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Levosimendan, a novel Ca^{2+} sensitizer, activates the glibenclamide-sensitive K^+ channel in rat arterial myocytes

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Abstract

The electrophysiological effect of levosimendan, a novel Ca^{2+}-sensitizing positive inotropic agent and vasodilator, was examined on rat mesenteric arterial myocytes using the patch clamp technique. Resting potential was significantly hyperpolarized with levosimendan, with an EC_{50} of 2.9 μM and maximal effect (19.5 ± 3.5 mV; $n = 12$) at 10 μM. Levosimendan (10 μM) significantly increased the whole-cell outward current. The currents intersected close to the calculated E_K (-84 mV), suggesting that the activated current was a K^+ current. Hyperpolarization and stimulation of K^+ current by levosimendan were not prevented by 30 μM H-7 (a non-specific inhibitor of protein kinases) and 100 nM charybdotoxin (a blocker of Ca^{2+}-activated K^+ channels), but were abolished by 10 μM glibenclamide. In single-channel current recording in open cell-attached patches, two types of K^+ channels were observed having conductances of 26 and 154 pS. The 154 pS channels were not affected by levosimendan and glibenclamide. The 26 pS channels were evoked in one-fourth of the patches when 10 μM levosimendan (and 0.1 mM UDP) was added (at -60 mV) and channel activity was abolished by glibenclamide. The mean open probability of the 26 pS channels was 0.094 ± 0.017 ($n = 9$), and the mean open time (at -60 mV) was 6.6 ms in the presence of UDP and levosimendan. Although significant hyperpolarization (4.7 ± 1.5 mV, $n = 8$) was observed at 1 μM levosimendan, the same concentration did not affect Ca^{2+} channel currents ($n = 10$). In summary, levosimendan hyperpolarized the arterial myocytes, probably through activation of a glibenclamide-sensitive K^+ channel. This mechanism may contribute to the vasodilating action of levosimendan.

Keywords: Levosimendan; K^+ channel; K^+ channel, ATP-sensitive; Patch clamp technique; Mesenteric artery, rat

1. Introduction

Vasodilator therapy is one of the most effective strategies for treatment of heart failure (Cohn et al., 1986; The CONSENSUS Trial Study Group, 1987; Packer, 1988; The SOLVD Investigators, 1991; Cohn et al., 1991) and optimal usage of vasodilators reduces both preload and afterload, resulting in reduced energy consumption by the heart. On the other hand, the Ca^{2+}-sensitizing agents, such as pimobendan, EMD 53998, MCI-154 and levosimendan, might also be favorable in terms of energy saving. This is because their positive inotropic action does not require enhanced intracellular Ca^{2+}, thereby extra energy would not be needed to pump or sequester the elevated Ca^{2+} (Packer, 1988; Nielsen-Kudsk and Aldershvile, 1995). The novel Ca^{2+} sensitizer levosimendan has an unique property that it binds to cardiac Troponin C in a Ca^{2+}-dependent manner and stabilizes the conformational change of troponin C produced by Ca^{2+} (Pollesello et al., 1994; Haikala et al., 1995; Nielsen-Kudsk and Aldershvile, 1995).

Furthermore, levosimendan acts as a vasodilator both in experimental animal models (Harkin et al., 1995; Pagel et al., 1995; Udvary et al., 1995; Vegh et al., 1995) and in clinical studies (Vegh et al., 1995; Sundberg et al., 1995; Lilleberg et al., 1995). For example, levosimendan decreased systemic vascular resistance, and increased coronary blood flow in conscious dogs (Harkin et al., 1995). Although levosimendan (0.1 or 0.3 μM) was suggested to produce phosphodiesterase inhibition in cardiac tissues (Edes et al., 1995), it remains unclear whether PDE inhibition is involved in the vasodilation produced by levosimendan.

It is suggested that hyperpolarization produced by K^+ channel-opening drugs makes an important contribution to vasorelaxation (Quast et al., 1994). It has also been re-

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ported that vasorelaxation induced by K^+ channel-opening drugs is abolished by glibenclamide (Edwards and Weston, 1993; Quayle and Standen, 1994; Nelson and Quayle, 1995). However, there are only a few studies which have identified a target K^+ channel by single-channel recording. In addition, single ATP-sensitive K^+ (K_{ATP}) channels in vascular smooth muscle cells have not been clearly defined and it remains controversial whether their conductance is small or large (Edwards and Weston, 1993; Quayle and Standen, 1994; Nelson and Quayle, 1995). Therefore, we examined the effects of levosimendan on membrane potentials and membrane currents in myocytes from rat mesenteric artery using the patch-clamp technique: whole-cell and single-channel recordings. Levosimendan hyperpolarized arterial myocytes and our data suggests that this occurred through opening of the 26 pS gibenclamide-sensitive K^+ channel, possibly the K_{ATP} channel.

2. Materials and methods

2.1. Cell isolation

Freshly isolated single vascular smooth muscle cells were prepared from peripheral segments of rat superior mesenteric artery, as previouly described (Ohya and Sperelakis, 1989a; Yokoshiki et al., 1997a). In brief, rats of either sex (weighing 250–350 g) were decapitated and bled under CO_2 anesthesia. From the vascular bed of the jejunum, peripheral segments of superior mesenteric artery (prearteriole; diameter < 300 μm) were dissected out and placed in Krebs–Ringer solution which had the following composition (mM): NaCl, 120.7; KCl, 5.9; $NaHCO_3$, 15.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.5; glucose, 11.5; bubbled with 95% O_2–5% CO_2. Connective tissues were carefully removed with surgical microscissors under a dissecting microscope.

The tissues were then transferred to a Ca^{2+}-free solution containing (mM): NaCl, 140; KCl, 6.0; glucose, 10; HEPES, 10 and pH adjusted to 7.3 with tris-(hydroxymethyl)aminomethane (Tris). The lumen was flushed with the Ca^{2+}-free solution to remove the blood cells. The tissues were then cut into small pieces (1–2 mm). Tissue pieces were incubated in the Ca^{2+}-free solution for about 15 min at 36°C. The incubation solution was exchanged to Ca^{2+}-free solution containing 0.25% collagenase (Wako, Osaka, Japan), 0.05% papain (Sigma, St. Louis, MO, USA), 0.05% trypsin inhibitor (type II-S; Sigma) and 0.3% bovine serum albumin (essentially free of fatty acids; Sigma). After about 50 min of incubation, the collagenase containing solution was washed out with fresh Ca^{2+}-free solution. Digested tissues were agitated gently with a blunt-tipped glass pipette to disperse the single cells. The debris was removed with a fine nylon mesh. Finally, the cells were placed in a stock solution having the following composition (mM): NaCl, 137; KCl, 6.0; $MgCl_2$, 0.5; $CaCl_2$, 0.5; glucose, 10; HEPES, 10; 0.2% trypsin inhibitor, 0.3% bovine serum albumin and pH adjusted to 7.3 with Tris. The cell suspension was stored in an ice-cold bath and used within 4 h of the cell dispersion. Only spindle-shaped elongated cells were used for experiments. All experiments were performed at room temperature (20–22°C).

2.2. Whole-cell recordings

The standard patch-clamp technique was applied in the whole-cell configuration with a patch-clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, CA, USA). Membrane potentials and whole-cell currents were measured in current-clamp or voltage-clamp mode, respectively. Voltage-clamp experiments were performed by applying either voltage ramp or step pulses. The patch electrodes (2–5 MΩ) were made from borosilicate glass capillary tubing (World Precision Instruments). The cell suspension was placed into a small chamber (0.5 ml) on the stage of an inverted microscope (TMD-Diaphot; Nikon, Tokyo, Japan). The bath was superfused with the following extracellular (bath) solution (mM): NaCl, 141; KCl, 4.7; $MgCl_2$, 1.2; $CaCl_2$, 1.8; glucose, 10; HEPES, 10 and pH adjusted to 7.4 with NaOH. The internal (pipette) solution for the whole-cell experiments was consisted of the following composition (mM): KCl, 125; $MgCl_2$, 4; HEPES, 10; EGTA, 10; ATP-Na_2, 5 and pH adjusted 7.2 with KOH.

To isolate Ca^{2+} channel current (Ba^{2+} current), the pipette was filled with high Cs^+ solution of the following composition (mM): CsOH, 100; CsCl, 30; EGTA, 10; HEPES, 10; L-glutamate, 112; ATP-Na_2, 5; free Mg^{2+}, 1 and pH adjusted 7.2 with CsOH. The bath solution was isotonic Ba^{2+} solution containing (mM): $BaCl_2$, 100; glucose, 10; HEPES, 10 and pH adjusted 7.3 with Tris. Leak current and residual capacitive current were subtracted using P/N protocol in the pCLAMP software (version 5.05, Axon Instruments).

Current and voltage signals were filtered at 1 kHz and digitized by an A/D converter (TL-1, Axon Instruments) and analyzed on a personal computer (IBM-compatible computer system) using the pCLAMP software (version 5.05). The membrane capacitance was determined from the current amplitude elicited in response to a hyperpolarizing voltage ramp pulse of 0.2 V/s from a holding potential of 0 mV (duration 25 ms, peak amplitude -5 mV) to avoid interference by any time-dependent ionic currents. Average cell capacitance was 12.6 ± 0.5 pF ($n = 39$).

2.3. Single-channel recordings

Single-channel current recordings were made in open cell-attached patch configuration (Kakei et al., 1985; Ohya and Sperelakis, 1989b) with the same patch-clamp amplifier used in the whole-cell experiments. In brief, after making cell-attached patch with recording pipette, one end of the cell was mechanically disrupted using another glass

pipette containing the bath solution. The tip of the patch electrode was coated with Sylgard (Dow Corning, Ann Arbor, MI, USA) and its resistance ranged from 2 to 6 MΩ when it was filled with the following pipette (extracellular) solution (mM): NaCl, 80; KCl, 60; $MgCl_2$, 1.3; $CaCl_2$, 1.7; HEPES, 10 and pH adjusted 7.4 with NaOH. The bath (intracellular) solution contained (mM): NaCl, 9; KCl, 117; KOH, 13; $MgCl_2$, 3; HEPES, 18; EGTA, 5; glucose, 10 and pH adjusted 7.3 with KOH. Currents signals were filtered at 1 kHz, sampled at 1 kHz and stored in a personal computer. The storing of the digitized signals was carried out using AxoTape (version 2, Axon Instruments), and analysis was performed by pCLAMP software (version 6.02).

2.4. Drugs and chemicals

Levosimendan was a gift from Orion Pharma (Espoo, Finland). Various nucleotide diphosphates, nucleotide triphosphates, glibenclamide, charybdotoxin and 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine dihydrochloride (H-7) were purchased from Sigma.

2.5. Data analysis

All data are given as mean ± SEM. Differences between the mean values of multiple subgroups were evaluated by analysis of variance (ANOVA), and intergroup comparisons were performed by the adjusted *t*-test within ANOVA (Bonferroni method). Paired data of change in membrane potentials were analyzed by Student's *t*-test. Significance was established at $P < 0.05$.

3. Results

3.1. Levosimendan hyperpolarizes rat mesenteric arterial cells via stimulating K^+ currents

Membrane potential in a myocyte from rat mesenteric artery was recorded in current-clamp mode (Fig. 1A). The average resting potential value was -44.3 ± 2.2 mV ($n = 36$). Extracellular application of 10 μM levosimendan produced hyperpolarization. The hyperpolarization usually started 1–3 min after exposure to levosimendan and reached steady-state level within about 5 min. The degree of hyperpolarization produced by 1 and 10 μM levosimendan was 4.7 ± 3.5 mV ($n = 8$, $P < 0.05$) and 19.4 ± 3.5 mV ($n = 12$, $P < 0.05$), respectively. This hyperpolarization could be reversed in about half of the cells tested, but it required more than 5 min from the beginning of washing out (Fig. 1A).

The dose–response relationship for hyperpolarization by levosimendan are shown in Fig. 1B. The degree of

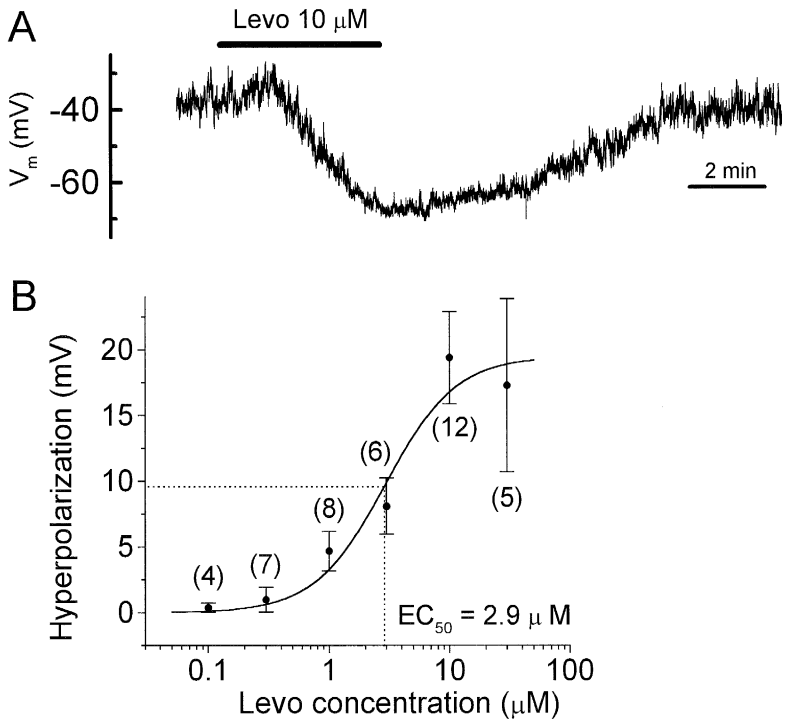

Fig. 1. Hyperpolarizing effect of levosimendan on resting membrane potential in rat mesenteric arterial myocytes. (A) Under whole-cell recording conditions, extracellular application of 10 μM levosimendan produced hyperpolarization. The hyperpolarization usually started within 1–3 min and reached steady-state level within about 5 min. (B) Dose–response relation for hyperpolarization by levosimendan. Degree of hyperpolarization is plotted against each levosimendan concentration. Number of cells studied are shown in parentheses. Data points were fitted by the Hill equation. The EC_{50} value was 2.9 μM and the Hill coefficient (n_H) value was 1.5. Levo, levosimendan.

hyperpolarization was plotted against each concentration. Data points were fitted to the Hill equation, hyperpolarization (mV) $= [x^{n_H}/(x^{n_H} + EC_{50}^{n_H})] \times E_{max}$, where x is the concentration of levosimendan, E_{max} is the maximal hyperpolarization, n_H is the Hill coefficient and EC_{50} is the concentration for half-maximal effect. The EC_{50} value was 2.9 μM and the n_H value was 1.5. The E_{max} was calculated to be 19.5 mV.

Superimposed current traces evoked by 300 ms test pulses from a holding potential of −70 mV are shown in Fig. 2A. The test potentials were from −120 to +80 mV, and applied every 10 s in 20 mV increments. Levosimendan (10 μM) stimulated currents to the outward direction at potentials positive to −70 mV (upper traces) and to the inward direction at potentials negative to −70 mV (lower traces). The current–voltage relationships before (open circles) and about 5 min after exposure to 10 μM levosimendan (closed circles) are shown in Fig. 2B ($n = 4$). The currents plotted were measured at the end of the test pulses (300 ms). Levosimendan elicited an increase in membrane conductance typical of K^+, i.e., a potential where these two current–voltage curves cross (about −70 mV) is close to the K^+ equilibrium potential (E_K) (about −84 mV in this experimental condition).

3.2. Glibenclamide inhibition of levosimendan-induced hyperpolarization and K^+ currents

We next examined the effect of glibenclamide, a blocker of ATP-sensitive (K_{ATP}) K^+ channels, on levosimendan-induced hyperpolarization. Glibenclamide (10 μM) alone depolarized the myocytes from rat mesenteric artery by 19.8 ± 4.6 mV ($n = 6$). One example of this depolarization is shown in Fig. 3A. Membrane potentials of the cells exposed to 10 μM glibenclamide were not affected by subsequent application of 10 μM levosimendan (Fig. 3A). In the presence of glibenclamide, the change in membrane potentials by levosimendan (10 μM) was 0.7 ± 1.9 mV ($n = 6$, $P = 0.72$). On the other hand, levosimendan was still capable of producing hyperpolarization in a myocyte

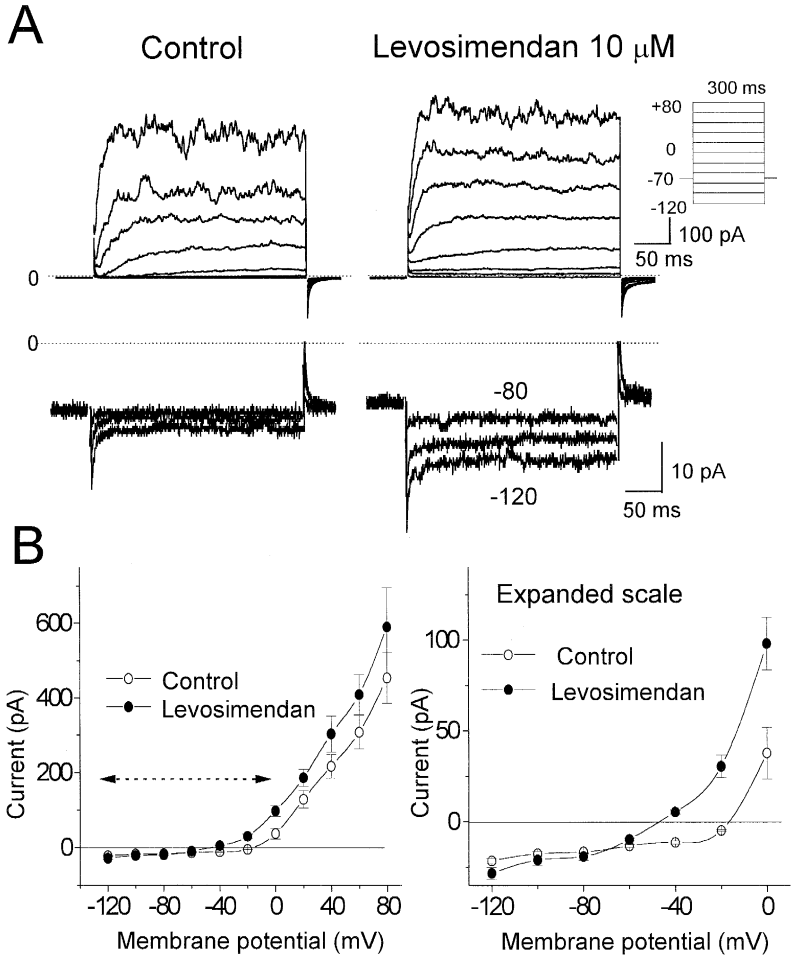

Fig. 2. Stimulation of K^+ currents by levosimendan in rat mesenteric arterial myocytes. (A) Superimposed current traces evoked by 300 ms pulses from a holding potential of −70 mV to test potentials ranging from −120 to +80 mV in 20 mV increments. (B) The current/voltage relationships before (open circles) and about 5 min after exposure to 10 μM levosimendan (closed circles) ($n = 4$). The current/voltage relationships shown on an expanded scale are given in the right diagram. The currents plotted were measured at the end of the pulse.

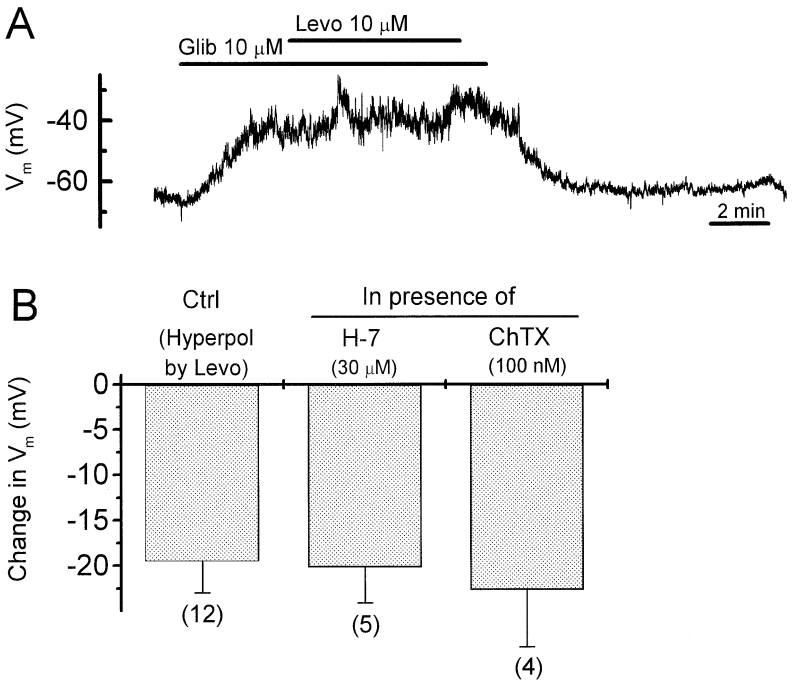

Fig. 3. Glibenclamide inhibition of levosimendan-induced hyperpolarization. (A) Levosimendan-induced hyperpolarization was abolished in presence of 10 μM glibenclamide. Initial application of glibenclamide produced depolarization. (B) Summary data showing the degree of hyperpolarization produced by levosimendan. Levosimendan (10 μM) was applied in the presence H-7, or charybdotoxin. In H-7 experiments, cells were also pre-incubated for more than 30 min. Values are given as changes in membrane potentials from those obtained during control period. Levo, levosimendan; Glib, glibenclamide; ChTX, charybdotoxin.

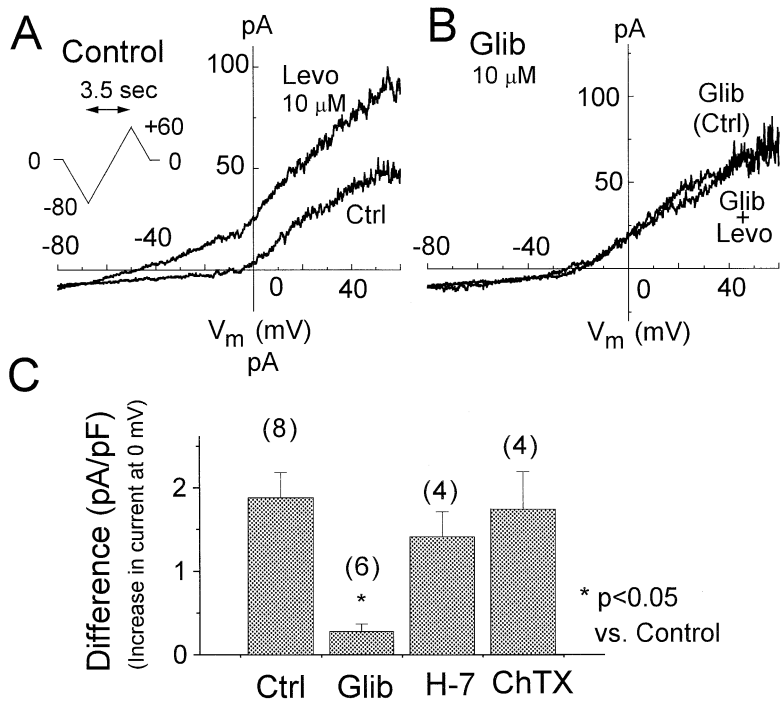

Fig. 4. Effect of H-7 and K^+ channel blockers on levosimendan-activated K^+ current. (A) Superimposed current traces evoked by voltage-clamp ramps (40 mV/s) between −80 mV to +60 mV are given in control (A), in presence of glibenclamide (B). Glibenclamide prevented the stimulation of the K^+ current produced by levosimendan (B). (C) Summary of changes in K^+ current produced by levosimendan. Differences in currents measured at 0 mV between after and before application of levosimendan in each condition are given. Number of cells studied are shown in parentheses. * $P < 0.05$ versus control. Ctrl, control; Levo, levosimendan; Glib, glibenclamide; ChTX, charybdotoxin.

pre-equilibrated (more than 30 min) with 30 μM H-7, a non-specific inhibitor of protein kinases or in presence of 100 nM charybdotoxin, a blocker of large conductance Ca^{2+}-activated K^+ channel (K_{Ca}) (Fig. 3B).

Fig. 4 shows superimposed current traces evoked by voltage-clamp ramps (40 mV/s) between −80 mV to +60 mV in control (A), in presence of glibenclamide (B). Fig. 4C summarizes the differences in currents measured at 0 mV after and before application of levosimendan in each condition. Levosimendan 10 μM was still capable of stimulating a K^+ current in the presence of a non-specific inhibitor of protein kinases, H-7 30 μM (and preincubated in bath), or a blocker of K_{Ca} channels, charybdotoxin 100 nM. However, glibenclamide 10 μM, prevented the stimulation of the K^+ current produced by levosimendan.

3.3. Levosimendan activates glibenclamide-sensitive K^+ channel in rat mesenteric arterial cells

Single K^+ channel currents were recorded in an open cell-attached patch (60 mM K^+ in the pipette (external surface) and 130 mM K^+ in the bath (internal surface); $E_K = -19.8$ mV) in myocytes from rat mesenteric artery. The membrane potential was held at −60 mV. When open cell-attached patches were made in ATP-free solution, no channel activities were usually observed. However, a channel having relatively large unit amplitude appeared frequently (approx. 80% of the patches) when membrane was held at potentials positive to 0 mV (Fig. 5B). This channel was strongly voltage-dependent (data not shown) and its conductance was 154 pS (Fig. 5C). According to its large conductance and voltage-dependency, it is similar to large conductance Ca^{2+}-activated K^+ (K_{Ca}) channel (i.e., 'big' K_{Ca} channel or 'maxi' K_{Ca} channel (Nelson and Quayle, 1995)). The K_{Ca} channels recorded at +40 mV were not affected by levosimendan (10 μM) (data not shown). Subsequent application of 10 μM glibenclamide had also no effect on the K_{Ca} channel ($n = 4$).

Cell membranes held at −60 mV in open-cell attached patches were quiescent and no distinct channel activities appeared after bath application of 10 μM levosimendan ($n = 13$). However, levosimendan evoked channel activities in the presence of 0.1 mM UDP (Fig. 6A). The mean open probability (P_o) of this channel was 0.094 ± 0.017 ($n = 9$) (calculated from continuous 30–100 s recording) in the presence of both UDP and levosimendan and the

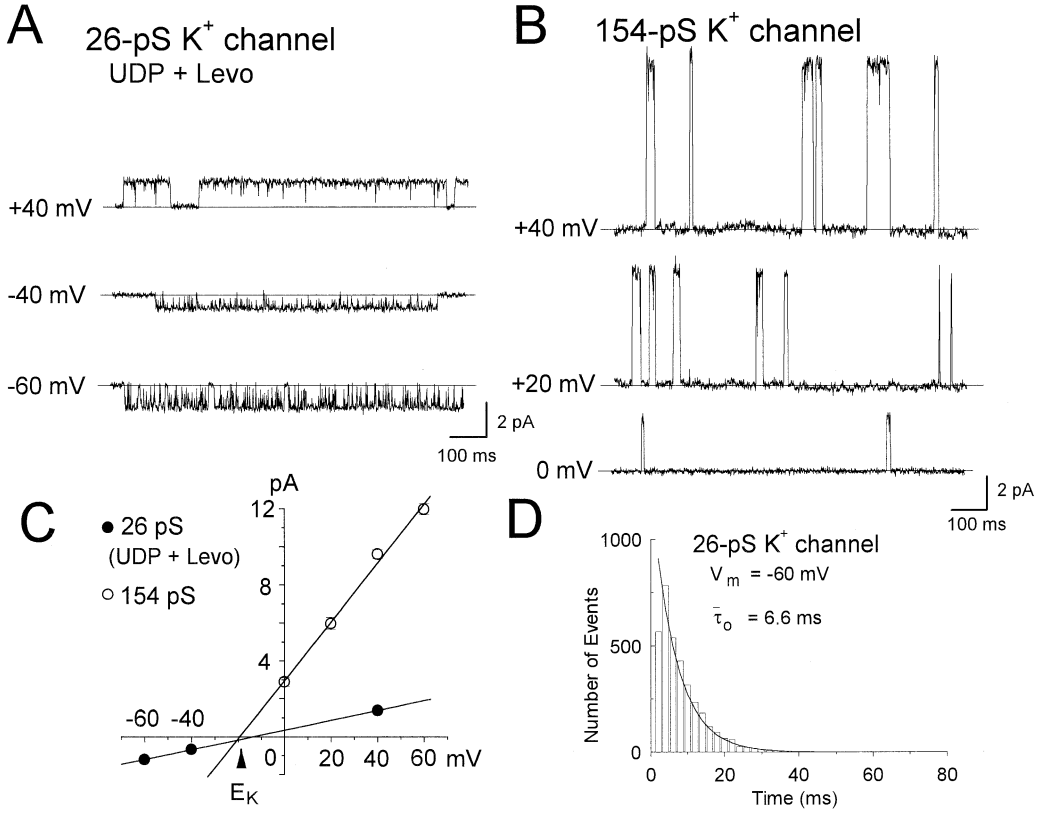

Fig. 5. Conductance and kinetic properties of the two types of K^+ channels in open cell-attached patches. (A) The K^+ channel currents evoked in presence of 0.1 mM UDP and 10 μM levosimendan at different membrane potentials are given. Its conductance was 26 pS as shown in (C). (B) The large conductance K^+ channel currents recorded at depolarized potentials are given. Its conductance was 154 pS as shown in (C). (C) The current/voltage relationships of the two types of K^+ channels. The slope conductance of unitary inward current was 26 pS ($n = 4$–5) and 154 pS ($n = 4$), respectively. (D) Open-time histograms of the unitary 26 pS K^+ channel currents at −60 mV. The histogram at −60 mV was fitted to a single exponential curve with a time constant of 6.6 ms.

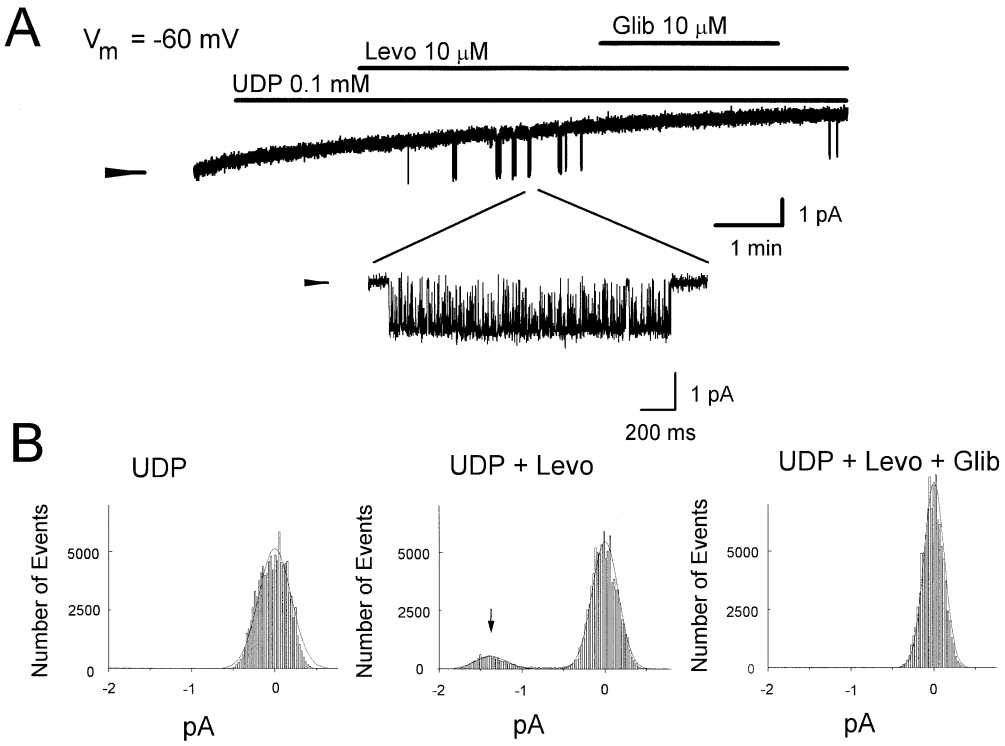

Fig. 6. Stimulation of the 26 pS K^+ channel by levosimendan in presence of UDP. (A) When a open-cell attached patch was formed in ATP-free solution, no distinct channel activity appeared. Although UDP (0.1 mM) alone had no effect, subsequent application of levosimendan (10 μM) evoked the 26 pS K^+ channel. The activated channel were abolished by glibenclamide (10 μM). Membrane potential (V_m) was held at −60 mV. (B) Amplitude histograms obtained from continuous 100 s-recording are given in each condition. The amplitude of unitary current was 1.38 pA (at −60 mV) (arrow in the middle inset). Levo, levosimendan; Glib, glibenclamide.

channel was abolished by 10 μM glibenclamide ($n = 5$). The channel had a unit amplitude of 1.38 pA at −60 mV (Fig. 6B) and its slope conductance was 26 pS ($n = 4$–5) (Fig. 5C). The opening of the channels appeared in bursts (Fig. 6A) and the flickerings within bursts decreased when membrane was depolarized (Fig. 5A). The channels of the

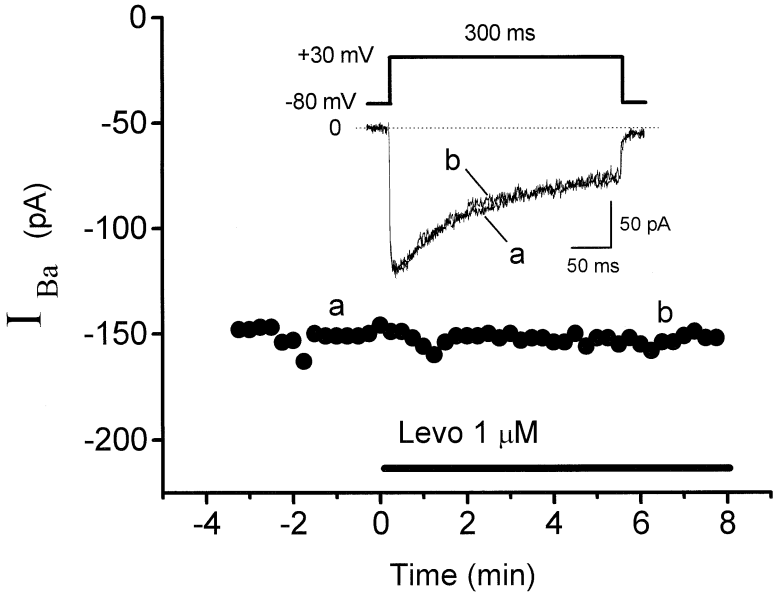

Fig. 7. Lack of effect of levosimendan on Ca^{2+} channel currents. Ba^{2+} currents (I_{Ba}) through Ca^{2+} channels were evoked by 300-ms depolarizing test pulses to +30 mV from a holding potential of −80 mV. Bath application of 1 μM levosimendan for 6–8 min produced no significant changes of I_{Ba} ($n = 10$). Upper inset shows superimposed current tracings of I_{Ba} during the control period (a) and 7 min after application of levosimendan (b). Levo, levosimendan.

same unit amplitude appeared in 9 out of 39 patches when levosimendan was added in the presence of UDP. Only one functional channel was observed in 9 patches. The open-time histograms at −60 mV were fitted by a single exponential curve with a time constant of 6.6 ms (Fig. 5D). In 39 patches, UDP (0.1 mM) alone did not produce any channel activities for at least 1 min-observation period.

3.4. Lack of effect of levosimendan on Ca^{2+} channel currents

Ca^{2+} channel currents (total) were recorded under the conditions of high Cs^+ solution in the pipette (to block K^+ channels) and isotonic Ba^{2+} solution in the bath. Ba^{2+} currents (I_{Ba}) through Ca^{2+} channels were evoked every 15 s by 300 ms depolarizing test pulses to +30 mV from a holding potential of −80 mV. The maximal I_{Ba} was obtained at this test potential (data not shown), as previously reported (Ohya and Sperelakis, 1989a; Yokoshiki et al., 1997a). As shown in Fig. 7, I_{Ba} was not affected by 1 μM levosimendan (which produced significant hyperpolarization (Fig. 1)). The amplitude of I_{Ba} after 6–8 min application of 1 μM levosimendan was 99.1 ± 3.8% (n = 10) of control. Therefore, the slow (L-type) Ca^{2+} channels are not affected by the concentration of levosimendan that has substantial effects on membrane potentials.

4. Discussion

The present study demonstrated that the new Ca^{2+}-sensitizing positive inotropic agent and vasodilator, levosimendan, hyperpolarized rat mesenteric arterial cells via activation of a glibenclamide-sensitive K^+ current. The maximal hyperpolarization (19.5 ± 3.5 mV) was produced by 10 μM levosimendan and the EC_{50} value was 2.9 μM. The hyperpolarization was rapid, reaching a steady-state within about 5 min. This effect could be reversed in many cells. Single-channel recordings showed that levosimendan activated a 26 pS glibenclamide-sensitive K^+ channel (in the presence of UDP).

Hyperpolarization reduces excitability and lowers intracellular calcium ($[Ca]_i$) by decreasing the open probability of the L-type Ca^{2+} channels and by stimulating the forward mode of the Na^+/Ca^{2+} exchanger (i.e., 3 Na^+ in/1 Ca^{2+} out) (Quast et al., 1994). The former action would decrease Ca^{2+} influx into the cell, whereas the latter would increase Ca^{2+} efflux. Both effects act to lower $[Ca]_i$ and thereby promote relaxation of the vascular smooth muscle cell. In addition, hyperpolarization by K^+ channel-opening drugs reduces sensitivity of the contractile elements to Ca^{2+}, inhibits Ca^{2+} store refilling and attenuates agonist-induced increase in IP_3 (Quast et al., 1994). All these actions favor relaxation of smooth muscle cells. Thus, the hyperpolarization by opening of the 26 pS glibenclamide-sensitive K channels would contribute to the vasodilating action of levosimendan.

In the present study, the average resting potential of the cells was approx. −45 mV. This value is in agreement with that (approx. −40 mV) in a recent report for rat pulmonary arterial myocytes (recorded in similar intracellular and extracellular solutions) (Yuan, 1995). The high concentration of Cl^- (133 mM) in the pipette solution, resulting in an E_{Cl} value of −3.3 mV, may cause the recorded resting potential to be lower than normal (i.e., somewhat depolarized), because the Cl^- conductance of arterial myocytes is relatively high (Nelson and Quayle, 1995). A possible contribution of Ca^{2+}-activated K^+ (K_{Ca}) channels in setting the resting potential may be masked by the high EGTA (10 mM) in the pipette solution. The cells we studied were in good condition because the Ca^{2+} channels, which were found to be regulated by intracellular ATP in the same preparation (Yokoshiki et al., 1997a), exhibited only little run-down over a period of 10 min (Fig. 7).

Depolarization of arterial myocytes by glibenclamide alone observed in the present study has been described in various intact arterial smooth muscle tissues, including rat and rabbit small mesenteric artery (Nelson et al., 1990; McPherson and Angus, 1991; Murphy and Brayden, 1995; Weidelt et al., 1997), guinea-pig coronary artery (Eckman et al., 1992) and rabbit vertebral artery (Nagao et al., 1996). For example, in rabbit vertebral artery, glibenclamide (1 μM) depolarized by 23 mV and markedly potentiated the agonist-induced contraction (Nagao et al., 1996). Thus, the depolarization produced by glibenclamide is not the result of metabolic impairment of the cells. Furthermore, injection of glibenclamide into the coronary artery of anesthetized dogs increased coronary resistance and reduced coronary blood flow (Imamura et al., 1992; Samaha et al., 1992), suggesting that functioning K_{ATP} channels are present and can be one of the regulators of basal vascular tone (Daut et al., 1994).

In myocytes from rabbit main pulmonary artery (Clapp and Gurney, 1992), the glibenclamide-sensitive K^+ current was not present unless ATP in the pipette was lowered to 1 mM or less. Inclusion of 0.1 mM ADP in the pipette solution evoked K_{ATP} current even when 5 mM ATP was present (Pfrunder et al., 1993). Single-channel recording from cell-attached patches (i.e., quasi-physiological condition) revealed that K_{ATP} channels were active with a very low open probability (0.00093–0.002) (Kajioka et al., 1991; Zhang and Bolton, 1995). There are different regulatory mechanisms as well as different densities of K_{ATP} channels in various vascular smooth muscles (Leech and Faber, 1996; Nagao et al., 1996; Nakae et al., 1996). Furthermore, there are different sub-types of sulfonylurea receptor, including the pancreatic type (1) (Aguilar-Bryan et al., 1995; Inagaki et al., 1995), the cardiac type (2A) (Inagaki et al., 1996) and the smooth muscle type (2B) (Isomoto et al., 1996).

Although large-conductance (100–258 pS) K_{ATP} channels were reported to be present in arterial myocytes

(Standen et al., 1989; Lorenz et al., 1992; Furspan and Webb, 1993), several studies showed that the conductance of the K_{ATP} channels in vascular smooth muscle cells were small or intermediate (15–50 pS) (Kajioka et al., 1991; Beech et al., 1993a,b; Dart and Standen, 1993, 1995; Kubo et al., 1994; Zhang and Bolton, 1995, 1996). These relatively small conductance K^+ channels were inhibited by glibenclamide, and were activated by K^+ channel-opening drugs, nucleotide diphosphates and adenosine (Kajioka et al., 1991; Beech et al., 1993a,b; Dart and Standen, 1993, 1995; Kubo et al., 1994; Zhang and Bolton, 1995, 1996). Channel activity could be evoked by severe hypoxia or metabolic inhibition (Beech et al., 1993a; Dart and Standen, 1995; Zhang and Bolton, 1995) and was inhibited by ATP (Kajioka et al., 1991; Zhang and Bolton, 1996). One of the most surprising features of K_{ATP} channels of vascular smooth muscle cells is that channel activity does not appear when inside-out patches are formed in ATP-free solution (Kajioka et al., 1991; Zhang and Bolton, 1995, 1996), in agreement with the present study. Possible run-down of the channels following formation of the patch seems unlikely, because the run-down of K_{ATP} channels in cardiac tissues or pancreatic β-cells generally occurs only after they initially exhibit vigorous activity (in ATP-free condition).

In myocytes from rabbit portal vein, the 15 pS K_{ATP} channels (in quasi-physiological K^+ gradient) were evoked only when both GDP and pinacidil were present during inside-out patch recording and the activated channels were inhibited by glibenclamide or ATP (Kajioka et al., 1991). The IC_{50} value for the ATP effect on channel activity was 29 μM, which is close to that for cardiac K_{ATP} channels (Terzic et al., 1995). The 20–24 pS K channel is the channel that is activated by nucleotide diphosphates, K^+ channel-opening drugs and metabolic inhibition, and is inhibited by glibenclamide (Beech et al., 1993a,b; Zhang and Bolton, 1995). These pharmacological responses and channel characteristics, such as no voltage-dependence or time-dependence, are similar to the cardiac K_{ATP} channels (Terzic et al., 1995). However, abrupt removal of ATP (inside-out patches) did not produce any channel activity. Thus, nucleotide diphosphates (NDPs) (instead of ATP) are more important regulators of these K_{NDP} channels (Beech et al., 1993a,b; Zhang and Bolton, 1995).

The present study identified the 26 pS glibenclamide-sensitive K^+ channel as a target for levosimendan to hyperpolarize rat mesenteric arterial myocytes. Levosimendan did not activate the channel unless UDP was present. This characteristic of the channel is quite similar to that of the 20–24 pS K_{NDP} channel (Beech et al., 1993a,b; Zhang and Bolton, 1995) or the 15 pS K_{ATP} channel (Kajioka et al., 1991), in terms of the requirement of nucleotide diphosphates for channel activation by pinacidil. We recently found, in both whole-cell and single-channel recordings, that levosimendan, as with vascular smooth muscle cells, also activated cardiac K_{ATP} channels synergistically with nucleotide diphosphates (Yokoshiki et al., 1997b). Thus, the 26 pS glibenclamide-sensitive K^+ channel recorded in the present study could be referred to as a K_{ATP} channel.

Levosimendan acts as a phosphodiesterase inhibitor because relatively high doses increase cAMP level in cardiac tissues (Edes et al., 1995). K_{ATP} channels in vascular smooth muscle cells are stimulated by protein kinase A or cGMP (presumably via protein kinase G) (Miyoshi and Nakaya, 1993; Kubo et al., 1994; Miyoshi et al., 1994; Quayle et al., 1994; Murphy and Brayden, 1995). Thus, the vasodilation produced by levosimendan may be mediated through its hyperpolarizing action and its phosphodiesterase-inhibitory action. The hyperpolarizing action of levosimendan cannot be attributed to a phosphorylation mechanism because H-7, a non-specific inhibitor of protein kinases, did not prevent the effects of levosimendan in the present study.

In patients with moderate left ventricular dysfunction, a high dose (4 mg) of levosimendan decreased systemic vascular resistance and pulmonary capillary wedge pressure and increased cardiac output (Lilleberg et al., 1995), suggesting both vasodilatory and positive inotropic actions. However, low doses (0.25–0.5 mg) of levosimendan increased cardiac output without significant change in systemic vascular resistance and heart rate (Lilleberg et al., 1995). Therefore, the vasodilatory action of levosimendan may be achieved at a higher dose compared to that for positive inotropy.

In summary, levosimendan hyperpolarized arterial smooth muscle cells, probably through activation of a glibenclamide-sensitive K^+ channel. This action may, at least in part, contribute to the vasodilating action of levosimendan. However, a word of caution concerns the fact that glibenclamide also exerts some non-specific effects that could complicate the interpretation (Sheppard and Welsh, 1992; Beech et al., 1993b; Tominaga et al., 1995).

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