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THE RESPONSE OF THE CIRCULAR MUSCLE LAYER OF THE GUINEA-PIG ISOLATED VAS DEFERENS TO TRANSMURAL ELECTRICAL STIMULATION

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1 Four preparations are described for the isolation of the response of the circular muscle of the guinea-pig vas deferens. These are the 'Furchgott' strip, the 'Vane' strip, the chain preparation and the perfused preparation.

2 The four preparations were stimulated transmurally with pulses of supramaximal voltage. The threshold pulse width to which the strips and the perfused preparation responded was 0.025 ms and the maximum responses occurred at 0.1 ms. The threshold frequency was 2 Hz for strip and perfused preparations, the maxima being 20 or 50 Hz for strip preparations and 100 Hz for perfused preparations. The effect of varying the number of pulses per train was also investigated on the perfused vas. Responses occurred to train lengths of 8, 16, 32, 128 pulses, the maximum response being given at 128 pulses at 100 Hz; 256 pulses per train did not produce a further increase in response. The perfused preparation exhibited an after-response at certain frequencies and train lengths.

3 Tetrodotoxin and the local anaesthetics, procaine and lignocaine, reversibly abolished the responses of strip and perfused preparations to transmural stimulation.

4 The response to intramural nerve fibre stimulation was abolished by guanethidine or bethanidine; this abolition was reversed by dexamphetamine. Noradrenaline contracted strip preparations of circular muscle and raised the pressure in perfused preparations; noradrenaline was competitively antagonized by thymoxamine. The major part of the motor innervation of the circular layer seems to be noradrenergic.

Introduction

In transverse section the vas deferens of the guinea-pig is seen to possess two well-defined layers of smooth muscle. Beneath the serous coat is a layer of smooth muscle in which the fibres have been cut transversely and so appear to be disposed along the length of the vas. This is referred to as the longitudinal muscle layer (LM). Next, towards the lumen is a layer of smooth muscle in which the fibres are disposed in a circular direction around the lumen. This is referred to as the circular muscle layer (CM). This layer may merge into a third less well-defined and much thinner muscle layer, next to the mucosa, in which the fibres appear to be disposed in longitudinal, circular and oblique directions.

Since Huković's original description of the isolated vas deferens-hypogastric nerve preparation (Huković, 1961) many studies have been made of its physiology and pharmacology. Those which have been based on contraction in response to electrical stimulation or to drugs have involved lengthwise mounting of the vas in a tissue bath, the vas being attached at one end to an isotonic or isometric recording system. Contractions of such a preparation are measured as a shortening of the vas and thus the response which is recorded would seem to be due to shortening of the longitudinal muscle fibres. Any contraction of the circular muscle is unlikely to contribute much to the recorded response. Nevertheless, the presence of this relatively large bulk of circularly-disposed muscle may need to be taken into account when the effects of electrical stimulation or drugs are being interpreted, since it may indirectly influence the response. We have now investigated the possibility of recording the responses of the circular layer of muscle of the vas deferens and have measured the effects of adrenergic neurone blocking and α-adrenoceptor blocking drugs on these responses.

Several methods have previously been devised by other workers for recording the responses of the circular smooth muscle component of tubular organs. These include spirally-cut preparations for small arteries (Furchgott & Badrakom, 1953), the method used by Vane for the rat fundus (Vane, 1957) which was also used by Harry for the guinea-pig ileum

(Harry, 1963); perfused preparations for arteries such as the central ear artery of the rabbit (de la Lande & Rand, 1965) and the mesenteric arteries of the rat (McGregor, 1965); the chain preparation for the trachea (Castillo & de Beer, 1947); and the circular strip preparation for the ileum of the cat (Gasser, 1926) and the ileum of the rabbit (Tweeddale, 1968).

In an organ such as the guinea-pig caecum, a physical separation of the circular muscle layer from the longitudinal layer (taenia) can be made (Akubue, 1966) so that a discrete strip consisting of circular muscle alone can be used. A similar preparation has been used for the human colon (Fishlock & Parks, 1963). For the cat jejunum, the outer longitudinal muscle layer and nerve plexus can be stripped off to leave the ganglion-free circular muscle (Evans & Schild, 1953).

The outer longitudinal muscle of the vas deferens is not easily separable from the inner circular muscle and the organ is too small to use the circular strip method conveniently. The methods which we have used in attempting to isolate the response of the circular muscle in the presence of longitudinal muscle are the strip preparations, a chain preparation and a perfused preparation. Responses to transmural electrical stimulation and to noradrenaline were obtained which were attributed to contraction of circular muscle and these responses were antagonized by guanethidine, bethanidine and thymoxamine.

Methods

Male albino guinea-pigs weighing over 600 g were killed by stunning and bleeding and the two vasa deferentia were removed without the hypogastric nerves.

'Furchgott' strips

Strips were prepared from individual vasa in a manner similar to that described by Furchgott & Badrakom (1953) for the preparation of arterial strips. The vas was pushed onto a glass rod of suitable diameter and a tightly-wound spiral strip was cut with the aid of fine scissors and forceps. Such a strip, of about 3 mm width and 30 mm length, was mounted in the conventional manner between parallel wire electrodes (Birmingham & Wilson, 1963) in a jacketed organ bath containing 20 ml of Krebs solution at 32°C bubbled with 95% O_2 and 5% CO_2. The strip was attached to an isotonic transducer under a load of 0.5 g; shortening of the strip in response to electrical stimulation or to drugs was recorded on a potentiometric recorder. Transmural stimulation was applied with pulses of supramaximal voltage for periods of 20 s every 4 min and the parameters of frequency (Hz) and pulse width (ms) were varied to obtain log frequency-response and log pulse width-response curves.

'Vane' strips

Strips were prepared by the method devised by Vane (1957) for the preparation of rat fundus strips. The vas was opened along its length and the resulting sheet of smooth muscle pinned out on cork under Krebs solution. A series of cuts was made at right angles to the long axis of the vas in alternate directions, each cut not completely severing the sheet of vas, the interval between the cuts being about 2 to 3 mm. Such a strip could be mounted in the conventional manner and stimulated transmurally as described above for the Furchgott preparation.

Chain preparation

Vasa were cut transversely into 6 or 7 tubular segments, each about 3 mm long. These were linked together by cotton thread to form a chain in a manner similar to that employed by Castillo & de Beer (1947) for the trachea. Such a chain was mounted as described for the strip preparations. Responses to electrical transmural stimulation or to drugs were recorded as a shortening of the total length of the chain which was due to a decrease in calibre of each individual segment. These responses were measured isotonically and displayed on a potentiometric recorder.

Perfused preparation

Each end of the vas was intubated by a small stainless steel tube of suitable diameter held in place by a ligature. This intubated vas was then mounted vertically between parallel wire electrodes in a jacketed organ bath containing 100 ml Krebs solution at 32°C bubbled with 95% O_2 and 5% CO_2. The upper and lower stainless steel tubes were fixed at the resting length of the vas to prevent it from shortening. The system was continuously perfused with Krebs solution (previously bubbled with 5% CO_2 in 95% O_2, at 32°C) by means of a constant flow roller pump. The effluent from the lower end of the vas was not allowed to mix with the bath fluid. The perfusion system was kept free of gas bubbles.

The pressure withing this perfusion system was measured by means of a pressure transducer. Any reduction in calibre of the vas increased the resistance to flow of the perfusate which resulted in an increase of pressure in the system, detected by the pressure transducer. This pressure signal was amplified and displayed on an ultra-violet recorder. It was established that maximal changes in pressure were pro-

duced by the tissue when the flow rate was constant at 0.85 ml/min and the outflow pressure head was 0 mmHg.

Perfused vasa were stimulated transmurally with pulses of supramaximal voltage for periods of 20 s every 8 min and the frequency (Hz) and pulse width (ms) parameters were varied to obtain log frequency-response and log pulse width-response graphs. In one series of experiments a pulse gating unit was used to investigate the effect of varying the total number of pulses in a stimulus train. Stimulus train lengths from 8 to 256 pulses were investigated at frequencies from 2 to 100 Hz.

Histological procedure

After all experiments the strip preparations were examined histologically. Strips were fixed in Souza's fixative, embedded in wax, sectioned at 6 μm, and stained with haematoxylin and eosin.

Drugs

The following drugs (obtained from the usual commercial sources) were used: procaine hydrochloride, lignocaine hydrochloride, tetrodotoxin, noradrenaline bitartrate, guanethidine sulphate, bethanidine sulphate, dexamphetamine sulphate and thymoxamine hydrochloride. All drug concentrations are expressed as final concentrations in mol per litre.

The Krebs solution used had the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 0.9, $NaHCO_3$ 25 and glucose 11.1.

Results

Strip preparation

When strips were stimulated transmurally with pulses of supramaximal voltage (140 V) at a frequency of 10 Hz for 20 s every 4 min, there was an increase in the shortening of the strip with increase in pulse width from 0.025 ms to 0.1 ms; further increase in pulse width to 0.2, 0.5 or 1.0 ms showed little change in response. When stimulated transmurally with pulses of supramaximal voltage at a pulse width of 0.1 ms, an increase in frequency from 2 to 100 Hz produced an increase in the shortening of the strip which was maximal at 20 or 50 Hz. Results for the strip preparations prepared by either of the two methods (Furchgott or Vane) were virtually identical.

Histology

Both types of strip preparation were examined histologically after all experiments. After careful orientation of the block, secretions were cut along the length of the strip and transversely across the strip. The fibres of the circular muscle layer were disposed along the length of the strip and the fibres of the longitudinal muscle layer were across the strip. In some cases these longitudinal muscle fibres were seen to run slightly obliquely.

Perfused preparation

When stimulated transmurally with pulses of supramaximal voltage at a frequency of 10 Hz for 20 s every 8 min, there was an increase in perfusion pressure with increase in pulse width from 0.025 to 0.1 ms (Figure 1a); further increase in pulse width showed a slight decline in response. When stimulated transmurally with pulses of supramaximal voltage at a pulse width of 0.1 ms for 20 s every 8 min, there was an increase in response with increase in frequency from 2 to 100 Hz (Figure 1b); frequencies above 100 Hz gave smaller responses. The shapes of the pulse width-response and frequency-response curves were similar to those obtained with strip preparations. When the 10 Hz stimulus was switched off there was always an after-response, a brief but large rise in pressure. This after-response was often present at higher frequencies but usually did not have such a spike-like form (Figure 2).

The effect on the frequency-response relation of varying the number of pulses in a stimulus train was investigated. By the use of a pulse gating unit which allowed a predetermined number of pulses to be delivered at any given frequency, frequency-response graphs (Figure 3a) were plotted for trains of 8, 16, 32, 64, 128 and 256 pulses. Trains of 2 or 4 pulses did not give responses at any frequency. It was found that the magnitude of the pressure rise at frequencies above 2 Hz increased with increase in train length from 8 to 128 pulses per train and that doubling this number to 256 produced no further increase in response. This confirmed that the number of pulses delivered when the vas was stimulated at a single frequency of 10 Hz for 20 s (200 pulses) was sufficient for the vas to develop a maximum response at that frequency. The effect of train length on the incidence of occurrence of the after-response was also investigated (Figure 3b). At a train length of 32 pulses an after-response appeared at 50 and 100 Hz only and was absent at all frequencies at train lengths shorter than 32. Increasing the train length to 64 increased the size of the after-response at 50 and 100 Hz and brought it in also at 20 and 10 Hz. A further increase in size of after-response occurred when there were 128 pulses in a train but it was still absent at 2 and 5 Hz. With a 256 pulse train length the after-response was absent at 2, 5 and 10 Hz and at 20, 50 and 100 Hz was smaller than that seen at these frequencies when

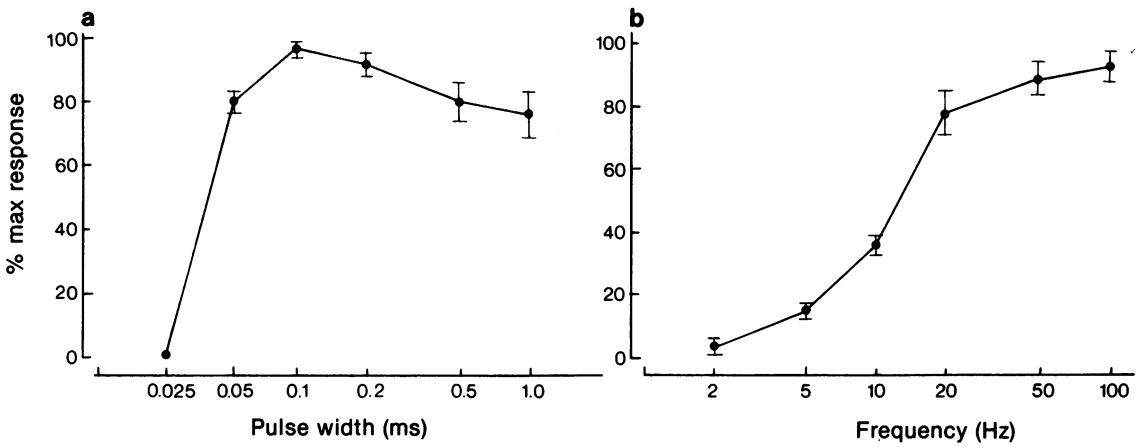

Figure 1 (a) Log pulse width-response curve and (b) log frequency-response curve for transmurally stimulated perfused guinea-pig vasa. Each point is the mean of measurements made on vasa from 5 guinea-pigs. Vertical lines show s.e. means. Stimuli at supramaximal voltage for 20 s (a) 10 Hz (b) 0.1 ms. Abscissae: (a) pulse width in ms on a log scale; (b) frequency in Hz on a log scale. Ordinates: rise in pressure expressed as % of the maximum response obtained.

the train length was 128 pulses. It therefore appears that for the train lengths used in this investigation, those with 128 pulses per train were optimal for the pressure rise produced during the passage of the train of stimuli at any frequency from 2 to 100 Hz and for the second pressure rise occurring after cessation of the train of stimuli at frequencies of 10, 20, 50 and 100 Hz.

Local anaesthetics and tetrodotoxin

Strip and perfused preparations were stimulated transmurally with pulses of supramaximal voltage at a frequency of 10 Hz and a pulse width of 0.1 ms for 20 s periods every 4 min and 8 min respectively. After several control responses had been recorded, tetrodotoxin (5×10^{-7} M) added to the tissue bath abolished the response to transmural stimulation but noradrenaline (2×10^{-4} M) added to the bath produced a shortening of the strips or a pressure rise from the perfused preparations (Figure 4). When the tetrodotoxin was washed out, normal responses to transmural stimulation gradually reappeared and eventually reached control size. Similar results were obtained with the local anaesthetic drugs, lignocaine (1×10^{-3} M) and procaine (1×10^{-3} M) but the blocking effect was more gradual than with tetrodotoxin.

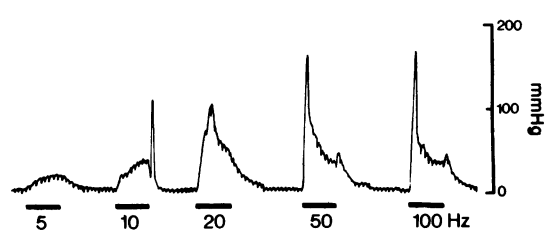

Figure 2 The effect of increasing the frequency of stimulation (Hz) on the pressure rise produced by a transmurally-stimulated perfused vas deferens of a guinea-pig. Pulse width 0.1 ms, supramaximal voltage (140 V); duration of stimulus train indicated by horizontal bars (20 s). The largest after-response occurred at 10 Hz.

Guanethidine and bethanidine

The effects of the adrenergic neurone blocking drugs guanethidine and bethanidine on the 'Furchgott' strip and whole perfused vas preparations were investigated. 'Furchgott' strip preparations were stimulated transmurally with trains of pulses of supramaximal voltage, 10 Hz and 0.1 ms for 20 s periods every 4 minutes. Whole perfused vas preparations were stimulated transmurally with trains of pulses of supramaximal voltage, 10 Hz and 0.1 ms for 20 s periods every 8 minutes. After several control responses to transmural stimulation had been recorded, guanethidine (2×10^{-5} M) completely abolished the responses of both preparations. Prolonged washing did not reverse this effect. Noradrenaline (final bath concentration 1×10^{-4} M) contracted the preparations during this period of block by guanethidine. Dexamphetamine added to the bath to give a final bath concentration of 2×10^{-5} M restored the responses to transmural stimulation to the original level, or above. Similar results were obtained with bethanidine (Figure 5).

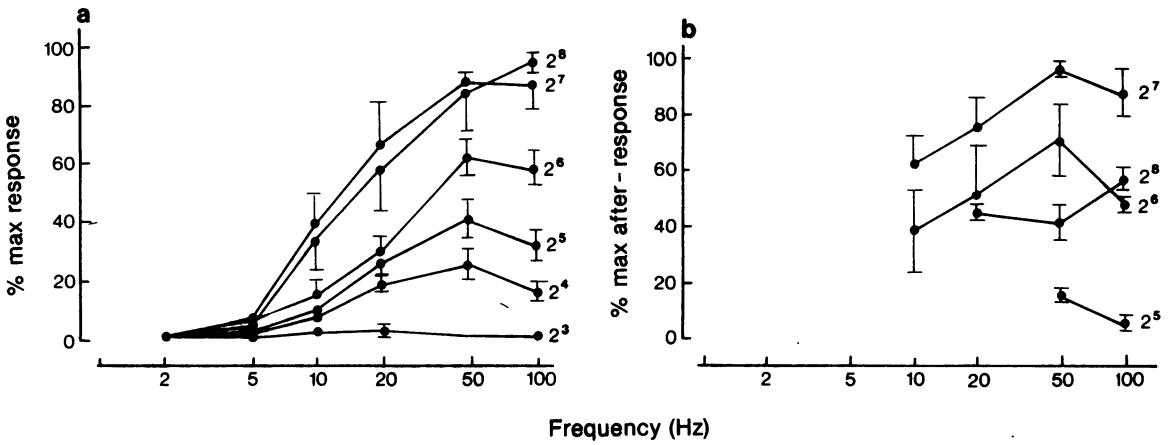

Figure 3 (a) Log frequency-response graphs for transmurally stimulated perfused guinea-pig vasa at train lengths from $8(2^3)$ to $256(2^8)$ pulses per train. Each point is the mean of measurements made on vasa from 6 guinea-pigs; vertical lines show s.e. means. Pulse width 0.1 ms supramaximal voltage. Abscissa scale: frequency in Hz on a log scale. Ordinate scale: rise in pressure expressed as a % of the maximum response obtained. (b) Log frequency—*after* response for transmurally stimulated perfused guinea-pig vasa at train lengths from $32(2^5)$ to $256(2^8)$ pulses per train. Each point is the mean of measurements made on vasa from 6 guinea-pigs; vertical lines show s.e. means. Pulse width 0.1 ms, supramaximal voltage. Abscissa scale: frequency in Hz on a log scale. Ordinate scale: rise in pressure expressed as a % of the maximum response obtained.

In another series of experiments the frequency of stimulation was varied to obtain three-point log frequency-response curves for the two preparations. The effects of guanethidine on the responses of strip and perfused preparations to stimulation at 5, 10 and 20 Hz (supramaximal voltage and 0.1 ms pulse width constant) were measured. Guanethidine (in Krebs solution to give a final concentration of 1×10^{-5} M) completely abolished the response to 5 Hz and considerably reduced that to 10 Hz and 20 Hz (Figure 6). Prolonged washing with guanethidine-free Krebs solution reversed this effect only very slightly at 20 Hz. However, 2×10^{-5} M dexamphetamine restored the log frequency-response curves near to, or

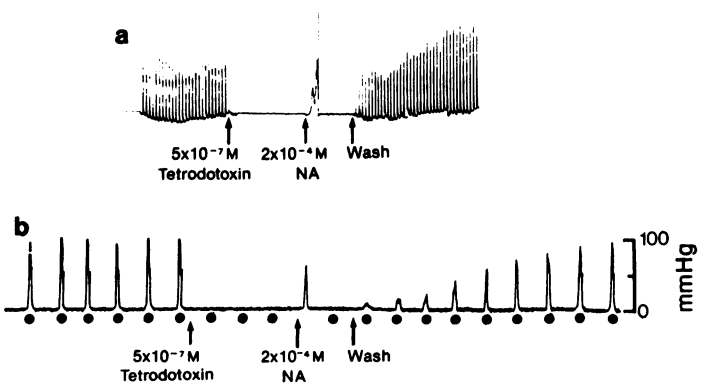

Figure 4 The effect of tetrodotoxin (5×10^{-7}M, final bath concentration) on the response of the 'Furchgott' strip preparation of the guinea-pig vas (a) and the perfused vas preparation (b) to transmural stimulation, every 4 and 8 min respectively (indicated by black dots in (b)). The voltage applied across the electrodes was 140 V, the pulse width was 0.1 ms and the frequency of stimulation was 10 Hz. The duration of the stimulus train was 20 s (200 pulses). At NA, noradrenaline was added to give a final bath concentration of 2×10^{-4}M, and washed out one minute later. At Wash, the Krebs solution in the tissue bath was replaced with fresh Krebs solution eight times.

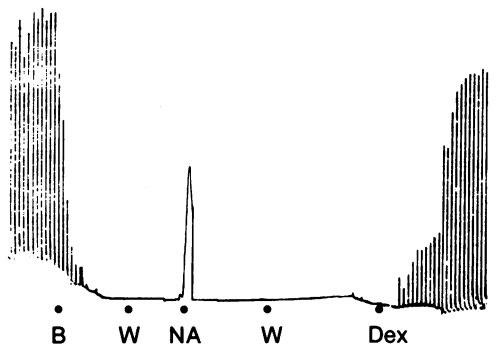

Figure 5 The effect of bethanidine (2×10^{-5} M at B) on the response of a Furchgott strip preparation to transmural stimulation (0.1 ms, 10 Hz for 20 s). At NA, noradrenaline was added to give a final bath concentration of 1×10^{-4} M. At W, the bath fluid was changed twice every 4 min over a period of 32 min. At Dex, dexamphetamine was added (2×10^{-5} M).

even above, control levels. The restoration for the perfused preparations was less complete than that for the strips.

It was noticed in about half of the perfused preparations that the after-response was more resistant to guanethidine blockade than the response. An example of this is shown in Figure 7. The response to transmural stimulation was reduced far more quickly than the after-response. However, the after-response was always eventually abolished by this concentration of guanethidine (1×10^{-5} M).

Noradrenaline

The effect of noradrenaline on 'Furchgott' strips and whole perfused preparations was investigated.

'Furchgott' strip. Noradrenaline contracted 'Furchgott' strips, an increase in concentration gave an increase in height of response from 8×10^{-6} M (threshold concentration) to 1×10^{-3} M (maximum) (Figure 8a).

Perfused preparation. Noradrenaline was administered to whole perfused vasa by two different routes; it was either added to the bath (extraluminal) or perfused (intraluminal). For these experiments, in addition to measurement of pressure change in the perfusion system, the upper stainless steel tube was freed from the clamp and was attached by thread to an isometric transducer to measure tension developed by the longitudinal muscle.

Extraluminal noradrenaline. Noradrenaline produced no pressure response at concentrations up to 10×10^{-4} M and at concentrations above this (2 and 4×10^{-4} m) the pressure response was very small ($n = 5$).

A graded tension response was given to noradrena-

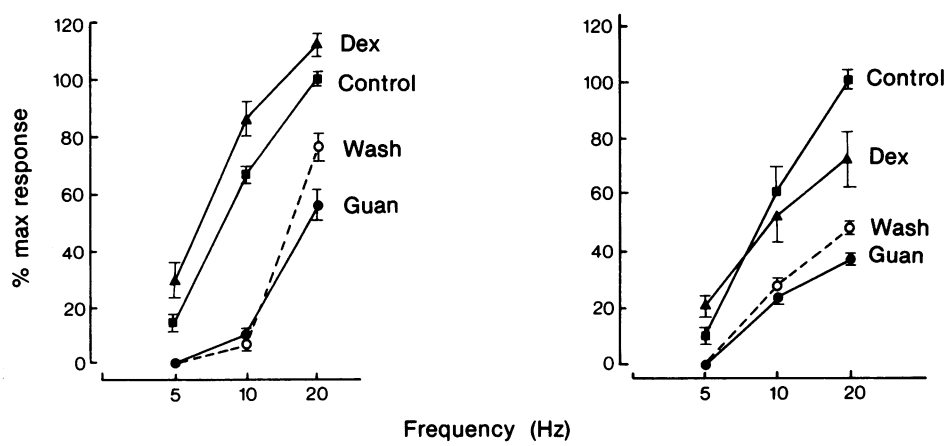

Figure 6 The effect of guanethidine (Guan, 2×10^{-5} M final bath concentration), washing (Wash, i.e. replacement of guanethidine-containing Krebs solution by guanethidine-free Krebs solution six times in 8 min) and dexamphetamine (Dex, 2×10^{-5} M final bath concentration) on the log frequency-response curves of the transmurally-stimulated 'Furchgott' strip and perfused preparations of the guinea-pig vas. Each point is the mean of measurements made on vasa from three guinea-pigs; vertical lines show s.e. means. The voltage applied across the electrodes was 140 V and the pulse width was 0.1 ms. Abscissa scale: frequency of stimulation in Hz on a log scale. Ordinate scale: contractile response expressed as a % of the maximum response obtained.

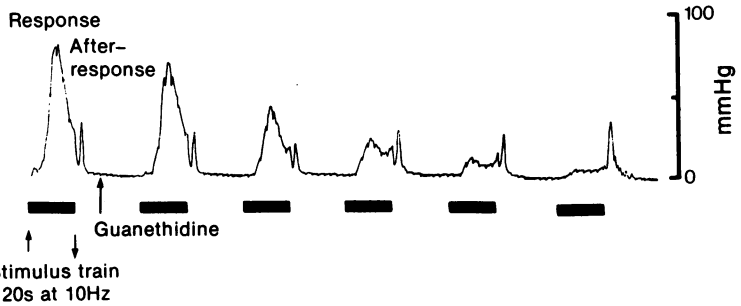

Figure 7 The effect of guanethidine (1×10^{-5} M final bath concentration) on the response and the after-response of the transmurally-stimulated perfused vas preparation. The voltage applied across the electrodes was 140 V, the pulse width was 0.1 ms and the frequency of stimulation was 10 Hz. The horizontal black bars represent the duration of the stimulus period (20 s) which was delivered every 8 minutes.

line added to the bath, from 4×10^{-7} M (threshold) to 4×10^{-4} M.

Intraluminal noradrenaline. When noradrenaline was perfused for 1 min periods, small concentration-dependent pressure responses were given to 10^{-7} M, 10^{-6} M, 10^{-5} M and 10^{-4} M noradrenaline ($n = 3$). These responses, although small, were larger than those given to noradrenaline added to the bath.

No tension response was obtained to perfused noradrenaline, even at a concentration of 10^{-4} M noradrenaline.

Thymoxamine

Log dose-response curves to noradrenaline were established for 'Furchgott' strip preparations in the absence and in the presence of the α-adrenoceptor blocking agent, thymoxamine, in concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M ($n = 5$). Thymoxamine produced parallel shifts of the log dose-response curves to the right and this antagonism was surmountable by high concentrations of agonist (Figure 8b).

Discussion

When strips of vas deferens were prepared by the method of Furchgott & Badrakom (1953) or of Vane (1957), the response to transmural electrical stimulation was a shortening of the length of the strip when isotonic recording was used or an increase in tension

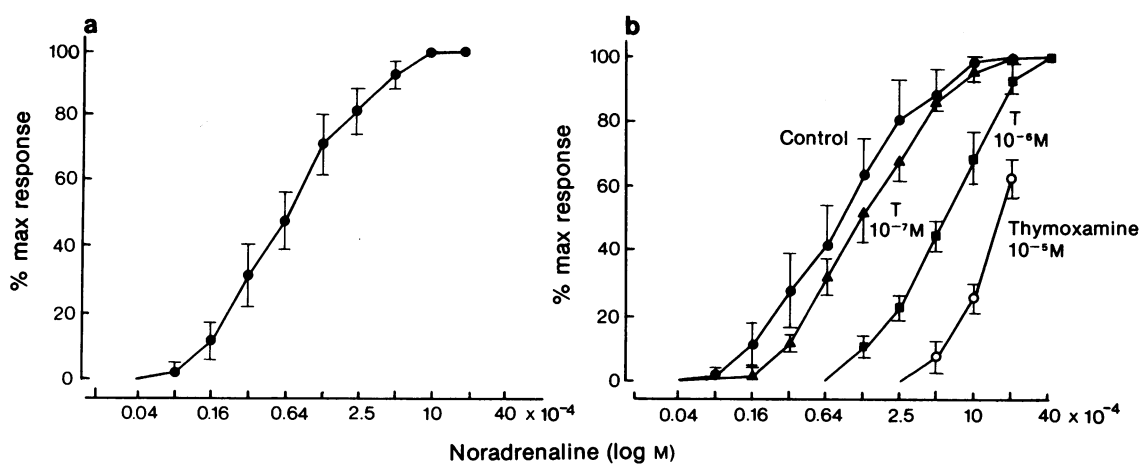

Figure 8 (a) Log dose-response graph for noradrenaline on the 'Furchgott' strip preparation. Each point is the mean of measurements made on the vasa from 5 guinea-pigs; vertical lines show s.e. means. (b) The effect of thymoxamine (T), 10^{-7} M (▲), 10^{-6} M (■) and 10^{-5} M (○) on the log dose-response curve to noradrenaline (●) for the 'Furchgott' strip preparation. Each point is the mean of measurements made on vasa from 5 guinea-pigs; vertical lines show s.e. means. Abscissae: concentration of noradrenaline on a log scale. Ordinates: contractile response expressed as % of the maximum response obtained.

when isometric recording was used. Histological examination of each strip showed that the majority of the smooth muscle fibres most likely to contribute to this response were running along the length of the strip and were derived from the layer of muscle referred to as the circular layer in transverse sections. A partial contribution from obliquely-disposed fibres from the longitudinal layer cannot be excluded but it is likely to be minor. Chains of rings, (Castillo & de Beer, 1947), prepared by tying together short lengths (2 to 3 mm) cut from a vas at right angles to its length, also shortened or produced tension when stimulated transmurally and the inference was that each ring decreased in diameter when its circular muscle contracted to contribute to the overall response of the chain. The method of preparation was tedious and seemed to offer no advantage over the strip preparations. The method of recording involving least interference with the integrity of the vas was the constant-flow luminal perfusion method of de la Lande & Rand (1965). The vas was fixed, at its resting length, at either end by the perfusion tubes to prevent shortening of the longitudinal fibres. Under these conditions transmural electrical stimulation produced a rise in perfusion pressure presumably due to a rise in resistance to flow, consequent upon a decrease in the bore of the lumen which seems most likely to be due to constriction produced by contraction of the circular fibres. The longitudinal muscle would also of course, be contracting, though not shortening, under these conditions and could conceivably contribute to the change in resistance. Experiments in which multiple transverse cuts distributed along the length of the vas and at a depth sufficient to sever the longitudinal fibres but not the circular fibres, produced no change in response and suggest that any effect of longitudinal muscle contraction is small.

The parameters of electrical stimulation necessary to produce the responses of the strip or the perfused preparations were very similar. Maximal responses for either technique were obtained at 0.1 ms pulse duration and at this value the log-frequency-response relations for the Furchgott strip or the Vane strip were virtually identical and were very similar to those for the perfused preparation. The reversible abolition by tetrodotoxin or by local anaesthetics of the response to transmural stimulation and the short duration of pulses necessary to elicit a response strongly argue in favour of the conclusion that transmural stimulation of strip preparations or of the perfused preparation produced responses due to stimulation of intramural nerve terminals in the circular muscle. These nerve terminals seem to have very similar characteristics to those which initiate the responses of the vas deferens set up in the conventional manner, when presumably the longitudinal layer of muscle mainly contributes to the response, as judged by the similarity of parameters of stimulation and frequency-response graphs (Day, 1965; Birmingham, 1966).

The results obtained with guanethidine and bethanidine indicate that the innervation of the circular fibres is, at least in part, by noradrenergic terminals. The adrenergic neurone blocking agents, guanethidine or bethanidine, gradually reduced and eventually abolished the contractile responses of strip preparations of circular muscle to transmural electrical stimulation. During the blockade, noradrenaline was still effective in contracting the muscle. The blockade had the characteristics of adrenergic neurone blockade in that it was not reversed by prolonged washing to remove the drugs but was quickly and completely reversed by dexamphetamine which is known to displace adrenergic neurone blocking agents from nerve terminals (Day & Rand, 1963). The frequency response curves for strip and for perfused preparations were both depressed by guanethidine; this depression was not reversed by washing but was antagonized by dexamphetamine.

Noradrenaline contracted the strip preparations and the competitive α-adrenoceptor blocking agent, thymoxamine, produced parallel shifts to the right of the log concentration-effect curve. The antagonism was surmountable with increased concentrations of agonist. These results indicated the presence of α-adrenoceptors on the circular smooth muscle fibres. The responses of the perfused vas deferens to noradrenaline depended on the route of administration. Noradrenaline produced concentration-dependent pressure rises when perfused intraluminally but not when applied extraluminally. On the other hand, tension increases were obtained from the longitudinal muscle on extraluminal application but not on intraluminal application of noradrenaline. These results confirm the expectation that it would be easier to produce effective concentrations at the circular muscle layer when the noradrenaline does not first have to traverse the longitudinal muscle layer.

We conclude from our results that it is possible to record the response of the circular muscle of the guinea-pig vas deferens by adoption of one or other of the techniques formerly used for other tubular organs containing smooth muscle. Some caution is necessary in the interpretation of the results because of the continued presence of the longitudinal fibres. It must be remembered when interpreting any results of an investigation of the possible neurotransmitters involved that the longitudinal muscle, though probably not involved to any large extent in the mechanical response, could be influencing the circular muscle indirectly by releasing from its innervation substances which diffuse to act on the circular muscle. It may be that the explanation of the after-response lies wholly or partly in this mechanism. The final solution

to this problem may only come with successful stripping of the longitudinal from the circular muscle.

It is known from fluorescence histochemical studies that the circular layer has a ground plexus of varicose nerve terminals as dense as that of the longitudinal layer and our results are consistent with the view that this is a noradrenergic innervation. However, this is not the only innervation; Gosling & Dixon (1972) have demonstrated a population of fluorescence-negative, cholinesterase-positive fibres predominantly in the circular layer. The significance of this innervation has yet to be determined and isolation of longitudinal and circular muscle responses may make this possible. The technique for recording of circular muscle response of the vas deferens by luminal perfusion (Anstey, 1971) has been used by Illés, Rónai & Knoll (1976) to show that the response to transmural stimulation with short trains of pulses is reduced by prostaglandin E_1 as has been demonstrated for some other noradrenergically-innervated tissues. They also confirmed the response of the circular muscle to noradrenaline and the antagonism by guanethidine of the response to electrical stimulation. More recently Anton, Duncan & McGrath (1977) used combined recording of longitudinal tension and the pressure response to luminal perfusion in a detailed analysis of the origin of the biphasic nature of the response of the rat vas deferens. Anton & McGrath (1977) also used luminal perfusion of the human isolated vas deferens in their investigation of the responses of the longitudinal and circular muscle layers to provide further evidence for adrenergic innervation.

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