THE POSTGANGLIONIC SYMPATHETIC FIBRE; ITS PHYSIOLOGY AND PHARMACOLOGY

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(Received March 5, 1962)

The advance in our knowledge of the sympathetic postganglionic fibre since 1958 is an excellent example of the progress in physiology which can follow an advance in pharmacology. For the advance in question has been the result of the isolation of reserpine as an important alkaloid from the plant *Rauwolfia serpentaria*. The first account of the properties of this substance was published in 1953 by Bein, Gross, Tripod and Meier, and for the next three years attention was directed in the main to a study of its action in the brain, because of its well known tranquillizing property. But in 1956 after Holzbauer and Vogt had found that reserpine caused a fall in the concentration of noradrenaline in the hypothalamus of the cat, two pieces of evidence were published which concerned the periphery. Carlsson and Hillarp (1956) showed that reserpine reduced the concentration of adrenaline and noradrenaline in the adrenal medulla, and Bertler, Carlsson and Rosengren (1956) showed that reserpine reduced the concentration of noradrenaline in the rabbit heart. These discoveries opened the gate to the subsequent advance. In the course of that advance, many observations hitherto unconnected have fallen into place like the pieces of a jig-saw puzzle, and now a picture has emerged which represents an important addition to our knowledge.

The sympathomimetic amines. In 1910 Barger and Dale published an account of the action of many amines which they described as sympathomimetic, because they imitated in their action the effect of sympathetic stimulation Of these adrenaline was the best known since it was a natural constituent of the body, and the others were for the most part simpler in structure. One of them was tyramine, the amine obtained by the removal of the carboxyl group from the amino-acid tyrosine. All these amines had similar properties and the differences between them which Barger and Dale recorded were quantitative rather than qualitative. The mode of action of all of them appeared to be the same, though the simpler amines were much less potent.

The first suggestion that this general similarity of mode of action was misleading came from the effect of cocaine. In 1910 Förhlich and Loewi

showed that when cocaine was injected into an animal, the rise of blood pressure caused by adrenaline was greater than before; that is to say cocaine potentiated the action of adrenaline. Then in 1927 Tainter and Chang showed that cocaine had the opposite effect on the action of tyramine; when cocaine was injected the rise of blood pressure caused by tyramine was less than before, or even was abolished. Cocaine was thus a means of demonstrating that sympathomimetic amines fell into two groups, one group the action of which was increased by cocaine, and a second group the action of which was reduced by cocaine. Ephedrine was a member of this second group.

A second indication that the mode of action of all sympathomimetic amines was not the same came from the results of denervation. Meltzer (1904) showed that when the iris of the cat's eye was denervated by removal of the superior cervical ganglion, and when time was allowed for the postganglionic fibres to degenerate, then the denervated iris was much more sensitive to the action of adrenaline than the normal iris. An injection of adrenaline which had no effect on the normal iris caused dilatation of the denervated iris. In 1931 Burn and Tainter showed that the opposite was true of tyramine. The denervated pupil was unaffected by doses of tyramine many times larger than the dose which dilated the normal pupil. Again what was true for tyramine was also true for ephedrine.

The third suggestion that the mode of action of all sympathetic amines was not the same came from the responses after giving reserpine. While the pressor action of adrenaline was greater than usual, Carlsson, Rosengren, Bertler and Nilsson (1957) showed that the pressor effect of tyramine was abolished. Thus in all three cases, after cocaine, after sympathetic denervation and after treatment with reserpine, the action of adrenaline and of noradraline was increased,while the action of tyramine was reduced or abolished. What was the common factor in the three cases?

Tissue stores of noradrenaline. As already pointed out, Bertler, Carlsson and Rosengren showed that when reserpine was injected into a rabbit, the amount of noradrenaline which could be extracted from the heart was reduced to a very low figure. Whereas hearts from normal rabbits contained 1.57 μg/g, hearts from rabbits injected with reserpine on the day before contained only 0.03 μg/g. Burn and Rand therefore examined the effect of reserpine on the amount of noradrenaline in other tissues as shown in Table 1, all these tissues having a sympathetic innervation. They observed the same effect in each, namely a great reduction in the amount of extractable noradrenaline.

TABLE 1 (Burn and Rand, 1957, 1959)

Change in the mean amount (μg/g) of extractable noradrenaline in organs after treating the animal with reserpine

Organ	Normal	After reserpine.
Aorta (rabbit)	0.47	0.11
Aorta (dog)	0.95	0.03
Spleen (cat)	1.13	0.12
Iris (cat)	0.17	0.01
Tail skin (cat)	0.35	0.06
Ear skin (rabbit)	0.12	0.02

These observations called to mind the findings of Euler and Purkhold (1951). Euler (1946) demonstrated the presence of noradrenaline in the splenic nerves of the ox, and then later his colleague Schmiterlöw (1948) showed that the arteries and veins of the ox and the horse also contained noradrenaline. Goodall (1951) found noradrenaline in the heart and discovered that the amount was greatly reduced by excision of the stellate ganglia and degeneration of the sympathetic accelerator fibres. Then Euler and Purkhold in the same year showed that noradrenaline was present in the spleen, the liver, the kidney and the salivary glands, and that in each of these organs the amount was greatly reduced when the sympathetic fibres degenerated.

Euler (1956) discussed these findings, and pointed out that if the noradrenaline which could be extracted from a normal spleen disappeared after degeneration of the sympathetic nerves, then the noradrenaline must be present in the sympthetic nerve endings in the spleen, despite the fact that calculation suggested that the amount in the endings might be as much as 3 to 30 mg per g.

The situation was thus reached that treatment with reserpine and sympathetic denervation were shown to have one effect in common, namely that they reduced the amount of noradrenaline in the heart and blood vessels, which was presumably present in sympathetic nerve endings. Could the disappearance of noradrenaline from sympathetic nerve endings be the cause of the increased pressor action of adrenaline and noradrenaline, and of the abolition of the pressor action of tyramine and ephedrine ?

Intravenous infusion of noradrenaline. In the hope of answering this question, Burn and Rand (1958c) carried out experiments in which they took cats which had been treated with reserpine, and having made a spinal preparation, recorded the effect on the blood pressure of various sympathomimetic amines. They observed for example that the pressor action of noradrenaline was larger than usual, while that of tyramine was very small. They then gave a slow intravenous infusion of noradrenaline at a uniform rate using a syringe driven by a motor. The total amount infused varied from 0.25-0.5 mg, this being given during the course of 20 to 40 min. At the end of this time they stopped the infusion and allowed the blood pressure to return to the initial level. When this was reached they injected noradrenaline in the same amount as before and saw that the rise of blood pressure was much less; they also injected tyramine and saw that the rise of blood pressure was much greater. Thus the infusion of noradrenaline had the effect of restoring the pressor action of tyramine. This observation suggested that the failure of tyramine to cause a rise of pressure in the cat after treatment with reserpine was actually due to the absence of noradrenaline from the sympathetic nerve endings, and that the normal mode of action of tyramine was to release noradrenaline from those endings. It further suggested that sympathetic nerve endings could take up and retain noradrenaline which was circulating in the blood.

Evidence of uptake of noradrenaline. The conception that sympathetic nerve endings could take up noradrenaline from the blood was novel. Hitherto most people had assumed that noradrenaline which disappeared from the blood was removed by enzymic destruction, apart from a small amount excreted by the kidneys. Further experiments were needed to support the idea that noradrenaline could be taken up and retained for subsequent release.

In the first place it was found that not only noradrenaline could restore the pressor action of tyramine when infused into the reserpine-treated rat, but that precursors of noradrenaline such as dopamine, L-dopa and phenylalaline were also able to do this. Adrenaline was much less effective than noradrenaline (Burn and Rand 1960a).

Evidence was however required that an infusion of noradrenaline led to an actual rise in the amount of noradrenaline which could be extracted from organs with a sympathetic innervation. Pennefather and Rand (1960) decided to examine the kidney and the horn of the uterus because these were

paired organs. They made their experiments on spinal cats which were eviscerated, and removed one kidney and one horn of the uterus first of all. An intravenous infusion of 1 mg noradrenaline was then given during 40 min, and 20 min later the second kidney and the second horn were removed. The amount of noradrenaline in the two kidneys and two horns was then estimated. In nine experiments the amount of noradrenaline was increased after the infusion, the average result being that there was 2.3 times as much noradrenaline in the kidney as in the control and that there was 1.3 times as much in the uterine horn as in the control. The increase was seen in the organs of both normal cats and of cats treated with reserpine. In other experiments an infusion of 25 mg dopamine was given, and in some animals this also caused an increase in the amount of extractable noradranaline as judged by the increase in the pressor activity of the extract.

These results were confirmed by experiments in rats, in which the noradrenaline in the heart was measured. In control rats, the heart contained 0.6 μg/g noradrenaline. In rats which received an intravenous infusion of 20 μg noradrenaline, the hearts contained 1.05 μg/g. This amount declined slowly, and when rats were killed 1 hr after the infusion, the hearts contained 0.8 μg/g (Muscholl 1960).

Further confirmation was obtained by Whitby, Hertting and Axelrod (1960) who injected labelled (tritiated) noradrenaline into cats; at the end of 2 hr the cats were killed, and the spleen, heart, adrenal glands were examined for the presence of the labelled noradrenaline. They found a considerable uptake in these organs, and they were able to show that this was noradrenaline itself, and not the substance produced by methylating the—OH group in the 3 position of the ring. These different results obtained by different methods in various organs thus established the fact that noradrenaline could be taken up by the tissues from the blood stream and held there.

Evidence that degeneration of sympathetic nerves prevented the uptake was obtained by Burn and Rand (1960a) who prepared cats in which the superior cervical ganglion of one side had been excised two weeks previously. They gave each cat reserpine and on the following day, under chloralose anaesthesia, injected tyramine. There was no dilatation of the pupil in either eye. They infused noradrenaline during 25 min and then injected tyramine again. Tyramine then caused dilatation of the normal eye, but not of the denervated eye. They also observed that when the vessels of the cat's foreleg had been denervated by removal of the stellate ganglion, as a result o

which the injection of tyramine had no constrictor action (Burn, 1932), an infusion of noradrenaline failed to restore the constrictor action. Thus it was evident that in tissues in which the sympathetic nerves had degenerated, there was no uptake of noradrenaline from the blood. This conclusion also was confirmed by the experiments of Hertting, Axelrod, Kopin and Whitby (1961) who found that the denervated salivary gland and the denervated lachrymal gland took up only a very small fraction of the tritiated noradrenaline which was taken up by the innervated glands.

Tyramine as a substance releasing noradrenaline. The evidence that tyramine acted by releasing noradrenaline gained confirmation from two other investigations. Lockett and Eakins (1960) working on cats under chloralose, which were given hexamethonium and from which the adrenal glands were removed, found that when they injected tyramine there was a rise in the amount of noradrenaline in the plasma withdrawn from the lower end of the aorta. The normal amount of noradrenaline was found to be 0.4 μg per 100 ml plasma, but after the injection of tyramine, the average figure was 2.5 μg per 100 ml plasma, that is a sixfold increase.

Vane (unpublished) has confirmed these results by the use of an elegant method in which the blood of a cat leaves the body by way of the carotid artery and then drips over an isolated organ in a bath. From the bath the blood is once more pumped into the vein of the cat. Vane used as an isolated organ a strip of the stomach of a rat which had been treated with reserpine. This stomach strip relaxed when noradrenaline was injected into the cat. When tyramine was injected into the cat the stomach strip contracted, this being also the response of the strip to tyramine when tyramine was added directly to the bath. This showed that no detectable amount of noradrenaline had been released into the plasma by tyramine. However when hexamethonium, which potentiates the action of noradrenaline, was injected into the cat as in the experiments of Lockett and Eakins, the injection of tyramine into the cat was followed by relaxation of the stomach strip, showing that noradrenaline was now released into the plasma. It appears however that in the absence of hexamethonium the noradrenaline released by tyramine does not enter the plasma.

Schümann and Weigmann (1960) made a homogenate of the adrenal medulla, and having removed the cell debris by low speed centrifugation, then carried out high speed centrifugation in a sucrose density gradient. They obtained a fraction which contained granules in which noradrenaline was

bound to adenosine triphosphate. They took the granules and found that, when oxygenated at 37°C, they released noradrenaline at a slow rate. When tyramine was added in concentrations which might be as low as 15 μg/ml, though they were usually higher, the rate of release from the granules increased by 150 per cent. Ephedrine was found to have the same action as tyramine, but dopamine did not have this action. The authors obtained similar results when they prepared granules from the splenic nerves. The authors considered that tyramine, being a stronger base, was able to displace noradrenaline from combination with adenosine triphosphate.

The foregoing results may be said to have established the theory that tyramine and other amines like ephedrine and amphetamine, which are not catecholamines, do not act themselves when they produce sympathomimetic effects, but liberate noradrenaline from binding sites in sympathetic nerve endings. This theory explains the failure of tyramine and related amines to act when the sympathetic nerves have degenerated, and it also explains their failure to act when reserpine has been given, when the noradrenaline in the sympathetic nerve endings has disappeared. It does not explain the effect of cocaine.

Block of uptake by coacine. Work by Trendelenburg (1959) has shown that when noradrenaline is injected into the blood stream, the rate of disappearance of noradrenaline from the plasma is greatly reduced in the presence of cocaine. He injected 25 μg/kg noradrenaline into spinal cats and measured the amount in the plasma after 1 min, after 2 min and after 3 min. When cocaine was given beforehand the amount in the plasma at the end of 1 min was twice as great, and at the end of 4 min nearly four times as great as in the absence of cocaine. Thus the rate of disappearance of noradrenaline was much slower in the presence of cocaine, and it was for this reason that the effect of noradrenaline on the blood pressure was greater and lasted longer. Macmillan (1959) suggested that cocaine might produce this effect by blocking the uptake of noradrenaline by the sympathetic nerve endings. Muscholl (1960) obtained evidence that this was so by measuring the uptake of noradrenaline by the rat heart when noradrenaline was infused, and determining the effect of cocaine upon the uptake. He found that when cocaine was given beforehand the uptake was almost entirely prevented. Similar results were obtained by Whitby, Hertting and Axelrod (1960) who showed that cocaine prevented the uptake of tritiated noradrenaline by the heart, spleen and adrenal glands.

Thus the potentiation of the pressor action of noradrenaline by cocaine occurs because the uptake of noradrenaline by the sympathetic nerve endings is prevented, and therefore the action on the blood vessels is greater and more prolonged. Since tyramine is an amine similar to noradrenaline it is probable that the uptake of tyramine by the sympathetic nerve endings is also prevented. If tyramine cannot enter the nerve endings in the presence of cocaine, it cannot release noradrenaline from the nerve endings, and therefore it is unable to exert a pressor action. Thus the one property of cocaine, that of preventing the entry of sympathomimetic amines into the sympathetic nerve endings accounts both for the potentiation of the action of noradrenaline and also for the suppression of the action of tyramine.

The effect of the uptake of noradrenaline on sympathetic stimulation. The physiological importance of the entry of noradrenaline into the sympathetic nerve ending has been shown by the effect on the response to sympathetic stimulation. When the hindleg of a dog which had been treated with reserpine was perfused with heparinized blood, stimulation of the postganglionic sympathetic fibres failed to cause vasoconstriction, it caused vasodilatation. When atropine was injected the vasodilatation was no longer seen, and stimulation had no effect. In the preparation from the dog treated with reserpine only cholinergic fibres were active.

When noradrenaline was added drop by drop to the reservoir from which blood was taken by the pump for delivery into the leg vessels, the tone in the vessels rose, but some time after the addition of noradrenaline was discontinued, the tone declined again. At this point the observation was made that stimulation of the sympathetic fibres caused vasoconstriction. The vasoconstrictor fibres which had no effect on the reserpine-treated preparation were restored to activity (Burn and Rand 1958c).

Similar effects were obtained in animals which were not given reserpine beforehand. The dog hindleg was perfused as before, and the lumbar sympathetic was stimulated so as to give a small constriction. After the addition of about 0.5 mg noradrenaline to the 700 ml of blood in circulation, the addition being gradual over a period of 30 min, then when the tone had fallen to the level before the addition of noradrenaline, sympathetic stimulation caused a much greater constriction than before. This increase in effect was not however of long duration and disappeared after stimulation was applied for two or three times.

More lasting effects were obtained when the vasoconstriction was recorded by the use of a plethysmograph applied to the hindleg of a dog under chloralose anaesthesia. A series of stimuli of different strengths was applied to the lumbar sympathetic chain, and the threshold strength for vasoconstriction was determined as well as the effect of greater stimuli. After the slow intravenous infusion of noradrenaline, strengths of stimulation which were previously subthreshold now produced vasoconstriction. In seven experiments the threshold itself fell to 40 per cent of its previous value, and in some experiments it fell to 16 per cent of its previous value. The increased effect of sympathetic stimulation persisted during the next hour or two up to the termination of the experiment (Burn and Rand 1960c).

Finally it was observed that the effect of stimulating the postganglionic fibres to the nictitating membrane, first reduced to small dimensions by treating the cat with reserpine beforehand, was restored by the intravenous infusion of dopamine (Burn and Rand 1960a).

The ordinary view has been hitherto that the noradrenaline liberated by sympathetic impulses from the adrenergic fibre is synthesized within the fibre. Holtz and Westermann (1956) showed that the enzyme dopa decarboxylase is present in splenic nerves, and Schümann showed that dopamine was present (1958). Thus it was accepted that noradrenaline was formed by synthesis. However the new evidence shows that noradrenaline can be taken up from the blood, into which it is ordinarily secreted by the adrenal medulla. Bülbring and Burn (1949) showed that splanchnic stimulation released noradrenaline as well as adrenaline from the adrenal medulla, and the function of this noradrenaline has now been demonstrated.

The action of nicotine and acetylcholine. Acetylcholine exerts actions in the body in the presence of atropine which are similar to those exerted by nicotine. Hitherto these actions have been associated in the main with the sympathetic ganglion and the neuromuscular junction, but there are many others which may be described as sympathomimetic because they reproduce the effect of sympathetic stimulation.

For example, when the rabbit ear, cut off from the rabbit, was perfused with Locke's solution through the central artery, Burn and Dutta (1948) found that acetylcholine, when injected into the perfusing fluid, at first caused dilatation. But when the perfusion had continued for a few hours, acetylcholine caused constriction, just as adrenaline caused constriction. The cons-

triction caused by adrenaline was reversed to dilatation when tolazoline (Priscol) was added to the perfusion fluid, and the constriction caused by acetylcholine was also reversed by tolazoline. Further observations were made by Kottegoda (1953) who showed that not only after a few hours but at the beginning of a perfusion, acetylcholine caused constriction when atropine was given first. He suggested that acetylcholine caused constriction by liberating an adrenaline-like substance, just as sympathetic stimulation caused constriction.

Finally Burn and Rand (1958b) showed that if a rabbit was first treated with reserpine, then the injection of acetylcholine in the presence of atropine into the fluid flowing through the ear no longer caused vasoconstriction ; it had no effect, and nicotine likewise had no effect. Thus in the vessels of the rabbit ear, acetylcholine in the presence of atropine acts like a sympathetic impulse ; it causes constriction by liberating noradrenaline. The action is peripheral, beyond the site of any known ganglion.

Another example is given by the pilomotor response in the cat's tail. Most of the hair of the tail can be removed by shears, leaving a series of 8 or 10 tufts on the dorsal aspect of the tail. If the lumbar sympathetic chain is stimulated, these tufts are erected. But the injection of acetylcholine into the skin at the base of a tuft also causes that tuft to be erected. So does the injection of nicotine, or of adrenaline or noradrenaline. Now noradrenaline is present in the skin of the cat's tail, and it can be greatly reduced by injecting the cat with reserpine. When a cat is given reserpine and acetylcholine or nicotine is then injected into the skin at the base of a tuft of hair, it has little effect in causing erection of the tuft, just as sympathetic stimulation also has little effect. Noradrenaline or adrenaline are however more active than before. This evidence indicates that acetylcholine causes erection of the tuft by liberating noradrenaline in the same way as sympathetic stimulation (Burn, Leach, Rand and Thompson, 1959).

There are several other examples of this action. In the presence of atropine, acetylcholine causes an increase of rate and amplitude of isolated rabbit atria (Kottegoda, 1953). This action like that of nicotine, which also increases the rate and amplitude, is absent if the atria are taken from a rabbit treated with reserpine. The action is therefore due to the release of noradrenaline, as is the accelerating effect of stimulating the nerves from the stellate ganglion (Burn and Rand 1958a).

A further example of a peripheral sympathomimetic action of acetylcholine in the presence of atropine is provided by the spleen. When the sympathetic fibres to the spleen are stimulated, the spleen contracts. When acetylcholine is injected into the splenic artery after atropine has been injected first, the acetylcholine causes contraction. This was observed by Daly and Scott (1961) who also showed that when the sympathetic fibres were cut and allowed to degenerate, then the injection of acetylcholine into the splenic artery did not cause contraction. Euler and Purkhold (1951) showed that when the sympathetic fibres degenerated, the noradrenaline in the spleen was greatly diminished in amount, and this finding was confirmed by Burn and Rand (1959). That is to say acetylcholine failed to cause contraction when the noradrenaline had largely disappeared, and therefore the action of acetylcholine in causing contraction of the normal spleen was almost certainly due to the liberation of noradrenaline.

In the intestine Gillespie and Mackenna (1960) have shown that nicotine in low concentration causes inhibition of an isolated loop, and that the stimulant action normally attributed to nicotine is only seen with higher concentrations. The inhibitory action of low concentrations of nicotine was found to be absent in loops of intestine taken from animals treated with reserpine, but it could be restored by adding noradrenaline to the bath for some time, after which it was washed out. The inhibitory action of low concentrations of nicotine then returned.

Sympathetic cholinergic fibres. The foregoing account has shown that acetylcholine in the presence of atropine has an action similar to that of sympathetic stimulation in the blood vessels, the heart, the pilomotor muscles of the cat's tail, the spleen and probably also in the intestine, though in this case the evidence is only complete for nicotine.

Has this action of acetylcholine a physiological significance? Everyone knows that the postganglionic sympathetic fibres to the sweat glands are cholinergic, but many do not realize that the presence of cholinergic fibres is much more widespread. Euler and Gaddum (1931) showed that the sympathetic fibres leaving the superior cervical ganglion in the dog caused flushing of the upper lip and neighbouring mucous membranes ; when the muscle of the upper lip was sensitized to the action of acetylcholine by degeneration of the motor nerve, then stimulation of the superior cervical ganglion caused contraction of the lip. They said that the fibres leaving the ganglion were pharmacologically parasympathetic, though anatomically sympathetic ; the

fibres released acetylcholine. In 1935 Bülbring and Burn showed that there were cholinergic fibres in the lumbar sympathetic chain of the dog, leading to the muscles of the hindleg; in the same year Bacq and Fredericq showed that there were cholinergic fibres in the sympathetic supply to the nictitating membrane, and Sherif showed that there were cholinergic fibres to the uterus of the dog. In 1948 Folkow, Frost, Haeger and Uvnäs showed that there were cholinergic fibres in the nervi accelerantes from the stellate ganglion to the heart.

Except for the fibres to the sweat glands no functional significance has been attached to these cholinergic fibres, though attempts have been made to offer an explanation for their function in the vessels of the hindleg. The cholinergic fibres have been disregarded by physiologists. However the evidence that acetylcholine has a sympathomimetic action dependent on the release of noradrenaline, suggests the possibility that cholinergic fibres in the sympathetic supply might liberate acetylcholine and that this in turn might release noradrenaline.

Hitherto an adrenergic fibre has been conceived as a fibre in which the nerve impulse directly releases noradrenaline. The possibility now outlined is that there is a cholinergic link in the release of noradrenaline, so that the nerve impulse releases acetylcholine as in all other peripheral motor nerves. In the adrenergic fibre however this acetylcholine, perhaps by increasing the permeability of the ending of the nerve fibre, allows noradrenaline to pass through, and the nerve is therefore adrenergic in its effect.

Cholinergic fibres to the spleen. To test this it was necessary to demonstrate that there were cholinergic fibres in sympathetic nerves in which they had not previously been demonstrated, as for example in the splenic nerves. Experiments were carried out in cats treated with reserpine. When atropine has not been given acetylcholine causes dilatation of the spleen, and stimulation of the splenic nerves in cats treated with reserpine caused dilatation of the spleen. This dilatation was greater when eserine was given, and was abolished by atropine; these results suggested that stimulation of the nerves liberated acetylcholine (Burn and Rand, 1960b).

Observations were also made with hemicholinium. This substance has the property of interfering with the synthesis of acetylcholine. In the presence of hemicholinium the acetyl group of acetyl-CoA cannot be handed on to choline, and therefore acetylcholine cannot be formed; the action of hemi-

cholinium can however be neutralized by giving excess of choline (MacIntosh, Birks and Sastry, 1956). The spleen of the cat was therefore perfused, hemicholinium being added to the perfusing fluid. In its presence the effect of successive periods of stimulation of the nerves in producing contraction of the spleen grew less and less, until there was no response. When choline was added to the perfusion fluid, the contractions were again evoked. Thus the effect of stimulating the postganglionic fibres to the spleen, which were adrenergic fibres, was gradually reduced by hemicholinium until it disappeared ; the effect was however restored by choline. This indicated that hemicholinium was acting by preventing the formation of acetylcholine. Thus there was a cholinergic link in the liberation of noradrenaline by the splenic nerves.

Final proof of the release of acetylcholine by stimulation of the splenic nerves was obtained by perfusing the spleen from a cat treated with reserpine, and allowing the fluid leaving the vein to run over a strip of guinea-pig ileum, of which the contractions were recorded. When the splenic nerves were first stimulated, the strip of ileum was not affected. Neostigmine was then added to the perfusing fluid, and when the splenic nerves were stimulated again, there was a large contraction of the ileum; this contraction was similar to that caused by injected 0.2 μg acetylcholine into the splenic artery. When the strip of ileum was treated with atropine, stimulation of the splenic nerves no longer evoked a contraction (Brandon and Rand, 1961). In view of these results it was clear that there were cholinergic fibres in the postganglionic supply to the spleen. The observations with hemicholinium not only demonstrated that cholinergic fibres were present, but since hemicholinium reduced the effect of stimulation to zero, the observations also demonstrated that probably all the fibres were cholinergic.

Cholinergic fibres to the vessels of the rabbit ear. The presence of cholinergic fibres in the postganglionic sympathetic fibres to the vessels of the rabbit's ear has been demonstrated by Burn and Rand (1960b) who showed that when the ear was perfused with Locke's solution containing eserine, stimulation of the postganglionic fibres caused the release of acetylcholine, since the fluid leaving the vein caused contraction of the muscle of the leech. They also observed that stimulation of the postganglionic fibers in the presence of eserine caused a greater constriction than in the absence of eserine. If the postganglionic fibres released acetylcholine, which in turn released noradrenaline, then eserine would increase the amount of acetycholine which was able to release noradrenaline. Finally they observed that when the rabbit ear was perfused with locke's solution containing acetylcholine, the effect of sympathetic sti-

mulation was reduced to zero, although an injection of noradrenaline still caused vasoconstriction. Chang and Rand (1960) showed that when hemicholinium was added to the fluid perfusing a rabbit's ear, the effect of postganglionic stimulation gradually failed until it had no more effect. Nicotine however had a greater effect than before. When the hemicholinium was removed from the perfusion fluid and injections of choline were given, the effect of stimulation was restored.

Cholinergic fibres to the isolated atria. It will be sufficient to mention one further case, namely that of the sympathetic fibres to the rabbit atria. Hukovic′ (1959) was able to prepare the atria as an isolated organ with the sympathetic fibres going to them. Normal atria responded to stimulation of the sympathetic fibres by an increase in the rate and force of the beat. However when he prepared atria from rabbits previously injected with reserpine so as to remove all noradrenaline, stimulation of the sympathetic fibres then caused inhibition of the atria. The inhibition was increased in the presence of eserine and abolished by atropine. Thus the stimulation of the sympathetic fibres released acetylcholine.

Later Chang and Rand (1960) studied the action of stimulating the stellate ganglion on the atria of the kitten. When hemicholinium was added to the bath, at the end of $4\frac{1}{4}$ hr the response to stimulation was almost abolished. The response was restored by the addition of choline. The evidence indicated that almost all of the fibres must have been cholinergic.

The action of TM10 and bretylium. In recent years substances have been discovered which block the action of sympathetic postganglionic fibres so that stimulation is ineffective. The first of these was the 2 : 6-xylyl ether of choline, known as TM10, which was introduced by Hey and Willey (1954). The second was bretylium introduced by Boura and Green (1959). Both these substances block the release of noradrenaline, though they do not interfere with the action of noradrenaline. Exley (1957) showed that their action is exerted at the end of the postganglionic fibre, and that the idea that they block conduction along the course of the postganglionic fibre is incorrect. Burn and Rand (1960b) showed that when sympathetic impulses were made ineffective in the presence of bretylium, tyramine released noradrenaline better than before. Hukovic′ (1960) showed that both TM10 and bretylium blocked the action of acetylcholine on the isolated atria of the rabbit heart and on the vessels of the rabbit ear in concentrations which also blocked the response to sympathetic stimulation. Thus the action of these substances is explained by the hypothesis that they block the action of the

acetylcholine released by the cholinergic fibres, so that the acetylcholine is no longer able to release noradrenaline. Since these substances block sympathetic stimulation completely, the conclusion must be drawn that all sympathetic fibres are cholinergic. The same conclusion follows from the evidence that hemicholinium blocks the effect of sympathetic stimulation, not in part but completely or almost completely.

Bretylium and TM10 are antagonists of acetylcholine of a new kind. At the parasympathetic endings, atropine is the best antagonist; at the neuromuscular junction tubocurarine is the best antagonist; at the ganglia, hexamethonium is the best antagonist, and now at the sympathetic postganglionic termination, bretylium is the best antagonist. Guanethidine appears to combine the actions of reserpine and bretylium,

We do not yet know the mode of action of the acetylcholine liberated by the sympathetic fibres. It is possible that when liberated it makes the end of the nerve fibre permeable to noradrenaline, so that noradrenaline is released. Bretylium and guanethidine thus prevent acetylcholine from increasing the permeability of the nerve fibre.

SUMMARY

Noradrenaline is not only present in sympathetic nerves but can be extracted from the organs they innervate. Noradrenaline disappears from organs like the spleen when the sympathetic nerves degenerate and also when the animal is injected with reserpine. Some sympathomimetic amines like tyramine, ephedrine and amphetamine have no action in denervated organs or in the innervated organs of an animal treated with reserpine. It has been shown that these amines act by liberating noradrenaline.

In animals treated with reserpine, sympathetic stimulation has very little effect. If a slow intravenous infusion of noradrenaline is given, sympathetic stimulation becomes more effective. It is concluded that noradrenaline can be taken up by the terminations of the sympathetic fibres from the blood stream, and then impulses passing down the fibres have a greater effect than before. It seems likely that noradrenaline is secreted into the blood by the adrenal medulla to maintain the efficiency of the sympathetic fibres

Acetylcholine (in the presence of atropine) and nicotine have many peripheral effects like those of sympathetic stimulation. Thus they constrict the vessels of the perfused rabbit ear. If animals are treated with reserpine, these

actions of acetylcholine (in the presence of atropine) and of nicotine are no longer seen. Thus these substances must act by liberating noradrenaline

The sympathetic postganglionic fibres to the sweat glands are cholinergic, but it is often forgotten that cholinergic fibres have been demonstated in the postganglionic fibres to the dog and cat hindleg, to the nictitating membrane, to the heart and to the uterus of the dog. They have now been demonstrated in the postganglionic fibres to the spleen, to the vessels of the rabbit ear, in the hypogastric nerves to the vas deferens, and indeed in all places where a search has been made. It is clear that acetylcholine liberated by a cholinergic fibre can release noradrenaline, and thus there may be a cholinergic link in all sympathetic postganglionic fibres. Evidence in favour of the cholinergic link is given by the effects of hemicholinium and bretylium.

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