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THE SYMPATHOMIMETIC EFFECT OF ACETYLCHOLINE ON THE SPLEEN OF THE CAT

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Evidence has accumulated that acetylcholine (ACh) is capable of acting on neurones at sites other than the subsynaptic membrane. It has been shown for instance that it can excite mammalian sensory nerve fibres in the skin and mesentery (Brown & Gray, 1948; Douglas & Gray, 1953), carotid sinus pressure receptors (Diamond, 1955) and C fibres carrying activity from cutaneous touch receptors (Douglas & Ritchie, 1960). Among the first to suggest that acetylcholine might excite post-ganglionic sympathetic fibres were Coon & Rothman (1940), who injected acetylcholine into the skin and obtained sympathetic effects which were prevented by ergotamine and by degeneration of the sympathetic supply to the area. Recently, Brandon & Rand (1961) and Daly & Scott (1961) have reinvestigated the sympathomimetic effect of acetylcholine on the spleen, previously described by Farber (1936). They showed that close arterial injection of acetylcholine into the spleen produced contraction of that organ and this effect could be prevented by reserpine, by adrenergic blocking agents, by hexamethonium and by degeneration of the sympathetic supply. It seemed possible that this sympathomimetic effect of acetylcholine might be due to its exciting the sympathetic post-ganglionic adrenergic C fibres, and experiments were carried out to test this hypothesis. Some of the results of these experiments have already been communicated to the Physiological Society (Ferry, 1962).

METHODS

Cats were anaesthetized with ethyl chloride and ether and then with intravenous chloralose, 80 mg/kg. The abdominal cavity was opened by a mid-line incision and the spleen and its arterial supply were separated from the stomach and omentum by tying and cutting the hepatic, left gastric and gastro-epiploic arteries. In some experiments the stomach was removed to gain better access to the arterial supply of the spleen.

Acetylcholine solution was injected into the splenic artery through a polythene cannula placed in a suitable side branch and pointing toward the heart. The arteries used for retrograde cannulation were the hepatic artery, the smaller of the two divisions of the splenic artery, or a small branch of the splenic artery just before it enters the spleen. In one experiment one of the gastro-epiploic arteries was used. The arrangement of the cannula was such that injections could be made without interrupting the natural blood supply during the injection.

Records were made of activity in many bundles of the splenic nerve or in one bundle or in a fine filament stripped off the bundle. The splenic nerve was cut at a point between the bifurcation of the splenic artery and the coeliac ganglion, and then the peripheral end was prepared for stimulation. Bright platinum wires were used for stimulating and recording electrodes. The activity in the nerve fibres was amplified with an a.c. amplifier and displayed on a cathode-ray tube. The abdominal cavity was filled with warm liquid paraffin.

For experiments with degenerated nerves, the splanchnic nerves had been cut by the following technique. The cat received 1 mg atropine sulphate subcutaneously and was then anaesthetized with ethyl chloride and ether. An incision was made in the flank and the splanchnic nerve exposed and divided just where it emerged beneath the diaphragm. In order to avoid death of the cat, which is often a consequence of simultaneous bilateral splanchnicotomy, the right splanchnic was cut in one operation, and about 10 days later the left splanchnic was cut in a second operation. The final experiment was performed after at least a further 10 days.

Acetylcholine chloride was made up as a 10^{-3} g/ml. solution in saline of the following composition (mM): $NaHCO_3$ 12; NaCl 137·8; KCl 4; KH_2PO_4 1; $CaCl_2$ 2; $MgCl_2$ 1; glucose 11. The solution was gassed with CO_2 5% + O_2 95%.

In the final experiment cats were often given 1 mg atropine sulphate by intravenous injection.

The adrenergic blocking agent Hydergine (Sandoz) is a preparation of dihydroergocornine, dihydroergocristine and dihydroergokryptine.

RESULTS

The injection of acetylcholine into the blood entering the spleen of seven normal cats was followed after a delay of about 1 sec by a brisk but evanescent discharge of impulses in the peripheral cut end of the splenic nerve, the discharge lasting for 10–20 sec. This response could be evoked many times in each cat and in seven experiments some 70 responses were obtained. Control injections of the saline solution were ineffective.

The dose of acetylcholine needed to evoke centripetal impulses varied and depended more on the rate of injection than on the amount injected. Thus responses were obtained with injections of 10 μg acetylcholine given fairly rapidly, while a slower injection of much larger amounts was ineffective. Figure 1 shows the discharge in the peripheral cut end of a single bundle of the splenic nerve about 15 mm from the spleen.

The nature of the fibres activated. The splenic nerve of the cat contains a small number of myelinated and a large number of non-myelinated nerve fibres (Utterback, 1944; Kuntz & Jacobs, 1955). The compound action potential shows a small group of fibres with a conduction velocity of about 16 m/sec and a much larger group with a conduction velocity of less than 1 m/sec. In order to discover which fibres carried the centripetal activity induced by acetylcholine the collision technique of Brown & Pascoe (1952) and Douglas & Ritchie (1957) was used. Stimulating electrodes were placed centrally on the splenic nerve and one bundle of the nerve was dissected off the artery between the stimulating electrodes and the spleen. A centrifugal volley was set up by regular maximal stimuli at

a frequency of one or two per second, and acetylcholine was injected into the splenic blood supply. The result of such an experiment is shown in Fig. 2. The acetylcholine provoked asynchronous activity in the fibres over the electrodes, as shown by a disturbance of the base line, and the C fibre spike was reduced in size. The fast fibres gave only a very small spike which was mixed with the stimulus artifact. It was not possible to discover any reduction of the fast spike, as it was masked by the asynchronous discharge. It was concluded from this type of experiment that acetylcholine could produce activity in C fibres.

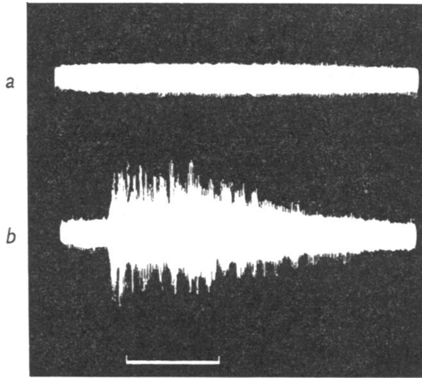

Fig. 1. Cat, 3·1 kg. Chloralose and atropine. Records from the peripheral cut end of one bundle of the splenic nerve about 15 mm from the spleen: the records have been retouched. Injections were made into the splenic arterial blood via the hepatic artery: *a*, 0·5 ml. saline; *b*, 250 μg ACh in 0·25 ml. saline. Time 5 sec.

The effect of drugs on the response. The appearance of impulses in the peripheral end of the splenic nerve after close arterial injection of acetylcholine into the spleen was not affected by the intravenous administration of atropine sulphate 1 mg or by the adrenergic blocking agent 'Hydergine' 0·5 mg/kg. The discharge evoked by acetylcholine was prevented, however, by intravenous hexamethonium bromide 10 mg/kg given 1–2 min previously. The injection of 1 or 2 mg hexamethonium into the splenic arterial blood reduced the effect of acetylcholine for about 5 min (Fig. 3).

The sensory innervation of the spleen. It has been shown above that acetylcholine is capable of producing activity in the C fibres of the splenic nerve. Although it is likely that many of these are the sympathetic postganglionic fibres to the spleen, the possibility of the presence of afferent C fibres cannot be excluded. It is known from the work of Ranson & Billingsley (1918) that the splanchnic nerves contain sensory C fibres and some of these might innervate the spleen. In addition, the splenic nerve might contain C fibres which ascend in the vagus.

The technique of the pupillary dilator response to nerve stimulation described by Bain, Irving & McSwiney (1935) was used to trace visceral afferent fibres. A cat was anaesthetized with ethyl chloride and ether and with intravenous chloralose 70 mg/kg. The splenic nerve was dissected off

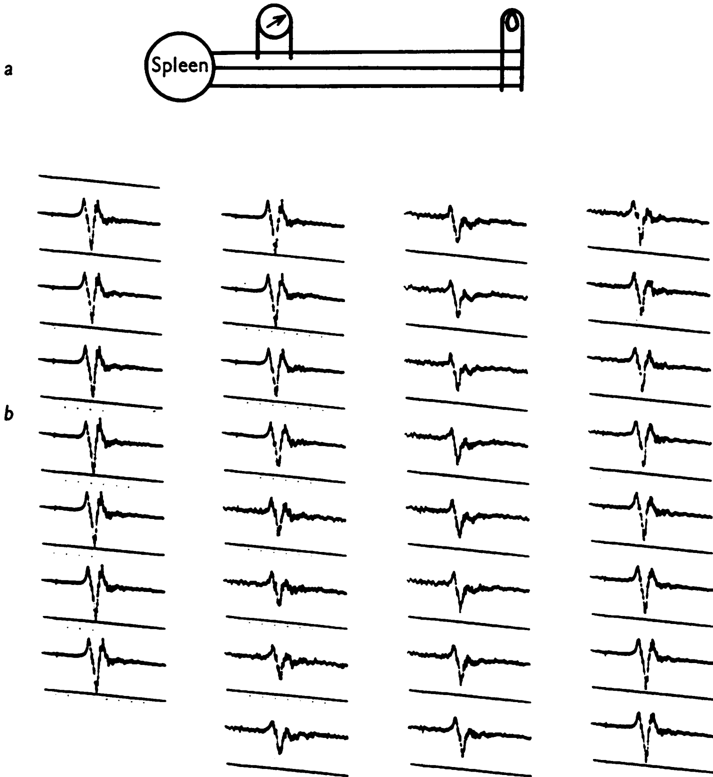

Fig. 2. *a*. Diagram showing the arrangement of the electrodes on the splenic nerve. Records were made of activity in a single bundle which contained nerve fibres cut central to the stimulating electrodes, but in continuity from these, over the recording electrodes, to the spleen. *b*. Cat. Chloralose and atropine. The left-hand column shows the control size of the C spike produced by stimulation at 2/sec with supramaximal stimuli. The second column shows records made while 200 μg ACh was injected into the splenic arterial blood via the hepatic artery. Conduction distance 30 mm; time 10 msec. The records are continuous.

the artery just central to the bifurcation, tied and cut. Blood pressure was recorded from the left femoral artery. The cat was left untouched for 40 min after the dissection was complete, to allow constriction of the pupils. After this rest, stimulation of the central cut end of the splenic nerve with a train of 50 supramaximal stimuli at a frequency of 10/sec caused an immediate dilatation of the pupils to 5 mm from the resting

size of 0·5 mm, and twitching of the left side of the abdominal wall. The blood pressure rose slightly and then fell. Section of the left splanchnic nerves caused a fall in blood pressure. Subsequent stimulation of the central cut end of the splenic nerve produced a pupillary dilatation of about the same magnitude as before; there was little twitching of the left side of the abdominal wall and no disturbance of the lowered blood pressure. Section of the right splanchnic nerves abolished the pupillary dilatation in response to stimulation of the splenic nerve. As a control,

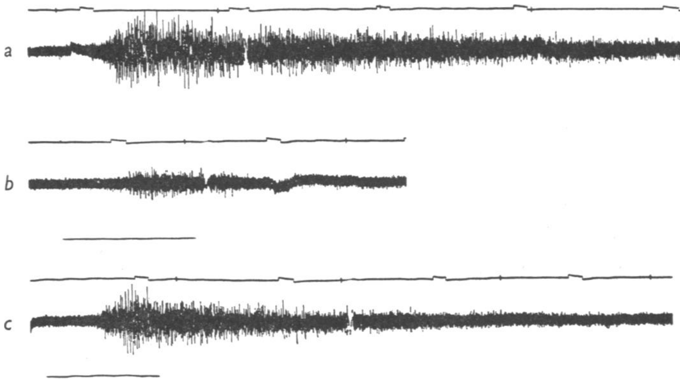

Fig. 3. Cat, 3·2 kg. Chloralose and atropine. Hydergine 0·5 mg/kg was given 30 min before taking this record of activity in the peripheral cut end of a single bundle of the splenic nerve. *a*. Response to 200 μg ACh injected into the spleen via the smaller division of the splenic artery. Between taking this record and the next 1 mg hexamethonium was injected via the hepatic artery. *b*. 200 μg ACh injected 2 min after the first injection. *c*. 200 μg ACh injected 7 min after the first injection. The period of the injection is indicated by a horizontal line below the record. Time, 1 sec.

electrodes were placed on the central cut end of the left splanchnic major nerve and pupillary dilatation and twitching of the abdominal muscles were obtained on stimulation. When the coeliac branch of the abdominal vagus was stimulated, no response of the pupil was seen; but when the ventral gastric branch of the abdominal vagus was stimulated, the pupillary dilatation described by Harper, McSwiney & Suffolk (1935) was observed.

It was concluded that the sensory innervation of the spleen runs in the left and right splanchnic nerves, and that the coeliac branch of the abdominal vagus does not contain sufficient afferent fibres to produce pupillary dilatation. Therefore, cutting and degeneration of the left and right splanchnic nerves should remove the afferent fibres to the spleen which cause pupillary dilatation. Such a procedure would leave the motor innervation intact, apart from decentralization of the cells of the coeliac ganglion.

The effect of acetylcholine after degenerative section of the splanchnic nerves. Two cats were used. In one the right splanchnic had been cut 23 days and the left splanchnic 10 days before the final experiment; and in the other cat the intervals were 39 and 28 days.

When the abdominal cavities of these animals were opened the spleen was large and engorged. There was no twitch of the abdominal muscles when the splenic nerve was tied, indicating that the sensory supply to the spleen was not functional. The preparations were set up in the usual way, but two sets of recording electrodes were placed under a single bundle of splenic nerve fibres which was continuous from the stimulating electrodes, over the recording electrodes and into the spleen. The two recording channels were set at different amplifications; the channel near to the spleen had a high gain, to record adequately the asynchronous activity, whereas that near the stimulating electrodes had a lower gain, to record the centrifugal volley produced by a maximal stimulus at the stimulating electrodes.

The upper records of Fig. 4 show the effect of the first injection of 80 μg acetylcholine. There is a reduction in the height of the C spike coincident with a burst of asynchronous activity in the same fibres. The lower part of Fig. 4 shows the second injection of 100 μg acetylcholine in the same preparation. The gain was doubled in the peripheral recording channel. This record shows that the lowering of the C spike is due to collision between the centrifugal volley and the asynchronous acetylcholine-induced activity, for the C spike is lowered only when the centrifugal volley and the acetylcholine induced discharge are present in the nerve fibres at the same time.

A second splanchnicotomized cat showed the same results (Fig. 6) as the other decentralized preparation, and there seemed to be no difference in the response to acetylcholine between deafferented preparations and normal preparations.

The site of action of acetylcholine. In the experiments shown in Figs. 2, 4 and 6, in which the nerve was intact from the stimulating electrodes to the nerve endings, it is possible that the asynchronous activity engendered in C fibres by acetylcholine might have been due to the excitation of ganglion cells which are known to occur along the splenic nerve (Kuntz & Jacobs, 1955). Therefore, in the experiments in which two recording channels were placed on one bundle of the splenic nerve which was continuous from the stimulating electrodes to the spleen, the bundle was cut between the two sets of recording electrodes (between I and II in Fig. 4). Injection of acetylcholine then produced activity in the peripheral cut end but not in the central cut end, and the C spike elicited by stimulation and recorded by the more central channel was not reduced in size. This experiment was carried out on normal cats and on the splanchnicotomized animals. It was concluded that the discharge in C fibres found in these experiments was centripetal

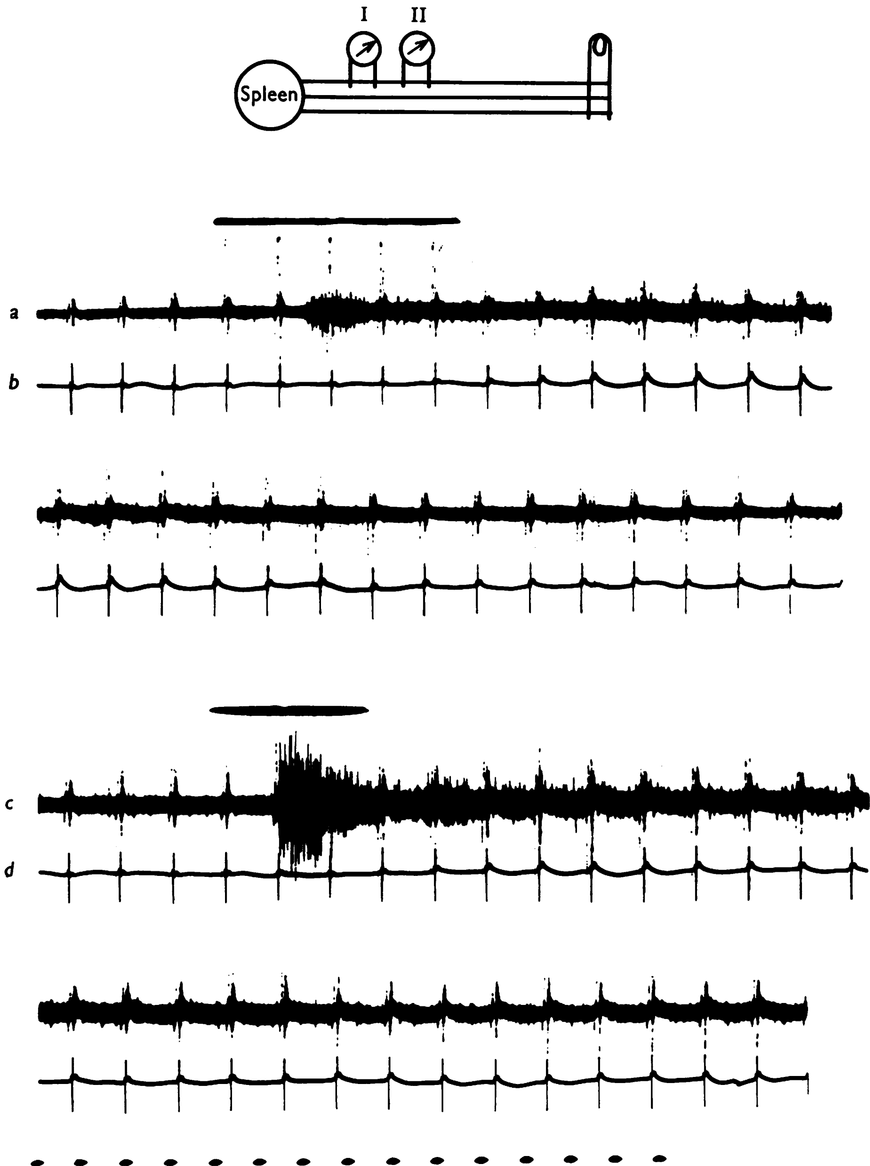

Fig. 4. Diagram showing the arrangement of a bundle of the splenic nerve over two pairs of recording electrodes and one pair of stimulating electrodes. The nerve fibres were cut central to the stimulating electrodes. Cat, 2·5 kg. Chloralose and atropine. Right splanchnic nerve cut 23 days and left splanchnic nerve cut 10 days previously. Effect of 2 injections of 80 μg and 100 μg acetylcholine into small branch of the splenic artery. *a* and *c* show records from electrode pair I, that at *c* being at twice the amplification of *a*. *b* and *d* show records from electrode pair II, at low amplification to show action potential propagated from stimulating electrodes after supramaximal stimuli at 2/sec. Time 0·5 sec.

and not due to the excitation of ganglion cells by acetylcholine, unless these ganglion cells had centrally directed axons like those found by Douglas, Lywood & Straub (1960), in the cat's superior cervical ganglion and preganglionic trunk.

In one experiment records were taken about 1 cm from the spleen from the peripheral cut end of a single nerve bundle which ran, without branching, along a small artery into the spleen. The injection of acetylcholine caused activity to appear in this bundle; occluding the small artery did not prevent this, but the discharge was abolished when the nerve was cut between the spleen and the electrodes. It was concluded that acetylcholine did not act on cut ends of nerve bundles, nor on the fibres running in the extrasplenic portions of the splenic nerve. The site of action of acetylcholine lies within the spleen.

The slow potentials seen after acetylcholine. If the recording channel had an adequate low-frequency response, the record of the compound action potential of the centrifugal volley produced by electrical stimulation showed slow waves after the injection of acetylcholine (Figs. 4–6) in both deafferented and normal preparations. Figure 5 shows the effect in a normal cat. In addition to showing slow waves, the C fibre spike was often increased above the control height, this effect being particularly well shown in Fig. 6. The increased spike size is correlated with the size of the post-spike potentials and may amount to 110 % of the control spike. If the bundle was cut between the two recording channels, as described above, the changes in spike size and post-spike potential were not seen. Changes in the size of C fibre action potentials due to repetitive activity in the same fibres have been described by Gasser (1950), Brown & Holmes (1956) and Ritchie & Straub (1956). It is likely that the cause of the increased spike and post-spike potentials was the burst of asynchronous impulses evoked by the acetylcholine injection in the fibres over the recording electrodes, and not a direct action of acetylcholine on the fibres at that place. If acetylcholine were to cause repetitive activity in individual C fibres, the centripetal train of impulses would condition the nerve and produce the observed effects when a synchronous centrifugal volley was elicited.

Records from fine filaments of the splenic nerve. Attempts were made to record the discharge of a single unit in the peripheral end of a splanchnicotomized preparation responding to the close arterial injection of acetylcholine. This was found to be difficult, as even extremely fine filaments contained many active fibres. The technique used was to desheath a bundle and tear it down with fine forceps to give a filament about 5 or 6 mm long, which was placed over platinum wire electrodes. This particular method kills many of the fibres present in the filament, but, even so, a

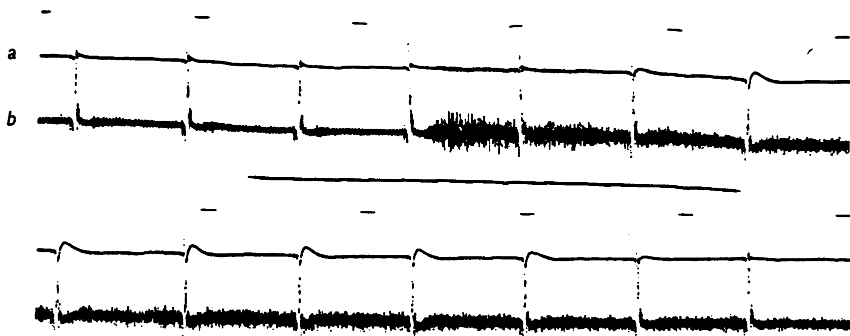

Fig. 5. Cat, 3·2 kg. Chloralose and atropine. Electrodes were arranged as in Fig. 4. *a*. Record from the central recording electrodes. *b*. Record from the peripheral recording electrodes. Acetylcholine 200 μg was injected into the blood entering half the spleen through a cannula placed in the smaller division of the splenic artery. Supramaximal stimulation at 2/sec. Time 0·5 sec. The records are continuous.

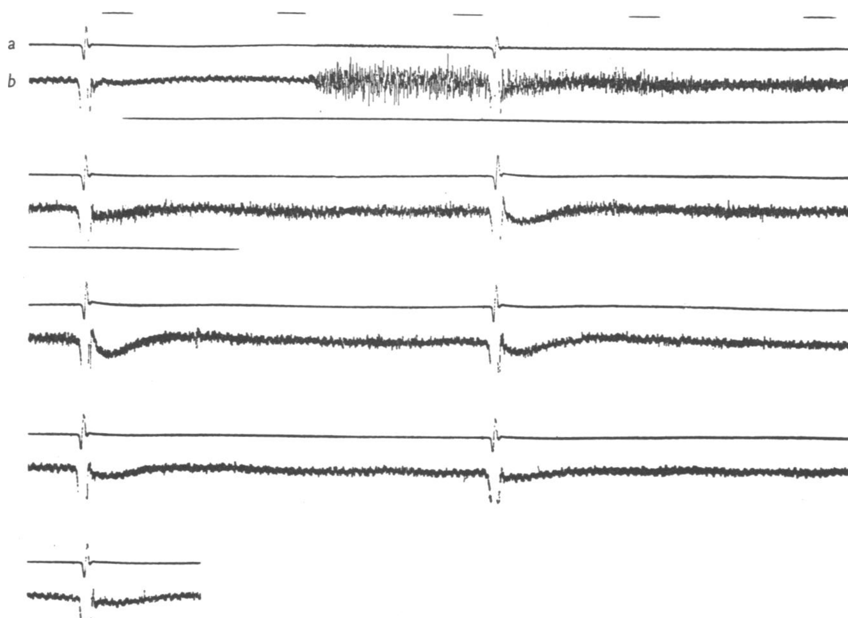

Fig. 6. Cat, 3 kg. Chloralose and atropine. Right splanchnic cut 39 days, left splanchnic cut 28 days before this experiment. Electrodes arranged as in Fig. 4. *a*. The C fibre spike produced by supramaximal stimulation of the nerve at a frequency of 1/sec recorded by the central pair of electrodes. *b*. The record from the peripheral pair of electrodes: 200 μg ACh injected into half the spleen through the smaller branch of the splenic artery. Time marker, 0·5 sec intervals. The records are continuous.

multifibre response to acetylcholine was always obtained. The results from one filament are shown in Fig. 7. This was a cat in which the splanchnic nerves had been cut and allowed to degenerate. Several sizes of action potential can be distinguished in the record, each having a duration of about 2 msec. The two smallest spikes and three slightly larger ones appeared at the beginning of the discharge at a frequency of about 40/sec. The spikes seen on the lower part of the record appeared irregularly for 2 sec at frequencies varying between 1/sec and 25/sec. It seems that acetylcholine can excite many fibres and causes a short high-frequency train of impulses in some fibres, and, in others, a longer irregular train of lower frequency.

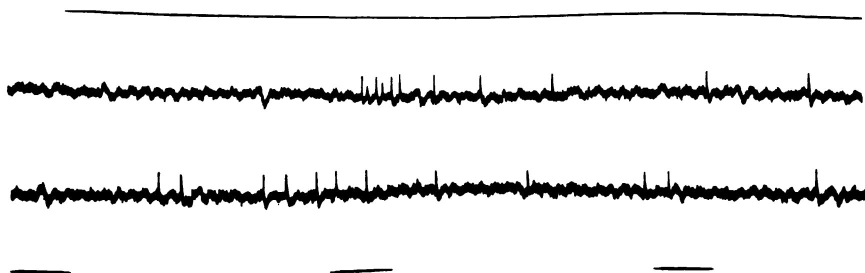

Fig. 7. Same preparation as in Fig. 4. Records from a fine filament in the peripheral end of the splenic nerve. Effect of 200 μg ACh injected into the splenic arterial blood via one branch of the splenic artery. Time 0·5 sec. The records are continuous.

The delayed response to nerve stimulation. In one cat, when the splenic nerve was stimulated and records taken from a bundle continuous from the stimulating electrodes to the spleen, the centrifugal volley was followed by a burst of impulses with a latency of 120 msec. This can be seen in Fig. 5, which is a record from this preparation. When the bundle was cut and records were taken from the peripheral cut end, the centrifugal volley disappeared but the asynchronous centripetal burst of impulses was still present. This centripetal activity followed each volley passing peripherally from the stimulating electrodes and stopped with cessation of stimulation. It was present after resting the preparation and showed an increase over the first few stimuli of a train. It was not affected by atropine or Hydergine. The injection of acetylcholine seemed to increase this asynchronous burst of impulses. The possible causes of this centripetal burst after a volley of impulses entering the spleen are either fibres from one bundle looping and running centrally in another bundle, or preganglionic fibres in one bundle relaying with a post-ganglionic neurone sending its axon centrally (Douglas, Lywood & Straub, 1960), or some ephaptic transmission between C fibres. The latency of this burst of impulses is compatible with activity entering

and leaving the spleen in C fibres. This effect was looked for in a number of other normal preparations but was never seen, and no further experiments have been carried out to determine more exactly the conditions under which it appears.

DISCUSSION

The excitation of C fibres by acetylcholine

The evidence for the excitation of C fibres by acetylcholine is twofold. First, the collision technique shows that in normal and splanchnicotomized animals there is a lowering of the amplitude of the slowly conducted action potential. Secondly, post-tetanic enhancement of the recorded spike potential and post-spike potentials is a property restricted to C fibres (Brown & Holmes, 1956), and the observed enhancement of the C spike can be explained by a conditioning discharge in C fibres evoked by acetylcholine. The other explanation that acetylcholine might act directly on the C fibres to produce a bigger spike can be discounted, as Armett & Ritchie (1960) found that acetylcholine acting directly on C fibre trunks produced a decrease in the action potential recorded with the sucrose-gap apparatus. It is unlikely that the increased spike and post-spike potentials could be due solely to the action of acetylcholine on the nerve over the recording electrodes, as suggested by Douglas & Ritchie (1960) in their experiments on sensory C fibres. The enhancement is not shown if the acetylcholine-induced impulses are prevented from reaching the electrodes recording the compound action potential by cutting the nerve peripheral to these electrodes. It seems quite clear, therefore, that acetylcholine excites C fibres. The centripetal discharge is still present after atropine and Hydergine. These substances would reduce or abolish any direct effect of acetylcholine on smooth muscle or the effect of any catecholamines released by acetylcholine, and would therefore prevent the excitation of mechanoreceptors by splenic contraction, so that it is unlikely that acetylcholine acts on some structure other than the C fibres. The abolition of the acetylcholine-induced discharge by hexamethonium cannot be due to a conduction block in the C fibres produced by the drug, for Daly & Scott (1961) report that the contraction of the spleen after nerve stimulation is not affected by hexamethonium. The excitant action of acetylcholine must be specifically blocked and this is entirely in keeping with the findings of other workers investigating the excitation of nerve fibres by acetylcholine (Gray & Diamond, 1957; Douglas & Ritchie, 1960).

The conclusion that acetylcholine acts within the spleen and not on C fibre trunks agrees with the suggestion of Armett & Ritchie (1960) that the nerve endings of C fibres are more sensitive than the axons. Perhaps the site of action of acetylcholine is at the point where the axis cylinders

emerge from the sheath of Schwann cells. In this respect the finding of Diamond (1959), that only the tips of regenerating myelinated fibres were sensitive to acetylcholine, is of interest.

The composition of the splenic nerve

The C fibres found in the splenic nerve of the cat could be sympathetic post-ganglionic nerve fibres, vagal fibres, afferent C fibres and perhaps preganglionic sympathetic fibres (Duncan, 1932). Agostoni, Chinnock, Daly & Murray (1957) have shown that many of the vagal efferent and afferent fibres running to the abdominal viscera are non-myelinated, and Iggo (1957) has recorded activity in vagal C fibres after stimulation of gastric and duodenal receptors. It is possible that some C fibres in the splenic nerve might be of vagal origin, but the work of Utterback (1944) indicates that if there is any vagal innervation of the spleen it cannot be very great. The experiment with the technique of Bain *et al.* (1935) suggests that all the splenic visceral afferent fibres travel in the splanchnic nerves, but it is possible that some visceral afferents might not cause pupillary dilatation, and their presence would not be detected by this experiment. However, Evans (1957) has shown that C fibres are the most potent of the various afferent fibres in producing pupillary dilatation, and it seems likely that many of the afferent C fibres would have been detected in the experiment reported above. Therefore section of the splanchnic nerves and degeneration of the cut fibres would leave a splenic nerve consisting of post-ganglionic C fibres arising in the coeliac ganglion; non-myelinated fibres from other sources must be very few, if any are present. The conclusion that the acetylcholine-induced discharge is carried in sympathetic post-ganglionic C fibres is valid only if the fibres cut in the splanchnics have degenerated and if regenerating afferent C fibres are not present in the portion of the nerve over the recording electrodes, for Diamond (1959) has shown that regenerating nerve fibres can be excited by acetylcholine. It seems that 10 days is adequate for degeneration, as Ranson (1912), Cajal (1928) and Machida (1929) found that C fibres had degenerated after about 7–10 days. Heinbecker, Bishop & O'Leary (1932) found that many C fibres stopped conducting impulses 90 hr after section, and McLennan & Pascoe (1954) found that C fibres did not conduct impulses 5 days after section. There is little information on the rate of regeneration in C fibres, but it seems that no regeneration begins until 14 days after section (Ranson, 1912; Machida, 1929). Thus it seems impossible for the cut axons of C fibres to be still functional or to have regenerated in the cat in which the left and right splanchnic nerves had been cut 10 and 23 days, respectively. In the other cat the splanchnic nerves had been cut 28 and 39 days and the possibility of regeneration

must be considered. In order to reach the recording electrodes the regenerating fibres must have grown at about 3 mm per day after the initial 14 days' latency. The nerves had been cut and allowed to retract and it seems reasonable to assume that, under these conditions, regenerating fibres had not reached the position of the recording electrodes.

Another possibility is that the non-myelinated axons regenerating from cut myelinated fibres carry some of the acetylcholine discharge. These begin regenerating almost immediately and their presence in the section of nerve over the electrodes cannot be excluded. However, the number of myelinated fibres in a normal splenic nerve is about 5 % of the total axons (Utterback, 1944) and, even if regeneration was 100 %, and if the non-myelinated portion carried impulses at the same rate as the sympathetic C fibres, the impulses carried by these regenerating myelinated fibres could only be a very small proportion of the impulse traffic in fibres conducting at 1 m/sec or less.

The number of fibres activated by acetylcholine

The reduction in size of the C spike produced by collision between a centrifugal volley and the asynchronous discharge induced by acetylcholine gives some idea of the number of fibres activated. From the evidence of records from a few fibres, there can be little doubt that acetylcholine provokes repetitive discharges in C fibres, and this is supported by the enhancement of the action potential of the centrifugal volley. The reduction in height of the C spike is a sufficient indication of the number of collisions between the centrifugal volley and the centripetal discharge, for experiments showed that the width of the C spike did not alter after the injection of acetylcholine (see Fig. 2).

The centrifugal volley was usually reduced to about 66 % of the control size and in one experiment (Fig. 2) to less than 50 %. This reduction could only be due to collisions in 34 and 50 % of the C fibres and thus fixes the minimal number of fibres which had been excited by the acetylcholine injection. In the experiments illustrated in this paper the collision between the centrifugal volley and the centripetal acetylcholine-induced activity occurred 0·5–1·5 sec after the onset of the latter and at a time when it had passed its peak intensity. Thus the estimate that a minimum of 34 % of the C fibres were excited by acetylcholine may be low. It is conceivable that many more than one third of the fibres might be excited, for the conduction distance was about 30 mm, and collision between a centripetal train and a centrifugal volley could be guaranteed only if the fibres were carrying impulses at a frequency higher than 16/sec. Therefore a 33 % reduction in size of the centrifugal volley would be expected if 33 % of the fibres carried impulses at a frequency of 16/sec, or, if all the fibres

carried impulses at a constant frequency of 5/sec. An assessment of the frequency of impulses in individual fibres might assist in the estimation of the number of fibres excited by acetylcholine: the results of an experiment designed to investigate this point are shown in Fig. 7. The results indicate that the frequency of impulses was variable, lying within the range 1–40/sec, and it was not possible to make an accurate estimate of the number of fibres activated. Figure 7 shows, however, that the spikes occur at a frequency of about 10/sec and that these are due to activity in at least three fibres. Thus the average frequency is nearer to 5/sec than to 16/sec. The injection of 200 μg of acetylcholine might excite many more than the absolute minimum of 34 % of the C fibres in the splenic nerve. It is unlikely that activity of this degree should be carried in the few non-sympathetic fibres which might remain in the splanchnicotomized animal or aberrant ganglion cells with centrally directed axons. The only conclusion which can be drawn is that acetylcholine excites the sympathetic post-ganglionic C fibres in the splenic nerve of the splanchnicotomized preparations and, presumably, in the normal cat.

The sympathomimetic effect of acetylcholine

If acetylcholine excited C fibres at a point somewhere near the nerve endings, then the impulse might be expected to propagate along the nerve fibres in both directions. Antidromic impulses have been recorded in these experiments. The orthodromic impulses would cause the release of noradrenaline and a sympathetic effect would be produced. A sympathomimetic effect of acetylcholine on the spleen has been reinvestigated by Brandon & Rand (1961) and Daly & Scott (1961) and their findings are fully compatible with the excitation of sympathetic post-ganglionic fibres by a nicotinic action of acetylcholine. My experiments have been carried out on the spleen only, but there are many reports of sympathomimetic effects of acetylcholine or nicotine in other organs. These effects could be prevented by adrenergic blocking agents, by curare, by hexamethonium, by excess acetylcholine or nicotine, by sympathetic degeneration or by pre-treatment of the animal with reserpine. Coon & Rothman (1940) and Burn, Leach, Rand & Thompson (1959) report a sympathomimetic effect of acetylcholine and nicotine on cutaneous structures. Kottegoda (1953*b*), Hilton (1954), Burn & Rand (1958*b*), Burn *et al.* (1959) and Strömblad (1959) show effects on the peripheral circulation and Millson (1959) and Setnikar & Ravasi (1959) on isolated rabbit aorta. Sympathomimetic effects on gut have been found by Ambache (1951), Ambache & Edwards (1951), Gillespie & Mackenna (1960) and Jarrett (1962). A stimulant action of acetylcholine or nicotine on cardiac muscle has been found by Hoffman, Hoffman, Middleton & Talesnik (1945), Kottegoda (1953*a*),

Burn & Rand (1958*a*) and Lee & Shideman (1959). Thompson (1958) showed that nicotine caused contraction of the isolated nictitating membrane, and a sympathomimetic effect of the same drug in bronchial muscle was shown by Hawkins & Paton (1958). Many of these workers concluded that the effects of acetylcholine and nicotine were due to the release of noradrenaline from the sympathetic nerves in the organ they studied. It is quite conceivable that acetylcholine does this by exciting the post-ganglionic adrenergic fibres in a way similar to the splenic C fibres. Thus it may be a property of all post-ganglionic sympathetic fibres that they can be excited by acetylcholine, a property shared with many sensory fibres. It is pertinent to ask if this has any importance in the operation of the sympathetic nervous system and it may be significant that it is considered unlikely that this phenomenon has any physiological role in the working of sensory systems (Gray & Diamond, 1957).

The alleged cholinergic link in the post-ganglionic adrenergic pathway

Burn & Rand (1960) and Burn (1961) have suggested that there is a 'cholinergic link' in the sympathetic post-ganglionic adrenergic pathway. They suggest that the post-ganglionic neurone releases acetylcholine at its endings and that this releases noradrenaline from a store near the nerve endings. Some of the evidence for this hypothesis is provided by the sympathomimetic effect of acetylcholine or nicotine on many sympathetically innervated organs, and it has been suggested that the mechanism of this effect is the excitation of the cholinergic link by these drugs. Brandon & Rand (1961) have offered their results on the sympathomimetic effect of acetylcholine on the spleen of the cat as evidence in favour of the presence of a 'cholinergic link' in this organ. Brandon & Boyd (1962) have shown that noradrenaline appears in the splenic venous blood after the close arterial injection of acetylcholine into the spleen, and suggest this is due to the activation of the 'cholinergic link'. Acetylcholine has now been shown to excite the splenic C fibres and this action must be considered in interpreting the effect of this drug on the spleen. The results of Daly & Scott (1961) are relevant to this point: they found that the sympathomimetic effect of acetylcholine on the spleen of the dog could be prevented by hexamethonium, but that the effects of nerve stimulation could not. This indicated that the proposed 'cholinergic link' must be surrounded by barriers impermeable to acetylcholine and hexamethonium. Furthermore, it suggests that acetylcholine injected into the spleen might act in one way only to produce its sympathomimetic effect, and this could well be the excitation of the post-ganglionic adrenergic C fibres described in this paper.

SUMMARY

1. Injection of 10–250 μg of acetylcholine into the blood supply of the spleen evoked a vigorous centripetal discharge in the C fibres of the splenic nerve of the chloralosed cat.

2. The discharge was not affected by atropine or Hydergine but was abolished by the administration of hexamethonium.

3. The discharge could still be elicited undiminished after the sensory innervation of the spleen had degenerated.

4. It is concluded that acetylcholine excites the sympathetic post-ganglionic nerves of the spleen somewhere near their endings.

5. This finding explains the frequency with which sympathomimetic effects have been produced by substances with nicotine-like actions.

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