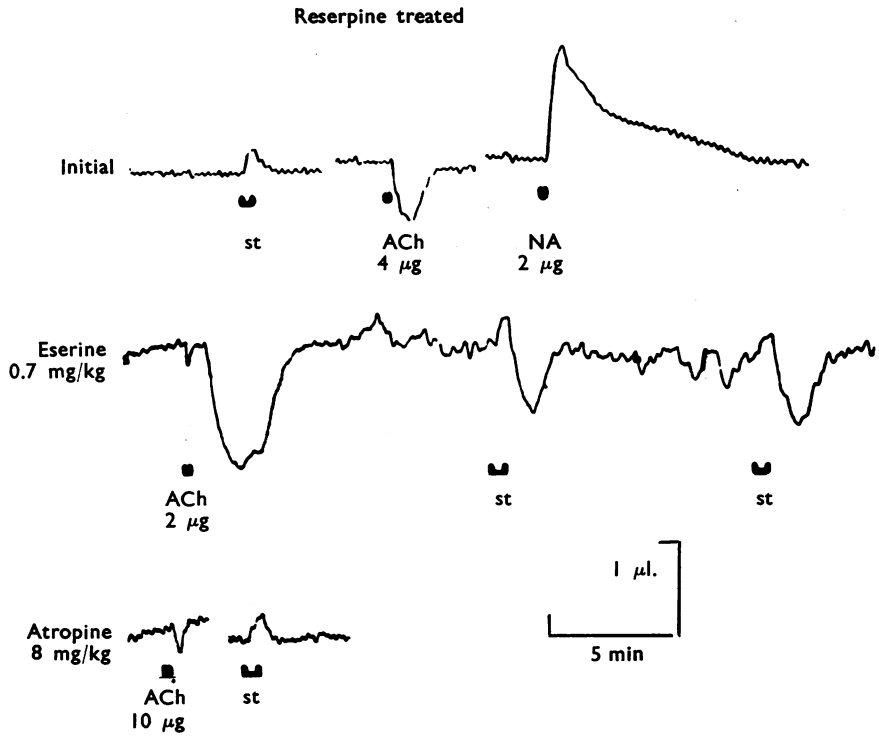

Fig. 6. Responses of ear vessels in a reserpine-treated rabbit. Sympathetic stimulation with 2 msec pulses at 20/sec for 15 sec at *st*; intravenous injections of acetylcholine at *ACh* and noradrenaline at *NA* in the doses indicated.

Degeneration of the pre-ganglionic sympathetic nerves

In eight rabbits the pre-ganglionic cervical sympathetic nerve was cut 5 to 17 days before the experiment. This was originally done to ensure that the dilator response to sympathetic nerve stimulation was not due to acetylcholine released from the endings of pre-ganglionic fibres which might have passed through the superior cervical ganglion and impinged on aberrant ganglion cells distal to the stimulating electrodes, since anatomical variations of this sort are thought to occur (Mitchell, 1956). It was found that not only was the vasodilator phase of the response to sympathetic stimulation still present, but it was enhanced. The vasoconstrictor phase was present, but relative to the vasodilatation it was less marked than in unoperated rabbits. Some of the decentralized ears were hypersensitive to noradrenaline, but this was not a constant finding and in any case the increased sensitivity was less marked than in unoperated rabbits after reserpine or guanethidine. The vasodilatation of the ear vessels in response to acetylcholine was usually greater than in unoperated rabbits. In four experiments carried out 12 to 17 days after decentralization the mean deflection of the pen in response to 2 μg of acetylcholine was 3 times greater than that in unoperated rabbits, but in 1 rabbit tested 5 days after operation there was no increase in sensitivity to acetylcholine.

Fig. 7 shows the results from an experiment on a rabbit in which the superior cervical ganglion had been decentralized 12 days previously. The response to stimulation using the usual parameters of stimulation resulted in a constriction followed by such a pronounced dilatation that the recording apparatus could not accommodate it. When the strength of the pulses was reduced to 1.5 V and the duration of application reduced to 9 sec the constriction was very slight and was followed by a pronounced dilatation as shown in Fig. 7. Atropine abolished this dilatation and the dilatation produced by acetylcholine. In this experiment the

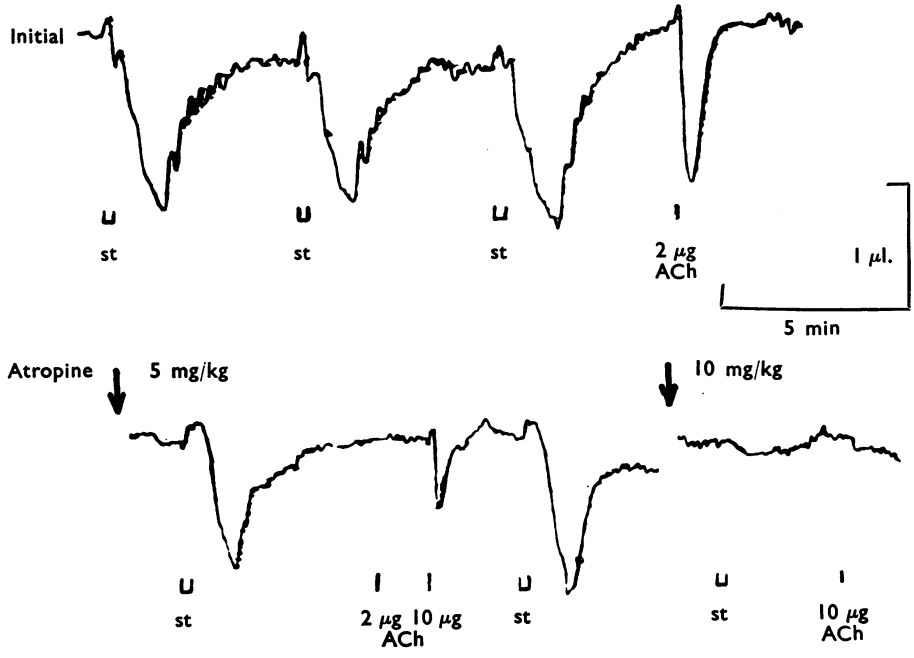

Fig. 7. Responses of the ear vessels 12 days after section of the pre-ganglionic sympathetic and greater and lesser auricular nerves. At *st*, stimulation with 2 msec pulses at 20/sec for 9 sec; at *ACh*, acetylcholine in the doses in μg indicated. After 5 mg/kg atropine the sympathetic dilatation was decreased and the response to 2 μg acetylcholine was blocked, but not the response to 10 μg. After an additional 10 mg/kg atropine the responses to 10 μg acetylcholine and to sympathetic stimulation were completely blocked.

greater and lesser auricular nerves had degenerated as well as the pre-ganglionic sympathetic, but similar results were obtained in other experiments in which only the pre-ganglionic sympathetic nerve had degenerated. The hypersensitivity to acetylcholine and to sympathetic vasodilatation may therefore be attributed to decentralizing the sympathetic ganglion.

Reactive hyperaemia

When the blood supply to the ear was occluded by clamping the carotid artery or the central artery at the base of the ear there was an upward movement of the

pen record indicating that there was less blood in the ear. On restoring the blood flow there was vasodilatation. This pattern of events, shown in Figs. 1 and 5, resembled the biphasic response to sympathetic stimulation. It seemed possible that the secondary vasodilatation seen after sympathetic stimulation might have been wholly or partly due to hyperaemia reactive to the decrease in blood supply to the ear during the primary vasoconstriction. However, there are a number of pieces of evidence which indicate that this is not the whole explanation. Fig. 2 shows the results from an experiment in which stimulation produced only constriction initially, but 2 hr later produced a biphasic response ; the vasodilatation of reactive hyperaemia did not alter during an experiment. The vasodilator component of the response to sympathetic stimulation was enhanced after eserine and decreased after atropine ; the hyperaemia following mechanical obstruction of the blood flow was not affected by these drugs. Vasodilatation in response to sympathetic stimulation was observed after procedures which reduced or abolished the initial vasoconstriction ; in these circumstances a reactive hyperaemia cannot account for the dilatation. For example, in Fig. 5 dilator responses persisted after the constriction was blocked by guanethidine ; in Fig. 6 the response after eserine in a reserpine-treated rabbit was almost entirely a dilatation ; in Fig. 7 the dilator component of the response was marked although constriction was insignificant.

DISCUSSION

The results show that stimulation of the post-ganglionic sympathetic nerves to the rabbit's ear produces vasoconstriction followed by dilatation. There is evidence that acetylcholine is involved in causing the vasodilator component of the response, although in certain circumstances the vasodilatation may be in part a reactive hyperaemia. The evidence in favour of a cholinergic mechanism derives mainly from experiments with eserine and atropine. The vasodilatation after sympathetic stimulation was always enhanced by eserine and abolished or decreased by atropine, as would be expected were acetylcholine responsible.

Vasodilatation is readily obtained on antidromic stimulation of sensory nerves to the rabbit's ear. Before concluding that the vasodilatation described in this paper was due to sympathetic nerves, it was necessary to exclude any possibility of antidromic vasodilatation. The sympathetic vasodilatation was unaltered by acute section of the greater and lesser auricular nerves, and was particularly well marked in those rabbits in which the nerves had degenerated (see Fig. 7). Therefore it is unlikely that sensory fibres which could produce antidromic vasodilatation were stimulated when the sympathetic nerves were stimulated. Sympathetic vasodilatation may also be distinguished from antidromic vasodilatation by its different sensitivity to eserine and by the relationship between the number of shocks and the duration of the response. Thus sympathetic vasodilatation is enhanced whereas antidromic vasodilatation is decreased after eserine (Holton & Perry, 1951 ; Holton, 1953). Antidromic vasodilatation occurs after a single shock which has no discernible effect when applied to the sympathetic ganglion. A train of 300 shocks which causes sympathetic vasodilatation lasting a few minutes would result in antidromic vasodilatation lasting 30 min or more were it applied to sensory nerves (compare Holton

& Perry, 1951 ; Hilton & Holton, 1954). It is therefore justifiable to conclude that antidromic vasodilatation did not contribute to the responses obtained when the superior cervical ganglion was stimulated.

The results after decentralizing the sympathetic ganglion supplying the nerves to the ear provide additional evidence for acetylcholine release from the post-ganglionic sympathetic nerves. The object of this procedure in the first place was to investigate whether the dilatation could have been due to acetylcholine released from the endings of pre-ganglionic cholinergic neurones which terminated beyond the superior cervical ganglion, since aberrant ganglion cells of this type have been described (see Mitchell, 1956). Cutting the cervical sympathetic trunk would produce degeneration of these as well as the pre-ganglionic neurones terminating in the superior cervical ganglion. However, our evidence in favour of post-ganglionic cholinergic sympathetic neurones not only remained but was strengthened by the results from these experiments. In these rabbits the ear vessels were hypersensitive to the dilator action of acetylcholine, and also sympathetic stimulation was more effective in producing vasodilatation than in unoperated rabbits. These two findings together are a strong indication that the transmitter responsible for the sympathetic dilatation is acetylcholine. The hypersensitivity is probably an example of the sensitivity of denervated structures which is known to occur not only as a result of true denervation, but also as a result of decentralizing the autonomic ganglia (Cannon & Rosenblueth, 1949). It seems reasonable to conclude that this is evidence that post-ganglionic sympathetic neurones normally release acetylcholine in the vicinity of the blood vessels in the rabbit's ear.

The use of reserpine and guanethidine enabled us to obtain responses with little or no constrictor component. Reserpine depletes the stores of noradrenaline from the skin of the rabbit's ear (Burn & Rand, 1958). After reserpine the vessels are already dilated and the sympathetically induced dilator response was only obtained after eserine. Guanethidine blocked the constrictor phase of the response to sympathetic stimulation but not the dilator phase. Presumably the constriction is caused by noradrenaline and the dilatation by acetylcholine. The rapid blocking action of guanethidine is not associated with depletion of noradrenaline, although some hours later depletion does occur (Cass & Spriggs, 1961). Probably the adrenergic blockade after guanethidine is due to prevention of noradrenaline release as has been shown for the other adrenergic nerve-blocking drugs xylocholine (Exley, 1957) and bretylium (Boura & Green, 1959). There are other observations that guanethidine blocks the adrenergic response to sympathetic nerve stimulation and then reveals cholinergic responses. McCubbin, Kaneko & Page (1961) and Bogaert, Schaepdryver & Vleeschhouwer (1961) found that, after guanethidine had blocked vasoconstriction in the dog's hindleg in response to sympathetic stimulation, there was a vasodilator response which was blocked by atropine. Day & Rand (1961) found that the normal effects of stimulating sympathetic nerves to the rabbit's intestine and to the cat's atria were reversed after guanethidine to resemble responses to acetylcholine which were then blocked by atropine. A possible explanation for these findings is that the normal role of the acetylcholine from cholinergic sympathetic post-ganglionic fibres is to release noradrenaline from stores at the nerve

endings. When this release is impossible because of blockade by guanethidine, or because of depletion of the stores by reserpine, then the acetylcholine can exhibit its muscarinic action on the receptors; in the rabbit's ear this results in vasodilatation. The fact that dilatation is almost always seen as a component of the response of the ear to sympathetic stimulation suggests that some of the acetylcholine normally acts directly on the blood vessels.

The relevance of our results to the hypothesis that the release of noradrenaline is cholinergically mediated is that they provide further evidence for the existence of cholinergic sympathetic nerve fibres. Evidence for sympathetic cholinergic vasodilator fibres to blood vessels of the skin has previously been lacking (for example, see Gaskell, 1956), although it has long been known that there are such fibres innervating muscle blood vessels (Burn, 1938).

Some of this work was done during the tenure of a Research Fellowship of the Wellcome Foundation by M.J.R. The expenses of the work were met by grants to P.H. from St Mary's Hospital Endowment Fund and from the Medical Research Council. The authors are grateful to Professor A. St G. Huggett for allowing M.J.R. to work at St Mary's, and to Professor G. A. H. Buttle for allowing him to work away from the School of Pharmacy.

REFERENCES

AMBACHE, N. (1955). The use and limitations of atropine for pharmacological studies on autonomic effectors. *Pharmacol. Rev.*, 7, 467–494.

ARMIN, J., GRANT, R. T., THOMPSON, R. H. S. & TICKNER, A. (1953). An explanation for the heightened vascular reactivity of the denervated rabbit's ear. *J. Physiol. (Lond.)*, **121**, 603–622.

BOGAERT, M., SCHAEPDRYVER, A. F. DE & VLEESCHHOUWER, G. R. DE (1961). On the pharmacology of guanethidine. *Arch. int. Pharmacodyn.*, **34**, 224–236.

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Brit. J. Pharmacol.*, **14**, 536–548.

BURN, J. H. (1938). Sympathetic vasodilator fibres. *Physiol. Rev.*, **18**, 137–153.

BURN, J. H. & DUTTA, N. K. (1948). The action of antagonists of acetylcholine on the vessels of the rabbit's ear. *Brit. J. Pharmacol.*, **3**, 354–361.

BURN, J. H. & RAND, M. J. (1958). Noradrenaline in artery walls and its dispersal by reserpine. *Brit. med. J.*, **i**, 903–908.

BURN, J. H. & RAND, M. J. (1960). Sympathetic postganglionic cholinergic fibres. *Brit. J. Pharmacol.*, **15**, 56–66.

BURN, J. H. & RAND, M. J. (1962). A new interpretation of the adrenergic nerve fiber. In *Advances in Pharmacology*, Vol. 1, pp. 1–30. Ed. S. GARATTINI & P. A. SHORE. New York: Academic Press.

BURN, J. H., RAND, M. J. & WIEN, R. (1962). Communication to Brit. Pharmacol. Soc., Oxford, July (to be published).

BUSSELL, L. J. (1940). The relation of atropine to adrenaline and the sympathetic system. *J. Pharmacol. exp. Ther.*, **69**, 128–139.

CANNON, W. B. & ROSENBLUETH, A. (1949). *The Supersensitivity of Denervated Structures.* New York: Macmillan.

CASS, R. & SPRIGGS, T. L. B. (1961). Tissue amine levels and sympathetic blockade after guanethidine and bretylium. *Brit. J. Pharmacol.*, **17**, 442–450.

CHANG, V. & RAND, M. J. (1960). Transmission failure in sympathetic nerves produced by hemicholinium. *Brit. J. Pharmacol.*, **15**, 588–600.

DAY, M. D. & RAND, M. J. (1961). Effect of guanethidine in revealing cholinergic sympathetic fibres. *Brit. J. Pharmacol.*, **17**, 245–260.

EXLEY, K. A. (1957). The blocking action of choline 2 : 6-xylyl ether bromide on adrenergic nerves. *Brit. J. Pharmacol.*, **12**, 297–305.

GASKELL, P. (1956). Are there sympathetic vasodilator nerves to the vessels of the hands? *J. Physiol. (Lond.)*, **131**, 647–656.

HILTON, S. M. & HOLTON, P. (1954). Antidromic vasodilatation and blood flow in the rabbit's ear. *J. Physiol. (Lond.)*, **125**, 138–147.

HOLTON, P. (1953). Antidromic vasodilatation and inhibitors of cholinesterase. *J. Physiol. (Lond.)*, **120**, 95–104.

HOLTON, P. & JONES, M. L. (1960). Some observations on changes in the blood content of the cat's pancreas during activity. *J. Physiol. (Lond.)*, **150**, 479–488.

HOLTON, P. & PERRY, W. L. M. (1951). On the transmitter responsible for antidromic vasodilatation in the rabbit's ear. *J. Physiol. (Lond.)*, **114**, 240–251.

HUKOVIĆ, S. (1960). The action of sympathetic blocking agents on isolated and innervated atria and vessels. *Brit. J. Pharmacol.*, **15**, 117–121.

KOTTEGODA, S. R. (1953). The action of nicotine and acetylcholine on the vessels of the rabbit's ear. *Brit. J. Pharmacol.*, **8**, 156–161.

MCCUBBIN, J. W., KANEKO, Y. & PAGE, I. H. (1961). The peripheral cardiovascular actions of guanethidine in dogs. *J. Pharmacol. exp. Ther.*, **131**, 346–354.

MITCHELL, G. A. G. (1956). *Cardiovascular Innervation*. Edinburgh and London : Livingstone.