Effects of the novel potassium channel opener, UR-8225, on contractile responses in rat isolated smooth muscle


Department of Pharmacology, School of Medicine, University Complutense of Madrid, 28040-Madrid, Spain and *Centro de Investigación Uriach, Degà Bahí, 59-67, 08026 Barcelona, Spain

1. The effects of UR-8225 [(1,2-dihydro-4-(1,2-dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-1-oxanthalened-6-carbonitrile)] and levocromakalim were studied on the electrical and contractile responses induced by noradrenaline and KCl and on ⁸⁶Rb⁺ efflux in rat aortic rings and on spontaneous mechanical activity in rat portal vein segments.

2. UR-8225 and levocromakalim, 10⁻⁴ M–10⁻³ M, relaxed the contractile responses induced by noradrenaline (IC₅₀ = 2.7 ± 0.4 x 10⁻⁶ M and 6.6 ± 1.3 x 10⁻⁷ M, respectively) or 30 mM KCl (IC₅₀ = 1.4 ± 0.2 x 10⁻³ M and 9.4 ± 1.3 x 10⁻⁴ M, respectively) more effectively than those induced by 80 mM KCl. The relaxant effect on noradrenaline-induced contractions was independent of the presence or absence of functional endothelium.

3. The vasorelaxant effect of UR-8225 and levocromakalim can be competitively antagonized by glibenclamide, an ATP-sensitive K⁺ channel blocker. There were no differences in the calculated pA₂ values for glibenclamide to inhibit UR-8225- and levocromakalim-induced relaxations (7.61 ± 0.08 and 7.69 ± 0.10, respectively). The slope of the Schild plot yielded values not significantly different from unity (0.95 ± 0.06 and 0.96 ± 0.05, respectively).

4. UR-8225 (10⁻⁴ M) hyperpolarized the resting aortic membrane potential from −50.7 ± 0.7 mV to −66.0 ± 2.0 mV and stimulated ⁸⁶Rb⁺ efflux.

5. UR-8225 and levocromakalim inhibited the contractions induced by Ca²⁺ in aortae incubated in Ca²⁺-free PSS containing methoxycavepamil in the presence of noradrenaline.

6. Both drugs inhibited the amplitude of spontaneous activity in portal veins (IC₅₀ = 5.1 ± 1.4 x 10⁻⁴ M and 1.5 ± 0.7 x 10⁻⁴ M, respectively), this effect being competitively antagonized by glibenclamide.

7. These results indicated that UR-8225 exhibited qualitatively similar, but slightly less potent, vasorelaxant effects than those exerted by levocromakalim, which suggests that they can be related to its ability to activate ATP-sensitive K⁺ channels in vascular smooth muscle cells.

Keywords: UR-8225; levocromakalim; rat aorta; portal vein; potassium channels; vascular smooth muscle

Introduction

Potassium channel openers constitute a class of vasodilator drugs with a novel mechanism of action. The vasorelaxant properties of this class of drugs have been initially attributed to the activation of ATP-sensitive potassium channels and the subsequent hyperpolarization of the smooth muscle membrane which prevents the opening of voltage-activated Ca²⁺ channels (Quast & Cook, 1989; Hamilton & Weston, 1989; Edwards & Weston, 1990). As potent peripheral vasodilators these drugs are expected to be useful in the treatment of several cardiovascular disorders, such as hypertension, angina pectoris, peripheral arterial diseases, cerebral ischaemia and congestive heart failure (Cook, 1988; Hamilton & Weston, 1989; Escande & Caveró, 1992; Sanguinetti, 1992).

UR-8225 is a new compound [(1,2-dihydro-4-(1,2-dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-1-oxanthalened-6-carbonitrile)] that stems from a structure-activity study carried out at the Uriach Research Center (Almansa et al., 1992). The key feature of the molecule is a naphtalenedione ring replacing the conventional benzyopryl nucleus present in levocromakalim (formerly BRL 38227) and other related compounds (Figure 1). In preliminary experiments, it has been found that UR-8225 exhibits potent vasodilator properties possibly related to its potassium channel opener properties (Garcia-Rafanell et al., 1992). Therefore, the purpose of the present paper was: (1) to analyze the vasorelaxant effects of UR-8225 in rat isolated vascular smooth muscle, and (2) to compare its effects with those of levocromakalim. A preliminary report of some of the results of this study has already been published (Casis et al., 1993).

Methods

Experimental procedure

Sprague-Dawley rats (either sex, 250–300 g) were killed by a blow on the head. The descending thoracic aorta and portal veins were rapidly dissected and placed in a physiological saline solution (PSS) of the following composition (mM): NaCl 118, KCl 4.75, NaHCO₃ 25, MgSO₄ 1.2, CaCl₂ 1.8, KH₂PO₄ 1.2 and glucose 11. After excess of fat and connective tissue were removed, the aortae were cut into rings (4–5 mm in length). Aortic rings were mounted under the tension of 1 g by two parallel L-shaped stainless-steel holders inserted into the lumen and longitudinal portal vein segments (15 mm in length) were mounted vertically under the basal tension of 1 g in 20 ml organ baths containing PSS and attached to a force-displacement transducer (Grass FT07) to measure isometric contractile force as previously described (Pérez-Vizcaino et al., 1991; 1993). The tissue bath was maintained at 37°C and bubbled with 95% O₂:5% CO₂ gas mixture. For the experiments in which Ca²⁺-free medium was used, Ca²⁺ was omitted from normal PSS and 0.03 mM EDTA was added. For most of the experiments care was taken not to damage the endothelium. In some experiments, endothelial cells were gently removed by rubbing the internal surface of the vessels with a small metal rod. The absence of functional endothelium was confirmed by the inability of the
initially contracted with $10^{-5}$ M noradrenaline. After washing, rings were incubated in Ca^{2+}-free PSS containing $10^{-5}$ M methoxyverapamil and 0.03 mM EDTA for 10 min. At this time, the addition of $10^{-5}$ M noradrenaline induced a transient contraction. After 30 min, when the basal tension was reached, the concentration of Ca^{2+} in the bathing media was increased to 2 mM Ca^{2+} and a tonic contraction was recorded. In experimental muscles, UR-8225 or levromakalim were added 30 min before the addition of Ca^{2+}. Results are expressed as a percentage of the initial noradrenaline-induced contraction.

(c) To study the effects of UR-8225 and levromakalim on the spontaneous portal vein contractions, cumulative concentration-response curves were obtained in the absence or in the presence of glibenclamide.

Appropriate parallel control experiments were always carried out in order to correct for the possible effects caused by vehicle alone.

**Measurement of membrane potential**

Cleaned, endothelium-free, aortic segments were pinned down in a Lucite chamber with the lumen side up. The muscle was continuously superfused with oxygenated PSS maintained at 34°C. Membrane potentials were recorded conventionally through glass microelectrodes filled with 3 M KCl (tip resistance 30–50 MΩ) as previously described (Deplon et al., 1992). The microelectrode was connected via Ag–AgCl wire to high-input capacity neutralizing amplifiers (WPI model 701, World Precision Instruments Inc., New Haven, CT, U.S.A.). Membrane potential was displayed on a storage oscilloscope (Tektronix 5104N, Tektronix Inc., Beaverton, OR, U.S.A.) and photographed with a Kymographic Grass camera (Model C-4, Grass Instrument Company, Quincy, MA, U.S.A.).

**$^{86}$Rb⁺ efflux**

The effects of UR-8225 on $^{86}$Rb⁺ efflux were determined as described by Tulenko & Cox (1991). Aortic rings were equilibrated for 10 min in a PSS of the following composition (mm): NaCl 140, KCl 4.75, CaCl₂ 1.5, MgSO₄ 1.0, glucose 11 and HEPES 10 at pH 7.4 bubbled with 100% O₂ at 37°C. Then rings were loaded for 3 h in PSS containing $^{86}$Rb⁺ (5 mM-¹). Afterwards the muscles were dipped quickly into PSS to remove excess radioactivity and then transferred through a series of vials (every 10 min for the first 30 min and every 3 min thereafter) each containing 0.8 ml of PSS for the first 51 min and PSS containing UR-8225 (10⁻⁶ M or 10⁻⁵ M) thereafter. At the end of the experiment, the radioactivity remaining in the aorta was determined by dissolving the vessel in 200 μl of a solution containing equal parts perchloric acid (37% w/v) and H₂O₂ (30 volumes) heated for 15 min at 75°C. After cooling, 5 ml of Aquasol-2 (Dupont, Boston, MA, U.S.A.) was added. The $^{86}$Rb⁺ activity in the vials and that extracted from the tissues were measured by Cerenkov counting. The results were expressed in terms of efflux rate constants which reflect the permeability of the cell membrane to Rb⁺. Rate constants (k) during each time interval were calculated using the following equation: \( k = \frac{\ln (A1/A2)}{(t2-t1)} \), where A₁ and A₂ represent the total tissue counts at time points t₁ and t₂, respectively.

**Drugs**

The following drugs were used: UR-8225 (Laboratorios, Uriach, Barcelona), levromakalim (SmithKline Beecham Pharmaceuticals, Betchworth, U.K.), (--)-noradrenaline bitartrate and glibenclamide (Sigma Ltd. Co., London), methoxyverapamil (D600, Knoll AG, Ludwigshafen/Rhein, Germany). Glibenclamide was diluted in dimethyl sulphoxide to make a stock solution of $10^{-2}$ M. All other drugs were dissolved in distilled deionized water to prepare a $10^{-2}$ M stock solution and further dilutions were made in PSS. The final concentra-
tion of solvent had no measurable effect on contractile responses or \(^{86}\)Rb\(^+\) efflux. Ascorbic acid (10\(^{-5}\) M) was added to each stock solution of noradrenaline, made up freshly each day.

**Statistics**

Throughout the paper values are expressed as mean ± s.e.mean and statistical analysis was performed with Student’s \(t\) test. The differences between control and experimental values were considered significant when \(P < 0.05\). Dose-response slopes were analyzed to give the concentration of UR-8225 or levromakalim producing a 50% inhibition of the maximal contractile response (IC\(_{50}\)) using a linear regression analysis over the response range of 20 to 80% of the maximal inhibition. pA\(_2\)-values were calculated by Schild-plot analysis (Arunlakshana & Schild, 1959).

**Results**

**Effects on spontaneous and noradrenaline-induced contractions in the portal vein**

In 16 portal vein segments the control amplitude of spontaneous contractions was 784.3 ± 133.4 mg. Figure 2 shows that UR-8225 and levromakalim (10\(^{-9}\) M–10\(^{-7}\) M) inhibited the amplitude of these contractions in a concentration-dependent manner and at 2 \(\times 10^{-7}\) M and 10\(^{-7}\) M, respectively, they suppressed the spontaneous activity. In 5 muscles, the IC\(_{50}\) values for UR-8225 and levromakalim to inhibit the myogenic activity were 5.1 ± 1.4 \(\times 10^{-7}\) M (\(n = 5\)) and 1.5 ± 0.7 \(\times 10^{-8}\) M (\(n = 5\)), respectively. The ability of glibenclamide to reverse the inhibitory effects of UR-8225 and levromakalim on the amplitude of spontaneous contractions was studied in 6 portal veins. In the presence of glibenclamide (3 \(\times 10^{-7}\) M, 10\(^{-6}\) M and 3 \(\times 10^{-5}\) M) there was a rightward shift of the curve for UR-8225 and levromakalim (Figure 2). Thus, in the presence of 3 \(\times 10^{-6}\) M glibenclamide the IC\(_{50}\) values for UR-8225 and levromakalim were 1.5 ± 0.5 \(\times 10^{-7}\) M and 3.2 ± 0.6 \(\times 10^{-7}\) M, respectively. There were no differences in the calculated pA\(_2\)-values for glibenclamide to inhibit UR-8225- and levromakalim-induced inhibitions (6.76 ± 0.05 and 6.80 ± 0.18, respectively). The slope of the Schild plot yielded values not significantly different from unity (1.14 ± 0.07 and 1.07 ± 0.22, respectively) which indicates that the inhibition was competitive. Addition of 10\(^{-7}\) M noradrenaline to portal vein segments induced a tonic contraction averaging 1312 ± 193 mg (\(n = 6\)). Cumulative addition of UR-8225 (10\(^{-7}\) M–10\(^{-5}\) M) induced a concentration-dependent inhibition of these contractions, the IC\(_{50}\) value being 6.9 ± 3.3 \(\times 10^{-8}\) M.

![Figure 2](image1.png)  
**Figure 2** Effects of UR-8225 (a) and levromakalim (b) added in a cumulative fashion on the amplitude of spontaneous contractions in rat portal vein segments. Results were obtained in the absence (○) and in the presence of glibenclamide 3 \(\times 10^{-7}\) M (■), 10\(^{-6}\) M (▲) and 3 \(\times 10^{-5}\) M (●). Ordinate scale: percentage of control values. Abscissa scale: log UR-8225 or levromakalim concentration (M). Each point represents the mean ± s.e.mean of 6 experiments. Insets: Schild-plot analysis. Ordinate scale: log(dose ratio – 1); abscissa scale: negative logarithm of glibenclamide concentration (M).

![Figure 3](image2.png)  
**Figure 3** Effects of UR-8225 (a) and levromakalim (b) added in a cumulative fashion on the 30 mM KCl-induced contractions in rat aortic rings. Results were obtained in the absence (○) and in the presence of glibenclamide 10\(^{-7}\) M (■), 3 \(\times 10^{-6}\) M (▲) and 3 \(\times 10^{-5}\) M (●). Ordinate scale: percentage of control values. Abscissa scale: log UR-8225 or levromakalim concentration (M). Each point represents the mean ± s.e.mean of 6 experiments. Insets: Schild-plot analysis. Ordinate scale: log(dose ratio – 1); abscissa scale: negative logarithm of glibenclamide concentration.
Relaxant effects on KCl- and noradrenaline-induced contractions

At concentrations up to \(10^{-5}\) M, UR-8225 or levcromakalim had no effect on baseline tension in aortic rings. In 14 aortae the contractile response produced by 20 mM KCl averaged 862.1 ± 107.9 mg. UR-8225 and levcromakalim, \(10^{-5}\) M, produced a concentration-dependent inhibition of this contractile response, the IC\(_{50}\) values being 9.2 ± 6.1 \(\times 10^{-8}\) M (\(n = 8\)) and 3.2 ± 0.3 \(\times 10^{-8}\) M (\(n = 6\)).

As shown in Figure 3, UR-8225 and levcromakalim also relaxed the contractions previously induced by 30 mM KCl, the IC\(_{50}\) values being 1.4 ± 0.2 \(\times 10^{-7}\) M (\(n = 6\)) and 9.4 ± 1.3 \(\times 10^{-8}\) M (\(n = 6\)). The figure also shows that glibenclamide (\(10^{-5}\) M–\(3 \times 10^{-6}\) M) shifted to the right these concentration-relaxation curves for UR-8225 and levcromakalim against these contractile responses. Thus, in the presence of \(3 \times 10^{-6}\) M glibenclamide, the IC\(_{50}\) values for UR-8225 and levcromakalim were 1.4 ± 0.4 \(\times 10^{-5}\) M and 1.1 ± 0.1 \(\times 10^{-5}\) M, respectively. There were no differences in the calculated pA\(_{2}\) values of glibenclamide to inhibit UR-8225- and levcromakalim-induced relaxations (7.61 ± 0.08 and 7.69 ± 0.10, respectively). The slope of the Schild plot yielded values not significantly different from unity (0.95 ± 0.06 and 0.96 ± 0.05, respectively) which indicates that the inhibition was competitive.

Addition of KCl (80 mM), noradrenaline (\(10^{-5}\) M) or both, to aortic rings produced a contractile response which averaged 1756 ± 254 mg (\(n = 15\)), 2183 ± 530 mg (\(n = 15\)) and 2831 ± 342 (\(n = 10\)), respectively. Figure 4 shows the relaxant effects of UR-8225 and levcromakalim (\(10^{-8}\) M–\(10^{-5}\) M) when added cumulatively to aortic rings previously contracted with these agonists. UR-8225 and levcromakalim inhibited in a concentration-dependent manner the contractile responses induced by \(10^{-5}\) M noradrenaline in endothelium-intact rings, the IC\(_{50}\) being 2.7 ± 0.4 \(\times 10^{-8}\) M (\(n = 7\)) and 6.6 ± 1.3 \(\times 10^{-7}\) M (\(n = 8\)), respectively. In endothelium-denuded rings, UR-8225 also relaxed noradrenaline-induced contraction, the IC\(_{50}\) being 1.9 ± 1.1 \(\times 10^{-6}\) M (\(n = 4\), not significantly different compared to endothelium-intact rings). Pretreatment with glibenclamide (\(10^{-5}\) M–\(3 \times 10^{-6}\) M) also shifted the concentration-responses to the right (not shown). In contrast, at \(10^{-5}\) M, both drugs inhibited the 80 mM KCl-induced contractions by only 11.7 ± 4.5% (\(P > 0.05\), \(n = 6\)) and 20.5 ± 4.3% (\(P < 0.05\), \(n = 6\)), respectively. In another group of experiments, the muscles were firstly exposed to 80 mM KCl and when the contractile response reached a steady-state, \(10^{-5}\) M noradrenaline was added to the bathing media. Figure 4 shows that under these conditions, UR-8225 or levcromakalim, \(10^{-5}\) M–\(10^{-3}\) M, only slightly inhibited these contractions: thus, at \(10^{-5}\) M these responses were inhibited by 8.7 ± 1.3% (\(n = 6\)) and 9.4 ± 2.0% (\(n = 6\)), respectively. These results indicated that both agents were not only almost ineffective against 80 mM KCl-induced contractions but also that a strong depolarization inhibited the effects of both drugs on noradrenaline-induced contractions.

Effects on concentration-response curves to KCl

Potassium channel openers relax contractions induced by 20 mM KCl but are ineffective against those induced by
80 mM KCl (Hamilton & Weston, 1989). Cumulative increases in KCl concentration (15–85 mM) to aortic rings in a Ca²⁺-containing PSS induced a concentration-dependent increase in developed tension. Figure 5 shows that both UR-8225 and levromakalim (10⁻⁷ M – 10⁻³ M), produced a concentration-dependent inhibition of these contractile responses, but this inhibitory effect was more marked against the responses induced by low concentrations of KCl (≤ 30 mM) which were almost abolished, than against the contractions induced by 45 or 85 mM KCl. Thus, the greater the KCl concentration the less the effect induced by UR-8225 and levromakalim.

Effects on noradrenaline-induced contractions in Ca²⁺-free solution

In another group of experiments, the effects of UR-8225 or levromakalim were studied on the contractile responses induced by CaCl₂ (2 mM) in aortic rings incubated in Ca²⁺-free PSS containing 0.03 mM EDTA and 10⁻³ M methoxyverapamil. Under these conditions addition of 10⁻⁵ M noradrenaline induced a phasic contraction resulting from the release of intracellular Ca²⁺. After 30 min, 2 mM CaCl₂ was added to the bathing media resulting in a tonic contractile response which averaged 54.0 ± 6.1% of the initial noradrenaline-induced contraction in the absence of EDTA and methoxyverapamil. In some aortic rings run in parallel, UR-8225 or levromakalim (10⁻⁷ M – 10⁻³ M) was added 30 min before the addition of CaCl₂. As is shown in Table 1, both drugs inhibited these tonic contractile responses induced by Ca²⁺, but levromakalim was significantly more potent than UR-8225 (P < 0.05).

Table 1 Effects of UR-8225 and levromakalim on the contractions induced by addition of 2 mM CaCl₂ to a Ca²⁺-free (0.03 mM EDTA) medium in the presence of 10⁻⁷ M noradrenaline and 10⁻⁴ M methoxyverapamil expressed as a percentage of the contraction of control rings

<table>
<thead>
<tr>
<th></th>
<th>10⁻¹ M</th>
<th>10⁻⁴ M</th>
<th>10⁻³ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>UR-8225</td>
<td>83.1 ± 11.0</td>
<td>83.0 ± 15.1</td>
<td>61.1 ± 13.3*</td>
</tr>
<tr>
<td>Levromakalim</td>
<td>92.5 ± 22.6</td>
<td>54.0 ± 14.0*</td>
<td>27.1 ± 9.1**</td>
</tr>
</tbody>
</table>

Each value is the mean ± s.e.mean of 7–8 rings.

*P < 0.05, **P < 0.01.

Effects of UR-8225 on membrane potential

The resting membrane potential of aortic smooth muscle cells averaged −50.2 ± 0.7 mV (n = 7). Addition of 10⁻⁵ M UR-8225 hyperpolarized the cells by almost 16 mV (−66.0 ± 2.0 mV, n = 7). Upon washing, the cells slowly repolarized to their normal resting potential.

Effects of UR-8225 on ⁸⁶Rb⁺ efflux

The magnitude of the hyperpolarization produced by UR-8225 strongly suggested that it could be due to an increase in K⁺ conductance. To study this possibility ⁸⁶Rb⁺ was used as a substitute for ⁴⁺K⁺. UR-8225 produced a concentration-dependent increase in the rate constant of ⁸⁶Rb⁺ efflux from the rat aorta (Figure 6). The rate of onset of the effect was also concentration-dependent.

Discussion

In the present study we have compared in isolated vascular smooth muscle of the rat the effects of UR-8225, a novel vasodilator agent, to those of levromakalim, a drug which relaxes vascular smooth muscle by opening K⁺ channels (Weston, 1989; Weston et al., 1990). The results indicated that the vasorelaxant effects of UR-8225 were qualitatively similar, but slightly less potent, than those exerted by levromakalim. Thus, in rat isolated aorta UR-8225: (1) inhibits the contractile responses induced by noradrenaline or low KCl concentrations (≤ 30 mM) more effectively than those induced by high (80 mM) KCl. These vasorelaxant effects do not appear to depend critically on the release of endothelial factors since the same inhibition was observed in the presence and absence of functional endothelium. (2) Decreases the contractile responses induced by Ca²⁺ in aortae incubated in Ca²⁺-free PSS containing methoxyverapamil, a calcium channel blocker, in the presence of noradrenaline; (3) hyperpolarizes the aortic membrane potential; (4) stimulates ⁸⁶Rb⁺ efflux. Furthermore, UR-8225 suppresses the spontaneous activity as well as the contractile response induced by noradrenaline in portal veins. In addition, the vasorelaxant effect of UR-8225 and levromakalim can be competitively antagonized by glibenclamide, an ATP-sensitive K⁺ channel blocker (Ashcroft, 1988). All of these results indicated that, as previously suggested with levromakalim (Weston, 1989; Weston et al., 1990) the vasorelaxant effects of UR-8225 could be related to its ability to activate ATP-sensitive K⁺ channels in vascular smooth muscle cells.

Potassium channel openers increase the permeability of the vascular smooth muscle cell to K⁺, resulting in membrane hyperpolarization (Cook, 1988; Weston, 1989). In rat aortic smooth muscle cells, UR-8225 induced a hyperpolarization of up to 16 mV shifting the membrane potential towards the predicted K⁺ equilibrium potential for the rat aorta (Hirst & Edwards, 1989) but far from the potential at which depolarization (voltage)-dependent L-type Ca²⁺ channels are activated (~45 mV). Thus, the hyperpolarization induced by UR-8225 may reduce the intracellular concentration of free Ca²⁺ and cause vasorelaxation by preventing the opening of voltage-activated calcium channels by excitatory agonists (Chiu et al., 1988; Nelson et al., 1988). To confirm whether the hyperpolarization produced by UR-8225 was due to an increase in membrane permeability to K⁺ the effects of the drug were studied on ⁸⁶Rb⁺ efflux. UR-8225 produced a concentration-dependent increase in the rate of ⁸⁶Rb⁺ efflux from rat aorta, which confirmed that UR-8225 hyperpolarizes the membrane potential and causes vascular relaxation in aortic smooth muscle through an increase in outward K⁺ conductance.

In addition, the sulphonylurea glibenclamide, a potent and selective blocker of ATP-sensitive K⁺ channels in vascular
smooth muscle (Ashcroft, 1988; Standen et al., 1989), competitively antagonized UR-8225- and levcromakalim-induced vasorelaxation. In fact, the pA2 values for glibenclamide to inhibit the relaxations induced by UR-8225 and levcromakalim were very similar, indicating that both drugs probably act at the same site. These results further support the contention that the population of potassium channels involved in the vasodilatation induced by UR-8225 could be the ATP-sensitive K+ channels (Standen et al., 1989; Quast & Cook, 1990).

If the hypothesis that the vasorelaxant effect of UR-8225 is due to the opening of K+ channels leading to hyperpolarization and alteration in the magnitude of agonist-induced depolarization is correct, it should be markedly reduced under circumstances where the membrane potential is maintained constant. In fact, a major characteristic of K+ channel openers is that they inhibit the contractions induced by 10–30 mM KCl, whereas they are almost ineffective against the contractions induced by 80 mM KCI or noradrenaline plus high KCl (Lawson & Cavero, 1989; Weston et al., 1990). At low KCl concentrations UR-8225, like other K+ channel openers, hyperpolarized the membrane potential decreasing the open state probability of L-type Ca2+ channels. In fact, cromakalim inhibited the increase in intracellular Ca2+ concentration induced by low concentrations (<30 mM) of KCl in coronary arterial smooth muscle due to the closure of voltage-activated Ca2+ channels (Yanagisawa et al., 1990). At high extracellular KCl concentrations the cell membrane is depolarized to a level far from the K+ equilibrium potential (approximately −20 mV in the presence of 80 mM KCl, Hamilton & Weston, 1989). Under these conditions K+ channel openers do not hyperpolarize the smooth muscle cells and therefore, their vasorelaxant effect is negligible (Hamilton et al., 1986; Bray et al., 1987). In addition, the finding that UR-8225 has no effect on high KCl-induced contractions suggests that it does not act as a conventional Ca2+ channel blocker and excludes that its vasorelaxant effect can be related to a direct effect on contractile proteins.

The rat portal vein exhibits spontaneous myogenic activity which is due to depolarization induced by the influx of Na+ and Ca2+ and is insensitive to tetrodotoxin (Johansson & Somlyo, 1980). Both levcromakalim and UR-8225 inhibited the frequency and the amplitude of spontaneous contractions. The ionic event terminating electrical excitation is a K+ outward current through voltage and/or Ca2+-dependent K+ channels (Johansson & Somlyo, 1980). Therefore, the opening of potassium channels and the subsequent hyperpolarization can be responsible for the inhibitory effect of UR-8225 and levcromakalim on myogenic activity of rat portal veins (Hamilton et al., 1986).

In rat aorta, the tonic component of noradrenaline-induced contractions is due to the activation of Ca2+ entry from the extracellular space via dihydropyridine-sensitive and insensitive pathways (Cauvin & Malik, 1984). UR-8225 and levcromakalim inhibited the tonic contraction induced by adding Ca2+ to a Ca2+-free medium containing EDTA and methoxyverapamil in the presence of noradrenaline, which suggests that both drugs inhibit the agonist-induced Ca2+ entry (Cook, 1988; Bray et al., 1991). Since this tonic contraction was generated in the presence of methoxyverapamil, it can be concluded that the inhibitory effect of UR-8225 and levcromakalim on K+ conductance does not require the entry of Ca2+ through dihydropyridine-sensitive channels (Kreye & Weston, 1986). The inhibitory effect on noradrenaline-induced tonic contractions can be explained because noradrenaline increases open state-probability of single voltage-activated Ca2+ channels (Nelson et al., 1988; Pacaud et al., 1991), whereas K+ channel openers oppose this action by hyperpolarizing the membrane potential (Hamilton et al., 1986; Bray et al., 1991). Other possible explanations are that the hyperpolarization induced by UR-8225 and levcromakalim may inhibit the ability of depleted intracellular Ca2+ stores to refill after Ca2+ release has occurred and/or the synthesis of inositol 1,4,5-trisphosphate (IP3) by noradrenaline. The former possibility has been previously reported with cromakalim in rabbit aorta (Chiu et al., 1988; Bray et al., 1991) and the latter with levcromakalim in rabbit mesenteric arteries (Ito et al., 1991). In fact, in skinned skeletal muscles IP3 induced Ca2+ release from the sarcoplasmic reticulum is voltage-dependent (Donaldson et al., 1988).

In conclusion, the present results demonstrate that in rat vascular smooth muscle UR-8225 produced vasorelaxant responses qualitatively similar to those of levcromakalim. This vasorelaxant action seems to be mediated via hyperpolarization of the membrane by activation of ATP-activated K+ channels.

We thank SmithKline Beecham Pharmaceuticals for the gift of levcromakalim. Financial support was provided by Cicyt Grant (SAF-92-0157) and by laboratorios Uriach S.A.

References


(Received February 26, 1993 Revised June 9, 1993 Accepted July 8, 1993)