Levosimendan: Effects of a Calcium Sensitizer on Function and Arrhythmias and Cyclic Nucleotide Levels during Ischemia/Reperfusion in the Langendorff-Perfused Guinea Pig Heart

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ABSTRACT

The majority of clinically used inotropes act by increasing cytosolic calcium levels, which may hypothetically worsen reperfusion stunning and provoke arrhythmias. We tested the hypothesis that the calcium sensitizer levosimendan (levo) given during ischemia alone or ischemia and reperfusion would improve reperfusion function without promoting arrhythmias. The Langendorff-perfused guinea pig heart, subjected to 40-min low-flow ischemia (0.4 ml/min) with or without levo (10–300 nM) during ischemia or ischemia/reperfusion was used. Left ventricular developed pressure (LVDP) was used as an index of mechanical function. The effect of levo (300 nM) or dobutamine (0.1 μM) on the incidence of ischemia/reperfusion arrhythmias was also investigated. Control hearts (vehicle-perfused) had LVDPs of 69.4 ± 2.1 mm Hg whereas hearts treated with levo during ischemia and reperfusion (300 nM) had LVDPs of 104.5 ± 2.7 mm Hg (p < .05). Hearts treated with levo during ischemia alone (10 nM) had reperfusion LVDPs of 95.8 ± 4.2 mm Hg (p < .05) after 30-min reperfusion. Hearts treated with both levo and 10 μM glibenclamide (K\textsubscript{ATP} channel blocker) during ischemia had reperfusion LVDPs of 73.4 ± 4.3 mm Hg after 30-min reperfusion. Of control hearts, 25% developed reperfusion ventricular tachycardia but not ventricular fibrillation. Levo-treated hearts had no ischemia/reperfusion arrhythmias whereas 83% (p < .05 versus control) of dobutamine-treated hearts developed ventricular tachycardia and 33% (p < .05 versus levo) developed reperfusion ventricular fibrillation. Levo improved reperfusion function without promoting arrhythmias in this model. This was possibly achieved by opening the K\textsubscript{ATP} channels during ischemia and sensitizing myocardial contractile apparatus instead of elevating cytosolic calcium levels in reperfused hearts.

The reduced contractile function associated with heart failure has been linked to reduced responsiveness of the myofilaments to calcium (Marban et al., 1990). The treatment of heart failure with inotropic agents such as catecholamines or by phosphodiesterase (PDE) inhibition, which acts primarily by increasing cyclic adenosine monophosphate (cAMP) levels and cytosolic calcium levels, is effective but has the risk of worsening the oxygen demand/supply balance and of being pro-arrhythmic. Recent experimental evidence suggests that interventions aimed at increasing cytosolic calcium during ischemia and early reperfusion may exacerbate myocardial ischemic and reperfusion injury (Nayler et al., 1979; Kusuoka et al., 1987; Steenbergen et al., 1987; Du Toit and Opie, 1992).

Calcium sensitizers belong to a new class of positive inotropic agents that has generated renewed interest in the treatment of heart failure. In contrast to most of the routinely used inotropes, these compounds when given in low concentrations do not increase cytosolic calcium concentrations but rather increase the sensitivity of the contractile apparatus to calcium (Haikala et al., 1995a,b, 1997). Levosimendan (levo) is also a PDE inhibitor (Edes et al., 1995) and a K\textsubscript{ATP} channel opener (Yokoshiki et al., 1997) when used in higher concentrations. Haikala and coworkers (1995b) recently demonstrated that levo increases myocardial calcium sensitivity without increasing myosin ATPase activity. This would suggest that hearts treated with levo should be capable of performing more work under normal and ischemic conditions without consuming additional ATP. Levo could, therefore, by virtue of its potential ATP sparing and its K\textsubscript{ATP} channel-opening properties, be a cardioprotective inotrope. It may protect the ischemic myocardium while at the same time improving reperfusion mechanical function without elevat-

ABBREVIATIONS: PDE, phosphodiesterase; levo, levosimendan; dobut, dobutamine; LVDP, left ventricular developed pressure; glib, glibenclamide; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; VT, ventricular tachycardia; VF, ventricular fibrillation; HR, heart rate; CF, coronary flow; LDH, lactate hydrogenase; PCR, phosphocreatine.
ing cytosolic calcium levels as is the case with conventional inotropes.

We have developed a hypothesis that the increase in cytosolic Ca\(^{2+}\) levels during ischemia can be lessened by increasing tissue cyclic guanosine monophosphate (cGMP) levels in the ischemic heart (Du Toit et al., 1998). This proposal is supported by data showing that increased cGMP levels reduce the inward calcium current in isolated myocytes (Hartzell and Fischmeister, 1986; Sumii and Sperelakis, 1995).

We tested the hypotheses that levo protects the ischemic heart and improves reperfusion function and arrhythmias. We also proposed that this cardioprotection was due to the opening of the sarcolemmal K\(_{ATP}\) channels, and/or levo-induced changes in the cAMP-to-cGMP ratios in the ischemic heart. We investigated the effects of levo during ischemia or during ischemia and reperfusion on ischemic and reperfusion left ventricular developed pressure (LVDP) and on ischemic and reperfusion arrhythmias. We also measured ischemic tissue cAMP, cGMP, and tissue ATP, phosphocreatine (PCr), and lactate levels in these hearts.

**Materials and Methods**

Guinea pigs weighing 250 to 300 g were sacrificed by cervical dislocation and hearts were rapidly excised and placed in ice-cold Krebs-Henseleit buffer. Hearts were transferred to the Langendorff perfusion apparatus where they were perfused with a Krebs-Henseleit buffer equilibrated with 95% CO\(_2\) and 5% O\(_2\) at 37°C (121.5 mmol/l NaCl, 3.8 mmol/l KCl, 1.2 mmol/l MgCl\(_2\), 15.5 mmol/l NaHCO\(_3\), 1.2 mmol/l KH\(_2\)PO\(_4\), 2.0 mmol/l Na-6H\(_2\)O, 2.5 mmol/l CaCl\(_2\), 15.5 mmol/l NaHCO\(_3\), 2.0 mmol/l Na-pyruvate, 11.0 mmol/l glucose, 16.0 mmol/l mannitol) at a perfusion pressure of 75 cm of H\(_2\)O. All animals received humane care in accordance with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health publication no. 80–23, revised 1978).

**Experimental Model and Protocol Used**

Hearts were mounted on a Langendorff perfusion apparatus and retrograde perfusion was initiated within 30 s of excision of the heart from the animal. After a 10-min equilibration period, the experiment was commenced. During equilibration, the left ventricle was fitted with a “cling-film” left ventricular balloon inflated to a diastolic pressure of 4 to 6 mm Hg. The balloon was attached to a Gould P23ID pressure transducer that was linked to a Lectromed Multitrace 2 chart recorder (Rue-Fondon, Channel Islands.).

Left ventricular diastolic and systolic pressure, coronary flow (CF), and heart rate (HR) were measured at 10, 20, and 30 min. After 30 min, hearts were subjected to 40-min low-flow ischemia (flow rate 0.4 ml/min). During low-flow ischemia hearts were perfused (constant flow) with buffer (placebo) or buffer containing a selected concentration of levo (Orion Pharma, Espoo, Finland). At the end of ischemia, hearts were reperfused in the Langendorff mode (constant pressure) and function was measured at 10-, 20-, and 30-min reperfusion.

To monitor the incidence of ischemic and reperfusion arrhythmias, the second channel of the chart recorder was used to monitor the electrocardiograph throughout the experiment in selected series of hearts. Electrocardiograph electrodes were attached to the aorta and the apex of the heart and connected to the chart recorder.

All chemical compounds were made up on the day of the experiment and levo (0.01, 0.03, 0.1, and 0.3 μM), dobutamine (dobut; 10 or 0.1μM; Eli Lilly, Johannesburg, South Africa), or glibenclamide (glib; 10 μM; Hoechst Pharmaceuticals, Frankfurt, Germany), or vehicle was infused into the aortic cannula directly above the heart by means of a Gilson (Worthington, OH) low-flow infusion pump.

Levo and glib were dissolved in dimethyl sulfoxide (highest concentration 0.01%, v/v).

**Levo Concentration-Response Curve (Fig. 1).** The concentration-response curve for levo in the guinea pig heart was constructed by consecutive 5-min infusions of levo (1.0–1000 nM) to the perfusion solution directly above the heart (n = 8).

**Effects of Levo on Ischemic and Reperfusion Function**

1. Levo (10, 30, 100, or 300 nM) was infused during low-flow ischemia and on reperfusion (n = 6–14).
2. Levo (10, 30, 100, or 300 nM) or dobut (0.1 μM) was given during low-flow ischemia and withdrawn from the perfusion buffer on reperfusion (n = 6–8).
3. To determine whether the cardioprotective properties of levo are related to its proposed ability to open the K\(_{ATP}\) channel, hearts were perfused with glib (10 μM; a K\(_{ATP}\) channel blocker) plus levo (10 or 30 nM) during ischemia alone (n = 6).
4. A series of hearts were treated with levo (300 nM) and glib (10 μM) during ischemia and reperfusion (n = 6).
5. Hearts that were treated with dobut (0.1 μM) during ischemia and reperfusion.

**Istemic Contracture.** During ischemia, left ventricular diastolic pressure was measured to monitor the percentage of ischemic contracture.

**Arrhythmia Study.** A series of experiments were performed where the electrocardiograph was monitored throughout the experiment (n = 6–8). Three groups of hearts were done:

1. Control hearts where no drug was used.
2. Hearts that were treated with levo (300 nM) during ischemia and reperfusion.
3. Hearts that were treated with dobut (0.1 μM) during ischemia and reperfusion.

**Collection of Tissue Samples for Biochemical Assays.** In a separate series of experiments, hearts were perfused for the stabilization period (30 min) before low-flow ischemia was initiated. After 10, 20, 30, or 40 min of low-flow ischemia with vehicle, levo (30 nM), the approximate EC\(_{50}\) for levo), or levo (30 or 10 nM) plus glib (10 μM). Hearts were freeze-clamped with Wollenberger tongs at −40°C for the determination of tissue cAMP, cGMP, ATP, PCr, and lactate levels in these hearts (n = 6–8).

**Coronary Effluent Sampling.** For the last 5 min of the preischemic perfusion and throughout ischemia and reperfusion, coronary effluent samples were taken for the determination of coronary effluent lactate dehydrogenase (LDH) content. Sample 1, 25 to 30 min; sample 2, 31 to 45 min; sample 3, 46 to 60 min; sample 4, 61 to 70 min; sample 5, 71 min; sample 6, 72 and 73 min; sample 7, 74 and 75 min; sample 8, 76 to 80 min; sample 9, 81 to 100 min.

**Biochemical Analysis**

Myocardial tissue ATP and PCr levels were determined by enzymatic methods (Lamprecht and Trauthold, 1963) on a Cobas-Fara spectrophotometer (Roche, Switzerland) using Sigma (St. Louis, MO) chemicals and Roche (Nutley, NJ) assay kits. The cAMP and cGMP levels were determined using radioimmunooassay kits obtained from Amersham Corp. (Amersham, UK).

For cGMP assays, freeze-clamped hearts were freeze-dried and 10 to 15 mg of dry tissue was extracted in 5% trichloroacetic acid. The extracted sample was ether-washed three times for 5-min wash cycles. These samples were diluted 1:10 (v/v) and acetylated for the \(^{125}\)I-labeled cGMP assay. The IC\(_{50}\) for the cGAMP assay was 25 pmol/tube.

For the cAMP assays, 10 to 15 mg freeze-dried samples were extracted with perchloric acid, neutralized, and assayed. The IC\(_{50}\) for this assay was 1.92 nmol/tube.

**Statistical Methods**

Significance between groups was determined by ANOVA followed by the Bonferroni’s test for multiple comparisons. For incidence of
arrhythmias, the Fisher's exact test was used. For paired comparisons the Student's $t$ test was used. $p < .05$ was considered significant.

**Definition of Arrhythmias**

Ventricular tachycardia (VT) was defined as three or more consecutive, morphologically similar ventricular extrasystoles. Ventricular fibrillation (VF) was defined as more than six consecutive ventricular complexes showing complete morphological irregularity (Du Toit and Opie, 1993).

**Results**

**Concentration Response (Fig. 1)**

Levo had positive inotropic effects in the concentration range between 1 and 300 nM (approximate EC$_{50}$, 30 nM) in the isolated guinea pig heart. At 300 nM, levo increased the LVDP from 108.6 ± 0.9 to 131.2 ± 3.1 mm Hg.

**Effect of Levo Perfusion on Reperfusion Function**

**Effect of Levo or Dobut Given during Ischemia and Reperfusion on Reperfusion Function** (Fig. 2). Levo given during ischemia and reperfusion improved reperfusion LVDP when used at concentrations of 300 and 100 nM. Control hearts developed a left ventricular pressure of 66.2 ± 4.4 mm Hg after 10-min reperfusion, 69.5 ± 1.9 mm Hg after 20-min reperfusion, and 69.3 ± 2.0 mm Hg after 30-min reperfusion. Hearts perfused with 300 nM levo during ischemia and reperfusion had reperfusion LVDPs of 100.1 ± 1.1, 102.6 ± 1.5, and 101.3 ± 1.8 mm Hg ($p < .002$ versus control at all three time points), respectively.

Dobut (0.1 μM, equipotent inotrope to 300 nM levo) given during ischemia and reperfusion had no effect on reperfusion function. Reperfusion LVDPs were 81.5 ± 5.0, 79.8 ± 5.1, and 78.4 ± 5.6 mm Hg after 10-, 20-, and 30-min reperfusion, respectively.

All concentrations of levo increased reperfusion CF rates when given during ischemia and reperfusion (Table 1).

Both levo (100 and 300 nM) and dobut (0.1 μM) given during ischemia and reperfusion increased reperfusion HR compared with control hearts.

**Effect of Levo or Dobut Given during Ischemia Alone on Reperfusion Function** (Fig. 3). Levo at concentrations of 10 and 30 nM given during ischemia alone improved reperfusion function. Levo (10 nM) improved reperfusion LVDP from 63.8 ± 7.1 mm Hg in control hearts to 97.8 ± 5.7 mm Hg ($p < .05$) in levo-treated hearts at 10-min reperfusion, 69.8 ± 2.8 to 95.8 ± 4.6 mm Hg ($p < .002$) at 20-min reperfusion, and from 70.1 ± 3.2 to 95.8 ± 4.2 mm Hg ($p < .05$ versus control) at 30-min reperfusion. Dobut (0.1 μM) given during ischemia alone had no effect on reperfusion function (LVDP).

Reperfusion CFs and HRs were no different in control hearts and hearts treated with levo or dobut during ischemia alone.

The use of both levo (10 or 30 nM) and glib (10 μM, a $K_{ATP}$ channel blocker) during ischemia completely abolished the cardioprotective effect of levo (Fig. 4). Glib had no inotropic effect on the nonischemic heart at the concentrations used in this study.

Levo (300 nM) and glib (10 μM) given during ischemia and reperfusion resulted in a small but nonsignificant improvement in reperfusion function when compared with control hearts (Fig. 4).
Ischemic Contracture

Ischemic diastolic pressure was 2.7 ± 1.6 mm Hg in control hearts, and 6.5 ± 3.3 mm Hg (10 nM), 8.2 ± 2.3 mm Hg (30 nM), 4.8 ± 1.3 mm Hg (100 nM), and 2.4 ± 0.9 mm Hg (300 nM) for each of the groups treated with the drug during ischemia. These elevated diastolic pressures during ischemia returned to baseline (4 mm Hg) within the first 5 min of reperfusion.

Effect of Levo (30 nM) or Dobut (0.1 µM) on Incidence of Reperfusion Arrhythmias (Fig. 5).

None of the hearts monitored for arrhythmias developed ischemic arrhythmias. Control hearts developed reperfusion VT but not VF. The incidence of VF was 25% and the time in VT was 2.6 ± 1.9 s (n = 8). Hearts treated with levo had no ischemic or reperfusion arrhythmias. The incidence of both reperfusion VT and VF was 0% (n = 6). Heart treated with dobut had no ischemic arrhythmias but developed both reperfusion VT and VF. Incidence of VT was 83% (*p < .05 versus control) and time in VT was 7.0 ± 2.6 s; incidence of VF was 33% (*p < .05 versus control) and time in VF was 5.0 ± 3.4 s (*p < .05 versus control).

Effect of Levo (30 nM) Alone or Levo (30 nM) and Glib (10 µM) on Ischemic cAMP, cGMP, Lactate, ATP, and PCr Levels

cAMP (Fig. 6). Levo increased cAMP levels in the ischemic guinea pig heart from 0.71 ± 0.05 nmol/g in control hearts to 1.02 ± 0.08 nmol/g (p < .05) in levo-treated hearts after 10-min ischemia and from 0.64 ± 0.04 nmol/g in controls to 0.93 ± 0.06 nmol/g (p < .05) in treated hearts after 20-min ischemia. After 40-min ischemia, cAMP levels were 0.44 ± 0.04 nmol/g in control hearts and 0.67 ± 0.04 nmol/g (p < .05) in levo-treated hearts.
Glib caused a delay in the levo-induced increase in ischemic cAMP levels. After 40-min ischemia, however, these hearts had cAMP levels similar to those of levo-treated hearts.

Ischemic tissue cGMP levels were unaltered by levo. The combination of levo and glib increased cGMP levels to \(26.13 \pm 1.28\) pmol/g after 20-min ischemia, \(27.63 \pm 0.69\) pmol/g after 30-min ischemia (\(p < .05\)), and \(28.69 \pm 0.55\) pmol/g after 40-min ischemia (\(p < .05\)).

**Tissue ATP and PCr Levels (Table 2).** Levo delayed the ischemia-induced decrease in tissue ATP levels. Tissue ATP levels tended to be lower in control hearts than levo-treated hearts after 10-min ischemia. After 20-min ischemia, tissue ATP levels were \(3.53 \pm 0.19\) \(\mu\)mol/g in levo-treated hearts.
Fig. 5. Incidence (\%; presented in box above bar graph) and duration (s) of reperfusion VT and VF in control hearts (n = 8) and hearts treated with levo (300 nM; n = 6) or dobut (0.1 µM; n = 6) during ischemia and reperfusion (*p < .05).

Fig. 6. Ischemic tissue cAMP levels for control hearts (n = 8) and hearts treated with levo (30 nM; n = 8) or levo and glib (10 µM; n = 6). *p < .05.
and 2.76 ± 0.29 μmol/g in control hearts (p < .05). After 30-min ischemia, ATP levels were 2.93 ± 0.28 μmol/g for controls and 3.52 ± 0.28 μmol/g for levo-treated hearts. After 40-min ischemia, ATP levels were 2.98 ± 0.16 μmol/g for controls and 3.52 ± 0.23 μmol/g for levo-treated hearts (p < .05).

**Tissue Lactate (Table 2).** Ischemic tissue lactate levels were higher in control hearts than in levo-treated hearts. After 10-min ischemia, lactate levels were 8.2 ± 0.6 mmol/g in controls and 6.2 ± 0.4 mmol/g (p < .05) in levo-treated hearts. By 20-min ischemia, these values were 9.8 ± 0.3 mmol/g and 8.4 ± 0.3 mmol/g (p < .05), respectively.

**LDH Release during Ischemia and Reperfusion**

There was a direct dose-dependent increase in LDH release in the coronary effluent of levo-treated hearts during reperfusion (Fig. 8). At 300 nM, levo increased LDH release during reperfusion compared with controls, although not significantly. LDH release during the 4th and 5th min of reperfusion of 10 nM levo-treated hearts (121.6 ± 15.5 mU/ml/min) was reduced compared with controls (170.0 ± 16.9 mU/ml/min; p < .05).

**Discussion**

The data obtained from this study indicate that levo, notwithstanding its positive inotropic effects, has cardioprotective properties in the isolated ischemic guinea pig heart. Hearts perfused with levo during ischemia, or ischemia and reperfusion, had improved reperfusion LVDP when compared with control-untreated hearts. The improved reperfusion function was not at the expense of the electrical stability of these hearts. Although dobut treatment increased the in-
cidence of reperfusion arrhythmias, levo had no effect on the incidence of either ischemic or reperfusion arrhythmias. Levo also decreased ischemic tissue lactate levels and increased ischemic tissue ATP levels when compared with concurrent controls, which may suggest an energy-sparing effect of levo.

Myocardial stunning, which is defined as a reversible decrease in mechanical function after mild or brief ischemia (Bolli, 1990), is thought to be caused by an insensitivity of the myofilaments to calcium (Kihara et al., 1989; Kusuoka et al., 1990). This decrease in mechanical function can be reversed by conventional inotropes that act by increasing cytosolic calcium levels (Mercier et al., 1982; Becker et al., 1986). However, previous studies (Mercier et al., 1982; Du Toit and Opie, 1992) suggest that increasing cytosolic calcium levels during ischemia and/or early reperfusion may jeopardize the reperfused heart by increasing the severity of reperfusion-induced calcium overload and subsequent reperfusion stunning (Du Toit and Opie, 1992) and arrhythmias (Fitzpatrick and Karmazyn, 1984). Based on these data, we proposed that inotropes acting by sensitizing the myocardial contractile apparatus in the stunned heart may be more effective in preserving myocardial function and a normal heart rhythm in the ischemic and reperfused heart than conventional inotropes.

Levo is a calcium sensitizer at low concentrations (Häkala et al., 1995a, 1997) and has PDE inhibitory (Edes et al., 1995) and K\textsubscript{ATP} channel-opening properties (Yokoshiki et al., 1997a,b) at higher concentrations. It may also be classed as an inodilator (Opie, 1986) because it has both inotropic and vasodilatory properties. The results in this study suggest that, in addition to these known properties of levo, it may also be anti-ischemic because it delayed the decline in tissue ATP and accumulation of tissue lactate in hearts during 40-min low-flow ischemia.

It could be reasoned that these anti-ischemic effects were the direct result of vasodilation by levo during low-flow ischemia. However, during this period perfusion rate (CF) was controlled by an infusion pump. When hearts were reperfused with levo, reperfusion CFs were elevated, which could have contributed to the improved reperfusion function in these hearts. Hearts perfused with levo during ischemia alone, however, had normal reperfusion CFs but showed improved postischemic recoveries. These data suggest that although the increase in reperfusion CFs could have contributed to the improved recoveries in the former series of hearts, it played no role in the latter. Although we eliminated the vasodilatory benefits of levo by infusing the perfusate at a fixed rate during low-flow ischemia, we could not control or monitor flow distribution through the heart during levo treatment. We cannot exclude the possibility that levo caused a flow redistributed during ischemia and early reperfusion which then favored preservation of function in these hearts. We propose that, in addition to its vasodilatory benefits, levo also acts by some other anti-ischemic mechanism to reduce ischemic and reperfusion damage.

Recent findings showing that calcium-sensitizing drugs decrease the energy cost of excitation-contraction coupling (Goto et al., 1996) and, when used in high concentrations, is an ATP-sensitive K\textsuperscript{+} channel opener (Yokoshiki et al., 1997a), possibly go some way toward explaining the anti-ischemic properties of levo.

Cardioprotective Properties of Levo. The cardioprotective properties of K\textsubscript{ATP} channel openers such as cromakalim and pinacidil given to the isolated heart model

![Figure 8](image-url)
before or during ischemia are well documented (Grover et al., 1990; Cole et al., 1991; Galinanes et al., 1992). The exact mechanism underlying the KATP channel openers ability to confer protection to the heart is not clear but may be related to its ability to inhibit ischemic depolarization (Grover et al., 1990) and shorten action potential duration (Auchampach et al., 1992; Edwards and Weston, 1993). Both of the effects of these openers would be expected to reduce calcium entry and subsequent calcium overload via voltage-operated Ca2+ channels. KATP channel openers have been shown to reduce intracellular calcium levels and prevent calcium overload in the ischemic and reperfused myocardium (Behling and Malone, 1995). The absence of a negative inotropic effect with K+ channel openers in the nonischemic heart (Grover, 1994), however, suggest that these compounds do not have marked effects on cytosolic calcium levels in the nonischemic heart.

ATP-sensitive K+ channel openers have also been shown to preserve the energy status of the heart during ischemia (Grover et al., 1991; Hosoda et al., 1992). How these compounds decrease ATP consumption during ischemia is not clear but appears to be independent of vasodilation. In our study, levo increased ischemic ATP and decreased lactate formation without altering PCr. The latter was unexpected but the conservation of ATP is in agreement with those of Grover and coworkers (1991). K+ channel openers do not have significant cardiodepressant effects (Grover, 1994) and would not be expected to decrease energy expenditure on this basis. They act by some unexplained mechanism, possibly by opening the recently characterized mitochondrial KATP channels to increase the efficiency of oxygen and energy utilization of these hearts. Alternatively, the protection against an increase in cytosolic calcium levels during ischemia and reperfusion (Behling and Malone, 1995) may reduce the activity of sarclemmal calcium ATPase pumps needed to maintain calcium homeostasis in the ischemic heart.

In this study the use of the KATP channel blocker glib reversed the cardioprotective effects of levo (Fig. 4). Hearts treated with both levo and glib had reperfusion LVDPs similar to controls. These data suggest that one of the cardioprotective mechanisms of levo was the opening of the KATP channel.

Although glib plus levo (300 nM) given during ischemia and reperfusion resulted in reduced reperfusion function when compared with concurrent levo (300 nM)-treated hearts, the KATP channel blocker did not completely abolish the inotropic effect of levo. This would suggest that closing the KATP channels (presumably opened by levo) during ischemia neutralized the cardioprotective properties of levo during ischemia but did not affect the inotropic properties of the drug during reperfusion.

Evidence for Anti-Ischemic Effects with PDE Inhibitors. Besides its KATP channel-opening properties, levo also has PDE-inhibitory properties (Edes et al., 1995). Some studies (Jentzer et al., 1981; Rump et al., 1993, 1994) have demonstrated anti-ischemic effects associated with PDE inhibition. Jentzer and coworkers (1981) found that the PDE inhibitor amrinone reduced oxygen consumption in the in vivo failing dog heart. More recently, Rump and coworkers (1994) proposed that PDE inhibitors may act by increasing myocardial perfusion and that levo has oxygen-sparing properties. They could not explain the mechanism behind the anti-ischemic properties of these compounds. The evidence for a cardioprotective role for PDE inhibitors is outweighed by evidence to show that elevated cAMP inhibitors contribute to cytosolic calcium overload and exacerbate myocardial ischemic/reperfusion stunning (Du Toit et al., 1998) and arrhythmias (Fitzpatrick and Karmazyn, 1984; Lubbe et al., 1992).

Effect of a Conventional Inotrope and Levo on Ischemic and Reperfusion Arrhythmias. The pro-arrhythmic effect of elevated cAMP (Lubbe et al., 1992) and cytosolic calcium levels is well documented (Fitzpatrick and Karmazyn, 1984; Coetzee and Opie, 1988). The incidence of reperfusion arrhythmias was increased in hearts treated with dobut during ischemia and reperfusion. This positive inotrope, like most conventionally clinically used compounds, increases cAMP and cytosolic calcium levels, which promotes arrhythmias. Levo had no pro-arrhythmic properties, and no ischemic or reperfusion arrhythmias were documented in these hearts. These findings are possibly related to the fact that notwithstanding the PDE-inhibitory properties of levo, it also has KATP channel-opening properties that may reduce/prevent the elevation in cytosolic calcium levels associated with ischemia and reperfusion. These may negate the detrimental effects of PDE inhibitor-induced elevations in cAMP levels.

Effect of Levo on Cyclic Nucleotide Levels. Our findings indicating that relatively low concentrations of levo (30 nM) increase cAMP levels in the ischemic heart are in disagreement with the findings of Edes and coworkers (1995) who reported a nonsignificant increase in cAMP levels in levo-treated (30 nM) nonischemic hearts. This discrepancy in findings suggests that ischemia, which increases cAMP levels per se, may potentiate the PDE-inhibitory properties of this compound. We would suggest that the levo-induced elevations in cAMP levels documented in the present study were insufficient to play a significant role in ischemic/reperfusion injury. In studies currently being conducted in this laboratory (Du Toit et al., 1998), nitric oxide donors given to the ischemic heart elevate both cAMP and cGMP levels yet still confer protection to these ischemic hearts. This protection is independent of nitric oxide’s vasodilatory properties. Surprisingly, reperfusion function of hearts treated with levo during ischemia alone were inversely proportional to the concentration of the drug used (Fig. 3). The higher the concentration of levo used, the lower the reperfusion function recoveries were. These data suggest that when given during ischemia, the protective properties of the compound are countered by a simultaneous detrimental effect in a dose-dependent manner. This may be explained by the PDE-inhibitory properties of the compound. If levo is a PDE inhibitor, then the drug may elevate the cAMP levels in the ischemic heart in a dose-dependent manner. This could explain why the lowest concentration of levo (when given in ischemia alone) confers more protection than the highest concentration of the drug. These high concentrations would have elevated ischemic/CAMP levels more and cause more ischemic damage than concurrent hearts treated with low concentrations of the compound. Again, this elevation in cAMP was clearly not sufficient to neutralize the protective properties of the compound, as even the highest concentrations of levo still improved reperfusion function in these hearts.

The cGMP levels in levo-treated hearts were increased toward the end of 40-min ischemia when compared with
controls. These data are consistent with previous findings (Edes et al., 1995) albeit in the nonischemic heart in that particular study. The increase in cGMP levels seen in hearts treated with both levo and glib were unexpected and the significance of this increase in cGMP levels is not known. Data from this study would suggest that, contrary to our hypothesis, the cardioprotective properties of levo are not directly related to its ability to increase tissue cGMP levels.

Levo- and Dobut-induced Tissue Damage. LDH released from the ischemic and reperfused heart is often used as an index of the severity of ischemic and reperfusion injury. We found that the lower concentrations of levo reduced coronary effluent LDH levels, albeit to a nonsignificant extent. There was, however, a tendency (again not significant) for levo to increase LDH release during reperfusion in a concentration-dependent manner. This may suggest that, although the high concentration of levo in the reperfused heart increased heart function during reperfusion by virtue of its inotropic properties, it may also have caused some damage during reperfusion by increasing mechanical stress on the reperfused heart. Speculatively, this increase in the workload imposed on the heart by levo may be detrimental to a heart during more severe/prolonged ischemia. The use of dobut during ischemia and reperfusion increased coronary effluent LDH levels during reperfusion. The increase in LDH release was accompanied by a poor reperfusion function [relative to levo (300 nm)-treated hearts; Fig. 2] and an increase in the incidence of reperfusion arrhythmias. Although dobut had a positive inotropic effect on the reperfused heart, it also increased ischemic and reperfusion tissue damage, possibly by increasing tissue cAMP and calcium levels during ischemia and reperfusion.

We conclude that levo, whether given during ischemia alone or during ischemia and reperfusion, improves reperfusion function and arrhythmias in the isolated guinea pig heart. The cardioprotective effects of this compound are independent of its vasodilatory properties and appear to be unrelated to its effects on ischemic tissue cAMP and cGMP levels. The ATP-sensitive K⁺ channel-opening and possibly the PDE-inhibitory properties of this compound may be implicated.

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References


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