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PREGANGLIONIC AND POSTGANGLIONIC STIMULATION OF THE GUINEA-PIG ISOLATED VAS DEFERENS PREPARATION

BY

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The isolated vas deferens of the guinea-pig contracted when stimulated transmurally with parallel wire electrodes. These contractions persisted in concentrations of hexamethonium, pentolinium, nicotine and mecamylamine which at the same time abolished the responses to hypogastric nerve stimulation. Procaine and lignocaine in local anaesthetic concentrations abolished the responses to transmural stimulation but potentiated the contractions produced by added noradrenaline. Guanethidine and bretylium in concentrations specific for adrenergic neurone blockade abolished the contractions due to transmural stimulation without impairing the responses of the muscle to added noradrenaline or acetylcholine. In contrast, high concentrations of the adrenergic-blocking agents phentolamine and dihydroergotamine were needed to block the contractions due to transmural stimulation; these concentrations also blocked the response to added noradrenaline but simultaneously reduced the responses to added acetylcholine or potassium chloride. Preparations from guinea-pigs previously treated with reserpine at first responded normally to transmural stimulation; thereafter the contractions diminished progressively but were never abolished. Hyoscine and atropine produced a small decrease in the response to transmural stimulation when present in concentrations up to 1×10^{-5} and a larger decrease only in concentrations of 1×10^{-4} or greater. Hemicholinium produced a small decrease of the contractions due to transmural stimulation in concentrations up to 1×10^{-4}; concentrations of 5×10^{-4} present for 1 hr produced only a slightly greater reduction in response. These experiments show that when the guinea-pig vas deferens is removed without the hypogastric nerve and stimulated transmurally by the method described, contractions are produced mainly by excitation of postganglionic adrenergic nerves.

The isolated hypogastric nerve-vas deferens preparation of the guinea-pig (Huković, 1961) has often been used to investigate the actions of drugs which block the contractions produced by sympathetic nerve stimulation. Many of the conclusions reached have been based on the assumption that the hypogastric nerve in the male guinea-pig contains mainly postganglionic nerve fibres. Available evidence now suggests that this assumption is invalid.

Sjöstrand (1962) has shown that the contractions of the vas deferens elicited by hypogastric nerve stimulation are inhibited by the ganglion-blocking drugs hexamethonium bromide, tetraethylammonium, azamethonium bromide, lobeline and nicotine. Furthermore, Ohlin & Strömblad (1963) have described a blocking action of hexamethonium on this preparation. These findings have been interpreted to

mean that there may be ganglionic synapses in the hypogastric nerve and that, in the Huković preparation, the contractions are initiated mainly by stimulation of preganglionic nerve fibres.

The experiments here reported show that the vas deferens contracts when it is stimulated transmurally and that these contractions are due to stimulation of postganglionic nerve fibres.

METHODS

Male guinea-pigs weighing from 400 to 850 g were stunned by a blow on the head and bled. The abdomen was opened in the midline and the vasa deferentia were dissected and set up as described below. Organ-baths containing 20 or 75 ml. of Krebs solution at 32° C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide were used. Contractions were recorded with isotonic frontal writing levers at loads of 0.75 to 1.0 g with magnifications of six- to ten-times. Throughout this work Multitone "Ten Pulse" stimulators were used.

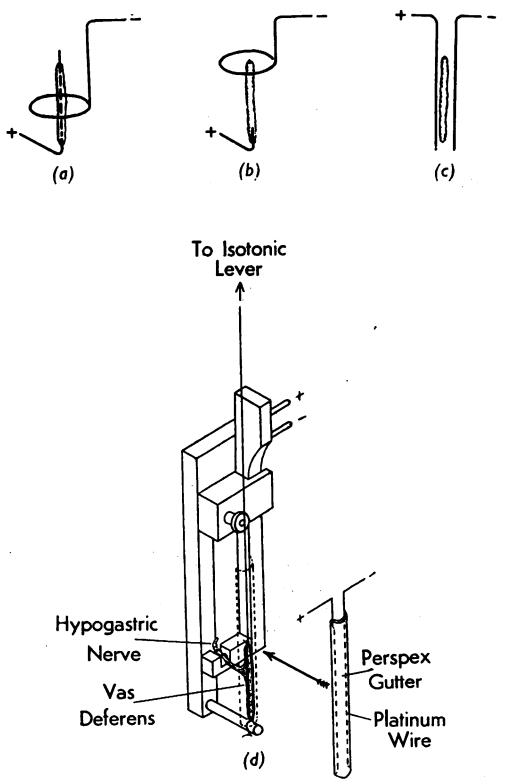

Fig. 1. Electrode arrangements for transmural stimulation of the isolated vas deferens. (*a*) An intraluminal platinum wire as one electrode with a coaxial circle of wire as the other electrode. (*b*) A short length of platinum wire just entering the lower end of the lumen as one electrode with a coaxial circle of wire as the other electrode at the upper end of the vas. (*c*) Two parallel lengths of platinum wire with the vas suspended between them. The wires are cemented to the edges of a Perspex gutter so that they are held parallel and about 4 mm apart. (*d*) The hypogastric nerve–vas deferens preparation set on a Perspex holder so that the nerve may be stimulated by means of unshielded electrodes or the vas may be stimulated transmurally with the parallel wire electrodes. The Perspex gutter is held in the position indicated by the arrow.

The isolated vas deferens preparation stimulated transmurally. The vasa deferentia were removed by severing the testicular and prostatic ends and cutting the mesenteries close to the organs. One end of a vas was tied to a supporting hook and the other (upper) end attached to the recording lever. The electrodes were two parallel lengths of 0.020 in. diameter platinum wire cemented to the edges of a Perspex gutter so that the vas could be suspended between them (Fig. 1, *c* and *d*). Stimulation was for periods of 15 sec at 3 min intervals at a frequency of 25 shocks/sec with a pulse duration of 0.1 msec and at supramaximal voltage (usually 90 to 120 V).

In some early experiments the vas deferens was stimulated transmurally by methods modelled on Paton's (1955) technique for the small intestine (Fig. 1, *a* and *b*); although the vas contracted well the intraluminal electrode often impeded relaxation.

The isolated vas deferens preparation stimulated alternately through the hypogastric nerve and transmurally. A method was devised for recording the contractions of the vas deferens stimulated alternately through the hypogastric nerve and transmurally. With the vas dissected by the method described by Huković (1961) and set up on a Perspex holder, conventional unshielded electrodes were used to stimulate the hypogastric nerve and the parallel wire electrodes were used for transmural stimulation (Fig. 1, *d*). For both methods the stimulus parameters were identical at a frequency of 25 shocks/sec and a pulse width of 0.1 msec; supramaximal voltage for transmural stimulation was usually 90 to 120 V and for the hypogastric nerve was usually 30 to 60 V. The interval between nerve stimulation and transmural stimulation was 4 min.

Drugs. These were acetylcholine chloride, atropine sulphate, bretylium tosylate, dexamphetamine sulphate, dihydroergotamine methane sulphonate, guanethidine sulphate, hemicholinium bromide (Dr J. P. Long), hexamethonium bromide, hyoscine hydrobromide, lignocaine hydrochloride, mecamylamine hydrochloride, nicotine hydrogen tartrate, (−)-noradrenaline bitartrate, pentolinium tartrate, phentolamine methane sulphonate, potassium chloride and procaine hydrochloride. All concentrations are quoted as final bath concentrations in g/ml. and, except those of procaine hydrochloride, lignocaine hydrochloride and potassium chloride, are expressed as base. Each drug was tested on separate preparations from at least four guinea-pigs.

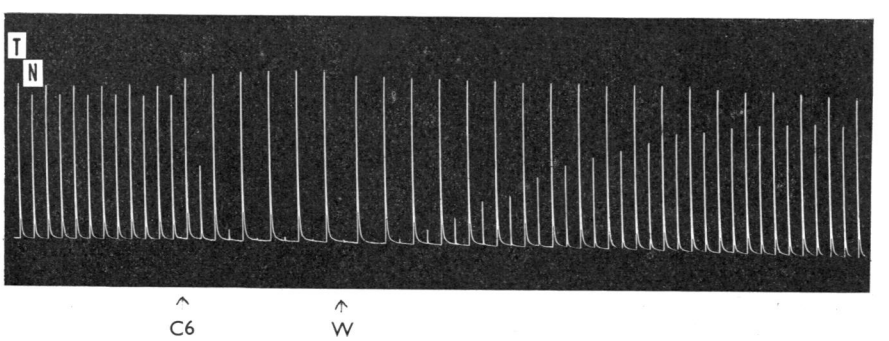

Fig. 2. The action of hexamethonium on the alternately stimulated vas deferens. At T the vas was stimulated transmurally at a frequency of 25 shocks/sec with 0.1 msec duration at supramaximal voltage for 15 sec. At N the hypogastric nerve was stimulated at a frequency of 25 shocks/sec with pulse duration 0.1 msec at supramaximal voltage for 15 sec. The transmural stimulation (T) and nerve stimulation (N) were alternated throughout the experiment. The interval between T and N was 4 min. At C6, hexamethonium was added (1×10^{-4} final bath concentration) and gradually blocked the response to hypogastric nerve stimulation leaving the response to transmural stimulation unimpaired. At W, with the stimulators switched off, the drug was washed out with six changes of bath fluid in 20 min. On recommencing stimulation there was a gradual recovery of the response to hypogastric nerve stimulation.

RESULTS

The isolated vas deferens preparation stimulated alternately through the hypogastric nerve and transmurally

The vas deferens gave reproducible contractions for 5 to 6 hr when stimulated alternately through the nerve and transmurally. Almost always the response to transmural stimulation was slightly greater than that to hypogastric nerve stimulation (Fig. 2). Reproducible contractions were not obtained if the intervals between stimuli were shortened or the pulse width for transmural stimulation was greater than 0.3 msec.

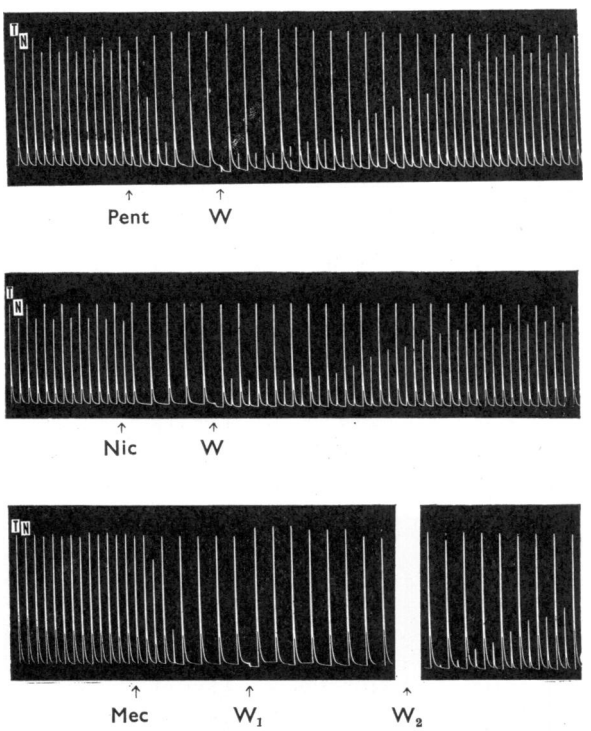

Fig. 3. The actions of pentolinium, nicotine and mecamylamine on the alternately stimulated vas deferens. At T the vas was stimulated transmurally and at N the hypogastric nerve was stimulated. The parameters and intervals of stimulation were the same as in Fig. 2. In the upper tracing, at Pent, pentolinium was added to a concentration of 5×10^{-5} and blocked only the response to hypogastric nerve stimulation. The drug was washed out six times during 20 min at W, then further stimulation was accompanied by a gradual recovery to nerve stimulation. In the middle tracing, nicotine (5×10^{-5} final concentration added at Nic) abolished only the response to hypogastric nerve stimulation. After washing out the drug (six washes in 20 min) there was a return of response to nerve stimulation. In the lower tracing, at Mec, mecamylamine was added (1×10^{-5} bath concentration) and blocked only the response to hypogastric nerve stimulation. After washing the preparation six times in 20 min at W_1, there was no return of contraction to nerve stimulation. The bath fluid was changed a further four times during 1.5 hr at W_2 after which there was a gradual return of response to hypogastric nerve stimulation.

Ganglion-blocking agents. Hexamethonium, added to the alternately stimulated preparation in concentrations from 1×10^{-5} to 1×10^{-4}, blocked the response to nerve stimulation leaving the contractions due to transmural stimulation unimpaired and usually slightly increased (Fig. 2). The block was readily reversed by washing.

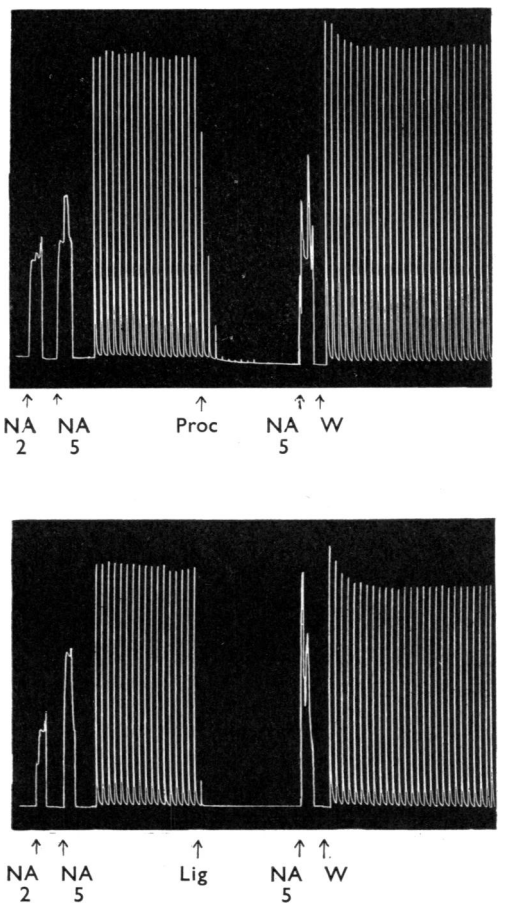

Fig. 4. The effects of local anaesthetics on the transmurally stimulated vas deferens. The vas deferens was removed without the hypogastric nerve from a guinea-pig and stimulated transmurally for 15 sec every 3 min at 25 shocks/sec with a pulse duration of 0.1 msec at supramaximal voltage. In the upper record with the stimulator switched off, two doses of noradrenaline were used to contract the vas. At NA2 the concentration of noradrenaline was 2×10^{-4}, and at NA5 the concentration was 5×10^{-4}; in each case the contact time was 1 min. before washing out the drug. With the stimulator switched on, a series of normal responses to transmural stimulation was obtained; then at Proc, procaine hydrochloride to a concentration of 2×10^{-4} was added and gradually abolished the contractions due to transmural stimulation. With the procaine still present and the stimulator off, at NA5 the higher dose of noradrenaline was repeated. At W the preparation was washed six times in 15 min. On recommencing transmural stimulation there was an immediate return of response. The lower tracing shows an identical experiment in which lignocaine (2×10^{-4} final concentration) was added at Lig.

Pentolinium (1 to 5×10^{-5}), nicotine (5×10^{-6} to 5×10^{-5}) and mecamylamine (5×10^{-6} to 5×10^{-5}) also reduced and finally blocked the responses to nerve stimulation without reducing the responses to transmural stimulation (Fig. 3). Mecamylamine produced a long-lasting block.

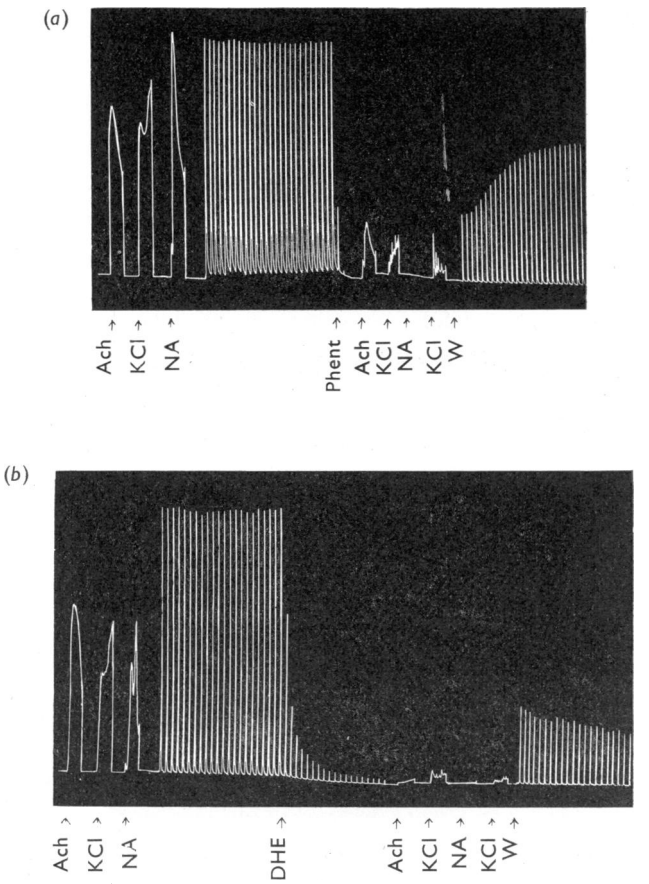

Fig. 5. The effect of adrenergic-blocking agents on the transmurally stimulated vas deferens. The vas was stimulated transmurally with the parameters and intervals of stimulation as for the experiments in Fig. 4. On the upper record with the stimulator off, the contractions of the vas to added drugs were recorded. At Ach, acetylcholine (1×10^{-4} final bath concentration) was added for 1 min contact. Potassium chloride (5×10^{-3} concentration) was added at KCl. At NA, noradrenaline was added for 1 min in a concentration of 5×10^{-4}. Next, a series of normal responses to transmural stimulation was recorded, then at Phent, phentolamine (2×10^{-4} final concentration) was added and the transmural contractions were quickly abolished. In the presence of the phentolamine the addition of drugs was repeated: the response to noradrenaline (NA) was abolished and the responses to acetylcholine (Ach) and potassium chloride (KCl) were reduced. At W the preparation was washed twenty times in 20 min. On recommencing stimulation there was a gradual but incomplete recovery of response to transmural stimulation. The lower tracing shows an identical experiment in which dihydroergotamine (3×10^{-4} final concentration) was added at DHE.

The isolated vas deferens preparation stimulated transmurally

On transmural stimulation of the vas deferens the contractions increased with increasing stimulus frequency from 3 shocks/sec to a maximal response at 50 shocks/sec. At 25 shocks/sec uniform contractions were obtained for 5 to 6 hr.

Local anaesthetics. Procaine and lignocaine, in concentrations of 1 to 2×10^{-4}, reduced and finally abolished the responses to transmural stimulation without reducing the response of the tissue to added noradrenaline (Fig. 4). In most experiments procaine potentiated the response to added noradrenaline. The action of

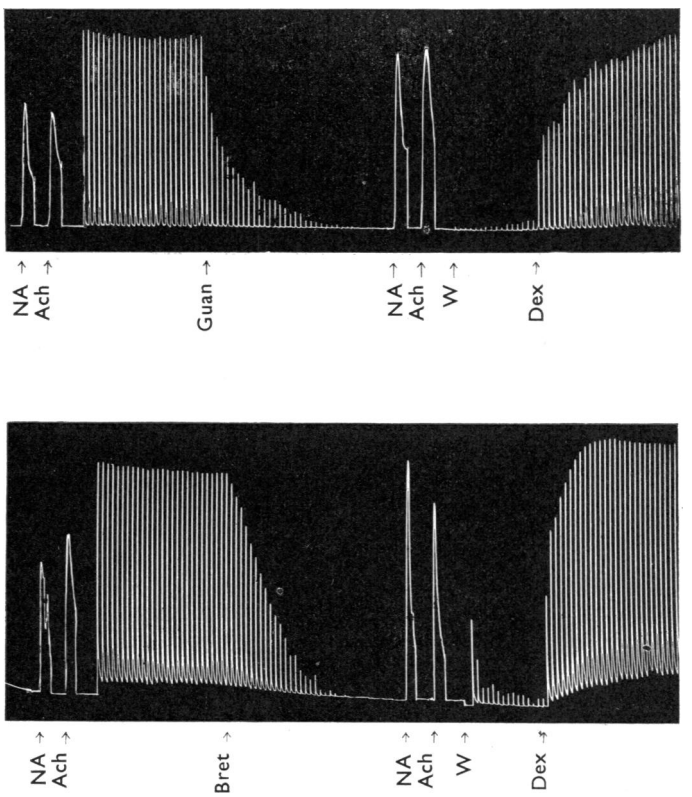

Fig. 6. The effects of adrenergic neurone-blocking agents on the vas deferens stimulated transmurally. The parameters and intervals of stimulation were as described for Fig. 4. In the upper record the unstimulated vas was contracted first by noradrenaline (5×10^{-4} concentration for 1 min) at NA, then by acetylcholine (2×10^{-4} concentration for 1 min) added at Ach. A series of normal responses to transmural stimulation was then recorded until at Guan, guanethidine (4×10^{-6} final bath concentration) was added. The transmural contractions were gradually abolished by the guanethidine and in its presence the doses of noradrenaline and acetylcholine gave increased responses. At W, the preparation was washed thirty times in 45 min. Stimulation after washing showed very little return of response until at Dex, dexamphetamine (5×10^{-6} concentration) was added and produced a gradual recovery to normal. The lower tracing shows a similar experiment using bretylium (8×10^{-6} final concentration) added at Bret.

the local anaesthetics was readily reversed by washing: in the experiments illustrated, the transmural responses were restored to normal after six changes of bath fluid in 15 min.

Prolonged exposure to concentrations of procaine and lignocaine greater than 3×10^{-4} often resulted in the onset of spontaneous contractions.

Adrenergic-blocking agents. Phentolamine (5×10^{-5} to 2×10^{-4}) abolished the contractions elicited by transmural stimulation and at the same time blocked the response to added noradrenaline. But the responses of the preparation to acetylcholine or potassium chloride were also impaired (Fig. 5, *a*). On washing out the drugs there was a slow and often incomplete recovery of the response to transmural stimulation.

Prolonged contact with dihydroergotamine (3×10^{-4}) usually almost completely blocked the response to transmural stimulation and always abolished the response to added noradrenaline. At the same time the responses to acetylcholine and potassium chloride were almost blocked (Fig. 5, *b*). Higher concentrations of dihydroergotamine could not be reached because of limited solubility in Krebs solution. The recovery of the response to transmural stimulation after washing was again slow and incomplete.

Adrenergic neurone-blocking agents. Guanethidine (1 to 4×10^{-6}) or bretylium (2 to 8×10^{-6}) progressively reduced and finally abolished the contractions produced by transmural stimulation. In the presence of full blocking concentrations of these drugs the contractions produced by noradrenaline or acetylcholine were unimpaired and often potentiated (Fig. 6). Repeated washing of the preparation gave only slight recovery of the response to transmural stimulation, but dexamphetamine (5×10^{-6}) produced a rapid return of the contractions to their original level.

Reserpine. Four animals had been previously treated for 2 days by intraperitoneal injections of either 2 or 3 mg/kg of reserpine in a 20% solution of ascorbic acid. In all four experiments the vasa initially gave full-sized contractions on transmural stimulation, but thereafter the responses gradually declined to a level approximately one-sixth of the original; with no preparation did the responses disappear.

Hyoscine and atropine. A concentration of 1×10^{-7} of hyoscine acting for 45 min usually produced less than a 5% reduction in the height of the contractions due to transmural stimulation. When the concentration was increased by ten-fold increments at 45 min intervals a large reduction in transmural contractions was seen only when concentrations of 1×10^{-4} or greater were present (Fig. 7). The contractions of the vas to added acetylcholine (1×10^{-4}) were blocked by hyoscine (1×10^{-7}). Atropine (1×10^{-7} or 1×10^{-6}) gave at the most a 5% reduction in the response to transmural stimulation. Increasing the concentration to 1×10^{-5} produced a 30% reduction in the response at the end of 45 min contact. A similar period of contact with atropine (1×10^{-4}) increased the reduction in response to 60%. The response of the vas to acetylcholine (5×10^{-4}) was abolished in the presence of atropine (1×10^{-7}).

Hemicholinium. A concentration of 1×10^{-5} present for 1 hr produced no reduction in the response to transmural stimulation. A concentration of 1×10^{-4}, in

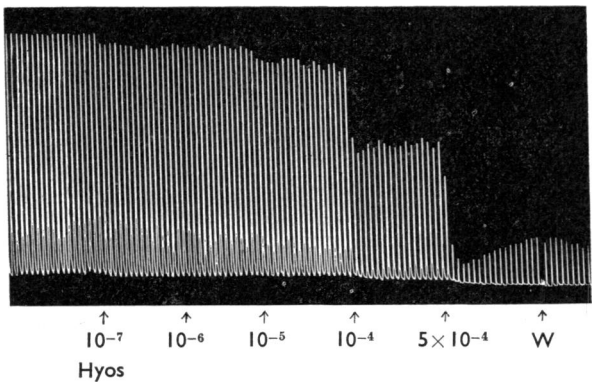

Fig. 7. The action of increasing concentrations of hyoscine on the transmurally stimulated vas deferens. The parameters and intervals of stimulation were as described for Fig. 4. At Hyos, hyoscine was added to a final bath concentration of 1×10^{-7} and allowed to act for 45 min, then without changing the bath fluid the concentration was increased to 1×10^{-6}, 1×10^{-5} and 1×10^{-4} at 45 min intervals; finally the concentration was increased to 5×10^{-4} for 45 min. Concentrations up to 1×10^{-4} gave less than a 15% reduction in response. At W, the preparation was washed six times in 15 min but showed no recovery of transmural response.

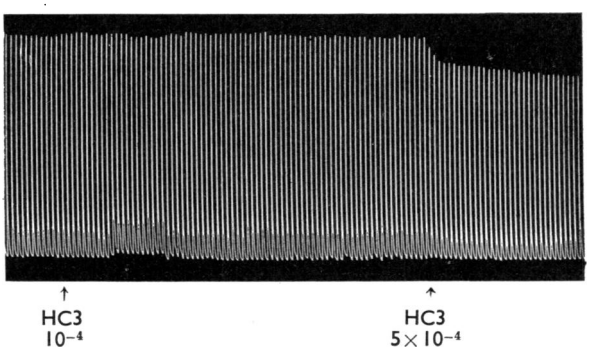

Fig. 8. The effect of hemicholinium on the transmurally stimulated vas deferens. The parameters and intervals of stimulation were as described for Fig. 4. At HC3, hemicholinium was added in a final bath concentration of 1×10^{-4} for 2 hr 40 min and produced no reduction in response. When the concentration was increased to 5×10^{-4} for a further 1 hr, the responses were reduced by 15%.

contact with the vas for periods up to 2.5 hr, gave less than a 5% reduction in height of contractions due to transmural stimulation. A 15% reduction was produced by hemicholinium (5×10^{-4}) at the end of 1 hr contact (Fig. 8).

DISCUSSION

The vas deferens contracts when stimulated transmurally by the method we have described and we have shown, using the alternately stimulated preparation, that these contractions persisted in the presence of concentrations of hexamethonium, pentolinium, nicotine and mecamylamine which simultaneously abolished the

responses to hypogastric nerve stimulation. Is the site of action of these drugs at a ganglionic synapse in the hypogastric nerve and, if so, does transmural stimulation excite postganglionic nerve fibres or does it stimulate the smooth muscle cells directly ?

The pulse duration used throughout the experiments was 0.1 msec: Paton (1955) showed for the transmurally stimulated guinea-pig ileum that such short pulse durations stimulate nervous structures but do not stimulate smooth muscle. Much wider pulse widths are needed to stimulate smooth muscle directly ; it therefore seemed likely that we were stimulating nerve fibres.

More direct evidence was obtained by the use of the local anaesthetic drugs procaine and lignocaine which abolished the responses of the vas to transmural stimulation in concentrations which did not reduce the contractions to added noradrenaline. These results show that the blocking action of the local anaesthetics is not produced by impairing the ability of the muscle to contract ; they allow the conclusion that the contractions due to transmural stimulation are produced by excitation of nerves.

We now have the evidence needed for localizing the site of action of the ganglion-blocking agents. If nerves are still excitable in concentrations of hexamethonium, pentolinium, nicotine and mecamylamine which abolish the responses to hypogastric nerve stimulation, these drugs must block by an action at a ganglionic synapse rather than by one of the alternative mechanisms (Sjöstrand, 1962) of an action on peripherally-located chromaffin cells or a nonspecific depression of nerve terminals.

We have now established that transmural stimulation excites postganglionic nerves. Are these nerves adrenergic or cholinergic, or do they contain both types of fibre ?

For the vas deferens stimulated through the hypogastric nerve, most of the available evidence seems to show that these nerves are adrenergic. In his original description of the preparation, Huković (1961) described a potentiating action of cocaine and a reduced response of the preparation after treatment of the guinea-pigs with reserpine. Boyd, Chang & Rand (1960) have reported a blocking action of several adrenergic-blocking agents and the same authors (1961) showed that the contractions of the vas are also abolished by bretylium.

We have used a similar range of drugs to investigate the chemical class of the postganglionic nerves. Dihydroergotamine and phentolamine were selected as two of the most specific adrenergic-blocking drugs and were found to abolish the responses to postganglionic nerve stimulation and to added noradrenaline. Although these results seem to support an adrenergic innervation, the reduced responses to added acetylcholine and potassium chloride indicate that the high concentrations of dihydroergotamine and phentolamine necessary to block the contractions due to transmural stimulation were nonspecific, since the drugs also impaired the ability of the muscle to contract in response to direct chemical stimulation.

The adrenergic neurone-blocking agents guanethidine and bretylium were without this depressant action on the smooth muscle ; they abolished the contractions due to transmural stimulation in concentrations approximately the same as those used

by Day (1962) for adrenergic neurone blockade in the rabbit Finkleman preparation and did not reduce the sensitivity of the vas to added acetylcholine or noradrenaline. This specific blocking action of guanethidine and bretylium provides good evidence that the fibres excited by transmural stimulation are adrenergic, but does not exclude the presence of other nerve fibres.

Additional evidence that the postganglionic nerve fibres are adrenergic was obtained using vasa from reserpine-treated guinea-pigs. Huković (1961), using doses of reserpine which had been shown by others to deplete the tissue stores of noradrenaline, found that the responses of the vas deferens to hypogastric nerve stimulation were decreased but never abolished. Using animals which had been previously treated with more than twice these amounts of reserpine we obtained equivalent results with the transmurally stimulated preparation. As Huković (1961) suggested, it seems that the noradrenaline stores in these adrenergic nerves are reduced but not depleted by these doses of reserpine.

Our experiments so far have shown that the postganglionic nerve fibres to the vas deferens are mainly adrenergic. Is there any evidence for the presence of cholinergic postganglionic fibres ? The results with hyoscine and atropine suggest that excitation of cholinergic nerves is responsible for only a very small part of the response to transmural stimulation. This conclusion is supported by experiments in which hemicholinium, in concentrations of 10^{-4} applied for 1 hr, was without effect or gave a just discernible reduction of the contractions due to transmural stimulation.

We may now consider what use may be made of the sympathetically innervated guinea-pig vas deferens for distinguishing the sites of action of blocking drugs. It is clear that the vas deferens stimulated through the hypogastric nerve by the method of Huković (1961) does not discriminate between ganglion blockade and adrenergic neurone blockade. Although Ohlin & Strömblad (1963), using this preparation with the nerve electrodes moved to a point 1 to 5 mm away from the organ, produced contractions which resisted ganglion-blocking concentrations of hexamethonium, they did not show whether these contractions were due to stimulation of nerve or muscle. For the study of adrenergic neurone blockade uncomplicated by a possible ganglion-blocking action, the vas deferens removed without the hypogastric nerve from the guinea-pig and stimulated transmurally seems to be the preparation of choice.

Since our findings were reported to the British Pharmacological Society in July, 1963, Bentley & Sabine (1963) have published their results with a preparation stimulated transmurally by an electrode arrangement similar to that illustrated in Fig. 1, *b* but using different stimulus parameters and the vas stripped of its mesentery and maintained at 36° C. They produced contractions which were resistant to block by ganglion-blocking agents and which were abolished by adrenergic neurone-blocking drugs. In contrast to our findings, procaine hydrochloride in concentrations up to 1×10^{-4} produced little more than a 50% reduction of the responses to transmural stimulation. In our experience with this drug and with lignocaine, concentrations greater than 1×10^{-4} will abolish contractions due to transmural stimulation of the vas deferens if the contractions are due entirely to stimulation of nerve fibres.

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