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EFFECTS OF SOME ORGANIC CALCIUM ANTAGONISTS AND OTHER PROCEDURES AFFECTING Ca^{2+} TRANSLOCATION ON KCl-INDUCED CONTRACTIONS IN THE RAT VAS DEFERENS

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1 Both phasic and tonic responses to KCl 160 mM were reduced by Ca^{2+} deprivation. After 90 min, the phasic response was abolished but $13 \pm 1.5\%$ of the tonic response remained. This resistant component was still present if the Ca^{2+}-free solution contained EGTA 0.1 mM. The tonic response was more resistant to deprivation in the prostatic half, while the phasic was more resistant in the epididymal half. KCl-induced contractions were completely restored 5 min after readmission of Ca^{2+}.

2 Both the phasic and the tonic responses were reduced on lowering, and increased on raising $[Ca^{2+}]_o$. In 0.1 mM Ca^{2+}, the phasic response was abolished, but $23 \pm 4\%$ of the tonic response remained (mainly attributable to the prostatic half). These resistant contractions indicate that some of the extracellular Ca^{2+}, especially in the prostatic half, is bound with high affinity, probably to the plasma membrane.

3 Incubation with $LaCl_3$ (0.3–10 mM) for 15 min inhibited the phasic response more than the tonic. After incubation for 1 h, 3 mM $LaCl_3$ abolished both phases. It is concluded that La^{3+} blocks Ca^{2+} channels most readily when they are opened during the spike. Hydralazine (0.76–5.1 mM) resembled $LaCl_3$ in that it reduced the phasic response with little effect on the tonic.

4 $MnCl_2$ (0.3–10 mM) reduced the phasic but increased the tonic response at all concentrations. The augmenting effect may be due to release of intracellular Ca^{2+} or to inhibition of Ca^{2+} efflux.

5 The tonic response was inhibited more than the phasic response by nifedipine (0.002–0.01 μM), methoxyverapamil (0.06–2 μM), verapamil (0.2–1 μM), flunarizine (0.2–100 μM) and diazoxide (22–650 μM). With higher concentrations, only flunarizine remained selective for the tonic response. It is concluded that flunarizine blocks Ca^{2+} channels most readily when opened during sustained spike-free depolarization.

6 Methoxyverapamil 48 μM and verapamil 100 μM virtually abolished both phases of the contraction to KCl 160 mM, but no more than 80% inhibition could be produced with nifedipine. It is concluded that voltage-sensitive Ca^{2+} channels exist in two sub-types, one of which is blocked by nifedipine, and both are blocked by verapamil, methoxyverapamil and flunarizine. Nitroprusside 17 μM had no effect on the phasic response but inhibited the nifedipine-resistant component of the tonic response.

7 Increasing $[Ca^{2+}]_o$ reversed the effects of verapamil, methoxyverapamil, nifedipine and $MnCl_2$, but not the effects of $LaCl_3$.

8 Dantrolene sodium (1.25–25 μM) had no effect on KCl-induced contractions.

Introduction

In smooth muscle, K^+ contractions are caused mainly by increasing membrane permeability to Ca^{2+} as a result of membrane depolarization. Depending on the type of smooth muscle and the concentration of K^+, spikes may also be initiated. In addition, K^+ may also release bound Ca^{2+} directly. Finally, the rise in intracellular cation concentration produced by any of these processes may secondarily mobilize intracellular calcium stores (Bolton, 1979).

Potassium (50 mM or more) causes a biphasic contraction of the rat vas deferens (Syson & Huddart, 1973). Immediately after addition there is a burst of action potentials which causes the phasic response. As depolarization continues, spiking is inhibited and tension is maintained at a lower level (tonic response) (Shimodan & Sunano, 1981). These responses are antagonized by calcium-free conditions, by lanthanum and by the organic calcium antagonists

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nifedipine, verapamil and methoxyverapamil (Swamy, Triggle & Triggle 1976; Triggle, Swamy & Triggle, 1979; Shimodan & Sunano, 1981). These results show that in the vas deferens, K^+ primarily utilizes extracellular calcium for contraction.

Our experiments were designed to study the sources of calcium involved in KCl contractions in the rat intact and bisected vas deferens. We have found that part of the response is remarkably resistant to calcium deprivation and also that there are differences among different calcium antagonists as inhibitors of these responses.

Preliminary accounts of some of these results have been given (Hay & Wadsworth, 1980; Hay & Wadsworth, 1981).

Methods

Vasa deferentia were removed from Wistar rats (250–450 g body wt.), in some experiments bisected (Pennefather, Vardolov & Heath, 1974; Anton, Duncan & McGrath, 1977) and suspended under 0.5 g tension in the appropriate physiological solution which was maintained at 36–38°C. Contractions were recorded isometrically. The standard physiological solution was Krebs-Henseleit of the following composition (mM): Na^+ 144, K^+ 5.8, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 25, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, Cl^- 128.6 and glucose 11.1 which was bubbled with 95% O_2:5% CO_2. Modifications of this solution were made containing various different concentrations of $CaCl_2$. To study the effect of $[Ca^{2+}]_o$ on contractions produced by addition of KCl, the solution contained 0.1–6 mM Ca^{2+}; preparations were left for 15 min before challenge with KCl after each increase in $[Ca^{2+}]_o$. For Ca^{2+}-deprivation experiments, either $CaCl_2$ was omitted (nominally Ca^{2+}-free) or $CaCl_2$ was omitted and EGTA 0.1 mM added.

In experiments where the effects of La^{3+}, Mn^{2+} and high Ca^{2+} were investigated, Krebs-HEPES 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 and HEPES 5 or 10. The solution was titrated to pH 7.4 and bubbled with O_2 or air.

KCl was added hypertonically and concentrations quoted refer to the amount added (in addition to 5.8 mM already present in the standard Krebs-Henseleit solution). To study the effects of antagonists, preparations were stabilized by multiple additions of KCl (contact period = 20 min) at intervals of 40 min. Subsequent responses are expressed as a percentage of the final contraction in this control series. The contact period for the antagonists was 15 min, unless otherwise stated.

$CaCl_2$ concentration-response curves were obtained by adding $CaCl_2$ cumulatively (each increase in concentration being left in contact until tension stabilized) using a depolarizing solution of the following composition (mM): Na^+ 25, K^+ 128, Mg^{2+} 1.2, Cl^- 155.4, glucose 11.1, HEPES 5, and EGTA = 0.03. The solution was titrated to pH 7.4 and bubbled with O_2 or air. After completion of each concentration-response curve, the vas deferens was relaxed using Ca^{2+}-free Krebs-Henseleit containing 0.1 mM EGTA. Following 20 min in this solution, the tissue was washed 3 times at 1 min intervals with the depolarizing solution and then allowed to relax before addition of $CaCl_2$ for the next concentration-response curve. A contact period of at least 15 min was used for the calcium antagonists.

The following drugs were used: verapamil HCl, methoxyverapamil HCl(D600), sodium nitroprusside, hydralazine HCl (all dissolved in distilled water); nifedipine and dantrolene sodium (2.89 mM and 2.51 mM respectively in propylene glycol); flunarizine 2 HCl (2.10 mM in 0.02 M tartaric acid); diazoxide and ethyleneglycol-bis (β-aminoethyl ether) N,N′-tetraacetic acid = EGTA (43.3 and 1 mM respectively in 0.2 M NaOH); N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES).

Results are given as mean ± s.e.mean. In the graphs, standard errors are shown by the vertical bars (except where less than the thickness of the symbol). Student's *t* test was used for statistical analysis.

Results

Ca^{2+} deprivation and readmission

Examples of responses in intact vasa deferentia under control conditions, and after Ca^{2+} deprivation and readmission are shown in Figure 1a-c.

Ca^{2+} deprivation reduced both phasic and tonic responses. With KCl 125 or 160 mM, responses declined rapidly at first, but then more slowly (Table 1). After 90 min deprivation, the phasic response was abolished, but a significant component of the tonic response was still present (Figure 2a and b). Addition of EGTA 0.1 mM to the Ca^{2+}-free solution had no effect on the rate of decline of the phasic response, but hastened the decline of the tonic at earlier times. EGTA did not abolish the resistant component of the tonic response remaining after 60 or 90 min deprivation (Figure 2d). However, nifedipine 0.29 μM almost abolished this residual response.

Since KCl was added hypertonically in these experiments, and since hypertonic solutions contract the portal vein, aorta and trachea (Andersson, Hellstrand, Johansson & Ringberg, 1974; Kirkpatrick, Morrow & Tomita, 1980) we performed control

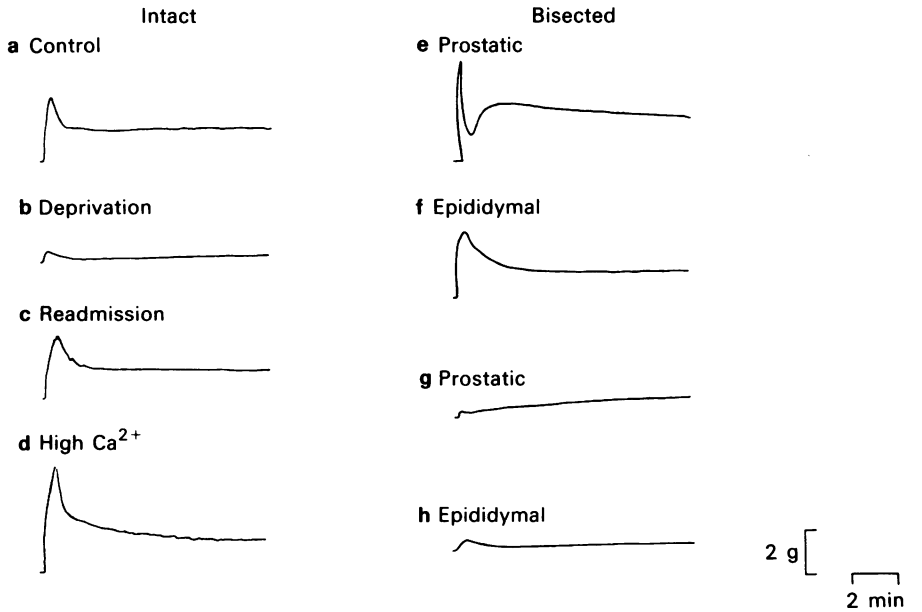

Figure 1 Contractions produced by KCl 160 mM in an intact (a–d) and in prostatic (e,g) and epididymal (f,h) halves of a bisected vas deferens. (a) Control contraction, $[Ca^{2+}]_o = 2.5$ mM; (b) after 15 min in Ca^{2+}-free Krebs-Henseleit containing EGTA 0.1 mM; (c) 20 min after readmission of 2.5 mM Ca^{2+}; (d) in high Ca^{2+} (6 mM); (e) and (f) control responses of prostatic and epididymal halves respectively in $[Ca^{2+}]_o = 2.5$ mM; (g) and (h) responses of prostatic and epididymal halves respectvely after 15 min in Ca^{2+}-free Krebs-Henseleit solution containing EGTA 0.1 mM.

experiments using sucrose. Sucrose 320 mM alone in some experiments caused a small contraction. The sucrose and KCl contractions were expressed as a percentage of the control tonic contraction to KCl in 2.5 mM Ca^{2+}. After 90 min, Ca^{2+} deprivation in the presence of EGTA 0.1 mM, KCl 160 mM produced a contraction of $12.7 \pm 2.1\%$ ($n = 8$) and sucrose 320 mM produced a contraction of $6.4 \pm 1.4\%$ ($n = 8$). These results are significantly different ($P < 0.05$) and furthermore the sucrose contractions developed more slowly than the residual tonic response to KCl, showing that hypertonicity makes only a small contribution to this residual response.

Bisection of the vas deferens showed that the prostatic and epididymal halves respond differently to Ca^{2+} deprivation (Figure 1e-h). The tonic response is more resistant to deprivation in the prostatic half than in the epididymal half. The residual response of the intact vas deferens after 90 min Ca^{2+} deprivation is mainly attributable to the prostatic part. However, the phasic response survives longer during Ca^{2+} deprivation in the epididymal half than in the prostatic half (Figure 2e and f). The sucrose-induced contraction is present in the prostatic part only.

Measurement of the tonic phase was made after approximately 10 min since although the time for stabilization was variable in different preparations, all had stabilized at this time. During calcium depri-

Table 1 Effect of calcium deprivation on responses to KCl in the intact and bisected vas deferens

Conditions	*Duration of deprivation*			*n*
	50% inhibition	*75% inhibition*		
	Phasic response (s)	Phasic response (min)	Tonic response (min)	
Prostatic half (KCl 160 mM, EGTA 0.1 mM)	23 ± 4	1.0 ± 0.12	69.6 ± 23.0	4
Epididymal half (KCl 160 mM, EGTA 0.1 mM)	209 ± 33	8.1 ± 1.3	16.0 ± 2.1	4
Intact vas (KCl 160 mM, EGTA 0.1 mM)	70 ± 10	4.7 ± 0.6	20.0 ± 8.9	8
Intact vas (KCl 160 mM, nominal Ca^{2+}-free)	54 ± 8	8.3 ± 2.0	31.5 ± 5.3	6–8
Intact vas (KCl 125 mM, nominal Ca^{2+}-free)	100 ± 51	5.8 ± 1.0	25.7 ± 6.0	5–8

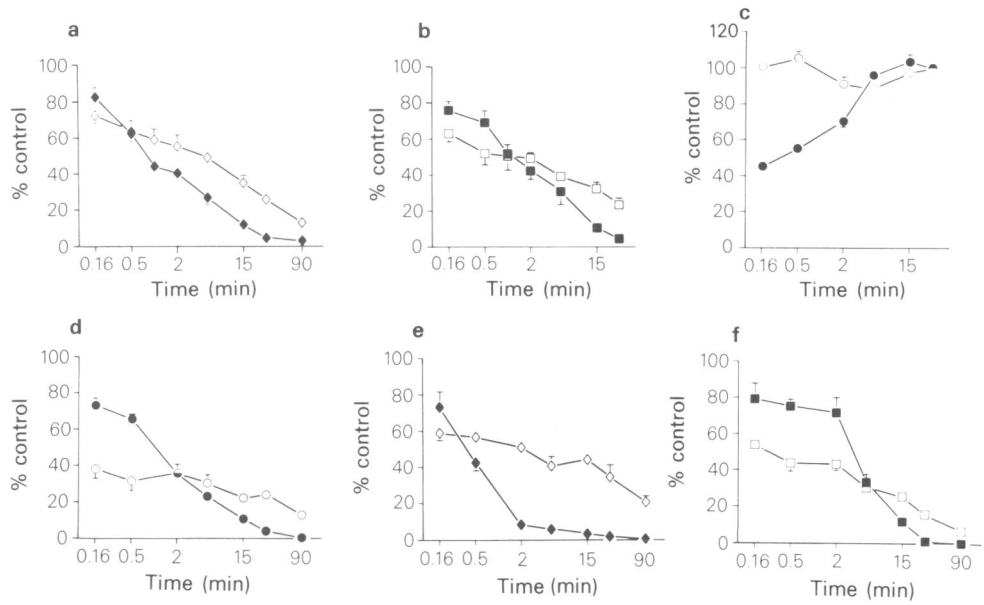

Figure 2 Effects of Ca^{2+} deprivation and readmission on KCl contractions in rat vas deferens. (a) KCl 160 mM, Ca^{2+} deprivation, nominally Ca^{2+}-free solution; (b) KCl 125 mM, Ca^{2+} deprivation, nominally Ca^{2+}-free solution; (c) readmission of 2.5 mM Ca^{2+} on responses to KCl 160 mM. The effects of Ca^{2+} deprivation in the presence of EGTA 0.1 mM on responses to KCl 160 mM are shown for (d) the intact vas deferens, (e) the prostatic half and (f) the epididymal half. Filled symbols = phasic response, open symbols = tonic response. Responses are expressed as a percentage of the corresponding phasic or tonic control response either (a,b, d–f) before deprivation or (c) 30 min after readmission. Note that times plotted on the abscissa scales are the times from the start of deprivation or readmission to the addition of KCl, but the tonic response is measured approx. 10 min after addition of KCl. (a) and (b): $n = 6-8$. (c), (e) and (f): $n = 4$. (d): $n = 8$.

vation (10 s–30 min) the tonic phase was smaller but the form of the response was unchanged, i.e. the response did not decay at a faster rate. The tonic response was determined by the $[Ca^{2+}]_o$ at the time of addition of KCl, even though $[Ca^{2+}]_o$ would have fallen further at the time of measurement. We have therefore measured all tonic responses at 10–15 min, including those during calcium deprivation (Figure 2).

Readmission of Ca^{2+} rapidly restored responses. With KCl 160 mM, the phasic response returned to control level 5 min after readmission of Ca^{2+} (Figure 1c and 2c).

Effect of varying $[Ca^{2+}]_o$

Both the phasic and the tonic responses were reduced on lowering, and increased on raising the calcium concentration in the Krebs-Henseleit solution (Figure 1d and 3a). At 1 mM and above, the phasic and tonic responses increased proportionately and the concentration-response curves, expressed as a percentage of the contractions occurring in 2.5 mM Ca^{2+}, are superimposed (Figure 3a). However, in the epididymal half, high $[Ca^{2+}]_o$ caused a progressive rise in the tonic response while the phasic reached a plateau (Figure 3c).

As would be expected from the deprivation experiments, a component of the tonic response remained in low Ca^{2+} solutions. With 0.1 mM Ca^{2+} the phasic response was abolished, but $23 \pm 4\%$ of the tonic response remained (Figure 3a). This residual component is primarily attributable to the prostatic half (Figure 3b).

Lanthanum and manganese

Lanthanum caused a dose-related inhibition of both phases of the KCl-induced contraction (Figures 4 and 5a). After 15 min incubation with $LaCl_3$ the phasic response was reduced more than the tonic response and even with 10 mM La^{3+}, the tonic response was still more than half of its control size (Figure 5a). However, after 1 h incubation, $LaCl_3$ 3 mM almost abolished both phases. These effects were essentially irreversible on washing out the La^{3+} or by increasing the Ca^{2+} concentraiton.

Manganese also reduced the phasic response but,

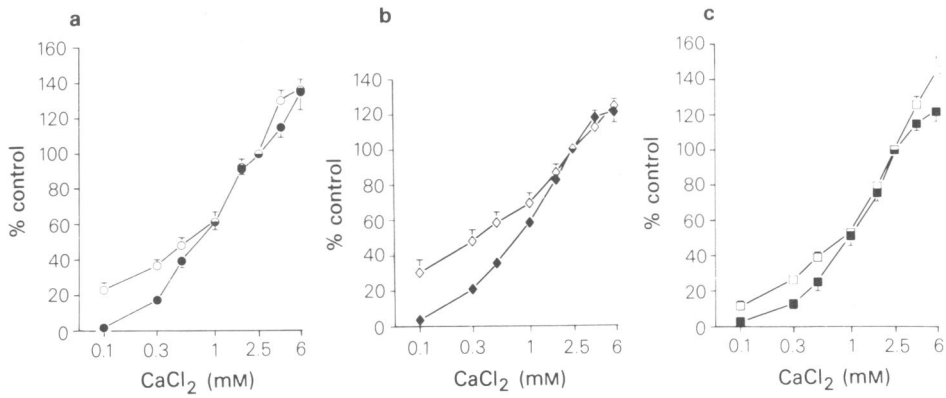

Figure 3 Effect of various Ca^{2+} concentrations on responses to KCl 160 mM: (a) intact vas deferens; (b) prostatic and (c) epdidymal half of bisected vas deferens. Filled symbols = phasic response, open symbols = tonic response. Responses are expressed as a percentage of the contraction given with 2.5 mM Ca^{2+}. (a): $n = 8$. (b) and (c): $n = 6$.

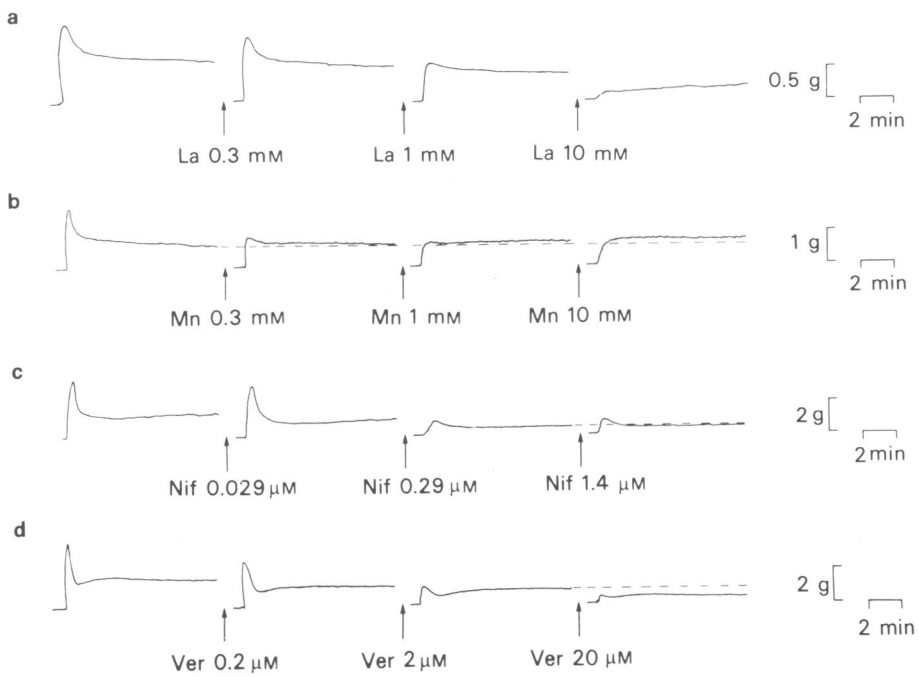

Figure 4 The effects of $LaCl_3$, $MnCl_2$, nifedipine (Nif) and verapamil (Ver) on responses to KCl 160 mM in the intact vas deferens. In each part, the first response is a control, this is then repeated in the presence of increasing concentration of the antagonist: (a) 15 min incubation with $LaCl_3$ 0.3, 1 and 10 mM reduced the phasic response more than the tonic; (b) $MnCl_2$ 0.3, 1 and 10 mM reduced the phasic response but augmented the tonic; (c) nifedipine 0.029, 0.29 and 1.4 μM, the highest concentration causing almost no additional effect; (d) verapamil 0.2, 2 and 20 μM progressively reduced the responses as the concentration was increased.

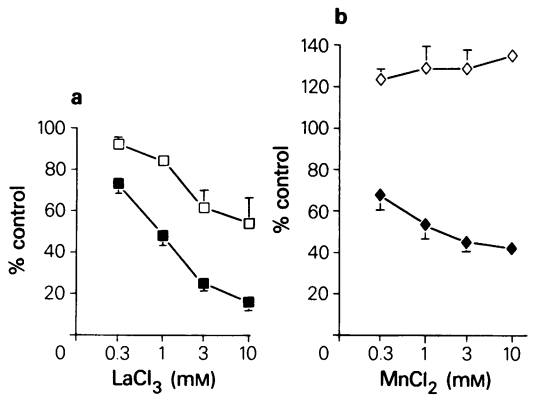

Figure 5 Effect of 15 min incubation with (a) $LaCl_3$ or (b) $MnCl_2$ on responses to KCl 160 mM in the intact vas deferens. Filled symbols = phasic response, open symbols = tonic response. In the presence of $MnCl_2$ 3 mM and 10 mM there may be an overestimate of the phasic responses since they merged with the augmented tonic respnses. (a) and (b): $n = 4$.

in marked contrast to La^{3+}, the tonic response was increased by all concentrations of $MnCl_2$ tested (Figures 4, 5b). Increasing $[Ca^{2+}]_o$ by 4 or 10 times (to 10 or 25 mM) reversed both the inhibitory and potentiating effects of $MnCl_2$. Control responses could also be restored readily by wash out of Mn^{2+}.

Organic calcium antagonists

All the organic calcium antagonists tested reduced both the phasic and the tonic responses to KCl 160 mM. Sample traces showing the effects of nifedipine and verapamil are given in Figure 4c and d. Low concentrations were selective for the tonic phase, though the concentration producing this effect depended on the potency of the antagonist (nifedipine 0.002–0.01 μM; methoxyverapamil 0.06–2 μM; verapamil 0.2–1 μM; flunarizine 0.2–100 μM; diazoxide 22–650 μM) (Figure 6). With higher concentrations, three different types of effects were observed, depending on the drug used. (1) With verapamil or methoxyverapamil, both phases were progressively reduced and with 100 μM, virtually extinguished (Figure 6b and d). (2) Nifedipine 1 μM produced about 80% block of both phases and even when the concentration was increased to 14 μM about 20% of both phasic and tonic responses remained (Figure 6a). This residual contraction was substantially reduced by verapamil 2 μM and was abolished by nitroprusside (67–168 μM). (3) In contrast to nifedipine, verapamil and methoxyverapamil, flunarizine was selective for the tonic phase even at the concentrations producing nearly complete inhibition (Figure 6e). Table 2 shows the IC_{50} values for all the calcium antagonists tested.

In control experiments, the effects of the solvents for the calcium antagonists were examined. Tartaric acid and NaOH had no effect. Propylene glycol had no effect in low concentrations, but above 0.01% (equivalent to that in nifedipine 0.29 μM) there was some reduction in the tonic phase. Thus the apparently greater inhibition by nifedipine 1.4–14 μM of the tonic response (Figure 6a) is in fact probably due to this solvent effect.

Bisection of the vas deferens showed that the epididymal and prostatic portions respond similarly to nifedipine or verapamil (Figure 7). The resistant component of the responses in the presence of nifedipine 1.4 μM was present in both halves (Figure 7a and b). Appropriate concentrations of nifedipine or verapamil, which selectively inhibit the tonic phase in the intact vas deferens, did not show this selectivity in either part of the bisected vas (Figure 7).

The effects of nifedipine, verapamil and methoxyverapamil were similar when lower concentrations

Table 2 Effects of various calcium antagonists on responses to KCl 160 mM in the rat vas deferens

	IC_{50} (μM)						
	Intact		*Prostatic*		*Epididymal*		
	Phasic	Tonic	Phasic	Tonic	Phasic	Tonic	n
Nifedipine	0.090 ±0.023	0.052 ±0.025	0.084 ±0.017	0.079 ±0.026	0.066 ±0.0064	0.090 ±0.032	4–6
Verapamil (KCl 160 mM)	1.47 ±0.35	0.55 ±0.26	3.10 ±0.86	7.84 ±2.10	1.08 ±0.20	1.22 ±0.29	4–6
Verapamil (KCl 125 mM)	1.85 ±1.04	0.94 ±0.31					6
Methoxyverapamil	1.99 ±0.38	0.88 ±0.32					8
Flunarizine	27.9 ±7.5	2.79 ±1.03					6
Diazoxide	1939 ±156	2117 ±48					4

of KCl (125 or 80 mM)) were used. KCl 125 mM produced a smaller phasic response than KCl 160 mM and this was inhibited to a greater extent by verapamil (0.2–0.8 μM). The tonic responses to KCl 125 mM and 160 mM were similar, and verapamil (0.2–0.8 μM) had a similar effect on them. The result is that verapamil showed less selectivity for the tonic phase when the concentration of KCl was lowered from 160 to 125 mM (Figure 6c).

The effects of nifedipine, verapamil and methoxyverapamil on Ca^{2+} concentration-response curves in K^+-depolarized vasa deferentia are shown in Figure 8. Progressive addition of calcium to depolarized tissues produced a gradual increase in tension which was allowed to stabilize (0.16–4 min) before being measured.

All three calcium antagonists depressed the curves in the middle of the Ca^{2+} concentration range, but sufficient increase in $[Ca^{2+}]_o$ ultimately restored or almost restored the original maximal response. How-

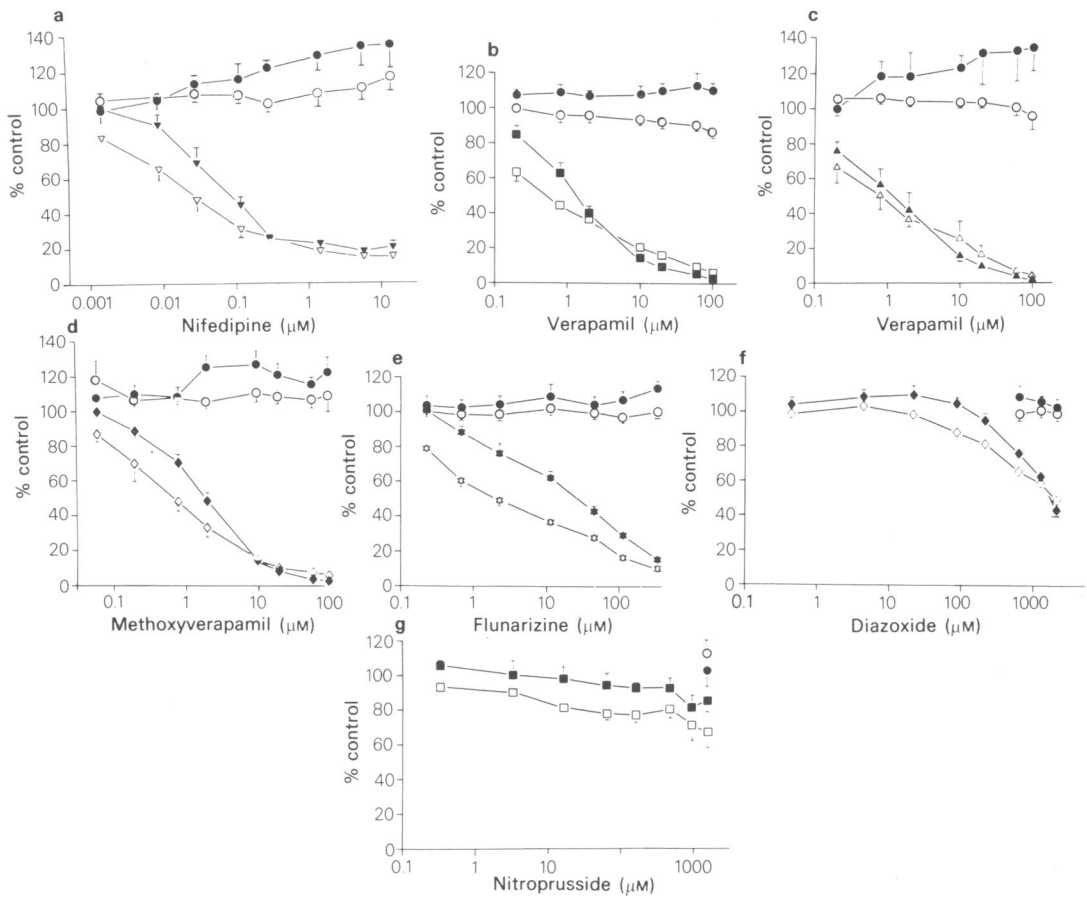

Figure 6 Effects of nifedipine, verapamil, methoxyverapamil, flunarizine, diazoxide and nitroprusside on responses to KCl in the intact vas deferens. Responses are expressed as a percentage of the corresponding phasic or tonic response before addition of antagonist, which was added cumulatively. In each experiment the contralateral vas deferens was treated in the same way except that no antagonist was administered; these control responses are shown in each part as (●) phasic, (○) tonic. For clarity some control points have been omitted from (f) and (g). (a) Effect of nifedipine on responses to KCl 160 mM: (▼) phasic, (▽) tonic. The residual response with nifedipine 14 μM was seen in all experiments, including those where control tissues showed no increase during the experiment, $n = 4–8$. (b) Effect of verapamil on responses to KCl 160 mM: (■) phasic, (□) tonic, $n = 6$. (c) Effect of verapamil on responses to KCl 125 mM: (▲) phasic, (△) tonic, $n = 6$. (d) Effect of methoxyverapamil on responses to KCl 160 mM: (◆) phasic, (◇) tonic, $n = 4–8$. (e) Effect of flunarizine on responses to KCl 160 mM: (★) phasic, (☆) tonic, $n = 6$. (f) Effect of diazoxide on responses to KCl 160 mM: (◆) phasic, (◇) tonic, $n = 4$, (g) Effect of nitroprusside on responses to KCl 160 mM: (■) phasic, (□) tonic, $n = 6$.

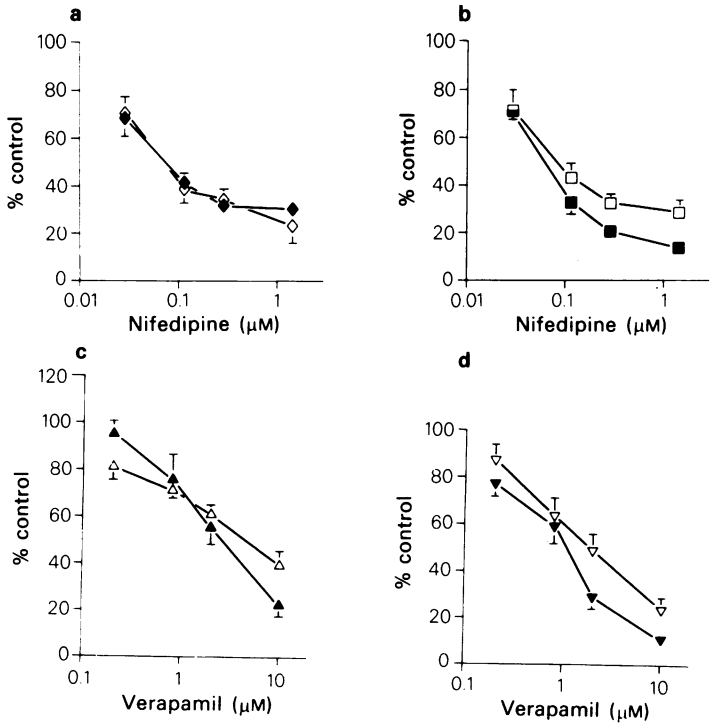

Figure 7 Effect of nifedipine and verapamil on responses of the bisected vas deferens to KCl 160 mM. (a) Nifedipine, prostatic; (b) nifedipine, epididymal; (c) verapamil, prostatic; (d) verapamil, epididymal. Filled symbols = phasic response, closed symbols = tonic response. (a) and (b): $n = 3-5$. (c) and (d): $n = 4-5$.

ever, this required high concentrations of Ca^{2+}, the contractile effect of which was preceded by inhibition, associated with an inflection in the concentration-response curve. Similarly, inhibition by nifedipine, verapamil or methoxyverapamil of the responses to KCl 160 mM (in 2.5 mM Ca^{2+}) was reversed by increasing $[Ca^{2+}]_o$ to 10–25 mM.

Of the calcium antagonists tested, flunarizine was the most persistent: after 2 h washing only 10% of the control response returned. After 2 h washing in the case of verapamil or methoxyverapamil, about 25% of the control response returned. In comparison, the effects of diazoxide were easily washed out, and after 25 min responses returned to control values.

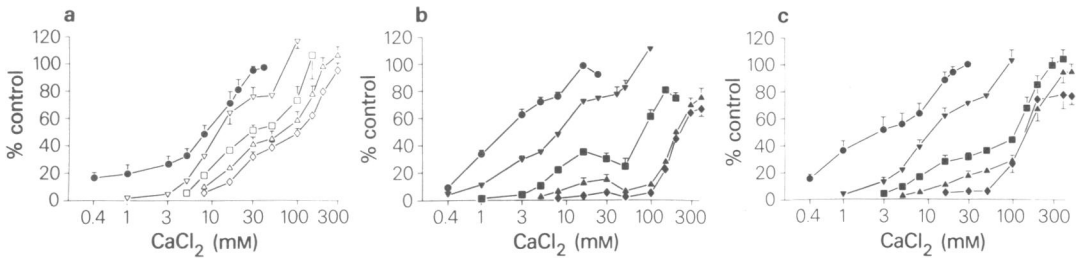

Figure 8 Effects of nifedipine, verapamil and methoxyverapamil on concentration-response curves to $CaCl_2$ in depolarized intact vasa deferentia. In each tissue, responses at each calcium concentration are expressed as a percentage of the maximum control response for that tissue before addition of the antagonist. (a) (●) Control; (▽) nifedipine 0.0087 μM; (□) nifedipine 0.029 μM; (△) nifedipine 0.087 μM; (◇) nifedipine 0.29 μM. (b) (●) Control; (▼) verapamil 0.2 μM; (■) verapamil 2 μM; (▲) verapamil 20 μM; (◆) verapamil 102 μM. (c) (●) Control; (▼) methoxyverapamil 0.19 μM; (■) methoxyverapamil 1.9 μM; (▲) methoxyverapamil 9.6 μM; (◆) methoxyverapamil 58 μM. (a): $n = 3-4$; (b): $n = 4-11$; (c) $n = 4$.

Nitroprusside, hydralazine and dantrolene

Nitroprusside 17 μM inhibited by about 20% the tonic response to KCl 160 mM; little further effect occurred with higher concentrations of nitroprusside. Nitroprusside had practically no effect on the phasic response (Figure 6g).

Hydralazine (0.76–5.1 mM) reduced the phasic response to KCl 160 mM with little effect on the tonic.

Dantrolene sodium (1.25–25 μM) had no effect on KCl contractions.

Discussion

Components of the KCl response resistant to Ca^{2+} deprivation

In agreement with Swamy *et al.* (1976), Triggle *et al.* (1979) and Shimodan & Sunano (1981), we have found that lanthanum, verapamil and methoxyverapamil completely block KCl contractions. Since it is well established that these drugs inhibit the movement of Ca^{2+} into smooth muscle, this is strong evidence that both phases of the KCl contraction in the rat vas deferens use extracellular Ca^{2+}. Despite this, we have found that a component of the tonic phase is resistant to Ca^{2+} deprivation and survives when $[Ca^{2+}]_o$ is lowered to 0.1 mM. From this we conclude that some of the extracellularly located Ca^{2+} is bound with high affinity, but nevertheless is available to activate contraction when the muscle is depolarized with KCl. This conclusion is supported by the observation that the residual response is antagonized by nifedipine. These extracellular binding sites must be mainly confined to the prostatic half since most of the deprivation-resistant tonic contraction occurs here. This component of the tonic response was also present when 125 mM KCl was used though not, according to Swamy *et al.* (1976), with 80 mM KCl.

In the rat vas deferens, about 25% of the phasic response to KCl can be attributed to release of neuronal noradrenaline, but the remainder appears to be caused by a direct effect on the smooth muscle. The contribution of endogenous noradrenaline release is substantial in the epididymal half but negligible in the prostatic half (Hay & Wadsworth, 1982). The phasic response is more resistant to Ca^{2+} deprivation in the epididymal half and this may be becaue of the greater contribution made by noradrenaline to the phasic response in this part of the vas.

Selectivity of drugs for phasic and tonic response

Shimodan & Sunano (1981) have shown that in the guinea-pig vas deferens stimulated with KCl, the phasic response is caused by a burst of spikes, while the tonic response is caused by sustained, spike-free depolarization. Flunarizine (and, at certain concentrations, nifedipine, verapamil, methoxyverapamil and diazoxide) has some selectivity for the tonic response, suggesting that Ca^{2+} channels opened under conditions of sustained depolarization are more sensitive to the organic calcium antagonists. To some extent the effect of the organic calcium antagonists on Ca^{2+} concentration-response curves is surmountable, but we found that the original maximal response could be regained only with the use of 100–400 mM Ca^{2+}. It is probable that these high concentrations of calcium cause contraction by a different mechanism, especially since they are markedly hypertonic and there is an inflection in the concentration-response curve. We therefore conclude that the effect of nifedipine, verapamil and methoxyverapamil is not completely surmountable, as has also been found in the rabbit mesenteric artery (Schümann, Görlitz & Wagner, 1975). Presumably this indicates that a given concentration of a calcium antagonist occludes a certain fraction of the ion channels, but to establish a quantitative relationship requires a knowledge of the relationship between $[Ca^{2+}]_i$ and tension and between $[Ca^{2+}]_i$ and Ca^{2+} extrusion rate.

$LaCl_3$ has the opposite profile to the organic calcium antagonists, being more active against the phasic than against the tonic response (Figure 5; Swamy *et al.*, 1976). This probably indicates that La^{3+} has greater affinity for the calcium channels when they are opened during the spike than when opened during sustained depolarization. Alternatively, it is possible that La^{3+} non-selectively depresses both the spike and spike-free activation of calcium channels, the tonic phase being spared due to a Ca-release process as was found with Mn^{2+} (see below). Hydralazine also appears to be a selective inhibitor of contractions associated with the spike in the vas deferens. This effect is presumably unconnected with the inhibitory action of hydralazine on arterial muscle which occurs at much lower concentrations (Chevillard, Saiag & Worcel, 1981; Khayyal, Gross & Kreye, 1981).

$MnCl_2$ also inhibits the phasic contraction but (unlike $LaCl_3$) augments the tonic contraction. Manganese has similar actions on the taenia coli (Imai & Takeda, 1967) and in the uterus (Ogasawara, Kato & Osa, 1980). This augmenting effect may be due either to Mn^{2+} inhibiting Ca^{2+} efflux (Osa, 1974) or to Mn^{2+} displacing intracellular bound Ca^{2+} (Imai & Takeda, 1967). Reversal of the effects of $MnCl_2$ by Ca^{2+} probably indicates that Ca^{2+} can displace Mn^{2+} from its sites of action within the muscle cells.

Component of KCl response resistant to nifedipine

In sufficient concentration, verapamil, methoxyverapamil and flunarizine all inhibit completely the KCl contraction but nifedipine spares part (about 20%) of both the phasic and the tonic responses. Since this component is antagonized by verapamil, we conclude that there are two sub-types of the Ca^{2+} channel, only one of which is blocked by nifedipine, but both are blocked by verapamil. Golenhofen (1981) has proposed that there are two types of calcium activation mechanism, one being blocked by nifedipine and the other by nitroprusside. Our results support this hypothesis since the combination of nifedipine and nitroprusside (but neither on its own) abolished the response to KCl.

The component of the KCl response resistant to nifedipine is not the same as the component resistant to Ca^{2+} deprivation, for the following reasons: both halves of the vas deferens respond similarly to nifedipine (and to verapamil) but the effects of Ca^{2+} deprivation were different in the two halves. Furthermore, nifedipine blocked the KCl response remaining after Ca^{2+} deprivation.

Dantrolene has been reported to have inhibitory effects in some smooth muscle preparations (Ellis, Butterfield, Wessels & Carpenter, 1976; Bowman & Khan, 1977; Graves, Dretchen & Kruger, 1978) but we did not observe any action on KCl responses in the vas deferens.

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References

ANDERSSON, C., HELLSTRAND, P., JOHANSSON, B. & RINGBERG, A. (1974). Contraction in venous smooth muscle induced by hypertonicity. Calcium dependence and mechanical characteristics. *Acta physiol Scand.*, **90,** 451–461.

ANTON, P.G., DUNCAN, M.E. & McGRATH, J.C. (1977). An analysis of the anatomical basis for the mechanical response to motor nerve stimulation of the rat vas deferens. *J. Physiol.*, **273,** 23–43.

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

BOWMAN, W.C. & KHAN, H.H. (1977). Effects of dantrolene sodium on isolated skeletal, smooth and cardiac muscle of the guinea-pig. *J. Pharm. Pharmac.*, **29,** 628–629.

CHEVILLARD, C., SAIAG, B. & WORCEL, M. (1981). Interactions between hydralazine, propildazine and purines on arterial smooth muscle. *Br. J. Pharmac.*, **73,** 811–817.

ELLIS, K.O., BUTTERFIELD, J.L., WESSELS, F.L. & CARPENTER, J.F. (1976). A comparison of skeletal, cardiac and smooth muscle actions of dantrolene sodium – a skeletal muscle relaxant. *Archs int. pharmacodyn.*, **244,** 118–132.

GOLENHOFEN, K. (1981). Differentiation of calcium activation processes in smooth muscle using selective antagonists. In: *Smooth Muscle* eds Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 157–170. London: Edward Arnold.

GRAVES, S., DRETCHEN, K.L. & KRUGER, G.O. (1978). Dantrolene sodium: effects on smooth muscle. *Eur. J. Pharmac.*, **47,** 29–35.

HAY, D.W.P. & WADSWORTH, R.M. (1980). Effect of verapamil on rhythmic contractions in isolated rat vasa deferentia. *Br. J. Pharmac.*, **68,** 182–183P.

HAY, W.D.P. & WADSWORTH, R.M. (1981). Potassium contractures in the rat isolated vas deferens: the role of noradrenaline release and of extracellular calcium. *Br. J. Pharmac.*, **72,** 143–144P.

HAY, D.W.P. & WADSWORTH, R.M. (1982). KCl contractions in the rat intact and bisected vas deferens: contribution of endogenous noradrenaline release. *Clin. exp. Pharmac. Physiol.* (in press).

IMAI, S. & TAKEDA, K. (1967). Actions of calcium and certain multivalent cations on potassium contracture of guinea-pig's taenia coli. *J. Physiol.*, **190,** 155–169.

KHAYYAL, M., GROSS, F. & KREYE, V.A.W. (1981). Studies on the direct vasodilator effect of hydralazine in the isolated rabbit renal artery. *J. Pharmac. exp. Ther.*, **216,** 390–394.

KIRKPATRICK, C.T., MORROW, R.J. & TOMITA, T. (1980). The contractile response of smooth muscle to immersion in hypertonic solutions. *Clin. exp. Pharmac. Physiol.*, **2,** 147–158.

OGASAWARA, T., KATO, S. & OSA, T. (1980). Effects of estradiol-17β on the membrane response and K-contracture in the uterine longitudinal muscle of ovariectomized rats studied in combination with the Mn action. *Jap. J. Physiol.*, **30,** 271–285.

OSA, T. (1974). Modification of the mechanical response of the smooth muscles of pregnant mouse myometrium and guinea-pig ileum by cadmium and manganese ions. *Jap. J. Physiol.*, **24,** 101–117.

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. Pharm. Physiol.*, **1,** 451–462.

SCHÜMANN, H.J., GÖRLITZ, B.D. & WAGNER, J. (1975). Influence of papaverine, D600 and nifedipine on the effects of noradrenaline and calcium on the isolated aorta and mesenteric artery of the rabbit. *Naunyn-Schmiedebergs Arch. Pharmac.*, **289,** 409–418.

SHIMODAN, M. & SUNANO, S. (1981). The initiation of phasic and tonic contraction by potassium and the effect of calcium, multvalent cations and Ca-antagonist on potassium contracture in guinea-pig vas deferens. *Jap. J. Physiol.*, **31,** 15–27.

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.

SYSON, A.J. & HUDDART, N. (1973). Contracture tension in rat vas deferens and ileal smooth muscle and its modification by external calcium and the tonicity of the medium. *Comp. Biochem. Physiol.*, **45A,** 345–362.

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.

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