J. Pharm. Pharmacol. 2000, 52: 213–217
Received July 19, 1999
Accepted September 28, 1999

Comparison of the Vasorelaxing Effect of Cromakalim and the New Inodilator, Levosimendan, in Human Isolated Portal Vein

JÁNOS PATARICZA, JÓZSEF HÕHN*, ANDRÁS PETRI*, ÁDÁM BALOGH* AND JULIUS GY. PAPP†

*Department of Pharmacology and Pharmacotherapy, *Department of Surgery, Albert Szent-Györgyi Medical University and †Research Unit for Cardiovascular Pharmacology, Hungarian Academy of Sciences, H-6701, Szeged, POB 115, Hungary*

Abstract

In the present study the vasorelaxing capacity of cromakalim, an ATP-sensitive potassium-channel (K_{ATP} channel) activator, and that of levosimendan, a new positive inotropic and vasodilating drug with calcium sensitizing and potassium-channel-activating properties, were compared in human isolated portal vein.

Based on the 50% effective concentrations (EC50), levosimendan was found to be about 16-fold more potent ($EC50 = 0{\cdot}281 \pm 0{\cdot}03\ \mu M$) as a relaxing agent than cromakalim ($EC50 = 4{\cdot}53 \pm 0{\cdot}12\ \mu M$) in noradrenaline-precontracted portal venous preparations. Glibenclamide, the known inhibitor of K_{ATP} channels, was able to prevent the cromakalim-induced venodilation completely. Glibenclamide ($15\ \mu M$) decreased the quasi-maximal effect of levosimendan (at $1{\cdot}27\ \mu M$ by about 60%) and also the effects of those submicromolar concentrations of the inodilator (at $0{\cdot}1\ \mu M$ by 23%, at $0{\cdot}3\ \mu M$ by 27% and at $0{\cdot}7\ \mu M$ by 19%, on average) which were therapeutically effective in preliminary human studies.

These findings indicate that, in the human portal vein, both cromakalim and levosimendan are powerful vasorelaxants and that a considerable part of the relaxing effect induced by levosimendan is of cromakalim type.

Drugs inducing vasodilation in the portal circulation of the liver would be important adjuvant tools for the management of portal hypertension. A recent study has provided evidence for the involvement of hyperpolarizing potassium channels in decreasing portal venous tone under physiological and pathological conditions (Mathie et al 1996). Potassium channels comprise a large group of ionic channels among which the ATP-sensitive type (K_{ATP} channels) appears to have functional importance in the regulation of portal venous tone (Kau et al 1994; Perez-Guerrero et al 1997). Hyperpolarization of the cell membrane by activation of these K_{ATP} channels leads to the closure of voltage-dependent calcium channels and to a consequent decrease of intracellular calcium concentration and smooth muscle tone (Nelson & Quayle 1995). At present, no information is available about the effect of potassium-channel activators in human portal vein. In the current experiments, the venodilating effect of cromakalim, an activator of K_{ATP} channels, and that of levosimendan, an inodilator drug of novel type (Haikala et al 1997) with potassium-channel activating property (Yokoshiki et al 1996), were evaluated on the tone of human isolated portal veins. The possible inhibitory effect of glibenclamide, a known inhibitor of K_{ATP} channels, on the relaxation induced by the two drugs was also investigated.

Correspondence: J. Pataricza, Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical University, H-6701, Szeged, POB 115, Hungary.

Materials and Methods

Drugs

Levosimendan was obtained from Orion-Pharma, Espoo, Finland. Noradrenaline bitartrate, cromakalim and glibenclamide were purchased from Sigma (St Louis, MO). The components of the Krebs–Henseleit solution were obtained from Reanal (Budapest, Hungary). Noradrenaline was dissolved in 0·9% NaCl with 10 mM ascorbic acid, resulting in 1 mM noradrenaline in the stock solu-

tion (Reanal, Budapest, Hungary). Cromakalim and glibenclamide were dissolved in distilled water containing 20% dimethylsulphoxide and 20% ethanol. The concentrations of both cromakalim and glibenclamide were 0·45 mM and 1 mM in the stock solutions, respectively. Levosimendan was dissolved in 70% ethanol, resulting in a solution of 0·21 mM concentration.

Tissue preparation

The protocols used in this study were approved by the Ethical Review Board of the Albert Szent-Györgyi Medical University (No. 51-119/1998 OEsz). The 21 portal veins used for the study were prepared from liver transplants of patients, aged 17 to 62 years (average age: 44 ± 14 years) who had died accidentally. Segments (2·2 cm long) of portal vein were removed from those livers deemed unsuitable for transplantation and were stored for up to 4 h in Bretschneider solution (NaCl (15 mmol L^{-1}), KCl (9 mmol L^{-1}), potassium hydrogen-2-oxoglutarate (1 mmol L^{-1}), $MgCl_2$ $6H_2O$ (4 mmol L^{-1}), histidine HClx H_2O (18 mmol L^{-1}), histidine (180 mmol L^{-1}), tryptophan (2 mmol L^{-1}) and mannite (30 mmol L^{-1})). Only macroscopically healthy veins were used. Veins were placed into an ice-cold Krebs–Henseleit solution of the following composition (in mM): NaCl 120, KCl 4·2, $CaCl_2$ 1·5, $NaHCO_3$ 20, $MgCl_2$ 1·2, KH_2PO_4 1·2 and glucose 11. The venous segments were prepared in the clinical surgery and transported to the experimental laboratory within 15 min. The vessels were then dissected free of perivascular tissue and cut into rings (5 mm long).

Experimental protocol

Two rings of each portal vein were mounted separately in water-jacketed baths containing 2 mL of Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2 at 37°C. These rings were suspended between a force-displacement transducer (Hugo-Sachs Elektronic, Type F30, Germany) and an anchor, and recorded on a polygraph (KUTESZ, Hungary). The preparations were stretched until near-maximum contractile responsiveness to noradrenaline was reached, at 40 mN basal tension. Each ring was allowed to equilibrate for 45 min before initiation of experimental procedures and during this period the incubation medium was changed every 15 min and the resting tension was readjusted.

In experiments designed to determine if cromakalim and levosimendan caused the human isolated portal vein to relax, after equilibration of the pairs of ring segments, contractions to 10 μM noradrenaline were induced. When the steady-state contraction amplitude produced by noradrenaline had developed, one of the rings was cumulatively exposed to levosimendan (0·01–1·27 μM) and the other ring was treated with the corresponding volumes of the solvent (1–64 μL). The same procedure was used with another pair of venous rings obtained from the same liver, except that, instead of levosimendan, cromakalim (0·2–47 μM) and its solvent (0·5–125 μL) were administered cumulatively at the steady state of noradrenaline-induced contraction. In the latter case, the largest volume of solvent produced relaxations in some cases an effect which was deduced from comparisons of the magnitude of relaxation induced by cromakalim.

In another series of experiments the capacity of glibenclamide (1·5 and 15 μM) to decrease relaxations caused by cromakalim and levosimendan was assessed. The protocol was similar to that described above, except that concentration–response curves for either cromakalim or levosimendan were determined in the presence and absence of 1·5 μM glibenclamide. One of the portal rings was incubated with the low concentration of glibenclamide, the other ring with the solvent of the blocker 30 min before the addition of noradrenaline. After completing the agonist–response curves for cromakalim and levosimendan, the ring not treated with glibenclamide was washed three times and incubated with 15 μM glibenclamide for 30 min. Then, steady-state contraction was induced by noradrenaline and another agonist–response curve was obtained.

Statistical analysis

The decrease of the venous tone caused by cromakalim or levosimendan was expressed as the percent of the noradrenaline-induced steady-state contraction amplitude. Results are expressed as mean ± s.e.m. and n refers to the number of venous rings obtained from different liver transplants. One-way analysis of variance with repeated measures was used to determine if significant differences existed between groups. When analysis of variance showed significant differences, the Newman–Keuls test was performed to determine differences between individual mean values. Values for 50% effective concentration (EC50) were obtained by fitting the exponential equation of $100/\{1 + \exp[b*(x-c)]\}$ to mean values.

Results

Effect of cromakalim and levosimendan on the tone of human isolated portal vein

Both cromakalim and levosimendan relaxed the noradrenaline-precontracted portal vein prepara-

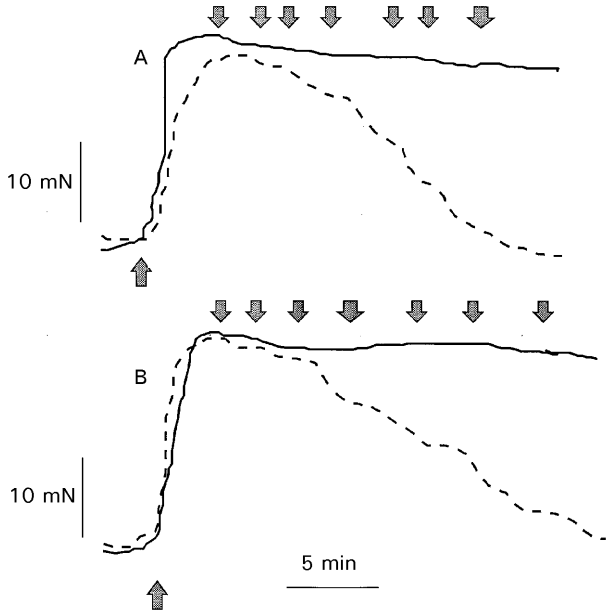

Figure 1. Typical concentration–response tracings for the relaxations induced by cromakalim (A) and levosimendan (B) in human isolated portal vein. Contractions of venous rings were induced by 10 μM noradrenaline (upward arrows). Downward arrows represent the addition of cumulative concentrations of cromakalim (dotted line A: 0·2, 0·9, 2·5, 5·7, 12·0, 24·2 and 47·2 μM, respectively) and levosimendan (dotted line B: 0·01, 0·03, 0·07, 0·15, 0·31, 0·63 and 1·3 μM, respectively) or the corresponding volumes of solvent (solid lines A and B). The original registrations are representatives of 7 independent experiments for each curve.

tions almost completely (Figure 1). Typical concentration–response registrations were derived from one out of seven independent experiments in the case of both cromakalim and levosimendan (Figure 1A and 1B, respectively). On the basis of the EC50 values, levosimendan was 16·1-fold more potent (EC50 = 0·281 ± 0·03 μM, n = 7) than cromakalim (EC50 = 4·53 ± 0·12 μM, n = 7) under identical experimental conditions.

Effect of glibenclamide on the venodilating action of cromakalim and levosimendan

Another series of experiments was aimed at determining the capacity of glibenclamide to modify the relaxations induced by cromakalim and levosimendan (Figure 2). Pretreatment of the portal preparations with 1·5 μM glibenclamide for 30 min caused a significant leftward shift in the cromakalim concentration–response curve, but had no effect on the maximum relaxation response (Figure 2A). However, a ten-times-higher glibenclamide concentration (15 μM) inhibited the relaxation by cromakalim (up to 48 μM) almost completely (Figure 2A).

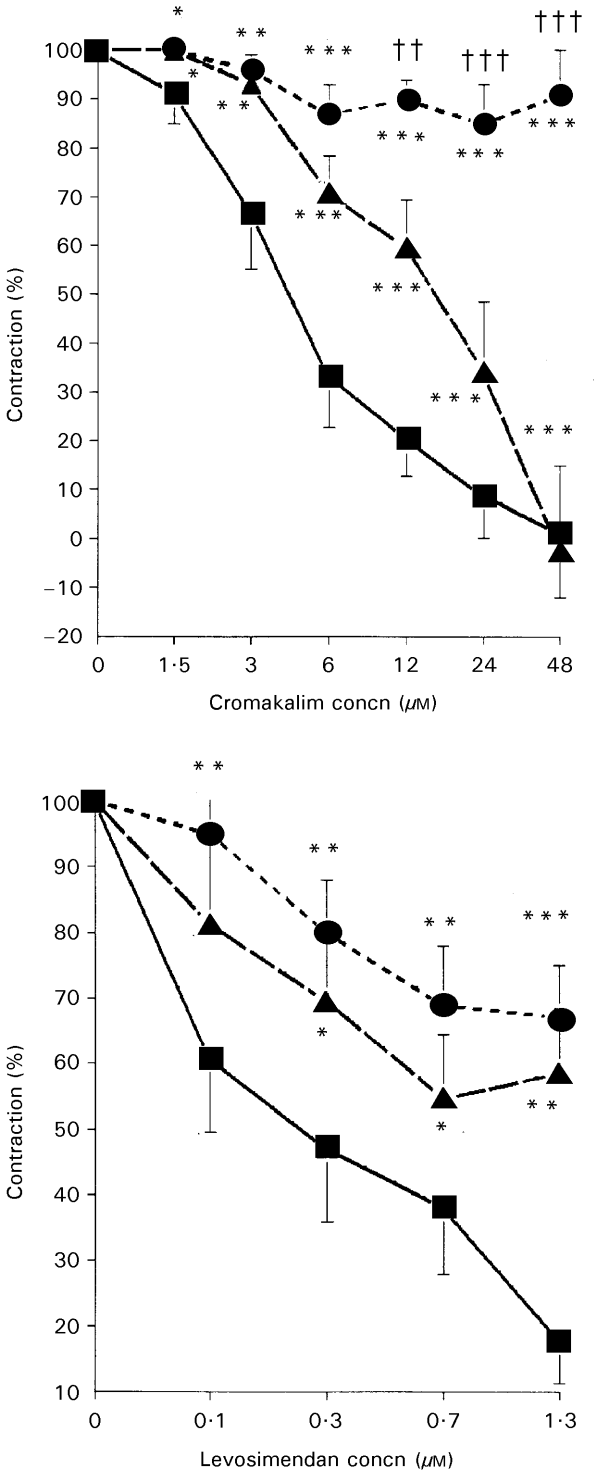

Figure 2. Effect of 1·5 μM ▲ and 15 μM ● glibenclamide on the relaxations induced by cromakalim (A, ■) and levosimendan (B, ■) in noradrenaline-precontracted human portal vein. Magnitude of contractions was expressed as percent of noradrenaline-induced tone. Data represent mean values ± s.e.m of 7 independent experiments in the case of cromakalim and 6 in the case of levosimendan. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with cromakalim or levosimendan alone; †† $P < 0.01$, ††† $P < 0.001$ compared with 1·5 μM glibenclamide treatment.

A low concentration of glibenclamide (1·5 μM) also decreased levosimendan-induced relaxation and, unlike cromakalim-induced relaxation, the maximum amplitude of the relaxation was also depressed (Figure 2B). A high concentration of glibenclamide (15 μM) further decreased the effect of levosimendan, but this effect did not differ significantly from that produced by the low glibenclamide concentration (Figure 2B).

Effect of glibenclamide on the basal tone and on the noradrenaline-induced contraction

The basal tone, which was set up to 40 mN (about 4 g), remained unchanged during the entire equilibration period (45 min). Even the large concentration of glibenclamide (15 μM) applied did not influence this tone until the end of the 30 min incubation period (change in basal tone: $0{\cdot}13 \pm 0{\cdot}01$ mN, $n = 7$). Similarly, noradrenaline-induced contractions were not significantly affected by 15 μM glibenclamide ($17{\cdot}3 \pm 1{\cdot}6$ mN vs $17{\cdot}4 \pm 2{\cdot}6$ mN in the presence and absence of glibenclamide, respectively, $n = 7$).

Discussion

In the present study we have demonstrated for the first time that the K_{ATP}-channel activator, cromakalim, was able to relax noradrenaline-contracted human isolated portal vein. Levosimendan, which was found to increase the open probability of K_{ATP} channels in isolated arterial myocytes (Yokoshiki et al 1996), was also found to be effective and to be a more potent relaxant than cromakalim in human portal venous preparations. Glibenclamide, a K_{ATP}-channel blocker, inhibited the cromakalim-induced relaxation of the portal vein, while it partially decreased the effect of levosimendan. Glibenclamide did not change the basal tone of portal veins and also did not significantly affect the contractions induced by noradrenaline. This finding supports the specificity of interactions between glibenclamide and cromakalim or levosimendan. It is worth mentioning, however, that the glibenclamide-sensitive component of the levosimendan action might also be related to mechanisms other than opening K_{ATP} ion channels (Tominaga et al 1995).

An increase in sympathetic nervous activity has been recognized in cirrhosis and in heart failure associated with portal hypertension or congestion. Furthermore, portal vasoconstriction to noradrenaline was shown to be enhanced in experimental cirrhosis (Mathie et al 1996). In the present study, both cromakalim and levosimendan antagonized the contractile effect of noradrenaline in the portal vein, although neither of the two drugs have adrenergic-receptor-blocking activities (Harkin et al 1995). The possible significance of administering cromakalim and levosimendan in portal hypertension would be production of a selective effect on the portal circulation as compared with the effect of these drugs on the splanchnic arterial bed. We have no functional evidence for this assumption, but the low potency of levosimendan for opening K_{ATP} channels in mesenteric arterial myocytes ($EC50 = 2{\cdot}9$ μM; Yokoshiki et al 1996) strongly suggests this possibility. Furthermore, it has been demonstrated that the tone of noradrenaline-contracted portal vasculature was sensitive to glibenclamide (Mathie et al 1996) while the splanchnic circulation was not affected (Ralevic et al 1996). Our present experimental observations points to the role of a cromakalim-type vasodilator mechanism in the human portal vein which could be activated by drugs involving glibenclamide-sensitive potassium channels.

The inodilator drug, levosimendan, has been shown previously to decrease the preload of the heart in intact conscious dogs (Harkin et al 1995) and in dogs with failing heart (Udvary et al 1995). The maximum inotropic effect of levosimendan was attained at 0·3 μM (Haikala et al 1997), corresponding to the effective concentrations of the drug in human portal vein. This supports the contribution of a direct venodilating effect of levosimendan to the diminished preload and to the consequent beneficial haemodynamic effect of the drug in congestive heart failure.

Acknowledgements

This work was supported by the Hungarian National Research Foundation (OTKA T030301) and by the ministry of Public Welfare (ETT T03043/99). We thank Mrs Authné Mária Fehér and Mrs Süliné, Zsuzsanna Tóth for technical assistance and (Orion-Farmos Orion-Pharma, Espoo, Finland) for supplying levosimendan.

References

Haikala, H., Kaheinen, P., Levijoki, J., Lindén, I.-B. (1997) The role of cAMP and cGMP-dependent protein kinases in the cardiac actions of the new calcium sensitizer, levosimendan. Cardiovasc. Res. 34: 536–546

Harkin, C. P., Pagel, P. S., Tessmer, J. P., Warltier, D. C. (1995) Systemic and coronary hemodynamic actions and left ventricular functional effects of levosimendan in conscious dogs. J. Cardiovasc. Pharmacol. 26: 179–188

Kau, S. T., Zografos, P., Do, M. L., Halterman, T. J., McConville, M. W., Yochim, C. L., Triverdi, S., Howe, B. B., Li, J. H. (1994) Characterization of ATP-sensitive potassium channel-blocking activity of ZENECA ZM181,037, a eukalemic diuretic. Pharmacology 49: 238–248

Mathie, R. T., Ralevic, V., Burnstock, G. (1996) Portal vascular responsiveness to sympathetic stimulation and nitric oxide in cirrhotic rats. J. Hepatol. 25: 90–97

Nelson, M. T., Quayle, J. M. (1995) Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268: C799–C822

Perez-Guerrero, C., Suarez, J., Herrera, M. D., Marhuenda, E. (1997) Vasodilating effect of tetrazepam in isolated vascular smooth muscles: comparison with cromakalim and diltiazem. Pharmacol. Res. 36: 237–242

Ralevic, V., Mathie, R. T., Moore, K. P., Burnstock, G. (1996) Vasoconstrictor responsiveness of the rat mesenteric arterial bed in cirrhosis. Br. J. Pharmacol. 118: 435–441

Tominaga, M., Horie, M., Sasayama, S., Okada, Y. (1995) Glibenclamide, an ATP-sensitive K^+ channel blocker, inhibits cardiac cAMP-activated chloride conductance. Circ. Res. 77: 417–423

Udvary, É., Papp, J.Gy., Végh, Á. (1995) Cardiovascular effects of the calcium sensitizer levosimendan, in heart failure induced by rapid pacing in the presence of aortic constriction. Br. J. Pharmacol. 114: 656–661

Yokoshiki, H., Katsube, Y., Sunagawa, M., Sperelakis, N. (1996) The vasodilator levosimendan hyperpolarizes and activates a glibenclamide-sensitive potassium channel in vascular smooth muscle cells. Eur. J. Pharmacol. 333: 249–259