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EXPERIMENTAL ASPECTS

The discovery of uptake of adrenaline in 1930-1933 and the development of the adrenergic fibre from a cholinergic fibre

J. H. BURN*

Emeritus Professor of Pharmacology, University of Oxford

THE DISCOVERY OF UPTAKE OF ADRENALINE 1930–1933

When Barger and Dale published their paper on the 'Chemical Structure and Sympathetic Action of Amines' in 1910, it appeared that sympathetic amines were similar in their properties, and that the differences between them were quantitative, but not qualitative. This view remained until 1926. It had been shown by Fröhlich & Loewi (1910) that the pressor action of adrenaline was increased by cocaine, but in 1926 Tainter & Chang showed that the pressor action of tyramine was abolished by cocaine.

A study of the action of tyramine was therefore begun by Burn. Together with Tainter (1931) he found that there was another important difference between adrenaline and tyramine. In the cat, after removal of the superior cervical ganglion of one side, and after allowing time for degeneration of the postganglionic fibres, the injection of tyramine was completely without effect on the denervated iris, although it dilated the normal iris. Adrenaline, as was expected, dilated the denervated iris more widely than the normal iris. Later, Burn (1932b) found the same difference between the denervated and normal iris after removing them from the body and examining their behaviour when they were immersed in oxygenated Ringer solution. Tyramine had no action on the denervated iris, while adrenaline had more action on the denervated iris than on the normal iris.

Burn (1932b) also examined the action of tyramine on the vessels of the foreleg of the cat. He removed the stellate ganglion of one side, and one week later made a spinal preparation and placed both forelegs in plethysmographs, to measure the volume changes which injections of adrenaline and of tyramine might cause. He found that in all experiments the injection of tyramine caused constriction of the leg volume on the normal side, but led only to passive dilatation on the denervated side. Adrenaline on the other hand caused diminution of leg volume on both sides, that on the denervated side being greater than or as great as that on the normal side. With the foreleg vessels as with the iris, the action of tyramine depended on the presence of a normal sympathetic innervation, whereas the action of adrenaline was usually greater and never less on the side without a sympathetic supply. When the nerves had gone, the action of tyramine had gone as well.

Burn also studied the action of tyramine in perfusion experiments. For these he used the Dale-Schuster pump (1928) to perfuse the hindleg of a dog. The pump was a double pump, one barrel of which was used to perfuse the lungs, while the second barrel was used to perfuse the hindleg. In making the preparation the hindleg was left without a circulation of blood for about 40 min during the time in which the lungs were prepared for perfusion. When the perfusion of the lungs and of the hindleg finally began, the blood pressure in the hindleg was extremely low, and while an injection of adrenaline had a constrictor action of the usual magnitude, tyramine had very little constrictor action. In the spinal cat the pressor action of tyramine is 1/40th of that of adrenaline, but at the beginning of the perfusion it was only 1/1400th of that of adrenaline.

The cause of the feeble action of tyramine was, however, not the low blood pressure, for when a solution of posterior pituitary extract was added, drop by drop to the reservoir of blood until the pressure was raised to a normal value, the effect of tyramine was not increased. Its action on the blood pressure remained very small. In other experiments, however, when adrenaline was added to the reservoir of blood, the normal constrictor action of tyramine gradually returned, until it was the same relative to

* Correspondence: 3 Squitchey Lane, Oxford, U.K.

adrenaline as that seen in the spinal cat. These results could be explained by supposing that during the period when the hindleg had been without a circulation, the tissues of the leg suffered from a lack of oxygen and the sympathetic nerves lost their store of sympathetic transmitter. If the action of tyramine was to release the transmitter from the sympathetic terminals, then when the transmitter was absent, tyramine would have no effect. However when adrenaline was added to the blood steadily, it might be taken up by the nerve terminals, and then tyramine might produce its normal effect. What was necessary was to study the response to sympathetic stimulation in the perfused hindleg.

Sympathetic stimulation

The hindleg was perfused as before and stimulation was applied to the lumbar sympathetic chain. At the beginning of the perfusion, stimulation, like an injection of tyramine, had very little effect. However when adrenaline was added drop by drop to the reservoir of blood, a response to stimulation appeared. This response was one that was quite unexpected, for instead of being a rise of pressure, indicating a vasoconstriction, it was a dilatation (Burn 1932a). The dilator response was always the earliest response to be observed.

In 1931, von Euler & Gaddum had shown that when the superior cervical ganglion was stimulated, there was a flushing of the buccal mucous membrane in the dog and a curious slow contraction of the facial muscles of one side when the facial nerve to that side had been cut a week before. They showed that these effects were due to the liberation of acetylcholine from the sympathetic postganglionic nerves. The dilatation observed as the early response to stimulation of the lumbar sympathetic chain in the vessels of the perfused dog hindleg was shown by Bülbring & Burn in 1935 to be also due to the release of acetylcholine. In the same year, Sherif, working under Gaddum, showed that the sympathetic fibres to the uterus of the dog, released acetylcholine when stimulated.

The early response of dilatation in the perfused dog hindleg when the sympathetic fibres were stimulated was of course not the only response. After a further period in which adrenaline continued to be added to the blood reservoir, Burn observed that the response depended on the length of time for which the stimulus was applied. The stimulation was given by induction shocks from a secondary coil, the shocks being break shocks timed by the rotary contact breaker designed by Sir Thomas Lewis. Fig. 1 shows

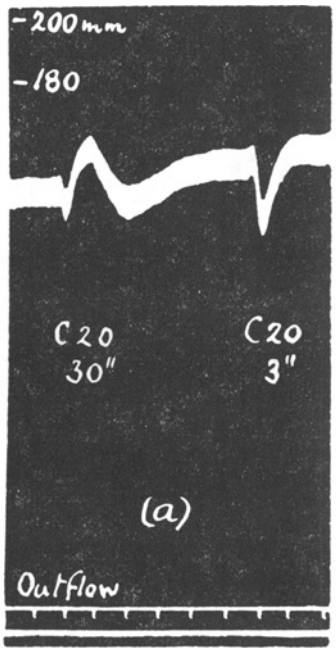

FIG. 1. Record of the pressure in the arteries of the dog hindleg when perfused with blood to which adrenaline was added drop by drop. The lumbar sympathetic chain was stimulated by break shocks from a secondary coil 20 cm distant from the primary. When the stimulation was for 30 s the response was mainly constrictor. When the stimulation was for 3 s, the response was dilator (Burn, 1932a, *J. Physiol.*, with permission).

two responses to stimulation when the secondary coil was 20 cm distant from the primary. On the left is the mainly constrictor response seen when stimulation was applied for 30 s. On the right is the dilator response when the same stimulation was applied for only 3 s. At the stage of the adrenaline infusion shown in Fig. 1, it is recorded in the original paper that 'it was found that at any strength of stimulus, a brief stimulus caused vaso-dilatation, while a prolonged stimulus favoured vaso-constriction'.

However, when the addition of adrenaline to the perfusing blood was continued for a longer time, the vasodilator responses disappeared and ordinary vasoconstrictor responses occurred regularly, as shown in Fig. 2 (Burn, 1933). This was taken from another perfusion experiment, showing the result of two stimulations given (a) after infusion of adrenaline for 45 min and (b) after infusion of adrenaline for 105 min. The two stimulations in (a) were the same in both strength and duration as those in (b). In (a) the two stimulations each resulted in a small vasodilatation. In (b) the two stimulations each produced clear vasoconstrictor responses. Evidently the

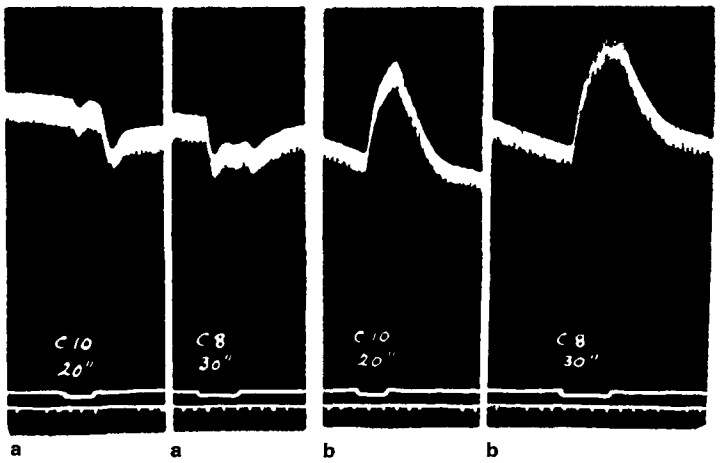

FIG. 2. Record of pressure in arteries of dog hindleg in another perfusion experiment. In (a) observations were made after adrenaline was added to the perfusing blood for 45 min. In (b) the adrenaline had been added to the blood at the same rate for 105 min. The two stimulations in (b) were exactly the same as in (a). The responses in (a) were dilatations, in (b) were constrictions (Burn, 1933, *Proc. Roy. Soc. Med.*, with permission).

amount of adrenaline released by each stimulation in (b) was much greater than was released by each stimulation in (a), and this was presumably because of the long period between (a) and (b) during which more adrenaline was taken up from the blood by the nerve endings. These observations, made in the period 1930 to 1933 and published in four papers, established the occurrence of adrenaline uptake as a physiological process.

Burn said in 1933 '. . . the adrenaline in circulation enables a store to be maintained at the sympathetic nerve ending of the chemical substance which is liberated when an impulse travels down the nerve'.

THE DEVELOPMENT OF AN ADRENERGIC FIBRE FROM A CHOLINERGIC FIBRE

It is surprising that so few have paid attention to the development of the adrenal medulla. Its cells are derived from the embryonic nervous system. Thus, Boyd (1960) has pointed out that at an early stage the intra-adrenal cells are very similar to those in the sympathetic ganglia. By the 30 mm crown-rump 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. *It follows that since acetylcholine is involved in the release of adrenaline and noradrenaline from the adrenal medulla, it may also be involved in the release of noradrenaline from sympathetic postganglionic fibres.*

For example, in Fig. 1 the two stimulations of the sympathetic chain were of the same strength and differed only in duration. When the stimulation was for 3 s, acetylcholine alone was released causing vasodilatation. When the stimulation was for 30 s, acetylcholine must have been released in larger quantity, but there was almost no sign of a dilator effect. The acetylcholine must have reached a concentration which caused vasoconstriction due to the release of noradrenaline, by the same mechanism as acetylcholine from the splanchnic nerves releases adrenaline and noradrenaline from the adrenal medulla.

Sympathetic fibres in teleost fishes

It is not true that the sympathetic fibres of all creatures release noradrenaline. Young (1936) examined the splanchnic nerves supplying the intestine of two teleost fishes. *Lophius piscatorius* and *Uranoscopus scaber*. He found that stimulation of the nerves caused contraction and not inhibition, though adrenaline itself caused inhibition. Burnstock (1958) studied another teleost fish, the trout, and obtained a similar result. He found that the splanchnic nerves were motor to the intestine, and concluded they were cholinergic, since the response to stimulation was blocked by atropine.

Sympathetic fibres in chickens

Since birds may be regarded as intermediate between fishes and mammals, Burn (1968) made preparations from the intestine of chickens about 5 months old similar to the preparation of the rabbit ileum described by Finkleman (1930), in which stimulation of the sympathetic fibres which run as periarterial nerves in the mesentery causes inhibition of the intestine, an inhibition which is blocked by bretylium. Stimulation of the periarterial nerves in the chicken did not produce inhibition of the intestine, but caused a powerful contraction. This contraction did not occur in the presence of atropine. While sympathetic stimulation did not cause inhibition, noradrenaline caused inhibition in concentrations from 2×10^{-8} g ml^{-1}.

Sympathetic fibres in new-born mammals

If in the course of evolution from teleost fishes to chickens and then to mammals, the sympathetic fibre has changed from cholinergic to adrenergic, then there was the possibility that ontogeny might recapitulate phylogeny, and that if, for example, the new-born puppy was examined, it would be found that there was a change in the early days or weeks of life from sympathetic fibres which were cholinergic to sympathetic fibres which were adrenergic. The first workers to study this were Boatman, Shaffer & others who published their paper in 1965. They perfused the hindleg vessels of new-born puppies with blood, and found that stimulation of the sympathetic postganglionic fibres did not cause constriction, but dilatation, despite the fact that the blood pressure was low, being 30 mm on the first day of life. The dilatation was abolished by the injection of atropine, 0·2 mg kg^{-1}. An injection of 0·2 μg adrenaline into the perfusion cannula caused vasoconstriction. Sympathetic stimulation caused vasodilatation not only on the first day of life, but also during the next two weeks; however, although the blood pressure of the puppies rose as the puppies grew older, stimulation in those 4 weeks old or over produced vasoconstriction.

Observations in new-born rabbits

Unaware of the results which Boatman and his colleagues had obtained in puppies, Burn began to study the rabbit intestine of new born rabbits using the Finkleman preparation he had used for the chicken intestine. He had used this preparation in over three hundred experiments and stimulation of the periarterial nerves had always caused inhibition when the loops of ileum were taken from adult rabbits. The results in two experiments each on a rabbit 3 days old are shown in Fig. 3. In the upper record the responses to stimulation at 3, 5, 10 and 20 Hz were motor, and the loop contracted to each stimulus. In the lower record, the experiment being carried out on a different 3 day old rabbit from another litter, the responses to frequencies of 3 and 5 Hz were motor, but the responses to 10 and 20 Hz were inhibitor, though the inhibitions were slight. In this experiment the addition of atropine (0·1 μg ml^{-1}) to the bath was shown to abolish the motor response to stimulation at 3 Hz. The responses of the ileum of a 12 day old rabbit were all inhibitor. Thus, sympathetic fibres which were inhibitor in the adult rabbit, were motor in the first 3 days of life, sometimes at all frequencies, sometimes only at low frequencies, and the change to inhibitor action for all frequencies took place before the 12th day.

Further observations

In 1974, Panchal (working under Prof. O. D. Gulati in Baroda) confirmed and extended these observations, using the Finkleman preparation and determining the response of an isolated loop of ileum to stimulation of the periarterial fibres supplying the loop. Panchal carried out experiments on rabbits on the first day of life and then on each successive day up to the 12th day. On each day of life he made observations on 3 or 4 rabbits and measured the mean response. Until the 3rd day the responses to stimulation at all frequencies were motor, but after the 3rd day the responses to the higher frequencies became inhibitor, and by the 7th day the responses to the lowest frequency only remained motor. The

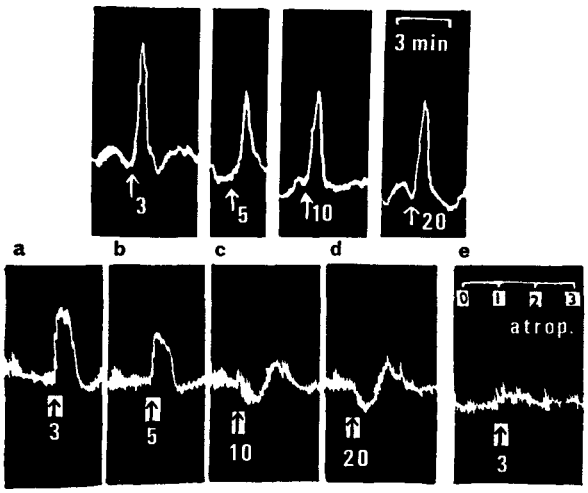

FIG. 3. Above are shown the contractions of a loop of intestine from a 3-day-old rabbit in response to stimulation of the periarterial nerves in the mesentery. Stimulation at 3, 5, 10 and 20 Hz in all cases gave a motor response instead of the inhibitory response always seen in loops taken from rabbits 12 days old or older, whatever the frequency. Below are observations from a 3-day-old rabbit from another litter. Note that in (a) and (b) when the stimulations were 3 and 5 Hz, the responses were motor, but in (c) and (d) when the stimulations were 10 and 20 Hz, an inhibitory response was developing. In (e) stimulation was at 3 Hz again, but in the presence of atropine there was no response (Burn, 1968, *Br. J. Pharmac.*, with permission).

results on the 2nd, 4th, 7th and 11th days are shown in Table 1. Stimulation was applied at 1, 2, 5, 10 and 20 Hz. For example in strips of ileum taken from rabbits on the 4th day of life, frequencies of 1, 2 and 5 Hz caused a contraction of the strips, but those of 10 and 20 Hz caused inhibition.

Table 1. (Panchal, 1974). *Response of strips of rabbit ileum to stimulation of sympathetic fibres.*

Rabbit, age in days	No. of rabbits	Size of responses in mm to stimulations of: 1 Hz	2 Hz	5 Hz	10 Hz	20 Hz
2	3	+10	+14	+16	+21	+26
4	3	+13	+15	+3	—11	—18
7	4	+1	—5	—14	—33	—37
11	3	—5	—7	—11	—28	—31

+ means motor response. — means inhibitor response.

Time of uptake

In 1964, the time at which adrenaline uptake occurs was studied in the rat heart by Glowinski, Axelrod & others. They found that the uptake of noradrenaline was deficient at birth, but that it increased rapidly after 8 days. In the second week after birth there was a three-fold increase in the capacity of the rat heart to take up noradrenaline. In the young rat, however, up to three weeks old, 50% of what was taken up was not retained. Retention did not reach adult level until 6 weeks of age.

Panchal also measured the uptake of noradrenaline in strips of ileum taken from rabbits on the 1st to 12th days of life by suspending the strips in an oxygenated solution containing noradrenaline 1 μg ml^{-1} for 20 min at 35°. The results in Table 2 indicate a slowly increasing uptake for the first 6 days and then a rapid increase till the 9th day where apparently it stayed at the same level. He also studied the uptake of similar strips when the solution contained cocaine, and found that the uptake throughout was negligible.

Table 2. (Panchal, 1974). *Amount of noradrenaline taken up by strips of ileum in a solution containing noradrenaline 1 μg ml^{-1} for 20 min at 35°.*

Rabbit, age in days	No. of rabbits	Amount of noradrenaline taken up by strip (μg g^{-1}) Absence of cocaine	Presence of cocaine
1	6	0·23	0·01
2	5	0·54	0·01
3	4	0·70	0·02
6	4	1·08	0·01
7	4	2·85	0·19
9	4	3·12	0·47
12	4	3·26	0·01

Conclusion

For evidence that acetylcholine is concerned in the release of noradrenaline the clearest results are those obtained by Malik (1970) in the perfused mesenteric arteries of the rat, in which stimulation of postganglionic sympathetic fibres caused a rise of perfusion pressure as seen in Fig. 4. The importance of this preparation lay in the fact that successive stimuli gave the same response for long periods. The perfusions were carried out in the absence of atropine at 30°. When the stimuli were applied at frequencies

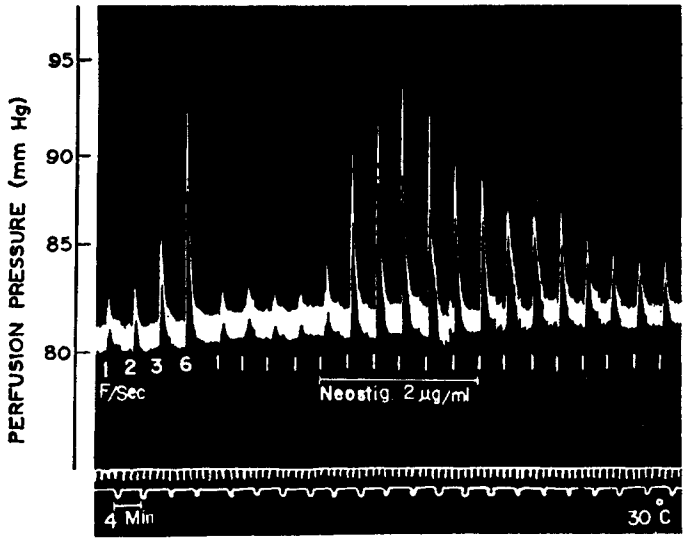

FIG. 4. Record obtained by perfusing the mesenteric arteries of the rat with Tyrode at 30°, and stimulating the sympathetic postganglionic fibres. The effect of neostigmine is shown on the responses to stimulation at 1 Hz for 30 s. On the left are responses to stimulation at 1, 2, 3 and 6 Hz in the absence of neostigmine. All other responses were elicited at 1 Hz. The infusion of neostigmine, 2 μg ml^{-1}, potentiated the response to stimulation at 1 Hz so greatly that the height of the response was equal to the height of the response recorded at 6 Hz in the absence of neostigmine (Malik, 1970, *Circulation Res.*, with permission).

of 1, 2 and 3 Hz, the anticholinesterases, neostigmine (2 μg ml^{-1}), DFP (2 μg ml^{-1}) and physostigmine (6 μg ml^{-1}) caused a large increase in the response to stimulation, the increase being greatest at the lowest frequency. The action of these substances can only be explained by the existence in the sympathetic fibres running to these mesenteric vessels of a cholinergic link such as is found between the splanchnic fibres and the adrenal medulla.

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