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

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

2 UR-8225 and levcromakalim, 10^{-9} M–10^{-5} M, relaxed the contractile responses induced by noradrenaline ($IC_{50} = 2.7 \pm 0.4 \times 10^{-6}$ M and $6.6 \pm 1.3 \times 10^{-7}$ M, respectively) or 30 mM KCl ($IC_{50} = 1.4 \pm 0.2 \times 10^{-7}$ M and $9.4 \pm 1.3 \times 10^{-8}$ 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 levcromakalim can be competitively antagonized by glibenclamide, an ATP-sensitive K^+ channel blocker. There were no differences in the calculated pA_2 values for 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).

4 UR-8225 (10^{-5} M) hyperpolarized the resting aortic membrane potential from -50.7 ± 0.7 mV to -66.0 ± 2.0 mV and stimulated $^{86}Rb^+$ efflux.

5 UR-8225 and levcromakalim inhibited the contractions induced by Ca^{2+} in aortae incubated in Ca^{2+}-free PSS containing methoxyverapamil in the presence of noradrenaline.

6 Both drugs inhibited the amplitude of spontaneous activity in portal veins ($IC_{50} = 5.1 \pm 1.4 \times 10^{-8}$ M and $1.5 \pm 0.7 \times 10^{-8}$ 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 levcromakalim, which suggests that they can be related to its ability to activate ATP-sensitive K^+ channels in vascular smooth muscle cells.

Keywords: UR-8225; levcromakalim; 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 Ca^{2+} 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; Weston, 1989; Escande & Cavero, 1992; Sanguinetti, 1992).

UR-8225 is a new compound [(1,2-dihydro-4-(1,2-dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-1-oxonaphthalen-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 naphthalenone ring replacing the conventional benzopyrane nucleus present in levcromakalim (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 (García-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 levcromakalim. 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, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 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-Vizcaíno *et al.*, 1991; 1993). The tissue bath was maintained at 37°C and bubbled with 95% O_2:5% CO_2 gas mixture. For the experiments in which Ca^{2+}-free medium was used, Ca^{2+} 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

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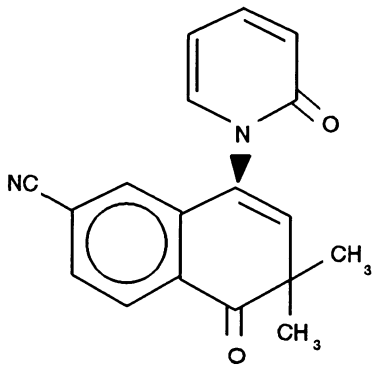
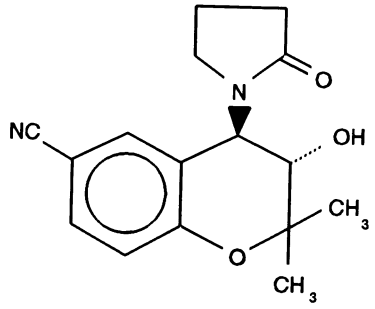


UR-8225



Levcromakalim

Figure 1 Chemical structure of UR-8225 and levcromakalim.

preparation precontracted with 10^{-5} M noradrenaline to relax in response to 10^{-6} M acetylcholine. Each preparation was allowed to equilibrate for at least 90 min, prior to initiation of experimental procedures, and during this period the incubation media were changed every 30 min before addition of drugs.

After equilibration the following experiments were performed: (a) in some experiments, aortic rings were exposed to single submaximal concentrations of KCl (20 mM). Control contractile responses were obtained at the beginning of the experiment every 30 min until two successive responses were almost identical in height. This was followed by exposure to UR-8225 or levcromakalim for 30 min before the addition of KCl. The results of these experiments are expressed as a percentage of the maximal control agonist-induced contractile responses. (b) Aortic rings were contracted by addition of noradrenaline (10^{-5} M) or KCl (30 or 80 mM). When the contractile tonic response to either agonist was stable, cumulative inhibitory concentration-response curves were obtained for UR-8225 or levcromakalim. The relaxant effect of each concentration was allowed to reach a stable level before the next addition was made. The ability of glibenclamide, a blocker of ATP-sensitive K^+ channels (Ashcroft, 1988), to antagonize the relaxant responses of UR-8225 was tested on the contractions induced by 30 mM KCl. Glibenclamide was added to the bath after the high KCl contraction had developed and 20 min before the addition of UR-8225 or levcromakalim. In another group of experiments, muscles were exposed to 80 mM KCl and when the contractile response reached the steady-state, 10^{-5} M noradrenaline was added to the bathing media. These results were expressed as a percentage of the maximal control agonist-induced responses. (c) In additional experiments, after equilibration, cumulative concentration-response curves were obtained for KCl (15–85 mM). Once the contractile response curve for a given agonist became stable, preparations were exposed to different concentrations of UR-8225 or levcromakalim for 30 min and a new concentration-response curve was obtained. (d) The effects of UR-8225 and levcromakalim on noradrenaline-stimulated Ca^{2+} entry were studied according to the following experimental protocol. Aortic rings were 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 levcromakalim were added 30 min before the addition of Ca^{2+}. Results are expressed as a percentage of the initial noradrenaline-induced contraction. (e) To study the effects of UR-8225 and levcromakalim 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 (Deplón *et al.*, 1992). The microelectrode was connected via Ag–AgCl wire to high-input impedance 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, $CaCl_2$ 1.5, $MgSO_4$ 1.0, glucose 11 and HEPES 10 at pH 7.4 bubbled with 100% O_2 at 37°C. Then rings were loaded for 3 h in PSS containing $^{86}Rb^+$ (5 μCi ml^{-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 H_2O_2 (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: $k = \ln(A1/A2)/(t2-t1)$, 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), levcromakalim (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^{-3} 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 levcromakalim 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 levcromakalim (10^{-9} M–10^{-5} M) inhibited the amplitude of these contractions in a concentration-dependent manner and at 2×10^{-7} M and 10^{-7} M, respectively, they suppressed the spontaneous activity. In 5 muscles, the IC_{50} values for UR-8225 and levcromakalim to inhibit the myogenic activity were $5.1 \pm 1.4 \times 10^{-8}$ M ($n = 5$) and $1.5 \pm 0.7 \times 10^{-8}$ M ($n = 5$), respectively. The ability of glibenclamide to reverse the inhibitory effects of UR-8225 and levcromakalim on the amplitude of spontaneous contractions was studied in 6 portal veins. In the presence of glibenclamide (3×10^{-7} M, 10^{-6} M and 3×10^{-6} M) there was a rightward shift of the curve for UR-8225 and levcromakalim (Figure 2). Thus, in the presence of 3×10^{-6} M glibenclamide the IC_{50} values for UR-8225 and levcromakalim were $1.5 \pm 0.5 \times 10^{-6}$ M and $3.2 \pm 0.6 \times 10^{-7}$ M, respectively. There were no differences in the calculated pA_2 values for glibenclamide to inhibit UR-8225- and levcromakalim-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^{-5} 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 $6.9 \pm 3.3 \times 10^{-6}$ M.

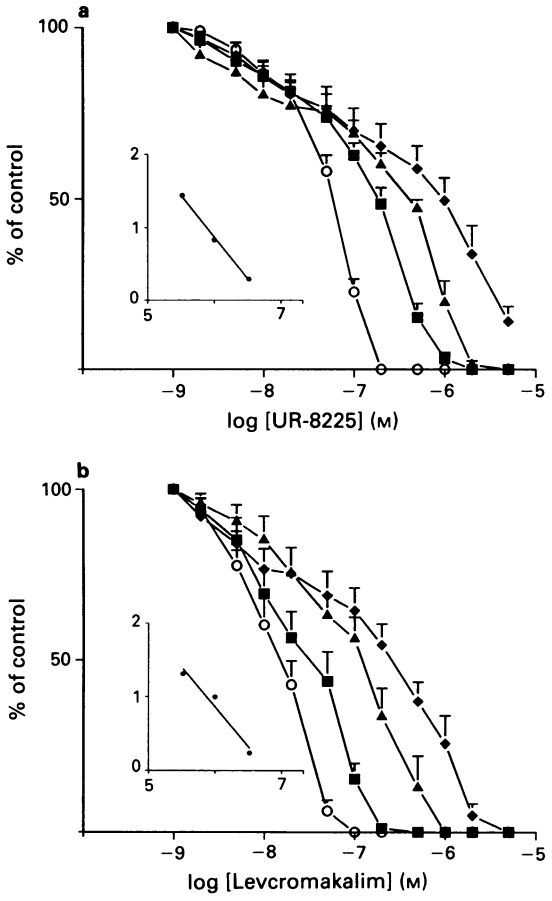


Figure 2 Effects of UR-8225 (a) and levcromakalim (b) added in a cumulative fashion on the amplitude of spontaneous contractions in rat portal vein segments. Results were obtained in the absence (○) and in the presence of glibenclamide 3×10^{-7} M (■), 10^{-6} M (▲) and 3×10^{-6} M (◆). Ordinate scale: percentage of control values. Abscissa scale: log UR-8225 or levcromakalim 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).

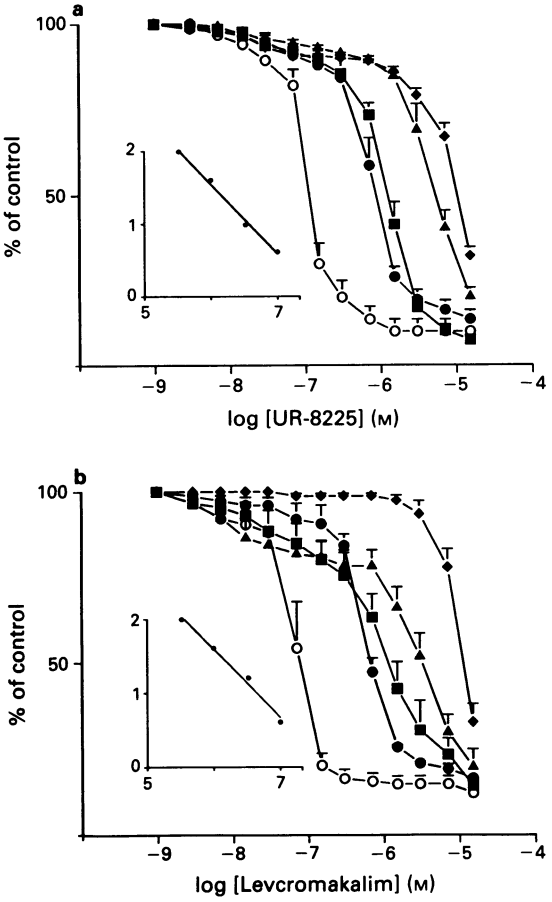


Figure 3 Effects of UR-8225 (a) and levcromakalim (b) added in a cumulative fashion on the 30 mM KCl-induced contractions in rat aortic rings. Results were obtained in the absence (○) and in the presence of glibenclamide 10^{-7} M (●), 3×10^{-7} M (■), 10^{-6} M (▲) and 3×10^{-6} M (◆). Ordinate scale: percentage of control values. Abscissa scale: log UR-8225 or levcromakalim 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.

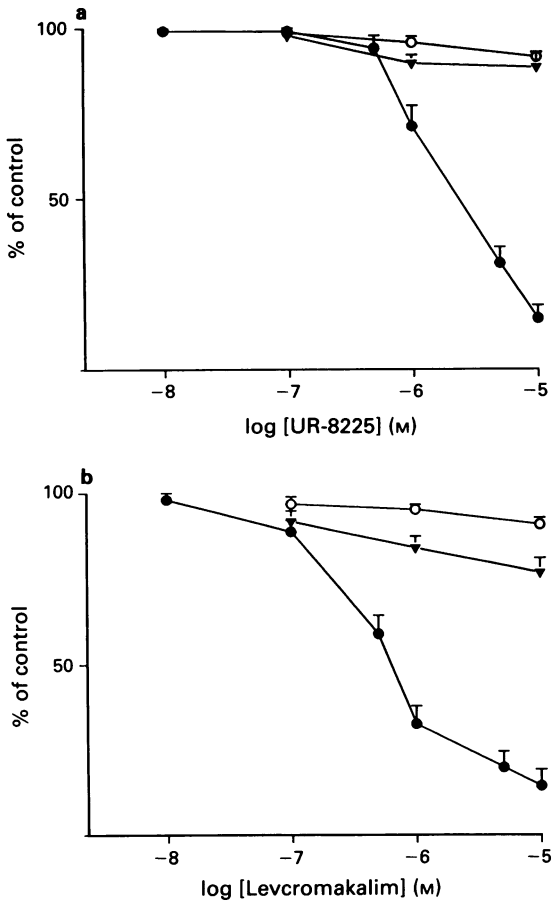


Figure 4 Effects of UR-82225 (a) and levcromakalim (b) added in a cumulative fashion on the contractions induced by 80 mM KCl (▼), 10^{-5} M noradrenaline (●) or 80 mM KCl plus 10^{-5} M noradrenaline (○) in rat aortic rings. Ordinate scale: percentage of control values. Abscissa scale: log UR-8225 or levcromakalim concentration (M). Each point represents the mean ± s.e.mean of 6–8 experiments.

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^{-9} M–10^{-5} M, produced a concentration-dependent inhibition of this contractile response, the IC_{50} values being $9.2 \pm 6.1 \times 10^{-8}$ M ($n = 8$) and $3.2 \pm 0.3 \times 10^{-8}$ 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 $1.4 \pm 0.2 \times 10^{-7}$ M ($n = 6$) and $9.4 \pm 1.3 \times 10^{-8}$ M ($n = 6$). The figure also shows that glibenclamide (10^{-7} M–3×10^{-6} M) shifted to the right these concentration-relaxation curves for UR-8225 and levcromakalim against these contractile responses. Thus, in the presence of 3×10^{-6} M glibenclamide, the IC_{50} values for UR-8225 and levcromakalim were $1.4 \pm 0.4 \times 10^{-5}$ M and $1.1 \pm 0.1 \times 10^{-5}$ 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 $2.7 \pm 0.4 \times 10^{-6}$ M ($n = 7$) and $6.6 \pm 1.3 \times 10^{-7}$ M ($n = 8$), respectively. In endothelium-denuded rings, UR-8225 also relaxed noradrenaline-induced contraction, the IC_{50} being $1.9 \pm 1.1 \times 10^{-6}$ M ($n = 4$, not significantly different compared to endothelium-intact rings). Pretreatment with glibenclamide (10^{-7} M–3×10^{-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^{-7} M–10^{-5} 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

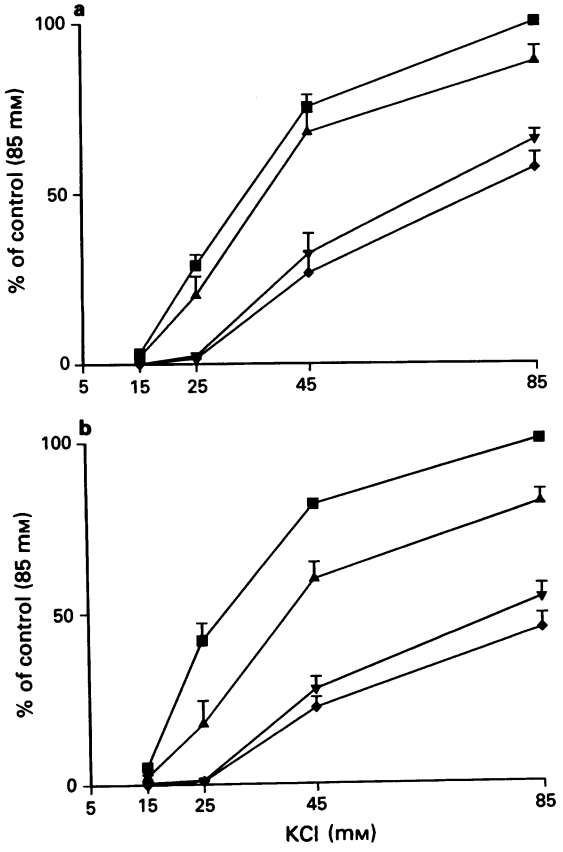


Figure 5 Effects of UR-8225 (a) and levcromakalim (b) on the contractions of aortic rings by addition of KCl (15–85 mM). Ordinate scale: percentage of the maximum control contraction obtained with 85 mM KCl in each experiment. Abscissa scale: KCl concentration (mM). Each point represents the mean ± s.e.mean of 6 experiments. (■) Controls; after UR-8225 or levcromakalim, 10^{-7} M, (▲), 10^{-6} M (▼) and 10^{-5} M (◆).

80 mM KCl (Hamilton & Weston, 1989). Cumulative increases in KCl concentration (15–85 mM) to aortic rings in a Ca^{2+}-containing PSS induced a concentration-dependent increase in developed tension. Figure 5 shows that both UR-8225 and levcromakalim (10^{-7} M–10^{-5} 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 ($\leq$ 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 levcromakalim.

Effects on noradrenaline-induced contractions in Ca^{2+}-free solution

In another group of experiments, the effects of UR-8225 or levcromakalim were studied on the contractile responses induced by $CaCl_2$ (2 mM) in aortic rings incubated in Ca^{2+}-free PSS containing 0.03 mM EDTA and 10^{-5} M methoxyverapamil. Under these conditions addition of 10^{-5} M noradrenaline induced a phasic contraction resulting from the release of intracellular Ca^{2+}. After 30 min, 2 mM $CaCl_2$ 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 levcromakalim (10^{-7} M–10^{-5} M) was added 30 min before the addition of $CaCl_2$. As is shown in Table 1, both drugs inhibited these tonic contractile responses induced by Ca^{2+}, but levcromakalim was significantly more potent than UR-8225 ($P<0.05$).

Table 1 Effects of UR-8225 and levcromakalim on the contractions induced by addition of 2 mM $CaCl_2$ to a Ca^{2+}-free (0.03 mM EDTA) medium in the presence of 10^{-5} M noradrenaline and 10^{-5} M methoxyverapamil expressed as a percentage of the contraction of control rings

	10^{-7} M	10^{-6} M	10^{-5} M
UR-8225	83.1 ± 11.0	83.0 ± 15.1	61.1 ± 13.3*
Levcromakalim	92.5 ± 22.6	54.0 ± 14.0*	27.1 ± 9.1**

Each value is the mean ± s.e.mean of 7–8 rings.
*$P<0.05$, **$P<0.01$.

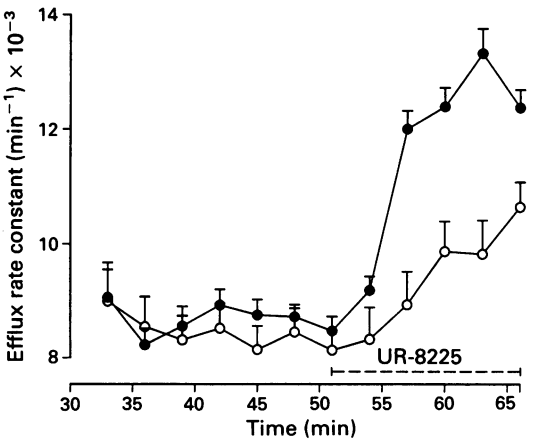


Figure 6 Effects of UR-8225 on the $^{86}Rb^+$ efflux in rat non-stimulated aortic rings. Ordinate scale: efflux rate constant ($min^{-1} \times 10^{-3}$ M). $^{86}Rb^+$ loaded rings were placed in PSS for the first 51 min and thereafter in PSS containing UR-8225, 10^{-6} M (○) or 10^{-5} M (●). Abscissa scale: time (min). Each point represents the mean ± s.e.mean of 6 experiments.

Effects of UR-8225 on membrane potential

The resting membrane potential of aortic smooth muscle cells averaged − 50.7 ± 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 $^{86}Rb^+$ 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 $^{86}Rb^+$ was used as a substitute for $^{42}K^+$. UR-8225 produced a concentration-dependent increase in the rate constant of $^{86}Rb^+$ 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 levcromakalim, 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 levcromakalim. Thus, in rat isolated aortae UR-8225: (1) inhibits the contractile responses induced by noradrenaline or low KCl concentrations ($\leq$ 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 Ca^{2+} in aortae incubated in Ca^{2+}-free PSS containing methoxyverapamil, a calcium channel blocker, in the presence of noradrenaline; (3) hyperpolarizes the aortic membrane potential; (4) stimulates $^{86}Rb^+$ 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 levcromakalim can be competitively antagonized by glibenclamide, an ATP-sensitive K^+ channel blocker (Ashcroft, 1988). All of these results indicated that, as previously suggested with levcromakalim (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 Ca^{2+} channels are activated (− 45 mV). Thus, the hyperpolarization induced by UR-8225 may reduce the intracellular concentration of free Ca^{2+} 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 $^{86}Rb^+$ efflux. UR-8225 produced a concentration-dependent increase in the rate of $^{86}Rb^+$ 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 pA_2 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, 1989).

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 KCl 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 Ca^{2+} channels. In fact, cromakalim inhibited the increase in intracellular Ca^{2+} concentration induced by low concentrations (< 30 mM) of KCl in coronary arterial smooth muscle due to the closure of voltage-activated Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+}-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 Ca^{2+} 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 Ca^{2+} to a Ca^{2+}-free medium containing EDTA and methoxyverapamil in the presence of noradrenaline, which suggests that both drugs inhibit the agonist-induced Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} stores to refill after Ca^{2+} release has occurred and/or the synthesis of inositol 1,4,5-trisphosphate (IP_3)-induced 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 IP_3 induced Ca^{2+} 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.

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