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AN ANALYSIS OF THE ANATOMICAL BASIS FOR THE MECHANICAL RESPONSE TO MOTOR NERVE STIMULATION OF THE RAT VAS DEFERENS

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SUMMARY

1. An anatomical basis was sought for the biphasic motor nerve response of the rat vas deferens. The motor nerve pathway to the tissue was stimulated at different points between the vertebral outflow and the intramural fibres, in the pithed rat and in isolated tissues, to examine the possibility of two anatomically separate groups of neurones. Different preparations of the isolated tissue were devised to detect whether different groups of smooth muscle fibres contributed to the two phases.

2. The fibres mediating both phases of the response arose from the upper lumbar vertebral outflows. Both phases were elicited by pre- or post-ganglionic stimulation and could be depressed by hexamethonium. In the pithed rat or with hypogastric nerve stimulation in the isolated tissue, however, the initial 'twitch' phase was relatively resistant to such blockade.

3. When the rat vas deferens was perfused through the lumen *in situ* or *in vitro*, the perfusion pressure response to motor nerve stimulation exhibited two phases similar to those of the longitudinal contractile response.

4. Isolated rat vasa were bisected into portions, each of which was stimulated and longitudinal tension was recorded. The proportions of the two phases of the response varied along the length of the tissue. At the prostatic end the total response was relatively weak with a dominant 'twitch' and at the epididymal end the two phases were comparable in magnitude. The distribution of adrenergic nerve terminals within the muscle layers also varied along the length of the rat vas deferens.

5. The effects of drugs were investigated on the motor responses of the above preparations. The 'twitch' phase was relatively susceptible to blockade by reserpine and lysergic acid diethylamide and the 'secondary'

phase to phentolamine with both equally sensitive to guanethidine. Each phase had similar susceptibilities to blockade irrespective of which part of the tissue was involved.

6. It was concluded that two types of nerve-muscle transmission may be involved in the rat vas deferens with the proportion of each varying along the length of the tissue but both displaying pharmacological characteristics of adrenergic fibres.

INTRODUCTION

Langley & Anderson (1895) demonstrated in several species that stimulation of the upper lumbar nerves produced contraction of both longitudinal and circular muscle of the ipsilateral vas deferens and that this motor pathway ran via the hypogastric nerve. Subsequently it was found that the ganglia in this pathway lay along the hypogastric nerve within a few cm of the effector tissue (Sjostrand, 1962; Kuriyama, 1963; Ferry, 1967) so that although the pathway was anatomically sympathetic, the post-ganglionic fibres were unconventionally short. In most species examined, the smooth muscle of the vas deferens receives a dense plexus of adrenergic fibres (Sjostrand, 1965), noradrenaline contracts the tissue (Hukovic, 1961) and stimulation of the hypogastric nerve results in an overflow of endogenous noradrenaline from the tissue concomitant with the contraction of the longitudinal muscle (Hughes, 1972). Together with the observation that adrenergic neurone blockers, such as guanethidine, block this motor response (Bentley & Sabine, 1963; Birmingham & Wilson, 1963) these findings indicate that in this tissue, as in the majority of others the transmitter released from the post-ganglionic sympathetic nerve terminals is noradrenaline.

Swedin (1971), however, demonstrated that in rat and guinea-pig the mechanical response of the vas deferens to motor nerve stimulation is complex, consisting of two phases, an initial rapid 'twitch' response which declines to be replaced by a slower better maintained 'secondary' response. These two responses display differences in their pharmacological properties, being differentially blocked by prostaglandins (Euler & Hedquist, 1969), phentolamine (Swedin, 1971), reserpine (Gillespie & McGrath, 1974) and lysergic acid diethylamide (Gillespie & McGrath, 1975). Both components are blocked by guanethidine (Swedin, 1971). In addition the two components are differentially affected by modification of the bath temperature when the tissues are examined *in vitro* (Birmingham & Freeman, 1976).

The purpose of the present investigation was to determine whether there is an anatomical basis for the two components of the mechanical

response in the rat vas deferens. First the motor nerve pathway was examined by observing the response of the whole vas deferens to electrical stimulation of the nerves at different sites. Secondly, the possibility was investigated of the longitudinal and circular muscle layers being differentially involved in the production of the biphasic longitudinal response to motor nerve stimulation. Thirdly, the tissue was cut transversely into portions to determine whether the two components of the response were present in equal proportions along its length. With each preparation the effects of some blocking drugs on the responses were analysed.

A preliminary communication of some of these results has been published (Duncan & McGrath, 1976).

METHODS

Pithed rat

Male Wistar rats (250–300 g) were pithed by the method of Gillespie, MacLaren & Pollock (1970) under nitrous oxide/halothane anaesthesia.

The moveable 10 mm long stainless steel electrode at the tip of the pithing rod was placed to stimulate the appropriate vertebral outflows as described in the Results. (stimulation parameters; 1 msec pulses, supramaximal voltage, frequencies in text). The position of the electrode within each rat was estimated during the experiment and verified post-mortem by radiography.

The isometric tension in the anococcygeus muscles was recorded as described by Gillespie & McGrath (1973). Systemic blood pressure was recorded from one femoral artery and heart rate was extracted electronically from this.

Isometric tension in one or both vasa deferentia was recorded from the freed epididymal end. The prostatic end was fixed to the underlying cork board by means of a steel pin. In some experiments the isometric tension in one vas was recorded and a polythene cannula was introduced into the lumen of the contralateral vas at the epididymal end allowing perfusion towards the prostatic end. Krebs bicarbonate solution was perfused at a constant rate of 20 or 40 μl./min using a Palmer slow injection apparatus and perfusion pressure was recorded from a side branch 1 cm from the vas using a Statham P23A pressure transducer. The perfusate usually escaped via the penis without obstruction, the vas itself providing almost all of the resistance, but in a few cases an incision was made at the base of the adjacent seminal vesicle to allow egress of the perfusate.

Throughout the experiment both vasa lay within a pool of prewarmed liquid paraffin formed within the abdomen. Drugs were administered via a cannulated femoral vein. Pancuronium bromide (1 mg/kg; I.V.) was given to prevent skeletal muscle twitching. All parameters were displayed on a Grass Polygraph.

Isolated vasa deferentia

Whole vasa were isolated from male Wistar rats (250–300 g) killed by a blow on the head and exsanguination.

All vasa were placed either whole or divided transversely into sections, as described in the Results, in a Perspex bath (cross-sectional area 16 mm^2) heated to 37 °C, through which Krebs bicarbonate solution preheated to 37 °C and gassed with 95% O_2:5% CO_2 was perfused at a constant rate of 1–5 ml./min. In this way the solution bathing the tissues was constantly renewed. Threads were sewn through the wall of the vas at each end so that the lumen was not obstructed. One end was

attached to a fixed point near the inflow of Krebs and the other to either an isometric tension transducer (initial resting tension of 0·5–1·0 g) or to an isotonic length transducer (Mercury Electronics; load 0·5–1·0 g, sufficient to return preparation to pre-stimulus length without constant lengthening).

Where appropriate, vasa were cannulated via the fixed end with polyethylene cannulae (o.d. 0·75 or 1·02 mm) and Krebs bicarbonate solution pre-warmed to 37 °C was perfused through the lumen using a slow injection apparatus. In this case a pressure transducer with a small volume dome (Elcomatic EM 750) was placed in series with the flow of the perfusate in order to reduce the dead-space.

Drugs were administered by pre-mixing them to the required value in Krebs solution and substituting this for the normal flow through the organ bath.

Field stimulation of the intramural nerves was achieved via parallel silver:silver chloride plate electrodes placed on either side of the tissue using a stimulator with a low output impedance (0·2 msec pulses, supramaximal voltage). This type of electrode was employed since preliminary experiments indicated that ring electrodes were unsuitable due to the variation in the response along the length of the tissue. In consequence it was necessary to ensure that the electrode ran parallel to the full length of the tissue to obtain a maximal response.

In six experiments rat vasa deferentia were removed with the hypogastric nerve attached, as described by Graham, Al Katib & Spriggs (1968). The vasa were placed in the bath in the usual way with the prostatic end fixed but the nerve was drawn through a side hole over bipolar platinum electrodes for stimulation (0·1 msec pulses, supramaximal voltage). In this way the tissue could be stimulated in alternate periods via the hypogastric nerve or by field stimulation. All responses were recorded on a Devices M2 recorder.

Histology

In order to identify the direction of the smooth muscle layers, tissues were stained with haematoxylin and eosin. Fluorescent adrenergic nerve terminal were located by exposure of freeze-dried tissues to formaldehyde vapour by the method of Hillarp and Falck as modified by Gillespie & Kirpekar (1966).

Measurement of responses

The maximum tensions developed by the 'twitch' and 'secondary' responses were measured separately as shown in Text-fig. 1*B*. In the whole vas and in the absence of drugs this was straightforward. Where the two components were not clearly distinguishable, the two measurements were taken at the same time after the start of stimulation as was found in control responses for that tissue. In measuring each of the 'twitch' and 'secondary' responses part of both components will be contained in each measurement. This results in a distortion not only of the height but also of the time of the maxima, especially for the 'twitch'. For this and other reasons, when the tissue is bisected into segments the 'twitch' and 'secondary' responses cannot be simply summed for comparison with the 'whole' vas response.

An adequate perfusion rate and sensitivity of the recording system was critical for the development of the two phases of the perfusion pressure response to motor nerve stimulation. In some preliminary experiments, where the 'dead space' volume between the perfusion syringe and the tissue was greater and the perfusion pressure was monitored via a side tube, no clear 'twitch' component was detected. In an earlier study in anaesthetized rats, Chang, Chang & Su (1967) measured the intraluminal pressure in a blind sac of vas deferens as described by Langley & Anderson (1895) and found only one component to the response during hypogastric nerve stimulation. Evidently this latter preparation also did not respond rapidly

enough to detect the twitch component. An analogous situation applies to the interpretation of longitudinal muscle responses when they are recorded isotonically. Whether or not the two phases are distinctly separable will depend on the frequency response characteristics of the recording system and on the relative rate of rise and magnitude of the two components of the response. As Fig. 4 shows, isometric recording gives a clearer measure of each response in the rat vas deferens. The 'twitch' response may, however, still be submerged within the 'secondary' response if the latter is larger and this will particularly apply to the epididymal end of the tissue. The suitability of the recording system is thus crucial when interpreting the relative responses obtained between species and by different workers. In the present case the high frequency 'twitch' component of the isometric response signal was unaffected by a single stage Sallen and Key low-pass filter of 3 db down at 33 Hz but was attenuated and prolonged by a similar filter of 3 db down at 10 Hz. No filters of lower frequency than the former example were, therefore, employed.

Statistical comparison between groups was made with Student's *t* test. The numbers of tissues employed were as mentioned in the text or where qualitative results are quoted were verified on tissues from at least four animals.

The following drugs were used and concentrations are expressed as grams of the base in the experiments in the pithed rat and as molar in the experiments *in vitro*: pancuronium bromide (Organon), reserpine (crystalline, Koch-Light), guanethidine suphate (Ciba), hexamethonium bromide (Koch-Light), lysergic acid diethylamide (Sandoz), noradrenaline bitartrate (Koch-Light) and phentolamine mesylate (Ciba).

The Krebs bicarbonate solution employed had the following composition (mM): NaCl, 119; KCl, 4·7; $MgSO_4$, 1·0; KH_2PO_4, 1·2; CaCl, 2·5; $NaHCO_3$, 25·0; glucose, 11·1; and was gassed with 95% O_2:5% CO_2.

RESULTS

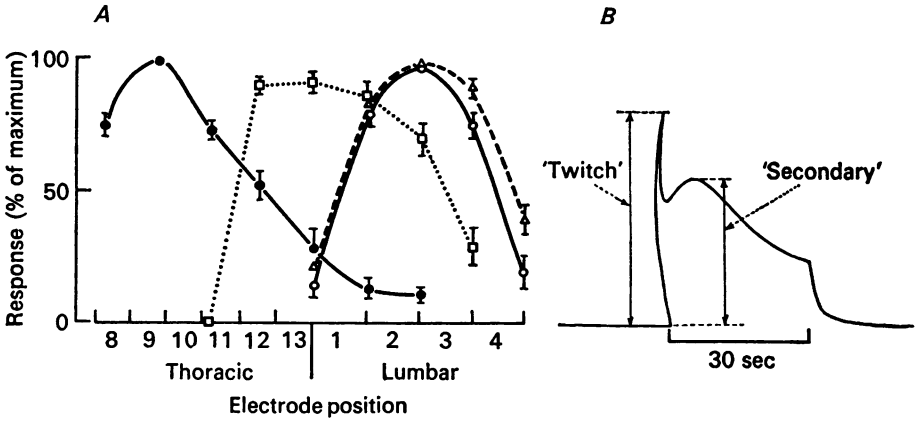
Anatomical origin of the motor pathway

Pithed rat – vertebral origin of motor response

The vertebral outflows were stimulated by placing the pithing rod electrode at successive 10 mm intervals along the spinal canal and stimulating at each position for 10 sec with 1 msec pulses at 30 Hz. Records were made of the response of vas deferens, of the anococcygeus muscles and of the arterial blood pressure. Each response at each position was expressed as a percentage of the maximum response. In the case of the vas deferens the two components of the response were analysed separately. Text-fig. 1 shows that the position for the maximum responses were different for the blood pressure (T9), anococcygeus (T12–L1) and vas deferens (L2–3) but that the two components of the vas deferens response were evoked from a common location. The only difference between the two components was that at L4 a relatively larger proportion of the 'twitch' component was present ($0{\cdot}01 > P > 0{\cdot}001$).

In five rats the lumen of one vas deferens was perfused with Krebs solution at a constant rate and the perfusion pressure recorded, while in the contralateral vas the isometric longitudinal tension was recorded. Text-fig. 2 shows that under these conditions, both responses of both

vasa were biphasic and shared an optimum vertebral origin. Also visible in Text-fig. 2 is a late rise in perfusion pressure at L1–2 and T12–L1 which parallels a rise in heart rate. This is a response due to the release of catecholamines from the adrenal medulla and at more rostral positions within the thoracic vertebrae, where no direct response to stimulation is found, is the only vas deferens response.



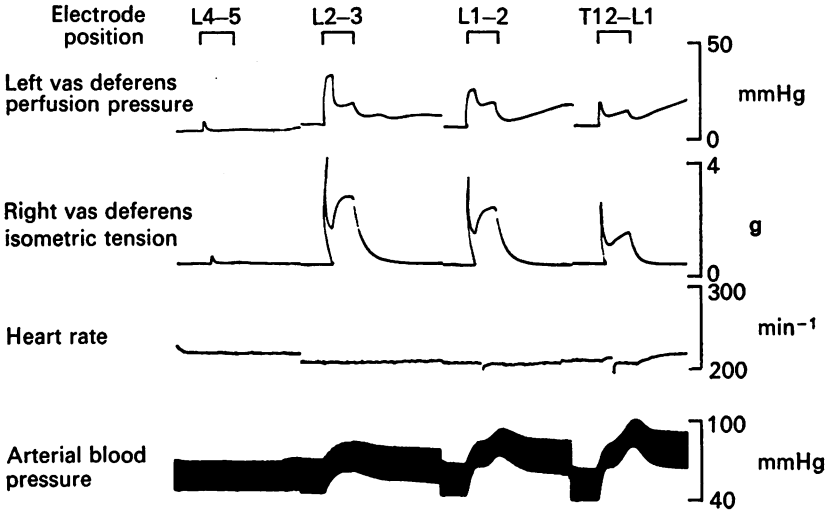
Text-fig. 1. *A*, pithed rat. Stimulation of vertebral outflow at different levels giving pressor responses and longitudinal isometric responses of anococcygeus and vas deferens represented as a percentage of maximal responses. Stimulation; 10 sec at 30 Hz (n = 6). ●—●, mean arterial blood pressure; □.....□, isometric anococcygeus tension; △---△, isometric, longitudinal vas deferens tension, 'twitch'; ○—○, isometric, longitudinal vas deferens tension, 'secondary'. *B*, typical longitudinal isometric response of rat vas deferens. Stimulation 30 sec at 30 Hz at L2–3. Measurement of 'twitch' and 'secondary' responses is indicated.

Effects of blocking drugs

Blocking drugs were tested against the isometric tension responses of the vas deferens to stimulation at the optimal position: L2–3.

Reserpine (3 mg/kg) and lysergic acid diethylamide (200 μg/kg) preferentially inhibited the initial 'twitch' component of the response (Text-fig. 3*A*, *B*). In the case of reserpine this blockade was not complete until two to five stimulation periods after administration. Repetitive stimulation after reserpine resulted in a reduction and change of shape of the 'secondary' response also (Text-fig. 3*A*). Hexamethonium (1 mg/kg) preferentially inhibited the 'secondary' component of the response but recovery was rapid (Text-fig. 3*C*). Guanethidine (10 mg/kg) or hexamethonium (5 mg/ kg) depressed both components of the response and phentolamine (2 mg/kg) preferentially inhibited the 'secondary' component.

As a control on the efficacy of these blocking drugs, responses of the anococcygeus muscles and blood pressure to upper lumbar sympathetic stimulation were always recorded. These responses were either abolished or considerably reduced by the doses employed of hexamethonium, guanethidine and phentolamine but were not acutely affected by reserpine or LSD.



Text-fig. 2. Pithed rat. Comparison of longitudinal isometric response with perfusion pressure response in vas deferens. Stimulation at 30 Hz for 10 sec at 5 min intervals with electrode in different vertebral positions. Trace was interrupted between periods of stimulation.

Isolated rat hypogastric nerve–vas deferens in vitro

Motor responses (isometric longitudinal) were produced in isolated vasa deferentia by alternately stimulating the hypogastric nerve (20 Hz 0·1 msec pulses, 20 sec) and stimulating the intramural nerves by field stimulation (20 Hz, 0·2 msec pulses, 20 sec). Both components of the response to hypogastric nerve stimulation were inhibited by hexamethonium in a dose dependent manner but the initial 'twitch' component was relatively more resistant to blockade. In contrast neither component of the response to field stimulation was inhibited by hexamethonium in doses up to 10^{-4} M (Table 1).

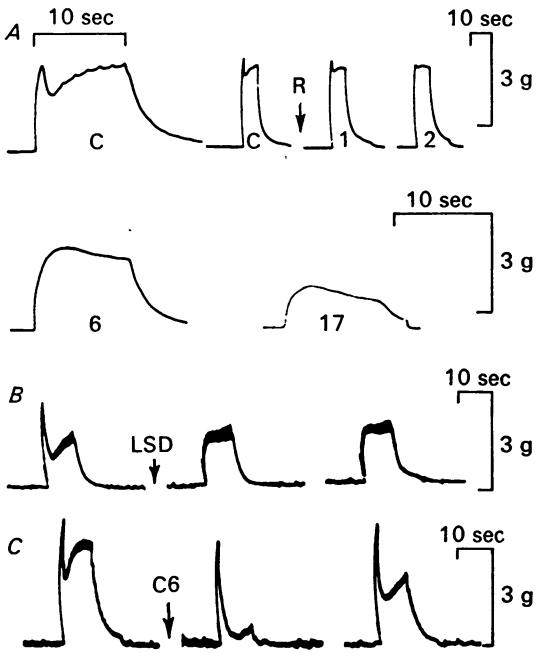
Comparison of the size of responses following different methods of stimulation

Table 2 shows the size of both components of the isometric longitudinal response of vasa to stimulation at 30 Hz at different points in the motor pathway.

The responses in the pithed rat and to field stimulation *in vitro* were of similar magnitude whereas those to stimulation of the hypogastric nerve were smaller and were more variable.

Longitudinal compared with circular responses

In the pithed rat the vas deferens perfusion pressure response to field stimulation was biphasic (Text-fig. 3). The biphasic nature of this response was dependent on the rate of perfusion and hence on the sensitivity of the recording system as shown *in vitro* in Text-fig. 4. At low rates of



Text-fig. 3. *A*, the effect of reserpine (R: 3 mg/kg, I.V.) on the response of the rat vas deferens (isometric longitudinal tension) to stimulation in the vertebral canal at L2–3, for 10 sec at 30 Hz every 4 min. Two control responses are shown (C) and the responses following reserpine are numbered (1–18). At the first control and nos. 6 and 17 the paper speed was increased to illustrate the time course of the response. *B*, the effect of lysergic acid diethylamide (LSD: 200 μg/kg, I.V.). Stimulation at L2–3 for 10 sec at 10Hz. *C*, the effect of hexamethonium (C6: 1 mg/kg, I.V.). Stimulation as in *A*.

perfusion the 'twitch' component was absent from the perfusion pressure records but not from the longitudinal response. As the perfusion rate was increased the 'twitch' component gradually emerged until an optimum was reached at which the 'twitch' and 'secondary' components were

present in similar proportions to those found in the longitudinal response. Further increases in perfusion rate beyond this level produced no further change in the response.

This difference in sensitivity of the 'circular' muscle response with different perfusion rates is similar to the difference in the longitudinal muscle response found when comparing isometric with isotonic recording. A comparison of Text-fig. 4*A* and *B* illustrates that with isotonic recording

TABLE 1. Effect of hexamethonium on the isometric longitudinal responses of the rat, isolated, whole vas deferens preparation to (1) hypogastric nerve stimulation, 20 Hz, 0·1 msec pulses, supramaximal voltage, 20 sec, (2) field stimulation, 20 Hz, 0·2 msec pulses, supramaximal voltage, 20 sec. Stimulation was alternately (1) or (2) with 4 min intervals. Steady-state responses are expressed as a percentage of pre-drug controls ± S.E. of mean (n = 6).

Concn. of hexamethonium (M)	Response (% of control)			
	(1)		(2)	
	Twitch	Secondary	Twitch	Secondary
10^{-8}	93 ± 4	90 ± 3	100 ± 2	99 ± 2
10^{-7}	99 ± 4	69 ± 4	98 ± 3	94 ± 2
10^{-6}	94 ± 3	61 ± 6	100 ± 2	98 ± 3
10^{-5}	83 ± 3	49 ± 4	102 ± 3	98 ± 2
10^{-4}	43 ± 6	24 ± 4	101 ± 3	99 ± 3

TABLE 2. Comparison of the amplitude of responses of the whole rat vas deferens to stimulation of its motor nerves at different sites. Stimuli were at 30 Hz for 10 sec. Mean responses ± S.E. of mean are indicated

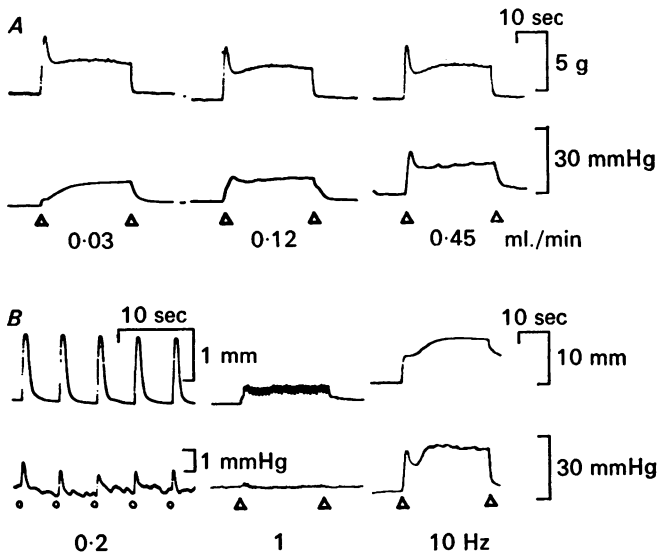
Preparation ...	Pithed rat	*In vitro*	*In vitro*
Site of stimulation ...	L2–3	Hypogastric nerve	Field stimulation
n	12	6	12
Twitch (g)	4·1 ± 0·3	2·9 ± 0·6	5·5 ± 0·4
Secondary (g)	3·3 ± 0·3	2·3 ± 0·8	3·7 ± 0·3

the separation of the two components of the motor response is less distinct. The two components are clearly visible by their different rates of rise and are obvious from the perfusion pressure record, but compared with the isometric recording in Text-fig. 4*A*, the 'twitch' component is greatly attenuated. It is to be expected that an isotonic record will respond less rapidly to a fast-onset, short-lived event such as the 'twitch' response and this emphasizes the importance of the recording method when investigating such phenomena.

The frequency/response characteristics of the 'longitudinal' and 'circu-

lar' muscle responses are illustrated in Text-fig. 4*B*. Both responses are produced by single pulses but, as a proportion of the maximal responses at high frequencies, the circular responses are relatively weak at low frequencies compared with the corresponding longitudinal responses. This was true whether the longitudinal response was isometric or isotonic.

Both circular and longitudinal muscle contracted in a dose dependent manner to noradrenaline 10^{-6}–10^{-4} M and these responses were inhibited

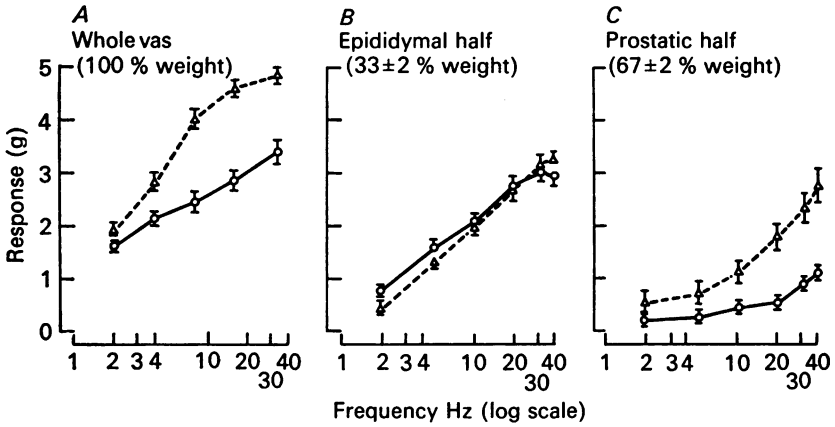


Text-fig. 4. The effect of perfusion rate and of stimulation frequency on the response of the isolated rat vas deferens to field stimulation between triangles. *A*, longitudinal isometric tension (upper) and perfusion pressure (lower) were recorded at various perfusion rates. Stimulation: 0·2 msec pulses at 10 Hz for 30 sec. The 'twitch' phase of the perfusion pressure response became apparent at higher perfusion rates. *B*, longitudinal isotonic shortening (upper) and perfusion pressure (lower) were recorded. Perfusion rate 0·45 ml./min. Stimulation: 0·2 msec pulses at 0·2–10 Hz for 30 sec. In the left hand panel the recorder sensitivity was increased to illustrate responses to single pulses (○). Note that the 'twitch' is less apparent in the isotonic recording compared with the isometric recording in *A*.

by phentolamine 10^{-6} M. The onset of the contraction to noradrenaline and of the blockade by phentolamine was relatively more rapid in the case of the longitudinal response as would be expected since the outer layer is of longitudinal muscle.

Lysergic acid dithylamide (10^{-7} M) preferentially inhibited the 'twitch' components, phentolamine (10^{-6} M) preferentially inhibited the 'secondary' components while guanethidine (10^{-7}–10^{-5} M) inhibited all components of the circular and longitudinal responses.

The perfusion pressure responses to field stimulation were similar whether the vas was perfused through the prostatic end or the epididymal end. If the vas was perfused through the prostatic end and the tissue was transversely bisected leaving only the prostatic half, no response or only a very small response to field stimulation could be obtained. In contrast if the vas was perfused through the epididymal end and the prostatic half was removed the response was hardly altered. This indicates that most of the 'circular' muscle response originates in the epididymal half.



Text-fig. 5. Frequency/response curve for the isolated rat vas deferens when whole or bisected transversely into two pieces of equal length. Field stimulation, 0·2 msec pulses, for 20 sec period. At all frequencies 20 sec was sufficient to allow maximal development of both phases of the isometric longitudinal tension response: △---△, 'twitch'; ○—○, 'secondary'. The relative mean weights of the portions are indicated above the graphs (n = 8). Bars represent S.E. of mean.

Variation of the motor response along the length of the rat vas deferens

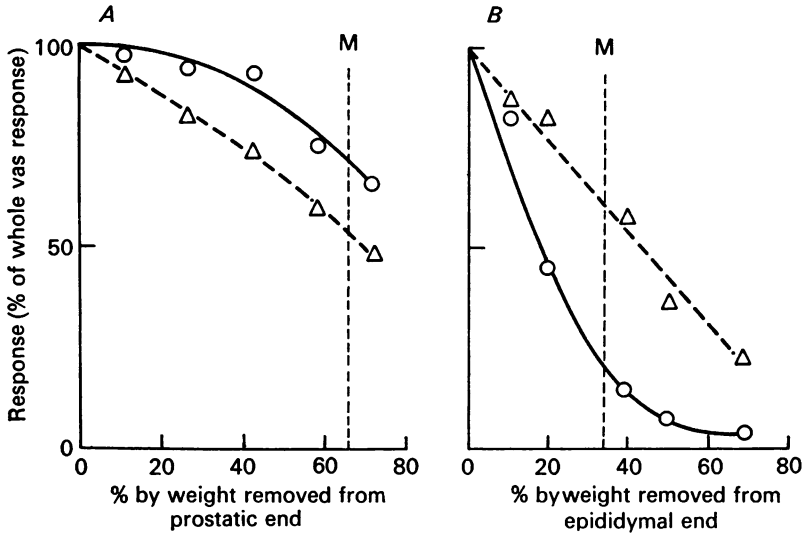
Transverse bisection

Since the perfusion pressure response to field stimulation in the rat vas had been found to be due mainly to the 'circular' muscle in the epididymal end of the tissue, vasa deferentia were transversely bisected into two equal lengths to examine the possibility of differences in the longitudinal responses. The contralateral vasa were left intact and frequency/response (longitudinal isometric) curves constructed comparing the whole vasa with the halves (Text-fig. 5). At each frequency the responses were largest in the whole vasa but comparing the two halves, the responses in the epididymal half were larger. The proportions of the two components of the response also differed markedly between preparations. In the whole

vasa the 'twitch' component dominated at all frequencies (Text-fig. 5*A*). In the epididymal half, however, the two components were of equivalent size with the 'secondary' component larger at all except the highest frequencies (Text-fig. 5*B*), while in the prostatic half, the 'twitch' component dominated (Text-fig. 5*C*).

Distribution of the response along the length of the vas deferens

The above established that the relationship between the two components of the motor nerve response was different in the two halves of

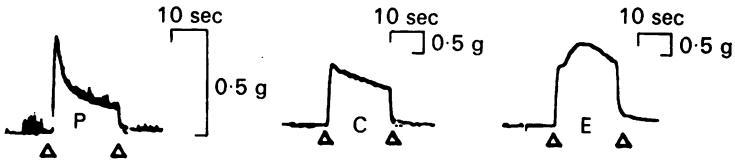


Text-fig. 6. The effect of removing portions from the rat vas deferens on the response of the remainder to field stimulation (20 Hz, 0·2 msec pulses for 20 sec period). Five portions were consecutively removed from each vas by transverse bisection, each approximately 15% of the weight of the whole vas. Tissue was removed from the prostatic end of one vas (*A*) and from the epididymal end of the contralateral vas (*B*). Responses of the isometric longitudinal tension are expressed as a percentage of those found initially in the whole vasa. Results from six rats were meaned with respect to the percentage response remaining and to the total weight of tissue removed at each stage. △---△, 'twitch'; ○—○, 'secondary'. The vertical dashed line (M) indicates the mean longitudinal linear mid-point of the vasa.

the tissue. In order to determine whether this relationship had two distinct forms or whether it changed gradually along the length of the tissue, whole vasa were set up and successive portions were removed from either the epididymal or prostatic ends. After each bisection, a response was produced to field stimulation of the remainder at 20 Hz for 20 sec and each component of the response was expressed as a percentage of the corresponding component found in the whole vas (Text-fig. 6).

Text-fig. 6 illustrates that as the tissue was removed from the prostatic end of the vas the 'secondary' component declined less and that when tissue was removed from the epididymal end, the 'secondary' component declined more than did the 'twitch' component. This demonstrates that both components are distributed along the length of the vasa, but that the 'secondary' component becomes relatively greater towards the epididymal end.

If the points are considered where half of the 'length' of the tissue has been removed it can be seen that when the epididymal half is left (Text-fig. 6*A*) a relatively large proportion of the 'secondary' component will remain, whereas when the prostatic half is left (Text-fig. 6*B*) only a small proportion of the 'secondary' component remains. This correlates with the observations in Text-fig. 5.



Text-fig. 7. The responses to field stimulation of isolated portions of rat vas deferens divided transversely into three portions of equal resting length. Recordings are of the isometric longitudinal tension. Stimulation parameters; 0·2 msec pulses at 5 Hz between triangles. P, prostatic third; C, central third; E, epididymal third. Note that the sensitivity of the recorder is greater for the prostatic third.

The gradually changing relationship of the two components of the response to field stimulation are illustrated in Text-fig. 7 which shows the responses obtained in three sections of equal length taken from one rat vas deferens.

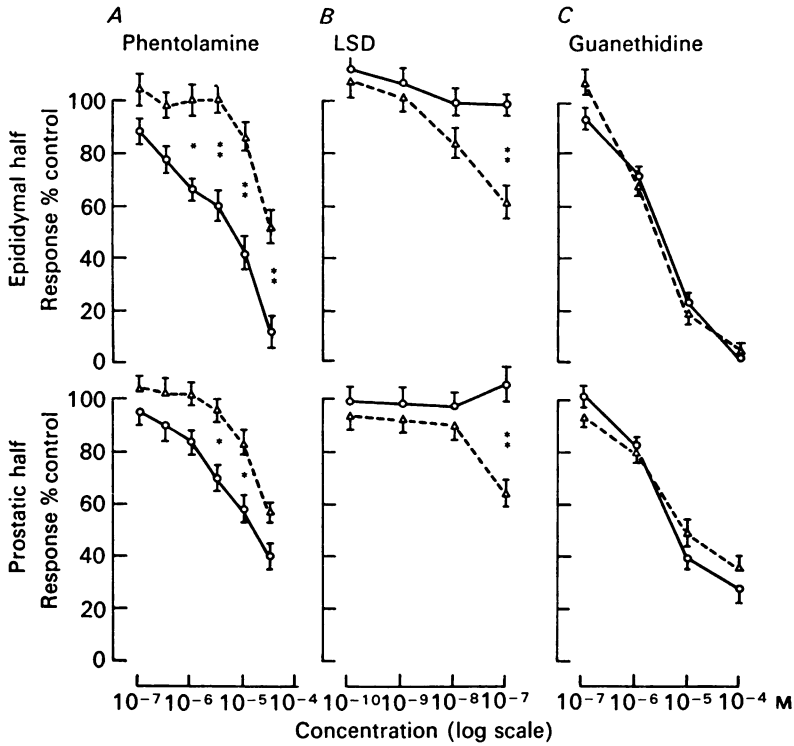
Effect of blocking drugs on the two halves of the vas deferens

In order to demonstrate whether the two components, 'twitch' and 'secondary' possessed similar pharmacological properties in the two halves of the vas, the effects of blocking drugs were assessed in the two halves against the longitudinal isometric responses to field stimulation at 5 and 20 Hz. The drugs chosen were phentolamine, lysergic acid diethylamide (LSD) and guanethidine since their effects on the whole tissue were known.

The effects of the drugs were similar against responses to 5 Hz and those to 20 Hz. Text-fig. 8 illustrates dose–response curves for the effects against 20 Hz stimulation.

Phentolamine had a similar effect on the responses in the two halves of

the tissue. Both components of the response to 20 Hz were inhibited in a dose dependent manner, but the 'secondary' response was inhibited by lower doses (Text-fig. 8 *A*). The responses to 5 Hz stimulation were inhibited in a qualitatively similar manner with the degree of inhibition of both components being greater at each dose.



Text-fig. 8. The effects of phentolamine, LSD and guanethidine on the responses of the two halves of isolated rat vas deferens to field stimulation (20 sec period, 20 Hz 0·2 msec pulses). Reproducible control responses were obtained before addition of drug and stimulation periods were repeated at 4 min intervals. Isometric, longitudinal tension responses were measured when equilibrium was reached between consecutive stimulation periods and are expressed as a percentage of the initial controls. △---△, 'twitch'; ○—○, 'secondary'. Asterisks denote the significance of the difference between the degree of inhibition of the 'twitch' and 'secondary' responses at each drug concentration. *, $0{\cdot}05 > P > 0{\cdot}01$; **, $0{\cdot}01 > P$. Bars represent S.E. of mean.

LSD had a similar effect on both halves of the tissue. The 'twitch' component to stimulation at 20 Hz was selectively inhibited by LSD 10^{-7} M (Text-fig. 8*B*). The 'twitch' response to 5 Hz stimulation was also selectively inhibited but the degree of inhibition at each dose was greater.

Guanethidine had a similar effect on both halves of the tissue. Both components of the response to stimulation at 20 Hz (Text-fig. 8*C*) and 5 Hz were equally inhibited in a dose dependent manner.

With each of the drugs tested, therefore, the effects on each component were as would be predicted from the effects on the whole vas.

Histological comparison of the two halves of vas deferens

Staining with H and E revealed two major differences between the epididymal and prostatic ends of the rat vas deferens. First, as would be expected from the external dimensions of the tisue the smooth muscle layers were thinner at the epididymal end (50–70 % of width at prostatic end). Secondly as has been demonstrated by previous authors (Dixon & Gosling, 1972; Pennefather, Vardolov & Heath, 1975) the inner circular and outer longitudinal layers were relatively discrete at the epididymal end while at the prostatic end bundles of circular and longitudinal fibres tended to be distributed within the longitudinal and circular layers respectively.

Formaldehyde-induced fluorescence revealed differences between the two ends of the tissue with respect to the distribution of adrenergic nerve terminals. At the prostatic end, a dense network of varicose fluorescent terminals was distributed evenly through the muscle layers. At the epididymal end however, the distribution of endings was less dense and concentrated towards the lumen so that the inner circular layer was relatively more densely innervated than the outer longitudinal layer (Pl. 1). The change in the distribution of the adrenergic terminals took place mainly within the epididymal half of the vas, the tissue in the centre of the organ being in this respect similar to that near the prostatic end.

DISCUSSION

These results demonstrate that in the rat vas deferens the biphasic mechanical response has an anatomical basis. The nerves mediating the two components are anatomically sympathetic sharing a similar vertebral origin. The smooth muscle at different points along the length of the vas however exhibits the two components in varying proportions, the 'secondary' component being relatively dominant at the epididymal end. The biphasic nature of the response is not due to differences in the circular and longitudinal muscle layers. The 'circular' muscle layer shows a biphasic response similar to that in the longitudinal layer and this response correlates with the longitudinal response at the corresponding point along the length of the vas. The two components of the response in the whole vas do not therefore reflect two post-synaptic events subsequent to release of transmitter from a single junction (Swedin, 1971) but rather indicate

differences in the nature of the junctional events at different points along the tissue.

Using the relatively specific pharmacological blockade of the 'twitch' component by LSD, the 'secondary' component by phentolamine and both components by guanethidine, each component exhibited a similar disposition towards blockade irrespective of (1) whether stimulation was pre- or post-synaptic, (2) whether recording was *in vivo* or *in vitro* and therefore whether the blocker was supplied via the blood stream or from diffusion from a physiological medium, (3) whether recording was of longitudinal or circular responses and (4) the relative proportions of the two components present at the given point along the length of the vas. This indicates that each component of the mechanical response has the same basis irrespective of where it occurs and suggests that the relative proportion of the two bases for the two components varies along the length of the tissue. The site of differentiation of the two components could, however, be either pre- or post-junctional.

Swedin (1971) proposed a post-junctional basis with the transmitter acting consecutively at two different sites on the smooth muscle. In this hypothesis the 'twitch' is due to receptors within the synaptic cleft while the 'secondary' response is due to more distant receptors on the muscle membranes. A major stumbling block to this latter hypothesis is the selective inhibition of the 'twitch' component by several drugs such as reserpine (Gillespie & McGrath, 1974) and lysergic acid diethylamide (Gillespie & McGrath, 1975) in the rat and prostaglandins (Euler & Hedqvist 1969) in the guinea-pig. These drugs are known to inhibit noradrenaline release from adrenergic nerves in vas deferens (Stjarne, 1973; Hughes, 1973), yet they can reduce the 'twitch' response but not the 'secondary' response. This suggests that there are differences in the pre-junctional events giving rise to the two components, those giving rise to the 'twitch' component being relatively sensitive to reserpine, LSD or prostaglandins. These pharmacological observations also reinforce the view that the 'twitch' as well as the 'secondary' response is mediated by noradrenaline.

Pre-junctional differences could exist either side by side in one type of nerve or in two nerve types as suggested by Ambache, Dunk, Verney & Zar (1972). The present results cannot finally answer this point. One observation which is difficult to explain in terms of one nerve type is however, the selective ganglionic blockade of the 'secondary' response by hexamethonium. This suggests that different ganglion cells are involved in the pathway leading to the 'secondary' response and that these are relatively sensitive to blockade by hexamethonium. The possibility of two anatomically distinct sets of ganglion cells is supported by the

observations of Graham *et al.* (1968) who found two groups of ganglion cells at different distances from the rat vas deferens: and differential sensitivity of different groups of ganglion cells to ganglion blockers is well documented (Paton & Zaimis, 1951; Hertzler, 1961; Volle, 1966).

The present observation with hexamethonium thus suggests the existence of two sets of post-ganglionic fibres mediating respectively the 'twitch' and 'secondary' components. Further investigation of the ganglion cells involved is indicated since Graham *et al.* (1968) demonstrated that 'twitch' responses of the rat vas deferens to hypogastric nerve stimulation were inhibited by atropine. Although this latter study did not distinguish between the two components, a muscarinic basis for the ganglion synapses mediating the 'twitch' cannot be excluded.

Ambache *et al.* (1972) suggested that the fibres mediating the 'twitch' are non-adrenergic whereas those mediating the 'secondary' component are adrenergic. While the present results confirm the relative resistance of the 'twitch' response to α-adrenoceptor blockers (Boyd, Chang & Rand, 1960) those drugs which did block the 'twitch' response, namely guanethidine, reserpine and LSD, are known to affect adrenergic mechanisms.

Guanethidine blocked the 'twitch' responses in doses identical to those blocking the 'secondary' response, which is assumed to be adrenergically mediated (Swedin, 1971; Ambache *et al.* 1972). Guanethidine is a highly specific drug owing part of its action to its accumulation by adrenergic terminals via the neuronal uptake process for noradrenaline (Chang, Costa & Brodie, 1965; Trendelenburg, 1972) and inhibits non-adrenergic neurones only in doses higher than those effective against adrenergic nerves (Dixit, Gulati & Gokhale, 1961).

The acute effect of reserpine was also indicative of an adrenergic mechanism for the 'twitch' response. Both in the present study with intravenous reserpine (3 mg/kg) to the pithed rat and in an earlier study (Gillespie & McGrath, 1974) where reserpine (200 μg/kg) was given intraperitoneally to the rat 4 hr before stimulation, the 'twitch' response was not abolished at once but declined with each subsequent train of pulses until it had disappeared. Von Euler (1969) made a similar observation on the 'twitch' response of guinea-pig vas deferens *in vitro* and suggested that reserpine prevented the refilling of a functional extra-granular noradrenaline pool from the granular pool, thereby explaining why this blockade was progressive. This might explain why the 'twitch' response declines after rapidly reaching its peak. With chronic administration of large doses of reserpine, both components of the vas deferens response are depressed and the residual response declines following each train of impulses (Hukovic, 1961; McGrath, 1963) as would be expected from the classical effects of reserpine.

The present finding that the motor fibres to the rat vas deferens originate from the upper lumbar nerves confirms the observation made by Langley & Anderson (1895) in other species, and also demonstrates that both mechanical components of the motor response are anatomically sympathetic. No clear separation of the two components of the vas deferens response could be made by selective stimulation of the vertebral outflow although responses in other tissues were clearly differentiated. One small difference was that with stimulation at L4 a relatively large 'twitch' component was found. This could indicate a difference in the origin of two types of fibre but the possibility of selective stimulation of fibres to the prostatic end of the tissue cannot be ruled out.

The response of the vas deferens to field stimulation was always larger than the corresponding response to hypogastric nerve stimulation in both the present study and whenever quantitative comparisons have been shown (Graham *et al.* 1968; Birmingham & Freeman, 1976). The responses of the pithed rat vas deferens to stimulation of the pre-ganglionic vertebral outflow were, however, of the same order as the responses of the isolated vas to post-ganglionic field stimulation, despite the contrasting mechanical and metabolic arrangements. This suggests that the relatively small responses from stimulation of the isolated hypogastric nerve are not a result of pre-ganglionic stimulation *per se* but due to the loss of part of the population of pre-ganglionic fibres during dissection.

The experiments with the perfused vas deferens were designed primarily to determine whether the two components of the longitudinal muscle response were also present within the circular layer. Macht (1917) demonstrated that circular or longitudinal strips cut from the vasa of several species react to spasmogens in a different manner from the whole organ. The present results, however, indicated no striking differences between the two layers in the rat vas, both 'twitch' and 'secondary' components being present.

Of the species tested so far, only human vas deferens displayed no 'twitch' component but rat, guinea-pig, mouse and rabbit vas deferens display similar distributions along their length of the two components of the longitudinal response with the 'secondary' response always dominant at the epididymal end and the 'twitch' component more obvious at the prostatic end (Anton & McGrath, 1977). Thus, whatever is the explanation for this, it is common to at least four species.

No simple correlation is evident between the mechanical response and either the thickness of the muscle layers or the distribution of adrenergic nerves through the tissue. For example the relative sparsity of adrenergic terminals in the longitudinal layer compared with the circular layer in the epididymal end of the rat vas deferens does not correlate with the powerful

adrenergic 'secondary' response in the longitudinal muscle. In addition, the noradrenaline content in $\mu g/g$ tissue of the epididymal half is only 70% of that in the prostatic half yet the former produces the larger motor nerve responses (J. C. McGrath & G. Powis, unpublished observations).

In conclusion, these results suggest that the biphasic motor nerve response of the rat vas deferens has an anatomical basis in that the time course of the response varies along the length of the tissue with two components being present to relatively different extents. The response of the whole vas depends, therefore, on the algebraic sum of these components. Two different types of synaptic transmission are suggested from the pharmacological experiments yet the properties of both are consistent with noradrenaline being the transmitter. Irrespective of the transmitter, however, caution is indicated in interpreting longitudinal responses of whole vas deferens in respect of the differences shown to exist along its length.

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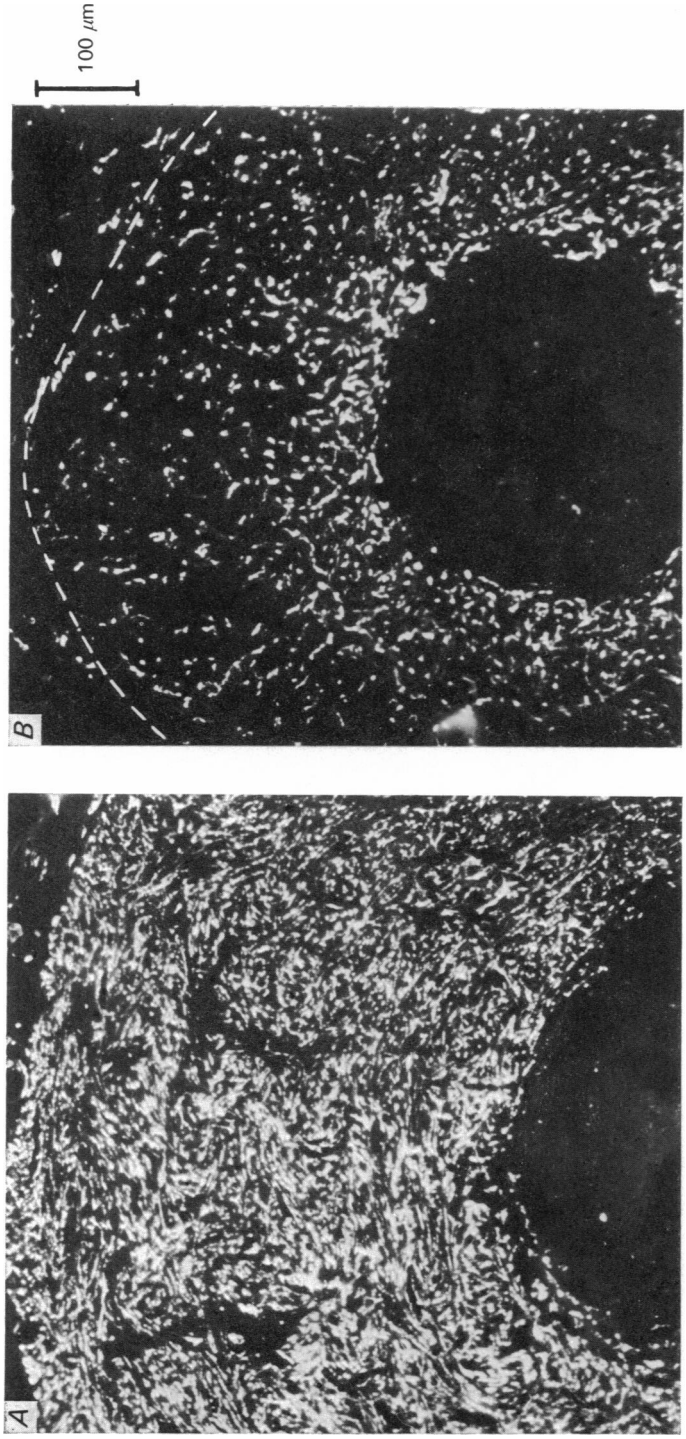
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EXPLANATION OF PLATE

Photomicrographs of the fluorescent adrenergic terminals in transverse sections from opposite ends of the rat vas deferens. *A*, tissue 5 mm from the prostatic end of the vas deferens. A dense plexus of adrenergic terminals extends evenly throughout the muscle layers. The boundaries of the muscle layers are marked by the limits of the fluorescence.

B, tissue 5 mm from the epididymal end of the same vas deferens as in *A*. Here the density of adrenergic terminals is greatest at the inner boundary of the smooth muscle layers and decreases towards the outside. The outer boundary of the smooth muscle is indicated by the dashed line. Both sections have the lumen visible at the bottom centre and were cut 6 μm thick.

Calibration for *A* and *B* 100 μm.