Papers and Originals

Release of Noradrenaline from the Sympathetic Postganglionic Fibre*

J. H. BURN,† M.D., F.R.S.

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It is now 45 years since Loewi, on the one hand, and Cannon and Uridil, on the other, laid the foundation for the theory of the chemical transmission of nerve impulses. On this foundation Dale and Gaddum began to build the main structure in 1930.

One of the earliest discoveries, and the first which came as a surprise, was that the splanchnic nerves did not directly release adrenaline from the adrenal glands. Feldberg and Minz (1933) noted that acetylcholine had been shown to release adrenaline from the glands, and were therefore led to investigate the transmission of splanchnic impulses; they found that acetylcholine transmitted the impulses to the adrenal medulla. There has long been general agreement that the cells of the adrenal medulla are derived from the embryonic sympathetic nervous system. At an early stage the intra-adrenal cells are very similar to those in the sympathetic ganglia. By the 30 mm. crown-rump length stage in the human embryo some of the cells differentiate towards the structure of chromaffin cells, but for some time most of the cells have the general appearance of sympathetic neuroblasts (Boyd, 1960).

Therefore, after the surprising demonstration of Feldberg and Minz, it might well have been asked whether there was another surprise in store, and whether acetylcholine was involved in the release of sympathin from the sympathetic postganglionic fibre. But no one asked this question at that time.

Evidence of Release of Acetylcholine by Sympathetic Nerves

Yet in 1931 Euler and Gaddum had made the discovery that sympathetic fibres released not only an adrenaline-like substance but also acetylcholine. Their finding arose from an investigation of the Rogowicz phenomenon, first described in 1885. This was a curious contraction of the denervated facial muscles of the dog which occurred when the cervical sympathetic nerve was stimulated. Euler and Gaddum showed that the contraction was due to the release of acetylcholine from the terminations of the postganglionic fibres coming from the superior cervical ganglion. Thus Euler and Gaddum established that fibres exerting their main effect by liberating what we now know to be noradrenaline also liberated acetylcholine.

This rather obscure phenomenon was generally forgotten when Dale and Feldberg in 1934 showed that the postganglionic fibres to the sweat glands of the cat's foot transmitted their impulses by acetylcholine alone. Since that time physiologists have paid little or no attention to evidence of acetylcholine release when this has been demonstrated in sympathetic fibres mainly concerned in the release of noradrenaline.

There were, however, by 1948 already several cases in which both noradrenaline and acetylcholine had been shown to be

released by stimulation of sympathetic fibres. Thus Bülbring and Burn (1935) found that both substances were released by the postganglionic fibres running to the vessels of the dog's hind leg. They showed that when the hind leg was perfused stimulation caused acetylcholine to appear in the venous effluent. In the same year Sherif (1935) found that acetylcholine was released by the sympathetic fibres to the dog uterus. In 1948 Folkow and Uvnäs found that both substances were released by the sympathetic fibres to the vessels of the cat's hind leg. The discovery that both substances were released by fibres innervating blood vessels was not very surprising, because it seemed reasonable that vessels should have an innervation which could cause vasodilatation as well as vasoconstriction.

Release of Acetylcholine by Sympathetic Nerves to the Heart

However, there was no such explanation for a second discovery made by Folkow, Frost, Haeger, and Uvnäs (1948). They showed that when the heart of the cat or of the dog was perfused stimulation of the stellate ganglion led to the appearance of acetylcholine in the fluid leaving the heart. The fibres carrying the impulses are the nervi accelerantes, and their function is to make the heart beat faster and more powerfully, which they accomplish by liberating noradrenaline. What then could be the purpose of the release of acetylcholine?

At this point it can be said that there is no doubt that the finding of Folkow and Uvnäs and their colleagues was correct. The release of acetylcholine has been confirmed three times. First by Huković (1959), who prepared the atria of the rabbit heart with sympathetic fibres attached. He set up the preparation in an isolated organ bath and stimulated the stellate ganglion; he observed an increase in the rate and force of the beat. He then injected rabbits with reserpine, and so was able to remove all noradrenaline from the sympathetic postganglionic fibres. When he prepared the atria from these rabbits he found that stimulation of the stellate ganglion caused inhibition; he found that the inhibition was greater in the presence of physostigmine, and that it was abolished by atropine. Thus stimulation caused liberation of acetylcholine.

Other experiments were carried out by Day and Rand (1961) on the isolated atria of the cat; they used guanethidine to prevent the release of noradrenaline, and found that stimulation of the stellate ganglion no longer caused a simple increase in force and rate, but that the response gradually changed to inhibition. Again, Boura and Green (1959), working on the cat anaesthetized with chloralose, found that if they prevented the release of noradrenaline by injecting bretylium, then stimulation of the inferior cardiac nerve caused slowing of the heart instead of acceleration, and that the slowing was blocked by atropine. The combined effect of the four pieces of evidence is to establish that acetylcholine is released when the accelerator nerves to the heart of the dog, the cat, and the rabbit are

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† Visiting Professor to Washington University, St. Louis.

stimulated, though under normal circumstances this acetylcholine exerts no effect on the heart rate.

Release of Acetylcholine from the Splenic Nerves

Further investigation has revealed that the sympathetic nerves to the spleen resemble those to the heart in releasing both noradrenaline and acetylcholine. Burn and Rand (1960) studied the response to stimulation of the splenic nerves in cats given reserpine previously to remove noradrenaline from the nerves. Stimulation did not evoke the usual response of contraction of the spleen, but on the contrary made the spleen dilate. This dilatation was shown to be due to the release of acetylcholine, because the dilatation increased after the injection of physostigmine and was abolished by the injection of atropine. More important, however, was the work of Brandon and Rand (1961), who perfused the spleens of cats which had been treated with reserpine. The postganglionic fibres of these cats contained little or no noradrenaline, and when they were stimulated (neostigmine being present in the perfusion fluid) Brandon and Rand found that acetylcholine was liberated in the venous effluent.

Amount of Acetylcholine in Splenic Nerves

These workers also measured the amount of acetylcholine and of noradrenaline in the nerves. They did this very ingeniously by following the method of Euler and Purkhold (1951), who estimated the noradrenaline in the splenic nerves of sheep by determining the amount in the normal spleen and the amount in the spleen after section and degeneration of the splenic nerves. Brandon and Rand made their experiments in cats. In the normal cat spleen the amount of noradrenaline was 1.13 $\mu\text{g./g.}$ and the amount of acetylcholine was 0.47 $\mu\text{g./g.}$ In spleens to which the sympathetic fibres had degenerated the amount of noradrenaline declined to 0.21 $\mu\text{g./g.}$ (18% of the amount in normal spleens) and the amount of acetylcholine declined to 0.1 $\mu\text{g./g.}$ (22% of the amount in normal spleens). Since there was almost the same percentage drop for the two substances, the conclusion was drawn that about 80% of the acetylcholine and 80% of the noradrenaline in normal spleens were present in the nerves, and that the amount of acetylcholine in the nerves was as high as 40% of the noradrenaline. Any impression that the amount of acetylcholine in postganglionic fibres is negligible must therefore be abandoned.

Other Sympathetic Nerves Releasing Acetylcholine

Stimulation of sympathetic postganglionic fibres has been shown to release acetylcholine into the venous effluent during perfusion of the skin vessels of the rabbit ear (Burn and Rand, 1960), and during perfusion of the pilomotor muscles of the cat's tail (Wolner, 1965). If to the cases described in which acetylcholine has been demonstrated in the venous effluent during perfusion experiments are added the original evidence of Euler and Gaddum (1931), and that obtained in the cat nictitating membrane by Burn, Rand, and Wien (1963), there are 10 tissues in which the evidence is very strong that stimulation of sympathetic postganglionic fibres releases acetylcholine as well as noradrenaline. Thus the evidence approaches, or has reached, a point at which it indicates a general rule.

Function of Acetylcholine

In order to consider the function of acetylcholine released by sympathetic stimulation the fact must be recalled that acetylcholine releases noradrenaline from sympathetic postganglionic

fibres. The first evidence came from Hoffmann, Hoffmann, Middleton, and Talesnik (1945), who showed that when they treated a rabbit heart, which was isolated and perfused, with atropine then acetylcholine injected into the heart was not inactive, as might have been expected, but caused an increase in the rate and force of the beat and also liberated an adrenaline-like substance into the fluid leaving the heart. Cabrera, Cohen, Middleton, Utano, and Viveros (1966) have proved that the noradrenaline came from the sympathetic postganglionic fibres.

Acetylcholine has been shown to release noradrenaline from the sympathetic fibres in the spleen (Daly and Scott, 1961), from the fibres to the vessels of the rabbit ear (Burn and Rand, 1958), and from the fibres to the pilomotor muscles of the cat's tail (Brücke, 1935; Coon and Rothman, 1940).

These observations might have been held to indicate the function of the acetylcholine released by sympathetic stimulation as being to release noradrenaline in its turn. However, Ferry (1963) put forward a different view. He showed that when acetylcholine was injected into the splenic artery of the cat it caused antidromic impulses to pass up the C fibres of the splenic nerve. He therefore presumed that it would also cause impulses to pass into the spleen. Thus he suggested that acetylcholine caused release of noradrenaline by first stimulating the postganglionic fibres, which in their turn released noradrenaline.

Evidence against this conclusion has now come from no fewer than three sources, which agree in finding that bretylium will block the release of noradrenaline by sympathetic stimulation before it blocks the release by acetylcholine. This observation makes it very difficult to suppose that acetylcholine releases noradrenaline by stimulating the sympathetic nerve. Hertting and Widhalm (1965), having injected labelled noradrenaline into cats, then perfused the spleen. They observed the release of labelled noradrenaline after (a) stimulation of the splenic nerves, and (b) injection of acetylcholine. The release by nerve stimulation was blocked by bretylium in concentrations from 2×10^{-6} g./ml. to 10^{-5} g./ml. The release by acetylcholine was blocked only by a concentration of bretylium of 5×10^{-5} g./ml. Wolner (1965) found a similar situation when he perfused the tail of the cat. Stimulation of sympathetic postganglionic fibres caused erection of the hairs, and so did injection of acetylcholine. When bretylium was added to the perfusing fluid the effect of sympathetic stimulation was blocked long before the effect of acetylcholine. Fischer, Weise, and Kopin (1966) also worked on the perfused cat spleen and found that when bretylium blocked the effect of stimulation in releasing noradrenaline it did not block the effect of acetylcholine.

Despite the evidence of these three studies the suggestion has been made that Ferry's view may still be right. If the action of bretylium is that of a local anaesthetic it might block the nerve fibres at points of branching before blocking the terminations themselves. One answer to this, which is alone sufficient, comes from Exley's (1957) observations on the action of xylocholine, which was the precursor of bretylium. He compared xylocholine with the otherwise identical compound in which the methyl groups attached to the nitrogen were replaced by ethyl groups. Xylocholine and the "ethyl" compound were equiactive as local anaesthetics, but the "ethyl" compound was devoid of action as an adrenergic blocking agent. The conclusion can therefore be drawn that acetylcholine releases noradrenaline from the postganglionic fibre by a direct action, as it does in the adrenal medulla, and not by stimulating the nerve.

Evidence that Acetylcholine Liberated by Sympathetic Stimulation Releases Noradrenaline

What was now required was evidence that acetylcholine, not injected but released by the sympathetic impulse, released noradrenaline in its turn. At first sight such evidence was not

easy to obtain. However, at the ending of the motor nerve in skeletal muscle the function of acetylcholine was accepted as being that of a humoral transmitter, mainly because (1) the motor nerve released acetylcholine on stimulation, (2) acetylcholine on "close-arterial" injection caused contraction, and (3) agents like physostigmine caused a single twitch to become a repetitive response, and depressed the contractions when the frequency of stimulation was high. So far as the postganglionic sympathetic fibre is concerned, the first two points have already been shown to apply. It remained to investigate the effect of anticholinesterases such as physostigmine.

Acetylcholine has an effect of its own on the end organ, and an anticholinesterase would increase this effect. The direct action of acetylcholine had to be excluded first of all, in order to see whether an anticholinesterase would affect the response of the organ to stimulation when only noradrenaline could act. Atropine or hyoscine was therefore injected; this also ensured that the anticholinesterase did not modify the response to noradrenaline (Burn, Philpot, and Trendelenburg, 1954). It seemed probable in advance that if the amount of noradrenaline released was increased by an anticholinesterase the greatest increase would be seen at the lowest frequency of stimulation, and that the increase would diminish as the frequency rose. For if the nerve impulse releases acetylcholine, then when a fixed number of pulses is given, and when the interval between pulses is long, there is more time for cholinesterase to destroy the acetylcholine between pulses, and so to prevent the concentration of acetylcholine from rising. The hypothesis assumes that as the concentration of acetylcholine rises more noradrenaline will be released. When the frequency of stimulation is higher there will be less time for cholinesterase to act between each pulse; therefore the concentration of acetylcholine will rise and more noradrenaline will be released. What then will be the effect of an anticholinesterase? It will surely be greatest at the lowest frequency when cholinesterase has its greatest effect. It will cause the response at the low frequencies to approximate to the response at the higher frequencies. The effect of the anticholinesterase will diminish as the frequency rises because the effect of the cholinesterase diminishes with the shortening of the time in which it can act.

Evidence has been obtained in five tissues. It was first obtained in the nictitating membrane of the cat (Burn *et al.*, 1963), and the results in two experiments are shown in Table I.

TABLE I.—Contractions of the Nictitating Membrane of the Cat in Response to Maximal Stimulation of Postganglionic Sympathetic Fibres After the Injection of Hyoscine. At Each Stimulation 100 Shocks Were Given. The Effect of Physostigmine 0.5 mg./kg. is Shown in Experiment 1 and of Neostigmine 0.5 mg./kg. in Experiment 2

Frequency per sec.	Experiment 1			Experiment 2		
	Control (mm.)	After Physostigmine (mm.)	% Inc.	Control (mm.)	After Neostigmine (mm.)	% Inc.
0.5	—	—	—	3.5	8	129
1	18	29	61	9.5	16.5	73
2	35	43	23	19.5	25.5	30
5	45	51	13	25	27	8
10	47	51	8	26.5	28	5
20	51	52	2	28	29	3

The height of the contraction of the membrane in response to postganglionic stimulation is shown, a fixed number of shocks being given at various frequencies. The observations were made after injecting hyoscine. In the first experiment the injection of physostigmine increased the contraction from 18 to 29 mm. at a frequency of 1 per second—that is, by 61%. But at a frequency of 5 per second the increase was only 13%. Moreover, at frequencies of 5, 10, and 20 per second the contractions in the presence of physostigmine remained constant and equal to the contraction at 20 per second in the absence of physostigmine. The results in this and in three other experiments agreed precisely with the expectation.

A second series of experiments were made in the taenia of the guinea-pig caecum by Ng (1966). The taenia can be dissected with sympathetic fibres which accompany the vessels running to it. The preparation is set up in an isolated organ bath and responds to acetylcholine by contraction and to noradrenaline by inhibition. Hyoscine was therefore always present in the bath to exclude the direct action of acetylcholine. Ng found that the addition of the anticholinesterases physostigmine, mipafox, or dyflos increased the inhibitory response to stimulation of low frequency, the increase becoming less as the frequency rose.

An experiment with mipafox is shown in Table II, in which the responses were measured one hour after its addition. With this anticholinesterase not only was an increased response to

TABLE II.—Inhibition of Isolated Taenia in Response to Stimulation of Perivascular Nerves at Different Frequencies. Observations in the Presence of Hyoscine. Concentration of Mipafox 10 µg./ml.

Frequency of Stimulation	Control (mm.)	In Presence of Mipafox (mm.)	% Change
2	15	30	+100
5	30	47	+56
10	46	63	+37
20	75	68	-9
50	57	50	-12

stimulation of low frequencies observed but a diminished response to stimulation of higher frequencies; this double effect was like that seen with physostigmine in the phrenic-nerve-diaphragm preparation (Bülbring, 1946). The effect of anticholinesterases on the response of the heart rate to stimulation of the fibres from the stellate ganglion has been studied by Huković (1966). He used the isolated heart of the rabbit perfused through the aorta with the sympathetic fibres attached. Atropine, 10⁻⁶ g./ml., was present in the perfusion fluid, and control observations were made stimulating the ganglion at frequencies from 0.2 to 5 per second for a period of 30 seconds. The heart rate was counted during one minute from the beginning of stimulation. When physostigmine or neostigmine, 10⁻⁶ g./ml., was added to the perfusion fluid the increase in heart rate in response to stimulation was much greater, as shown in the example given in Table III, and it was particularly

TABLE III.—Effect of Neostigmine 10⁻⁶ g./ml. on the Increase in Heart Rate Produced by Stimulation of the Stellate Ganglion for 30 Seconds at Various Frequencies. All Observations made in the Presence of Atropine 10⁻⁶ g./ml. and Choline 0.5 mg./ml. Figures are Beats per Minute

Frequency:	5/sec.	2/sec.	1/sec.	0.5/sec.	0.2/sec.
1st control	45	38	17	4	0
2nd "	—	39	17	0	—
3rd "	44	41	20	1	0
4th "	43	31	18	0	0
1st neostigmine ..	69	52	25	11	0
2nd "	67	53	41	24	9
3rd "	60	48	40	—	11

striking that at frequencies of 0.5 and 0.2 per second stimulation became effective in the presence of the anticholinesterase when it had been practically without effect in the control period.

The action of physostigmine has also been observed on the response of the retractor penis muscle of the dog to stimulation of the postganglionic fibres arising in the first sacral sympathetic ganglion (Armitage and Burn, in press). The observations were made in situ when the dog was anaesthetized with chloralose, and were made after the injection of hyoscine. The contractions observed before and after the injection of physostigmine in one experiment are shown in Table IV, in which it is seen that the response to stimulation of the lowest frequency was doubled after the injection of physostigmine, while the response to stimulation of the highest frequency was increased by 9% only. The increase for intermediate frequencies was graded, diminishing as the frequency rose.

TABLE IV.—*Effect of Physostigmine on Contractions of the Retractor Penis of the Dog in Response to Stimulation of Postganglionic Sympathetic Fibres Leaving the First Sacral Ganglion. Observations After the Injection of Hyoscine 0.15 mg./kg. Five Maximal Shocks Given at Each Stimulation*

Frequency of Stimulation	Contractions (mm.)		% Increase
	Control	After Physostigmine 0.5 mg./kg.	
0.2/sec. . . .	37	74	100
0.5/sec. . . .	44	69	57
1/sec.	51	71	39
2/sec.	64	70	9

The fifth set of observations were made by Bernard and De Schaepdryver (1964), who stimulated the sympathetic fibres to the dog's hind leg and measured the blood flow through the femoral artery. They first injected atropine. They used maximal stimuli at frequencies from 0.1 to 3 per second, and observed the effect of injecting physostigmine, neostigmine, and sarin. At stimulation of frequency 0.1 per second, the vasoconstriction was increased by 45% immediately after giving physostigmine, and later by 90%. For stimulation at the higher frequency of 3 per second the corresponding figures were smaller, being 16% and 27%. They observed such an effect in each of 14 experiments. These authors thought that their results might be explained by stimulation of fibres innervating chromaffin cells in the skin. However, Coupland and Heath (1961) have shown that the cells in the skin thought to be chromaffin cells are mast cells. Another explanation advanced by Bernard and De Schaepdryver was that some of the fibres they stimulated might have been preganglionic, and that the effect of the anticholinesterase was to improve ganglionic transmission. But anticholinesterases have not been found to improve ganglionic transmission when maximal stimuli are used. The evidence of these authors thus appears to be precisely similar to that provided by the four preceding investigations, and to support the view that acetylcholine is the mediator in the release of noradrenaline.

Action of Hemicholinium

Various substances have been shown to prevent the release of noradrenaline from the sympathetic postganglionic fibre, and one of these is hemicholinium. Birks and MacIntosh (1961) have shown that this substance causes a failure of transmission in the superior cervical ganglion of the cat when perfused with Locke's solution, and that this failure is due to interference with the synthesis of acetylcholine. They found that hemicholinium impedes the transport of choline to the intraneuronal site where acetylcholine is synthesized. They also observed that the block produced by hemicholinium was overcome by an excess of choline.

A study made by Rand and his colleagues (Chang and Rand, 1960; Brandon and Rand, 1961; Rand and Ridehalgh, 1965) has shown that hemicholinium not only blocks transmission through the sympathetic ganglion but also blocks the response to sympathetic postganglionic stimulation in the following tissues: the perfused vessels of the rabbit ear, the isolated atria of the cat, the perfused spleen of the cat, and the isolated colon of the guinea-pig. Rand found it necessary to allow hemicholinium to act for a long time, as shown in Table V, but in each case when the block was finally produced it was removed

TABLE V.—*Effect of Hemicholinium on Stimulation of Postganglionic Sympathetic Fibres*

Organ	Concentration of Hemicholinium	Time Required to Block	Effect of Choline
Spleen (cat) . . .	50 µg./ml.	263 min.	Block removed
Atria (cat) . . .	500 "	270 "	" "
Ear vessels (rabbit) . .	50 "	180 "	" "
Colon (guinea-pig) . .	50 "	335 "	" "

by choline. Various observers have failed to observe a block with hemicholinium, but they have not allowed sufficient time to cause a block. Others who have thought they produced some degree of block with hemicholinium failed to demonstrate that the block was removed by choline. The evidence of Rand thus gives quite unexpected support to the view that acetylcholine plays an essential part in the release of noradrenaline from the sympathetic fibre.

Action of Botulinum Toxin

Botulinum toxin is a substance which was shown by Burgen, Dickens, and Zatman (1949) to prevent the release of acetylcholine from the terminations of the motor nerves. Rand and Whaler (1965) have shown that botulinum toxin blocks the response to sympathetic postganglionic stimulation in the isolated intestine of the rabbit, the pilomotor muscles of the cat's tail, and the guinea-pig vas deferens. They observed the block in the rabbit ileum in 17 out of 19 experiments. Since botulinum toxin did not alter the action of noradrenaline itself, these observations also are in favour of the view that acetylcholine is an intermediary in the release of noradrenaline.

Agents Used to Block Noradrenaline Release

The first agent used clinically to block noradrenaline release was bretylium; it is no longer used, and guanethidine has taken its place. In discussing the action of these two substances, the observation of Brücke (1935) may be recalled that a large dose of acetylcholine, or of nicotine, injected into the skin of the cat's tail at the base of a tuft of hair blocked the response of this tuft to sympathetic stimulation, though not that of other tufts. The injection did not block the effect of adrenaline. Acetylcholine when perfused through the vessels of the rabbit ear has also been shown to prevent the constriction caused by sympathetic stimulation but not that caused by noradrenaline (Burn and Rand, 1960). Similarly, acetylcholine has been shown to block the accelerator action of sympathetic stimulation in the isolated atria of the rabbit heart (Huković, 1961).

Thus acetylcholine has two actions. In smaller concentrations it causes the release of noradrenaline from sympathetic postganglionic fibres, and in high concentrations it prevents the release of noradrenaline by sympathetic stimulation. Such a double action can be understood if acetylcholine is involved in the release of noradrenaline from the sympathetic fibre; it has some similarity to the action of acetylcholine at the neuromuscular junction, where small concentrations cause contraction, but high concentrations (in the presence of physostigmine when the frequency of stimulation is high) cause a progressive diminution of contractions.

The blocking agents bretylium and guanethidine were successors to xylocholine, and xylocholine is a choline derivative. Bretylium resembles choline in being a compound containing what is in effect trimethylammonium. Both bretylium and guanethidine block the response to sympathetic stimulation in low concentrations in a tissue suspended in a bath, but the effect of a first application of a large dose is to release noradrenaline. Thus bretylium and guanethidine have the same two actions as acetylcholine, and their relation to acetylcholine in the sympathetic fibre has some resemblance to the relation of decamethonium to acetylcholine at the neuromuscular junction. Decamethonium is mainly a blocking agent, but the first large dose stimulates. It is interesting that both Dixit, Gulati, and Gokhale (1961) and Burn and Seltzer (1965) found that bretylium and guanethidine have an action in the phrenic-nerve-diaphragm preparation similar to that of decamethonium, and of the same order of magnitude. Decamethonium was found to be five times more potent than bretylium and eight times more potent than guanethidine. We have then reason to

suppose that their immediate action in the sympathetic postganglionic fibre is to block the action of acetylcholine.

Comparison with the Adrenal Medulla

What is the action of acetylcholine which bretylium and guanethidine block in the sympathetic fibre? Douglas and Rubin (1963) have analysed the action of acetylcholine in the adrenal medulla, where they have shown that acetylcholine makes the membrane of the chromaffin cell permeable to calcium ions, which enter the cell and release adrenaline and noradrenaline. The amount of these substances released is proportional to the external calcium concentration. In the sympathetic postganglionic fibre the situation is similar. Burn and Gibbons (1965) have found that the response of the rabbit ileum to sympathetic stimulation is proportional to the external calcium concentration, and, further, they have found that the increase in rate of rabbit atria caused by acetylcholine (in the presence of atropine) is also proportional to the external calcium concentration.

Moreover, if the sympathetic fibres or the adrenal medulla are bathed for 20 minutes in a Ca^{++} -free medium the membrane (of the fibres or of the chromaffin cell) becomes permeable to Ca^{++} itself, which enters and releases the catecholamine without the intervention of acetylcholine. From this we can say that the function of the acetylcholine both in the adrenal medulla and in the sympathetic postganglionic fibre is the same—namely, to make the chromaffin cell membrane or the membrane of the postganglionic fibre permeable to calcium. Bretylium and guanethidine should then prevent acetylcholine from making the membrane of the postganglionic fibre permeable to calcium. Proof of this has now been obtained. When guanethidine or bretylium has blocked the response of the rabbit ileum to sympathetic stimulation a rise in the external calcium concentration removes the block. The block reappears when the external calcium concentration is reduced to its former value. Guanethidine affects the action of acetylcholine in the same way. Acetylcholine, in the presence of atropine, accelerates the rate of isolated rabbit atria. The acceleration depends on the calcium concentration in the bath, and increases as the concentration rises. In the presence of guanethidine the acceleration at a given calcium concentration is reduced or blocked, but the block is overcome if the calcium concentration is increased.

Conclusion

The evidence presented gives no picture of what the actual events may be at the termination of the postganglionic fibre. The anatomical relations are obviously quite different from those in the adrenal medulla, where the release of catecholamines by acetylcholine is a transynaptic event. In the sympathetic fibre it would not at first sight seem to be transynaptic. There are vesicles without granules, and vesicles with granules; the former may contain acetylcholine and the latter noradrenaline, which might suggest that acetylcholine releases noradrenaline within the fibre. But when postganglionic nerves are stimulated at low frequency acetylcholine as well as noradrenaline is released, and both act on the end organ. In the nictitating membrane their effects are additive, and in the rabbit ileum or guinea-pig taenia they are antagonistic (Burn, Dromey, and Large, 1963). There is no question that acetylcholine leaves the postganglionic fibre. The release of noradrenaline may therefore depend on acetylcholine, which has left the fibre, acting on the outside of the fibre as it acts on the chromaffin cell in the adrenal medulla. Thus again the event would be transynaptic. This conception is not more strange than was the finding at the time it was made that postganglionic fibres, whose function was to release sympathin, also took up adrenaline from the blood (Burn, 1933).

Those who hold to the view that the sympathetic impulse releases noradrenaline directly should remember that all postganglionic nerves so far investigated release acetylcholine as well as noradrenaline; that the amount of acetylcholine in the splenic nerves is as high as 40% of the amount of noradrenaline; that acetylcholine releases noradrenaline from sympathetic fibres when sympathetic impulses are blocked by bretylium, as three papers have shown; that anticholinesterases increase the response to postganglionic stimulation, as has been described in five tissues; and that hemicholinium and botulinum toxin block the release of noradrenaline from sympathetic fibres. The view that the sympathetic impulse releases noradrenaline directly has never been more than an assumption. The points mentioned show that this assumption needs reconsideration.

Summary

The view has been held for many years that the impulse which passes down the sympathetic postganglionic fibre releases noradrenaline directly. An account of evidence is given which indicates that the release is a more complicated process, and that it closely resembles the mechanism of release of adrenaline from the adrenal medulla. The impulse passing down the fibre first releases acetylcholine. This acetylcholine, probably after leaving the fibres, makes the membrane of the fibre permeable to calcium ions. These ions enter the fibre and release noradrenaline from the granules in which they are held.

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