ELSEVIER

European Journal of Pharmacology 286 (1995) 123–130

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Differential vasorelaxant effects of levcromakalim and P1060 in the isolated KCl- and RbCl-precontracted human saphenous vein: possible involvement of intracellular Ca^{2+} stores

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Received 3 July 1995; accepted 11 July 1995

Abstract

The influence of rubidium-substituted physiological salt solution (Rb-PSS) on the relaxant effects of K^+ channel openers was investigated in the human saphenous vein. In tissues precontracted with 20 mM KCl (in K-PSS) levcromakalim and P1060 produced complete, sustained relaxations. However, in Rb-PSS (containing 20 mM RbCl) these effects were inhibited and, although complete relaxations still occurred, were transient. When caffeine was applied at the beginning of this *fade* of levcromakalim-induced relaxation in Rb-PSS its contractile effect was potentiated. Similarly, the contraction to noradrenaline was potentiated when applied at the beginning of this fade of levcromakalim-induced relaxation, whereas this response was attenuated in control tissues bathed in 20 mM KCl (in K-PSS). Our results show that the relaxant effects of K^+ channel openers in human saphenous vein are inhibited in Rb-PSS, in agreement with previous studies in animal tissue, and suggest that an increased Ca^{2+} uptake into intracellular stores may be contributory to vasorelaxation.

Keywords: Levcromakalim; P1060; Saphenous vein, human; Rubidium ion

1. Introduction

Potassium channel openers (K^+ channel openers) may have therapeutic potential in a variety of cardiovascular disorders including hypertension, intermittent claudication and ischaemic heart disease. The majority of pharmacological studies so far have been performed in animal tissue, whilst there are comparatively few data regarding the actions of K^+ channel openers in human smooth muscle. We have previously reported that cromakalim is a potent relaxant of human saphenous vein in vitro, an action probably mediated via the opening of ATP-sensitive K^+ channels (K_{ATP}) (Soares de Moura and Jasbik, 1992). Current electrophysiological studies suggest that these drugs may act via the opening of relatively small conductance, glibenclamide-sensitive K^+ channels in both arterial and venous smooth muscle (Kajioka et al., 1990, 1991; Noack et al., 1992a,b; Beech et al., 1993; Criddle et al., 1994).

However, recent evidence has suggested that K^+ channel openers may possess other 'non-plasmalemmal' actions (for review see Quast, 1993). For example, in experiments using rat aorta in which the K^+ ions of the physiological salt solution (PSS) have been replaced by rubidium (Rb) ions, the vasorelaxant effects of the K^+ channel openers are inhibited, possibly due to blockade of membrane K^+ channels by Rb^+ since these drugs can no longer elicit hyperpolarisation (Greenwood and Weston, 1993). However, despite their inability to induce electrical changes under these conditions, K^+ channel openers can still produce a full relaxation of the precontracted rat aorta. Thus K^+ channel openers may possess actions in addition to membrane K^+ channel opening, although at present these remain undefined. Previous studies using fura-2 as a marker for intracellular Ca^{2+} have indicated an

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0014-2999/95/$09.50
SSDI 0014-2999(95)00426-2

ability of the K^+ channel openers to modulate intracellular Ca^{2+} changes induced by noradrenaline in the mesenteric artery of the rabbit and rat (Ito et al., 1991b; Itoh et al., 1992; Criddle et al., 1994). Moreover, levcromakalim inhibits the refilling of noradrenaline-sensitive calcium stores in rabbit aorta (Bray et al., 1991) and trachea (Chopra et al., 1992).

We have recently shown that the mechanoinhibitory effects of K^+ channel openers are inhibited in the presence of Rb^+ in the isolated human myometrium (Criddle and Soares de Moura, 1995). The aim of the present study was to ascertain whether such inhibitory effects of Rb^+ also exist in human vascular smooth muscle by comparing the mechanical effects of levcromakalim and P1060 (*N*-(*t*-butyl-*N*″-cyano-*N*′-3-pyridyl-guanidine), a pinacidil analogue, on human isolated saphenous vein in the presence and absence of Rb^+. Furthermore, we have also examined the possibility that the K^+ channel openers may be able to relax blood vessels in the presence of Rb^+ by an enhancement of Ca^{2+} uptake into intracellular storage sites.

A preliminary account of some of these results has been presented (Criddle and Soares de Moura, 1994).

2. Material and methods

2.1. Preparation of blood vessels

Segments of branches of the long saphenous vein (leftovers) were obtained from patients undergoing heart revascularisation surgery. Institutional approval for use of this tissue was obtained. On removal from the patient, the vessel segments were immediately placed in a physiological salt solution (K-PSS: a Krebs-Henseleit solution of composition (mM); 118.3 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 0.026 EDTA and 11.1 glucose), carefully cleaned of perivascular tissue and cut into rings approximately 0.3 cm long. All experiments were performed on the day of surgery.

2.2. Organ chamber experiments

Rings of saphenous vein were suspended in organ chambers filled with 30 ml of K-PSS solution bubbled with 95% O_2 5% CO_2 at 37°C. Each ring was suspended by two stainless steel stirrups passed through its lumen. One stirrup was anchored inside the organ chamber, the other connected to a force transducer (FTA 10; Hewlett-Packard Co., Palo Alto, CA, USA) for the measurement of isometric force on a Hewlett-Packard 7754A recorder. All rings were progressively stretched to the optimal point of the length-tension curve as determined by the response to 60 mM KCl and allowed to equilibriate for 60 min.

2.3. Effects of Rb-PSS on relaxant responses to K^+ channel openers

Following stabilisation, tissues were exposed to either normal K-PSS or a modified solution in which the K^+ salts had been replaced by their Rb^+ equivalents (Rb-PSS; KCl and KH_2PO_4 were omitted and replaced by RbCl 5.9 mM and NaH_2PO_4 1.2 mM) for 30 min. Each tissue received 3 washes throughout this period. Tone was then induced in the saphenous veins using either 20 mM KCl or 20 mM RbCl as appropriate, and cumulative relaxant concentration-response curves constructed to levcromakalim or P1060 (using a 5 min contact time at each concentration).

In other experiments designed to investigate the 'fade' mechanism, a single concentration of either K^+ channel opener (1 μM) was used to relax veins precontracted with either 20 mM K-PSS or 20 mM Rb-PSS in order to compare the duration of relaxation in the presence or absence of Rb^+. Following a plateau fade to a new level of tone a higher concentration of K^+ channel opener (10 μM) was applied to the vein. In separate experiments glibenclamide (3 μM) was applied following a stable fade of the relaxation induced by levcromakalim in veins contracted with 20 mM RbCl.

2.4. Effects of vasorelaxants on Ca^{2+} storage

In order to examine the possible involvement of intracellular Ca^{2+} stores in the fade of responses to the K^+ channel openers in Rb-PSS, the effects of levcromakalim on the contraction induced by 1 mM caffeine were investigated. The protocol adopted involved initially obtaining control contractions to caffeine, then exposing the test tissue to Rb-PSS as described above for 30 min (the control ring exposed only to K-PSS) and repeating the application of caffeine. Following this, the saphenous veins were contracted with 20 mM RbCl or 20 mM KCl as appropriate and when a plateau was reached 1 μM levcromakalim was added to relax the tissue. When a 'fade' in the relaxation began to appear in the tissue exposed to Rb-PSS, each vein was then re-exposed to caffeine and the ensuing contraction recorded. The effects of nifedipine were also assessed accordingly.

In addition, a similar protocol was adopted to investigate effects of levcromakalim on noradrenaline-induced contractions in the saphenous vein. A concentration of 3×10^{-7} M, which produces an approximate 50% maximal response in human saphenous vein (Soares de Moura and Jasbik, 1992), was used to allow any potentiation of the contractile response to noradrenaline to be manifested.

2.5. Drugs

Levcromakalim (SmithKline Beecham), P1060 (Leo), nifedipine (Bayer) and noradrenaline (Sigma) were made as stock solutions (10 mM) in absolute ethanol and diluted in distilled water on the day of the experiment. Glibenclamide (Sigma) was prepared as a stock solution (10 mM) in dimethyl sulphoxide (DMSO) and diluted in distilled water. Caffeine (Sigma) was dissolved directly into Krebs solution at the desired concentration.

2.6. Analysis of data

Data are expressed as the mean ± S.E.M. of n observations. Statistical analysis was performed using a paired Student's t-test with significance identified at $P < 0.05$. IC_{50} values were calculated for each experimental concentration-effect curve and expressed as the mean of n experiments together with the appropriate 95% confidence interval.

3. Results

3.1. Effects of Rb-PSS on K^+ channel opener-induced relaxations

Rb-PSS produced a gradual increase in tone of 0.75 ± 0.12 g ($n = 12$). Comparable increases in tension were induced by addition of 20 mM KCl and 20 mM RbCl to K-PSS and Rb-PSS (1.24 ± 0.7 g ($n = 12$) and 1.65 ± 0.28 g ($n = 12$), respectively). Levcromakalim elicited full, concentration-dependent relaxations of precontracted saphenous veins with a mean IC_{50} of 0.097 μM (range 0.07–0.12 μM, $n = 6$). In the presence of Rb-PSS the relaxant effects of levcromakalim were inhibited approximately 4-fold; the mean IC_{50} was 0.40 μM (range 0.3–0.5 μM, $n = 6$) (Fig. 1). P1060 was a more potent relaxant of human saphenous vein than levcromakalim, with a mean IC_{50} of 0.036 μM (range 0.021–0.051 μM, $n = 6$) and was inhibited approximately 11-fold in the presence of Rb^+ (mean IC_{50} of 0.4 μM (range 0.32–0.49 μM, $n = 6$)) (Fig. 1).

3.2. Duration of K^+ channel opener-induced relaxation in K-PSS and Rb-PSS

In separate experiments both levcromakalim and P1060, at a concentration of 1 μM, produced complete relaxations of saphenous veins precontracted with either 20 mM KCl or 20 mM RbCl. In K-PSS these relaxations were well maintained in excess of 2 h ($n = 6$); however, in Rb-PSS there was a gradual 'fade' of the relaxations induced by both K^+ channel openers (Fig. 2). For example, in veins exposed to levcromakalim (1 μM) the relaxation began to decline after 12.4 ± 3.1 min to a new plateau level of 44.0 ± 9.1% of the initial relaxation ($n = 7$). Often this fade was associated with the development of spontaneous activity in the vein, comprising oscillatory contractions and relaxations. Following this fade, a higher concentration of levcromakalim (10 μM) further relaxed the tissue ($n = 6$, Fig. 2). Similarly, the relaxation induced by P1060 (1 μM) was transient, with a fade occuring after 6.8 ± 0.8 min to a new level of vascular tone that was 35.9 ± 4.4% of the initial relaxation ($n = 6$). This was also further relaxed by addition of a higher concentration (10 μM) of P1060 ($n = 3$) or a higher concentration of levcromakalim (10 μM) applied after the 'fade' to P1060 ($n = 3$).

In separate experiments, glibenclamide (3 μM), applied when a stable fade to the relaxant effects of

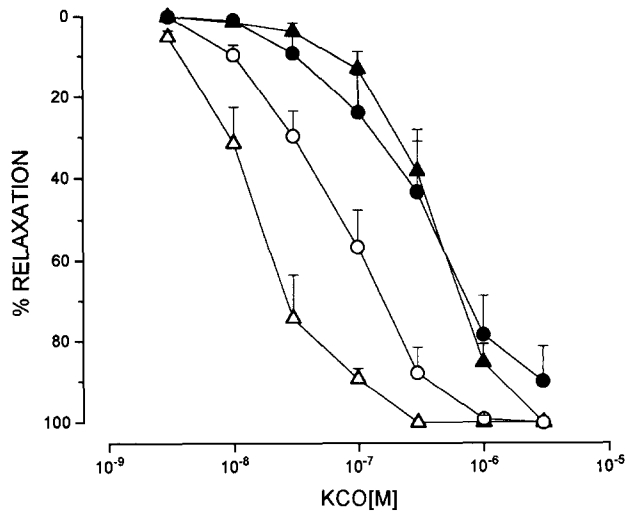


Fig. 1. Relaxant effects of levcromakalim (○) and P1060 (△) in human saphenous veins precontracted with either 20 mM KCl (in K-PSS; open symbols) or 20 mM RbCl (in Rb-PSS; filled symbols). Data are shown as the mean ± S.E.M., $n = 6$ for each data point.

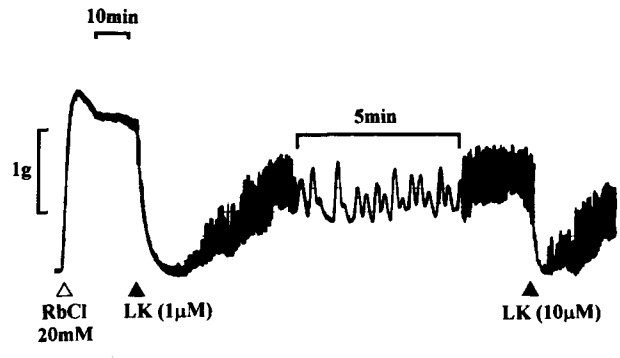


Fig. 2. Typical recording showing the transient relaxation induced by levcromakalim (1 μM) of human saphenous vein precontracted with 20 mM RbCl in Rb-PSS. A portion of the trace has been expanded to show the oscillatory nature of this fade. Note also that a higher concentration of levcromakalim (10 μM) was able to further relax the vein following the fade.

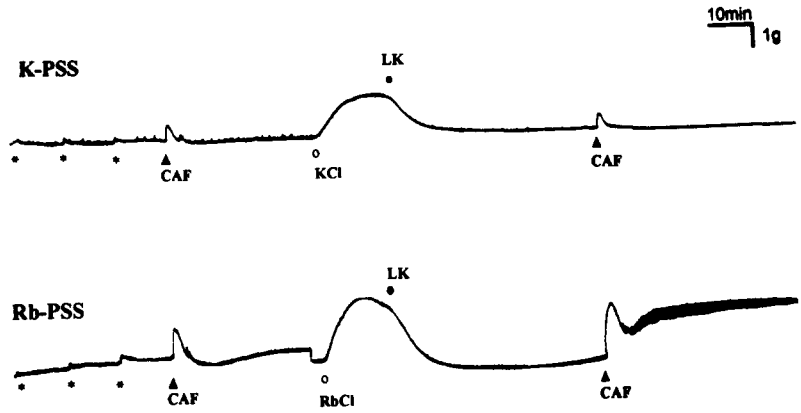
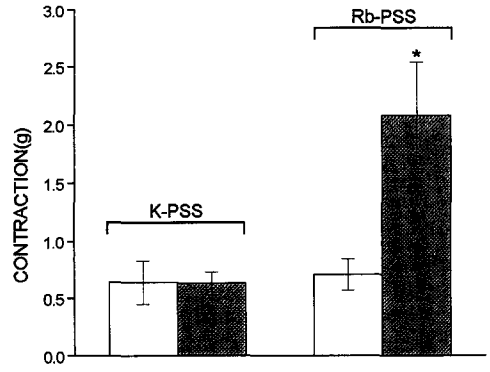


Fig. 3. Typical recording showing the effects of levcromakalim on caffeine-induced contractions of paired preparations from the same human saphenous vein in the presence of either 20 mM KCl (K-PSS; upper trace) or 20 mM RbCl (Rb-PSS; lower trace). All experiments were time-matched, with the second application of caffeine at the point where fade of the relaxant effects of levcromakalim in Rb-PSS was just beginning. Note: * denotes washes with Rb/K-PSS, and also offset of the lower trace prior to the addition of 20 mM RbCl.

i) LEVCROMAKALIM



ii) NIFEDIPINE

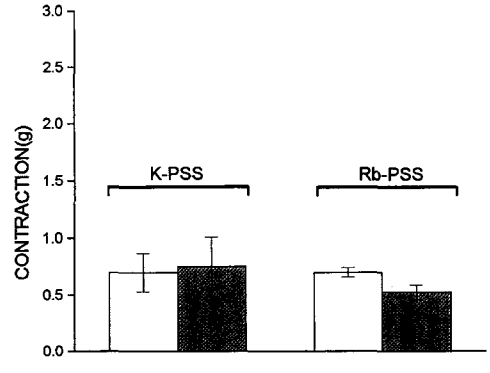


Fig. 4. Effects of (i) levcromakalim (1 μM) and (ii) nifedipine (1 μM) on caffeine-induced contractions of the human saphenous vein bathed with K-PSS or Rb-PSS. Open bars show the control contraction to 1 mM caffeine, and filled bars the contraction to caffeine following relaxation of 20 mM KCl-or 20 mM RbCl-induced tone by levcromakalim ($n = 8$) or nifedipine ($n = 4$).

levcromakalim (1 μM) in 20 mM Rb-PSS had occurred, reversed the remaining levcromakalim-induced relaxation ($n = 6$).

Since the relaxant profile of levcromakalim and P1060 in Rb-PSS was essentially the same, in further experiments designed to investigate the underlying mechanism(s) of the 'fade' we concentrated on the effects of levcromakalim alone.

3.3. *Effects of levcromakalim and nifedipine on caffeine-induced contractions*

In saphenous veins application of caffeine (1 mM) induced a rapidly developing, transient contraction in both K-PSS and Rb-PSS (18.8 ± 2.8% ($n = 8$) and 21.9 ± 7.7% ($n = 8$) of the maximal contraction induced by 60 mM KCl, respectively) that was reproducible following washout periods of 30 min. Often a secondary,

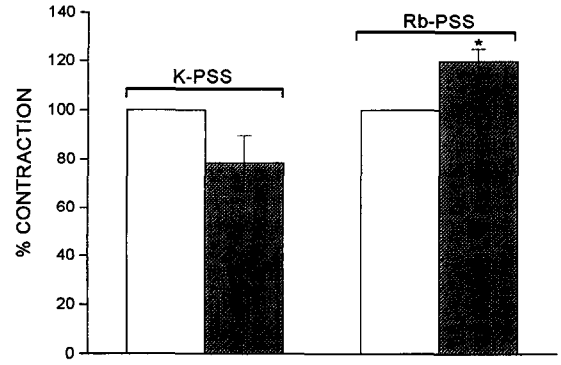


Fig. 5. Effects of levcromakalim (1 μM) on the contraction induced by noradrenaline (3×10^{-7} M) in human saphenous veins bathed with K-PSS or Rb-PSS. Open bars show the control contraction to noradrenaline, filled bars the contraction following relaxation of 20 mM KCl-or 20 mM RbCl-induced tone by levcromakalim ($n = 7$).

slower relaxant component to the caffeine response was apparent in both Rb-PSS and K-PSS groups (see control caffeine contraction in Rb-PSS; Fig. 3) although there was considerable variation between preparations. In addition, prolonged exposure to Rb-PSS (> 2 h) or an increase in basal tone induced by 20 mM RbCl did not potentiate caffeine-induced contraction ($n = 4$).

In order to examine the possibility that K^+ channel openers can promote the sequestration of Ca^{2+} into intracellular stores, the effects of levcromakalim on caffeine-induced contractions were investigated following its relaxation of vessels precontracted with either 20 mM RbCl (in Rb-PSS) or 20 mM KCl (in K-PSS). In a similar manner to the previously described experiments (see Section 3.1) the basal tone was increased in those tissues exposed to Rb-PSS (Fig. 3); however, the control contraction to caffeine (1 mM) was not altered (Fig. 4). Following a full relaxation of the contractions induced by either 20 mM RbCl or KCl by levcromakalim (1 μM), the response to levcromakalim slowly began to fade in Rb-PSS. When caffeine (1 mM) was reapplied at this point, the induced contraction was greatly potentiated in the veins exposed to Rb (Figs. 3 and 4). This response was biphasic comprising a rapid, initial phase and a secondary, more prolonged contraction that was sometimes phasic in nature (Fig. 3). However, the contractile response to caffeine was not potentiated by nifedipine (1 μM) in either K-PSS or Rb-PSS (Fig. 4).

3.4. Effects of levcromakalim on noradrenaline-induced contractions

The magnitude of the control responses to noradrenaline did not differ between K-PSS and Rb-PSS, the increase in tension being 2.10 ± 0.6 g ($n = 7$) and 2.05 ± 0.2 g ($n = 7$), respectively. In contrast to the caffeine response, the noradrenaline-induced contraction was slower, more sustained and was not biphasic. However, following a maximal relaxation of the saphenous veins precontracted with either 20 mM RbCl or 20 mM KCl by levcromakalim (1 μM), the response to noradrenaline differed between tissues exposed to K-PSS and Rb-PSS (Fig. 5). Thus the contraction to noradrenaline was attenuated in the presence of levcromakalim in veins bathed in K-PSS, whereas there was a small but significant potentiation in the presence of Rb^+.

4. Discussion

4.1. Inhibitory effects of Rb-PSS on K^+ channel opener-induced vasorelaxation

In accord with previous studies in animal smooth muscle (Morris and Taylor, 1989; Foster et al., 1989,1992; Greenwood and Weston, 1993), the relaxant effects of K^+ channel openers in human saphenous vein were inhibited in the presence of Rb^+ and were transient. In the present study we were not able to examine effects on membrane potential or Rb^{86} efflux; however, the profile of mechanical activity of the K^+ channel openers in the human blood vessel appeared essentially the same as that found previously in rat aorta (Greenwood and Weston, 1993).

For example, the vasorelaxant effects of levcromakalim were inhibited to the same degree in the presence of Rb^+ in human saphenous vein as in rat aorta (4-fold). Similarly, this relaxation was transient, faded to a new level of tone after a period of minutes and was glibenclamide-sensitive. Although P1060 was not tested in the previous study in rat aorta (Greenwood and Weston, 1993), in human saphenous vein it exhibited a similar profile of activity to levcromakalim, the only difference being a greater inhibition in Rb-PSS possibly suggesting more involvement of plasmalemmal K^+ channels in its actions. We have previously reported a similar difference in the inhibition induced by Rb-PSS of the mechanoinhibitory effects of levcromakalim and P1060 in the isolated human myometrium (Criddle and Soares de Moura, 1995). Thus it appears that the previous findings in animal vascular tissue are well correlated with the human situation. A discrepancy, however, relates to the ability of the K^+ channel openers to further relax the tissue following fade to a new level of tone in the presence of Rb^+; in contrast to the effects in rat aorta, in human saphenous vein a higher concentration of either K^+ channel opener was able to maximally relax the tissue anew following the initial fade. The reason for this anomaly is currently unclear but may relate to differences between species and/or the physiology of veins and arteries.

At present the mechanism underlying the effects of Rb-PSS on smooth muscle is not known. In isolated blood vessels Rb-PSS induces a slowly developing contraction which is unlikely to be due to blockade of K_{ATP} since glibenclamide, an inhibitor of K_{ATP} in smooth muscle (Noack et al., 1992b), has no effect on basal vascular tone per se (Yanagisawa et al., 1990; Soares de Moura and Jasbik, 1992; Greenwood and Weston, 1993). However, since inhibitory actions of Rb^+ on K_{ATP}, Ca^{2+}-dependent and inwardly rectifying K^+ channels exist in non-vascular tissues (Standen and Stanfield, 1980; Gallacher et al., 1984; Ashcroft et al., 1989) it is possible that the observed effects of Rb-PSS in saphenous vein may be the summation of many inhibitory events. Further experiments are required to clarify this issue.

In agreement with Greenwood and Weston (1993) our data support the proposal that K^+ channel openers possess additional non-plasmalemmal relaxant mechanisms (for review see Quast, 1993). However,

since the fade of the relaxant action of levcromakalim and P1060 in Rb-PSS was overcome by application of higher concentrations of drug, it would appear that a tachyphylaxis is not involved as previously suggested, but instead may reflect more direct intracellular changes.

4.2. K^+ channel opener-induced relaxation: a consequence of intracellular Ca^{2+} sequestration?

It has been reported that cromakalim is able to inhibit the release of noradrenaline-sensitive Ca^{2+} stores in rabbit aorta (Bray et al., 1991) and rat mesenteric vascular beds (Quast and Baumlin, 1991), possibly via a hyperpolarisation-induced reduction in $InsP_3$ production (Itoh et al., 1992). In addition, several reports have shown that K^+ channel openers are able to produce modest inhibitions of the refilling of noradrenaline-and $InsP_3$-sensitive intracellular Ca^{2+} stores (Bray et al., 1991; Chopra et al., 1992). This action appears to be independent of membrane hyperpolarisation and may underlie their vasorelaxant effects in Rb-PSS (Greenwood and Weston, 1993), but conclusive evidence is still lacking.

The contraction produced by RbCl in blood vessels may be mediated by both intracellular Ca^{2+} release and Ca^{2+} influx into the cell, since Ca^{2+}-induced contraction of guinea-pig aorta under depolarising conditions is in part ryanodine-sensitive (Ito et al., 1991a), whilst depolarisation-induced Ca^{2+} transients in guinea-pig bladder appear to be a consequence of both Ca^{2+}-induced Ca^{2+} release and Ca^{2+} influx (Ganitkevich and Isenberg, 1992). During exposure of saphenous vein to 20 mM KCl or 20 mM RbCl it is possible that Ca^{2+} release occurs and may thus result in a depletion of intracellular Ca^{2+} stores. Under these conditions it appears more likely that an inhibition of the RbCl-induced contraction by the K^+ channel openers would be mediated via a movement of Ca^{2+} into intracellular stores and/or from the cell, thereby decreasing free intracellular Ca^{2+}, rather than an inhibition of refilling. Interestingly, cromakalim and its active enantiomer levcromakalim are able to decrease basal intracellular Ca^{2+} levels in rabbit aorta and mesenteric artery, as determined using fura-2 epifluorescence techniques, via a glibenclamide-sensitive mechanism (Yanagisawa et al., 1990; Ito et al., 1991b).

The transient relaxation induced by K^+ channel openers in Rb-PSS is often associated with the development of rhythmic mechanical oscillations in blood vessels (Greenwood and Weston, 1993; present study) and may reflect interference with intracellular Ca^{2+} handling. The phenomenon of oscillations of intracellular Ca^{2+} is well documented in a number of cell types and may involve the periodic release of Ca^{2+} from intracellular stores (Berridge, 1991). Furthermore, a recent report indicates that rhythmic contractions can be induced by phenylephrine in ryanodine-pretreated rabbit mesenteric arteries (Omote et al., 1993), possibly as a consequence of an impaired sarcoplasmic reticulum buffering capacity (Van Breemen et al., 1986; Hwang and Van Breemen, 1987). If K^+ channel openers are promoting uptake of Ca^{2+} into stores then at some point these may become saturated and spontaneously discharge; this will cause contraction followed by relaxation as the stores empty and subsequently refill. Under conditions in which the veins are exposed to 20 mM RbCl and a K^+ channel opener concurrently, the tissues will be depolarised, since K^+ channel openers are no longer capable of eliciting hyperpolarisation (Greenwood and Weston, 1993), and Ca^{2+} entry into the cell will occur continuously via an activation of voltage-sensitive Ca^{2+} channels producing a slowly developing contraction or *fade* of relaxation.

A sequestrative action of K^+ channel openers is directly suggested from the potentiation of contractions to caffeine at a time during the fade at which we postulate that an increase in the loading of intracellular Ca^{2+} stores would have been induced. Caffeine is known to cause contraction in smooth muscle via the release of Ca^{2+} from intracellular storage sites (Leijten and Van Breemen, 1984), and accordingly was insensitive to the Ca^{2+} channel blocker nifedipine in saphenous vein. Previous studies in vascular smooth muscle have shown that K^+ channel openers either do not affect (Itoh et al., 1990; Ito et al., 1991b; Quast and Baumlin, 1991), increase (Yamagishi et al., 1992) or decrease (Bray and Weston, 1989; Wilson and Cooper, 1989) contractions to caffeine. In saphenous vein, we found that levcromakalim did not alter the magnitude of caffeine-induced contraction per se, but greatly potentiated it when caffeine was applied at the beginning of fade of levcromakalim-induced relaxation in Rb-PSS. Thus it would appear that K^+ channel openers can in some way augment the caffeine-releasable intracellular Ca^{2+} store. Interestingly this potentiated response to caffeine was also biphasic in nature, the latter phase of the contraction possibly an acceleration of the fade of the relaxant effects of the K^+ channel openers in Rb-PSS. It is conceivable that this may be due to a permeating effect on the sarcoplasmic reticulum induced by the continued presence of caffeine, thereby compromising the ability of the intracellular pools to store Ca^{2+} and thus interfering with any sequestering action of the K^+ channel openers.

In addition, the contractile response to noradrenaline was modified differently by levcromakalim depending upon whether the saphenous vein was exposed to 20 mM KCl or 20 mM RbCl. In the former case the contraction was attenuated, presumably at least partly due to K^+ channel opener-induced hyperpolarisation, whilst in the latter situation the contraction was poten-

tiated. This augmentation of the response to noradrenaline may be the result of an increase in the content of agonist-releasable Ca^{2+} stores; however, it also appears likely that there will be some contribution of Ca^{2+} influx into the cell under these circumstances. In this respect the data obtained with caffeine are more conclusive; however, the results with noradrenaline remain consistent with a sequestration effect of K^+ channel openers. Interestingly Ito et al. (1991b) have reported that in β-escin-skinned mesenteric arteries (which cannot generate a membrane potential), levcromakalim (0.1 μM) greatly increases both the rise in intracellular Ca^{2+} and tension induced by noradrenaline under Ca^{2+}-free conditions. Thus both protocols (Rb-PSS and β-escin pretreatment) which abolish the membrane-hyperpolarising action of levcromakalim result in an increased contraction to noradrenaline in the presence of the K^+ channel opener.

4.3. Conclusions

The mechanical effects of K^+ channel openers are inhibited in Rb^+-containing physiological solutions in human blood vessels in a similar manner to that detailed previously in animal tissue (Greenwood and Weston, 1993). The present study has extended these findings and our data suggest that, in addition to plasmalemmal K^+ channel opening, the K^+ channel openers may exert relaxant effects in blood vessels partly via an increased uptake of Ca^{2+} into intracellular stores. Since it was beyond the scope of this study to measure intracellular Ca^{2+} levels directly, there are obvious limitations to our findings and thus conclusions remain speculative at the present time. However, our data support the view that the mode of action of K^+ channel openers is more complex than previously thought.

Acknowledgements

We wish to thank Dr T.C. Hamilton (SmithKline Beecham) for the generous gift of levcromakalim.

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