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THE EFFECTS OF CASTRATION ON NEUROTRANSMISSION IN THE RAT VAS DEFERENS

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1 Responses to adrenoceptor agonist drugs and to field stimulation were examined in vasa deferentia from adult castrated or intact rats. Isometric tension was recorded *in vitro* from whole or transversely bisected vasa.

2 After castration vasa exhibited spontaneous contraction, noradrenaline no longer produced a 'tonic' contraction but increased the 'phasic' spontaneous activity and salbutamol inhibited spontaneous activity by a β-adrenoceptor-mediated mechanism.

3 After castration the 'adrenergic' components of the contractile responses to field stimulation were lost whereas 'non-adrenergic' responses remained, pre-junctional inhibition of field stimulation-induced contractions by either endogenous or exogenous activation was lost but adrenergic terminals could still be demonstrated microscopically.

4 Testosterone treatment partially reversed these effects of castration.

5 The relevance of these results to the nature of neurotransmission and to the genesis of spontaneous contraction in the vas deferens is discussed.

Introduction

Following castration, the effects of agonist drugs, including adrenaline, on the rat isolated vas deferens are altered. The response, which normally consists of a sustained contraction with superimposed rhythmic activity (Waddell, 1916; Macht, 1917), loses the sustained component. This effect can be prevented by testosterone treatment (Martins & Valle, 1939).

The contraction of rat vas deferens to transmural or extrinsic nerve stimulation consists of two elements of which the relative dominance varies along the length of the organ (Pennefather, Vardolov & Heath, 1975; Anton, Duncan & McGrath, 1977). With trains of pulses and frequencies of $\geqslant 2$ Hz, the response consists of an initial rapid component which declines to be replaced by a slower but better maintained, second phase (Swedin, 1971; Gillespie & McGrath, 1974). With single stimuli, separation of the phases is more complete. A rapid first component with a peak at 250 ms is dominant in the prostatic end of the organ and a second slower component with a peak at 650 ms is dominant in the epididymal end. The second component is modified by drugs which affect adrenergic transmission in a manner confirming that noradrenaline is the transmitter substance. The first component, however, is completely resistant to such manoeuvres (McGrath, 1977; 1978; Booth, Connell, Docherty & McGrath, 1978). From these results it can be concluded that both the transmitter substance and the post-junctional receptors involved in mediating the first component are different from those in adrenergic transmission. In addition, during trains of stimuli at $\geqslant 2$ Hz both components are restrained by activation of pre-junctional inhibitory α-adrenoceptors (Brown, McGrath & Summers, 1979).

The object of the present study was to examine how castration affected the response to noradrenaline and the responses to activation of the neurotransmission processes in the tissue and whether the consequent effects were reversed by testosterone treatment.

A preliminary account of these results has been published (Gilmore & McGrath, 1977).

Methods

Male Wistar rats were killed by a blow on the head and exsanguination. Vasa deferentia were isolated and set up (either whole or bisected transversely into two portions of equal lengths) through 'ring and hook' Ag:AgCl electrodes in a 30 ml organ bath containing Krebs bicarbonate solution at 37°C and gassed with 5% CO_2 and 95% O_2 (Anton *et al.*, 1977).

Isometric longitudinal tension was recorded on a Grass Model 7 polygraph, a Devices M2 recorder or a Tektronix D13 storage oscilloscope. Responses were elicited by field stimulation via the electrodes with

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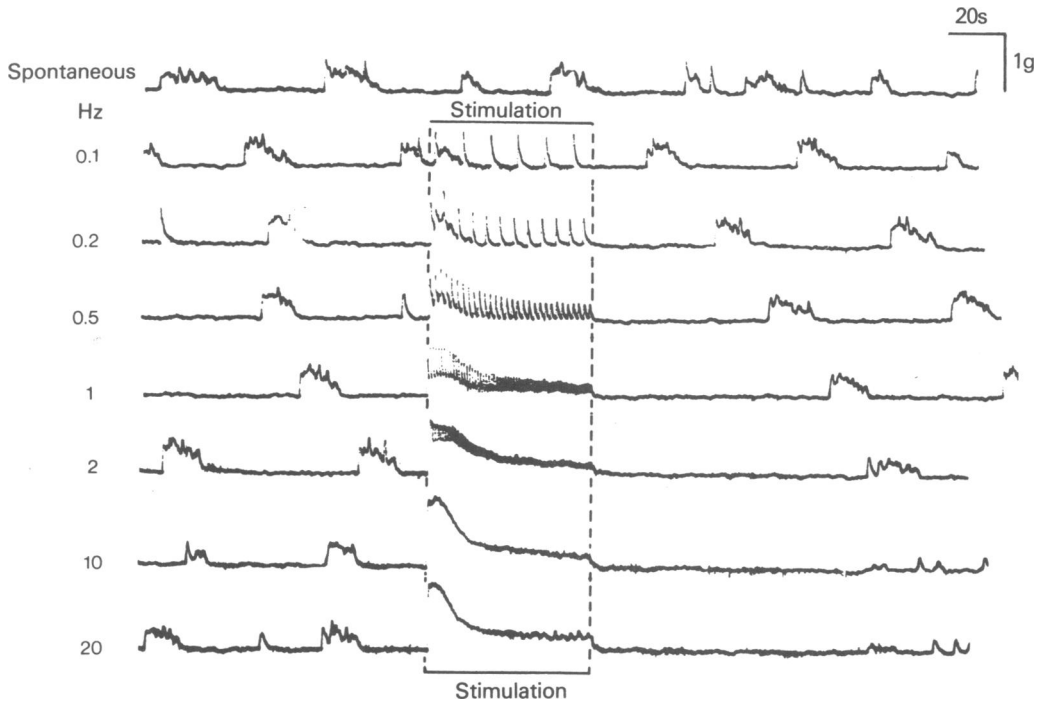

Figure 1 Isometric tension recording of the whole vas deferens from a castrated rat (24 weeks old, castrated for 12 weeks). Records are consecutive from top to bottom with 5 min intervals between. All increases in tension are spontaneous except for the responses in the centre of each line produced by field stimulation (supramaximal pulses, 0.5 ms, 60 s period limited by vertical dotted lines, frequencies indicated on the left). Note that a quiescent period, of which the length increases with increasing stimulus frequency, follows after field stimulation.

supramaximal pulses of 0.5 ms duration applied either individually or in trains at frequencies described in the text. Dose-response curves to noradrenaline were constructed by exposing the tissue to each concentration for a minimum of 5 min or until the response had reached equilibrium, whichever was the longer. The first concentration was 10^{-8} M and increasing concentrations up to 10^{-4} M were subsequently tested, with wash out to establish control conditions between each.

Effects of castration

Adult rats at least 12 weeks old, weighing 250 to 300 g were selected. They were divided into two groups. One group was castrated via an abdominal incision under ether anaesthesia. The other group was left as untreated controls. Further groups of operated controls were not investigated in this study since Martins & Valle (1939) demonstrated that sham-operated, vasoligated or unilaterally castrated rats did not develop the changes in sensitivity of the vas which were found with bilateral castration. After 10 weeks the castrated group was further subdivided. One subgroup was treated with testosterone propionate, 2 mg, subcutaneously in corn oil, daily for ten days. The remainder were untreated. The vasa from each rat were investigated 12 weeks after the date of castration.

Pharmacological treatments

(a) For comparison of the responses from vasa taken from castrated rats with the 'non-adrenergic' response in non-castrates, control rats were pretreated with reserpine (3 mg/kg, i.p., 18 h). This treatment reduces the noradrenaline content of the rat vas deferens to less than 1% of control values (Gillespie & McGrath, 1974) and selectively eliminates the adrenergic component of the contractile response to nerve stimulation (McGrath, 1978). (b) Chemical sympathectomy was produced by pretreatment with 6-hydroxydopamine; 2 × 50 mg/kg on day 1, 2 × 100 mg/kg on day 4, intraperitoneally, tissues taken on day 5 or 6 (Thoenen & Tranzer, 1968).

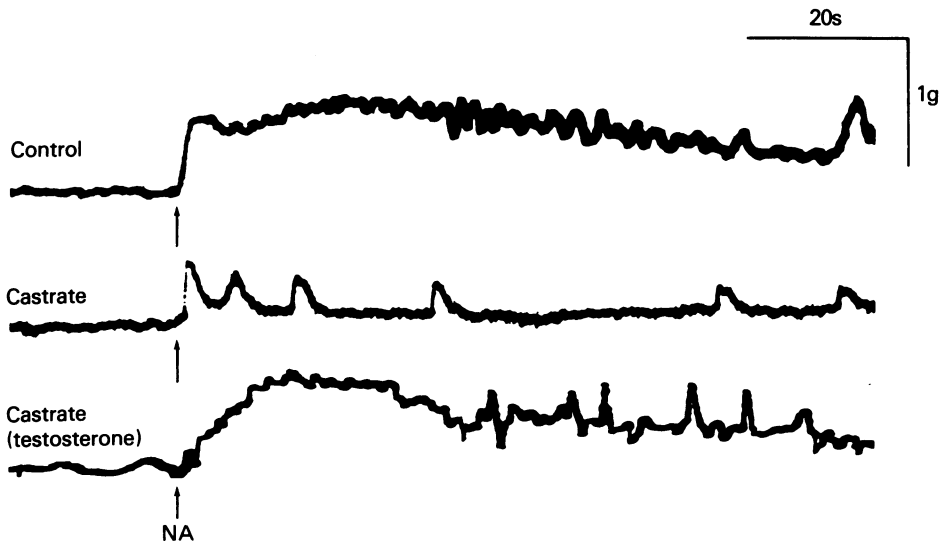

Figure 2 The effects of noradrenaline (NA, 10^{-6} M) on the isometric tension recordings from whole vasa taken from (top to bottom) a control rat, a castrate and a castrate which has been treated with testosterone (2 mg s.c. daily for ten days). Note that the tissue from a castrate is the same one as in Figure 1, recorded at a later time during relative quiescence.

Microscopy

After the various treatments, tissues were prepared for examination in the light or electron microscope. Segments 4 mm long were taken from points starting 5 mm from each end of the vas since the organisation of the muscle layers and density of adrenergic innervation vary along the organ (Anton *et al.*, 1977). To identify the mucosa and the smooth muscle layers, tissues were stained with haematoxylin and eosin (H&E) or Masson. Fluorescent adrenergic nerve terminals were located by exposure of freeze-dried tissues to formaldehyde vapour by the method of Hillarp and Falck as modified by Gillespie & Kirpekar (1966). For examination in the electron microscope tissues were fixed and prepared by the method of Tranzer & Richards (1976) which uses a modification of the chromaffin reaction to produce dense-cored vesicles in adrenergic terminals only when noradrenaline is present.

Drugs

Drugs were dissolved in 0.9% w/v NaCl solution (saline) and added to the organ bath in a maximum dilution of 1 in 100 to give the appropriate molar concentration. For intraperitoneal administration, reserpine was dissolved in 0.4% w/v ascorbic acid immediately before use; 6-hydroxydopamine was dissolved in de-oxygenated saline containing ascorbic acid (1 mg/ml) immediately before use.

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 and glucose 11.1, and was gassed with 95% O_2 and 5% CO_2. Drugs used were: 6-hydroxydopamine hydrobromide (Sigma); noradrenaline bitartrate (Koch-Light); oxymetazoline hydrochloride (Merck); prazosin hydrochloride (Pfizer); reserpine, crystalline (Koch-Light); salbutamol (Allen & Hanburys); sotalol hydrochloride (Duncan Flockhart); testosterone propionate (Sigma); yohimbine hydrochloride (Sigma).

Results

Spontaneous activity

Whole vasa from castrated rats invariably exhibited spontaneous activity as noted by Martins & Valle (1939). This activity consisted typically of either bursts of twitches separated by relatively quiescent periods as shown in Figure 1 or of random and intermittent twitches. In over 400 vasa from control rats comparable activity was never found. Vasa from reserpine-treated (18 h) rats did not but those from rats sympathectomized with 6-hydroxydopamine (5–6 days) did exhibit spontaneous activity. If left undisturbed in the bath, vasa from castrated rats continued to show spontaneous activity for several hours. Following trains of field stimuli, however, the onset of spontaneous activity was commonly delayed (Figure 1). In

the bisected vas from castrates, spontaneous activity was always found in both portions (12 vasa from 7 different rats). In the prostatic portions the activity consisted of frequent (1 to 4/min) but short lived (3 to 10 s) 'twitches' while in the epididymal portions activity consisted of less frequent (0.3 to 2/min) 'bursts' of activity similar to that shown in a whole vas in Figure 1 and which lasted from 10 to 30 s. Spontaneous activity was not reduced by tetrodotoxin (2×10^{-7} M), by prazosin (6×10^{-6} M) or by atropine (10^{-6} M).

Following testosterone treatment of castrates, spontaneous activity was not found in any of 8 vasa from five rats.

Noradrenaline and other adrenoceptor agonists

The sensitivity to noradrenaline of vasa from controls or from castrates was difficult to quantify.

(a) In controls the response to concentrations of noradrenaline which were just above the threshold for contraction (10^{-7} M to 10^{-6} M) commonly consisted of a series of twitches separated by a brief return to baseline. With higher concentrations the baseline was also elevated so that the response consisted of a maintained 'tonic' contraction with superimposed 'phasic' twitches as described by Martins & Valle (1939) for high concentrations of adrenaline (Figure 2).

(b) In castrates it was difficult to detect the threshold accurately due to the presence of spontaneous activity which was already present in drug-free Krebs and was similar to the effect produced in controls by low concentrations of noradrenaline. The threshold did not, however, appear to be significantly different from that found in controls. As the concentration of noradrenaline increased, the frequency and height of twitches increased but the 'tonic' phase was frequently absent e.g. Figure 2, and if it occurred at all, did so only at relatively high doses (Figure 3). While this is in general agreement with the findings of Martins & Valle (1939) one difference is that with the drugs which they tested no 'tonic' phase was present after castration while it did occur in some of our experiments. A quantitative comparison of the doses producing a 'tonic' response is shown in Figure 3.

(c) In castrates treated with testosterone the nature of the responses to noradrenaline returned towards control. In some cases the 'tonic' response could be restored to typical control values (Figure 2) but the mean recovery was variable and incomplete (Figure 3).

The effects of noradrenaline are not always a reliable guide to the functional state of post-junctional α-adrenoceptors, especially in a tissue like the vas deferens where the adrenergic innervation is dense, since the neuronal uptake of noradrenaline can influence the effective concentration at the receptors. This can be overcome, however, by using an agonist

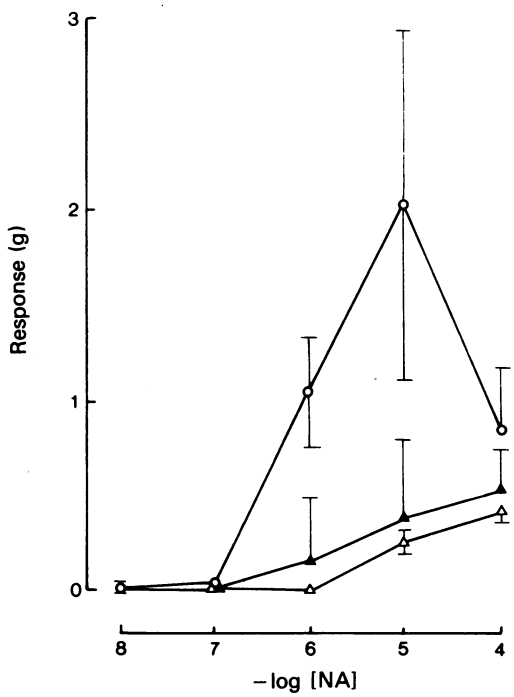

Figure 3 The effect of castration on the dose-response curves of the maintained contraction ('tonic' component) to noradrenaline (NA) of whole vasa. Control (○) $n = 8$; castrate (△) $n = 11$; castrate treated with testosterone (▲) $n = 6$. Vertical bars indicate s.e. mean. Note wide variation in responses indicated by s.e. mean.

such as oxymetazoline which is not a substrate for neuronal uptake (Birmingham, Paterson & Wojcicki, 1970). In control rats the threshold concentration of oxymetazoline for contraction of the epididymal portion of vas was 10^{-7} M ($n = 10$). In castrates the spontaneous activity of epididymal portions was first increased by oxymetazoline 10^{-7} M ($n = 5$). This additional spontaneous activity was attenuated by prazosin (6×10^{-6} M). In the prostatic portions of either controls or castrates, oxymetazoline, in concentrations of up to 10^{-7} M, produced no detectable contraction.

Spontaneous activity in the epididymal or prostatic portions was reduced in both height and frequency by the β_2-adrenoceptor agonist, salbutamol. The threshold for this effect was 10^{-7} M and almost complete cessation of activity occurred with 10^{-6} M. This effect was alleviated by sotalol (2×10^{-6} M) although sotalol itself did not affect the spontaneous activity when given in the absence of salbutamol.

Nerve stimulation

The contractile response of the whole vas to a train of

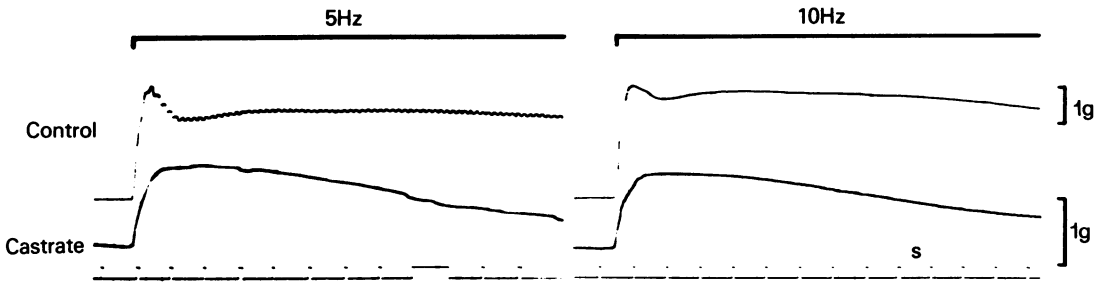

Figure 4 The effects of castration on the time course of the contractile response of the whole vas deferens to a train of field stimuli (supramaximal pulses, 0.5 ms, continuous stimulation at 5 or 10 Hz indicated by horizontal bar). Isometric tension recordings; top, control; bottom, castrate. Note the absence of two 'phases' in the castrate.

pulses Stimulation of the intramural nerve fibres in the whole vas deferens from normal rats at frequencies of $\geqslant 2$ Hz produced a biphasic response as described previously (Swedin, 1971; Gillespie & McGrath, 1974); this owes its unusual time course to the interaction and fusion of the biphasic responses produced by each individual impulse (McGrath, 1977; 1978).

In castrates, however, each individual impulse produced a rapid, short-lived, monophasic, 'twitch-like' response (Figure 1). With trains of impulses these individual responses fused in a manner which produced a monophasic response which reached a peak within 5 s and faded to a lower plateau within a further 10 s (Figures 1 and 4).

The magnitude of the isometric responses to stimulation over a wide frequency range is shown in Figure 5. The initial rapid 'peak' (produced by the first pulse or at higher frequencies by the fusion of the responses to the first few pulses) gave, in controls, a frequency-response curve which rose regularly over the range 1 to 40 Hz. After castration (12 weeks) these initial responses were smaller than in controls but a frequency-response relationship remained. The tension produced 15 s after the start of a continuous train of pulses at each frequency was taken as a measure of the 'secondary' component. In controls this produced an unusual frequency-response curve with a dip at 2 Hz but little difference between higher and lower frequencies. In castrates responses were smaller at all frequencies and the dip at 2 Hz was less prominent. The testosterone treatment of castrates produced movement of the frequency-response curves for each parameter to a value intermediate between castrates and controls (Figure 5).

In strictly quantitative terms these changes in the magnitude of the responses to trains of pulses could be explained simply by the reduction in the weight of the smooth muscle present; the wet weight of the whole vas was changed as follows: mg, mean $\pm$ s.e. mean ($n = 6$); control, 82 $\pm$ 5; castrate 29 $\pm$ 3; testosterone-treated castrate 68 $\pm$ 5. However, the time course of the response in tissues from castrates had only one component, compared with the two found in controls, and was similar to that found after reserpine or α-adrenoceptor blocking agents (Swedin, 1971; Gillespie & McGrath, 1974; Anton *et al.*, 1977; McGrath, 1978). Since in these earlier studies the basis for the two phases was simplified by bisecting the tissue and by employing only single stimuli, this latter method was applied to tissues from castrates.

The contractile responses of bisected vasa to single stimuli Single stimuli produced monophasic responses in both halves of the bisected vasa from castrates. In the prostatic portion this response was larger than in the epididymal portion and reached a peak at around 250 ms as is found in controls (Figure 6). In the epididymal end, in contrast, the response was small, monophasic and showed no sign of the dominant second component found in controls. The time courses of the responses in the vasa from castrates were similar to those found after reserpine treatment or α-adrenoceptor blockade (Figure 6).

These responses in castrates were not affected in either height or duration by prazosin (6×10^{-6} M) or cocaine (10^{-6} M). In vasa from control rats prazosin abolishes and cocaine potentiates the second component of the response (McGrath, 1978).

From these results it can, therefore, be concluded that a nerve-induced post-junctional α-adrenergic effect is absent in vasa from castrates.

The inhibitory effect of endogenously released noradrenaline during trains of stimuli It has been proposed that during field stimulation noradrenaline liberated from adrenergic nerves can act on inhibitory α-adrenoceptors located pre-junctionally on 'non-adrenergic' motor nerves (Ambache, Dunk, Verney & Zar, 1972). Consequently it should be possible to reveal this inhibitory effect of the adrenergic nerves by employing an α-adrenoceptor antagonist to remove

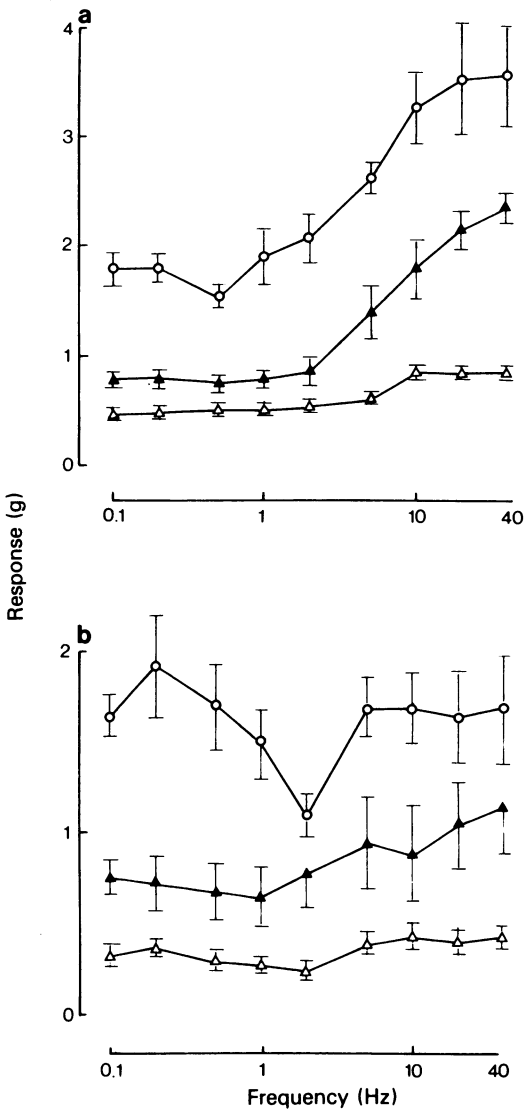

Figure 5 The effect of castration on the frequency-response curves of the contractile responses produced by field stimulation in whole vasa. (a) Tension at 1.5 s after the start of the stimulus train i.e. the peak of the initial rapid phase in controls; (b) tension at 15 s after the start of continuous stimulation i.e., an indication of the maintained response in each situation. Control (○) $n = 8$; castrate (△) $n = 11$; castrate treated with testosterone (2 mg s.c. daily for ten days) (▲) $n = 6$. Vertical bars indicate s.e. mean. The tissues are the same ones as in Figure 3.

the inhibition and hence increase the contractile response. Since the contractile effect of adrenergic nerve stimulation could not be found in castrates we sought the inhibitory effect. This search was not, however, straightforward and the method requires some explanation as follows.

In the dose range 10^{-7} M to 10^{-6} M, yohimbine can increase the response of control vasa to trains of field stimuli (≥ 2 Hz) by antagonizing the prejunctional inhibitory effect of endogenously released noradrenaline. Unfortunately concentrations of yohimbine above 10^{-7} M, which are necessary for a clear increase in the response, also antagonize post-junctional α-adrenoceptors and hence tend to reduce the adrenergic part of the response (Brown, McGrath & Summers, 1979). The clear separation of the pre- and post-junctional actions of endogenous noradrenaline with yohimbine is therefore not possible. One way round this problem, which was employed by Ambache & Zar (1971) while examining the effects of exogenous noradrenaline, is to block the post-junctional α-adrenoceptors from the start with a preferential antagonist. In this situation only the 'non-adrenergic' response is present post-junctionally and it is the prejunctional effects of noradrenaline on this residual transmission which will be uncovered by yohimbine. In the present study prazosin (6×10^{-6} M) was employed to remove the post-junctional effect of adrenergic nerve stimulation (McGrath, 1977; 1978). The presence of a pre-junctional effect during a train of 40 pulses at 8 Hz was then tested with yohimbine (6×10^{-7} M) (Figure 7).

In vasa from control rats, in the presence of prazosin, yohimbine consistently increased the response to a train of stimuli at 8 Hz, an effect which was most marked in the epididymal portion. In vasa from castrates, under identical conditions, no increase was found (Figure 7).

Since no endogenous activation of pre-junctional α-adrenoceptors could be uncovered, the presence of the receptors was tested by employing an exogenous agonist against the response to well separated (5 min) single stimuli. In the latter conditions no α-adrenoceptor-mediated feedback can be demonstrated so that conditions are ideal for the demonstration of the functional presence of pre-junctional α-adrenoceptors (Brown *et al.*, 1979). Oxymetazoline (10^{-10} to 10^{-7} M), which acts on pre-junctional α-adrenoceptors to inhibit the nerve-induced release of noradrenaline (Starke, Endo & Taube, 1975) and which inhibits the initial rapid contraction of the bisected vas (from normal rats) to trains of stimuli (Brown *et al.*, 1979), failed to produce any reduction in the response to single stimuli or to trains in vasa from castrates. Following oxymetazoline (10^{-8} M) the response of the prostatic portion to a single stimulus, expressed as a percentage of the drug-free response, was 32 ± 6.8 ($n = 6$) in controls and 100.5 ± 3.9 ($n = 4$) in castrates.

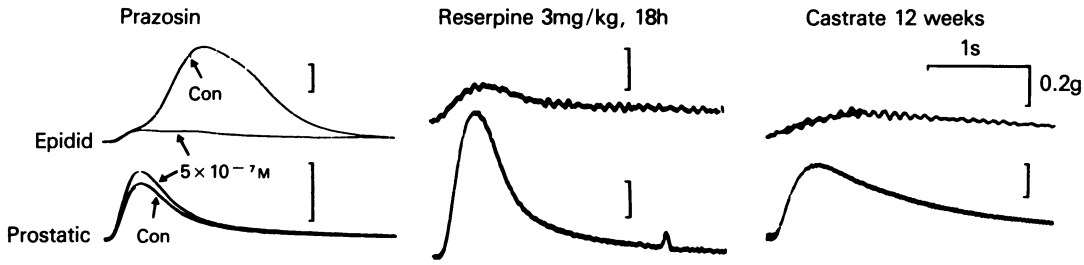

Figure 6 Comparison of the effects of castration and of drugs which block adrenergic nerve responses on the time course of the contractile responses of bisected portions of vas deferens to single field stimuli (supramaximal pulse, 0.5 ms). Isometric tension trace was triggered by stimulus. Upper traces, epididymal portions; lower traces, prostatic portions. Left panel shows the effect of prazosin (5×10^{-7} M) on a control response (Con) from an untreated rat. The centre panel shows responses from a rat pretreated with reserpine (3 mg/kg, i.p., 18 h). Right panel shows response from a castrate rat (12 weeks). The trace speed was constant. Vertical calibration (0.2 g) varies as indicated.

Microscopy

Staining with haemotoxylin and eosin or Masson confirmed previous observations that the secretory layers of the mucosa had almost completely degenerated and the smooth muscle layers had become smaller both in radial thickness and in overall radius in vas deferens of castrated rats. As judged by the number of nuclei, the total number of smooth muscle cells did not appear to be markedly reduced but the closer proximity of nuclei and the reduction in the quantity of visible cytoplasm indicated that the reduction in tissue mass was mainly caused by the reduction in size of individual smooth muscle cells. The outer sheath of connective tissue was thicker and more folded in castrates than in controls giving the impression that it had not been affected by castration and had simply bunched around the reduced mass of muscle. The smooth muscle layers in vasa from intact rats have a complex geometry of unknown functional significance, a distinct feature of which is the intrusion of longitudinal bundles of fibres into the circular layer. Such complex organisation was lacking in castrates, the layers being more uniformly circular or longitudinal. Following exposure of freeze-dried tissue to formaldehyde vapour, the fluorescence microscope revealed a dense adrenergic innervation in vasa from castrates similar to that found in controls.

Electron microscopy, using the method of Tranzer & Richards (1976), which employs a chromaffin reaction to produce dense cores in the vesicles of adrenergic nerves, also revealed a dense plexus of adrenergic terminals. No consistent population of terminals containing 'non-granular' vesicles could be found. The smooth muscle cells were more loosely packed than in controls and, perhaps as a consequence, the distance between varicose terminals and muscle cells tended to be greater.

Discussion

These results confirm the observation of Martins & Valle (1939) that drug-induced contractions of the vas deferens are influenced by testosterone status and extend this to the effects of noradrenaline. In addition the pre- and post-junctional effects of stimulation of the intramural nerve fibres were found to depend on testosterone status. After castration the adrenergic components of the responses to nerve stimulation were lost whereas a clear 'non-adrenergic' contraction remained which was resistant to the effects of α-adrenoceptor blockade, had a time course similar to that found in the presence of an α-blocker or after pretreatment with reserpine or 6-hydroxydopamine (McGrath, 1978; Booth *et al.*, 1978) and had a distribution along the organ similar to that of the 'non-adrenergic' response found in adult controls (Anton *et al.*, 1977; Booth *et al.*, 1978). The residual response was smaller in magnitude than in controls but this could be explained by the considerable reduction in the muscle mass following castration.

An increase in the response to agonists of vasa from castrated guinea-pigs has been found when the magnitude of the response was corrected for the loss in tissue weight (Greenberg, Kadowitz, Schedl & Long, 1973). We did not feel that this correction was justified in the present conditions due to the gross morphological changes produced by castration.

The loss of the contractile response to adrenergic nerve stimulation could be due to failure of neurotransmission of either pre- or post-junctional origin or to a failure in the contractile process. However, the change in the response to noradrenaline, which is usually a guide to this site, could not, in this case, resolve the problem.

α-Adrenoceptors are clearly present on the muscle cells since rhythmic activity is produced by noradre-

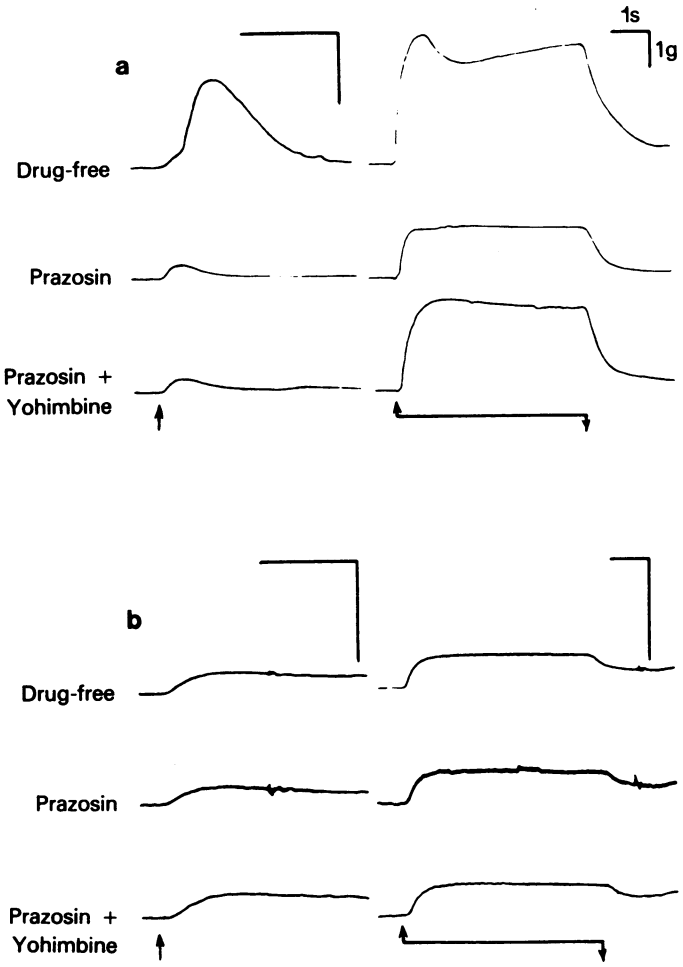

Figure 7 Demonstration of the loss, after castration, of endogenous α-adrenoceptor-mediated inhibition. The responses in epididymal portions of rat vasa to single stimuli (left panel, at arrow) or trains of stimuli at 8 Hz (right panel, at horizontal line) are shown: (a) control rat, (b) castrate rat. In each case, from top to bottom, are drug-free controls, in the presence of prazosin (6×10^{-6} M), in the additional presence of yohimbine (6×10^{-7} M). Note that in the control, prazosin reduced part of the response to either a single pulse or a train but yohimbine subsequently increased only the response to a train. In the castrate, however, neither drug had any detectable effect.

naline at a similar threshold concentration in castrates and controls. In our opinion, however, thorough pharmacodynamic analysis is not possible in either castrates or controls since the response is multiphasic, no equilibrium point exists at a given concentration and at different concentrations the 'phases' occur in different relationships with respect to time (see also Wadsworth, 1974). The results do, nevertheless, show that the relationship between activation of α-adrenoceptors and contraction is changed by castration. The site of this alteration could involve either excitation-contraction coupling or the contractile apparatus.

The results with single stimuli or noradrenaline might suggest that the tissue, after castration, has simply lost the ability to sustain a contraction. However, with trains of pulses (Figure 4) or during bursts of spontaneous activity (Figure 1) contractions can be maintained for longer than would be necessary to permit normal responses to single stimuli or noradrenaline. Although an effect on the contractile apparatus cannot be ruled out, this evidence suggests that a failure of some common stage in the excitation-coupling process for the tonic contraction to exogenous noradrenaline or the contraction to noradrenaline released from nerves is responsible for changes following cas-

tration. In contrast the 'non-adrenergic' transmission process and the rhythmic response to exogenous noradrenaline can survive castration, suggesting that excitation-contraction coupling for these latter processes employs a different route (or routes) which survives castration. Alternatively, a change in the receptor population could be responsible.

In the case of contraction to adrenergic nerve stimulation, of course, the above interpretation assumes that adrenergic nerves are (1) present and (2) capable of releasing noradrenaline in response to field stimuli. Since our observations confirm the presence of adrenergic terminals, the first criterion can be met. For the second criterion only negative evidence is currently available since neither the pre- nor the post-junctional effects of adrenergic nerve stimulation could be detected. In the case of the pre-junctional effect, which is normally mediated by α-adrenoceptors, however, the receptors do not appear to be functional in castrates. It is, therefore, possible that noradrenaline is being released but that the receptors or some steps subsequent to their activation are absent. The point is also not resolved by the post-junctional α-adrenoceptor effect of exogenously administered noradrenaline or oxymetazoline since the contractile response differs qualitatively from the response in controls (lacking a 'tonic' phase) and it is not possible to ascertain that the receptors producing their effects are accessible to endogenously released noradrenaline.

The one definite effect via adrenoceptors which was observed in castrates was the inhibitory β-effect. Inhibitory, β-adrenoceptor-mediated effects have previously been demonstrated in rat vasa (Ganguly & Bhattacharya, 1970). The present effects in rat vasa appear to be at least partly post-junctional since salbutamol reduced spontaneous muscle contraction.

Spontaneous contraction of the vas deferens *in vitro* has been previously reported in castrates (Martins & Valle, 1939) and in four other situations viz. after pretreatment over several days with reserpine, during withdrawal from prolonged morphine treatment (Pollock, Muir, MacDonald & Henderson, 1972), after surgical denervation (Lee, Westfall & Fleming, 1975) and after transplantation of the vas to a site adjacent to the wall of the colon (Jurkiewicz, Jurkiewicz, Gomes & Aucelio, 1977). The present results show that the selective elimination of the adrenergic nerve terminals by producing chemical sympathectomy with 6-hydroxydopamine will also induce spontaneous activity but that merely removing noradrenaline from the tissue within an 18 h period with reserpine will not. Another means of producing chemical sympathectomy, by prolonged treatment with guanethidine (Evans, Iwayama & Burnstock, 1973) also results in spontaneous activity in the vasa (authors, unpublished observations). In each case muscarinic or α-adrenoceptor antagonists or tetrodotoxin failed to abolish spontaneous activity suggesting a myogenic origin. A common factor, which was postulated for each of these situations in which spontaneous activity was found, is a functional adrenergic denervation which has been present for at least five days. A speculative explanation for the induction of spontaneous activity might, therefore, be the loss of a 'trophic' influence exerted by noradrenaline on the muscle. Another common factor which has been reported after reserpine, during morphine withdrawal (MacDonald, 1970), after castration or after chemical sympathectomy is that in the response to noradrenaline the 'phasic' component of the contraction is relatively more dominant when compared with controls. This supports the suggestion that some change in the excitation-contraction coupling process may have taken place in each case (see above).

Since the 'non-adrenergic' response remains after the treatments of castration, 6-hydroxydopamine, reserpine or chronic guanethidine, this element of the innervation is apparently incapable of preventing the changes which follow 'adrenergic denervation'. This in turn suggests that the adrenergic nerves exert a day-to-day trophic influence which cannot be taken over by the 'non-adrenergic' component although, superficially, the two appear similar in that they produce muscle contraction when artificially excited.

Testosterone treatment partly reversed the effects of castration on the vas. The schedule employed was similar to that of Martins & Valle (1939) although in this earlier study the treatment appears to have been started immediately after castration and was, therefore, used to 'prevent' the effects of castration. The present results indicate that the process can be partly 'reversed' but that this schedule may not have been of sufficient duration to enable complete restoration of normality. The abolition of spontaneous activity, a gain in muscle mass, and the return of an adrenergic nerve response were, however, achieved.

In conclusion, the function of the adrenergic nerves in the rat vas deferens depends on testosterone status. This has implications for the use of the tissue as a pharmacological tool, especially when employing pretreatments which alter hormonal balance, and may also be of significance for the physiological role of the organ. The selective survival of the 'non-adrenergic nerve' response provides another argument for separating this from the relatively straightforward adrenergic component.

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