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Effects of KCl on ^{45}Ca uptake and efflux in the rat vas deferens

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1 The effects of KCl 160 mM on ^{45}Ca uptake and efflux in the rat isolated vas deferens were investigated using a modification of the lanthanum method and a superfusion system respectively.

2 In the prostatic half, basal cellular ^{45}Ca uptake was 679 ± 21 nmol g^{-1} tissue wet weight and KCl 160 mM increased this by 155%. In the epididymal half, basal cellular ^{45}Ca uptake was 730 ± 28 nmol $^{45}Ca\ g^{-1}$ and KCl 160 mM increased this by 46%.

3 Verapamil 2.04 μM or nifedipine 0.29 μM had no or little effect on basal ^{45}Ca efflux or on basal ^{45}Ca uptake. The KCl-induced increase in ^{45}Ca uptake in both halves was inhibited by verapamil 2.04 μM or nifedipine 0.29 μM, concentrations which markedly reduce the contractile response. It is concluded that high K^+ contracts the rat vas deferens by stimulation of the entry of extracellular Ca^{2+} to the intracellular compartment.

4 KCl 160 mM produced a large, rapid and reversible increase in the rate of ^{45}Ca efflux into Ca^{2+}-containing and Ca^{2+}-free Krebs-Henseleit solutions, which was not inhibited by verapamil 2.04 μM, nifedipine 0.29 μM or nitroprusside 1,678 μM.

5 The relative size of the slow component of ^{45}Ca efflux was larger in the prostatic half compared to the epididymal half of bisected tissues, suggesting that the postulated high affinity binding sites are predominant in this region. However, the rates of the fast and slow components of ^{45}Ca efflux from prostatic and epididymal halves were identical. KCl 160 mM produced a similar increase in ^{45}Ca efflux in prostatic and epididymal halves.

Introduction

The effects of KCl on transmembrane ^{45}Ca movements have been examined in various smooth muscles. Thus, in agreement with the dependence of the contractile response on extracellular Ca^{2+}, high K^+ was demonstrated to increase ^{45}Ca uptake in several vascular smooth muscles (Van Breemen *et al.*, 1972; Thorens & Haeusler, 1979; Casteels & Droogmans, 1981; Godfraind & Dieu, 1981; Ozaki *et al.*, 1981). This KCl-induced stimulation of ^{45}Ca uptake was antagonized by the calcium channel inhibitors verapamil (Thorens & Haeusler, 1979), methoxyverapamil (Karaki & Weiss, 1979), diltiazem (Van Breemen *et al.*, 1981) or flunarizine (Godfraind & Dieu, 1981). However, the effects of high K^+ on ^{45}Ca efflux were less clearcut; this procedure was shown to increase, decrease or have no effect on ^{45}Ca efflux from vascular smooth muscle. The increase in ^{45}Ca efflux may originate from intracellular sites, which are displaced through a process of $^{40}Ca/^{45}Ca$ exchange by Ca^{2+} entering via membrane ion channels (Casteels & Droogmans, 1981; Deth & Lynch, 1981). In contrast, the decreased ^{45}Ca efflux observed in canine and rabbit renal arteries using effluxing conditions that minimize backflux and rebinding of ^{45}Ca probably reflects an inward shift of Ca^{2+} from membrane binding sites into deeper locations, subsequently activating the contractile machinery (Hester *et al.*, 1978).

In the rat isolated vas deferens KCl 160 mM produces a biphasic contraction that is inhibited by withdrawal of extracellular Ca^{2+}, by lanthanum or by calcium channel inhibitors including verapamil and nifedipine. These results suggest that contractions produced by high K^+ in this tissue are caused by the opening of voltage-dependent membrane channels and entry of extracellular calcium into the intracellular environment (Hay & Wadsworth, 1982b). We have now investigated this by studying ^{45}Ca uptake and efflux in the rat vas deferens, and the effects of verapamil and nifedipine on KCl-induced changes in transmembrane ^{45}Ca movements. Bisected vasa deferentia were used in some experiments since there is evidence for differences in excitation–contraction coupling mechanisms in the prostatic and epididymal

halves (Hay & Wadsworth, 1982b). A preliminary account of these results was given to the British Pharmacological Society (Hay & Wadsworth, 1982a).

Methods

Vasa deferentia from Wistar rats (270–425 g body weight) were removed and in some experiments transversely bisected (Pennefather *et al.*, 1974; Anton *et al.*, 1977).

^{45}Ca uptake was measured by a modified 'lanthanum method' (Van Breemen *et al.*, 1972), using a high La^{3+} concentration (50 mM) (Godfraind, 1976) incubated at a low temperature, 0.5°C (Deth, 1978). Tissues were equilibrated for approximately 45 min in 2.5 mM Ca Krebs-Henseleit solution at 37°C. They were then transferred to tubes containing ^{45}Ca 0.5 μCi ml^{-1} in 2.5 mM Ca Krebs-Henseleit solution for 30 min. Where appropriate KCl 160 mM was added for 20 min. The tissues were then transferred to Tris-buffered Krebs solution also containing KCl 160 mM and ^{45}Ca 0.5 μCi ml^{-1} for 5 min. Finally the tissues were incubated in $LaCl_3$ 50 mM, contained in Ca^{2+}-free Tris-buffered Krebs solution at 0.5°C for 60 min. Antagonists were added to some of the tissues and were present throughout the period of equilibration and uptake. Following digestion of the tissue overnight with EDTA 5 mM the radioactivity present was counted by liquid scintillation and the cellular ^{45}Ca content calculated.

^{45}Ca efflux was analysed using a superfusion method at 37°C. After 3 h loading with ^{45}Ca 2 μCi ml^{-1} in 2.5 mM $CaCl_2$ Krebs-Henseleit solution the tissues were suspended under 0.5 g tension and superfused with Ca^{2+}-containing or Ca^{2+}-free Krebs-Henseleit solution at a rate of 2.7 ml min^{-1}, after 90 min the superfusing solution was changed to an identical one containing KCl 160 mM. The superfusate was collected every 2 min and the radioactivity present was counted by a Packard Tri-Carb 460 CD Liquid Scintillation System. The results were calculated and plotted as Rate Coefficient and Desaturation plots (see Weiss, 1977). When studying the effects of antagonists, one vas deferens served as control and the contralateral tissue was treated with the antagonist, which was present throughout the period of efflux.

The composition of the Krebs-Henseleit solution was (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, glucose 11.1, aerated with 95% O_2: 5% CO_2 and maintained at 37 ± 0.5°C. The composition of the Tris-buffered Krebs solution was (mM): Na^+ 144, K^+ 5.8, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 157.2, glucose 11.1, Tris 5, aerated with O_2 or air.

Drugs

The following drugs were used: sodium nitroprusside (BDH) and verapamil HCl (Knoll) (both dissolved in distilled water); nifedipine (Bayer) (2.89 mM in propylene glycol); ethylenediaminetetraacetic acid disodium (EDTA) (Sigma) and ethyleneglycol-bis-(β-aminoethyl ether) N,N'-tetraacetic acid (EGTA) (Sigma) (both 0.1 M in 0.2 M NaOH); Tris-hydroxymethylaminomethane (Tris).

Solutions containing nifedipine and nitroprusside were protected from light at all times using aluminium foil.

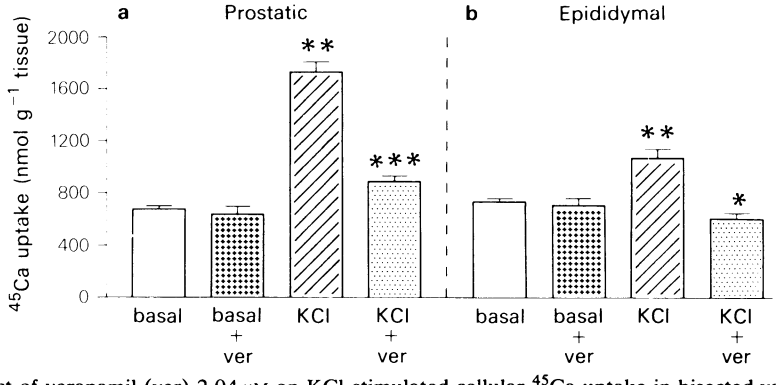

Figure 1 Effect of verapamil (ver) 2.04 μM on KCl-stimulated cellular ^{45}Ca uptake in bisected vasa deferentia. KCl 160 mM substantially increased ^{45}Ca uptake in the epididymal half (46% increase) and especially in the prostatic half (155% increase). Verapamil 2.04 μM markedly inhibited the increase in ^{45}Ca uptake produced by KCl 160 mM in the prostatic half and abolished the KCl-induced increase in ^{45}Ca uptake in the epididymal half. Verapamil 2.04 μM was without effect on basal uptake in either half. Columns are mean values ± s.e.mean, $n = 6–12$. Significantly different from basal ^{45}Ca uptake, $*P < 0.01$; $**P < 0.001$. Significantly different from basal and KCl-stimulated ^{45}Ca uptake, $***P < 0.001$.

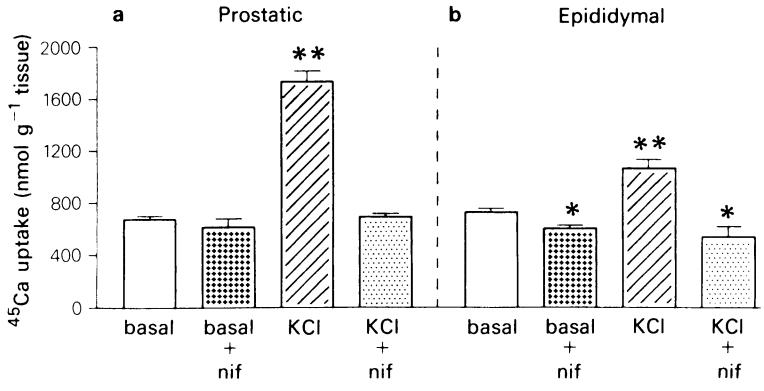

Figure 2 Effect of nifedipine (nif) 0.29 μM on KCl-stimulated cellular ^{45}Ca uptake in bisected vasa deferentia. Nifedipine 0.29 μM abolished the increase in ^{45}Ca uptake produced by KCl 160 mM in both halves. Nifedipine 0.29 μM had no effect on basal ^{45}Ca uptake in the prostatic half, but decreased basal ^{45}Ca uptake in the epididymal half. Columns are mean values ± s.e.mean, $n = 6$–12. Significantly different from basal ^{45}Ca uptake, $*P < 0.01$; $**P < 0.001$.

^{45}Ca was obtained from The Radiochemical Centre, Amersham.

Results are expressed as mean ± s.e.mean. In the graphs, standard errors, when indicated, are shown by vertical bars. Statistical analysis of the data was made using Student's t test and the 0.05 level of probability was regarded as significant.

Results

^{45}Ca uptake (cellular ^{45}Ca content)

La^{3+}-resistant ^{45}Ca uptake was measured in bisected vasa deferentia and the results are summarized in Figures 1 and 2. Although the basal ^{45}Ca uptake was larger in the epididymal half, KCl 160 mM produced a greater increase in ^{45}Ca uptake in the prostatic half: there was a 155% increase in the prostatic half from a basal value of 679 ± 21 nmol ^{45}Ca g^{-1} tissue wet weight, $n = 12$, to a KCl-stimulated value of 1734 ± 80 nmol ^{45}Ca g^{-1}, $n = 12$, $P < 0.001$, whereas in the epididymal half KCl 160 mM increased ^{45}Ca uptake by 46% from a control level of 730 ± 28 nmol ^{45}Ca g^{-1}, $n = 12$, to 1067 ± 67 nmol ^{45}Ca g^{-1}, $n = 12$, $P < 0.001$. KCl 160 mM caused a 15% reduction in tissue wet weight in the epididymal half: control weight = 40.2 ± 1.32 mg, $n = 12$, and weight after KCl incubation = 34.2 ± 1.29 mg, $n = 12$, $P < 0.01$. This decrease in tissue weight is not sufficient to account for the KCl-induced increase in ^{45}Ca uptake in this half. KCl 160 mM had no significant effect on tissue weight in the prostatic half (control weight = 56.0 ± 2.21 mg, $n = 12$; weight after KCl addition = 51.9 ± 2.01 mg, $n = 12$).

Verapamil 2.04 μM substantially reduced the KCl-induced increase in ^{45}Ca uptake in the prostatic half and abolished the increase in ^{45}Ca uptake in the epididymal half; verapamil 2.04 μM had no effect on basal uptake in either half (Figure 1). The KCl-induced stimulation of ^{45}Ca uptake in both halves was abolished by nifedipine 0.29 μM. Nifedipine 0.29 μM was without effect on basal ^{45}Ca uptake in the prostatic half, but significantly reduced basal uptake in the epididymal half: basal uptake in the epididymal half = 730 ± 28 nmol ^{45}Ca g^{-1} tissue wet weight, $n = 12$, and basal uptake in the presence of nifedipine 0.29 μM = 609 ± 21 nmol ^{45}Ca g^{-1}, $n = 6$, $P < 0.01$ (Figure 2).

^{45}Ca efflux

Intact vas deferens Addition of KCl 160 mM, 90 min after the start of efflux, produced a large, rapid increase in ^{45}Ca efflux into 2.5 mM $CaCl_2$ Krebs-Henseleit solution and also into nominally Ca^{2+}-free Krebs solution, which was maximal during the first collecting period (2 min), not maintained, and which was rapidly reversed on washout (after a 20 min contact period) (see control graphs in Figure 3). Superfusion of the tissue with KCl 160 mM in 2.5 mM $CaCl_2$ Krebs-Henseleit solution produced the characteristic biphasic contraction, whereas, as expected, in Ca^{2+}-free Krebs solution there was little or no tension developed. However, the maximum stimulation of ^{45}Ca efflux produced by KCl 160 mM was greater into nominally Ca^{2+}-free Krebs solution (735% increase from an unstimulated value of 0.68 ± 0.07% 2 min^{-1} to 5.0 ± 1.14% 2 min^{-1}, $n = 8$, $P < 0.01$) than into 2.5 mM $CaCl_2$ Krebs solution (411% increase from an unstimulated value of 0.47 ± 0.07% 2 min^{-1} to 1.93 ± 0.5% 2 min^{-1}, $n = 9$,

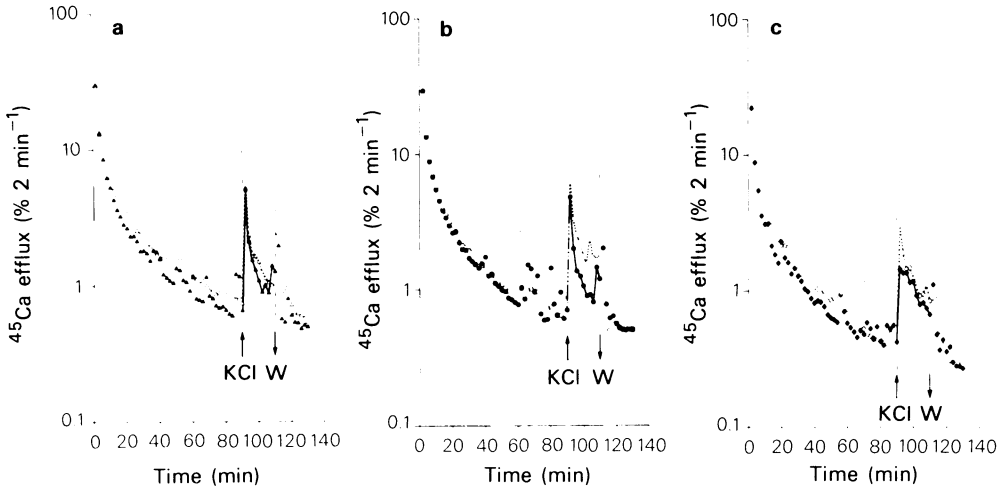

Figure 3 Rate coefficient curves of the effects of (a) verapamil, (b) nifedipine and (c) nitroprusside on KCl-stimulated ^{45}Ca efflux from intact vasa deferentia. KCl -60 mM increased ^{45}Ca efflux into (a, b) nominally Ca^{2+}-free Krebs-Henseleit solution, or (c) 2.5 mM $CaCl_2$ Krebs-Henseleit solution; this effect, which was maximal during the first 2 min collecting period, was rapidly reversed on washout (W) after 20 min exposure to KCl 160 mM. Closed symbols = control efflux, open symbols = efflux in the presence of the antagonist, which was present during the entire period of efflux. The KCl-stimulated increase in ^{45}Ca efflux was not antagonized by (a) verapamil 2.04 μM (in Ca^{2+}-free Krebs solution), (b) nifedipine 0.29 μM (in Ca^{2+}-free Krebs solution) or (c) nitroprusside 1678 μM (in 2.5 M $CaCl_2$ Krebs solution). The basal efflux of ^{45}Ca was not affected by verapamil, nifedipine or nitroprusside. The s.e.mean of all points was omitted for clarity; (a and b), $n = 4$; (c), $n = 5$.

$P < 0.02$); the later phase of efflux was similar in both solutions. These results indicate that KCl-induced stimulation of ^{45}Ca efflux was not due to mechanical distortions of the tissue produced by contractile activity. KCl 160 mM also increased ^{45}Ca efflux into Ca^{2+}-free Krebs-Henseleit solution plus EGTA 0.05 mM.

Verapamil 2.04 μM was without effect on the KCl-induced increase in ^{45}Ca efflux into 2.5 mM $CaCl_2$ Krebs-Henseleit solution and Ca^{2+}-free Krebs solutions (with or without EGTA 0.05 mM). Similarly, the stimulation of efflux produced by KCl 160 mM into 2.5 mM $CaCl_2$ Krebs solution or nominally Ca^{2+}-free Krebs solution was not inhibited by nitroprusside 1,678 μM or nifedipine 0.29 μM respectively. Verapamil 2.04 μM, nifedipine 0.29 μM or nitroprusside 1.678° μM did not significantly affect basal ^{45}Ca efflux (Figure 3).

Bisected vas deferens The rate of loss of the fast and slow components of ^{45}Ca efflux into 2.5 mM Krebs-Henseleit solution was identical from prostatic and epididymal halves of bisected tissues (Table 1 and Figure 4). However, the size of the slow component was larger in the prostatic half, e.g. after 90 min efflux the percentage of the original total activity remaining in the tissues was 48.4 ± 2.54%, $n = 4$, in the prostatic half and 39.9 ± 2.61%, $n = 4$, in the epididymal half (significantly different from each other, $P < 0.01$) (Figure 4). KCl 160 mM produced an increase in ^{45}Ca

Table 1 Parameters of washout components of ^{45}Ca efflux into 2.5 mM $CaCl_2$ Krebs-Henseleit solution from the bisected vas deferens

	Fast component			*Slow component*		
	% total activity in tissue	$t_{\frac{1}{2}}$ (min)	*Rate constant* (min^{-1})	*% total activity in tissue*	$t_{\frac{1}{2}}$ (min)	*Rate constant* (min^{-1})
Prostatic half	28	8	0.0866	56	414	0.00167
Epididymal half	30	8	0.0866	46	414	0.00167

The values are calculated from desaturation plots by the curve peeling method.
Rate constant, k, = $0.693 t_{\frac{1}{2}}$ (Weiss, 1977) where $t_{\frac{1}{2}}$ = the calculated half-time of the washout component.

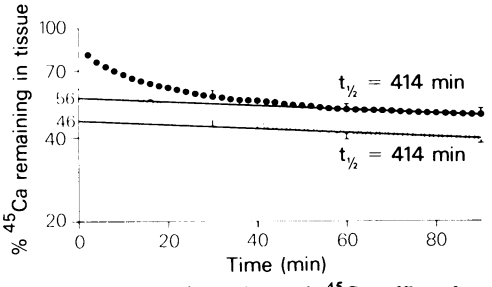

Figure 4 Desaturation plots of ^{45}Ca efflux from bisected vasa deferentia into 2.5 mM $CaCl_2$ Krebs-Henseleit solution. There was a parallel rate of loss of ^{45}Ca in (●) prostatic and (○) epididymal halves, although the slow component of washout was larger in the prostatic half. The s.e.mean of most points was omitted for clarity; $n = 4$.

efflux which was of comparable size and followed a similar pattern in both regions, e.g. the increase in efflux after the initial 2 min contact period with KCl was 388% in the epididymal half and 333% in the prostatic half. The KCl-induced increase in ^{45}Ca efflux in both halves was quickly reversed on washout (Figure 5).

Discussion

^{45}Ca uptake

The increase in ^{45}Ca uptake produced by KCl 160 mM in the prostatic and epididymal halves of bisected vasa deferentia confirms the conclusions reached after tension studies (Hay & Wadsworth 1982b) that addition of high K^+ to the rat vas deferens, as in other smooth muscles, produces tension development by stimulation of the entry of extracellular Ca^{2+} into the intracellular compartment. The much larger stimulation of ^{45}Ca uptake in the prostatic half may indicate that in this region compared with in the epididymal half: (1) A greater number of membrane calcium channels are activated, (2) Ca^{2+} extrusion mechanisms are less efficient, and/or (3) There is a substantially greater intracellular sequestration of Ca^{2+}. In support of (3), in a light and electron microscopic study of the rat vas deferens it was demonstrated that the amounts of smooth endoplasmic reticulum and mitochondria were greater in the prostatic region compared to the epididymal region (Flickinger, 1973).

The antagonism of KCl-induced increase in ^{45}Ca uptake in both halves of bisected vasa deferentia by verapamil and nifedipine confirms that these agents inhibited contractions produced by high K^+ by inhibition of Ca^{2+} entry (Hay & Wadsworth, 1982b), and reaffirms the sensitivity of smooth muscle membrane calcium channels activated by membrane depolarization to inhibition by calcium channel inhibitors (Bolton, 1979).

The greater sensitivity of verapamil for inhibition of KCl-induced stimulation of ^{45}Ca uptake in the epididymal half possibly lends support to the idea, postulated above, that KCl 160 mM activates more membrane calcium channels in the prostatic half, and, therefore, a higher concentration of verapamil would be required to occlude and inactivate all the calcium channels and abolish the increased ^{45}Ca uptake in this region. However, there are other possible reasons for this finding, including small differences in the affinity of the calcium channels in the two halves. The difference in sensitivity of the increased ^{45}Ca uptake produced by KCl 160 mM in both halves to verapamil, is in part agreement with the results from tension studies (Hay & Wadsworth, 1982b). How-

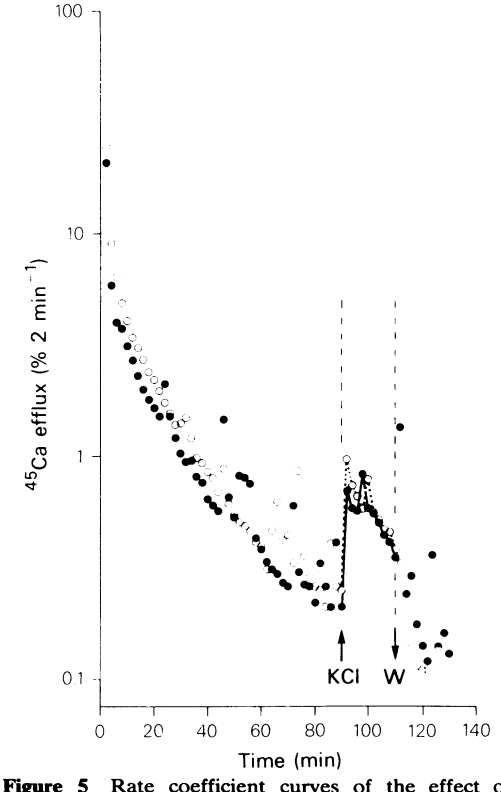

Figure 5 Rate coefficient curves of the effect of KCl 160 mM on ^{45}Ca efflux from bisected vasa deferentia into 2.5 mM $CaCl_2$ Krebs-Henseleit solution. KCl 160 mM increased efflux of ^{45}Ca to the same extent in (●) prostatic and (○) epididymal halves; the stimulatory effect of KCl 160 mM in both regions was rapidly reversed on washout (W). The s.e.mean of all points was omitted for clarity; $n = 4$.

ever, closer analysis and comparison of the effects of verapamil and nifedipine on contractions and stimulation of ^{45}Ca uptake produced by KCl 160 mM reveals, to some extent, an apparent lack of correlation between their effects on tension and on ^{45}Ca uptake. For example, in the prostatic half, verapamil 2.04 μM reduced both phases of the contraction to KCl by about 40% but caused 80% inhibition of the KCl-induced increase in ^{45}Ca uptake, and in the epididymal half verapamil 2.04 μM abolished the increase in ^{45}Ca uptake, but only reduced the phasic and tonic components of the contraction to KCl by approximately 70% and 50% respectively. Furthermore, the maximum inhibition of the two phases of the contraction to KCl 160 mM in both halves by nifedipine was about 80% (Hay & Wadsworth, 1983b), whereas nifedipine 0.29 μM abolished the KCl-induced increase in ^{45}Ca uptake in both halves, when one might have expected a residual component of increased ^{45}Ca uptake after addition of nifedipine. These discrepancies are probably largely due to the error and limit of detection involved in the measurement of ^{45}Ca uptake. This was estimated by Karaki & Weiss (1980a) to be 5–10%, using their experimental protocol which involved La^{3+} 80.8 mM at 0.5°C. In view of the magnitude of the levels of ^{45}Ca involved in the present study, an error of this size may represent a sufficient quantity of undetected KCl-induced increase in ^{45}Ca uptake to produce a significant contractile response in tension studies, and, furthermore, the postulated nifedipine-resistant component of the KCl-stimulated increase in ^{45}Ca uptake (if it exists) might not be detected.

Nifedipine 0.29 μM and verapamil 2.04 μM had no significant effect on basal ^{45}Ca uptake except with nifedipine in the epididymal half. In this group, basal ^{45}Ca uptake was only 18% less than in the control group and the difference was only just statistically significant. Furthermore, a higher concentration of nifedipine (14.4 μM) had no effect on basal ^{45}Ca uptake in either half of the rat vas deferens (Hay & Wadsworth, unpublished observations). We therefore believe that, despite this result, nifedipine and verapamil were not affecting passive transmembrane ^{45}Ca uptake in our experiments. This conclusion is in accord with many studies using various calcium channel inhibitors including nifedipine and verapamil, that show no effect on basal ^{45}Ca uptake (Hester *et al.*, 1979; Thorens & Haeusler, 1979; Godfraind & Dieu, 1981; Ozaki *et al.*, 1981; Van Breemen *et al.*, 1981).

^{45}Ca efflux

The possible mechanisms mediating the large, reversible KCl-induced increase in ^{45}Ca efflux which were considered were: (1) Stimulation of the uptake of ^{40}Ca which exchanges with ^{45}Ca, which was proposed for the methoxyverapamil-sensitive increase in ^{45}Ca efflux produced by high K^+ in the rabbit ear artery (Droogmans *et al.*, 1977; Casteels & Droogmans, 1981). However, this was concluded not to be the mechanism involved in the present study for two reasons: (i) the KCl-induced increase in ^{45}Ca efflux was unaffected by verapamil and nifedipine and (ii) the increased efflux of ^{45}Ca occurred into Ca^{2+}-free washout solutions. (2) Release of a store of ^{45}Ca from high affinity extracellular or membrane sites, which is not involved in the contractile response (and would not be inhibited by calcium channel inhibitors). In the rabbit aorta ^{45}Ca bound at high affinity superficial locations was rapidly released by washout into Ca^{2+}-containing solution, but only slowly depleted into Ca^{2+}-free solutions (Karaki & Weiss, 1980b). Therefore, it is possible that the larger stimulatory effect of high K^+ on ^{45}Ca efflux in the absence of Ca^{2+} than in its presence, which was observed in the present study, is because of the substantial release of a high affinity superficial ^{45}Ca store into Ca^{2+}-free solution with little or no release into Ca^{2+}-containing solution; the remainder of the increased ^{45}Ca efflux into Ca^{2+}-free or Ca^{2+}-containing solutions may be mediated by the displacement of slowly exchangeable low affinity ^{45}Ca (Karaki & Weiss, 1980b) and/or by mechanism (3). (3) Release by high K^+ of intracellular ^{45}Ca which is transported to the extracellular space via a separate population of membrane calcium channels (not blocked by calcium channel inhibitors) from that mediating Ca^{2+} entry. This hypothesis was originally proposed by Godfraind (1978) for noradrenaline-stimulated ^{45}Ca uptake (sensitive to cinnarizine) and efflux (resistant to cinnarizine) in the rat aorta. Alternatively, intracellularly released ^{45}Ca may enter the extracellular environment by a mechanism that does not involve transport through membrane calcium channels. In support of a KCl-induced release of intracellular ^{45}Ca, it is of interest that Saad & Huddart (1981) reported that high K^+ increased ^{45}Ca efflux from microsomes and mitochondria isolated from rat vas deferens. However, in contrast to the results demonstrated in this study they found that KCl had no stimulatory effect on ^{45}Ca efflux from the whole tissue; this discrepancy may have been partly because they used a lower concentration (100 mM) than that employed in the present study or because of differences in experimental protocol.

The mechanism of action of nitroprusside has not been fully clarified but may involve an intracellular action on Ca^{2+} sequestration and/or efflux (see review by Kreye, 1980). Therefore, it could be argued that the lack of effect of nitroprusside on KCl-induced increase in ^{45}Ca efflux suggests that this increased rate of loss of ^{45}Ca does not involve release of intracellular Ca^{2+}.

The larger slow component of ^{45}Ca efflux in the prostatic half compared with that in the epididymal half suggests that the postulated high affinity Ca^{2+}-binding sites are predominant in this region. These sites may include superficial high affinity Ca^{2+} sources involved in the contractile response to high K^+, which would be consistent with the tension studies which demonstrated that the component of the contraction to KCl 160 mM resistant to long periods of Ca^{2+}-deprivation was also predominant in the prostatic half (Hay & Wadsworth 1982b).

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