J. Physiol. (1967), **192**, *pp.* 463–478
With 10 *text-figures*
Printed in Great Britain

THE INNERVATION OF THE VAS DEFERENS OF THE GUINEA-PIG

By C. B. FERRY*

From the M.R.C. Group for Research in Adrenergic Mechanisms, University Laboratory of Physiology, Oxford

(*Received* 21 *March* 1967)

SUMMARY

1. The compound action potential of the hypogastric nerve of the guinea-pig contained two main elevations. The low-threshold fibres had a range of conduction velocities from 1·5 to 10 m/sec. The high threshold fibres conducted action potentials at less than 1 m/sec. The hypogastric nerve contained small myelinated fibres and non-myelinated fibres.

2. In the preparation *in vitro*, junctional potentials and contractions were elicited by stimulation of the rapidly conducting fibres alone. Trains of C fibre volleys were ineffective.

3. In the preparation *in vivo*, conduction from the hypogastric nerve to the vas deferens nerve was unidirectional and abolished by hexamethonium. After the administration of hexamethonium, the contraction produced by stimulation of the vas deferens nerve was unaffected.

4. The close arterial injection of acetylcholine (ACh) into the pelvic viscera caused centrifugal activity in the motor fibres of the vas deferens nerve, but no impulses were detected in the hypogastric nerve.

5. Ganglion cells are present in the last 2 cm of the hypogastric nerve.

6. It is concluded that there is a ganglionic relay between the hypogastric and vas deferens nerves.

INTRODUCTION

The nature and properties of adrenergic neuromuscular transmission is being investigated in a number of preparations, and the guinea-pig vas deferens-hypogastric nerve preparation, described by Huković in 1961, is one of the more convenient test objects. Before 1962 it had been assumed that the hypogastric nerve contained the post-ganglionic fibres innervating the vas deferens (Burnstock & Holman, 1961), but in that year Sjöstrand (1962*a*) found that the contractile response of the vas deferens was blocked

* Present address: Department of Pharmacology, The Medical School, University of Newcastle upon Tyne.

by a variety of ganglion blocking agents, and the noradrenaline content of the vas was not depleted after degenerative section of the hypogastric nerve (Sjöstrand 1962*b*). Furthermore, Burnstock & Holman (1962) found that after degenerative section of the hypogastric nerve, junctional potentials were still obtained in the cells of the vas deferens when the peripheral stump of the hypogastric nerve was stimulated. Electrophysiological evidence that the hypogastric nerve is the preganglionic innervation of the vas deferens was presented to the Physiological Society in 1962 (Ferry, 1963*a*) and 1963 (Kuriyama, 1963; Ferry 1963*c*). Experiments providing pharmacological evidence of a ganglionic relay were published during 1963 by Ohlin & Strömblad, by Bentley & Sabine and by Birmingham & Wilson.

This paper is a more complete account of the experiments that I have previously communicated and demonstrated to the Physiological Society (Ferry, 1963*a*, *c*).

METHODS

The hypogastric nerve–vas deferens preparation in vitro. Guinea-pigs weighing about 500 g were killed by dislocation of the neck. The vas deferens and hypogastric nerve were dissected as described by Huković (1961). The preparation consisted of the vas deferens, pieces of the blood vessels supplying the pelvic viscera associated with the hypogastric plexus, and the hypogastric nerve trunk up to a point 4–5 cm from the base of the vas deferens. The preparation was placed in a Perspex bath and irrigated with saline of the composition given below at 35–37° C. The hypogastric trunk was led into a side chamber, placed over platinum wire recording and stimulating electrodes and immersed in liquid paraffin. In most experiments the cut end of the hypogastric trunk was stimulated at a point some 4–5 cm from the vas deferens. The action potentials were recorded 1–2 cm nearer the vas deferens. The usual arrangement of the electrodes is shown diagrammatically in Fig. 1. The longitudinal tension of the vas deferens was recorded with an RCA 5734 transducer.

To record junctional potentials in the smooth muscle cells, a micropipette filled with 3 M-KCl and with resistance of 30–50 MΩ was suspended from a chlorided fine silver wire and inserted into the muscle. The cathode follower input stage was of conventional design.

The hypogastric nerve–vas deferens preparation in vivo. Guinea-pigs weighing about 500 g were anaesthetized by the subcutaneous injections of 6 ml. of 25% urethane. After allowing time for the absorption of the anaesthetic a tracheal cannula was inserted and the animals were eviscerated from the colon to the duodenum in order to leave space in the abdominal cavity. The epididymal end of the vas deferens was freed and attached to an RCA 5734 transducer to record contractions. The hypogastric nerve was cut near the inferior mesenteric artery and dissected to the point where it divided near the colon. Stimulating electrodes were placed on the cut end of the nerve, about 4–5 cm from the vas deferens. Recording electrodes on the hypogastric trunk about 3 cm from the vas deferens monitored the centrifugally conducted action potentials. For convenience, the preparation was usually made on the left side.

To record from the vas deferens nerves the connective tissue sheath investing the vas deferens was cut and one of the many fine bundles of nerve fibres coursing along the outside of the vas deferens was dissected and placed over recording electrodes. Usually the bundle was cut peripheral to the electrodes, but in some experiments it was left intact. The abdominal cavity was filled with warm liquid paraffin.

Close-arterial injections into the pelvic viscera were made through a cannula inserted retrogradely in the left external iliac artery peripheral to the origin of the internal iliac artery. The dorsally directed branches of the latter were tied and cut, as were the larger branches of the hypogastric artery and the vesical artery. The right common iliac artery was tied. Control injections of indian ink showed that injections reach the vas deferens, the prostate, the neck of the bladder and the caudal part of the ureter. The right jugular vein was cannulated for the intravenous administration of drugs. These were atropine sulphate, 1 mg; or hexamethonium bromide 5 mg. Acetylcholine chloride was dissolved in saline (composition given below) and doses refer to the salt. The saline used had 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% and O_2 95%.

RESULTS

The fibres in the hypogastric nerve. The action potential of the hypogastric nerve was recorded in every experiment. Usually the hypogastric nerve trunk was cut 3–5 cm from the vas deferens, the cut end was stimulated, and the centrifugal action potential was recorded diphasically as it passed the recording electrodes about 1 cm away from the stimulating electrode. In a few experiments monophasic action potentials were recorded in an isolated section of hypogastric trunk stimulated at the peripheral cut end or at the central cut end. The similarity between the centrifugal and centripetal action potentials suggested that conduction was bidirectional and that the hypogastric trunk contained no synapses.

The action potential of the hypogastric nerve comprised a rapidly conducted potential and a potential conducted at less than 1 m/sec. Three components were distinguished in the rapidly conducted action potential: the fastest had conduction velocities ranging from 7 to 10 m/sec, the major elevation had a conduction velocity ranging from 4 to 7 m/sec and the third, smallest elevation having conduction velocities from 1·5 to 4 m/sec (Fig. 1). The peak of the C fibre elevation was conducted at 0·5 m/sec. These conduction velocities were determined by measuring the difference in latency after moving the stimulating electrodes a known distance. The nerves were immersed in liquid paraffin at 35–37° C.

The thresholds of the rapidly conducting fibres overlapped from one component to another but the threshold of the C fibres was considerably greater than that of the rapidly conducting fibres. It was often possible to elicit a maximal or near maximal rapidly conducted action potential without exciting the C fibres (Fig. 1).

The hypogastric nerve trunk contained many non-myelinated nerve fibres and myelinated nerve fibres with diameters from 3 to 7 μ (Fig. 10). It is likely that these two groups of fibres are responsible for the C elevation and the rapidly conducted components of the compound action potential.

Nerve impulses and junctional potentials. It has been shown that the

responses of the smooth muscle of the vas deferens to stimulation of the hypogastric nerve are junctional potentials which summate to give spike potentials and an associated contraction of the muscle (Burnstock &Holman, 1961; Huković, 1961). The immediate task was to link these responses with activity in one or other of the groups of hypogastric nerve fibres.

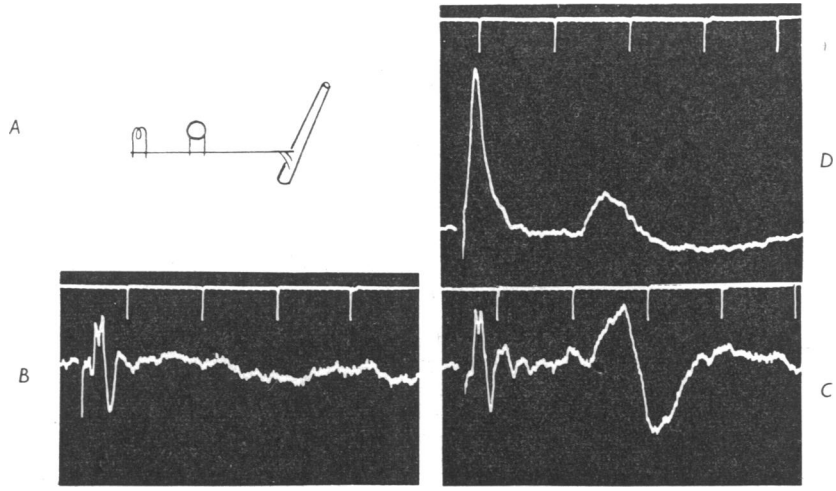

Fig. 1. Guinea-pig hypogastric nerve–vas deferens *in vitro*. Action potentials conducted 10 mm centrifugally and recorded in the hypogastric trunk 3 cm from the vas deferens.

A. Diagram of the arrangement of electrodes.

B. The action potential of the rapidly conducting fibres recorded diphasically.

C. The maximum compound action potential recorded diphasically.

D. As in *C*, but after the application of cocaine to the nerve over the peripheral recording electrode. Time 10 msec.

In these experiments the hypogastric nerve was stimulated at a frequency of 1/sec at a point 4–5 cm from the vas deferens. The junctional potentials were recorded with a micropipette inserted into cells of the outer longitudinal layer of smooth muscle. The first few junctional potentials in a train showed a progressive increase in amplitude, as described by Burnstock, Holamn & Kuriyama (1964). In the experiments reported in this paper, records were made after this initial facilitation was complete. In experiments of the type depicted in Fig. 2*B* junctional potentials were elicited by maximal stimulation of the rapidly conducting fibres at 1/sec, and, after increasing the frequency to 10/sec, the junctional potentials summated, action potentials appeared and the smooth muscle contracted. These responses were seen when only the rapidly conducting fibres in the hypogastric nerve were stimulated (Fig. 2*A*).

In some experiments the hypogastric action potential and the intracellular record from a smooth muscle cell were photographed simultaneously. Activity in all the rapidly conducting hypogastric fibres produced junction potentials (Fig. 2*C*). Increasing the stimulus to recruit the C fibres had little effect on the junctional potentials. In some experiments there was no change; in others there was a small hump on the falling phase of the large potential elicited by maximal stimulation of the rapidly conducting fibres (Fig. 5*a*, *b*, *e*).

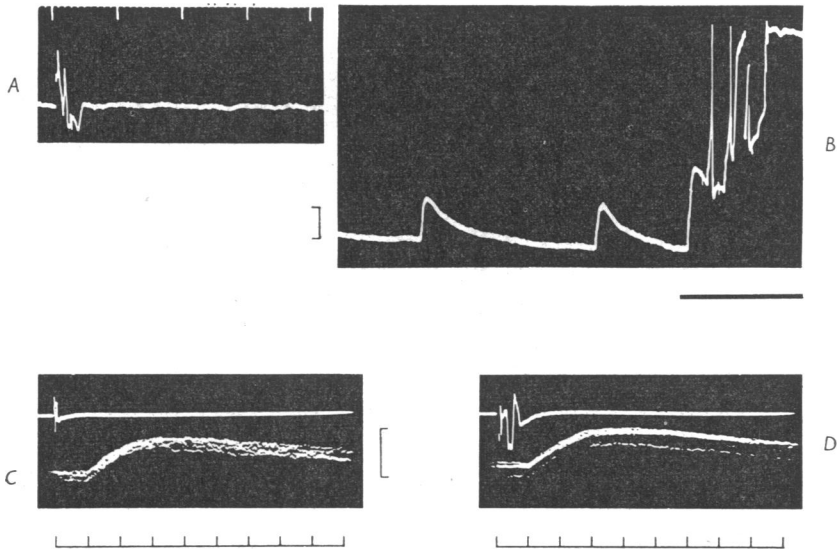

Fig. 2. Guinea-pig hypogastric nerve–vas deferens *in vitro*.

A. The action potential of the hypogastric nerve trunk recorded as in Fig. 1*B*. Time 10 msec.

B. Record of the transmembrane potential of a smooth muscle cell made with an intracellular micropipette. The hypogastric nerve was stimulated at a frequency of 1/sec, with the stimulus parameters as in *A*. The horizontal line indicates a period of stimulation at 10/sec.

C, *D*. Ten superimposed records from another cell showing the hypogastric action potential (top) and the intracellularly recorded junctional potential. Frequency of stimulation 1/sec. Time 50 msec. Voltage calibration in *B*, *C*, *D*, 10 mV. The spikes have been retouched.

The contraction of the longitudinal muscle of the vas deferens. Records were made of the longitudinal contractions of the vas deferens after stimulation of the rapidly conducting hypogastric fibres alone and after recruitment of the C fibres. The train of stimuli at 10/sec was maintained until a maximal contraction had been produced. In the experiment illustrated in Fig. 3, the hypogastric nerve action potentials and the isometric tension of the vas deferens were recorded simultaneously. The

record consists of many superimposed sweeps of the oscilloscope spot and, because the sweep velocity was much greater than the rate of development of tension, the contraction was recorded as a series of almost horizontal lines. Stimulation of the rapidly conducting hypogastric fibres caused a contraction which was not increased after recruitment of the hypogastric C fibres.

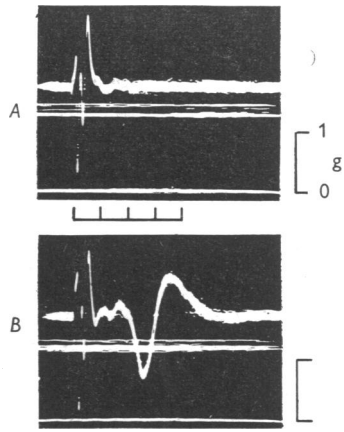

Fig. 3. Hypogastric nerve–vas deferens *in vitro*. Diphasic action potential of hypogastric nerve trunk (upper trace) and longitudinal tension of the vas deferens (lower trace). Tension calibration 1 g. Upward deflexion shows increased tension. Time 10 msec.

A. Many superimposed traces showing the rise of the tension of the vas deferens with repetitive stimulation at 10/sec.

B. As in *A*, the stimulus was increased to recruit the hypogastric C fibres.

Thus, the contractile response of the longitudinal muscle of the vas deferens *in vitro* is mediated by activity in the rapidly conducting fibres of the hypogastric nerve trunk.

The responses to impulses in the C fibres alone. The failure of stimulation of the hypogastric C fibres to evoke responses in the smooth muscle of the vas deferens could be explained if the fast fibres produced responses which were already maximal. Further experiments were performed, similar to those reported above but with the addition of a pair of Ag/AgCl-Ringer electrodes connected to a polarizing circuit and placed on the hypogastric nerve between the pairs of stimulating and recording electrodes. A maximum compound action potential was elicited and the strength of the polarizing current adjusted until the action potential of the rapidly conducting fibres was blocked. A C fibre volley alone was thus recorded and allowed to propagate towards the muscle. The effect of blocking the action potential of the rapidly conducting fibres is shown in Fig. 4. The polarizing current slowed conduction in the C fibres, for the massed action

potential was smaller in amplitude and more dispersed. The area of the potential was not reduced, which suggests that there was no block of conduction in the C fibres. The contractile response was abolished when the rapidly conducting fibres were blocked. There was a submaximal contraction with a less intense block which allowed conduction in some of the rapidly conducting fibres. The C fibre volleys alone were ineffective.

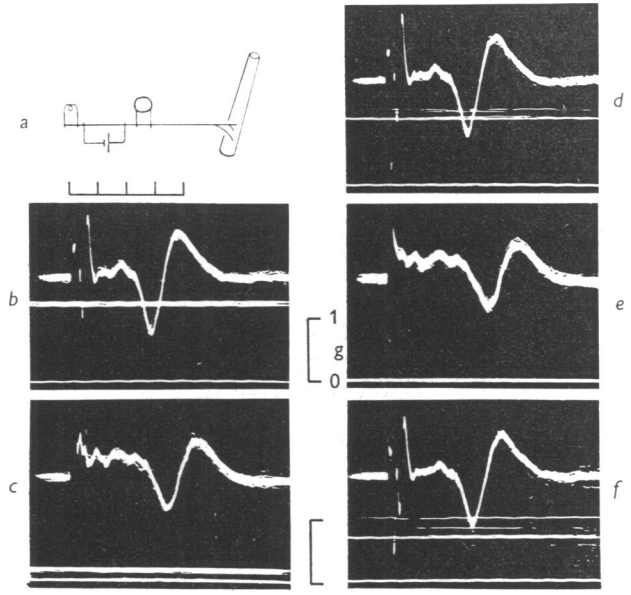

Fig. 4. The action potential of the hypogastric nerve trunk and the tension of the vas deferens recorded as in Fig. 3. Tension calibration 1 g.

(*a*) Diagram showing the arrangement of electrodes for stimulation, polarization block and recording the hypogastric action potential.

(*b*, *d*, *f*) Control responses to stimulation at 10/sec. No polarizing current.

(*c*, *e*) The responses to stimulation of the nerves while the polarizing current at various intensities was passing along the nerve.

The records were made in the sequence (*a*) to (*e*). Time 10 msec.

The effect of blocking conduction in the rapidly conducting hypogastric fibres on the electrical response of a smooth muscle cell is shown in Fig. 5. Absence of the rapidly conducted component from the compound action potential of the hypogastric nerve was associated with loss of the junctional potential. Activity in a few of these nerve fibres resulted in a small fragmented junctional potential.

From the experiments described above it was concluded that the outer longitudinal smooth muscle cells of the guinea-pig vas deferens *in vitro* were likely to be innervated by the rapidly conducting hypogastric nerve

fibres alone, the hypogastric C fibres made little, if any, contribution to the motor innervation of the muscle.

In view of the experiments of Sjöstrand (1962*a*, *b*) who found that the contractile response to hypogastric stimulation could be abolished by hexamethonium, it is considered that these hypogastric fast fibres represented the preganglionic innervation of the vas deferens.

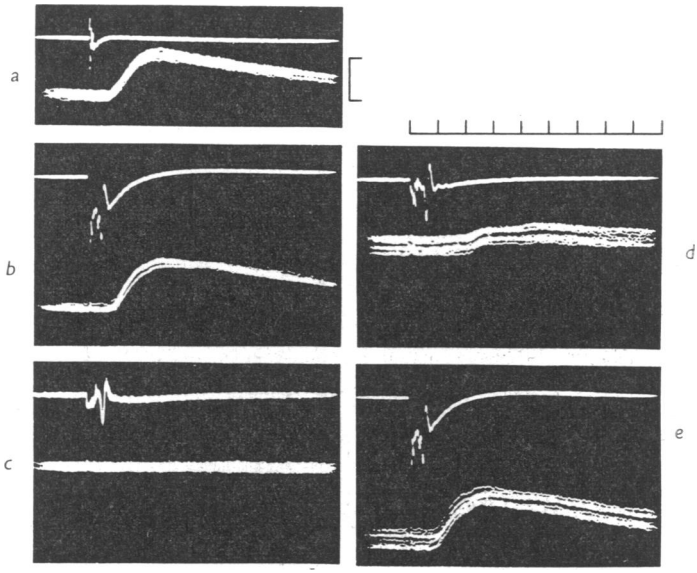

Fig. 5. The action potential of the hypogastric nerve trunk and junctional potentials in a smooth muscle cell recorded as in Fig. 2*C*, *D*. Polarizing electrodes were placed between the hypogastric stimulating and recording electrodes as in Fig. 4*a*.

(*a*) Junctional potentials elicited by stimulation of the rapidly conducting hypogastric nerve fibres.

(*b*) Junctional potentials elicited after stimulation of all hypogastric nerve fibres. Large stimulus artifact.

(*c*) Stimulus as in *b*, but the fast fibres have been blocked by the polarizing current.

(*d*) Small junctional potentials seen after reducing the polarizing current. There is some fast fibre activity.

(*e*) Control record as in *b*. No polarizing current. Time 50 msec. Voltage calibration 10 mV for lower trace only.

The hypogastric nerve–vas deferens preparation in vivo. There are many bundles of nerves 10–100 μ in diameter which run in the adventitia surrounding the vas deferens. These originate in the hypogastric plexus and eventually penetrate the smooth muscle of the vas deferens. In order to record activity in these vas deferens nerve bundles, a preparation *in vivo* was used in which a nerve bundle was lifted up into liquid paraffin. In

most experiments, the bundle was cut and the activity in the central cut end was recorded.

In one experiment about 1 cm of a bundle of vas deferens nerve was dissected free, and stimulating and recording electrodes placed on it. The action potential contained only one component which had a conduction velocity of less than 0·5 m/sec estimated from the spike latency and the separation of the electrodes. Histological examination of the vas deferens nerves showed that some bundles contained only non-myelinated fibres and some bundles contained, in addition, a few myelinated fibres (Fig. 10).

Activity in the vas deferens nerve after hypogastric nerve stimulation. Stimulation of the hypogastric nerve caused the appearance of a scattered discharge of action potentials in the vas deferens nerve with a latency of about 20 msec. In some experiments the latency of a submaximal vas deferens nerve discharge, produced by the excitation of only a few hypogastric fibres, was decreased when more hypogastric fibres were excited. In one experiment the vas deferens nerve was stimulated and a small

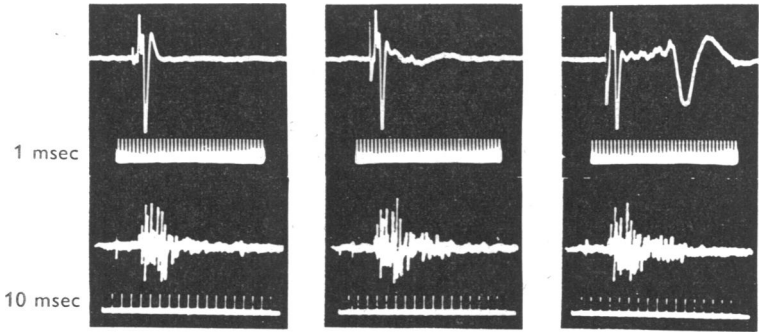

Fig. 6. Guinea-pig hypogastric nerve–vas deferens *in vivo*. Urethane. Activity in hypogastric and vas deferens nerves after hypogastric nerve stimulation. Upper half of record shows hypogastric action potential and 1 msec time trace. Conduction distance 10 mm. Lower half shows the activity in the central cut end of a bundle of the vas deferens nerve and 10 msec time trace. The records of activity were displayed on two oscilloscopes with differing sweep speeds and photographed simultaneously. The amplitude of the stimulating pulse was increased from left to right.

action potential was recorded ascending the hypogastric trunk with a conduction velocity of about 10 m/sec. In other experiments no centripetal activity was observed. These findings suggest that a synapse is interposed between the hypogastric and vas deferens nerve fibres.

The excitation of the most rapidly conducting hypogastric fibres led to an almost maximal discharge in the vas deferens nerve (Fig. 6). A small increment in the stimulus to excite the less rapidly conducting hypogastric fibres resulted in an increase in the later part of the vas deferens nerve

response. When the hypogastric C fibres were recruited, there was little or no further increase in the activity in the vas deferens nerve. Thus, it seems that the hypogastric C fibres did not project to the vas deferens nerves.

The contraction of the vas deferens in vivo. Experiments were carried out on the preparation *in vivo* which were similar to those performed *in vitro* and illustrated in Fig. 3. When the rapidly conducting fibres were stimulated at a frequency of 10/sec, the tension recorded was about 5 g—i.e. 5 times that of the *in vitro* preparation. It is possible that the response recorded might have been an underestimation of the strength of the contraction for the prostatic end of the vas deferens was not fixed other than

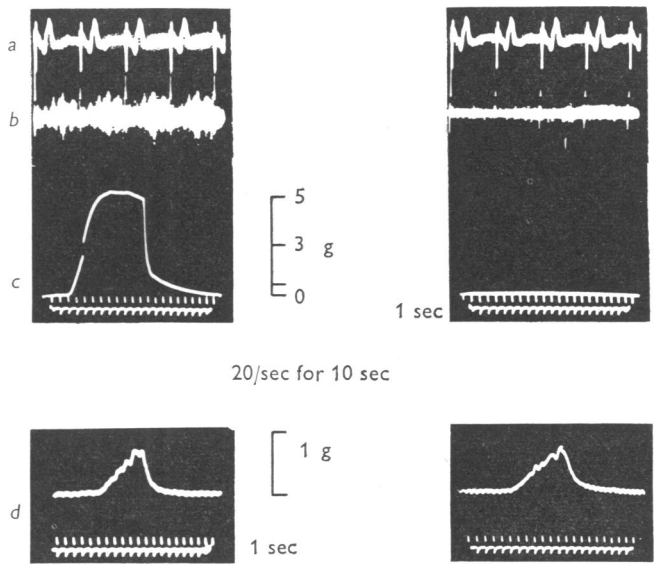

Fig. 7. Hypogastric nerve–vas deferens *in vivo*. Urethane. The responses to maximal stimulation of the hypogastric trunk at 20/sec for 10 sec.

(*a*) The action potentials of the hypogastric trunk.

(*b*) Activity in an uncut bundle of the vas deferens nerve. Duration of sweeps 200 msec.

The above records show many superimposed traces.

(*c*) A single sweep showing the contraction. This record is synchronous with *a* and *b*.

Time 1 sec. Tension calibration 0, 1, 3, 5 g.

(*d*) The electrodes on the vas deferens nerve used to record *b* were used to stimulate that bundle of the vas deferens nerve with a train of stimuli which caused the contraction recorded in this part of the figure. Time 1 sec. Tension calibration 1 g.

Records *a* and *b* were made on an oscilloscope with a rapid sweep speed. Records *c* and *d* were recorded from a second oscilloscope with a much slower sweep speed. Left—control responses. Right—shortly after the intravenous injection of hexamethonium bromide 5 mg.

by its natural attachments, and some shortening of the muscle occurred. Recruitment of the hypogastric C fibres usually had no effect on the contraction, but in one experiment there was 10% increase. It can be concluded that the longitudinal muscle of the vas deferens is innervated by the rapidly conducting hypogastric fibres. The hypogastric C fibres contribute little or nothing towards the motor innervation.

The effect of hexamethonium on the hypogastric–vas deferens pathway. The intravenous administration of 5 mg of hexamethonium bromide abolished or greatly reduced the discharge of action potentials in the vas deferens nerve after maximal hypogastric stimulation. The effect was not permanent; there was some recovery of transmission if the preparation was left unstimulated for 5–10 min. In one experiment the hypogastric nerve was stimulated regularly at 0·2/sec before, during and after the administration of hexamethonium. There was no recovery even 30 min after the injection of the drug, but a short train of eighty stimuli at 40/sec caused a temporary and incomplete restoration of transmission.

In another series of experiments, the usual preparation was made, but the bundle of the vas deferens nerve over the electrodes was not cut; it was allowed to proceed to the periphery, Stimulation of the hypogastric nerve at 10–20/sec caused a discharge in the vas deferens nerve bundle and contraction of the vas deferens (Fig. 7). The vas deferens nerve bundle was then stimulated through the electrodes previously used for recording and a smaller, slower contraction was produced. Hexamethonium was injected and the sequence repeated. The contraction and vas deferens nerve discharge following hypogastric stimulation were abolished, but the contractile response to vas deferens nerve stimulation was unaffected.

The close-arterial injection of acetylcholine. If the hypogastric trunk contains preganglionic fibres and the vas deferens nerve is post-ganglionic, injection of acetylcholine into the pelvic viscera might provoke a centrifugal discharge in the vas deferens nerves, but would not be expected to produce a response in the preganglionic fibres of the hypogastric trunk. Experiments showed this to be so and Fig. 8*A* shows the results in one of several preparations. Acetylcholine injected into the pelvic arterial blood of an atropinized guinea-pig caused a brisk discharge in the central cut end of a vas deferens nerve bundle, contraction of the vas deferens and usually little or no activity in the hypogastric nerve. Control injections of saline were ineffective. In some of the experiments reported earlier (Ferry, 1963*c*) a few spikes were recorded from the hypogastric nerve, but in these experiments the acetylcholine had been injected into the aorta via the right common iliac artery, and perhaps reached structures not accessible to injections made into the left external iliac artery as described in Methods.

The activity recorded in the central cut end of the vas deferens nerves could have arisen in several ways. Acetylcholine could have excited the ganglion cells which may be present on the motor pathway; or, it could have excited sensory C fibres (Douglas & Ritchie, 1960) or the post-ganglionic adrenergic nerves (Ferry, 1963*b*) near their peripheral endings

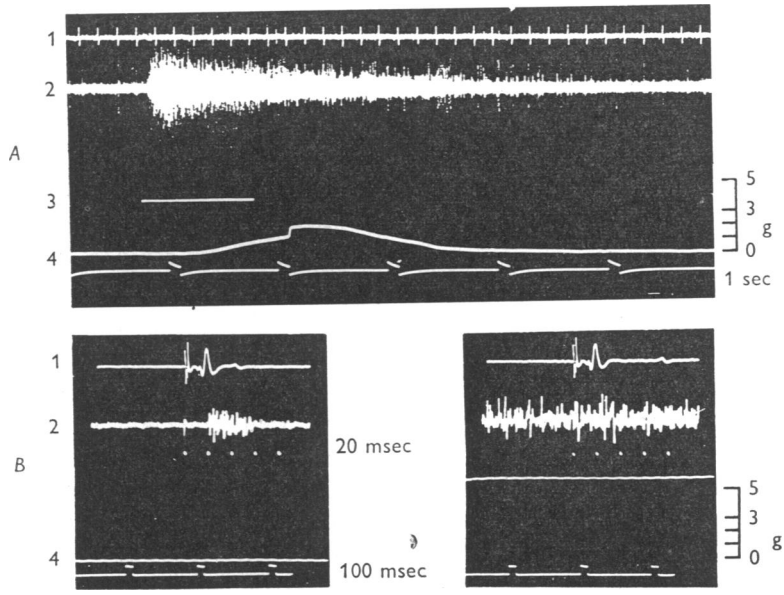

Fig. 8. Hypogastric nerve–vas deferens *in vivo*. Urethane and atropine sulphate 1 mg. Close arterial injections of acetylcholine chloride made into the pelvic viscera via the stump of the left external iliac artery.

(1) Record from the hypogastric nerve trunk (electrocardiogram displayed). (2) Record from central cut end of a bundle of vas deferens nerve. (3) Injection signal. (4) Tension of the vas deferens. Calibration 0, 1, 2, 3, 5 g. Time 1 sec.

A. The response to an injection of 5 μg ACh chloride as a 10^{-4} g/ml. solution.

B. 1, 2, 4 as above. Time mark, 100 msec for tension record, 20 msec for hypogastric and vas deferens nerve records. Stimulation at 1/sec.

Records 1, 2 were made from an oscilloscope with a rapid sweep. Record 4 was made simultaneously on a second oscilloscope with a slower sweep. Left—response to a single maximal stimulus to the hypogastric nerve. Right—response to nerve stimulation during the injection of 40 μg ACh chloride as a 10^{-4} g/ml. solution over 7 sec. See text for further details.

and thus produced an axon reflex in the central stump of the bundle of vas deferens nerve fibres from which records were made. If the motor fibres were excited at the ganglion, there ought to be mutual occlusion between the vas deferens nerve discharge following maximal hypogastric stimulation and the injection of a large dose of ACh. If the ACh-induced discharge were carried in sensory fibres or those motor fibres which

contributed a branch to the bundle from which recordings were made, the vas deferens nerve responses to hypogastric stimulation and ACh injection would partially summate.

The results of an experiment performed to elucidate this point is shown in Fig. 8*B*. The hypogastric nerve was stimulated repetitively at 1/sec and 40 μg of ACh was injected over a period of about 7 sec. There was a brisk discharge of impulses in the vas deferens nerve and contraction of the smooth muscle. There was no summation of the discharges produced by ACh and hypogastric stimulation. It was concluded that ACh acted on the normal motor pathway at a point between the hypogastric nerve trunk and the vas deferens nerves.

The histology of the innervation of the vas deferens. A guinea-pig was killed and the hypogastric nerve–vas deferens preparations dissected from both sides. One preparation was fixed in formol-saline and cross-sections

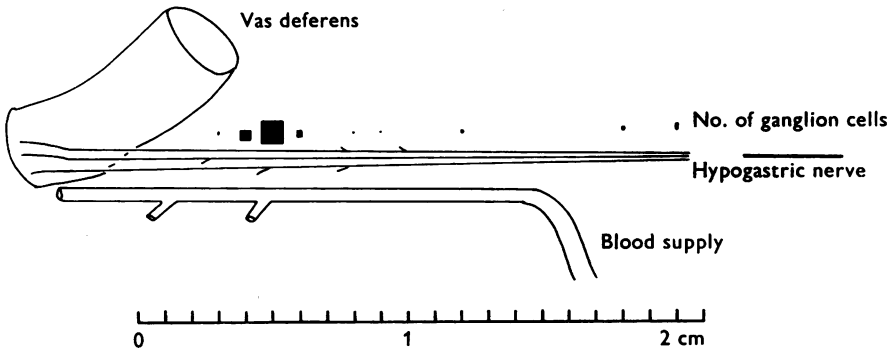

Fig. 9. Diagram of the motor innervation of the guinea-pig vas deferens. The hypogastric nerve trunk divides to form a plexus near the blood vessels supplying the pelvic viscera. Ganglion cells are embedded in this plexus. Serial sections were cut and examined at mm intervals. The number of ganglion cells in each section was estimated and plotted on the diagram as a black rectangle, the area of which represents the number of ganglion cells.

10 μ thick were cut. Every 100th section was stained with haematoxylin and eosin and examined. Ganglion cells were seen in the hypogastric plexus, the majority lying near the base of the vas deferens (Fig. 9).

The other preparation was fixed in osmic acid and cross-sections 5 μ thick were cut.

Figure 10 shows photomicrographs of sections of the vas deferens, the hypogastric plexus and the hypogastric nerve trunk. The latter contains myelinated fibres 2–7 μ in diameter and non-myelinated fibres. The hypogastric plexus contains both of these types of nerve fibre and also ganglion cell bodies. The vas deferens nerves contain mainly non-myelinated fibres. Some bundles are completely amyelinate. Those depicted in Fig. 10 had

the greatest number of myelinated fibres per bundle. These findings confirm those of K. C. Richardson (1962, personal communication); of Ohlin & Strömblad (1963), of Falck, Owman & Sjöstrand (1965) and of Merrillees, Burnstock & Holman (1963) that ganglion cells are present near the base of the vas deferens. The present findings indicate that the ganglionic region extends up to 2 cm from the vas deferens along the hypogastric plexus.

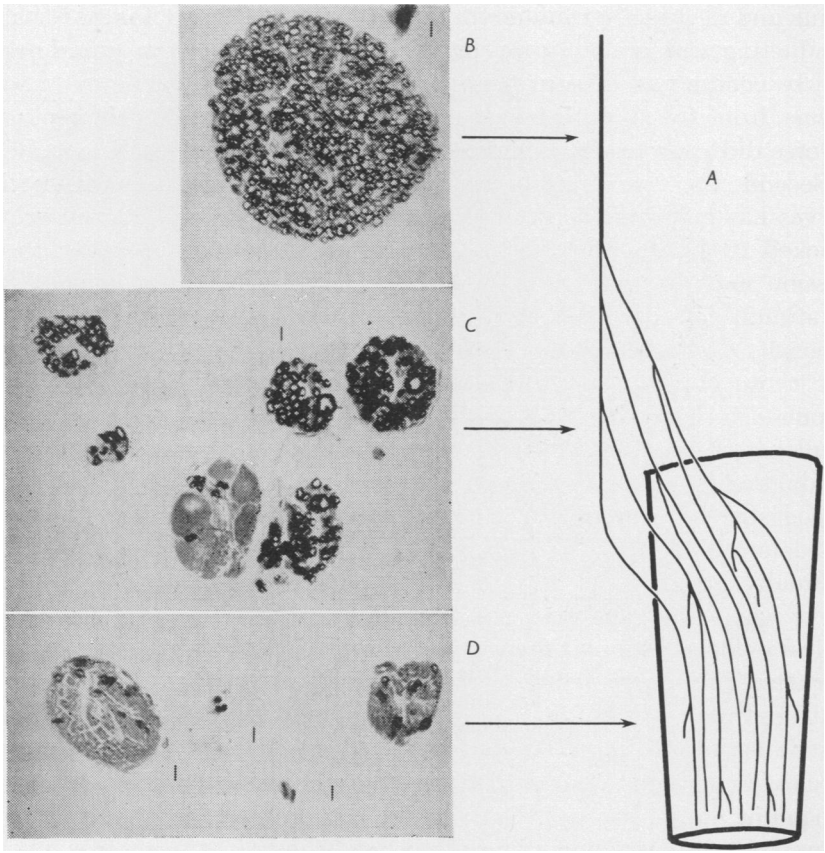

Fig. 10. Hypogastric nerve–vas deferens preparation, fixed in osmic acid and 5 μ sections cut.

A. Diagram showing the location of the other sections shown in this figure.

B. Section of the hypogastric nerve trunk. Calibration 10 μ. Myelinated fibres 2–7 μ.

C. Section through the hypogastric plexus showing five bundles with nerve fibres and one with ganglion cells and nerve fibres.

D. Two large and two small bundles of nerve fibres in the connective tissue around the vas deferens. These particular bundles form only a small part of the vas deferens nerves, but they are those with most myelinated fibres.

DISCUSSION

The main conclusion to be drawn from this work is that those fibres in the hypogastric nerve trunk which are motor to the vas deferens are almost entirely preganglionic, they form synapses with the post-ganglionic neurones in the peripheral part of the hypogastric nerve. The evidence for this conclusion is as follows.

First, there is a structural discontinuity between the hypogastric nerve trunk and the vas deferens nerves. The hypogastric motor fibres are rapidly conducting and probably myelinated, the vas deferens nerve fibres are slowly conducting non-myelinated fibres. Conduction of nerve impulses occurs from the hypogastric to the vas deferens nerves, but not in the reverse direction to any appreciable extent.

Second, the discontinuity between the hypogastric and vas deferens nerves has many of the properties of a ganglionic relay. Transmission is blocked by hexamethonium, and in such a blocked preparation, transmission may be temporarily re-established after a high frequency train of stimuli (cf. Larrabee & Bronk, 1947). Acetylcholine injected close-arterially excites the motor fibres in the vas deferens nerve, but probably not those of the hypogastric nerve. Because the discharge of nerve impulses in the hypogastric nerve following stimulation of the hypogastric trunk may be abolished by a suitable dose of hexamethonium, and because conduction is almost completely unidirectional, it would seem that all the hypogastric fibres motor to the vas deferens form synapses central to the vas deferens nerve.

Finally, ganglion cells have been found at that region of the hypogastric nerve where the ganglionic relay has been located by the electrophysiological evidence. Similar findings have been previously reported by other workers. The experiments described above show that the 'ganglion' is scattered over some 2 cm of the hypogastric plexus. Thus if the hypogastric nerve is stimulated less than 2 cm from the vas deferens, either preganglionic fibres alone or pre- and post-ganglionic fibres will be excited depending on the parameters of the stimulatory current pulse. In order to stimulate post-ganglionic fibres alone, the stimulating electrodes must be very close to the base of the vas deferens, or even more peripheral.

The histological work reported in this paper was carried out by Mr T. A. Marsland and the photomicrographs were made by Dr Sybil Cooper; I am deeply indebted to them. I should like to thank Mrs Hazel Parsons for technical assistance and Mr Gary Hoffman for help with some of the later experiments. I am most grateful to Sir Lindor Brown for his constant encouragement and to the Medical Research Council who support a group for Research in Adrenergic Mechanisms in this laboratory.

REFERENCES

BENTLEY, G. A. & SABINE, J. R. (1963). The effects of ganglion-blocking and postganglionic sympatholytic drugs on preparations of the guinea-pig vas deferens. *Br. J. Pharmac. Chemother.* **21**, 190–201.

BIRMINGHAM, A. T. & WILSON, A. B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Br. J. Pharmac. Chemother.* **21**, 569–580.

BURNSTOCK, G. & HOLMAN, M. E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.* **155**, 115–133.

BURNSTOCK, G. & HOLMAN, M. E. (1962). Effect of denervation and of reserpine treatment on transmission at sympathetic nerve endings. *J. Physiol.* **160**, 461–469.

BURNSTOCK, G., HOLMAN, M. E. & KURIYAMA, H. (1964). Facilitation of transmission from autonomic nerve to smooth muscle of guinea-pig vas deferens. *J. Physiol.* **172**, 31–49.

DOUGLAS, W. W. & RITCHIE, J. M. (1960). The excitatory action of acetylcholine on cutaneous non-myelinated fibres. *J. Physiol.* **150**, 501–514.

FALCK, B., OWMAN, C. H. & SJÖSTRAND, N. O. (1965). Peripherally located adrenergic neurons innervating the vas deferens and seminal vesicle of the guinea-pig. *Experientia* **21**, 98–100.

FERRY, C. B. (1963*a*). The innervation of the vas deferens of the guinea-pig. *J. Physiol.* **166**, 16*P*.

FERRY, C. B. (1963*b*). The sympathomimetic effect of acetylcholine on the spleen of the cat. *J. Physiol.* **167**, 487–504.

FERRY, C. B. (1963*c*). The post-ganglionic fibres of the vas deferens of the guinea-pig. *J. Physiol.* **169**, 72*P*.

HUKOVIC, S. (1961). Responses of the isolated sympathetic nerve—ductus deferens preparation of the guinea-pig. *Br. J. Pharmac. Chemother.* **16**, 188–194.

KURIYAMA, H. (1963). Electrophysiological observations on the motor innervation of the smooth muscle cells in the guinea-pig vas deferens. *J. Physiol.* **169**, 213–228.

LARRABEE, M. G. & BRONK, D. W. (1947). Prolonged facilitation of synaptic excitation in sympathetic ganglia. *J. Neurophysiol.* **10**, 139–154.

MERRILEES, N. C. R., BURNSTOCK, G. & HOLMAN, M. E. (1963). Correlation of fine structure and physiology of the innervation of smooth muscle in the guinea-pig vas deferens. *J. cell. Biol.* **19**, 529–550.

OHLIN, P. & STRÖMBLAD, B. C. R. (1963). Observations on the isolated vas deferens. *Br. J. Pharmac. Chemother.* **20**, 299–306.

SJÖSTRAND, N. O. (1962*a*). Inhibition by ganglion blocking agents of the motor response of the isolated guinea-pig vas deferens to hypogastric nerve stimulation. *Acta physiol. scand.* **54**, 306–315.

SJÖSTRAND, N. O. (1962*b*). Effect of reserpine and hypogastric denervation on the noradrenaline content of the vas deferens and the seminal vesicle of the guinea-pig. *Acta physiol. scand.* **56**, 376–380.