The Novel Calcium Sensitizer Levosimendan Activates the ATP-Sensitive K⁺ Channel in Rat Ventricular Cells

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ABSTRACT
Levosimendan, a new Ca⁺⁺-sensitizing and positive inotropic agent, was reported to act as a coronary vasodilator and protect ischemic myocardium. To elucidate the mechanisms of these actions, the possible electrophysiological effects of levosimendan on isolated rat ventricular cells were examined by the patch-clamp technique with whole-cell and single-channel recordings. Levosimendan (3 and 10 μM) markedly shortened action potential duration and activated an outward current at potentials positive to −70 mV. The increased current was abolished by glibenclamide, a blocker of the ATP-sensitive K⁺ (KATP) current. Stimulation of KATP current was dose dependent, with an EC₅₀ value of 4.7 μM; a maximal effect occurred at 30 μM. The L-type Ca⁺⁺ current was not affected by levosimendan (0.2–10 μM). In single-channel current recording in open cell-attached patches, KATP channels, which had been inhibited by 0.3 mM ATP, were activated by levosimendan. However, levosimendan did not stimulate the KATP channels that exhibited high spontaneous activity in ATP-free solution. Levosimendan also could not stimulate KATP channels that had rundown in ATP-free solution. However, levosimendan could stimulate rundown KATP channels that were reactivated by nucleotide diphosphates. KATP channels inhibited by 0.5 mM ADP; a nonhydrolyzable ATP analog, were not stimulated by levosimendan; however, the channels were stimulated by levosimendan in the presence of 30 to 50 μM ATP. Levosimendan stimulates cardiac KATP channels that are suppressed by intracellular ATP. It appears that levosimendan acts synergistically with nucleotide diphosphates. These properties of levosimendan may help protect ischemic myocardium because activation of KATP channels by levosimendan would likely occur in ischemic regions in which intracellular ADP concentration is increased and intracellular ATP concentration is decreased.

The treatment of heart failure is very important in the field of cardiovascular medicine. Recent clinical trials with angiotensin-converting enzyme inhibitors demonstrated the improvement of quality of life and the reduction of mortality in patients with severe chronic heart failure (CONSENSUS Trial Study Group, 1987; SOLVD Investigators, 1991). However, results with positive inotropic agents, such as PDE inhibitors and bete I agonists, were disappointing in terms of excess morbidity and mortality without producing important clinical benefits (Packer, 1988; Packer et al., 1991).

A new class of cardiovascular drugs, the myofilament Ca⁺⁺ sensitizers, has been developed for the treatment of both acute and chronic heart failure (Packer, 1988; Nielsen-Kudsk and Aldershvile, 1995). Compared with previous positive inotropic agents that enhance intracellular Ca⁺⁺, the Ca⁺⁺-sensitizing drugs, including pimobendan, EMD 53998, MCI-154 and levosimendan, increase myocardial contractility by producing more force for a given amount of intracellular Ca⁺⁺ (Nielsen-Kudsk and Aldershvile, 1995). Among these agents, levosimendan has a unique property in that it binds to cardiac cTnC in a Ca⁺⁺-dependent manner (Pollesello et al., 1994; Haikala et al., 1995a, 1995b; Nielsen-Kudsk and Aldershvile, 1995). The positive inotropic effect, due to increased Ca⁺⁺ sensitivity of the contractile proteins, has been reported to be exerted at concentrations of 0.03 to 10 μM in skinned fibers from guinea pig papillary muscles (Edes et al., 1995; Haikala et al., 1995b). In addition, levosimendan causes vasodilation in both experimental animal models (Harkin et al., 1995; Pagel et al., 1995; Vegh et al., 1995) and clinical studies (Lilleberg et al., 1995; Sundberg et al., 1995; Vegh et al., 1995). Although levosimendan (0.1–0.3 μM) was suggested to act as a PDE inhibitor (Edes et al., 1995), this drug had favorable preventive effects against ventricular tachyarrhythmias induced by ischemia-reperfusion (Kaszała et al., 1994).

Although levosimendan (5–10 μM) increased the I_Ca(L) in guinea pig ventricular cells, presumably by the PDE inhibition (Virag et al., 1996; Boknik et al., 1997), little data are available concerning the electrophysiological effects of levosimendan. In the present study, we explored the effects of

ABBREVIATIONS: KATP, ATP-sensitive K⁺; NDP, nucleotide diphosphate; cTnC, cardiac troponin C; PDE, phosphodiesterase; APD, action potential duration; RP, resting potential; APA, action potential amplitude; I_Ca(L), L-type Ca⁺⁺ current; KCO, K⁺ channel-opening drug.

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levosimendan on membrane currents and action potentials in rat ventricular cells using the patch-clamp technique of whole-cell and single-channel recordings. Levosimendan shortened APD, and our data suggest this occurred through opening of KATP channels.

Methods

Young (10–18 days old) Sprague-Dawley rats were used for this study. The rats were handled in accordance with the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

Cell preparation. Freshly isolated single cells were prepared from ventricles of young rats as previously described (Yokoshiki et al., 1996). In brief, the rats were decapitated while under CO₂ anesthesia, and the hearts were removed and rinsed in oxygenated Tyrode’s solution and then immersed in Ca²⁺-free Tyrode’s solution for 20 min. After the spontaneous beatings had ceased, the ventricles were dissected, and small pieces were enzymatically digested for 50 min (37°C) in Ca²⁺-free Tyrode’s solution containing collagenase (1 mg/ml; Wako Chemicals, Osaka, Japan). The cells were mechanically dispersed in the modified KB solution at room temperature using a Pasteur pipette. The cell suspensions were stored in a refrigerator (4°C) and were used for 2 to 6 hr after isolation. Usually, 40% to 60% of the isolated cells were rod shaped in Tyrode’s solution (containing 1.8 mM Ca²⁺). Rod-shaped cells with smooth surfaces and clear cross-striations were selected for experiments. All experiments were performed at room temperature (20–22°C).

Table 1

Summary of changes produced by levosimendan in action potentials in rat ventricular cells

The action potential parameters after exposure to 10 μM levosimendan were assessed from the action potentials 10 sec before the cells became inexcitable. The rats were 10 to 18 days old.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>RP</th>
<th>APA</th>
<th>APD₂₆₀</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>6</td>
<td>−74.4 ± 0.7</td>
<td>119 ± 4.4</td>
<td>132 ± 29</td>
</tr>
<tr>
<td>Levosimendan (3 μM)</td>
<td>6</td>
<td>−75.3 ± 0.5</td>
<td>115 ± 5.9</td>
<td>61 ± 29*</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>−76.3 ± 0.5</td>
<td>119 ± 4.6</td>
<td>110 ± 35</td>
</tr>
<tr>
<td>Levosimendan (10 μM)</td>
<td>7</td>
<td>−77.1 ± 0.5*</td>
<td>100 ± 5.9*</td>
<td>11 ± 3.4*</td>
</tr>
</tbody>
</table>

APD₂₆₀, APD at −60 mV.

* P < .05 vs. control.

Fig. 1. Shortening of APD by levosimendan in young (days 10–18) rat ventricular cells in current-clamp mode. A, Action potentials recorded in current-clamp. Extracellular application of 10 μM levosimendan caused shortening of APD; this effect could be reversed after washing out (W.O.). Arrows, peak levels of the action potentials in d and e. B, Time courses of changes in APA, APD at −60 mV (APD₂₆₀) and resting potential (RP). a–h, Time points at which action potentials in A were recorded.

Fig. 2. Stimulation of K⁺ current by levosimendan in rat ventricular cells. A, Superimposed current traces evoked by voltage-clamp ramps (−40 mV/sec) between +40 mV and −100 mV. The outward current was rapidly increased by levosimendan (b) and intersected with the abscissa at −75 mV. The reversal potential (Vrev) was close to the EK (−83 mV), suggesting that most of the current stimulated by levosimendan was a K⁺ current. Glibenclamide (1 μM) abolished the levosimendan-stimulated currents (c). B, Time courses of the currents measured at 0 and −100 mV. a–c, Time points at which the currents in A were recorded.

Fig. 3. Dose-dependent activation of K⁺ currents by levosimendan. A, Current-voltage relations for the currents stimulated by different doses of levosimendan. The currents were evoked by ramp pulses and measured at 0 mV when the currents were maximally activated. Difference in currents between the maximal activated and control (before application of levosimendan) were plotted against each concentration. Number of cells studied are shown in parentheses. Data points were fitted by the Hill equation. The EC₅₀ value was 4.7 μM, and the Hill coefficient (nH) value was 2.3.
Levosimendan Activates K⁺ Channel

Results

Shortening of APD. Action potentials in young (days 10–18) rat ventricular cells were recorded in current-clamp at a stimulation rate of 0.1 Hz (fig. 1). Bath application of 10 μM levosimendan produced shortening of APD with slight hyperpolarization of RP (table 1). This effect usually started 1 to 5 min after exposure to levosimendan and reached a maximum within 1 to 2 min from the onset of the response. As shown in traces d and e of figure 1A, the cells exposed to 10 μM levosimendan became inexcitable at its maximal effect. This effect could be reversed after washing out of the levosimendan (fig. 1A, right). Levosimendan (3 μM) also abbreviated the APD (similar to trace c) but did not abolish excitability [i.e., the overshoot of the action potential (above the zero potential) remained]. These effects on the action potentials are summarized in table 1.

Stimulation of K_ATP current. Suppressed current traces evoked by voltage-clamp ramps are shown in figure 2A, and the time courses of the currents (measured at 0 and −100 mV) are illustrated in figure 2B. The ramp pulses were applied every 15 sec from +40 to −100 mV at 40 mV/sec. The outward current was rapidly increased by levosimendan. The average reversal potential (V_rev) was −83 mV (table 1), which was close to the equilibrium potential for K⁺ (E_K) value (−83 mV) [E_K = −59 mV × log (140/5.4)], suggesting that most of the current stimulated by levosimendan was a K⁺ current. This effect usually started 1 to 5 min after exposure to levosimendan and reached a maximum within 1 to 2 min from the onset of the response. Glibenclamide (1 μM), a relatively specific inhibitor of K_ATP channels, abolished the levosimendan-stimulated current (n = 4), as shown in figure 2. Therefore, the levosimendan-stimulated current is similar to a K_ATP current. Figure 3 gives the current-voltage relations for the currents produced by different doses of levosimendan (fig. 3A) and the dose-response relation (fig. 3B). The maximal activated current at 0 mV was measured, and the difference between the maximal-activated and control currents (before application of levosimendan) was plotted against each concentration (fig. 3B). Data points were fitted to the Hill equation: current increase = [x^nH(x^H + EC50^nH)] × E_max, where E_max is the maximal stimulatory effect, n_H is the Hill coeffi-

Fig. 4. Lack of effects of levosimendan on rat cardiac I_{Ca(L)}. I_{Ca(L)} was evoked every 15 sec by a test potential to +10 mV from a holding potential of −40 mV. Levosimendan (10 μM) had no effects on I_{Ca(L)}, which was markedly stimulated by the subsequent application of 1 μM isoproterenol (ISO).

143, KCl 5.4, NaH₂PO₄ 0.33, MgCl₂ 0.5, CaCl₂ 1.8, glucose 5.5 and HEPES 5, pH adjusted to 7.4 with NaOH. The modified KB solution was K-glutamate 50, KOH 20, KCl 40, taurine 20, KH₂PO₄ 20, MgCl₂ 3, glucose 10, EGTA 0.5 and HEPES 10, pH adjusted to 7.4 with KOH.

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). Action potentials and whole-cell currents were measured under 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, Sarasota, FL). 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 normal Tyrode’s solution.

The composition of the internal (pipette) solution for the whole-cell stage of an inverted microscope (TMD-Diaphot; Nikon, Tokyo, Japan). The bath was superfused with the normal Tyrode’s solution.
cient and EC_{50} is the concentration for half-maximal effect. The EC_{50} value was 4.7 \mu M, and the n_H value was 2.3. The E_{\text{max}} was calculated to be 46.8 pA/pF.

**No effect on I_{Ca(L)}**. I_{Ca(L)} was recorded under the condition in which all K^+ currents were blocked by extracellular TEA and 4-AP and intracellular Cs^+. Fast Na^+ current was also blocked by substitution of extracellular Na^+ with TEA. I_{Ca(L)} was evoked every 15 sec by a test potential to +10 mV from a holding potential of −40 mV. As shown in figure 4, bath application of 10 \mu M levosimendan had no effect on I_{Ca(L)}; peak current amplitude after a 5-min exposure to levosimendan was 96.5 ± 0.8% of control (n = 4). In contrast, isoproterenol (1 \mu M) markedly stimulated I_{Ca(L)} (204 ± 16% of control). Lower doses of levosimendan [0.2 \mu M (n = 5) and 1 \mu M (n = 4)] also had no effect on I_{Ca(L)} (0.2 \mu M, 94.4 ± 1.8% of control; 1 \mu M, 97.7 ± 1.0% of control).

**Stimulation of K_{ATP} channels**. Single-channel K^+ currents were recorded in the open cell-attached patch mode (symmetrical 150 mM K^+) from rat ventricular cells at various membrane potentials (fig. 5A). The K^+ channel activities appeared within 30 sec after the open cell-attached patches were made by mechanical disruption of one end of the cell in bath solution containing no ATP. The opening of the channels appeared in bursts, and the flickerings within bursts decreased when the membrane was depolarized, as previously reported (Kakei et al., 1985; Tung and Kurachi, 1991). The current-voltage relation for this channel is shown in figure 5B. The conductance of the unitary inward current was 80 pS (n = 4), and a slight inward rectification was observed at positive membrane potentials. The open-time histograms (at −80 mV) was fitted by a single exponential curve with a time constant of 1.3 msec (fig. 5C). The conductance and kinetic properties of this K^+ channel are similar to those of the K_{ATP} channel previously reported for cardiac cells (Kakei et al., 1985; Tung and Kurachi, 1991).

The effects of levosimendan on these K_{ATP} channels were examined in various conditions. Membrane potential was held at −80 mV. Bath application of 10 \mu M levosimendan stimulated the K_{ATP} channels, which had been inhibited by 0.3 mM ATP, and the channel activity was abolished by 10

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**Fig. 5.** Conductance and kinetic properties of the K_{ATP} channel currents in open cell-attached patch. A, K_{ATP} channel currents recorded in open cell-attached patch (symmetrical 150 mM K^+) from rat ventricular cells at various membrane potentials are given. Bath solution contained no ATP. B, Current-voltage relationship of the K_{ATP} channel. The slope conductance of unitary inward current was 80 pS (n = 4). C, Open-time histograms of the unitary K_{ATP} channel currents at −80 mV. The histogram at −80 mV was fitted by a single exponential curve with a time constant of 1.3 msec. Data were filtered at 2 kHz and sampled at 10 kHz.
μM glibenclamide (fig. 6). However, K<sub>ATP</sub> channels exhibiting high spontaneous activity in ATP-free solution were not stimulated by levosimendan (fig. 7A). Levosimendan also could not stimulate K<sub>ATP</sub> channels that had rundown (i.e., channel activity almost disappeared) in ATP-free solution (fig. 7B). However, levosimendan could stimulate rundown K<sub>ATP</sub> channels that were reactivated by 50 to 100 μM ADP (fig. 8A) or 3 mM UDP (fig. 8B). In three of 13 patches tested, these NDPs did not stimulate the rundown channels, whereas subsequent application of levosimendan reactivated the channels. Although K<sub>ATP</sub> channels inhibited by 0.5 mM AMP-PNP, a nonhydrolyzable ATP analog, were not stimulated by levosimendan (fig. 9A), the channels were activated in the presence of 30 to 50 μM ADP (fig. 9B). A summary of the relative open probability values of the K<sub>ATP</sub> channels is given in table 2.

**Discussion**

In the present study, we demonstrated that the new Ca<sup>2+</sup>-sensitizing positive inotropic agent levosimendan activated K<sub>ATP</sub> channel currents in rat ventricular myocytes under physiological conditions. In contrast, levosimendan (0.2–10 μM) had no effect on I<sub>Ca(L)</sub>. Single-channel recordings (in open cell-attached patches) showed that levosimendan stimulated the K<sub>ATP</sub> channels in the presence of ATP, whereas stimulation was not observed in channels exhibiting a high degree of spontaneous activity in ATP-free condition. Levosimendan also could not stimulate rundown K<sub>ATP</sub> channels. Although the channels inhibited by AMP-PNP, a nonhydrolyzable ATP analog, could not be activated by levosimendan, the presence of NDPs restored the ability of levosimendan to potentiate the channel activity. Furthermore, levosimendan synergistically stimulated the rundown K<sub>ATP</sub> channels when NDPs were present.

In addition to the Ca<sup>2+</sup>-sensitizing positive inotropic effect, levosimendan (0.1–100 μM) increased the cAMP level in guinea pig cardiomyocytes (Edes et al., 1995; Boknik et al., 1997). On the basis of these findings, levosimendan was suggested to produce PDE inhibition, and levosimendan was found to stimulate I<sub>Ca(L)</sub> (Virag et al., 1996; Boknik et al., 1997). On the contrary, in the present study, I<sub>Ca(L)</sub> of rat cardiomyocytes was not affected by levosimendan (0.2–10 μM). This discrepancy might arise from the different species studied, which have different PDE isozymes (Polson, 1996). For example, in isolated rat ventricular cells, several selective PDE III inhibitors (to which milrinone belongs) had little or no effect on cAMP level, but a PDE IV inhibitor, Ro 20-1724, had a potent action in increasing cAMP (Kelso et al., 1993). Thus, cAMP could still be hydrolyzed by the PDE IV isozyme (shown to be abundant in rat ventricle; Bode et al., 1991), even when the PDE III was inhibited. In addition, the basal level of cAMP could be different in the two cases.

The activation of K<sub>ATP</sub> channels by levosimendan is not due to any metabolic impairment of the cell because levosimendan (30 μM) did not change the activity of the myofibrillar ATPase that consumes ATP for cross-bridge cycling (Haikala et al., 1995b). Furthermore, I<sub>Ca(L)</sub>, which is metabolically regulated in several tissues (Sperlakos and Schnei- der, 1976; Irisawa and Kokubun, 1983; O'Rourke et al., 1992; Yokoshiki et al., 1997), was not affected by levosimendan (0.2–10 μM) in the present study.

The Ca<sup>2+</sup>-sensitizing effect of levosimendan in skinned

![Fig. 6. Stimulation of K<sub>ATP</sub> channels by levosimendan in the presence of ATP.](image-url)
be reported to increase dose-dependently from 0.03 to 10 μM (Edes et al., 1995; Haikala et al., 1995b). However, in intact muscles (guinea pig papillary muscles), high doses (>1 μM or 10 μM) of levosimendan produced lesser stimulation or even negative inotropy (Haikala et al., 1995b; Boknik et al., 1997). Therefore, stimulation of KATP channels observed in the present study may account for both the lesser stimulation (or negative inotropy) at the higher doses and the vasodilatory action.

Although levosimendan produced PDE inhibition at the doses of 0.1 to 0.3 μM (Edes et al., 1995), this drug prevented ventricular fibrillation induced by ischemia-reperfusion in anesthetized dogs (Kaszala et al., 1994), thought to be due to Ca2+ overload and subsequent triggered activity (Janse and Wit, 1989). Levosimendan also reduced ischemic damage induced by ligation of the coronary artery in isolated rabbit hearts (Rump et al., 1994a, 1994b), an effect observed even at 0.1 μM (Rump et al., 1994b). At this concentration, the increase in the coronary flow was relatively small, and the authors speculated that factors beyond coronary dilation might contribute to the anti-ischemic effects of levosimendan. On the other hand, opening of KATP channels is generally considered to be cardioprotective against ischemia-related events (Hearse, 1995). Therefore, our results may in part account for the antiarrhythmic and anti-ischemic effects reported in the previous studies (Kaszala et al., 1994; Rump et al., 1994a, 1994b) because activation of KATP channels by levosimendan would likely occur in ischemic regions in which intracellular ADP concentration ([ADP]i) is increased and intracellular ATP concentration ([ATP]i) is decreased.

The therapeutic concentration of levosimendan as a positive inotropic agent is thought to be ~50 ng/ml (i.e., 0.18 μM) in patients with left ventricular dysfunction (Lilleberg et al., 1995; Sandell et al., 1995). This value is lower than that required for KCO action of levosimendan in the present study. Therefore, levosimendan at this therapeutic concentration may produce little or no activation of KATP channels in human hearts under normal conditions. However, levosimendan might more readily activate the KATP channels in ischemic myocardium because the ratio of [ADP]i to [ATP]i ([ADP]i/[ATP]i) may be increased, as mentioned above. Because the synergistic action of levosimendan with ADP could be observed at low concentrations (30–50 μM ADP), levosimendan might protect ischemic myocardium more effectively than nicorandil, which required higher ADP concentrations (0.5 mM (Shen et al., 1991) to 1 mM (Jahangir et al., 1994)) for activation of KATP channels. However, one must be careful in extrapolating these experimental results to the clinical setting.

The site of action of KCOs remains unclear (Henry and

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**Fig. 7.** Lack of effect of levosimendan on KATP channels in the absence of ATP. KATP channels exhibiting high spontaneous activity (A) or run-down (B) in ATP-free solution were not stimulated by 10 μM levosimendan.
It might be simplest to assume that there is competition between KCOs and ATP at the ATP-binding site that closes the channel. The parallel shift produced by KCOs in the concentration-response curve for ATP inhibition of the channels (Thuringer and Escande, 1989; Nakayama et al., 1990; Ripoll et al., 1990) may support this hypothesis. However, the possibility exists that ATP and KCOs act at different sites with opposing effects on the channel. Because levsimendan was not able to stimulate the channel that had been inhibited by AMP-PNP, the competition hypothesis at the ATP-binding site (inhibitory) would not account for the $K^+$ channel-opening action of levosimendan.

A synergistic activation of $K_{ATP}$ channels by the KCO drug diazoxide and ADP was reported in pancreatic $\beta$ cells (Larrson et al., 1993). The authors concluded that channel stimulation by diazoxide is dependent on the binding of Mg-ADP to a cytosolic regulatory constituent of the channel. Therefore, the synergistic action with NDPs might be shared by other KCOs as in the case of levosimendan. In addition, the functional classification of KCOs has recently been postulated based on their mechanisms of action (Terzic et al., 1995). According to their model, KCOs were classified into three types because they seem to have at least three sites of action: (1) the ATP-binding unit (inhibitory), (2) the NDP-binding unit (stimulatory) and (3) the transducer unit. Nicorandil, a NDP-acting drug (“type 3” in their classification), is considered to act in the presence of NDP by both enhancing the maximal channel activity and decreasing sensitivity of $K_{ATP}$ channels to inhibition by ATP (Shen et al., 1991; Terzic et al., 1995). With respect to mechanism of action, levosimendan may act similarly to nicorandil. One difference is that activation of the channel by nicorandil was not observed in the

**Fig. 8.** Levsimendan synergistically activates run-down $K_{ATP}$ channels with nucleotide diphosphates (NDPs). After run-down of $K_{ATP}$ channels, 100 $\mu$M ADP (A) and 3 mM UDP (B) could stimulate the channels. Subsequent application of levosimendan (10 $\mu$M) produced further stimulation of the channels.
presence of ATP (0.5 mM) unless ADP (0.5 mM) was also present (Shen et al., 1991). On the other hand, levosimendan could stimulate the channel in the presence of ATP alone, that is, in the absence of added ADP; however, some endogenous ADP was undoubtedly present. Because levosimendan could antagonize the channel inhibition by AMP-PNP if ADP (30–50 mM) were present, it might be possible that levosimendan synergistically activated the channels with a small amount of ADP generated by ATP hydrolysis in the vicinity of the channels. For example, hydrolysis of ATP occurs continuously within the sarcolemmal membrane in association with cellular activities such as Na⁺-K⁺ pump, Ca²⁺-ATPase (Sperelakis, 1995) and actin filament assembly (Stossel, 1993) to maintain homeostasis.

In summary, levosimendan shortened APD in rat ventricular cells, presumably through opening of K_{ATP} channels.

### TABLE 2
Summary of effects of levosimendan on K_{ATP} channels in rat ventricular cells recorded in open-cell attached patches

Relative P_o values were calculated by dividing the P_o value by that obtained in ATP-free solution. The P_o values were obtained from 45 to 60 sec of continuous recordings. Rats were 10 to 18 days old.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Levosimendan 10 μM</th>
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<tbody>
<tr>
<td>ATP-free</td>
<td>6 1.00 ± 0.00</td>
<td>0.87 ± 0.25</td>
</tr>
<tr>
<td>ATP 0.3 mM</td>
<td>6 0.10 ± 0.06</td>
<td>1.45 ± 0.27</td>
</tr>
<tr>
<td>AMP-PNP 0.5 mM</td>
<td>5 0.03 ± 0.02</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>AMP-PNP + ADP 30–50 μM</td>
<td>6 0.01 ± 0.01</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>Rundown</td>
<td>5 0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Stimulation of activity after rundown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP 50–100 μM</td>
<td>7 0.28 ± 0.17</td>
<td>1.08 ± 0.31</td>
</tr>
<tr>
<td>UDP 3 mM</td>
<td>6 0.34 ± 0.15</td>
<td>1.17 ± 0.25</td>
</tr>
</tbody>
</table>

P_o, open probability; n, number of patches tested.

* P < .05 vs. control (before application of levosimendan).

![Fig. 9.](image) Effect of levosimendan on K_{ATP} channels in the presence of AMP-PNP, a nonhydrolyzable ATP analog. A, Levosimendan (10 μM) had no effect on K_{ATP} channels inhibited by 0.5 mM AMP-PNP. However, subsequent application of 10 μM levosimendan in the presence of 0.3 mM ATP was capable of stimulating the channels. B, K_{ATP} channels inhibited by AMP-PNP were activated by levosimendan when ADP (30–50 μM) was present in the bath solution.
This stimulation of K\textsubscript{ATP} channels by levosimendan may result from the synergetic action with NDPs.

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References


