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The effects of calcium channel inhibitors and other procedures affecting calcium translocation on drug-induced rhythmic contractions in the rat vas deferens

D.W.P. Hay & R.M. Wadsworth

Department of Physiology & Pharmacology, University of Strathclyde, Glasgow G1 1XW

1 In the rat isolated vas deferens, methoxamine 8.1 μM produced an initial phasic response that declined towards baseline and was followed by rhythmic contractions that continued until wash-out. These responses were predominant in the epididymal half. $BaCl_2$ 1 mM produced a similar type of response which was not mediated by noradrenaline release or activation of α-adrenoceptors. The barium responses were similar in the epididymal and prostatic halves.

2 Incubation in nominally Ca^{2+}-free solution caused abolition or near abolition of rhythmic contractions produced by barium or methoxamine. The initial phasic response to methoxamine was abolished in Ca^{2+}-free solution, whereas that produced by barium persisted.

3 Rhythmic contractions produced by methoxamine or barium were inhibited by Mg^{2+} (2.4–20 mM) and by La^{3+} (1–5 mM). Mg^{2+} had selectivity for inhibition of the frequency of methoxamine- but not barium-induced rhythmic contractions.

4 Despite their dependence on $[Ca^{2+}]_o$, barium- and methoxamine-induced rhythmic contractions were resistant to inhibition by calcium channel inhibitors. Verapamil, nifedipine and flunarazine inhibited the amplitude of rhythmic contractions more readily than the frequency (methoxamine IC_{50} for verapamil: amplitude = 29.8 ± 5.40 μM, $n = 6$, frequency = 96.7 ± 31.0 μM, $n = 5$, for nifedipine: amplitude = 2.42 ± 0.34 μM, $n = 7$, frequency = 3.24 ± 0.75 μM, $n = 7$, and for flunarizine: amplitude = 15.9 ± 5.95 μM, $n = 7$, frequency = 153 ± 28.6 μM, $n = 7$). There was no differentiation between inhibition of methoxamine and barium-induced responses.

5 Like Mg^{2+}, methoxyverapamil selectively inhibited the frequency of methoxamine-induced contractions (IC_{50}: amplitude = 16.8 ± 2.86 μM, $n = 5$, frequency = 2.07 ± 0.81 μM, $n = 5$) but not barium-induced contractions (IC_{50}: amplitude = 13.9 ± 1.95 μM, $n = 5$, frequency = 48.5 ± 8.98 μM, $n = 5$).

6 Diazoxide (43.3–2167 μM) and nitroprusside (3.36–6712 μM) had only a small effect on barium contractions, but produced a dose-related reduction in the amplitude of methoxamine-induced responses. Diazoxide and nitroprusside caused methoxamine contractions to occur in groups, although they had no effect on their overall frequency.

7 It is concluded that barium- and methoxamine-induced rhythmic contractions in the rat vas deferens are mediated by the entry of $[Ca^{2+}]_o$ via membrane calcium channels that have a lower affinity (10–100 ×) for calcium channel inhibitors than those mediating the KCl response. Channels activated by methoxamine are concentrated in the epididymal half, whereas those opened by barium are evenly distributed. However, although responses to methoxamine and barium are similar in form, differences in the effects of some of the drugs tested, together with the results of previous studies, indicate that they produce contractions by different mechanisms.

Introduction

Recordings from the vas deferens of anaesthetized rabbits have detected spontaneous rhythmic contractions in the absence of nerve stimulation or drug administration (Melin, 1970; Prins & Zaneveld, 1980). It has also been suggested that the human vas deferens contracts spontaneously *in vivo* (Ventura, Freund, Davis & Pannuti, 1973). However, it is now firmly established that the mammalian vas deferens is

normally quiescent *in vitro*, although in an early study it was reported that the rabbit and rat vas deferens produced spontaneous rhythmic contractions (Waddell, 1916). Nevertheless, in certain conditions, e.g. after castration (MacDonald & McGrath, 1980) the tissue exhibits spontaneous activity. Addition of appropriate concentrations of noradrenaline and other α-adrenoceptor agonists to the rodent vas deferens produces rhythmic contractions that appear after the initial rapid response, and which persist until washout (Waddell, 1916; Ohlin & Strömblad, 1963; Hotta, 1969; Pennefather, Vardolov & Heath, 1974). Rhythmic activity is also produced in the mammalian vas deferens by histamine, muscarinic agonists and nicotine (Waddell, 1916; Ohlin & Strömblad, 1963), by cocaine (O'Donnell & Hecker, 1967; Cliff, 1968) and by local anaesthetics (Cliff, 1968). Barium was also found to induce rhythmic responses in vasa deferentia of several mammalian species (Waddell, 1916). Furthermore, α-adrenoceptor agonists induce rhythmic activity in human vasa deferentia (Ratnasooriya, Wadsworth & Gilmore, 1979), present in both longitudinal and circular muscle layers (Anton & McGrath, 1977).

Although analysis of the biphasic response to high concentrations of noradrenaline in the rat vas deferens has been performed, involving Ca^{2+}-deprivation studies and the use of La^{3+} and calcium channel inhibitors (Swamy, Triggle & Triggle, 1976; Triggle, Swamy & Triggle, 1979; Stone, 1981; Hay & Wadsworth, 1983b), there have been no investigations into the involvement of Ca^{2+} in the rhythmic contractions produced by α-adrenoceptor stimulation. Thus, the purpose of this study was to examine excitation-contraction coupling mechanisms involved in rhythmic activity in the rat vas deferens, using calcium channel inhibitors and analysis of the Ca^{2+}-dependence of the responses. We have also examined the effects on rhythmic responses of three other drugs known to affect calcium translocation, but whose mechanisms of action have not been clarified; diazoxide (Levy, 1975, Thorens & Haeusler, 1979), hydralazine (McLean, Du Souich, Barron & Briggs, 1978) and nitroprusside (Kreye, 1980). The sympathomimetic agent selected for analysis was methoxamine, which is a selective postjunctional α_1-adrenoceptor agonist having no indirect sympathomimetic action (Trendelenburg, Maxwell & Pluchino, 1970).

In addition to contractions induced by methoxamine, the mechanisms underlying the rhythmic activity produced by barium were investigated. The contractions produced by barium are unlikely to involve activation of α-adrenoceptor or other specific receptor systems, but may be mediated by an interaction with Ca^{2+} at an extracellular or intracellular level (Weiss, 1977). Thus, the mechanisms behind the rhythmic contractions produced by methoxamine and barium may be different, and consequently responses induced by either agent may be expected to show different sensitivities to the antagonists of calcium translocation mechanisms used in this study.

Methods

Vasa deferentia were removed from Wistar rats (215–460 g) and suspended under 0.5 g tension in Krebs-Henseleit solution at 36–38°C. Contractions were recorded isometrically. The Krebs-Henseleit solution contained (mM): Na^+ 144, K^+ 5.8, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO^-_3 25, $H_2PO^-_4$ 1.2, SO^{2-}_4 1.2, Cl^- 128.6 and glucose 11.1 and was bubbled with 95% O_2 plus 5% CO_2. The nominally Ca^{2+}-free solution was made the same way but omitting $CaCl_2$.

Calcium-dependence

To study the calcium-dependence of methoxamine- or barium chloride ($BaCl_2$)-induced contractions, tissues were incubated for 30 min in nominally Ca^{2+}-free Krebs-Henseleit solution before addition of methoxamine or $BaCl_2$. In the continuing presence of either agonist, increasing concentrations of $CaCl_2$ were then added cumulatively at 20 min intervals.

La^{3+} and high Ca^{2+}

In experiments involving the addition of La^{3+} or high Ca^{2+} a HEPES, Tris or EPPS-buffered Krebs solution of the following composition was used (mM): Na^+ 144, K^+ 5.8, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 157.2, glucose 11.1, HEPES 5–10 mM, Tris 1–10 mM, or EPPS 10 mM. The solution was titrated to pH 7.4 and bubbled with O_2 or air.

Reserpine and sympathetic denervation

Some rats were pretreated with reserpine, using a schedule of either (a) 5 mg kg^{-1} i.p. at 48 h followed by 3 mg kg^{-1} i.p. at 24 h before use, or (b) 2.5 mg kg^{-1} i.p. 24 h before use. Another group of animals was injected with guanethidine (25 mg kg^{-1} daily i.p. for 14 days followed by 50 mg kg^{-1} daily i.p. for 2 days) in order to destroy adrenergic nerves (Heath, Evans, Gannon, Burnstock & James, 1972). This treatment reduces the contraction to single pulse transmural stimulation to 9% of control (Hay & Wadsworth, 1983a) and the tissues are referred to in the text as denervated tissues.

Measurement of rhythmic activity and effects of antagonists

Both the amplitude and frequency of methoxamine- and barium-induced rhythmic contractions were measured. The mean amplitude was obtained by averaging the maximum tension produced by the last 20 contractions or by the contractions occurring during the last 12.5 min of the drug contact period. The mean frequency, expressed as responses min^{-1}, was calculated from the number of contractions present during the last 12.5 min of the drug contact period. In studying the effects of the antagonists, after an initial equilibration period of about 45 min, the antagonist was added cumulatively with each concentration left in contact with the tissue for approximately 20 min. The amplitude and frequency of the rhythmic contractions were expressed as a percentage of the control values before addition of the antagonist. It was found that in control preparations there was a gradual increase in the amplitude of the rhythmic responses which paralleled a reduction in their frequency during the period of the experiment (up to 4 h). Therefore, to allow correct qualitative analysis of the results and accurate measurement of antagonist IC_{50} values, in most experiments one vas deferens was used as the control and the contralateral vas deferens was treated with the antagonist, and control and treated tissues were compared simultaneously.

Drugs

The following drugs were used: atropine sulphate (BDH), guanethidine sulphate (Ciba), hydralazine HCl (Ciba), (±)-methoxamine HCl (Wellcome), (±)-methoxyverapamil HCl (Knoll), methysergide bimaleate (Sandoz), phentolamine mesylate (Ciba), sodium nitroprusside (BDH), (±)-verapamil HCl (Knoll) (all dissolved in distilled water); dantrolene sodium (Eaton) (2.50 mM in propylene glycol); diazoxide (Allen & Hanburys) (43.3 mM in 0.2 M NaOH); flunarizine 2HCl (Janssen) (2.09 mM in 0.02 M tartaric acid); nifedipine (Bayer) (2.89 mM in propylene glycol); reserpine (in 20% ascorbic acid); N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES); tris-hydroxymethylaminomethane (TRIS); 4-2-hydroxyethyl-1-piperazinepropanesulphonic acid (EPPS) (Sigma).

Statistics

Results are expressed as mean ± s.e.mean. In the graphs, standard errors are shown by vertical bars (except where less than the thickness of the symbol). Statistical analysis of the data was made using Student's *t* test, and the 0.05 level of probability was regarded as significant.

Results

Components of methoxamine- and barium-induced contractions

The concentrations of the agonists used in the experiments presented here (except in the few studies using HEPES, Tris or EPPS-buffered Krebs solution) were methoxamine 8.1 μM and $BaCl_2$ 1 mM. These produced characteristic maximal, or near maximal, rhythmic contractions and the rhythmic responses induced by methoxamine and barium were of similar amplitude and also of similar frequency; increases in the concentration of either agent produced little effect or caused a reduction in rhythmic activity. Typical responses to methoxamine 8.1 μM and $BaCl_2$ 1 mM in intact and in epididymal and prostatic halves of bisected vasa deferentia are shown in Figure 1.

Methoxamine 8.1 μM usually produced an initial phasic contraction that reached a maximum after 30–90s, and then declined towards the baseline. This was followed by rhythmic contractions (usually superimposed on a slightly elevated basal tone) which started 2–6 min after addition of methoxamine and continued until washout (up to 4 h). Both the initial phasic and the rhythmic components of the response were predominant in the epididymal half; rhythmic responses were of comparable amplitude but of much lower frequency in the prostatic half (Figure 1). Removal of the middle third portion of the vas deferens resulted in a greater difference in the responses between the two regions than when the tissue was simply bisected (Table 1).

$BaCl_2$ 1 mM in some experiments produced an initial phasic response which reached a peak after 20–90 s, and which was smaller than that produced by methoxamine 8.1 μM, but, unlike the methoxamine response, usually relaxed to the base line. Rhythmic contractions started during the phasic response, or at an equivalent time when this was not present, and continued until washout (up to 4 h). Perhaps the most interesting finding was that, in contrast to the response to methoxamine, both the initial phasic response and the amplitude and frequency of the barium-induced rhythmic contractions were similar in the epididymal and prostatic halves (Figure 1). Responses of the two regions of the vas deferens were also similar after removal of the central third of the tissue (Table 1).

Rhythmic responses to methoxamine 8.1 μM were substantially reduced by phentolamine (0.26–1.06 μM) and abolished by phentolamine 2.65 μM ($n = 24$). In contrast, phentolamine 2.65 μM was without effect on rhythmic contractions to $BaCl_2$ 1 mM (amplitude $+4.9 \pm 5.9\%$, frequency $+14.3 \pm 11.2\%$, $n = 7$) and an increase in their frequency was produced by phentolamine 13.2 μM. The

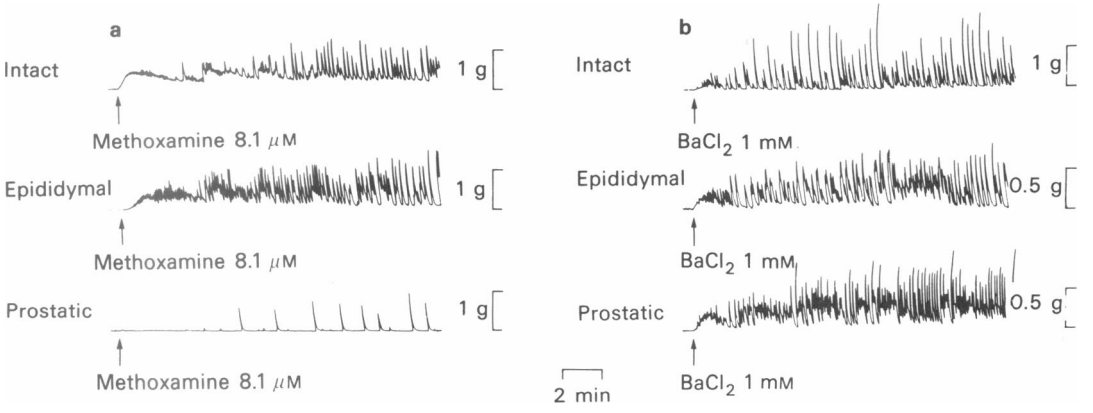

Figure 1 Contractions (initial phasic and rhythmic) induced by methoxamine or barium in intact and bisected vasa deferentia. (a) Responses produced by methoxamine (8.1 μM) were similar in the intact tissue and in the epididymal half, but there was little response in the prostatic half. (b) $BaCl_2$ (1 mM) produced similar contractions in the intact preparation and in the two regions of the bisected tissue.

amplitude of both the initial phasic response and the rhythmic contractions was increased after reserpine-treatment or chronic guanethidine treatment (Table 1), which was probably due to the non-specific super-sensitivity produced by these procedures in the rodent vas deferens (Kasuya, Goto, Hashimoto, Watanabe, Munakata & Watanabe, 1969; Westfall, 1970). In one experiment it was found that rhythmic responses to $BaCl_2$ 1 mM were unaffected by atropine 1.44 μM, propranolol 3.38 μM or methysergide 8.52 μM.

Ca^{2+}-dependence and effects of magnesium and lanthanum

Both the initial phasic contraction and rhythmic contractions to methoxamine 8.1 μM were abolished after incubation for 30 min in nominally Ca^{2+}-free Krebs-Henseleit solution. Addition of Ca^{2+} produced rhythmic contractions and the frequency of responses was found to be more sensitive than the amplitude to low $[Ca^{2+}]_o$: maximum frequency was obtained at $[Ca^{2+}]_o$ = 1 mM, and maximum amplitude

Table 1 Components of contractions produced by methoxamine 8.1 μM or by $BaCl_2$ 1 mM

	Phasic component *tension* (g)	*Rhythmic component* *tension* (g)	*frequency* (min^{-1})	n
Methoxamine 8.1 μM				
Intact	0.31 ± 0.02	0.83 ± 0.03	4.78 ± 0.13	94
Bisected: prostatic half	0.11 ± 0.03	1.28 ± 0.16	1.87 ± 0.24	10
epididymal half	0.44 ± 0.07[a]	1.53 ± 0.15[b]	4.46 ± 0.35[a]	10
Trisected: prostatic third	0.06 ± 0.01	1.11 ± 0.67	0.18 ± 0.11	4
epididymal third	0.03 ± 0.01	0.62 ± 0.22	3.56 ± 1.54	4
$BaCl_2$ 1 mM				
Intact	0.14 ± 0.02	0.79 ± 0.04	5.43 ± 0.15	124
Bisected: prostatic half	0.11 ± 0.02	0.84 ± 0.11	5.61 ± 0.43	19
epididymal half	0.10 ± 0.02[b]	0.71 ± 0.04[b]	5.94 ± 0.39[b]	19
Trisected: prostatic third	0.04 ± 0.01	0.52 ± 0.07	4.92 ± 0.12	4
epididymal third	0.06 ± 0.02	0.68 ± 0.08	6.64 ± 0.99	4
Denervated (intact)	0.33 ± 0.09	1.22 ± 0.25	6.29 ± 0.51	10
Reserpinised (intact)	0.30 ± 0.11	2.19 ± 0.64	5.81 ± 0.64	6

[a] Significantly different from the prostatic half, $P < 0.001$. [b] Not significantly different from the prostatic half. Results are expressed as mean ± s.e.mean.

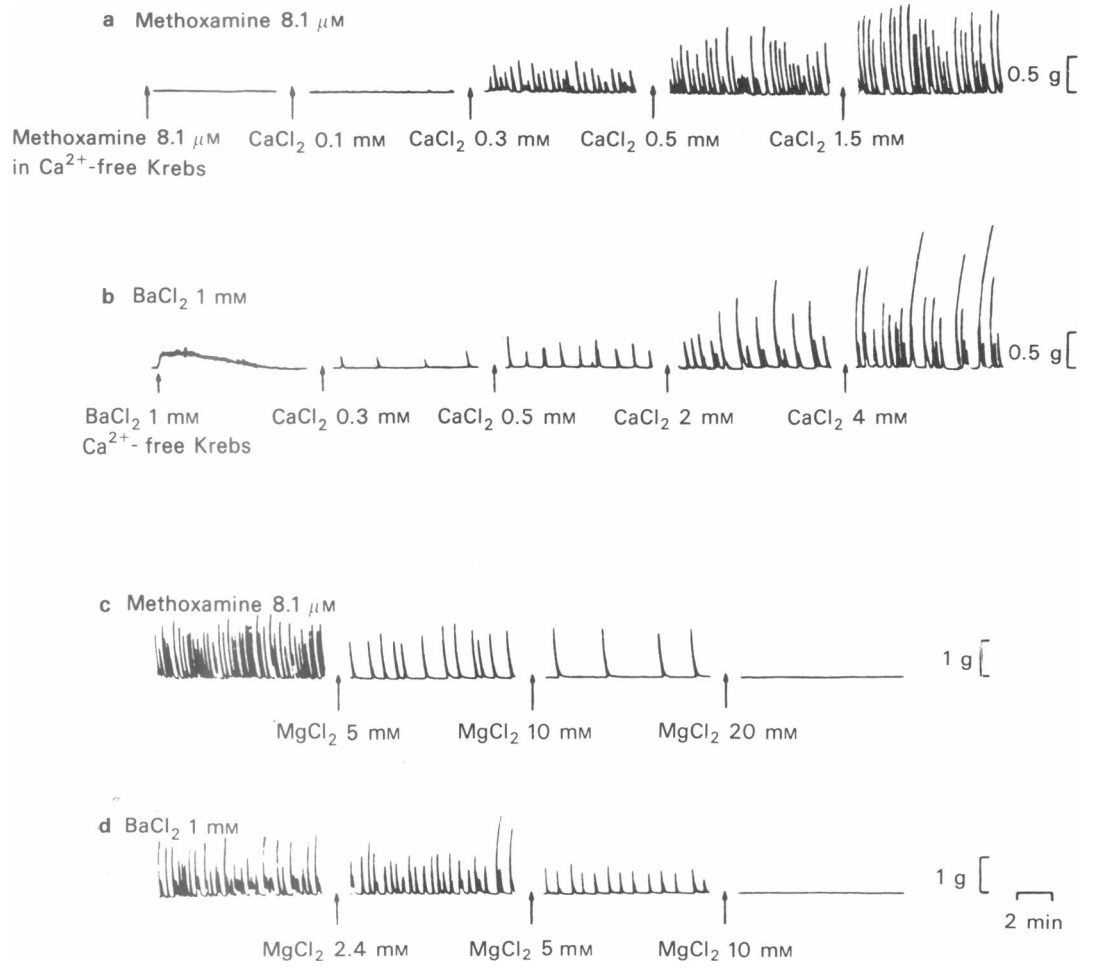

Figure 2 Effects of Ca^{2+}-deprivation and Ca^{2+}-readmission and of magnesium on methoxamine- or barium-induced rhythmic responses in the intact tissue. (a) Both the initial phasic and rhythmic responses to methoxamine (8.1 μM) were abolished in Ca^{2+}-free solution. Cumulative addition of $[Ca^{2+}]_o$ increased the amplitude and frequency of rhythmic contractions. (b) The initial phasic response to $BaCl_2$ (1 mM) was present in Ca^{2+}-free medium superimposed on which small rhythmic contractions occurred. Increasing the $[Ca^{2+}]_o$ produced a parallel increase in the amplitude and frequency of rhythmic responses. Experiments involving addition of magnesium were done in 2.5 mM $CaCl_2$ Krebs-Henseleit solution and the concentrations of Mg^{2+} quoted are the total, including the 1.2 mM originally present in the physiological solution. (c) Mg^{2+} selectively inhibited the frequency of rhythmic responses to methoxamine (8.1 μM). (d) The amplitude and frequency of rhythmic contractions produced by $BaCl_2$ (1 mM) were inhibited in parallel by Mg^{2+}.

occurred at $[Ca^{2+}]_o = 2$ mM (Figure 2a and 3a).

In contrast to methoxamine 8.1 μM, the initial phasic response to $BaCl_2$ 1 mM persisted in Ca^{2+}-free Krebs, and in 4 out of 9 experiments small rhythmic contractions were also present. In further contrast to methoxamine-induced responses, there was a parallel increase in the amplitude and the frequency of barium-induced rhythmic contractions on elevation of $[Ca^{2+}]_o$: maximum amplitude occurred at $[Ca^{2+}]_o = 4$ mM and frequency was maximal at 2 mM (Figures 2b and 3b). The rate of loss of rhythmic responses to $BaCl_2$ 1 mM was analysed by changing from 2.5 mM Krebs-Henseleit solution to Ca^{2+}-free Krebs in the continued presence of barium: the amplitude of the contractions was reduced by 50% of their control value after 14.1 ± 2.8 min, $n = 6$, in Ca^{2+}-free solution which is approximately the same rate of decay of the tonic component of the contraction to KCl 160 mM observed in Ca^{2+}-deprivation studies (Hay & Wadsworth, 1982a).

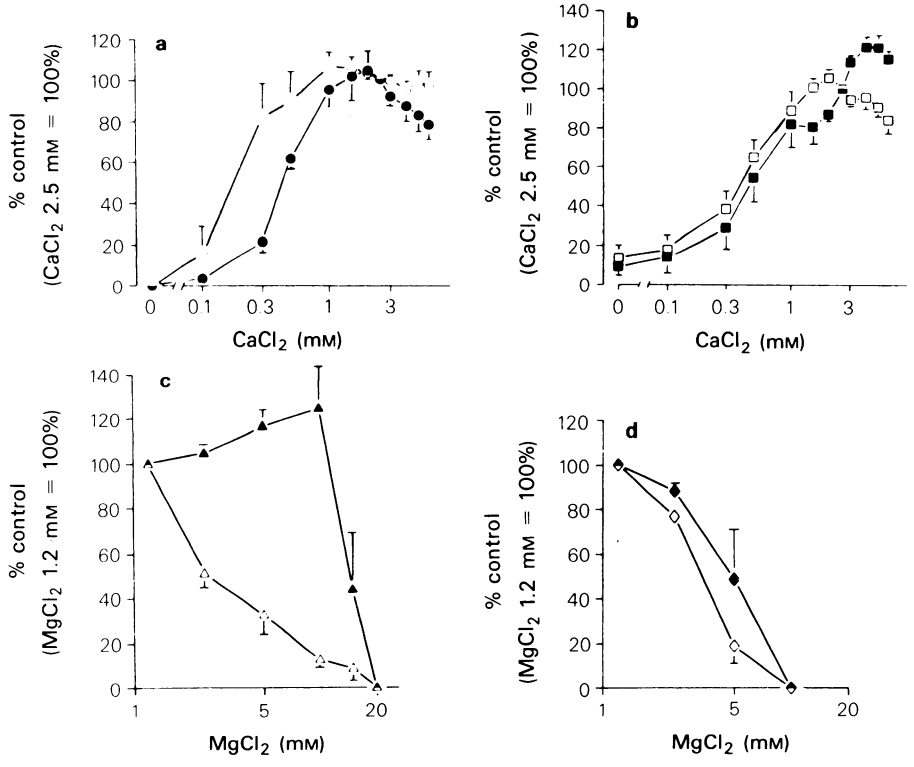

Figure 3 Effects of extracellular Ca^{2+} and Mg^{2+} on rhythmic contractions produced by methoxamine or $BaCl_2$. Closed symbols = amplitude, open symbols = frequency. (a), (b) Starting in a Ca^{2+}-free Krebs-Henseleit solution, $CaCl_2$ was added cumulatively in the presence of methoxamine (8.1 μM) (●, ○) or $BaCl_2$ (1 mM) (■, □). (c), (d) In 2.5 mM $CaCl_2$ Krebs-Henseleit Mg^{2+} was added cumulatively in the presence of methoxamine (8.1 μM) (▲, △), (c) or $BaCl_2$ (1 mM) (◆, ◇), (d). Each point refers to the total $[Mg^{2+}]_o$. (a) and (b): $n = 9$. (c) and (d): $n = 4$.

The amplitude and frequency of rhyrhmic responses to $BaCl_2$ 1 mM were reduced in parallel by Mg^{2+} (2.4–5 mM) and abolished by Mg^{2+} 10 mM. In contrast, the frequency of methoxamine-induced contractions was selectively reduced by Mg^{2+} (2.4–10 mM), with no effect on their amplitude. The amplitude of rhythmic responses to methoxamine 8.1 μM was reduced by Mg^{2+} 15 mM and abolished by Mg^{2+} 20 mM (Figure 2, c, d and Figure 3, c, d).

Both the amplitude and frequency of rhythmic contractions induced by methoxamine 8.1 μM or $BaCl_2$ 1 mM were reduced in Krebs solution buffered with HEPES, Tris or EPPS compared to those produced in normal Krebs-Henseleit solution, although there was no effect on the initial phasic response produced by either agent. In some experiments the inhibitory effect of the buffering compounds could be partly overcome by increasing the agonist concentration. $LaCl_3$ (1–5 mM) reduced both the amplitude and frequency of rhythmic responses produced by either agonist; the basal tone was increased by $LaCl_3$ 5 mM.

Effects of drugs

The effects of diazoxide, nitroprusside and hydralazine, as well as the calcium channel inhibitors nifedipine, verapamil, methoxyverapamil and flunarizine, on the rhythmic activity produced by methoxamine 8.1 μM or $BaCl_2$ 1 mM were investigated. In general, these agents had a greater inhibitory action on the amplitude rather than on the frequency of responses, although some qualitative differences existed in their effects and there were marked differences in their potencies; the IC_{50} values are indicated in Table 2. The effects of the drugs are described in more detail below.

Nifedipine, verapamil, methoxyverapamil and flunarizine Nifedipine (1.44–5.78 μM) was equally effective in reducing the amplitude of rhythmic contractions produced by either methoxamine 8.1 μM or $BaCl_2$ 1 mM. The frequency of responses was reduced by nifedipine 5.78 μM, with methoxamine-induced contractions more sensitive to inhibition, and re-

Table 2 Effect of calcium channel inhibitors, nitroprusside and hydralazine on rhythmic contractions produced by methoxamine (8.1 μM) or $BaCl_2$ (1 mM) in the intact vas deferens

	IC_{50} (μM)							
	Amplitude				*Frequency*			
	Methoxamine (8.1 μM)		*$BaCl_2$ (1 mM)*		*Methoxamine (8.1 μM)*		*$BaCl_2$ (1 mM)*	
Nifedipine	2.42 ± 0.34	(7)	3.00 ± 0.84	(6)	3.24 ± 0.75	(7)	6.87 ± 1.29	(6)
Verapamil	29.8 ± 5.40	(6)	35.6 ± 10.9	(7)	96.7 ± 31.0	(5)	134 ± 26.1	(6)
Methoxyverapamil	16.8 ± 2.86	(5)	13.9 ± 1.95	(5)	2.07 ± 0.81	(5)	48.5 ± 8.98	(5)
Flunarizine	15.9 ± 5.95	(7)	54.5 ± 20.0	(7)	153 ± 28.6	(7)	> 314	(7)
Diazoxide	432 ± 164	(6)	*269 ± 126	(7)	a	(6)	a	(6)
Nitroprusside	212 ± 110	(6)	b	(6)	4,086 ± 472	(6)	> 6,712	(6)
Hydralazine	348 ± 145	(3)	426 ± 267	(3)	c	(3)	4,391 ± 162	(3)

There was no significant difference between the sensitivity of the amplitude of methoxamine and barium-induced contractions to inhibition (except with nitroprusside). The amplitude of responses was more sensitive to inhibition than was the frequency, except with methoxyverapamil on methoxamine-induced responses, $P < 0.002$.

* In 2 out of 9 experiments diazoxide produced no inhibition.

a No effect with diazoxide up to a concentration of 2,186 μM; b nitroprusside increased the amplitude of barium-induced responses; c no effect with hydralazine up to a concentration of 5,086 μM.

Results are expressed as mean ± s.e.mean. Numbers in parentheses show the number of experiments.

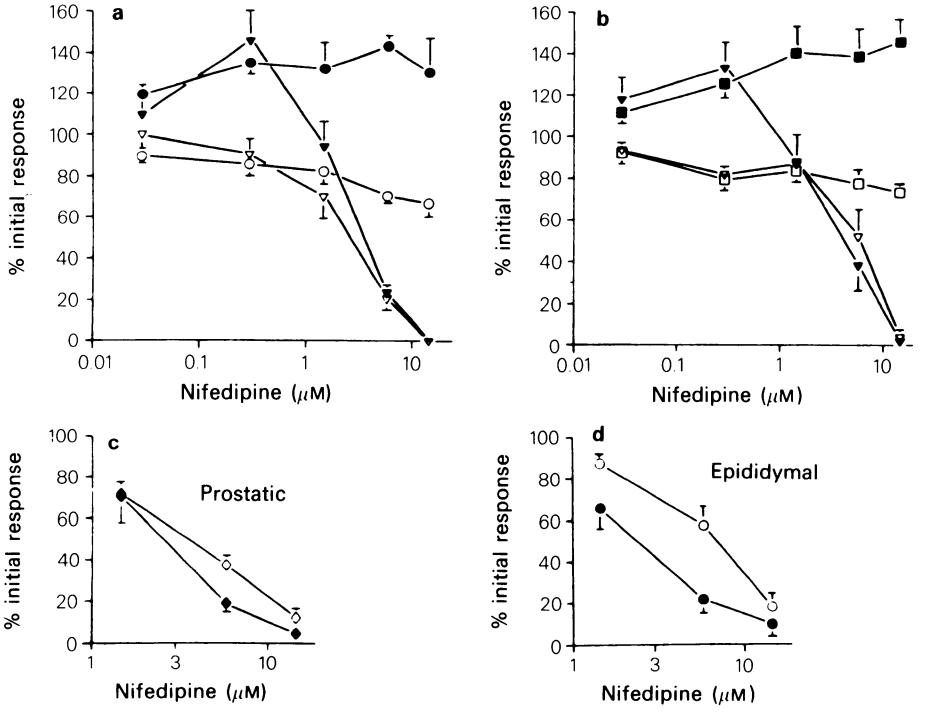

Figure 4 Effect of nifedipine on rhythmic contractions produced by methoxamine (8.1 μM) in (a) intact vas deferens and by barium ($BaCl_2$, 1 mM) in (b) intact and (c, d) bisected vasa deferentia. Closed symbols = amplitude of rhythmic contractions, open symbols = frequency of rhythmic contractions. (a) Methoxamine (8.1 μM) was added to control tissues (●, ○) and also to the contralateral tissues to which nifedipine was added cumulatively (▼, ▽). (b) $BaCl_2$ (1 mM) was added to control tissues (■, □) and also to the contralateral tissues to which nifedipine was added cumulatively (▼, ▽). Nifedipine was added cumulatively to (c) prostatic (◆, ◇) and (d) epididymal halves (●, ○) of bisected vasa deferentia in which rhythmic activity had been established with $BaCl_2$ (1 mM) (a): $n = 6-7$. (b): $n = 6$. (c) and (d): $n = 7$.

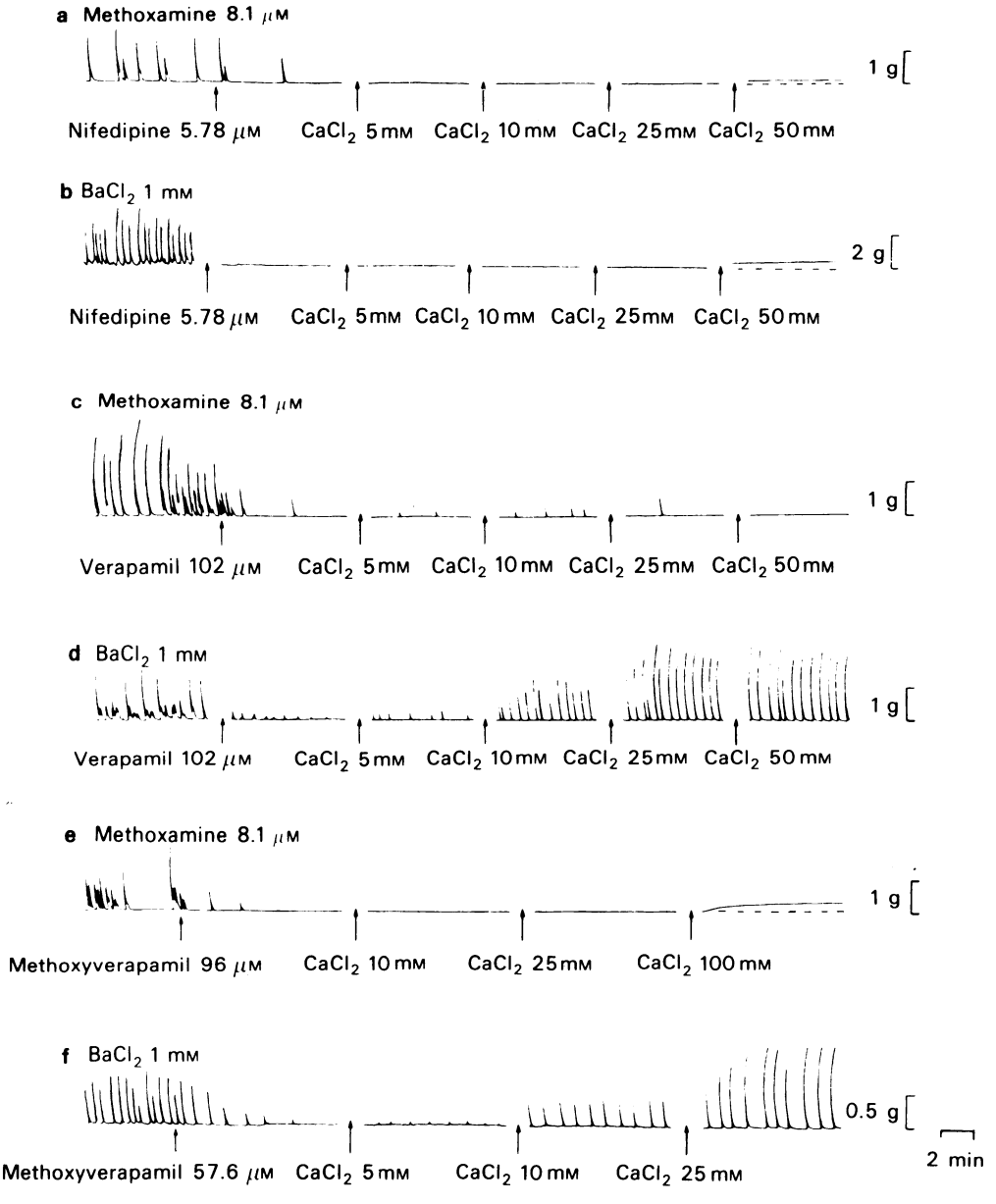

Figure 5 Effect of increasing $[Ca^{2+}]_o$ on the inhibitory actions of nifedipine, verapamil and methoxyverapamil on rhythmic contractions produced by methoxamine or $BaCl_2$. The first panel indicates control rhythmic responses to methoxamine 8.1 μM (a, c, e) or $BaCl_2$ 2 mM (b, d, f) in 2.5 mM $CaCl_2$ HEPES- (a, b, c, e, f) or Tris-buffered Krebs solution (d). Responses were inhibited by addition of (a, b) nifedipine 5.78 μM, (c, d) verapamil 102 μM, (e) methoxyverapamil 96 μM or (f) methoxyverapamil 57.6 μM (as shown in the second panel), and in the continued presence of the calcium channel inhibitors, $[Ca^{2+}]_o$ was added cumulatively (2.5–97.5 mM to give total concentrations of 5–100 mM as indicated). The inhibitory effect of nifedipine on (a) methoxamine-induced or (b) barium-induced responses was not reversed at all by increasing the $[Ca^{2+}]_o$. Elevation of $[Ca^{2+}]_o$ reversed the effects of verapamil (d) and methoxyverapamil (f) on responses to $BaCl_2$ (2 mM), but not their inhibitory actions on methoxamine-induced responses ((c) and (e) respectively). Note the increase in baseline tone produced by Ca^{2+} 50 and 100 mM (the original resting tension is indicated by the dotted line).

sponses produced by either agonist were abolished by nifedipine 14.4 μM (Figure 4, a, b; Table 2). The inhibitory effect of nifedipine (1.44 or 5.78 μM) on rhythmic responses produced by methoxamine 8.1 μM or $BaCl_2$ 2 mM in HEPES-buffered Krebs solution was not reversed by increasing the $[Ca^{2+}]_o$ from 5–50 mM (Figure 5, a, b). Responses to $BaCl_2$ 1 mM in prostatic and epididymal halves of bisected vasa deferentia were equally sensitive to inhibition by nifedipine (Figure 4, c, d).

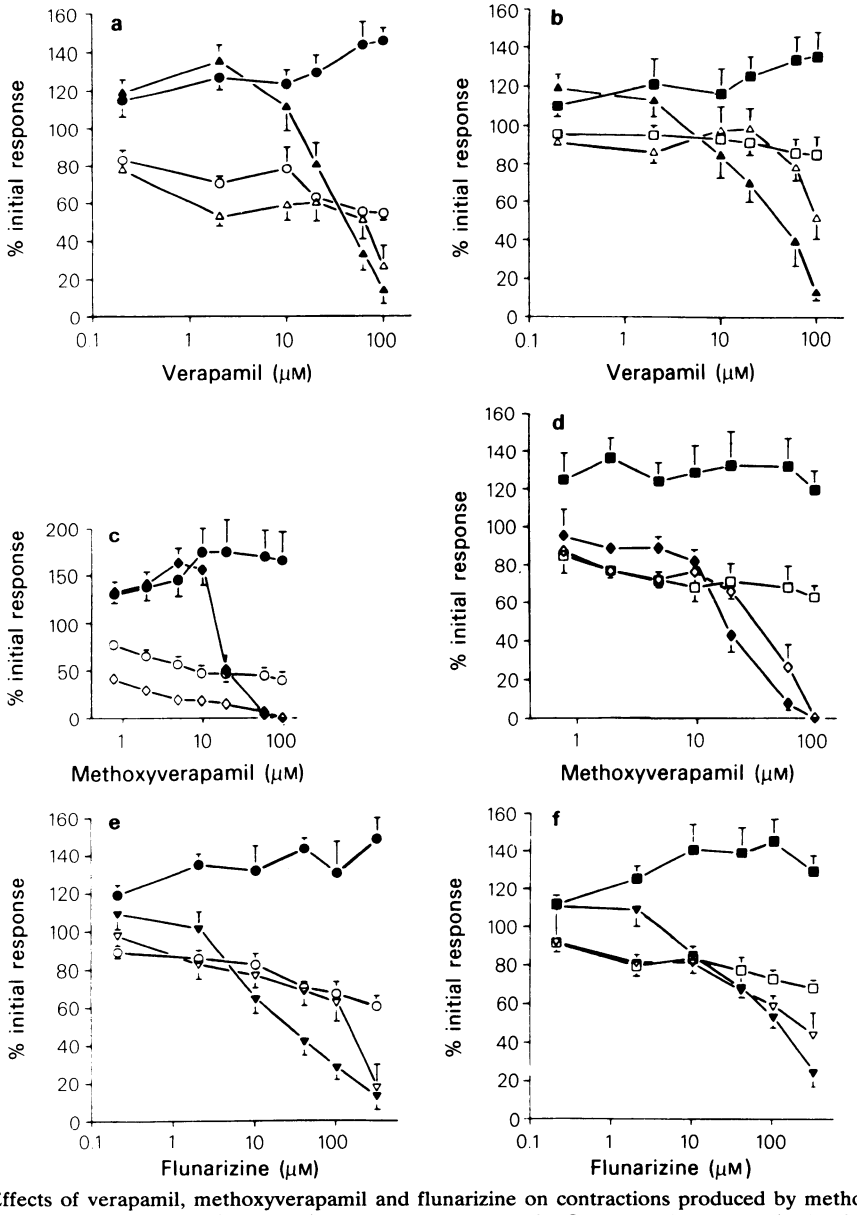

Figure 6 Effects of verapamil, methoxyverapamil and flunarizine on contractions produced by methoxamine, 8.1 μM (a, c and e), or $BaCl_2$, 1 mM (b, d and f), in intact vasa deferentia. Closed symbols = amplitude of rhythmic contractions, open symbols = frequency of rhythmic contractions. In each experiment, one vas deferens was used as the control (methoxamine, 8.1 μM ●, ○; $BaCl_2$, 1 mM ■, □), while cumulatively increasing concentrations of (a, b) verapamil (▲, △), (c, d) methoxyverapamil (◆, ◇) or (e, f) flunarizine (▼, ▽) were added to the contralateral vas deferens. (a): $n = 6$. (b): $n = 6–8$. (c, d): $n = 5$. (e, f): $n = 6–7$.

Verapamil produced quantitatively and qualitatively similar effects against rhythmic responses, produced by either agonist. Thus, verapamil (10.2–102 μM) reduced the amplitude of contractions, whereas verapamil 10.2–20.4 μM produced an increase in the frequency of responses in 50% of experiments, and a marked reduction in the frequency only occurred with verapamil 102 μM (Figure 6, a, b; Table 2). The inhibitory effect of verapamil (20.4 or 102 μM) on responses to $BaCl_2$ 2 mM was reversed by increasing the $[Ca^{2+}]_o$, whereas the inhibition of contractions induced by methoxamine 8.1 μM was not (Figure 5, c, d).

Methoxyverapamil (9.6–57.6 μM) reduced, but at 96 μM abolished, the amplitude of rhythmic contractions produced by $BaCl_2$ 1 mM. The frequency of responses was less sensitive to inhibition, and in 2 out of 5 experiments methoxyverapamil 9.6 μM increased the frequency of contractions (Figure 6d). However, in contrast to its effect on barium-induced responses, methoxyverapamil selectively blocked the frequency of contractions to methoxamine 8.1 μM: the frequency was approximately 8 times more sensitive to inhibition than was the amplitude. (Figure 6c; Table 2). Increasing the $[Ca^{2+}]_o$ reversed the inhibitory effect of methoxyverapamil (19.2–96 μM) on barium-induced responses, but was without effect on the inhibition of methoxamine-induced contractions produced by methoxyverapamil 96 μM (Figure 5, e, f).

Flunarazine (2.09–314 μM) reduced the amplitude of both methoxamine- and barium-induced

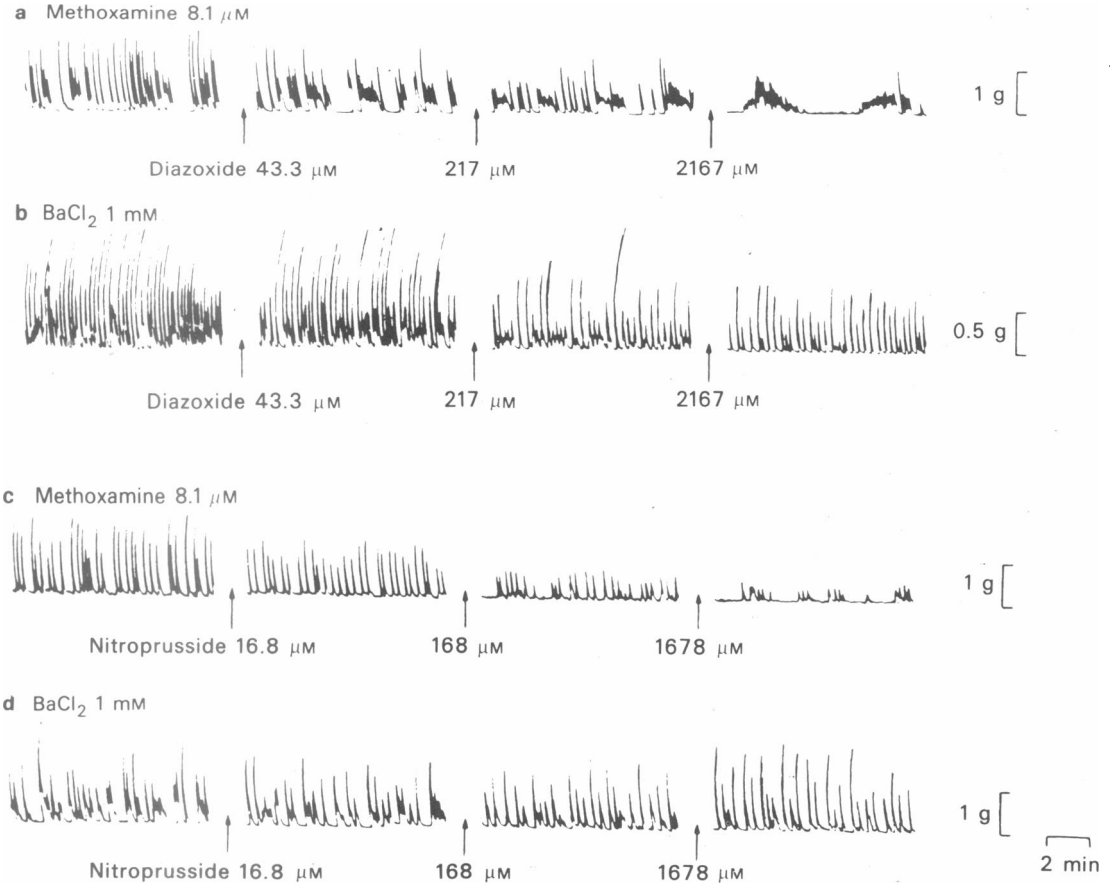

Figure 7 Effects of diazoxide and nitroprusside on rhythmic contractions produced by methoxamine (a and c) or $BaCl_2$ (b and d) in intact vasa deferentia. Control responses to (a, c) methoxamine, 8.1 μM and (b, d) $BaCl_2$, 1 mM are shown in the first panel of each section. Cumulatively increasing concentrations of diazoxide (a, b) or nitroprusside (c, d) were then added. (a) Diazoxide and (c) nitroprusside produced a dose-related inhibition of methoxamine-induced responses and caused the contractions to occur in groups. (b) Diazoxide caused some reduction in responses to $BaCl_2$ (1 mM) whereas (d) nitroprusside increased barium-induced contractions.

rhythmic contractions, but was without effect on the frequency of these responses except at the highest concentrations used (105 and 314 μM) (Figure 6, e, f; Table 2). Tartaric acid, the solvent for flunarizine, produced a slight decrease in the amplitude of contractions induced by methoxamine 8.1 μM and $BaCl_2$ 1 mM at a concentration equivalent to that in flunarizine 314 μM.

Diazoxide and nitroprusside Diazoxide and nitroprusside had a similar profile of action on both the amplitude and the frequency of methoxamine-induced contractions and also on responses, to barium. Contractions produced by methoxamine 8.1 μM were reduced in amplitude in a dose-related manner by diazoxide (43.3–2167 μM) (Figures 7a and 8a). In contrast, although diazoxide (43.3–217 μM) produced some reduction in the amplitude of responses to $BaCl_2$ 1 mM no further inhibition was produced by increasing the concentration up to 2167 μM (Figures 7b and 8b); in some preparations these higher concentrations of diazoxide increased the amplitude of barium-induced responses. NaOH, equivalent to that in diazoxide 2167 μM, caused some reduction in the amplitude and frequency of rhythmic responses, especially those produced by methoxamine.

Nitroprusside (16.8–6712 μM) produced a dose-dependent reduction in the amplitude of methoxamine-induced rhythmic contractions (Figures 7c and 8c). There was a small reduction in the amplitude of responses to $BaCl_2$ 1 mM with nitroprusside (3.36–168 μM) but in higher concentrations, (503–6712 μM), nitroprusside increased the amplitude of contractions (Figures 7d and 8d).

Both diazoxide (217–2167 μM) and nitroprusside (671–1678 μM) caused the methoxamine-induced rhythmic responses to occur in groups with no overall effect on their frequency. There was no effect by either drug on the frequency of contractions produced by $BaCl_2$ 1 mM (except with the highest concentration of nitroprusside, 6712 μM) and, unlike methoxamine-induced contractions, no grouping of responses occurred (Figure 7).

Hydralazine Hydralazine (25.4–5086 μM) produced a dose-related reduction in the amplitude of rhythmic responses produced by methoxamine 8.1 μM with no effect on their frequency. Contractions to $BaCl_2$ 1 mM were decreased in amplitude by hydralazine (102–2543 μM), although there was a corresponding increase in frequency in the same concentration range; responses were abolished by hydralazine 5086 μM (Figure 8, e, f).

Dantrolene In the one preparation examined, dantrolene (1.25–5.01 μM) was without effect on rhythmic responses produced by either methoxamine or barium, but higher concentrations (25.0–50.1 μM) decreased the amplitude with a parallel increase in the frequency of responses to either agonist.

Tetraethylammonium (TEA) TEA (1.63–5.44 mM) was found to produce rhythmic contractions that were not affected by phentolamine 2.65 μM ($n = 1$).

Discussion

Characteristics of rhythmic responses and effects of calcium channel inhibitors

The main result which emerged from this series of experiments was that although rhythmic contractions produced by either methoxamine or barium were dependent on $[Ca^{2+}]_o$, they were inhibited by the calcium channel inhibitors tested only in concentrations that were approximately 10–100 times those required to inhibit KCl-induced contractions (Hay & Wadsworth, 1982a). A general feature of the calcium channel inhibitors was that they inhibited the amplitude of the rhythmic responses more readily than their frequency, and exhibited no selectivity for inhibition of contractions produced by either methoxamine or barium. Further results consistent with this general pattern are the effects of verapamil and nifedipine on rhythmic contractions produced by 5-hydroxytryptamine, which are also dependent on $[Ca^{2+}]_o$ (Hay & Wadsworth, 1982b). These results suggest that the rhythmic contractions produced by both methoxamine and barium are mediated by the uptake of $[Ca^{2+}]_o$ via similar types of membrane calcium channel which have a lower affinity for the calcium channel inhibitors than those activated by high K^+. Both the methoxamine-operated channels and those stimulated by barium, which involve a mechanism other than α-adrenoceptor activation (see below), are probably similar to the receptor-operated channels postulated by Bolton (1979), which are insensitive to the calcium channel inhibitors. The mechanisms underlying the rhythmic responses produced by stimulation of α-adrenoceptors must be different from those mediating the biphasic response produced by a high concentration of noradrenaline (100 μM), both phases of which were inhibited by low concentrations of methoxyverapamil or nifedipine (< 1 μM) (Triggle *et al.*, 1979).

The epididymal half of the rat vas deferens has been shown to be more sensitive to α-adrenoceptor agonists than the prostatic half (Pennefather *et al.*, 1974; Kasuya & Suzuki, 1979). As expected from the results of these earlier studies, it was found that both the initial phasic response and rhythmic contractions produced by methoxamine were predominant in the

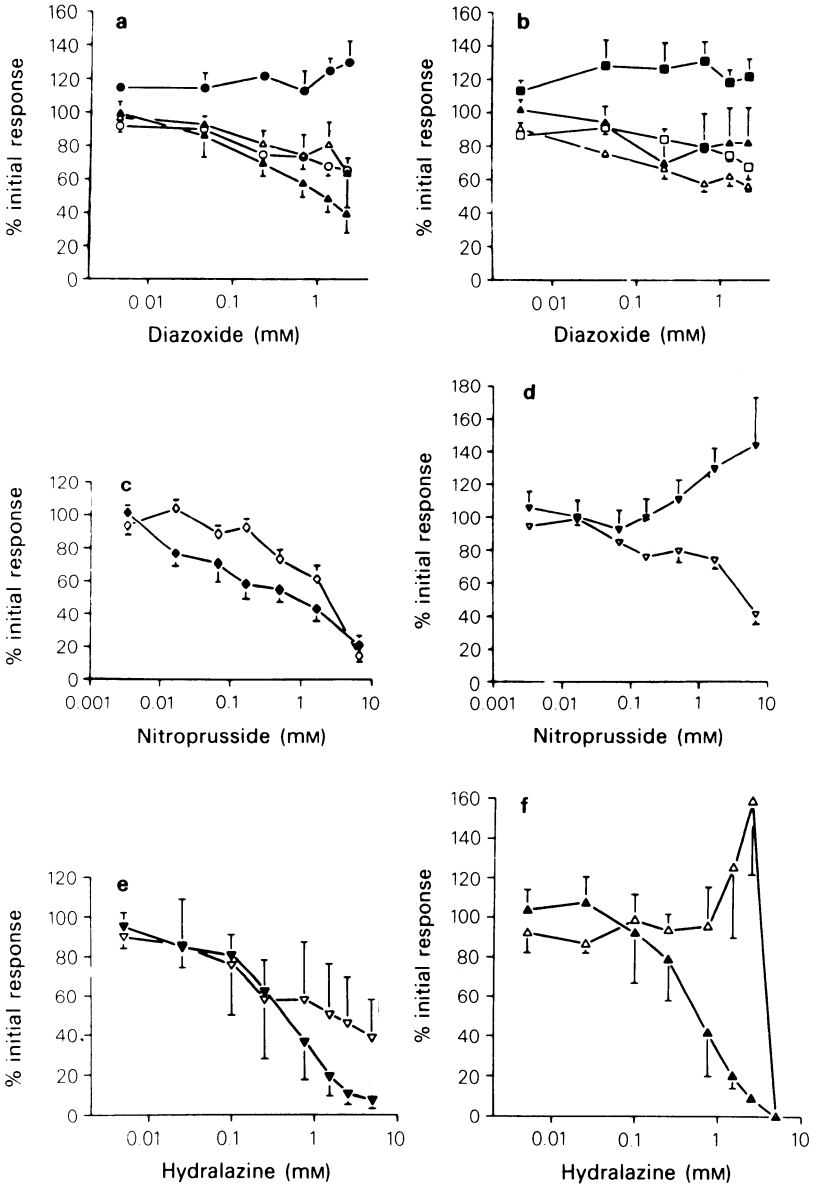

Figure 8 Effects of diazoxide, nitroprusside and hydralazine on contractions produced by methoxamine, 8.1 μM (a, c and e) or $BaCl_2$, 1 mM (b, d and f), in intact vasa deferentia. Closed symbols = amplitude of rhythmic contractions, open symbols = frequency of rhythmic contractions. (a, b) One vas deferens was used as the control, and rhythmic contractions were obtained with methoxamine, 8.1 μM (●, ○) or $BaCl_2$, 1 mM (■, □). The contralateral vas deferens was exposed to increasing concentrations of diazoxide (▲, △). (c, d) Nitroprusside was added cumulatively to vasa deferentia in which rhythmic contractions had been established with methoxamine, 8.1 μM (◆, ◇) or $BaCl_2$, 1 mM (▼, ▽). (e, f) Hydralazine was added cumulatively to vasa deferentia in which rhythmic contractions had been established with methoxamine, 8.1 μM (▼, ▽) or with $BaCl_2$ 1 mM (▲, △). (a): $n = 5-7$. (b): $n = 6-9$. (c, d): $n = 6$. (e, f): $n = 3$.

epididymal half (Table 1). However, in contrast to methoxamine, both the initial phasic and rhythmic components of the response to barium were present to an equal extent in both regions of the vas deferens indicating that the prostatic half has the ability to produce frequent rhythmic contractions. This suggests that the barium-activated calcium channels are evenly distributed throughout the vas deferens, and this hypothesis is reinforced by the demonstration that barium-induced responses in both regions were equally sensitive to nifedipine (Figure 4). In further support, barium-stimulated La^{3+}-resistant ^{45}Ca uptake is similar in both halves (Hay & Wadsworth, 1982c). The rhythmic contractions (and also the initial phasic response) produced by $BaCl_2$ were not mediated by the release of endogenous noradrenaline or activation of α-adrenoceptors, as they were not inhibited by phentolamine, reserpinisation or denervation. Furthermore, the lack of effect of atropine, propranolol or methysergide against these rhythmic responses rules out activation of muscarinic receptors, β-adrenoceptors or 5-hydroxytryptamine receptors respectively as being responsible for the barium-induced responses.

The electrical events underlying the rhythmic responses produced by methoxamine or barium are not known but they are likely to involve membrane depolarization, as has been shown for noradrenaline (Magaribuchi, Ito & Kuriyama, 1971; Sjöstrand, 1973b) and barium (Sjöstrand, 1973a) in the guinea-pig vas deferens, and may perhaps be associated with some sort of regenerative spiking activity. There is some evidence to suggest that the rhythmic responses, particularly those induced by $BaCl_2$, involve an effect on K^+ conductance. Thus, TEA, which inhibits K^+ currents (Hille, 1967), produced rhythmic contractions which, like barium-induced responses, were unaffected by phentolamine. Furthermore, TEA 10 mM initiated rhythmic activity in the normally quiescent rabbit ear artery which was caused by depolarization of the smooth muscle cell membrane and Ca^{2+}-dependent action potentials; TEA was also shown to reduce K^+ efflux, and the TEA-induced responses were inhibited by methoxyverapamil or Mn^{2+} (Droogmans, Raeymaekers & Casteels, 1977). In addition, barium was reported to decrease K^+ conductance in the rat portal vein (Hermsmeyer, 1976) which was probably responsible for the augmentation of the spontaneous rhythmic activity, also described by Uvelius & Sigurdsson (1981) who concluded that barium was acting both by increasing the spike activity and also by increasing the uptake or release of Ca^{2+} per spike. An inhibitory effect by barium, and possibly also methoxamine, on K^+ conductance, by delaying repolarization and thus promoting action potential discharge, could be responsible for the initiation and maintenance of the rhythmic contractions in the rat vas deferens observed in the present study.

The initial phasic response to $BaCl_2$, unlike the rhythmic activity, occurred in Ca^{2+}-free medium and was thus probably mediated by an intracellular Ca^{2+} source. The response to a much higher concentration of $BaCl_2$ (30 mM) in the rat vas deferens was also resistant to Ca^{2+}-deprivation and was thought to involve an intracellular Ca^{2+} store (Jurkiewicz, Markus & Picarelli, 1975). Release of intracellular Ca^{2+} by barium has been proposed in the rat portal vein (Uvelius & Sigurdsson, 1981), and another possible intracellular mechanism is direct activation of the contractile proteins, as barium has been shown to enter smooth muscle cells (Uvelius, Sigurdsson & Johansson, 1974) and can substitute for Ca^{2+} in maintaining contractile activity, albeit to a limited extent (Ebashi, 1971; Uvelius *et al.*, 1974). Alternatively, the initial response to $BaCl_2$ may be mediated by uptake of a high-affinity membrane bound Ca^{2+} source (which would be resistant to Ca^{2+}-deprivation) through Ca^{2+} channels sensitive to calcium inhibitors, as has been proposed for the initial contraction to barium in the guinea-pig ileum (Clement, 1981).

The increase in the frequency of methoxamine- and barium-induced responses produced by certain concentrations of verapamil (and also the small increase in frequency of barium response observed with methoxyverapamil) may be caused by membrane depolarization, as reported by Haeusler (1972) for verapamil in similar concentrations in the rabbit main pulmonary artery. An increase in the frequency of 5-HT-induced rhythmic contractions has been reported for verapamil in the identical concentration range (Hay & Wadsworth, 1982b).

Similar high concentrations of La^{3+} to those needed to inhibit KCl contractions (Hay & Wadsworth, 1982a) were required to inhibit rhythmic responses to methoxamine or barium, which indicates that responses of the rat vas deferens are generally insensitive to La^{3+}, and the inhibitory effects of La^{3+} are, therefore, probably mediated by a non-selective antagonism of the membrane calcium channels.

Differences in methoxamine- and barium-induced rhythmic contractions

Although both methoxamine- and barium-induced rhythmic contractions were similar in form, dependent on $[Ca^{2+}]_o$ and exhibited similar sensitivities to calcium channel inhibitors, there were several findings in this series of experiments which suggest that they produced responses by different mechanisms:

(1) Methoxyverapamil was alone amongst the calcium channel inhibitors in selectively inhibiting the

frequency of methoxamine-induced contractions. Elevation of $[Mg^{2+}]_o$ produced a similar effect on responses to methoxamine as methoxyverapamil. These results suggest that both methoxyverapamil and Mg^{2+} inhibited methoxamine-induced rhythmic contractions by a similar mechanism of action, perhaps involving inhibition of the excitation mechanism and the generation of pacemaker activity, rather than an effect on excitation-contraction coupling; Mg^{2+} has been reported to inhibit spontaneous rhythmic contractions of the rat portal vein, reducing the number of action potentials while having little effect on Ca^{2+} uptake (Sigurdsson & Uvelius, 1977). Magnesium produces complex effects in smooth muscle and may affect responses by competition with Ca^{2+} at several extracellular and intracellular binding sites (Weiss, 1977). The differential inhibitory effect of Mg^{2+} may thus reflect differences in the relative utilization of Ca^{2+} from the different locations by methoxamine or barium, although the possibility of a direct effect of Mg^{2+} on drug-receptor interactions was discussed by Weiss (1977).

(2) Elevation of the $[Ca^{2+}]_o$ reversed the inhibitory effect of verapamil and methoxyverapamil on barium-induced contractions but did not reverse their action on responses produced by methoxamine. It is possible that the lack of reversal of the inhibition of the methoxamine-induced responses may be because methoxyverapamil and verapamil were acting at a site other than the calcium channel, or that the binding of these agents to the channel did not involve competition with Ca^{2+}, or that verapamil and methoxyverapamil inhibited the channel by binding to a less accessible location, perhaps on the inner surface of the membrane. Alternatively, a more probable explanation is that at the concentrations of the antagonists used a sufficient fraction of the methoxamine-activated calcium channels, but not the barium-activated channels, were blocked to prevent restoration of the response by increasing the $[Ca^{2+}]_o$ (Van Breemen, Hwang & Meisheri, 1981). It is of interest that the inhibitory effect of nifedipine against barium-induced rhythmic contractions, unlike that produced by verapamil or methoxyverapamil, was not reversed by elevation of the $[Ca^{2+}]_o$. The reason(s) for this can only be speculated at but may indicate that nifedipine inactivates the calcium channel by a different mechanism and/or at a different site from verapamil or methoxyverapamil. Henry (1980) suggested that nifedipine may act at the outer surface of the membrane calcium channel, whereas methoxyverapamil and verapamil act on the inner surface of the channel.

(3) Diazoxide and nitroprusside produced substantial effects on the amplitude and also, unlike the other drugs tested, on the arrangement of rhythmic contractions to methoxamine (causing them to occur in groups) but both agents, especially nitroprusside, had little inhibitory effect on barium-induced contractions. In fact nitroprusside in high concentrations increased the amplitude of barium responses, perhaps as a consequence of a reduction in Ca^{2+} efflux as has been found with 100 μM in canine renal arteries (Hester, Weiss & Fry, 1979), or some other mechanism which increased the amount of intracellular Ca^{2+} available to the contractile machinery. Although diazoxide has been reported to inhibit KCl contractions (Levy, 1975), it has been proposed that diazoxide has a main mechanism of action other than inhibition of Ca^{2+} influx (Thorens & Haeusler, 1979). These workers found that noradrenaline contractions were more sensitive to inhibition by diazoxide than responses dependent on $[Ca^{2+}]_o$ and there was a large differentiation between the concentration of diazoxide required to inhibit KCl contraction and KCl-stimulated ^{45}Ca uptake. Nitroprusside produces relaxation of smooth muscle via a mechanism that is independent of Ca^{2+} influx and is not mediated through α- and β-adrenoceptors (Verhaeghe & Shepherd, 1976), but may involve interference with the intracellular activation of calcium perhaps involving cyclic nucleotides (Kreye, Baron, Lüth & Schmidt-Gayk, 1975; Schultz, Schultz & Schultz, 1977). However, evidence against an involvement of cyclic nucleotides has been provided by Diamond & Janis (1978), who showed that although nitroprusside increased the levels of cyclic GMP in the rat vas deferens it was without effect on developed tension. Thus, it is likely that diazoxide and nitroprusside inhibited methoxamine contractions by a similar mechanism, probably mediated through alterations in intracellular Ca^{2+} release and sequestration, and/or Ca^{2+} efflux rather than by inhibition of Ca^{2+} influx. This is supported by ^{45}Ca flux studies which indicated that activation by methoxamine but not barium in the rat vas deferens probably involves intracellular Ca^{2+} stores, and would thus be more susceptible to inhibition by diazoxide and nitroprusside (Hay & Wadsworth, 1982c). Furthermore, in the rat uterus, diazoxide has been reported to increase the levels of cyclic AMP (Johansson, Andersson & Wikberg, 1977), which has been postulated to be involved in Ca^{2+} extrusion and sequestration (Van Breemen, 1977; Van Breemen, Aaronson, Loutzenhiser & Meisheri, 1980); although nitroprusside has been shown to be without effect on the cyclic AMP levels in aortic smooth muscle (Kreye *et al.*, 1975).

Furthermore, the differential effects of methoxamine and barium on La^{3+}-resistant ^{45}Ca uptake (Hay & Wadsworth, 1982c) provides further evidence for these agents producing rhythmic contractions in the rat vas deferens by different mechanisms. Thus, it is concluded that methoxamine and barium

induce rhythmic activity by activating similar but distinct membrane calcium channels that permit the uptake of $[Ca^{2+}]_o$ to activate the intracellular contractile machinery; these channels have low affinity for calcium channel inhibitors.

Hydralazine

Although it was found that hydralazine inhibited the amplitude of both barium- and methoxamine-induced responses, in similar concentrations to those that reduced the KCl contraction in the same tissue (Hay & Wadsworth, 1982a) the concentrations required were over 1,000 times those reported to directly relax small arterial muscle (Worcel, 1978).

Equivalent high concentrations have been shown to block KCl contractions and KCl-stimulated ^{45}Ca uptake in rabbit aortic strips (McLean *et al.*, 1978) and hydralazine has low activity on responses to phenylephrine in the rat vas deferens (Brown, Chevillard & Worcel, 1982). Therefore it is likely that hydralazine inhibits rhythmic responses in the rat vas deferens via a non-specific inhibition of transmembrane Ca^{2+} uptake rather than by its specific action observed with low concentrations.

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References

ANTON, P.G. & McGRATH, J.C. (1977). Further evidence for adrenergic transmission in the human vas deferens. *J. Physiol.*, **273**, 45–55.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BROWN, N.L., CHEVILLARD, C. & WORCEL, M. (1982). In vitro actions of hydralazine on vascular and non-vascular smooth muscle: pre and post-junctional effects. *Br. J. Pharmac.*, **76**, 204P.

CLEMENT, J.G. (1981). $BaCl_2$-induced contractions in the guinea-pig ileum longitudinal muscle: role of presynaptic release of neurotransmitters and Ca^{2+} translocation in the postsynaptic membrane. *Can. J. Physiol. Pharmac.*, **59**, 541–547.

CLIFF, G.S. (1968). Drug-induced rhythmicity in smooth muscle. *J. Pharm. Pharmac.*, **20**, 579–580.

DIAMOND, J. & JANIS, R.A. (1978). Increases in cyclic GMP levels may not mediate relaxant effects of sodium nitroprusside, verapamil and hydralazine in rat vas deferens. *Nature*, **271**, 472–473.

DROOGMANS, G., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *J. gen. Physiol.*, **70**, 129–148.

EBASHI, S. (1971). Comparative aspect of structural proteins with particular reference to regularity proteins. In *Vascular Neuroeffector Systems*, ed. Bevan, J. A., Furchgott, R.F. & Somlyo, A.P. pp. 190–201. New York: Karger.

HAEUSLER, G. (1972). Differential effect of verapamil on excitation-contraction coupling in smooth muscle and on excitation-secretion coupling in adrenergic nerve terminals. *J. Pharmac. exp. Ther.*, **180**, 672–682.

HAY, D.W.P. & WADSWORTH, R.M. (1982a). Effects of some organic calcium antagonists and other procedures affecting Ca^{2+} translocation on KCl-induced contractions in the rat vas deferens. *Br. J. Pharmac.*, **76**, 103–113.

HAY, D.W.P. & WADSWORTH, R.M. (1982b). The contractile effects of 5-hydroxytryptamine on the rat isolated vas deferens. *Br. J. Pharmac.*, **77**, 605–613.

HAY, D.W.P. & WADSWORTH, R.M. (1982c). The effects of barium and methoxamine on calcium uptake in the rat bisected vas deferens. *Br. J. Pharmac.*, **76**, 197P.

HAY, D.W.P. & WADSWORTH, R.M. (1983a). KCl contractions in the rat intact and bisected vas deferens: contribution of endogenous noradrenaline release. *Clin. exp. Pharmac. Physiol.*, (in press).

HAY, D.W.P. & WADSWORTH, R.M. (1983b). The effects of calcium channel inhibitors on twitches and noradrenaline contractions of the rat bisected vas deferens. *Eur. J. Pharmac.*, (in press).

HEATH, J.W., EVANS, B.K., GANNON, B.J., BURNSTOCK, G. & JAMES, V.B. (1972). Degeneration of adrenergic neurons following guanethidine treatment: an ultrastructural study. *Virchows Arch. Abt. B Zellpath.*, **11**, 182–197.

HENRY, P.D. (1980). Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. *Am. J. Cardiol.*, **46**, 1047–1058.

HERMSMEYER, K. (1976). Ba^{2+} and K^+ alteration of K^+ conductance in spontaneously active vascular smooth muscle. *Am. J. Physiol.*, **230**, 1031–1036.

HESTER, R.K., WEISS, G.B. & FRY, W.F. (1979). Differing actions of nitroprusside and D-600 on tension and ^{45}Ca fluxes in canine renal arteries. *J. Pharmac. exp. Ther.*, **208**, 155–160.

HILLE, B. (1967). The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. gen. Physiol.*, **50**, 1287–1302.

HOTTA, Y. (1969). Some properties of the junctional and extrajunctional receptors in the vas deferens of the guinea-pig. *Agents and Actions*, **1**, 13–21.

JOHANSSON, S., ANDERSSON, R.G.G. & WIKBERG, J. (1977). Mechanical and metabolic effects of diazoxide in rat uterus. *Acta pharmac. Toxicol.*, **41**, 328–336.

JURKIEWICZ, A., MARKUS, R.P. & PICARELLI, Z.P. (1975). Effect of full agonists following calcium deprivation in rat vas deferens. *Eur. J. Pharmac.*, **31**, 292–304.

KASUYA, Y., GOTO, K., HASHIMOTO, H., WATANABE, H., MUNAKATA, H. & WATANABE, M. (1969). Non-specific denervation supersensitivity in the rat vas deferens in vitro. *Eur. J. Pharmac.*, **8**, 177–184.

KASUYA, Y. & SUZUKI, N. (1979). Variation in postjunctional natures along the length of the rat vas deferens as a cause of regional difference in the sensitivity to norepinephrine. *Archs int. pharmacodyn.*, **241,** 24–31.

KREYE, V.A.W. (1980). Sodium nitroprusside. In *Pharmacology of Antihypertensive Drugs*, ed. Scriabine, A. pp. 373–396. New York: Raven Press.

KREYE, V.A.W., BARON, G.D., LÜTH, J.B. & SCHMIDT-GAYK, H. (1975). Mode of action of nitroprusside on vascular smooth muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **288,** 381–402.

LEVY, J.V. (1975). Verapamil and diazoxide antagonism of agonist-induced contractions of aortic strips from normal and spontaneously hypertensive rats (SHR). *Res. Comm. Chem. Path. Pharmac.*, **11,** 387–404.

MacDONALD, A. & McGRATH, J.C. (1980). The effects of castration on neurotransmission in the rat vas deferens. *Br. J. Pharmac.*, **69,** 49–58.

McLEAN, A.J., Du SOUICH, P., BARRON, K.W. & BRIGGS, A.H. (1978). Interaction of hydralazine with tension development and mechanisms of calcium accumulation in K^+-stimulated rabbit aortic strips. *J. Pharmac. exp. Ther.*, **207,** 40–48.

MAGARIBUCHI, T., ITO, Y. & KURIYAMA, H. (1971). Effects of catecholamines on the guinea-pig vas deferens in various ionic environments. *Jap. J. Physiol.*, **21,** 691–708.

MELIN, P. (1970). In vivo recording of contractile activity of male accessory genital organs in rabbits. *Acta physiol. scand.*, **79,** 109–113.

O'DONNELL, S.R. & HECKER, S.E. (1967). Contraction of rat vas deferens by cocaine. *J. Pharm. Pharmac.*, **19,** 695.

OHLIN, P. & STRÖMBLAD, B.C.R. (1963). Observations on the isolated vas deferens. *Br. J. Pharmac.*, **20,** 299–306.

PENNEFATHER, J.N., VARDOLOV, L. & HEATH, P. (1974). Regional variation in the response of the rat vas deferens to field stimulation, to noradrenaline and to tyramine. *Clin. exp. Pharmac. Physiol.*, **1,** 451–462.

PRINS, G.S. & ZANEVELD, L.J.D. (1980). Contractions of the rabbit vas deferens following sexual activity: a mechanism for proximal transport of spermatozoa. *Biol. Reprod.*, **23,** 904–909.

RATNASOORIYA, W.D., WADSWORTH, R.M. & GILMORE, D.P. (1979). The effect of sympathomimetic drugs on contractility of the vas deferens *in vitro* and *in vivo*. *J. Reprod. Fert.*, **56,** 633–641.

SCHULTZ, K.-D., SCHULTZ, K. & SCHULTZ, G. (1977). Sodium nitroprusside and other smooth muscle-relaxants increase cyclic GMP levels in rat ductus deferens. *Nature*, **256,** 750–751.

SIGURDSSON, S.B. & UVELIUS, B. (1977). The effects of variations in extracellular magnesium concentration on electrical and mechanical activity in rat portal vein. *Acta physiol. scand.* **99,** 368–376.

SJÖSTRAND, N.O. (1973a). Effects of acetylcholine and some other smooth muscle stimulants on the electrical and mechanical responses of the guinea-pig vas deferens to nerve stimulation. *Acta physiol. scand.* **89,** 1–9.

SJÖSTRAND, N.O. (1973b). Effects of adrenaline, noradrenaline and isoprenaline on the electrical and mechanical responses of the guinea-pig vas deferens to nerve stimulation. *Acta physiol. scand.*, **89,** 10–18.

STONE, T.W. (1981). Differential blockade of ATP, noradrenaline and electrically evoked contractions of the rat vas deferens by nifedipine. *Eur. J. Pharmac.*, **74,** 373–376.

SWAMY, V.C., TRIGGLE, C.R. & TRIGGLE, D.J. (1976). The effects of lanthanum and thulium on the mechanical responses of rat vas deferens. *J. Physiol.*, **254,** 55–62.

THORENS, S. & HAEUSLER, G. (1979). Effects of some vasodilators on calcium translocation in intact and fractionated vascular smooth muscle. *Eur. J. Pharmac.*, **54,** 79–91.

TRENDELENBURG, U., MAXWELL, R.A. & PLUCHINO, S. (1970). Methoxamine as a tool to assess the importance of intraneuronal uptake of L-norepinephrine in the cat's nictitating membrane. *J. Pharmac. exp. Ther.*, **172,** 91–99.

TRIGGLE, C.R., SWAMY, V.C. & TRIGGLE, D.J. (1979). Calcium antagonists and contractile responses in rat vas deferens and guinea pig ileal smooth muscle. *Can. J. Physiol. Pharmac.*, **57,** 804–818.

UVELIUS, B. & SIGURDSSON, S.B. (1981). Stimulating effects of Ba^{2+} on contractile activity in the smooth muscle of the rat portal vein. *Acta physiol. scand.*, **113,** 201–205.

UVELIUS, B., SIGURDSSON, S.B. & JOHANSSON, B. (1974). Strontium and barium as substitutes for calcium on electrical and mechanical activity in rat portal vein. *Blood Vessels*, **11,** 245–259.

VAN BREEMEN, C. (1977). Calcium requirement for activation of intact aortic smooth muscle. *J. Physiol.*, **272,** 317–329.

VAN BREEMEN, C., AARONSON, P., LOUTZENHISER, R. & MEISHERI, K. (1980). Ca^{2+} movements in smooth muscle. *Chest*, **78,** (1, suppl.), 157–165.

VAN BREEMEN, C., HWANG, O. & MEISHERI, K.D. (1981). The mechanism of inhibitory action of diltiazem on vascular smooth muscle contractility. *J. Pharmac. exp. Ther.*, **218,** 459–463.

VENTURA, W.P., FREUND, M., DAVIS, J. & PANNUTI, C. (1973). Influence of norepinephrine on the motility of the human vas deferens: a new hypothesis of sperm transport by the vas deferens. *Fertility and Sterility*, **24,** 68–77.

VERHAEGHE, R.H. & SHEPHERD, J.T. (1976). Effect of nitroprusside on smooth muscle and adrenergic nerve terminals in isolated blood vessels. *J. Pharmac. exp. Ther.*, **199,** 269–277.

WADDELL, J.A. (1916). The pharmacology of the vas deferens. *J. Pharmac. exp. Ther.*, **8,** 551–559.

WEISS, G.B. (1977). Calcium and contractility in vascular smooth muscle. In *Advances in General and Cellular Pharmacology*, vol. 2, ed. Narahashi, T. & Bianchi, C.P. pp. 71–154 New York: Plenum Press.

WESTFALL, D.P. (1970). Non-specific supersensitivity of the guinea-pig vas deferens produced by decentralization and reserpine treatment. *Br. J. Pharmac.*, **39,** 110–120.

WORCEL, M. (1978). Relationship between the direct inhibitory effects of hydralazine and propildazine on arterial smooth muscle contractility and sympathetic innervation. *J. Pharmac. exp. Ther.*, **207,** 320–330.

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