J. Physiol. (1977), **273**, *pp.* 45–55
With 6 text-figures
Printed in Great Britain

FURTHER EVIDENCE FOR ADRENERGIC TRANSMISSION IN THE HUMAN VAS DEFERENS

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(*Received 31 March 1977*)

SUMMARY

1. Isolated portions of human vas deferens responded to field stimulation of the intramural nerve fibres or to exogenously applied noradrenaline with rhythmical contractions of both longitudinal and circular muscle layers.

2. In guinea-pig and rabbit vas deferens field stimulation produced an initial rapid 'twitch' response which was not found with human vasa.

3. Responses of the human vas to field stimulation were depressed by the α-adrenoceptor blocking agents phentolamine and yohimbine.

4. It is concluded that the motor innervation of the human vas deferens is adrenergic and the relevance of this to the physiological operation of the tissue is discussed.

INTRODUCTION

Birmingham (1968) demonstrated that isolated portions of human vas deferens *in vitro* contract to field stimulation of their intrinsic nerves. These responses, but not the corresponding contractions to exogenously applied noradrenaline, were abolished by guanethidine suggesting that the motor innervation was adrenergic.

More recently the adrenergic nature of part of the motor nerve response in the vas deferens of several rodent species has been questioned (Ambache & Zar, 1971; Jenkins, Marshall & Nasmyth, 1976; Hedqvist & Euler, 1976), mainly due to (1) the relatively weak contractions produced by NA compared with those to nerve stimulation and (2) pharmacological observations including the resistance to α-adrenoceptor blocking agents of an early rapid 'twitch' component of the response to nerve stimulation. This transient 'twitch' precedes a better sustained 'secondary' response which is susceptible to α-adrenoceptor blockade (Swedin, 1971) and these two components have been shown to be differentially distributed along the length of the rat vas deferens, the 'secondary' component originating

mainly in the epididymal end of the vas (Pennefather, Vardolov & Heath, 1975; Duncan & McGrath, 1976).

The purpose of the present study was to demonstrate that in human vas deferens this 'twitch' component is not found and that the response in this species is comparable with an adrenergic innervation. To this end, the responses to field stimulation of the motor nerves were monitored in the circular and longitudinal smooth muscle layers of isolated portions of vas deferens from humans and from rodents.

METHODS

Portions of human vas deferens 2–5 cm long starting from within 3 cm of the epididymis were obtained from healthy male patients (30–50 yr) undergoing elective vasectomy. The tissues were transferred to the laboratory in iced Krebs bicarbonate solution after which they were allowed up to 4 hr to re-equilibrate to the experimental conditions.

Whole vasa were isolated from guinea-pigs (500–600 g) or mice (40–50 g, Parkes strain) killed by a blow on the head and exsanguination, and from rabbits killed by an overdose of sodium pentobarbitone.

All vasa were set-up either whole or divided transversely into sections, as described by Anton, Duncan & McGrath (1977), in a Perspex bath at 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. Isometric longitudinal tension, isotonic longitudinal shortening and luminal perfusion pressure were recorded as appropriate.

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. In a few cases noradrenaline was administered by substituting an appropriate solution for the perfusate through the lumen of the vas.

Field stimulation of the intramural nerves was achieved via parallel silver:silver chloride plate electrodes (0·2 msec pulses, supramaximal voltage) as described by Anton *et al.* 1977).

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 or patients.

The following drugs were used, and concentrations are expressed as molar: noradrenaline bitartrate (Koch-Light), phentolamine mesylate (Ciba) and yohimbine hydrochloride (Baird Pharmaceuticals).

The Krebs bicarbonate solution employed had the following composition (mM): NaCl, 119; KCl, 4·7; $MgSO_4$, 1·0; KH_2PO_4, 1·2; $CaCl_2$, 2·5; $NaHCO_3$, 25·0; glucose, 11·1; and was gassed with 95% O_2:5% CO_2.

RESULTS

Human vas deferens

The specimens of human vas deferens tested differed from rat, rabbit, mouse and guinea-pig vas deferens in having no initial rapid 'twitch' component to the response of either the longitudinal (isometric or isotonic) or circular muscle (see Figs. 1 and 6). No human vas tested responded to a single pulse. The threshold frequency was 2–5 Hz, maximal responses

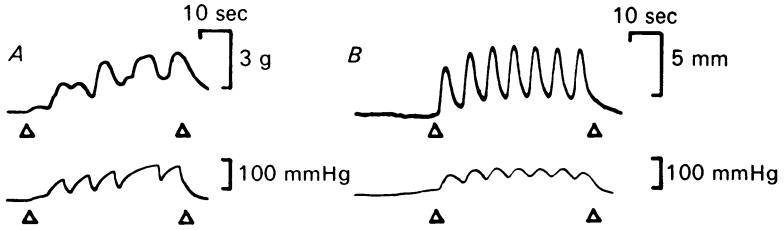

Fig. 1. The responses of isolated portions of human vasa deferentia to field stimulation. *A*, isometric longitudinal tension (upper record) and perfusion pressure (lower record). Field stimulation between the triangles produced rhythmic responses which were in phase on both recordings. Stimulation parameters: 0·2 msec pulses at 10 Hz. *B*, isotonic longitudinal shortening (upper record) and perfusion pressure (lower record). Field stimulation produced responses similar to those in *A*. Stimulation parameters: 0·2 msec pulses at 5 Hz. Stimulation for 20 sec between triangles. Perfusion rate was 0·45 ml./min.

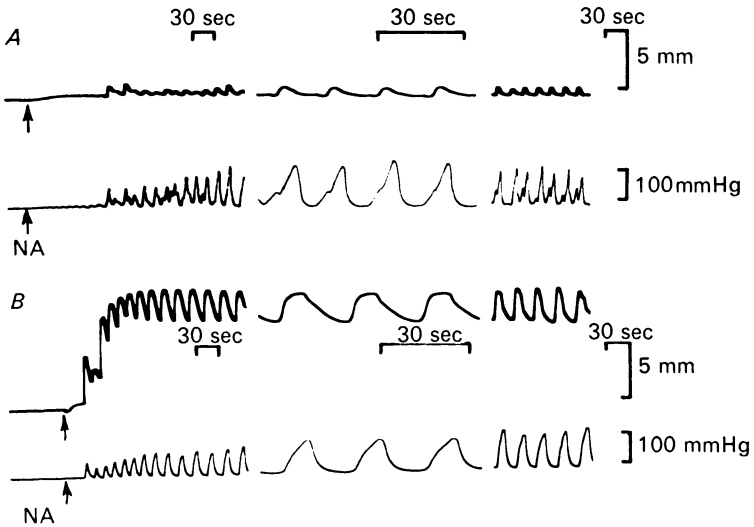

Fig. 2. The responses of an isolated portion of human vas deferens to noradrenaline (NA). This is the same tissue as in Fig. 1*B*. Isotonic longitudinal shortening (upper records) and perfusion pressure (lower records). The paper speed was increased in the centre panel. *A*, noradrenaline (10^{-5} M) was administered through the lumen in the perfusion fluid. Note the comparatively large response on the perfusion pressure. *B*, same tissue 30 min after return to control conditions. NA 10^{-5} M was administered via the Krebs solution bathing the outside of the tissue. Note that the longitudinal response is relatively large under these conditions. NA administered at arrows. Perfusion rate was 0·45 ml./min.

were obtained at 20–30 Hz (Fig. 3) and at all frequencies between 2 and 30 Hz the peak of the response was obtained after 10–30 sec (Fig. 1). A feature of human vas was, however, that on stimulation for prolonged periods the activity of the muscle occurred in slow waves rather than as a maintained contraction and these waves had a similar time scale in longitudinal and circular muscle.

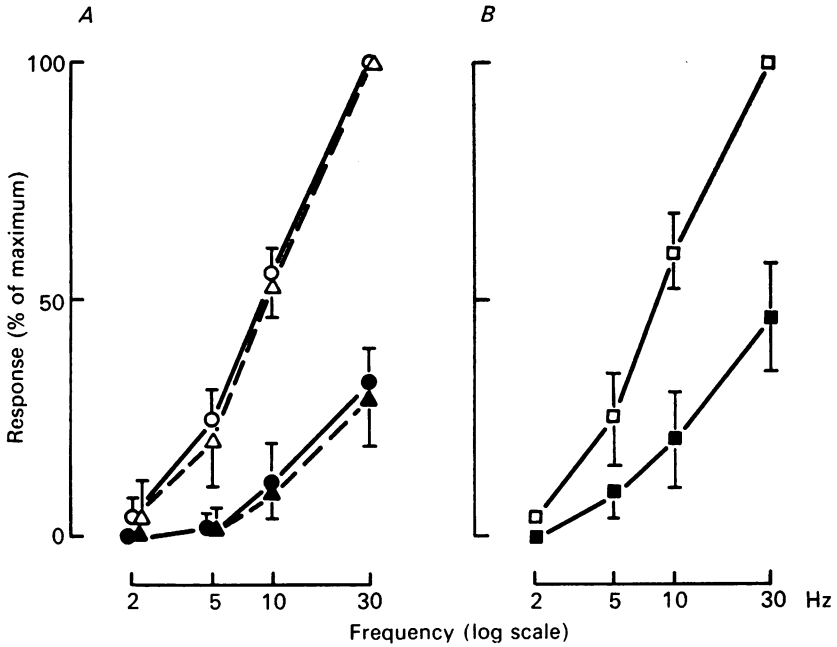

Fig. 3. Frequency/response curve for the effects of field stimulation on the human vas deferens. Stimulation at each frequency lasted 30 sec. For each parameter the area under the curve during the period of stimulation was integrated and the resultant responses are expressed as a % maximum response, i.e. response at 30 Hz. *A*, longitudinal responses; ○—○, isometric; △---△, isotonic. *B*, perfusion pressure response (□—□). Filled symbols represent responses in the presence of phentolamine 10^{-6} M. Bars indicate S.E. of mean. In each case $n = 6$.

As with the other species the human vas was contracted by noradrenaline (10^{-6}–10^{-4} M). The onset of the response to noradrenaline was more rapid in the longitudinal record if the noradrenaline was introduced to the outside of the tissue and more rapid in the perfusion record if the noradrenaline was perfused through the lumen. This latter observation also confirms that the longitudinal shortening and perfusion pressure response are reflecting respectively the activity mainly in the longitudinal (outer) layer or in the circular (inner) muscle layers. The response to noradrenaline

exhibited the same type of wave pattern as produced by nerve stimulation (Fig. 2*A* and *B*).

Both maintained level of tone and the frequency of the contractile waves increased with either increased frequency of nerve stimulation or increased concentration of noradrenaline. For this reason a quantitative measure of the responses was made by integrating the area between the

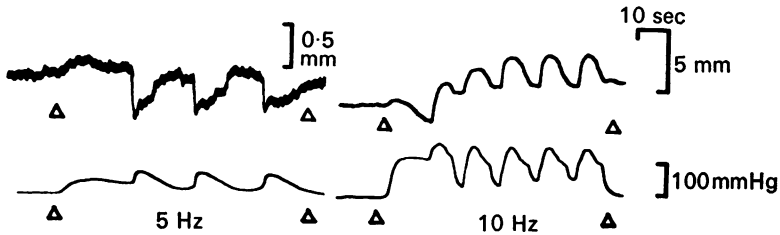

Fig. 4. Isotonic longitudinal response (upper record) and perfusion pressure response (lower record) in a portion of human vas deferens which exhibited lengthening during field stimulation. Stimulation parameters: 0·2 msec pulses, at 5 or 10 Hz between triangles. Note that the sensitivity of the isotonic recording was increased in the left panel. Perfusion rate was 0·45 ml./min.

response curve and the resting base line over a fixed period of time. Fig. 3 illustrates the frequency/response curve arrived at in this manner when the responses were expressed as a percentage of the relevant maximum and demonstrates the inhibitory effect of the α-adrenoceptor blocking agent phentolamine on these responses.

The effects of another α-adrenoceptor blocker, yohimbine, on the response to field stimulation were tested. At the low dose of 6×10^{-8} M a small potentiation was found whereas at higher doses (6×10^{-7}–6×10^{-6} M) the response was reduced.

Out of eighteen preparations studies in this series, two showed very little longitudinal response and two showed very little perfusion response. Histological examination with haematoxylin and eosin staining revealed that these specimens had little longitudinal muscle and circular muscle respectively. As well as indicating variability among tissues, this further demonstrated that the perfusion pressure and the longitudinal responses are registering respectively the responses of circular and longitudinal muscle. Fig. 4 illustrates another type of response which was found in three human vasa. Here the longitudinal axis lengthened during field stimulation concomitant with contraction of the circular muscle as indicated by the perfusion pressure.

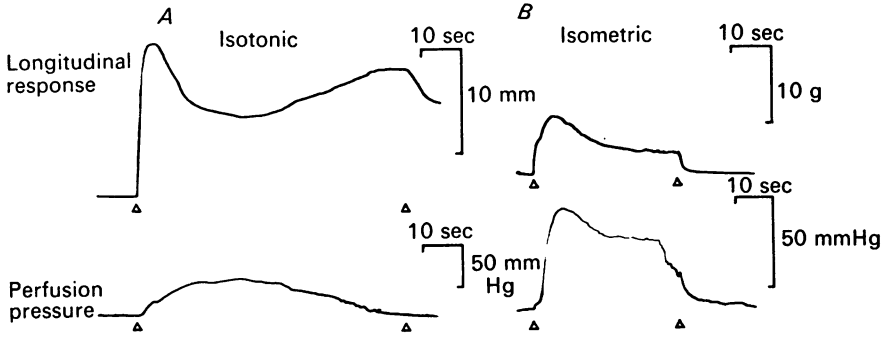

Fig. 5. Responses of the whole isolated guinea-pig vas deferens to field stimulation (0·2 msec pulses between triangles). *A*, isotonic longitudinal shortening and perfusion pressure. Stimulation frequency 10 Hz. *B*, isometric longitudinal tension and perfusion pressure in tissue from another guinea-pig. Stimulation frequency 25 Hz. Perfusion rate in both 0·45 ml./min.

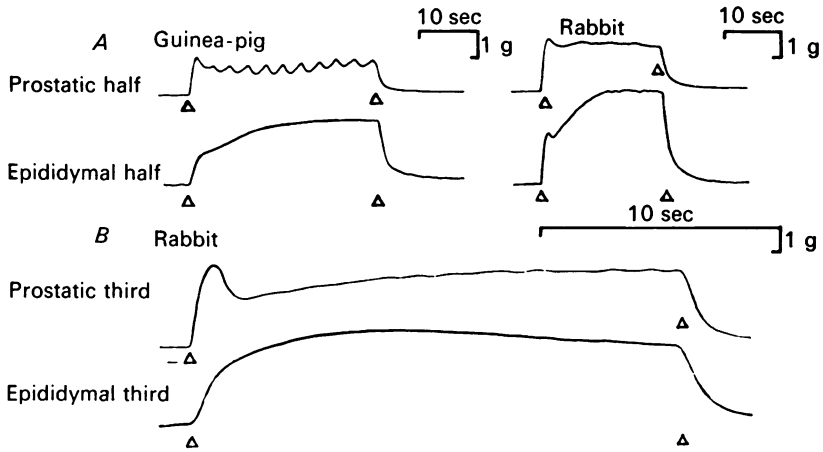

Fig. 6. The response of isolated portions of bisected vas deferens to field stimulation. All recordings are of the isometric longitudinal tension. Stimulation parameters; 0·2 msec pulses between triangles. *A*, guinea-pig vas deferens and rabbit vas deferens each divided transversely into two portions of equal length. Stimulation frequency 10 Hz. *B*, rabbit vas deferens divided transversely into three portions of equal length. Only the portions from the two ends were recorded. Stimulation frequency 10 Hz. Note that the epididymal third shows no evidence of a 'twitch' response in contrast to the epididymal half from the contra lateral vas which is shown in *A*.

Guinea-pig vas deferens

Field stimulation of the guinea-pig vas deferens produced a biphasic isometric or isotonic motor response in the longitudinal muscle. The response of the perfusion pressure however did not consistently show two components with a comparable time scale. In four out of ten guinea-pig vasa the perfusion response was monophasic with its maximum being attained at a time between that for the two longitudinal components (Fig. 5*A*). In the other six vasa however, the circular muscle response followed a time course similar to that for the longitudinal muscle as shown in Fig. 5*B*. As in the rat vas (see Anton *et al.* 1977), the longitudinal and circular muscle of the guinea-pig vas responded to single pulses although in the circular muscle responses to low frequencies of stimulation were relatively small as a percentage of maximal response compared with the longitudinal responses.

Transverse bisection of guinea-pig, mouse and rabbit vas deferens

If the vas deferens from guinea-pig, mouse or rabbit was bisected into two portions of equal length, the response of the longitudinal isometric tension to field stimulation was greater in the epididymal half and, as in the case of the rat, (Anton, *et al.* 1977) the responses in the two halves were qualitatively different, with the 'secondary' component dominating in the epididymal half.

In the two halves of the rabbit vas deferens the responses were similar to those in the guinea-pig with the 'secondary' component dominating in the epididymal half (Fig. 6). If the tissue was taken from the longitudinal 'thirds' at the ends of the vas, the epididymal end showed no 'twitch' component (Fig. 6*B*).

The rabbit, mouse and guinea-pig vas deferens therefore showed differences between the two ends analogous to those found in the rat with the 'secondary' component more dominant at the epididymal end.

DISCUSSION

The responses of the human vas deferens to field stimulation show three main differences from rodent vasa which concern whether the motor transmission is adrenergic. (1) The 'twitch' response, which is resistant to α-adrenoceptor blockade (Swedin, 1971; Ambache & Zar, 1971), is absent from the human vas. (2) The response which is found in human vas is sensitive to α-adrenoceptor blockade. (3) In human vas the responses to nerve stimulation and to noradrenaline are of a similar nature. In contrast, in several other species exogenously applied agonist drugs produce

a series of rhythmical contractions (Waddell, 1916; Macht, 1917; Martins & Valle, 1939) whereas continuous nerve stimulation produces a smooth contraction after the initial 'twitch' (Swedin, 1971; Anton *et al.* 1977).

Some of the obstacles to acceptance of noradrenaline as the motor transmitter in the vasa from other species are thus removed, confirming the conclusion of Birmingham (1968) that adrenergic transmission is found in human vas deferens. The possibility that non-adrenergic transmission or α-adrenoceptor resistant, adrenergic transmission exists in the prostatic end of human vas deferens cannot be entirely ruled out since only sections relatively near the epididymis were tested. As can be seen in Fig. 6, at the extreme epididymal end of rabbit vas deferens the 'twitch' is virtually absent and by analogy this may correspond to the human sections examined. On the other hand, confirmation of the adrenergic nature of the motor innervation in the epididymal end means that such nerves are likely to be involved in the initial transport of sperm from the epididymis during emission and thus be of physiological significance.

If the function of the smooth muscle and its motor innervation in the vas deferens is to propel sperm from the epididymis to the urethra by some process of centrally controlled peristalsis, then the longitudinal and circular muscle layers must interact in an organized fashion. In guinea-pig vas deferens there are histochemical differences between the circular and longitudinal muscle layers with the circular muscle containing a relatively high concentration of cholinesterase (Anstey, Birmingham & Tomlinson, 1974) corresponding to a population of nerve fibres exhibiting specific staining for acetylcholinesterase (Gosling & Dixon, 1972; Furness & Iwayama, 1972). Measurement of the longitudinal muscle responses alone might therefore reflect only part of the overall response with the possibility of an entirely different innervation in the circular layer. Both in the present study with human vas deferens and previously with rat vas deferens (Anton *et al.* 1977) the motor nerve response of the circular muscle (measured as the perfusion pressure) and of the longitudinal muscle (measured either as the isometric longitudinal tension or the isotonic longitudinal shortening) exhibited similar properties. Similar results were also found for the guinea-pig vas deferens in the present study although with this species the time course of the response varied considerably both between animals and in some cases between the circular and longitudinal layers in the same tissue, leaving open the possibility of different types of innervation.

The relationship between the responses observed with field stimulation of isolated tissues and the physiological operation of the vas deferens is not clear since the latter is not well documented. Simple contraction of the entire organ would expel only the sperm contained within the lumen

of the vas and this would not explain the number ejaculated (Freund & Davis, 1969). It is possible, therefore, that the longitudinal muscle might participate in the forward propulsion movement and might even act to increase the diameter of the lumen in front of a moving bolus. In seven out of fifty perfused vasa of various species some initial decrease in perfusion pressure below the resting baseline preceded the rise in pressure following field stimulation or vertebral sympathetic nerve stimulation. In addition as shown in Fig. 4, field stimulation of human vasa can produce an increase in the longitudinal axis of the tissue concomitant with contraction of the circular muscle. If gross stimulation of all the fibres can produce these effects, organized reflex stimulation might effectively produce peristalsis.

Since both longitudinal and circular layers remain intact in the preparation and since each layer is in fact slightly helical, it is possible that some small contribution to the perfusion pressure response might be made by the 'longitudinal' muscle and vice versa. This was checked as shown in Fig. 2 by administering noradrenaline either to the outside of the tissue so that it first contacted the longitudinal layer or via the fluid perfusing the lumen so that it first contacted the circular layer. In this way, either layer could be seen to contract independently as indicated by the longitudinal shortening (outer longitudinal layer) or perfusion pressure (inner circular layer) with only small accompanying responses from the other record which could have been due either to a helical response or to penetration of the noradrenaline. After several minutes contact, when the noradrenaline was administered from outside the tissue, the perfusion pressure also developed a large response. In contrast with the noradrenaline in the perfusate the longitudinal response remained small. This is as might be expected from the geometry of the respective situations. Noradrenaline diffusing from the relatively large surface area outside towards the smaller area of the inner circular layer would reach the circular muscle in a higher concentration than in the converse case where the noradrenaline diffusing from the small surface area of the lumen will gradually be diluted as it diffuses towards the outer longitudinal layer. The development of each response can thus be explained in terms of the penetration of noradrenaline through the tissue.

The type of response shown in Fig. 4 together with the predominance of one or the other of the muscle layers indicates caution in interpreting longitudinal responses alone in human vasa. Over-all, the perfusion pressure gave the most consistent and reproducible measure of smooth muscle activity in this species.

Although the circular and longitudinal responses of most specimens of human vasa showed a close correlation, no 'twitch' component was

present. In contrast, the responses to both field stimulation and to exogenously applied noradrenaline consisted of a series of slow waves which were synchronized in both circular and longitudinal layers. Since the distribution of adrenergic nerve terminals within the human vas deferens is more sparse than in the guinea-pig or rat (Birmingham, 1968; Anton, 1976), the absence of the 'twitch' may correspond to this morphological difference with transmission in the human vas being more dependent on diffusion to distant receptors rather than on a rapid efflux into a narrow synaptic cleft.

The inhibition of the motor nerve response by α-adrenoceptor blockade is of possible therapeutic significance although in the case of phentolamine the dose employed here was higher than is likely to be employed clinically. The opposite effect, potentiation of the motor nerve response, by another α-adrenoceptor blocker, yohimbine, is likely to be due to inhibition of presynaptic α-adrenoceptors which have been shown, in the sympathetic terminals of the guinea-pig atria, to play an inhibitory role in the release of noradrenaline (Langer, Adler-Graschinsky & Giorgi, 1977). Yohimbine is a relatively selective antagonist of presynaptic α-adrenoceptors (Starke, Borowski & Endo, 1975) and potentiates the motor nerve response of the vas deferens of the mouse (Marshall, Nasmyth, Nicholl & Shepperson, 1977) as well as of the guinea-pig, rat and rabbit (J. C. McGrath & R. J. Summers, unpublished observations). This poses the possibility that new α-adrenoceptor antagonists might either potentiate or depress motor nerve responses in the human vas according to the ratio of their pre- and post-synaptic effects. With drugs such as phentolamine which possess both effects (Drew, 1976) the resultant might be a balance with depression overcoming potentiation at high concentrations.

We are grateful to the Medical Research Funds of the University of Glasgow for apparatus and to the staff of the Urology Departments of Glasgow Western and Royal Infirmaries for arranging the supply of human vasa.

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