ELSEVIER

European Journal of Pharmacology 272 (1995) 293–296

ejp

Short communication

Inhibitory effects of Rb^+ on the responses to levcromakalim and P1060 in the isolated human myometrium

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Received 21 July 1994; revised 31 October 1994; accepted 18 November 1994

Abstract

The mechanoinhibitory effects of two structurally dissimilar K^+ channel openers, levcromakalim and P1060, and verapamil were compared in strips of human myometrium bathed in either K-PSS (normal Krebs solution) or Rb-PSS (K^+ salts replaced by Rb^+ equivalents). In Rb-PSS the effects of levcromakalim and P1060 on amplitude and frequency of spontaneous contractions were inhibited by more than 20- and 138-fold, respectively, whereas those of verapamil were unaltered. These results indicate that K^+ channel openers possess Rb-sensitive and Rb-insensitive mechanoinhibitory actions on the human uterus, the former being more important in the effects of P1060 than levcromakalim.

Keywords: Uterus, human; Rubidium; K^+ channel opener; Levcromakalim; P1060

1. Introduction

The relaxant effects of K^+ channel openers are inhibited by rubidium in a variety of animal smooth muscle preparations, including guinea-pig bladder (Foster et al., 1989), guinea-pig trachea (Morris and Taylor, 1989; Isaac et al., 1994), rat aorta (Greenwood and Weston, 1993) and also more recently in human vascular smooth muscle (Criddle and Soares de Moura, 1994). Under conditions in which the bathing solution contains Rb^+ ions instead of K^+, the K^+ channel openers can no longer elicit hyperpolarisation or stimulate transmembrane ^{42}K movement, yet can fully relax blood vessels suggesting that they possess actions that are not mediated via the opening of plasmalemmal K^+ channels (Greenwood and Weston, 1993). At present the mechanism(s) underlying these putative secondary effects is unknown.

The K^+ channel openers have been shown to exert inhibitory effects on both spontaneous and evoked contractions of the isolated uterus of the rat (Piper et al., 1990) and human (Cheuk et al., 1993; Morrison et al., 1993). These effects appear to be mediated via the activation of ATP-sensitive K^+ channels (K_{ATP}) since they are inhibited by glibenclamide. The aim of the present study was to ascertain whether inhibitory effects of rubidium on the actions of K^+ channel openers occur in the human uterus, and if differences exist between structurally different drugs by comparing the actions of the benzopyran levcromakalim with the cyanoguanidine pinacidil derivative P1060.

2. Materials and methods

2.1. Preparation of myometrial strips

Segments of uterus, taken from the anterior surface of the uterine body, were obtained from premenopausal patients, exhibiting no overt disease, undergoing hysterectomy due to myomatose. Institutional approval for use of this tissue was obtained. On removal from the patient, the segments were immediately placed in a Krebs-Henseleit solution (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) and were cut into strips approximately 2 cm long. These were suspended in organ chambers filled with 30

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Elsevier Science B.V.
SSDI 0014-2999(94)00706-3

ml of Krebs-Henseleit solution bubbled with 95% O_2-5% CO_2 at 37°C. One end of the strip was fixed to the bottom of the 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 strips were preloaded with 2 g tension and allowed to equilibriate for 60 min.

2.2. Protocols

Following stabilisation, tissues were exposed to either normal Krebs (K-PSS; control group) 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; test group) for 30 min. Each tissue received 3 washes throughout this period. The strips were then allowed to equilibrate for a further 30 min before cumulative relaxant concentration-response curves were constructed to levcromakalim, P1060 or verapamil (using a 30 min contact time at each concentration).

2.3. Drugs

Levcromakalim (SmithKlineBeecham, Harlow, UK), P1060 (Leo, Copenhagen, Denmark) and verapamil (Knoll, Rio de Janeiro, Brasil) were made as stock solutions (10 mM) in absolute ethanol and diluted in distilled water on the day of the experiment.

2.4. Analysis of data

Data are expressed as the mean ± S.E.M. of n observations. Statistical analysis was performed using a non-paired Student's t-test with significance identified at $P < 0.05$.

3. Results

3.1. Effects of Rb-PSS on spontaneous myometrial activity

During the equilibration period the myometrial strips developed spontaneous phasic contractions. In tissues bathed in K-PSS the basal amplitude and frequency of contraction were 1.78 ± 0.27 g and 0.18 ± 0.02 min^{-1}, respectively ($n = 12$). There was no significant difference in spontaneous myometrial activity between the K-PSS group and tissues exposed to Rb-PSS, the latter exhibiting a mean amplitude and frequency of contraction of 2.01 ± 0.27 g and 0.16 ± 0.02 min^{-1}, respectively ($n = 12$).

3.2. Effects of levcromakalim, P1060 and verapamil on spontaneous myometrial activity

Levcromakalim induced a concentration-dependent inhibition of the amplitude and frequency of contraction of uterine strips bathed in K-PSS, a maximal effect occurring at a concentration of 1 μM ($n = 6$, Fig. 1). In Rb-PSS these actions of levcromakalim were inhibited approximately 26- and 20-fold, respectively (Table 1). P1060 inhibited contractions of the uterus with a slightly greater potency than levcromakalim and the inhibitory concentration-effect curves of this drug were displaced to the right to a much greater extent in the presence of rubidium. Thus in Rb-PSS the effects of P1060 on the amplitude and frequency of contraction were shifted 138- and 160-fold, respectively (Fig. 1, Table 1).

In contrast, the Ca^{2+} channel entry blocker verapamil produced a concentration-dependent inhibition of the amplitude and frequency of contraction which was not significantly different between the K-PSS and Rb-PSS groups (Fig. 1, Table 1).

4. Discussion

Levcromakalim and P1060 are potent inhibitors of the spontaneous contractions of human isolated myometrium. The potency of levcromakalim observed in our study was the same as that previously reported in human uterus by Cheuk et al. (1993); however, these are the first data obtained in human myometrium using the pinacidil derivative P1060. This cyanoguanidine was slightly more potent than levcromakalim in its effects on the uterus, and we have previously described a similar difference regarding the vasorelaxant effects of these drugs in the human saphenous vein (Criddle and Soares de Moura, 1994).

Recently a study in rat aorta has indicated that the relaxant actions of a series of K^+ channel openers are attenuated in Rb-substituted physiological Krebs solution (Greenwood and Weston, 1993). Interestingly, the authors found that in Rb-PSS the K^+ channel openers could not induce hyperpolarisation or stimulate $^{42}K^+$ efflux from a preloaded tissue but were able to produce complete relaxation of precontracted aortae. They concluded that drugs such as levcromakalim may in fact possess mechanisms in addition to plasmalemmal K^+ channel opening. This topic has recently been extensively reviewed (Quast, 1993). In agreement with the findings in rat aorta (Greenwood and Weston, 1993) we found that the inhibitory effects of Rb-PSS in uterus were specific to the action of K^+ channel openers since the effects of verapamil remained unaffected.

In both rat aorta and human saphenous vein, the vasorelaxant effects of levcromakalim were inhibited approximately 4-fold in Rb-PSS (Greenwood and Wes-

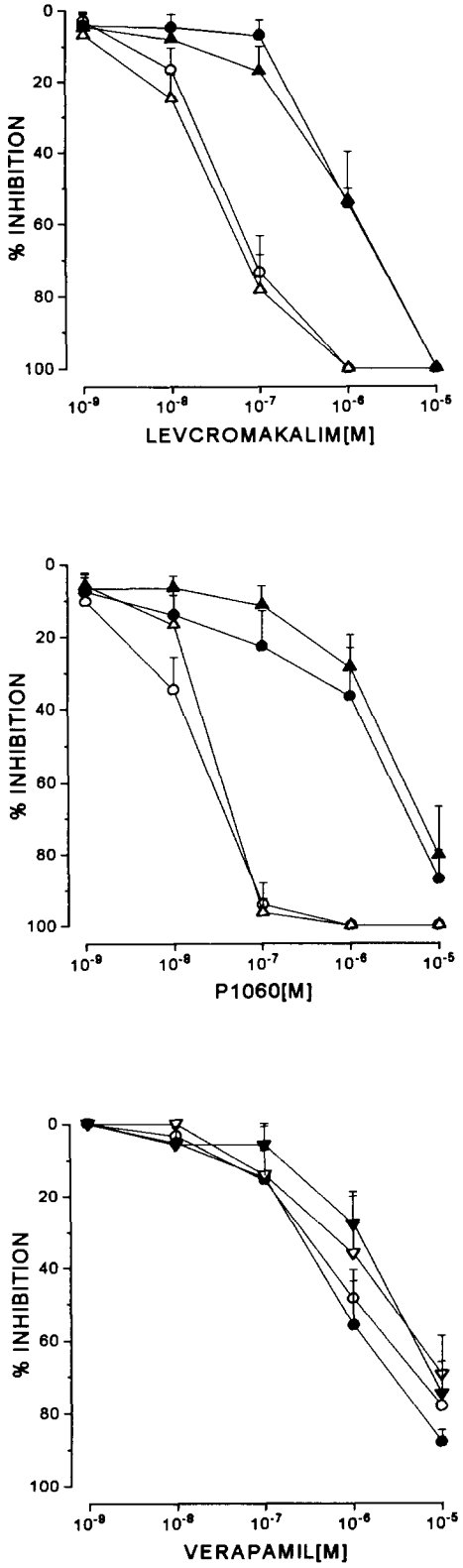

Fig. 1. Effects of levcromakalim, P1060 and verapamil on the amplitude (○) and frequency (▽) of spontaneous myometrial contractions, in the presence of K-PSS (open symbols) or Rb-PSS (filled symbols).

ton, 1993; Criddle and Soares de Moura, 1994); however, in human uterus the actions of levcromakalim were inhibited more than 20-fold, suggesting a greater role of a Rb-sensitive (putatively plasmalemmal K^+ channel-mediated (Greenwood and Weston, 1993)) relaxant mechanism in this tissue. Paradoxically, the hyperpolarisation associated with the relaxant action of the racemate cromakalim appears to be considerably less in the uterus (< 5 mV; Hollingsworth et al., 1987) than in aorta of the rat (~ 25 mV; Taylor et al., 1988) at an equivalent concentration. Such an effect may be indicative of selective actions of K^+ channel openers at pacemaker foci (Hollingsworth et al., 1987). However, in accord with our data in uterus, a profound inhibition by Rb-PSS of the relaxant effects of levcromakalim has also recently been described in quiescent, non-vascular smooth muscle (Isaac et al., 1994). Furthermore, in human uterus the mechanoinhibitory effects of P1060 were attenuated in excess of 130-fold in Rb-PSS and therefore would appear to indicate that the major part of its mechanoinhibitory action is mediated via the opening of Rb-sensitive K^+ channels. In agreement with this, the effects of this compound in saphenous vein are inhibited in Rb-PSS to a greater extent than are those of levcromakalim (Criddle and Soares de Moura, 1994).

At present the mechanism of inhibition by Rb-PSS of K^+ channel opener-induced effects is unknown. It seems likely that K^+ channel openers exert effects on the human uterus via an activation of K_{ATP}, since their action is inhibited by glibenclamide (Cheuk et al., 1993), a blocker of K_{ATP} in smooth muscle (Noack et al., 1992). However, as yet no inhibitory effect of Rb on K_{ATP} has been reported in smooth muscle, although millimolar concentrations of this ion block K_{ATP} in pancreatic β-cells (Ashcroft et al., 1989). Alternatively, the effects of Rb-PSS on K^+ channel opener action in the uterus may reside in an effect on the large conductance calcium-dependent K^+ channel (BK_{Ca}), since Rb is able to block BK_{Ca} in secretory cells (Gallacher et al., 1984). An approximate 100-fold potency difference between P1060 and cromakalim on the activation of this channel has been reported in rat portal vein (Hu et al., 1990) and recent evidence suggests that the action of pinacidil in the human pregnant myometrium is mediated, at least in part, via BK_{Ca} (Ashford et al., 1993), although this study did not demonstrate that the effects of pinacidil were glibenclamide-insensitive. However, an inhibitory effect of Rb on inwardly rectifying K^+ channels in skeletal muscle has also been reported (Standen and Stanfield, 1980) and it is possible that the effects of Rb-PSS are the summation of many inhibitory events.

In conclusion, Rb-PSS appears able to differentiate between the actions of structurally dissimilar K^+ channel openers in the human uterus. Since we were unable

Table 1
Mean IC_{50} values of levcromakalim, P1060 and verapamil for inhibition of the amplitude and frequency of spontaneous myometrial contractions

	K-PSS Mean IC_{50} (μM)		Rb-PSS Mean IC_{50} (μM)	
	Amplitude (g)	Frequency (min^{-1})	Amplitude (g)	Frequency (min^{-1})
Levcromakalim	0.041 ± 0.013	0.038 ± 0.010 ($n = 6$)	1.07 ± 0.50 *	0.75 ± 0.16 * ($n = 6$)
P1060	0.018 ± 0.004	0.026 ± 0.004 ($n = 6$)	2.48 ± 0.96 *	4.15 ± 2.67 * ($n = 6$)
Verapamil	2.19 ± 0.99	3.76 ± 2.10 ($n = 4$)	1.91 ± 1.27	6.02 ± 1.56 ($n = 4$)

Data expressed as mean ± S.E.M. (* $P < 0.05$).

to perform detailed electrophysiological experiments or radiolabelled ion flux studies, our conclusions can only remain speculative at present. However, the data from our study appear to support the view that the diverse group of smooth muscle relaxants designated as K^+ channel openers possess additional Rb-insensitive, putatively 'non-plasmalemmal' actions. Interestingly, there appears to be a large difference in the inhibition produced by Rb-PSS of the actions of the K^+ channel openers tested in our study and may indicate a future basis for development of selectivity of drug action in the uterus. Such a feature would be very desirable for possible use in conditions such as pre-term labour.

Acknowledgements

We wish to thank the staff of the Departmento de Obstetricia e Ginecologia, Hospital Universitario Pedro Ernesto, for their assistance with the supply of human uterine tissue and Dr. T.C. Hamilton for the generous gift of levcromakalim.

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