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# ADRENERGIC AND 'NON-ADRENERGIC' COMPONENTS IN THE CONTRACTILE RESPONSE OF THE VAS DEFERENS TO A SINGLE INDIRECT STIMULUS

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SUMMARY

1. The mechanical response of the longitudinal smooth muscle of the rat vas deferens to stimulation of its motor nerves by a single pulse has been examined. The motor nerves were stimulated *in vivo* via the spinal outflows in the pithed rat or *in vitro* by field stimulation.

2. The contraction in the whole vas consisted of two components, an initial, rapid, brief contraction reaching a maximum at 300 msec and a second, slower and more prolonged contraction reaching its maximum at 600 msec. When the isolated vas was divided into prostatic and epididymal halves the contribution of these two components varied. The initial rapid component was more prominent in the prostatic half and the slower, second component more prominent in the epididymal half. Lowering the bath temperature caused, in both halves, these two components to merge into a single, slow, prolonged response. Both components were more rapid and briefer than the equivalent response of rat anococcygeus.

3. The second, slow component was abolished by $\alpha$-adrenoceptor antagonist drugs, potentiated and prolonged by drugs which inhibit the neuronal uptake of noradrenaline and absent from tissues taken from rats pre-treated with reserpine, suggesting that the neurotransmitter for this component is noradrenaline.

4. These experiments were extended to the mouse or guinea-pig vas deferens. Both showed the same two component mechanical response as the rat vas and in both the second, slow component was preferentially inhibited by $\alpha$-adrenoceptor antagonists and potentiated by drugs blocking noradrenaline uptake.

5. Drugs known to reduce the response to repetitive nerve stimulation of the vas were examined for their effect on the response to a single stimulus. Lysergic acid diethylamide preferentially inhibited the second, slow phase of contraction whereas apomorphine preferentially inhibited the first rapid phase. Guanethidine inhibited responses but any differential effects could not be analysed due to its stimulant properties.

6. These results show that there are two components even to the response to a single stimulus. The second of these appears to be adrenergic while the transmitter responsible for the first remains to be determined.

## INTRODUCTION

The contractile response of the vas deferens from several species to repetitive stimulation of its motor nerves is complex. There are two distinct phases, an initial rapid 'twitch' and a slower, better maintained 'secondary' contraction, which, in the rat, only becomes visible with trains of pulses lasting at least 2–3 sec (Swedin, 1971; Gillespie & McGrath, 1975). The two phases show differential susceptibility to drugs, e.g. $\alpha$-adrenoceptor blockers such as phentolamine are relatively more effective against the 'secondary' phase (Swedin, 1971).

It has been suggested that both phases of transmission might be adrenergic but that the twitch is produced by a high concentration of noradrenaline (NA), in the narrow synaptic gap, which can overcome the competitive blockade produced by $\alpha$-adrenoceptor blockers (Swedin, 1971; Furness, 1974).

An alternative explanation first postulated by Ambache and co-workers, is that the motor nerves producing the 'twitch' are non-adrenergic (Ambache & Zar, 1971; von Euler & Hedqvist, 1976) and that the physiological function of the dense adrenergic innervation (Falck, Owman & Sjostrand, 1965) might be to modulate this non-adrenergic motor response (Ambache, Dunk, Verney & Zar 1972). In this latter hypothesis the 'secondary' response to hypogastric nerve or field stimulation only occurs with long trains of pulses and is due to overflow of NA from these 'inhibitory' adrenergic nerves.

Analysis of these components of the contractile response is complicated by two main factors: (1) both responses are present simultaneously and can only be separated when one or other is dominant and (2) at least two inhibitory feed-back processes, which modulate both the motor response and the release of NA, are present, involving prostaglandins and pre-junctional $\alpha$-adrenoceptors (Euler & Hedqvist, 1969; Swedin, 1971; Stjarne, 1973).

The present study demonstrates a method of overcoming these two factors. First, if the response to a single pulse is used then neither feed-back system has an opportunity to modulate transmitter release. Secondly, if the mechanical responses are examined in the two ends of the vas then the two components of the response vary. At the prostatic end the initial 'twitch' is dominant while at the epididymal end the slower, secondary component is dominant (Anton, Duncan & McGrath, 1977).

It is first demonstrated that a single pulse can produce a contractile response with two components in the rat whole vas deferens *in vivo* or *in vitro*. Secondly, the two components are further analysed in bisected portions of isolated vas deferens from three species with a view to distinguishing adrenergic and/or 'non-adrenergic' components and finally the effects are assessed of several drugs which are known to affect the overall response to trains of pulses.

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

## METHODS

Male Wistar rats (250–300 g) were pithed by the method of Gillespie, MacLaren & Pollock (1970). The movable pithing rod electrode was placed to stimulate the spinal outflows at L2–3, which is the optimal position for stimulation of the motor nerves to the vas deferens (Anton *et al.* 1977). The method of recording isometric tension from the *in situ* vas deferens has been described

(Gillespie & McGrath, 1973). Pancuronium bromide (1 mg/kg, I.V.) or gallamine (20 mg/kg) prevented skeletal muscle twitching on stimulation. Stimuli were of supramaximal voltage and 0·5 msec duration.

Vasa deferentia were isolated from male Wistar rats (250–300 g), guinea-pigs (500–600 g) and mice (Parkes strain, 40–50 g) killed by a blow on the head and exsanguination. All vasa were placed, either whole or halved transversely, in a Perspex bath and superfused with Krebs bicarbonate solution at 38 °C and gassed with 95 % $O_2$:5 % $CO_2$ as described previously (Anton *et al* 1977). Field stimulation was applied via parallel Ag:AgCl plate electrodes (Grass S88 stimulator, supramaximal voltage; pulse widths in text) (Anton *et al.* 1977).

Anococcygeus muscles from some of the rats were isolated and mounted in Ag:AgCl ring electrodes and stimulated with 0·3 msec pulses (supramaximal voltage) in a 10 ml. bath containing Krebs bicarbonate solution at 38 °C and gassed with 95 % $O_2$:5 % $CO_2$ (Gillespie, 1972). Where comparison was made with anococcygeus, vasa deferentia were also mounted and stimulated in this latter manner. When employing ring electrodes with the vas deferens, care is necessary to ensure that the whole tissue comes within the stimulating field since the characteristics of the response vary gradually along the length of the vas (Anton *et al.* 1977). In such experiments the cathode was a spiral of wire surrounding the upper half of the portion of tissue and the anode was the hook to which the lower end of the tissue was attached.

Vasa or anococcygeus were connected by thread to a Grass FT03 transducer and isometric tension was recorded on a Tektronix D13 dual beam storage oscilloscope and on a Devices MX2 pen recorder or an S.E. Laboratories 3006/DL U.V. oscillograph. The oscilloscope time trace was triggered simultaneously with the application of the stimulus pulse to the tissue.

The following drugs were used and were added to the Krebs solution superfusing the tissue or into the 10 ml. tissue bath to give the appropriate molar concentration. Apomorphine hydrochloride (Macfarlan Smith), azapetine (Roche), cocaine hydrochloride, guanethidine sulphate (CIBA), lysergic acid diethylamide (Sandoz), phentolamine mesylate (CIBA), prazosin hydrochloride (Pfizer), reserpine, crystalline (Koch-Light), yohimbine hydrochloride (Sigma).

Reserpine pre-treatment of rats: reserpine was dissolved in 2 % (w/v) ascorbic acid and, when appropriate, administered intraperitoneally (3 mg/kg) 18 hr before killing the rat. This schedule reduces the noradrenaline content of rat vas deferens by over 98 % (Gillespie & McGrath, 1974). The Krebs bicarbonate solution 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

### *Vas deferens in situ in pithed rats*

Stimulation of the spinal outflows at L2–3 with single pulses produced biphasic contractile responses in the whole vas deferens.

The first phase reached a peak at 280–340 msec, and the second phase at 560–650 msec after the stimulus. In four of five rats tested, the first phase was greater than the second (Fig. 1*A*) but in one rat the second phase was dominant. The temperature of the rat was critical for the characteristics of the response. The rectal temperature was normally maintained at 37 ± 0·5 °C but if it was allowed to fall below this range the contractions became slower to rise and more prolonged until by 35–33 °C the two phases merged and became indistinct.

### *Isolated rat vas deferens*

Single field stimulation of whole vas deferens produced biphasic responses similar to those found in the pithed rat. The first phase was greater in height and the second phase was normally present as a shoulder on the declining arm of the first phase rather than as a distinct peak (Fig. 1*B*).

In order to separate the two components, further analysis was carried out on vasa transversely bisected into two portions of equal length.

A single pulse (0·3 msec, supramaximal voltage) produced (1) in the epididymal half of the tissue a bisphasic response with the second phase dominant (Fig. 2), (2) in the prostatic half a response with only one clear peak (Fig. 2) but which had sometimes a shoulder on the declining arm of the peak corresponding in time with the second phase of the epididymal portion, e.g. Fig. 3*A*.

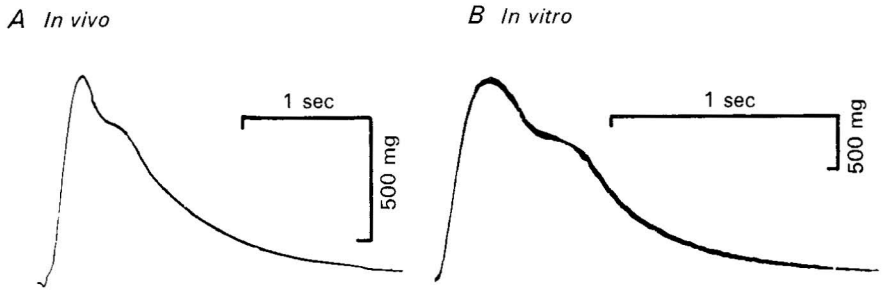


Fig. 1. Longitudinal isometric responses of the rat whole vas deferens *A*, *in situ* in the pithed rat to single pulse stimulation of the vertebral outflow at *L*2–3 (1 msec pulse, supramaximal voltage) and *B*, *in vitro*, to single pulse field stimulation (0·3 msec pulse, supramaximal voltage). Note that each response has two distinct phases, the second appearing as a shoulder on the declining arm of the first.

TABLE 1. Contractile responses of the isolated prostatic and epididymal portions of rat bisected vas deferens to single pulse field stimulation (0·3 msec, supramaximal voltage) ($n$ = 20). $P$ levels indicate the significance of the difference between the means for prostatic and epididymal portions assessed by Student's $t$ test

| Response | Prostatic | Epididymal | $P$ |
|---|---|---|---|
| Delay to first detectable contraction | — | — | — |
| Range (msec) | 45–60 | 65–75 | — |
| Time to peak, mean ± S.E. of mean (msec) | 270 ± 2 | 750 ± 5 | < 0·001 |
| Range (msec) | 260–290 | 720–790 | |
| Maximum tension, mean ± S.E. of mean (mg) | 380 ± 30 | 640 ± 35 | < 0·001 |
| Range (mg) | 220–600 | 430–900 | |

The prostatic response started to rise after a shorter delay, reached its maximum more rapidly and developed a lesser tension than the epididymal response (Table 1). In both portions the maximum tension and recovery to baseline were attained more rapidly than in the case of the isolated anococcygeus (Fig. 2*A*).

With the electrodes and stimulator employed, the responses in each end became maximal with a pulse width of 0·3 msec. Further increase from 3 to 10 msec did produce an increased response, presumably due to direct stimulation of the smooth muscle. No separation between the responses in the two portions or between the proportions of the two phases in each portion could be made by reducing the pulse width or voltage below maximal values. This is illustrated for pulse width in an epididymal portion in Fig. 2*B*. 0·3 msec pulses were employed throughout the subsequent investigation.

*Interval between pulses*

Since the object was to study the response to a single pulse, it was important to determine the interval between pulses necessary to avoid interaction. Initially 60 sec was chosen as the interval between pulses and at this interval reproducible responses could be obtained. As the interval was reduced below 60 sec the second component was at first reduced until the interval reached 30–20 sec, at which point both phases began to be subject to potentiation by the previous pulse. With further increase in the interval beyond 60 sec it also became clear that even with this interval the second phase of the response was attenuated. In subsequent experiments, therefore, 5 min intervals were left between pulses and no interaction was encountered.

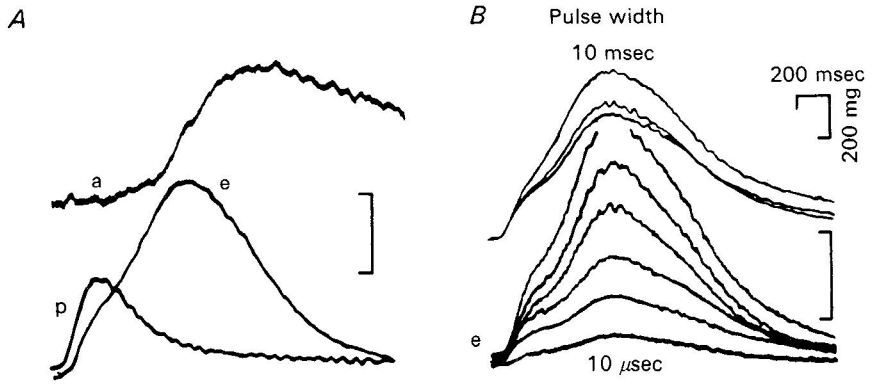


Fig. 2. Longitudinal isometric tension response of rat isolated tissues to single pulses of field stimulation. In each case the record was triggered simultaneously with the stimulus. *A*, comparison of the time course of responses in anococcygeus (*a*), bisected portions of vas deferens, epididymal portion (*e*), prostatic portion (*p*), supramaximal pulses, width 0·3 msec. *B*, effect of varying the pulse width on the response of an epididymal portion of vas deferens to stimulation with supramaximal (30 V) pulses. Pulse widths from bottom to top, 10, 20, 30, 50, 100, 300 μsec, 1, 3, 10 msec. Stimuli of increasing width applied at 5 min intervals.

*Temperature*

The temperature of the Krebs solution bathing the tissue was maintained within the limits 37–38 °C. When the temperature was lowered, in each half of the tissue, the mechanical responses to a single pulse became slower to rise, were longer in duration and the two components merged in such a way that they could not be distinguished. The threshold for discrimination of two phases in the epididymal end was 36–35 °C. The durations of the responses were considerably increased at low temperatures. At 12 °C a single stimulus could produce a response which did not decline to baseline until 30–60 sec.

*α-adrenoceptor blocking agents*

Four agents were tested and produced a dose-related inhibition of the second phase of the epididymal response in the following concentrations: phentolamine ($10^{-7}$ M–$10^{-5}$ M) prazosin ($5 \times 10^{-8}$ M – $5 \times 10^{-7}$ M), azapetine ($10^{-8}$ M – $10^{-7}$ M) and yohimbine ($3 \times 10^{-7}$ M–$10^{-5}$ M). In contrast, the first phase of the response, in both

halves of the tissue, was unaffected (Fig. 3). Concentrations of each agent which produced maximal inhibition of the second phase could also potentiate the first phase (Fig. 3). Azapetine was the most effective in this respect and at concentrations of $6 \times 10^{-6}$ M and above also produced rhythmic contractions of the tissue which could not be abolished by other $\alpha$-adrenoceptor blockers.

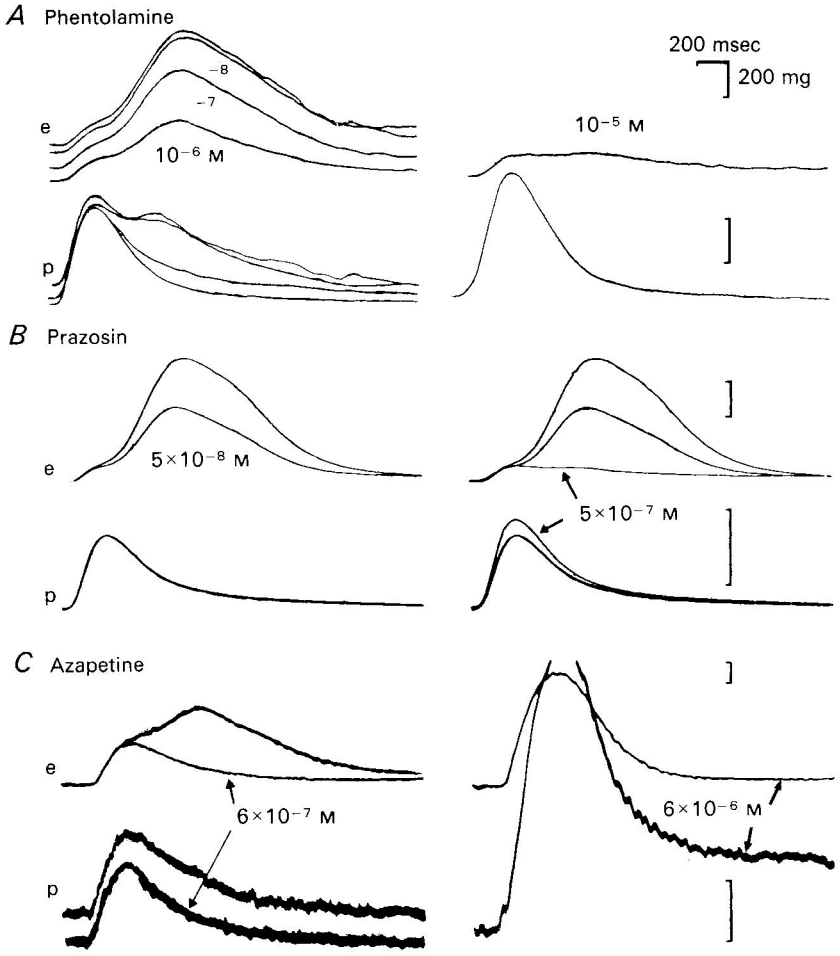


Fig. 3. Effects of $\alpha$-adrenoceptor blocking drugs on the responses of isolated portions of rat bisected vasa deferentia to single supramaximal stimuli (0·3 msec). Responses are of the epididymal (e) and prostatic (p) portions. Sequential equilibrium responses are shown with the control at the top and read from top to bottom except where otherwise stated. Any baseline shifts are due to staggering of the traces. *A*, left: control, in the presence of phentolamine $10^{-8}$ M, $10^{-7}$ M, $10^{-6}$ M; right: in the presence of phentolamine $10^{-5}$ M. *B*, left: control, in the presence of prazosin $5 \times 10^{-8}$ M; prostatic responses are coincident; right: as on the left plus a response in the presence of prazosin $5 \times 10^{-7}$ M. Note that in this case the latter response in the prostatic portion is above, i.e. is larger than, the control. *C*, left: control, in the presence of azapetine $6 \times 10^{-7}$ M; right: in the presence of azapetine $6 \times 10^{-6}$ M. The peak was lost from the prostatic trace due to the limits of the recording screen. Simultaneous paper recording indicated a further rise of 60 mg. Calibrations: horizontal 200 msec, vertical 200 mg.

*Apomorphine*

Apomorphine selectively inhibited the first phase of the single pulse response while leaving the second phase (Fig. 4*A* and *B*). A dose-related inhibition of the first phase was produced by $10^{-7}$–$10^{-4}$ M solutions. In contrast, the second phase of the response, as seen in the epididymal portion, was increased in height and duration by $10^{-5}$ M–$10^{-4}$ M-apomorphine (Fig. 4*A*). $10^{-4}$ M-Apomorphine also produced rhythmic contraction of the epididymal but not of the prostatic portion.

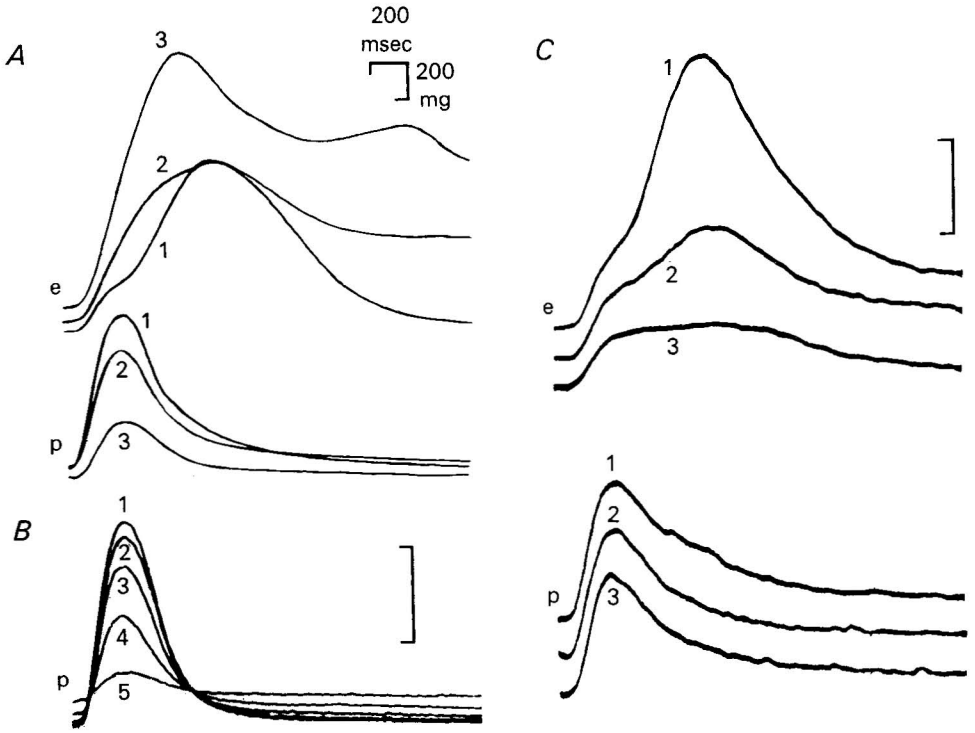


Fig. 4. Effects of apomorphine or LSD on the responses of isolated portions of rat bisected vasa deferentia to single supramaximal stimuli (0·3 msec). *A*, control (1), apomorphine $10^{-5}$ M (2), $10^{-4}$ M (3). Note that responses of the epididymal portion (e) are increased and prolonged while those of the prostatic portion (p) are decreased. *B*, control (1), apomorphine $10^{-7}$ M (2), $10^{-6}$ M (3), $10^{-5}$ M (4), $10^{-4}$ M (5). Prostatic (p) only. Baseline shifts in *A* and *B* represent actual changes in 'resting' tension due to the induction of spontaneous activity by apomorphine. *C* control (1), LSD $10^{-7}$ M for 10 min (2) and 25 min (3). Upper panel, epididymal (e); lower panel, prostatic (p). Calibrations; horizontal 200 msec, vertical 200 mg.

*Lysergic acid diethylamide (LSD)*

LSD ($10^{-7}$ M) inhibited the second phase of the response but did not inhibit the first phase (Fig. 4*C*).

*Guanethidine*

Guanethidine ($10^{-7}$ M–$10^{-5}$ M) produced a dose-related inhibition of the first phase of the response. Higher concentrations ($10^{-5}$ M and above) also produced rhythmic

contraction of the tissue and could potentiate nerve responses (Fig. 5). These stimulant actions of guanethidine are due to its known indirect sympathomimetic action in this tissue (Gillespie & McGrath, 1975). When this latter effect was prevented by the addition of prazosin ($5 \times 10^{-7}$ M) the response to a single pulse, in both halves of

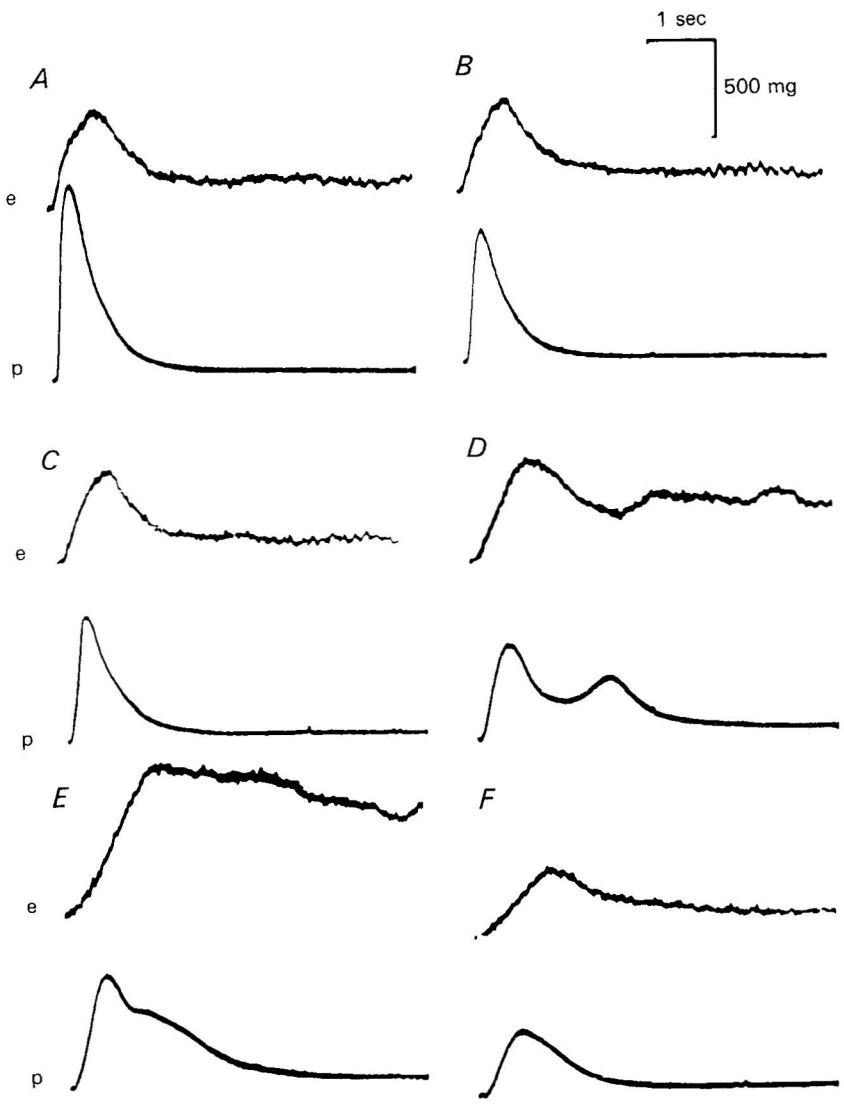


Fig. 5. Effects of guanethidine on the responses of isolated portions of rat bisected vas deferens to single supramaximal stimuli (0·3 msec). Responses are shown in pairs: epididymal (e), prostatic (p). *A*, control, *B*, in the presence of guanethidine $10^{-7}$ M, *C*, guanethidine $10^{-6}$ M, *D*, guanethidine $10^{-5}$ M, *E*, guanethidine $10^{-4}$ M, *F*, guanethidine $10^{-4}$ M plus prazosin $5 \times 10^{-7}$ M. All responses are equilibrium responses obtained at least 15 min after addition of drug. Note that the magnitude of the control prostatic response falls outside the range indicated for twenty experiments in Table 1. In every other noted respect, however, the experiment was typical of those providing the quantitative data in Table 2.

the tissue, was monophasic and was both smaller and more prolonged than in the absence of drugs (Fig. 5*E*). The residual response after guanethidine ($10^{-4}$ M) varied markedly in size between tissues from different rats. At concentrations of $10^{-5}$ or $10^{-4}$ M-guanethidine it was not possible to distinguish, due to the extreme variations in the time course of the responses, whether either component of the response within a particular portion was relatively susceptible to guanethidine. The percentage inhibitions by guanethidine of the overall height in the two portions are compared in Table 2. Each component of the response was reduced in a dose dependent manner by guanethidine. At high doses, however, the net effect was variable and appeared to depend on a balance between inhibition (probably of prejunctional origin) and potentiation (due to the post-junctional stimulant effect antagonised by prazosin). This could explain the wide variation in the net effect of $10^{-4}$ M-guanethidine (Table 2).

TABLE 2. Effects of guanethidine on the maximum height of the response of isolated portions of rat vas deferens to a single field stimulus. The responses remaining in the presence of guanethidine are expressed as a percentage of the corresponding control response

| Guanethidine concentration | Epididymal portion (Mean (%) ± S.E. of mean, $n = 7$) | Prostatic portion (Mean (%) ± S.E. of mean, $n = 8$) |
|---|---|---|
| $10^{-7}$ M | 85 ± 4 | 90 ± 4 |
| $10^{-6}$ M | 76 ± 7 | 60 ± 2 |
| $10^{-5}$ M | 48 ± 9 | 30 ± 4 |
| $10^{-4}$ M | 41 ± 23 | 40 ± 17 |

*Cocaine*

Cocaine ($10^{-6}$ M) increased the height and duration of the second phase of the response to a single pulse. Even in the prostatic portion where the second phase is normally small, it became dominant after cocaine (Fig. 6). The second phase also became rhythmic in nature although no spontaneous activity of the tissue was produced by $10^{-6}$ M-cocaine in the absence of stimulation. Due to the large potentiation of the second phase, it was not obvious whether cocaine had affected the first phase of the response. When an $\alpha$-adrenoceptor blocker was given, however, the second phase was abolished leaving the first phase. Figure 6*A* illustrates that after cocaine ($10^{-6}$ M) and phentolamine ($10^{-6}$ M) a single pulse produces a monophasic response similar in height to the initial first phase. Higher concentrations of cocaine ($6 \times 10^{-6}$ M and above) produced rhythmic contractions characteristic of its known indirect sympathomimetic activity in this tissue (O'Donnell & Hecker, 1967).

*Reserpine pre-treatment*

Reserpine (3 mg/kg, I.P., 18 h) removed the second component of the response to a single stimulus. In this case the response after the drug could not be compared with the control response from the same tissue so responses from rat vasa deferentia treated with reserpine were compared with responses from untreated controls. The responses were recorded on a fast paper speed to enable accurate measurement of the time course and the mean responses from groups of tissues were plotted. Fig. 7 illustrates that the second component of the response was absent after reserpine

leaving, in the epididymal portion, a monophasic response with an early peak. The response in the prostatic portion was not reduced following reserpine.

*Guinea-pig and mouse isolated vas deferens*

In the mouse vas deferens, even with the whole tissue present, two distinct phases were found (Fig. 8*B*). Cocaine ($10^{-6}$ M) selectively potentiated the second phase. Increasing the concentration of cocaine to $10^{-5}$ M reduced the size of the potentiation, indicating caution with this agent at high concentration. Phentolamine ($10^{-6}$ M). however, abolished the second phase, leaving the first phase intact. When the mouse

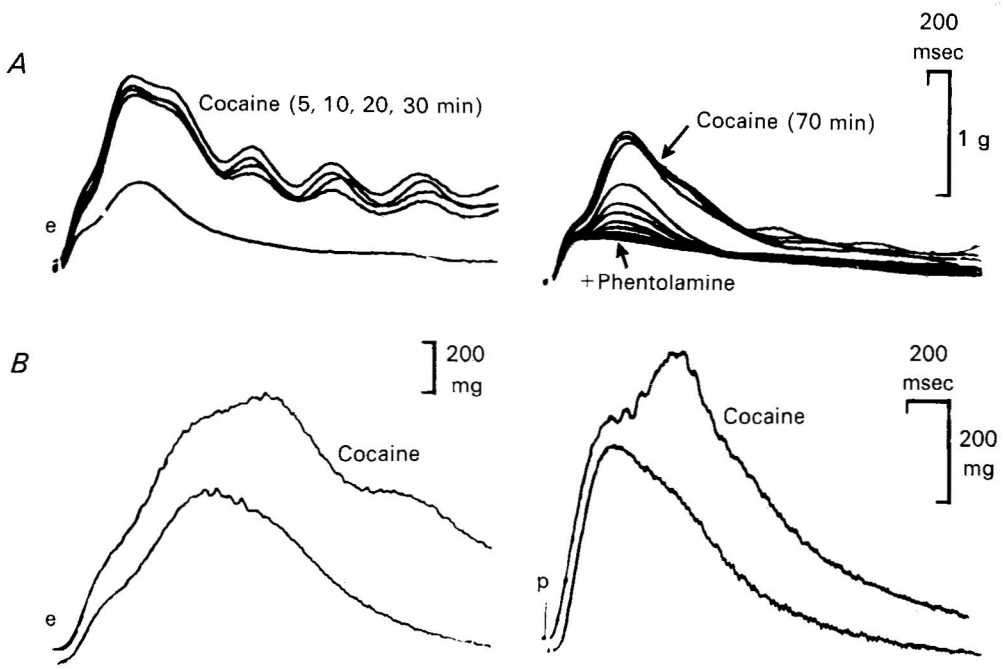


Fig. 6. Effects of cocaine on the responses of isolated portions of rat bisected vasa deferentia to single supramaximal stimuli (0·3 msec). *A*, epididymal portion only (e). Left, from bottom to top: control, in the presence of cocaine $10^{-6}$ M for 5, 10, 20 and 30 min; stimuli delivered at intervals of 5 min. Right: records are sequential with those on the left, from top to bottom (stimuli now at 1 min intervals), in the presence of cocaine for 70 min (four stimuli), in the additional presence of phentolamine $10^{-5}$ M (eight stimuli). *B*, epididymal portion (e), prostatic portion (p). Effects of cocaine $10^{-6}$ M. The prostatic traces are staggered horizontally by 30 msec.

vas was bisected into portions, the first phase could be seen in isolation in the prostatic half and the second phase became dominant in the epididymal half.

With a single pulse, the response in the guinea-pig vas showed two differences from those in rat and mouse. First the response was slower to rise and more prolonged, and secondly the distinction between the two phases was less obvious. Fig. 8*A* illustrates this in an epididymal portion of guinea-pig vas. In the presence of cocaine ($10^{-6}$ M), however, the second phase was potentiated and after phentolamine ($10^{-6}$ M to $10^{-5}$ M) was inhibited. In contrast neither drug affected the

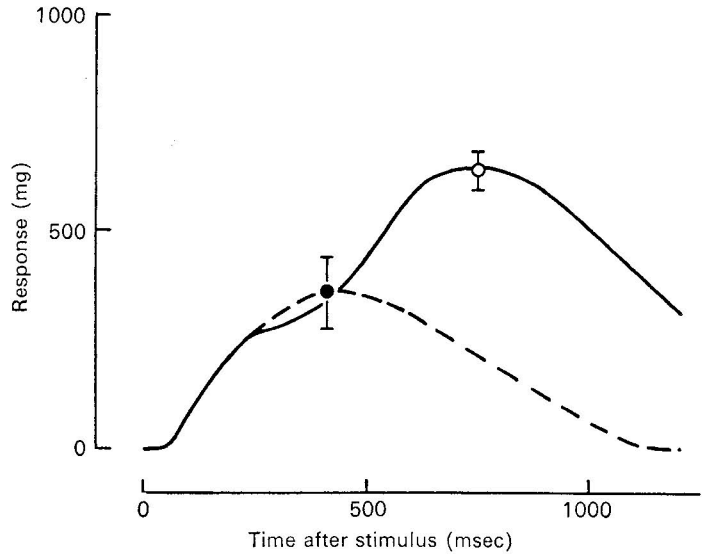


Fig. 7. Comparison of the time courses of the contractile responses of isolated epididymal portions of rat bisected vasa deferentia to single supramaximal stimuli (0·3 msec) in control and in tissue treated with reserpine (3 mg/kg, I.P. 18 hr). The response of each tissue was measured at sequential 50 msec intervals after the stimulus and the mean responses from each group plotted with respect to time. Symbols and vertical bars indicate means ± S.E. of mean for the peak tension and the time at which it occurs. Horizontal bars are smaller than symbols. (○) Continuous line, controls, $n = 20$; (●) broken line, treated with reserpine, $n = 9$. Intermediate points and error bars have been omitted for clarity.

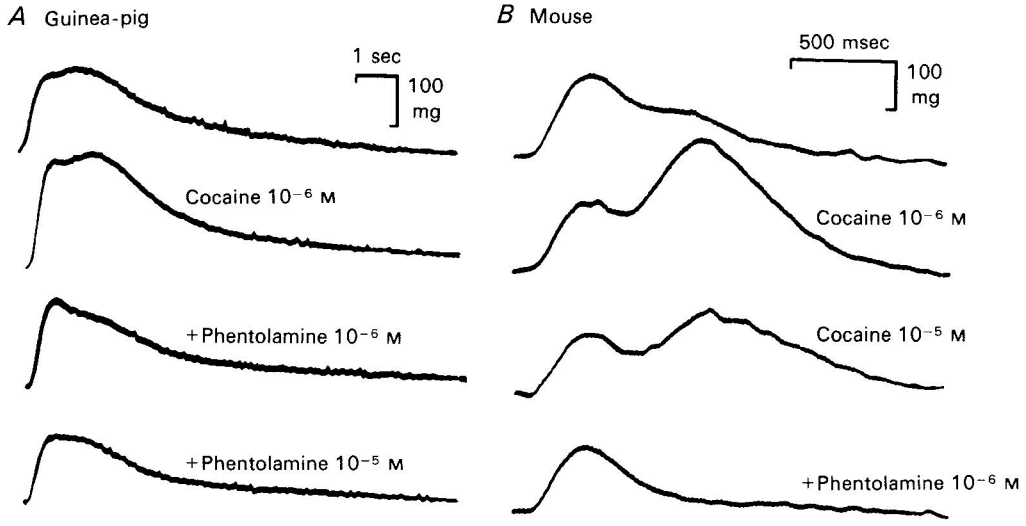


Fig. 8. Effects of cocaine and phentolamine on the responses of isolated portions of guinea-pig or mouse vasa deferentia to single supramaximal stimuli (0·3 msec). *A*, epididymal portion from a guinea-pig bisected vas deferens. Sequential responses are, from top to bottom: control, in the presence of cocaine $10^{-6}$ M, in the additional presence of phentolamine $10^{-6}$ M and $10^{-5}$ M. *B*, mouse (Parkes strain) whole vas deferens. Sequential responses are, from top to bottom: control, in the presence of cocaine $10^{-6}$ M, $10^{-5}$ M, in the additional presence of phentolamine $10^{-6}$ M. All responses are equilibrium responses obtained 15–20 min after addition of drugs. Note the difference in trace speed in *A* and *B*.

first phase. In the prostatic half of guinea-pig vas a second component to the response to a single stimulus could not be distinguished.

With both these species, therefore, a single pulse produced two phases, the second of which was predominant in the epididymal half, was potentiated by NA uptake blockade and was inhibited by $\alpha$-adrenoceptor blockade.

## DISCUSSION

### *The existence of two phases*

The response of rat vas deferens to a single stimulus exhibits two phases with differing time courses. Both phases are found if the response of the whole organ is recorded irrespective of whether stimulation is via the vertebral outflow or by field stimulation. A clearer separation is, however, achieved by bisecting the tissue. A rapid first phase is predominant in the prostatic end and a second slower phase dominates in the epididymal portion.

The presence of two phases within the response of the vas deferens to a single pulse has not previously been noted. In our own earlier work (Gillespie & McGrath, 1975) there were three main reasons for this. One was a failure to examine the time course of the response in sufficient detail by running the recorder too slowly. This was compounded by the dominance of the first phase when recording the response of the whole organ. Thirdly, responses to a single pulse were always recorded after first establishing supramaximal stimulation parameters and reproducible, 'equilibrium' responses with trains of pulses at 5–10 Hz. In such circumstances the response to a single stimulus is greatly attenuated compared with those in experiments where stimulation is applied more sparingly (J. C. McGrath, unpublished observations). In over 200 tissues tested where the recording system and stimulation protocol was appropriate, a biphasic response has invariably been found. Further experimental modifications which may also result in the fusion of the two phases to produce a monophasic recording include a temperature below 35–36 °C and unnecessary filtering of the signal. Compared with comparable smooth muscles, e.g. anococcygeus, both phases of the vas deferens contraction to a single pulse are extremely rapid. Imposition of a low pass filter of 2 db down at 3 Hz completely smooths out the response to a single pulse to a monophasic record. For this latter reason isotonic longitudinal responses to a single stimulus will also display only a single phase due to the initial delay before the isometric tension equals the pre-load (Anton *et al.* 1977).

With repetitive stimulation, Ambache & Zar (1971) found a different threshold for excitation of two postulated 'non-adrenergic' components to the response of guinea-pig vas deferens and Birmingham & Freeman (1976) could further differentiate the 'twitch' and 'secondary' components with rat or guinea-pig vasa by lowering the bath temperature. In the present experiments, however, the two phases of the response to a single pulse could not be further separated by changing the pulse width or lowering the bath temperature.

### *The effects of drugs*

The second component of the response to a single stimulus could be inhibited by $\alpha$-adrenoceptor blocking agents, was absent after reduction of the tissue noradrena-

line stores by reserpine and was potentiated by cocaine, procedures without effect on the first component. The second phase, therefore, seems to be due to noradrenaline released from nerves within the tissue, acting on $\alpha$-adrenoceptors on the smooth muscle and whose duration of action is limited by re-uptake into the nerves. The first component requires neither the presence of significant noradrenaline stores, the functional integrity of $\alpha$-adrenoceptors nor is it limited by the uptake process for noradrenaline. It may, therefore, be concluded that it is not due to noradrenaline or that if it is then the process of neurotransmission must differ considerably not only from that in other tissues but also from the adrenergic transmission simultaneously present in this tissue and responsible for the second component.

This relatively simple explanation of the origin of the first and second components of the response to a single pulse is at odds with at least two well established observations in the literature from experiments involving repetitive stimulation in rat vasa. First, reserpine or LSD, which in the present experiments reduced only the second component of the response to a single stimulus, are known to reduce the twitch or first component of the response to repetitive stimulation (von Euler, 1969; Ambache, Dunk, Verney & Zar, 1973; Hughes, 1973; McGrath, 1973; Gillespie & McGrath, 1974, 1975). Secondly, guanethidine, an adrenergic neurone blocking drug and which is known to block both components of the response to repetitive stimulation (Anton *et al.* 1977), did not abolish the response to a single stimulus.

An explanation of these apparent anomalies requires consideration of how the individual pulses in a train summate to produce the compound response consisting of 'twitch' and 'secondary' components. This in turn can be simplified by considering how the varying distribution of the two components of the single pulse response along the organ leads to corresponding differences in the response to repetitive stimulation. The relative dominance of the first and second components to a single stimulus in the prostatic and epididymal ends respectively is paralleled by a corresponding respective dominance of the 'twitch' and 'secondary' responses to repetitive stimulation (Anton *et al.* 1977). However, since a single pulse produces a distinct second phase to the response with a peak at around 600–800 msec then the 'twitch' to a train of pulses, with a rapid rise to a peak at 1000–2000 msec, must contain an element from the second phase *in addition to* the rapid first component found with a single stimulus. This may explain the apparent anomaly that drugs such as reserpine and LSD, which result in a reduction in the output of noradrenaline, can reduce the 'twitch' (von Euler, 1969; Hughes, 1973; Gillespie & McGrath, 1974) while $\alpha$-adrenoceptor blockers cannot abolish the 'twitch' (Swedin, 1971). The 'twitch' will be reduced, due to the loss of the adrenergic component, by any drug which selectively reduces the output of noradrenaline, but will never be abolished by such agents since the resistant first component will remain. It must also be borne in mind when extrapolating from the present results to those involving repetitive stimulation that the effects of each pulse in a train after the first pulse will be subject to feed-back processes and the corresponding contractile responses will be subject to post-junctional facilitation and/or inhibition processes.

The inhibitory effect of guanethidine on all components of the response to repetitive nerve stimulation has been one of the most difficult observations to reconcile with the hypothesis that a non-adrenergic component is present (Ambache & Zar,

1971; Swedin, 1971; Anton *et al.* 1977). Similarly the present results, which indicate that guanethidine can produce some inhibition of the first component to a single stimulus, are the only inconsistency in the argument that this part of the response is not adrenergic. Nevertheless, the additional complicating factor of the onset of the stimulant effect of guanethidine at $10^{-5}$ M, which is due to an indirect sympathomimetic action (Gillespie & McGrath, 1975), suggests caution in interpreting the net effects of this agent. Furthermore, the motor nerves of the rat vas deferens are relatively resistant to blockade by guanethidine compared, for example, with vasopressor nerves (Chang, Chang & Su, 1967). Even with $10^{-4}$ M-guanethidine the response of the prostatic portion of rat vasa deferentia to repetitive stimulation was not abolished (Anton *et al.* 1977), and in the present experiments a residual response remained to a single stimulus even after $10^{-4}$ M-guanethidine and $\alpha$-adrenoceptor blockade, pointing to a type of neurotransmission which, unlike other examples of sympathetic transmission, is attenuated by guanethidine, but not abolished by it.

This interpretation of the nature of transmission in the vas deferens therefore incorporates elements from the hypotheses for 'adrenergic' (Swedin, 1971) and 'non-adrenergic' (Ambache & Zar, 1971) motor transmission. One element which is, however, difficult to accept, on the present evidence, is the proposal that the rapid first phase, which forms part of the 'twitch' to repetitive stimulation, might be due to a high concentration of noradrenaline in the neuromuscular cleft which cannot be inhibited by competitive $\alpha$-adrenoceptor blockers (Swedin, 1971). If this was the case then this element of the response, being in such close association with the adrenergic nerves, should have been susceptible to potentiation by blockade of noradrenaline uptake. However, no such effect was found.

The role of the uptake of NA into adrenergic neurones in terminating the mechanical or electrophysiological response to motor nerve stimulation in the vas deferens has been the subject of apparently conflicting reports. Uptake blockers have been reported to potentiate (Hukovic, 1961; Bell, 1967), inhibit (Ambache *et al.* 1972; Marshall, Nasmyth & Shepperson, 1977) or not change (Wakade & Krusz, 1972) the post-junctional events following nerve stimulation. The present results employing single pulses suggest that the second phase is both potentiated in height and prolonged in duration by low concentrations of cocaine while the first phase remains undisturbed. With higher concentration of cocaine further effects may occur. For example, cocaine has an indirect sympathomimetic action (O'Donnell & Hecker, 1967). The resulting release of NA from the nerve terminals can thus act on post-junctional $\alpha$-receptors to contract the muscle (O'Donnell & Hecker, 1967) and on presynaptic $\alpha$-receptors to inhibit the responses to nerve stimulation (J. C. McGrath & R. J. Summers, unpublished observations). With trains of pulses, NA released by the nerve action potential will also produce these opposing actions making analysis even more complex. Analysis of the effects of cocaine on neurotransmission in this tissue is thus simplified by the use of single pulses and concentrations of cocaine sufficient to block noradrenaline uptake into nerves but low enough to avoid side-effects through release of noradrenaline.

The anatomical distribution and the differing time courses of the two phases were clear-cut in rat and mouse vasa deferentia whereas in guinea-pig vasa both phases of the response had a relatively slower time course and were less distinct from each other.

In each of these three species, however, the second phase was potentiated by cocaine and blocked by phentolamine, suggesting an adrenergic response, while the first phase was unaffected. Holman, Taylor & Tomita (1977) found in mouse vasa deferentia that single pulses of field stimulation produced weak responses compared to those found in the present study and in that of Henderson & Hughes (1976). Of three strains of mouse tested, the Parkes strain gave responses with the clearest separation of the two components. Qualitatively similar but smaller responses with a similar distribution along the vas deferens were found with TO and C57/BL strains (J. C. McGrath, unpublished observations). Responses of mouse vas deferens thus vary with the strain of mouse employed.

It has been suggested that dopamine might be a motor transmitter in rat vas deferens since apomorphine can reduce the contractile responses of the isolated tissue to dopamine and to trains of field stimulation but, in contrast, can increase responses to noradrenaline (Simon & Van Maanen, 1976). The present results demonstrate that the inhibitory action of apomorphine is specifically exerted against the first, 'non-adrenergic' phase, while the adrenergic component is potentiated and prolonged. This correlates, therefore, with the observation that apomorphine potentiates the response to exogenous noradrenaline (Simon & Van Maanen, 1976).

The mechanism by which apomorphine selectively inhibits the first phase of the nerve response is not clear. Another possibility in addition to antagonism at post-junctional dopamine receptors (Simon & Van Maanen, 1976) is that apomorphine is an agonist at prejunctional dopamine receptors which inhibit the release of motor transmitter. Apomorphine can stimulate dopamine receptors in the central nervous system (see Nichols, 1976) and dopamine can inhibit nerve-induced responses of the vas deferens by a prejunctional action (Tayo, 1977). The balance of evidence is, therefore, insufficient to justify the conclusion that dopamine is a motor transmitter in this tissue.

In conclusion, the response of rat, mouse or guinea-pig vas deferens to a single electrical stimulus applied to the intramural nerve fibres consists of two components. A wide range of drugs elicit differential effects from these components and the relative size of the two phases varies widely along the length of the organ.

The effects of drugs on these phases show that they are not directly analogous to the two components found when a train of pulses is applied since the latter depend on the summation, modified by feed-back processes, of the two phases arising from each pulse. When employing this tissue to examine neurotransmission mechanisms or pharmacological responses thereon, therefore, recognition of these properties will facilitate interpretation. The question of whether adrenergic or 'non-adrenergic' motor transmission is present within the tissue can also be resolved since responses can be found which are attributable to each.

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