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THE EFFECTS OF GANGLION-BLOCKING AND POSTGANGLIONIC SYMPATHOLYTIC DRUGS ON PREPARATIONS OF THE GUINEA-PIG VAS DEFERENS

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The contractions of the guinea-pig isolated vas deferens elicited by electrical stimulation of the hypogastric nerve were completely blocked by the following drugs: guanethidine, bretylium, dimethylphenylpiperazinium hydrochloride, nicotine, pempidine, hexamethonium, hemicholinium, D-tubocurarine and procaine. However, when the vas deferens was stimulated through an electrode in its lumen, the contractions in response to frequent, short stimuli (50 shocks/sec, 1 msec duration) were blocked by guanethidine, bretylium and dimethylphenylpiperazinium, but were not affected by the remaining drugs, except that procaine and hemicholinium each caused some reduction in the responses. When the preparation was stimulated transmurally with shocks of 200 msec duration at 1 shock/sec, the contractions were unaffected by any of the above drugs, except hemicholinium which again caused a slow reduction of up to 50% of the original response. It is concluded that nicotine, pempidine, hexamethonium, D-tubocurarine and hemicholinium probably block the response to stimulation of the hypogastric nerve by acting on peripheral ganglia in its pathway. Hemicholinium appears to have an additional effect in depressing the responses of the smooth muscle of the vas deferens to direct electrical stimulation, and procaine may act both on the ganglia and at the nerve terminals.

The isolated hypogastric nerve-vas deferens preparation of the guinea-pig (Huković, 1961) has been widely used for various physiological and pharmacological experiments (Chang & Rand, 1960; Burnstock & Holman, 1961, 1962). Much of this work appears to involve the assumption that the majority of fibres in the hypogastric nerve are postganglionic distal to the inferior mesenteric ganglion. Recent experiments by Sjöstrand (1962), however, have provided clear indications of the existence of synaptic relays in the hypogastric nerve, and histological studies by Merrillees, Burnstock & Holman (1963) have demonstrated ganglion cells in this nerve close to its entry into the vas deferens. It is important therefore to re-examine the effects of certain ganglion-blocking and postganglionic sympatholytic drugs in the light of this new knowledge. Accordingly a study was made of the actions of several drugs on the hypogastric nerve-vas deferens preparation and a comparison was made with their action on a preparation of the isolated vas deferens, stimulated from an electrode inserted into the lumen of the organ. Merrillees (personal communication) has never observed any ganglion cells in the wall of the vas deferens,

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and thus the transmural stimulation probably acts solely on postganglionic nerve endings or directly on the smooth muscle cells, according to the conditions.

METHODS

The guinea-pig isolated hypogastric nerve-vas deferens preparation. This was set up as described by Huković (1961). The tissue was suspended in an organ-bath of 75 ml. capacity, using the fluid formulated by Huković, and gassed with 5% carbon dioxide and 95% oxygen, at 36° C. Perspex holders held the nerve between annular platinum electrodes. Rectangular wave stimuli of 1 msec duration were delivered from a Grass Model S4D stimulator for periods of 5 sec every 2 min at a frequency of 50 shocks/sec. The voltage was varied so that maximal and submaximal responses alternated.

The guinea-pig isolated, stripped vas deferens preparation. The vas deferens taken directly from the body shows a low sensitivity to added drugs. However, if the mesenteric investment is carefully stripped away a much more sensitive preparation results, that gives larger and more rapid responses. In these experiments the vas deferens was mounted on a J-shaped Perspex holder containing a length of platinum wire. About 2 mm of this wire protruded through the lower arm of the J, and was inserted into the lumen of the vas deferens. A second electrode, also of platinum, formed a loop around the inner electrode, and thus around the vas deferens also at about 5 mm distance from the tissue. Two types of stimulation were used, (a) trains of shocks of 1 msec duration at 50 shocks/sec for 5 sec (subsequently referred to as fast stimulation), and (b) groups of five stimuli of 200 msec duration at 1 shock/sec (subsequently called slow stimulation). These patterns of stimulation alternated once every 4 min as it was found that if they were applied more frequently the size of the responses diminished rapidly. For both types of stimulation the voltage was adjusted to give maximal contractions. At the slow rate of stimulation very high voltages were needed to produce appreciable responses if the duration of each shock was less than 100 msec. Shock durations of 200 msec led to adequate responses with moderate voltage. Further increase of the duration above 200 msec gave larger responses, followed, however, by a prolonged relaxation of the preparation.

Paired preparations were always used, both taken from the same animal. One was set up as a hypogastric nerve-vas deferens, and the other as a stripped, transmurally-stimulated vas deferens. When the control contractions had reached a steady level the drug under test was added to both baths, and the responses were recorded until they were reduced to a constant level or, if no block occurred, for a minimum of 30 min. In some experiments the baths were then washed out to test for spontaneous recovery, and in others attempts were made to abolish the block by the addition of various drugs (Day, 1962; Bentley, 1962; Day & Rand, 1963). Each drug was tested on at least four separate preparations.

Drugs. These were guanethidine (Ismelin, 2% aqueous solution, Ciba), bretylium tosylate (Darenthin, Burroughs-Wellcome), nicotine hydrogen tartrate (B.D.H.), dimethylphenylpiperazinium iodide (Light & Co.), pempidine tartrate (May & Baker), hemicholinium, hexamethonium chloride (Light & Co.), D-tubocurarine chloride (Burroughs-Wellcome), procaine hydrochloride (Bayer Pharma Pty.), tyramine hydrochloride (Calbiochem) and carbachol (B.D.H.).

RESULTS

Hypogastric nerve-vas deferens preparation

This preparation gave regular and reproducible responses to stimulation of the hypogastric nerve.

Effects of tyramine and carbachol. The addition of tyramine (1 to 2×10^{-5}) potentiated both maximal and submaximal responses; these returned to the original levels after washing the preparation. No rise in the baseline of the trace was noted.

When carbachol was added to give a concentration of 5 to 50×10^{-7} a small contraction was seen, which slowly relaxed during the following 1 to 2 min. Both maximal and submaximal responses were immediately increased and this effect slowly disappeared after washing (Fig. 1).

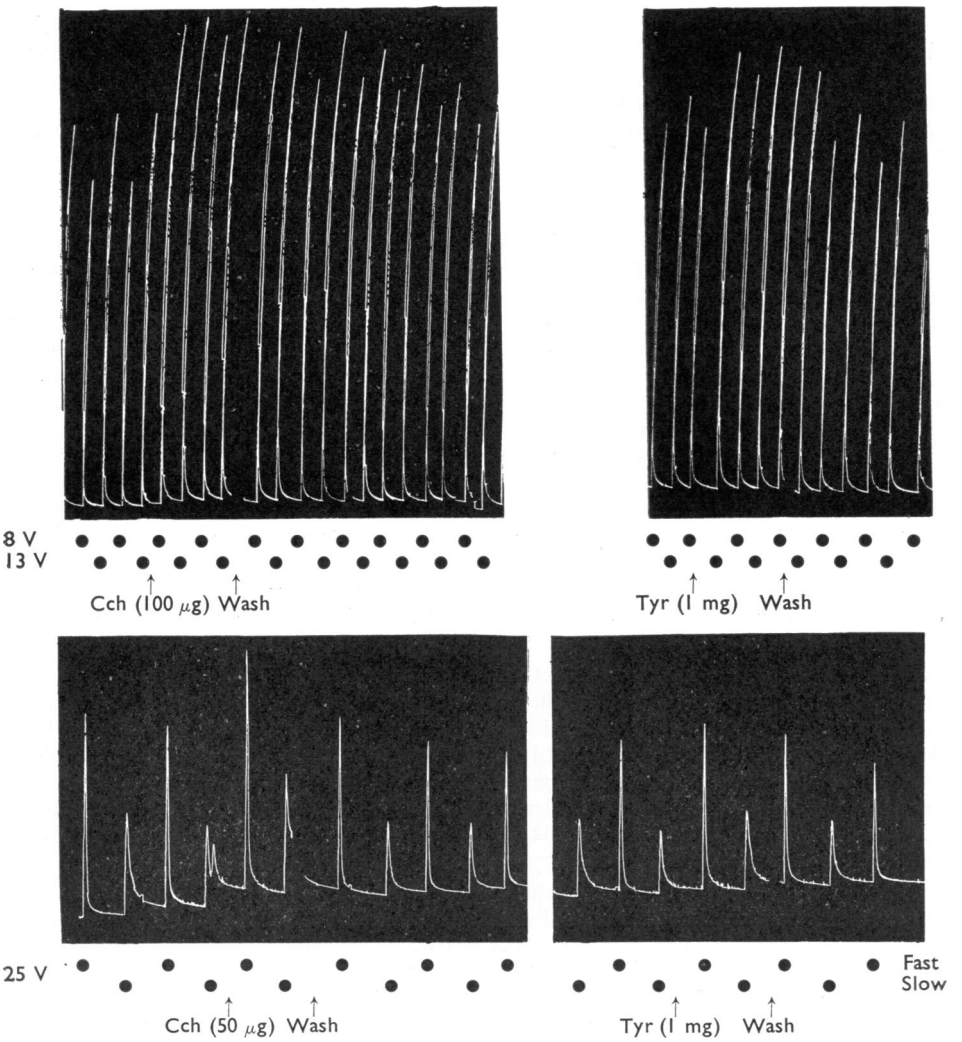

Fig. 1. The effects of carbachol and tyramine on vas deferens preparations. Upper record, hypogastric nerve-vas deferens; lower record, stripped vas deferens, transmural stimuli. Stimulation was applied at dots. For the hypogastric nerve-vas deferens preparation, alternate maximal and submaximal stimulations were used, at a stimulus frequency of 50 shocks/sec and with 1 msec duration, for 5 sec periods each 2 min. For the stripped vas deferens preparation, maximal stimuli were applied alternately at fast (50 shocks/sec, 1 msec duration) and slow (1 shock/sec, 200 msec duration) rates for 5 sec each 4 min. At Cch, carbachol was added (100 μg in upper record, 50 μg in lower). At Wash, the organ-baths were washed out. At Tyr, 1 mg of tyramine was added. Both drugs reversibly potentiated the responses to the two types of stimulation.

Effects of drugs acting on autonomic nerves. The following drugs completely blocked the response of the vas deferens to stimulation of the hypogastric nerve: guanethidine (5×10^{-6}), bretylium (1×10^{-5}), nicotine (1×10^{-5}), pempidine (2 to 5×10^{-5}), dimethylphenylpiperazinium (1×10^{-5}), hemicholinium (1×10^{-4}), procaine (1×10^{-4}) and hexamethonium (4×10^{-5}). With the exception of procaine, hexamethonium and nicotine, no spontaneous abolition of the block was seen up to 30 min after washing the drugs out of the bath. D-Tubocurarine (1 to 2×10^{-5}) produced only a small reduction in response and, in concentrations of 1×10^{-4}, the effect varied from complete block to less than 50% reduction of response. The actions of guanethidine, bretylium and dimethylphenylpiperazinium were abolished slowly in the presence of tyramine (1.5×10^{-5}), and more rapidly in the presence of carbachol (5 to 50×10^{-7}). Nicotine, pempidine and procaine were not antagonized by either drug. The blocks produced by D-tubocurarine, hexamethonium and dimethylphenylpiperazinium were partly abolished by carbachol, but not by tyramine (Table 1).

TABLE 1

THE EFFECTS OF VARIOUS DRUGS ON THE RESPONSES OF THE VAS DEFERENS TO STIMULATION TRANSMURALLY AND OF THE HYPOGASTRIC TRUNK

+ = abolition of block; ± = partial abolition; − = no abolition

		Effect on response to stimulation of				
			Wall of vas deferens		Abolition of block by	
Drug	Concentration	Hypogastric nerve	At 50 shock/sec, 1 msec duration	At 1 shock/sec, 200 msec duration	Tyramine	Carbachol
Guanethidine	5×10^{-6}	Block	Block	No action	+	+
Bretylium	1×10^{-5}	Block	Block	No action	+	+
Dimethylphenylpiperazinium	1×10^{-5}	Block	Block	No action	+	+
Procaine	5 to 10×10^{-5}	Block	Partial block	No action	−	−
Hemicholinium	1×10^{-4}	Block	Up to 50% reduction, delayed	Up to 50% reduction, delayed	−	+
Nicotine	1×10^{-5}	Block	No action	No action	−	−
Pempidine	1×10^{-5}	Block	No action	No action	−	−
Hexamethonium	2×10^{-5}	Partial block	No action	No action	−	
	4×10^{-5}	Complete block			−	±
D-Tubocurarine	5 to 10×10^{-5}	Block	Small increase	Occasional small increase	−	±

Stripped vas deferens, stimulated transmurally

Transmural stimulation at a rate of 50 shocks/sec produced regular contractions, only slightly smaller than those elicited by stimulation of the hypogastric nerve. However, the slow rate of stimulation caused smaller contractions, which tended to diminish spontaneously as time passed. Relaxation of the contractions produced by the slow stimulation was slower than with the fast stimulation, and was seldom complete in less than 2 min.

Effects of tyramine and carbachol. Tyramine (1.3×10^{-5}) and carbachol (2 to 4×10^{-7}) increased the responses to both fast and slow transmural stimulations. This effect was abolished when the drugs were washed out of the bath (Fig. 1).

Effect of drugs which block hypogastric nerve transmission. (1) Stimulation at 50 shocks/sec and 1 msec duration (fast stimulation) caused responses which were completely blocked by bretylium (1×10^{-5}, Fig. 2), guanethidine (5×10^{-6}) and dimethylphenylpiperazinium (1×10^{-5}). Nicotine (1×10^{-5}, Fig. 3) and pempidine (1×10^{-5}) had no action on these responses. D-Tubocurarine (1×10^{-4}) similarly had no blocking action, and in some experiments increased the responses (Fig. 4). Procaine (5×10^{-5}) rapidly reduced the responses to about half of the original level,

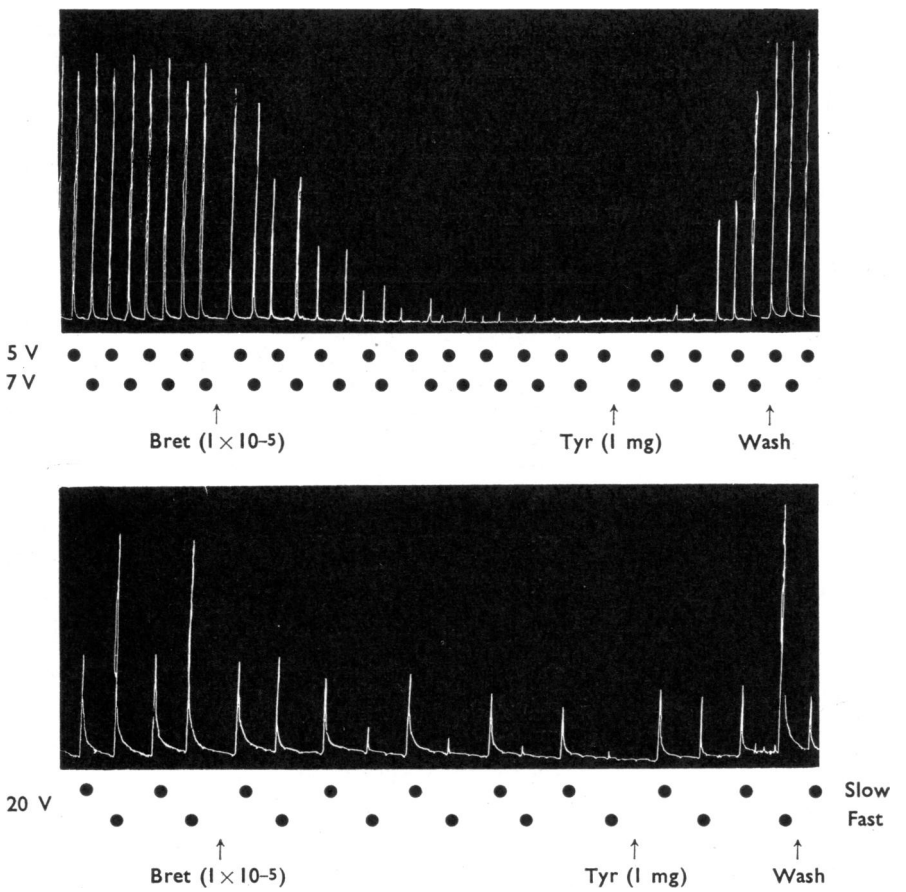

Fig. 2. The actions of bretylium on vas deferens preparations. Upper record, hypogastric nerve-vas deferens; lower trace, stripped vas deferens, transmural stimuli. Stimulation was applied at dots as in Fig. 1. At Bret, bretylium was added to 1×10^{-5}. At Tyr, 1 mg of tyramine was added. At Wash, the organ-bath was washed out. The response to stimulation of the hypogastric nerve was completely blocked, as was that to fast transmural stimulation and both these responses were restored by tyramine. The responses to slow transmural stimulation were but little affected by bretylium.

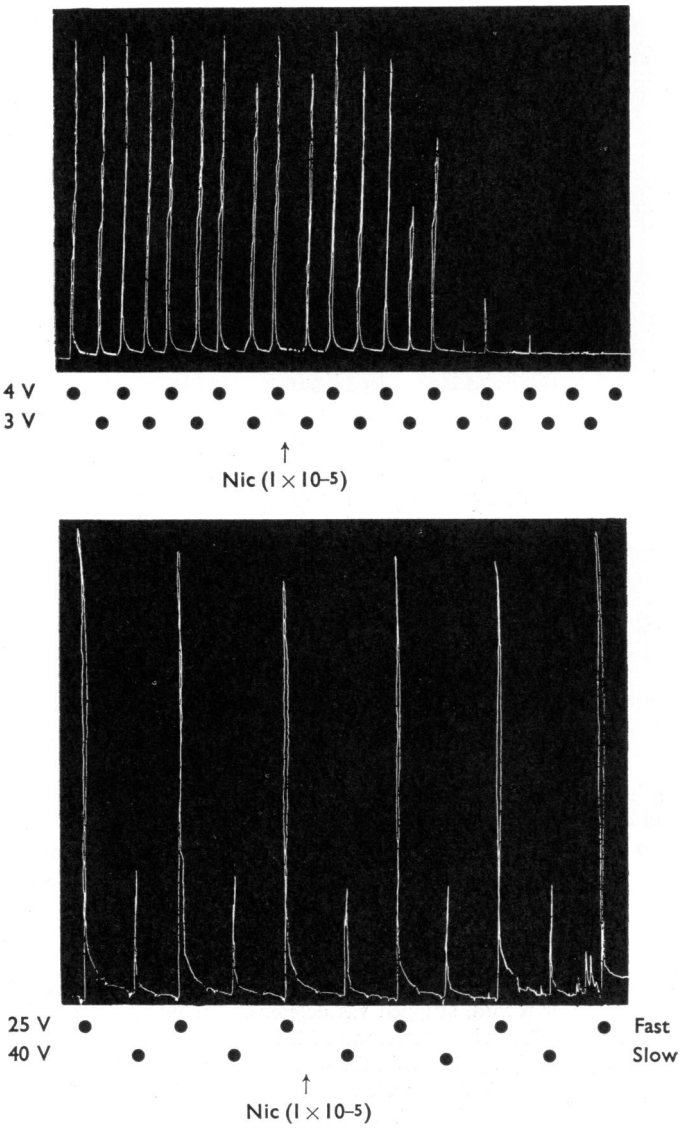

Fig. 3. The actions of nicotine on vas deferens preparations. Upper record, hypogastric nerve-vas deferens; lower record, stripped vas deferens. Stimulation was applied at dots, as in previous Figs. At Nic, nicotine was added to 1×10^{-5}. The response to stimulation of the hypogastric nerve was completely blocked, but that to transmural stimulation was not affected.

but an increase in the concentration of the drug to 1×10^{-4} caused little further reduction (Fig. 5). Hemicholinium (1×10^{-4}) produced a reduction of up to 50% which developed more slowly than when the hypogastric nerve was stimulated (Fig. 6). The block produced by bretylium, guanethidine or dimethylphenylpiper-

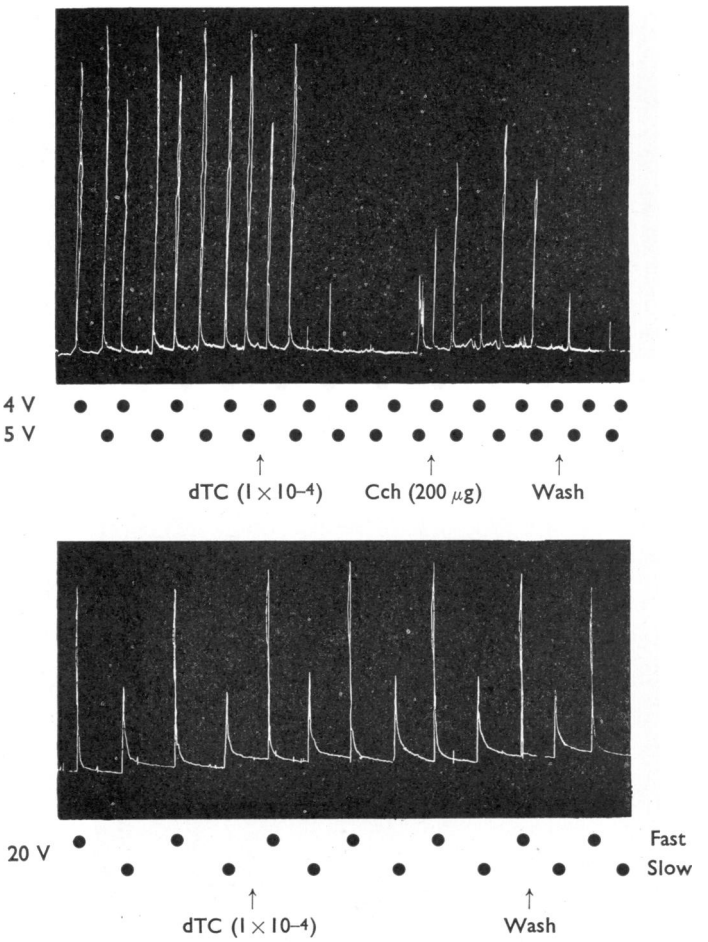

Fig. 4. The actions of D-tubocurarine on vas deferens preparations. Upper record, hypogastric nerve-vas deferens; lower record, stripped vas deferens. Stimulations were applied at dots, as in previous Figs. At dTC, D-tubocurarine was added to 1×10^{-4}. At Cch, 200 μg of carbachol was added (upper record only). At Wash, the organ-baths were washed out. The responses to stimulation of the hypogastric nerve were completely blocked and this block was reversibly antagonized in the presence of carbachol.

azinium could be reversibly antagonized by tyramine or carbachol. The partial block produced by procaine or hemicholinium was relieved to a small extent by carbachol, but not by tyramine.

(2) Stimuli at 1 shock/sec and 200 msec duration (slow stimulation) caused contractions which were clearly inhibited only by hemicholinium (although there was also a tendency to spontaneous reduction in response), and this drug caused a slow diminution of up to 50% of the original contractions, as it did on the response to fast stimulation (Fig. 6). D-Tubocurarine occasionally caused a small increase in these responses (Fig. 4). These results are summarized in Table 1.

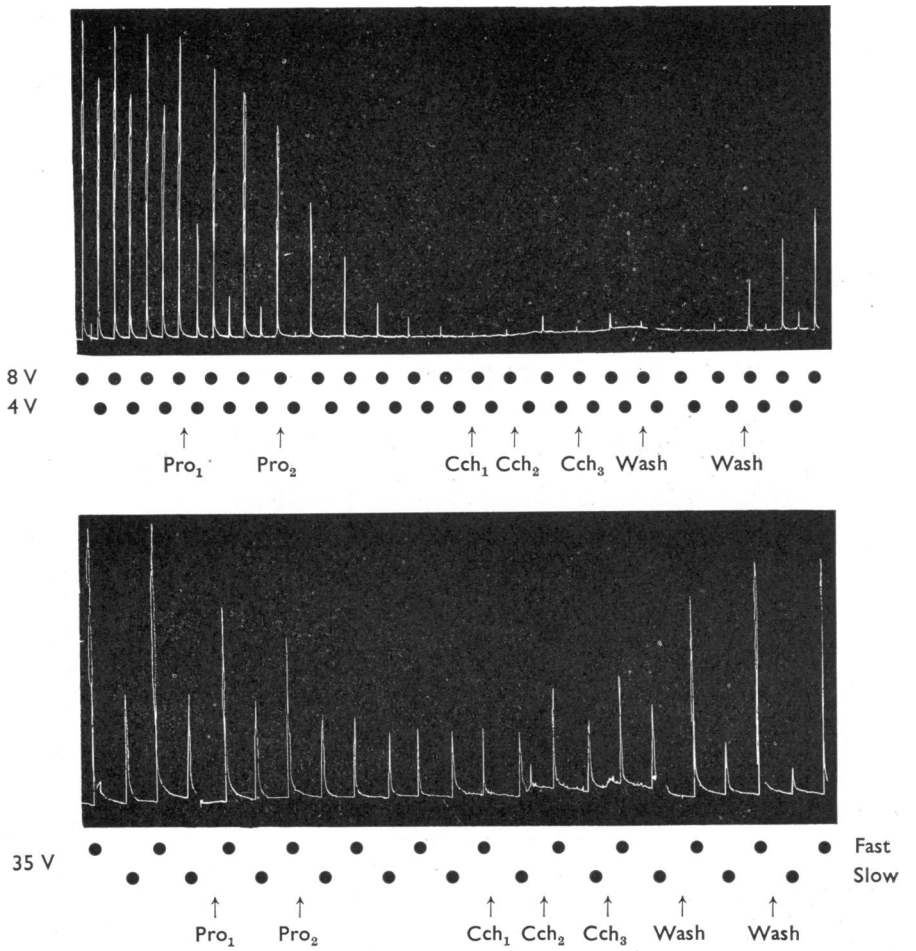

Fig. 5. The actions of procaine on vas deferens preparations. Upper record, hypogastric nerve-vas deferens; lower record, stripped vas deferens. Stimulations were applied at dots, as in previous Figs. At Pro_1, procaine was added to 5×10^{-5}, and at Pro_2 to 1×10^{-4}. At Cch_1, 50 μg of carbachol was added, at Cch_2 a further 100 μg was added, and at Cch_3 a further 200 μg was added. At Wash, the organ-baths were washed out. Note the complete block of responses to stimulations of the hypogastric nerve, and little reversal of this by carbachol. The responses to both fast and slow transmural stimulations were only partly blocked, and carbachol caused some reversal of this effect.

(3) With the stripped vas deferens, guanethidine, bretylium and dimethylphenylpiperazinium each strongly potentiated the responses of the vas deferens to added noradrenaline and thus the block produced by these drugs on the responses to high-frequency transmural stimulation could not be due to an interference with the effects of liberated transmitter on the smooth muscle. It seemed important to investigate whether hemicholinium or procaine, which partially blocked the responses to transmural stimulation, were acting by reducing the response of the smooth muscle to liberated transmitter. However, neither of these drugs reduced the

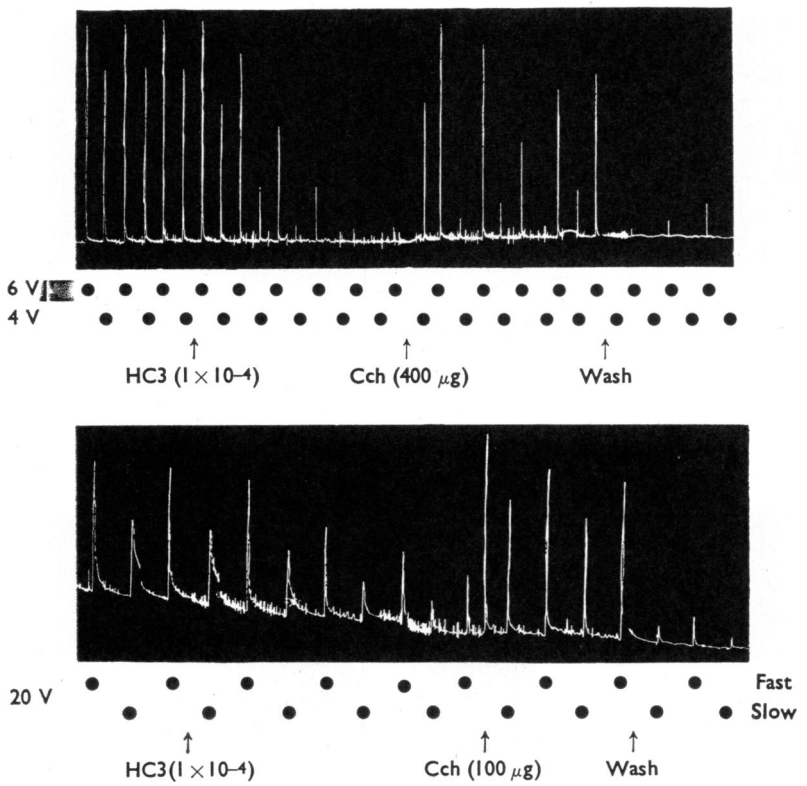

Fig. 6. The actions of hemicholinium on vas deferens preparations. Upper record, hypogastric nerve-vas deferens; lower record, stripped vas deferens. Stimulations were applied at dots, as in previous Figs. At HC3, hemicholinium was added to 1×10^{-4}. At Cch, carbachol was added, 400 μg in the upper record and 100 μg in the lower record. At Wash, the organ-baths were washed out. The response to stimulation of the hypogastric nerve was completely blocked, and those for the stripped vas deferens to both fast and slow stimulations were reduced. Carbachol reversibly antagonized the effects of hemicholinium.

contractions of the stripped vas deferens to added noradrenaline. In fact, hemicholinium (1×10^{-4}) caused a small increase in the response, while procaine (1×10^{-4}) approximately doubled it.

DISCUSSION

This paper has described the effects of nine drugs on the responses of the vas deferens to three types of stimulation. These are (i), frequent (fast), short stimuli (50 shocks/sec, 1 msec duration) to the hypogastric nerve; (ii), frequent (fast), short stimuli applied transmurally; and (iii), infrequent (slow), long stimuli (1 shock/sec, 200 msec duration) also applied transmurally.

The work of Sjöstrand (1962) and of Merrillees *et al.* (1963) together with the findings in the present paper provide clear evidence that the type (i) stimulation involves preganglionic fibres. However, fast transmural stimulation (type ii)

probably acts only on postganglionic nerve fibres in the walls of the vas deferens, for the following reasons: first, Merrillees (personal communication) has not found ganglion cells in the vas deferens, and, second, the contractions elicited by this type of stimulation are not affected by ganglion-blocking drugs, but are blocked by guanethidine, bretylium and dimethylphenylpiperazinium. The slow transmural stimulation may activate both nerve and smooth muscle fibres in the untreated preparation (G. Burnstock, personal communication) but after treatment with guanethidine or similar drugs, in doses which block the responses to fast stimulation, the vas deferens still responds to the slow stimulation, indicating that the muscle cells can be directly stimulated. Hence it may be concluded that any drug which blocks or reduces this type (iii) of response is probably acting directly on the muscle cells themselves, while drugs which block only the postganglionic nerve fibres would not reduce the response.

The situation in the vas deferens, therefore, appears to be analogous with that in the guinea-pig ileum, as described by Paton (1955). In this organ also, either autonomic nerve endings or smooth muscle cells may be stimulated at will by varying the stimulus parameters.

The ganglion-blocking drugs hexamethonium, pempidine, nicotine and D-tubocurarine had no blocking action on the response to transmural stimuli, yet at the same concentrations they completely blocked the contractions due to stimulation of the hypogastric nerve. This result suggests that these drugs have no postganglionic sympathetic blocking action, but act solely on peripheral ganglia in the hypogastric nerve. No explanation can be offered for the observation that D-tubocurarine often increased the response to transmural stimuli.

Hemicholinium produces only a partial block (less than 50%) of the response to fast transmural stimulation, and this block develops more slowly than the complete block the drug causes on the response to stimulation of the hypogastric nerve. At the same time hemicholinium reduces the response to slow transmural stimulation, which suggests that this drug directly depresses the response of the smooth muscle cells to electrical stimulation. On the other hand, hemicholinium does not reduce the contractions of the vas deferens produced by the addition of noradrenaline. Thus it seems probable that hemicholinium, like hexamethonium, produces most of its blocking action on the hypogastric nerve by blocking ganglia, and not by an action on sympathetic nerve terminals, as suggested by Chang & Rand (1960) and Rand & Chang (1960). This suggestion is supported by the finding of McIntosh, Birks & Sastry (1956) that hemicholinium can cause a failure of transmission in the superior cervical ganglion of the cat. If this drug does in fact block the hypogastric nerve by an action on the ganglion cells, this may explain why it does not block the inhibitory response to sympathetic stimulation in the Finkleman preparation (Bentley, 1962), and why it does not block contractions of the nictitating membrane in response to stimulation of the postganglionic cervical sympathetic nerve in the cat (Gardiner & Thompson, 1961).

Procaine (1×10^{-4}) completely and consistently blocks the response to stimulation of the hypogastric nerve. At a concentration of 5×10^{-5}, the drug reduces but does

not block the response to transmural stimulation, and even when the concentration in the bath is raised to 1×10^{-4} the drug does not completely abolish the response. This result suggests that procaine may have two sites of action, both on the ganglia (Harvey, 1939) and also at the nerve terminals. Since procaine potentiates the response of the vas deferens to added noradrenaline, the depressant action of this drug towards the electrically-induced contractions appears to involve only an effect on nerve.

The action of dimethylphenylpiperazinium requires further mention. It had been reported previously (Bentley, 1962) that this drug was of similar potency to guanethidine in blocking responses both of the hypogastric nerve-vas deferens and of the Finkleman preparation. Since dimethylphenylpiperazinium acts on ganglia the possibility existed that it might have had an action similar to that of nicotine. However, this does not seem probable, as the drug can also block the responses to fast transmural stimulation.

The block of responses to stimulation of the hypogastric nerve caused by procaine, hexamethonium and nicotine is abolished fairly rapidly after washing, but the other six drugs had a much more prolonged effect. The addition of tyramine or carbachol abolished the effects of guanethidine, bretylium and dimethylphenylpiperazinium, both on the hypogastric nerve-vas deferens and on the stripped vas deferens preparations. Hemicholinium, hexamethonium and D-tubocurarine were antagonized by carbachol, but not by tyramine, while the responses to procaine, pempidine and nicotine were not affected by either of these two latter drugs. These results have shown that there are two sites at which the drugs used may block the responses of the hypogastric nerve-vas deferens preparation. If allowance is made for their action at ganglia, the effect of these drugs at sympathetic nerve endings in the vas deferens appears to be similar to that at other sympathetic junctions.

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