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The role of potassium channels in the vasodilatory effect of levosimendan in human internal thoracic arteries

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Abstract

Objective: We investigated the role of potassium channels in vasodilatory effect of levosimendan in human internal thoracic arteries. **Methods:** Samples of redundant internal thoracic arteries obtained from patients undergoing a coronary artery bypass graft surgery were cut into 3 mm wide rings and suspended in 20 ml organ baths. Isometric tension was continuously measured with an isometric force transducer connected to a computer-based data acquisition system. **Results:** Levosimendan (10^{-8}–10^{-5} M) or cromakalim (10^{-8}–10^{-5} M) produced concentration-dependent relaxation responses in human internal thoracic arteries precontracted by 10^{-6} M phenylephrine. The relaxant responses to levosimendan did not differ significantly between endothelium-intact and endothelium-denuded preparations. Incubation of human internal thoracic artery rings with adenosine 3′,5′-triphosphate (ATP)-dependent potassium channel blocker glibenclamide (10^{-6} M) for 30 min significantly inhibited the relaxant responses to both levosimendan and cromakalim. The Ca^{2+}-activated potassium channel blocker iberiotoxin (10^{-7} M) also caused a significant but smaller inhibition on relaxant responses to levosimendan. Incubation of the rings with the voltage-dependent potassium channel blocker 4-aminopyridine (5 mM) for 10 min did not cause significant alterations in relaxant responses to levosimendan. **Conclusions:** The findings of this study suggested that levosimendan-induced relaxation responses in human internal thoracic arteries were depended on the activation of ATP-dependent and Ca^{2+}-activated potassium channels.
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Keywords: Levosimendan; Potassium channel; Human internal thoracic artery; Vasodilatation

1. Introduction

Levosimendan is a new positive inotrope that improves the sensitivity of myofilaments to Ca^{2+} (Ca^{2+} sensitizer) by selectively binding to troponin C [1]. It is a cardiovascular drug for the treatment of acute and decompensated heart failure with positive inotropic and anti-stunning effects [2,3].

Besides increasing the strength of cardiac contractions, levosimendan also interacts with potassium channels. In accordance, opening of adenosine 3′,5′-triphosphate (ATP)-dependent K^+ channels (K_{ATP}) by levosimendan has been observed in rat arterial [4] and ventricular myocytes [5]. Levosimendan was also shown to open the mitochondrial K_{ATP} in preparations of rat liver [6] and heart [7].

Stimulation of K_{ATP} in vascular smooth muscle cells probably contributes to the vasodilatory action of levosimendan [8]. Accordingly, it has been shown that levosimendan induces vasodilatation through the opening of K_{ATP} in several vascular tissues including guinea pig coronary vessels [9], human isolated portal [10] and saphenous vein [11], and pulmonary vascular bed of the cat [12].

Mechanisms other than K_{ATP} opening seem to play a role in vasodilatory effect of levosimendan, as well. For instance, activation of voltage-sensitive (K_V) and calcium-activated potassium channels (K_{Ca}) in porcine isolated coronary artery [13], K_{Ca} in human saphenous vein [11], cAMP-dependent and cAMP-independent mechanisms in coronary artery [14], nitric oxide production in pig coronary vessels [15], and calcium desensitization in porcine coronary artery [16] have all been reported to play a role in levosimendan-induced relaxations.

Internal thoracic artery (ITA) is frequently used for coronary artery bypass grafting. It is especially important to know the functional effects of levosimendan in this vessel. However, to our knowledge, the effect of levosimendan in ITA preparations has not been studied yet. Therefore, in this study, we investigated the role of potassium channels in the possible vasodilatory effect of levosimendan in human ITA.

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2. Materials and methods

2.1. Patients

Informed consent was obtained from 24 adult patients (64 ± 2 years) who underwent a coronary artery bypass graft surgery. Exclusion criteria were arrhythmias, congestive heart failure, dilated heart and anti-arrhythmic or oral hypoglycaemic medication. The study was approved by our institutional ethics committee.

2.2. Methods

The samples of the ITA were transported in cold (4 °C) physiological salt solution to the laboratory. They were cleaned of the connective tissue and cut into 3 mm wide rings. The endothelium was mechanically removed by gently rubbing the intimal surface with a stainless steel rod covered with a cotton swab. The rings were carefully suspended by two stainless steel clips passed through the vessel lumen in 20 ml organ baths filled with physiological salt solution (mM: NaCl 118, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.2) maintained at 37 °C gassed with 95% O_2 and 5% CO_2 to obtain a pH of 7.4. The rings were placed at the optimal point of length–tension relation by gradually stretching them until contraction induced by 20 mM of KCl was maximal at each level of distension. Isometric tension was continuously measured with an isometric force transducer (FDT10-A, Commat Ltd.), connected to a computer-based data acquisition system (TDA 97, Commat Ltd.).

Successful removal of the endothelium was confirmed by the inability of Ach to induce relaxation in phenylephrine-precontracted arteries. In preliminary studies we found that removal of endothelium did not significantly alter the relaxation responses to levosimendan (data not shown). Arterial rings contracted with 10^{-6} M phenylephrine were exposed to gradually increasing concentrations of levosimendan (10^{-8}–10^{-5} M) and responses of arterial segment were recorded. In order to evaluate the roles of K_{ATP}, K_V, and K_{Ca}, the effects of pre-incubation with blockers of these channels, namely, glibenclamide (10^{-6} M, 30 min), 4-aminopyridine (5 mM, 10 min), and iberiotoxin (10^{-7} M, 30 min) ($n = 10$ for each set), on responses to levosimendan in phenylephrine-precontracted vessels were investigated. In another set of experiments ($n = 7$), the effect of incubation with glibenclamide on relaxant response to K_{ATP} opener, cromakalim (10^{-7}–10^{-4} M) was studied in phenylephrine-precontracted ITA. Separate experiments were performed at intervals of at least 45 min.

2.3. Materials

Phenylephrine, glibenclamide, cromakalim, iberiotoxin, 4-aminopyridine, and the salts for the physiological salt solution were purchased from Sigma Chemical (St. Louis, MO, USA). Levosimendan was obtained from Abbott (Istanbul). Phenylephrine, 4-aminopyridine, and iberiotoxin were dissolved in distilled water. Glibenclamide and cromakalim were dissolved in distilled water containing 20% ethanol and 20% dimethylsulphoxide.

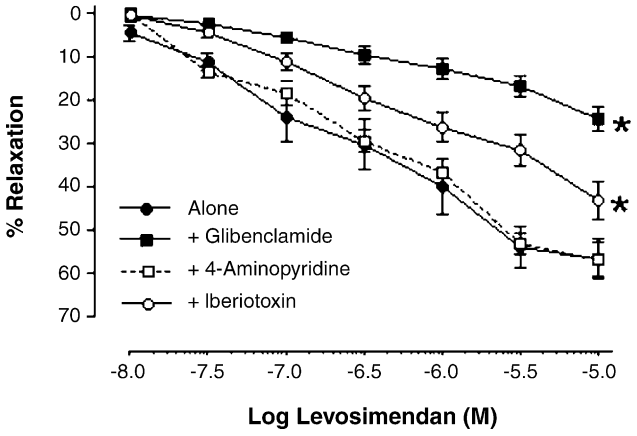


Fig. 1. Effects of levosimendan in human internal thoracic artery rings precontracted by 10^{-6} M phenylephrine. The effects of ATP-dependent potassium channel blocker glibenclamide, voltage-dependent potassium channel blocker iberiotoxin and Ca^{2+}-activated potassium channel blocker 4-aminopyridine. Each point represents the mean with SEM shown by vertical bars. $n = 10$ for all groups. $^*P < 0.05$ as compared with levosimendan alone values.

2.4. Statistical analysis

All values are expressed as mean ± SEM. Relaxation responses to levosimendan or cromakalim are expressed as percentages of the phenylephrine-induced contraction. The concentration of agonist which elicited a 50% maximal response (E_{max}) was designated as the EC_{50}, which was calculated by linear regression. Sensitivity was expressed as pD_2 ($-\log EC_{50}$). Statistical analysis of the results was performed using one-way analysis of variance and Student's *t*-test where appropriate. *P*-values lower than 0.05 were considered significant.

3. Results

Phenylephrine (10^{-6} M) caused stable and sustained contractions of human ITA rings. Both levosimendan

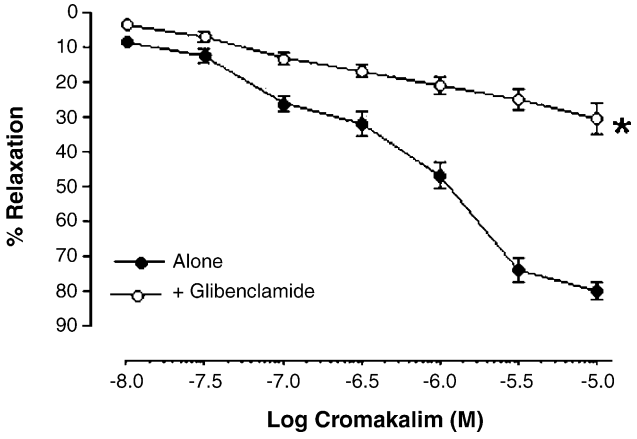


Fig. 2. Effects of cromakalim in human internal thoracic artery rings precontracted by 10^{-6} M phenylephrine alone and in the presence of ATP-dependent potassium channel blocker glibenclamide. Each point represents the mean with SEM shown by vertical bars. $n = 7$, $^*P < 0.05$ as compared with respective cromakalim values.

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Table 1
pD_2 (−log EC_{50}) and E_{max} (%) values for levosimendan ($n = 10$) and cromakalim ($n = 7$) on phenylephrine-induced contractions of human internal thoracic arteries

	E_{max}	pD_2
Levosimendan	57 ± 14.4	6.06 ± 0.09
Cromakalim	$80 \pm 6.8^{*}$	$6.49 \pm 0.19^{*}$

Numbers are given as mean ± SD.
* $P = 0.0013$ and 0.0026 for E_{max} and pD_2, as compared with respective levosimendan values.

(10^{-8}–10^{-5} M) (Fig. 1) and cromakalim (10^{-8}–10^{-5} M) (Fig. 2) caused concentration-dependent relaxation responses in human ITA rings precontracted by 10^{-6} M phenylephrine. Maximal responses and the sensitivities of human ITA rings to levosimendan and cromakalim differed significantly (Table 1).

The relaxant responses to both levosimendan and cromakalim were significantly inhibited by pre-treatment of tissues with K_{ATP} blocker glibenclamide (10^{-6} M) for 30 min (Figs. 1 and 2). The relaxant responses to levosimendan were significantly but, to a lesser degree, inhibited by pre-treatment of tissues with K_{Ca} blocker iberiotoxin (10^{-7} M for 30 min). Incubation of ITA rings with K_V blocker 4-aminopyridine (5 mM for 10 min) did not cause a significant alteration in relaxation responses to levosimendan (Fig. 1). Incubation with tested potassium channel blockers for 30 min did not cause significant effects on basal tones of ITA rings (data not shown).

4. Discussion

In this study, the prototype of K_{ATP} channel openers, cromakalim produced relaxations in human ITA. The vasodilatory effect of cromakalim was inhibited by glibenclamide at a specific concentration (10^{-6} M) for inhibiting K_{ATP} channels [13] indicating the presence of functional K_{ATP} channels in human ITA. The vasodilatory effect of levosimendan, which is antagonized by K_{ATP} blocker glibenclamide, has been demonstrated both in arterial [4] and venous [10] vascular beds, as well as in the coronary arteries [9]. Accordingly, in this study, we have presented, for the first time, the pharmacological evidence about both the functional relaxant effect of levosimendan and the role of K_{ATP} channels in this effect of levosimendan in human ITA. Our results indicated that levosimendan exerts a direct concentration-dependent relaxant effect on ITA in human that was significantly blocked by glibenclamide, the known inhibitor of K_{ATP} channels. On the basis of pD_2 and E_{max} values, cromakalim was approximately threefold more potent and 1.5-fold more efficacious than levosimendan in human ITA rings (Table 1) that was in accordance with the findings of Pataricza et al. [13] who reported a 4.1-fold lower EC_{50} value for cromakalim than for levosimendan in porcine isolated coronary artery rings.

There are conflicting results about the types of potassium channels involved in relaxant effect of levosimendan in different vascular tissues [9–13]. Yokoshiki and Sperelakis [8] have concluded that levosimendan seemed to preferentially stimulate the K_V and K_{Ca} in large conductance vessels, and K_{ATP} in small resistance arteries. In partial support of this view, in this study, K_{Ca} blocker iberiotoxin but not K_V blocker 4-aminopyridine also caused a significant inhibition in relaxant responses to levosimendan in human ITA rings that was lesser than that of glibenclamide, which indicated that both K_{ATP} and K_{Ca} were responsible for the relaxant effect of levosimendan in this tissue, albeit not in equal degrees.

In this study, levosimendan was required at concentrations of 0.01–10 μM to induce relaxation in human ITA rings with a pD_2 value of 6.06 ± 0.09. It has been reported that levosimendan exerts positive inotropic effect in isolated guinea pig heart in the concentration range of 0.03–1 μM and higher concentrations (0.3–10 μM) are needed in skinned myocardial fiber preparations [17]. We also recently showed that levosimendan produces increments in developed tension in isolated human atrial trabeculae with a pD_2 value of 6.82 ± 0.33 [18]. On the contrary, Gruhn et al. [14] reported a pD_2 value of 3.64 ± 0.05 for levosimendan in 30 mM phenylephrine-precontracted porcine coronary arteries and suggested that there might be differences in potency of levosimendan depending on the differences in species or vasoconstrictors used.

Although it has been suggested that levosimendan may have a higher potency to relax small coronary arteries in preference to large ones [14], neither the concentration ranges used nor the pD_2 values obtained in these studies, including the present one, seem satisfactory to produce effective vascular smooth muscle relaxation in levosimendan-treated patients. It has been reported that although levosimendan given in doses of 0.25 and 0.5 mg increased left ventricular function in patients with ischemic heart disease and left ventricular dysfunction, a significant decrease in total peripheral resistance was seen only after 2 and 4 mg [19]. A single 0.5 mg oral dose of levosimendan produce a peak plasma concentration of ~20 ng/ml (0.07 μM) in patients with congestive heart failure [20]. Therefore, the findings of this study also suggest that levosimendan can be used in clinical practice without unfavorable direct effects on ITA grafts in coronary bypass patients.

In conclusion, this study provided, for the first time, pharmacological evidence about the functional relaxant effect of levosimendan in human ITA. The findings suggested that levosimendan-induced relaxation responses in isolated human ITA were dependent on the activation of K_{ATP} and K_{Ca}.

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