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The levosimendan metabolite OR-1896 elicits vasodilation by activating the K_{ATP} and BK_{Ca} channels in rat isolated arterioles

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1 We characterized the vasoactive effects of OR-1896, the long-lived metabolite of the inodilator levosimendan, in coronary and skeletal muscle microvessels.

2 The effect of OR-1896 on isolated, pressurized (80 mmHg) rat coronary and gracilis muscle arteriole (~150 μm) diameters was investigated by videomicroscopy.

3 OR-1896 elicited concentration-dependent (1 nM–10 μM) dilations in coronary (maximal dilation: 66±6%, relative to that in Ca^{2+}-free solutions; pD_2: 7.16±0.42) and gracilis muscle arterioles (maximal dilation: 73±4%; pD_2: 6.71±0.42), these dilations proving comparable to those induced by levosimendan (1 nM–10 μM) in coronary (maximal dilation: 83±6%; pD_2: 7.06±0.14) and gracilis muscle arterioles (maximal dilation: 73±12%; pD_2: 7.05±0.1).

4 The maximal dilations in response to OR-1896 were significantly ($P<0.05$) attenuated by the nonselective K^+ channel inhibitor tetraethylammonium (1 mM) in coronary (to 34±9%) and gracilis muscle arterioles (to 28±6%).

5 Glibenclamide (5 or 10 μM), a selective ATP-sensitive K^+ channel (K_{ATP}) blocker, elicited a greater reduction of OR-1896-induced dilations in skeletal muscle arterioles than in coronary microvessels.

6 Conversely, the selective inhibition of the large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) with iberiotoxin (100 nM) significantly reduced the OR-1896-induced maximal dilation in coronary arterioles (to 21±6%), but was ineffective in skeletal muscle arterioles (72±8%).

7 Accordingly, OR-1896 elicits a substantial vasodilation in coronary and skeletal muscle arterioles, by activating primarily BK_{Ca} and K_{ATP} channels, respectively, and it is suggested that OR-1896 contributes to the long-term hemodynamic effects of levosimendan.
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Abbreviations: BK_{Ca}, large conductance Ca^{2+}-activated K^+ channel; GLI, glibenclamide; IBTX, iberiotoxin; K_{ATP}, ATP-sensitive K^+ channel; PSS, physiological salt solution; TEA, tetraethylammonium

Introduction

Levosimendan, the (−) enantiomer of {[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl]hydrazono}propanedinitrile, is a Ca^{2+}-sensitizing cardiotonic agent. Besides its direct positive effects on the myocardial contractility, part of the hemodynamic benefit following levosimendan administration has been ascribed to vasodilation in the coronary conductance and resistance arteries (Michaels *et al*., 2005).

Previous clinical studies demonstrated a long-term improvement in mortality following short-term levosimendan administration (Follath *et al*., 2002; Moiseyev *et al*., 2002). This suggests the involvement of long-lived metabolites in mediating certain of the levosimendan-induced cardiovascular effects. Approximately 5% of levosimendan is converted to OR-1855, the (−) enantiomer of 4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenylamine, in the large intestine, and then to OR-1896, the (−) enantiomer of *N*-[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl] acetamide. Levosimendan has an elimination half-life of only 1 h (Sandell *et al*., 1995), whereas that of OR-1896 is about 75–80 h (Kivikko *et al*., 2002a). Moreover, 40% of OR-1896 is bound to plasma proteins (Kivikko *et al*., 2003), as compared with 98% for levosimendan (Sandell *et al*., 1995), and thus the active metabolite has a considerably larger free fraction than that of the parent drug. These pharmacokinetic features provide plausible explanations for the prolonged hemodynamic effects of levosimendan metabolites, which last for up to 7–9 days after discontinuation of a 24-h infusion of levosimendan (Kivikko *et al*., 2002b).

OR-1896 appears to be pharmacologically active and to exert a positive inotropic effect *via* Ca^{2+} sensitization in the myocardium (Takahashi *et al*., 2000a, b; Takahashi & Endoh, 2002; Papp *et al*., 2004; Szilagyi *et al*., 2004). Although OR-1896 has been suggested to account for the sustained vasodilation long after levosimendan withdrawal (Kivikko *et al*., 2003), no experimental studies have as yet addressed the effects of OR-1896 on the vascular dynamics.

Accordingly, in this study, we set out to characterize the direct vascular effects of OR-1896, and hence to furnish evidence for the vasodilator role of OR-1896 during

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levosimendan treatment. Changes in the spontaneously developing vascular tone in the presence of increasing OR-1896 concentrations were monitored in arterioles isolated from the coronary tree and skeletal muscles of the rat. This approach allowed a comparative assessment of the OR-1896-induced effects in the microcirculation of the heart and of the periphery. As levosimendan-induced vasodilation has been associated with the activation of ATP-sensitive K^+ (K_{ATP}) channels, and possibly other types of K^+ channels (Yokoshiki *et al*., 1997a; Pataricza *et al*., 2003; Hohn *et al*., 2004), modulators of K^+ channel functions were also employed. This facilitated the recognition of postulated similarities in the mechanisms of action of OR-1896 and levosimendan.

Our results reveal OR-1896 to be a strong vasodilator in coronary and skeletal muscle microvessels with a pharmacological potency similar to that of levosimendan. The suggested activation of K_{ATP} and large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) upon OR-1896 administration resembles previous experimental findings with levosimendan (Pataricza *et al*., 2003; Hohn *et al*., 2004). Our data therefore indicate similar pharmacological profiles for levosimendan and OR-1896 in their effects on the microvascular tone, and identify OR-1896 as a potential mediator of the long-term cardiovascular effects of levosimendan not only in the myocardium, but also in the peripheral vascular system.

Methods

Experiments were carried out on male Wistar rats ($n=23$, weighing $\sim$350 g). The animals were housed in the animal care facility at our university, and were fed standard rat chow and drank tap water *ad libitum* with a 12-h light–dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Debrecen, in accordance with guidelines set by the Hungarian Animal Protection Law and in compliance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. After overnight fasting, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}) and the gracilis muscle was removed. The animals were then euthanized by an additional injection of sodium pentobarbital (150 mg kg^{-1}), and the hearts were excised.

Isolation of coronary and gracilis muscle arterioles

With the use of microsurgical instruments and an operating microscope, the second branches of the septal coronary artery and the first branches of the gracilis artery ($\sim$1.5 mm in length) were isolated simultaneously and transferred into organ chambers containing two glass micropipettes filled with physiological salt solution (PSS), composed of (in mM) 110.0 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 1.0 $MgSO_4$, 1.0 KH_2PO_4, 5.5 glucose and 24.0 $NaHCO_3$, equilibrated with a gas mixture of 10% O_2 and 5% CO_2, balanced with nitrogen, at pH 7.4. In this study, 10% of oxygen was used in the superfusion bath, as higher concentrations of oxygen may cause excess free radical production, as suggested previously (Rubanyi & Vanhoutte, 1986). The vessels were cannulated at both ends and the micropipettes were connected with silicone tubing to a pressure servo control system (Living Systems Instrumentation, Burlington, VT, U.S.A.) to set the intraluminal pressure to 80 mmHg. The temperature was set at 37°C by a circulating bath temperature controller (Cole Parmer, Vernon Hills, IL, U.S.A.). Images were collected with a digital camera (CFW1310, Scion Corp., Frederick, MO, U.S.A.) connected to a microscope (Nikon, Eclipse 80i). The internal diameter at the midpoint of the isolated arterioles was measured offline by Image J software (NIH Image, Bethesda, MD, U.S.A.) (Koller & Bagi, 2002; Koller & Bagi, 2004).

Experimental protocols

During an incubation period of 1 h, a spontaneous myogenic tone developed in the isolated arterioles in response to the intraluminal pressure of 80 mmHg. The arteriolar responses to ACh (0.1 μM) were then recorded to assess the viability of the endothelium. Isolated arterioles in which either spontaneous arteriolar tone was absent or ACh-induced dilation was less than 70% were excluded from the experiments.

In the first series of experiments, cumulative concentrations of levosimendan (1 nM–10 μM) or OR-1896 (1 nM–10 μM) were administered to the coronary and skeletal muscle arterioles and the changes in diameter were measured.

The second series of experiments was performed to assess the contribution of K^+ channels to the OR-1896-mediated vascular responses. To this end, arterioles were incubated in the presence of the nonselective K^+ channel blocker tetraethylammonium (TEA, 1 mM for 30 min) (Tammaro *et al*., 2004) and the arteriolar responses to cumulative concentrations of OR-1896 were then obtained again. Measurements of the arteriolar responses to ACh (0.1 μM) bracketed these assays.

In a separate set of experiments, the potential role of K_{ATP} channels in OR-1896-induced vasodilation was specifically tested. First, the arteriolar responses to cumulative concentrations of the K_{ATP} channel opener pinacidil (0.1–10 μM) were recorded in the presence of glibenclamide (GLI; 1, 5 or 10 μM, for 30 min), a selective inhibitor of K_{ATP} channels (Cseko *et al*., 2004; Wu *et al*., 2004). The vascular diameters were then recorded in the presence of OR-1896 (1 nM–10 μM) before and after the application of GLI (5 and 10 μM in the gracilis and coronary arterioles, respectively). The arteriolar responses to ACh (0.1 μM) were also assayed in the presence of GLI.

In another series of experiments, the possible role of the BK_{Ca} channels in the OR-1896-mediated arteriolar responses was investigated. OR-1896-induced arteriolar dilation was followed before and after the application of iberiotoxin (IBTX, 100 nM, for 10 min), a selective blocker of BK_{Ca} channels (Richards *et al*., 2001; Pataricza *et al*., 2003). In this set of experiments, the arteriolar responses to ACh (0.1 μM) were also reassessed.

Drugs

Levosimendan and OR-1896 were from Orion Pharma, Espoo, Finland. All other salts and chemicals were from Sigma-Aldrich Co. (St Louis, MO, U.S.A.). Levosimendan, OR-1896, pinacidil and GLI were prepared by dissolution in dimethylsulfoxide (100 mM stock solutions), which were further diluted with ethanol. TEA and IBTX were diluted in distilled water (1 M and 10 μM stock solutions, respectively). Solutions were prepared on the day of the experiment. Drugs were added to

the vessel chamber and the final concentrations are reported. The maximum concentration of DMSO was 0.015% and that of ethanol was 0.5% in the bath, the concentrations that had no significant effect on arteriolar diameter. Vascular diameter changes were recorded for 2 min following drug application and the peak arteriolar responses were selected to illustrate the drug-induced effects.

Data analysis

Drug-induced arteriolar diameter changes were expressed as percentages of the maximal dilations (obtained on assessment of the passive diameter at 80 mmHg intraluminal pressure in Ca^{2+}-free PSS). Data were then fitted assuming the sigmoid dose–response curves by using GraphPad Prism Software (version 4.00 for Windows, San Diego, CA, U.S.A.) and after fitting pD_2 (the negative logarithm to base 10 of the EC_{50}) values were calculated. The myogenic tone of the arterioles was calculated from the active (AD, in Ca^{2+}-containing PSS) and passive (PD, in Ca^{2+}-free PSS) diameters at 80 mmHg as follows: [(PD−AD)/PD], expressed as percentage. Data are expressed as means ± s.e.m. Statistical analyses were performed with Student's *t*-test. $P<0.05$ was considered statistically significant.

Results

In isolated coronary and skeletal gracilis muscle arterioles, a spontaneous tone developed in response to an intraluminal pressure of 80 mmHg without the use of any vasoactive agent (Table 1). The basal arteriolar tone was enhanced in the coronary arterioles after incubation and in the presence of 1 mM TEA, but not in the presence of 10 μM GLI or 100 nM IBTX. In the skeletal muscle arterioles, the same concentrations of TEA or IBTX had no effect on the basal tone, but this was however significantly enhanced by GLI (5 μM). The vasodilation to ACh (0.1 μM) was not affected significantly by K^+ channel inhibitors in the coronary arterioles, whereas in the gracilis arterioles it was significantly reduced by TEA, but not by GLI and IBTX (Table 1). These findings indicated that, although certain K^+ channels might play a role in the modulation of the spontaneous arteriolar tone in both coronary and gracilis muscle arterioles, the presence of various K^+ channel inhibitors did not essentially limit the dilator capacity of these microvessels.

Effects of OR-1896 and levosimendan on coronary and skeletal muscle arteriolar diameters

The arteriolar responses to cumulative concentrations of OR-1896 and levosimendan were first compared in isolated coronary and skeletal muscle arterioles. OR-1896 (1 nM–10 μM) elicited substantial dilations in both the coronary (maximal dilation: 66 ± 6%; pD_2: 7.16 ± 0.42) and the gracilis muscle arterioles (maximal dilation: 73 ± 4%; pD_2: 6.71 ± 0.42) in a concentration-dependent manner (Figures 1 and 2). The extent of the dilation induced by OR-1896 at a concentration of 10 nM was significantly greater in the coronary than in the gracilis muscle arterioles (17 ± 4 and 9 ± 2%, respectively, $P<0.05$), whereas the degrees of dilation at any other

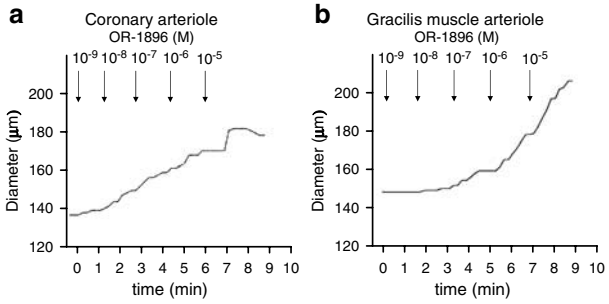

Figure 1 Representative traces of dilations of coronary (a) and skeletal (gracilis) muscle (b) arterioles in response to cumulative concentrations of OR-1896 (1 nM–10 μM).

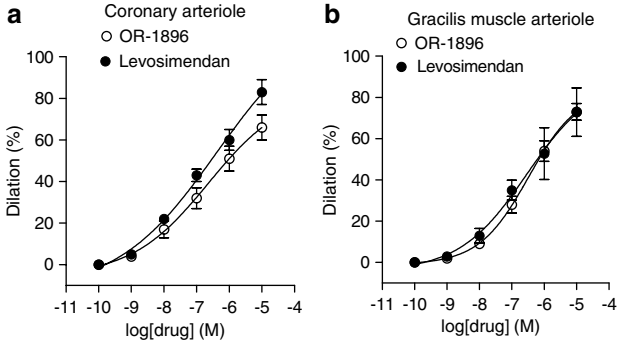

Figure 2 Percentage dilations of coronary (a) and skeletal muscle (b) arterioles in response to cumulative concentrations of OR-1896 ($n=17$–22) or levosimendan ($n=5$–6). The lines represent regression lines. Data are means ± s.e.m.

Table 1 Arteriolar diameters, calculated myogenic tone and ACh-induced responses in the absence and presence of various inhibitors of K^+ channels

	Ca^{2+}− n = *17–22*	*Ca^{2+}+* n = *17–22*	*TEA* n = *6–7*	*GLI* n = *6–7*	*IBTX* n = *5*
Coronary arteriolar diameter (μm)	197 ± 14	119 ± 11	90 ± 4	117 ± 27	88 ± 8
Myogenic tone (%)	—	40 ± 2	50 ± 4*	46 ± 6	40 ± 4
Dilation to ACh (0.1 μM, %)	—	90 ± 4	74 ± 12	57 ± 11	90 ± 5
Gracilis arteriolar diameter (μm)	224 ± 8	158 ± 9	166 ± 19	119 ± 13*	140 ± 5
Myogenic tone (%)	—	29 ± 2	28 ± 6	49 ± 3*	37 ± 7
Dilation to ACh (0.1 μM, %)	—	82 ± 2	59 ± 7*	70 ± 5	88 ± 5

Values are means ± s.e.m., *$P<0.05$ vs control diameter (Ca^{2+}+). Ca^{2+}−: in the absence of extracellular Ca^{2+}; Ca^{2+}+: in the presence of extracellular Ca^{2+}; TEA: tetraethylammonium; GLI: glibenclamide; IBTX: iberiotoxin.

concentration of OR-1896 were similar in the two types of vessels. The effective concentrations of OR-1896 were not significantly different in the coronary and skeletal muscle arterioles. Moreover, the magnitudes and effective concentrations of the OR-1896-induced dilations were not significantly different from those induced by levosimendan (1 nM–10 μM) either in the coronary (maximal dilation: 83±6%; pD_2: 7.06±0.14) or in the gracilis muscle arterioles (maximal dilation: 73±12%; pD_2: 7.05±0.1) (Figure 2).

Role of K^+ channels in mediating OR-1896-induced arteriolar dilation

In order to elucidate the role of K^+ channels in the OR-1896-induced arteriolar dilation, a nonselective K^+ channel inhibitor, TEA, was employed. In the presence of 1 mM TEA, the magnitude of the dilation in response to OR-1896 was attenuated markedly in both the coronary and the gracilis muscle arterioles (Figure 3).

In the next series of experiments, an attempt was made with selective inhibitors to specify the K^+ channel types involved in the OR-1896-induced arteriolar dilations. First, the role of K_{ATP} channels was addressed. Arteriolar responses to the K_{ATP} channel opener pinacidil (0.1, 1 or 10 μM) were obtained in the presence of different concentrations of GLI (1, 5 or 10 μM), a selective blocker of K_{ATP} channels (Figure 4a and b). These experiments related to the potential influence of K_{ATP} channel openings and their pharmacological inhibition on the microvascular tone. In the coronary arterioles the presence of 10 μM GLI, and in the gracilis arterioles the presence of 5 μM GLI resulted in comparable and significant reductions of the pinacidil-evoked arteriolar dilations (the maximal dilation after GLI administration in the coronary arterioles decreased from 68±7 to 35±6%, and in the gracilis arterioles it decreased from 90±2 to 22±5%). In the subsequent tests, therefore, the above GLI concentrations were used to test the OR-1896-evoked K_{ATP} channel openings. In the coronary

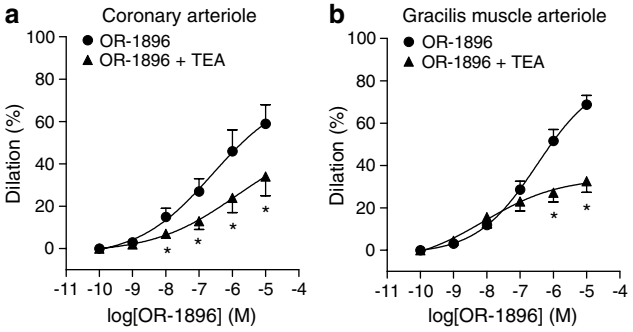

Figure 3 Percentage dilations of coronary (a) and skeletal muscle (b) arterioles in response to cumulative concentrations of OR-1896 before and after incubation with TEA (1 mM, $n=6$ in each group). The lines represent regression lines. Data are means±s.e.m. Asterisks indicate significant differences ($P<0.05$).

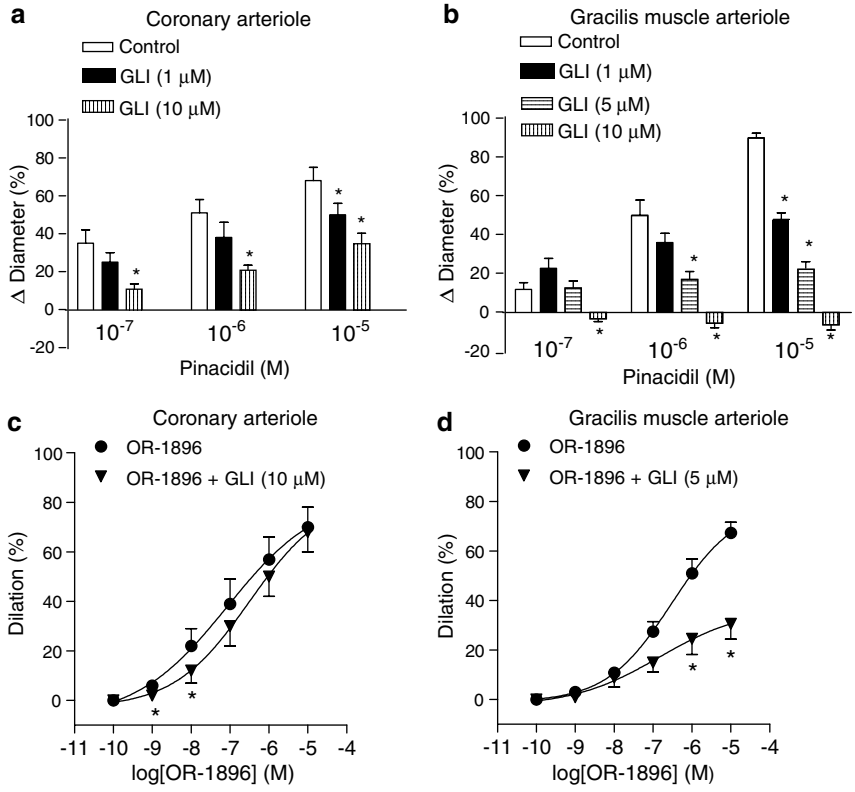

Figure 4 Percentage dilations of coronary (a) and skeletal muscle (b) arterioles in response to cumulative concentrations of pinacidil (0.1–10 μM) before and after incubation with GLI (1, 5 or 10 μM, $n=6$ in each group). Percentage dilations of coronary (c) and skeletal muscle (d) arterioles in response to OR-1896 before and after incubation with the selected concentrations of GLI (5 and 10 μM). The lines represent regression lines. Data are means±s.e.m. Asterisks indicate significant differences ($P<0.05$).

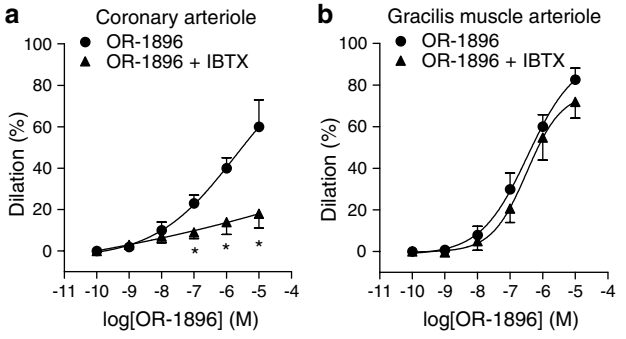

Figure 5 Percentage dilations of coronary (a) and skeletal muscle (b) arterioles in response to cumulative concentrations of OR-1896 before and after incubation with IBTX (0.1 μM, $n=5$ in each group). The lines represent regression lines. Data are means$\pm$s.e.m. Asterisks indicate significant differences ($P<0.05$).

arterioles, GLI shifted the OR-1896-evoked concentration–response curve to the right, and this resulted in significant reductions in the magnitudes of the vasodilation at OR-1896 concentrations of 1 and 10 nM (Figure 4c). In comparison with the coronary arteriolar responses, however, GLI gave rise to greater reductions in the magnitudes of the OR-1896-induced dilations in the gracilis muscle arterioles at OR-1896 concentrations of 1 and 10 μM (Figure 4d).

To assess the potential role of BK_{Ca} channels in the OR-1896-induced vasodilation, in another series of experiments, the selective BK_{Ca} channel inhibitor IBTX (100 nM) was used. IBTX markedly reduced the magnitude of the OR-1896-induced dilations in the coronary arterioles, but it had no effect on the dilations of the skeletal muscle arterioles (Figure 5).

Discussion

Clinical observations indicated that short-term levosimendan administration is followed by long-term hemodynamic changes that parallel not the plasma level of levosimendan, but rather the levels of its by-products (Kivikko *et al*., 2003). The present study revealed a pronounced vasodilator potential for the long-lived levosimendan metabolite OR-1896 in the coronary and skeletal muscle microvessels of the rat. Our data provide a plausible explanation for the long-lasting vascular effects that follow levosimendan application.

Most previous preclinical investigations on the vascular effects of levosimendan were conducted on pharmacologically preconstricted large vessels (i.e. arteries and veins) and relatively little attention has been devoted to OR-1896. In our model, arterioles with a spontaneous myogenic tone were selected and the levels of vasodilation in response to levosimendan and OR-1896 were systematically compared. The observed levosimendan- and OR-1896-induced changes in diameter of arterioles having spontaneous tone are likely to be more directly related to the microcirculation than findings obtained in those of large, preconstricted vessels. The data presented in this study suggest that both levosimendan and OR-1896 have substantial potential to decrease the vascular resistance and to modulate the microcirculation, both in the coronary and in the skeletal muscle. The extent of vasodilation reached similar maxima in the coronary and skeletal muscle arterioles at an OR-1896 concentration of 10 μM. However, at a therapeutically more relevant concentration (i.e. 10 nM), OR-1896 appeared to be somewhat more potent in the coronary than in the gracilis muscle arterioles. These features of OR-1896 were reminiscent of those of levosimendan when their vasodilator effects in the two different microvessels were compared. Interestingly, other studies suggested that the characteristics of levosimendan-evoked vasodilations also depend on the type of vessel chosen for the experiments. For example, the vasodilatory effects of levosimendan were not identical in the pulmonary and peripheral circulation in experiments on open-chest pigs (Leather *et al*., 2003). Our results suggest that OR-1896 at lower submicromolar concentrations has more potent vasodilator effects on isolated coronary microvessels than on skeletal muscle arterioles.

The marked similarity in the concentration dependences of OR-1896 and levosimendan suggested common cellular mechanisms of action. Previous studies have shown that levosimendan-induced vasodilation is associated with the activation of K_{ATP} channels (Pataricza *et al*., 2003; Hohn *et al*., 2004). The opening of K_{ATP} channels by levosimendan was first identified by voltage- and current-clamp studies in isolated rat vascular smooth muscle cells (Yokoshiki *et al*., 1997a) and isolated ventricular cardiomyocytes (Yokoshiki *et al*., 1997b). As levosimendan-stimulated K_{ATP} channel activity was abolished by GLI, it was concluded that levosimendan hyperpolarized the vascular smooth muscle cells, probably through the activation of a GLI-sensitive K^+ channel. Subsequent experiments in the presence of pharmacological blockers of the K_{ATP} channels in different vascular preparations provided further evidence of the interaction between levosimendan and K_{ATP} channel opening (Pataricza *et al*., 2000; De Witt *et al*., 2002). Also, in guinea-pigs, levosimendan has the potential to induce coronary vasodilation *via* membrane hyperpolarization following the activation of K_{ATP} channels (Kaheinen *et al*., 2001). Studies additionally suggested that the vasodilator responses to levosimendan are only partially dependent on the activation of K_{ATP} channels (De Witt *et al*., 2002). The involvement of more than a single K^+ channel subtype in the levosimendan-induced vasorelaxation was supported by experiments on human saphenous veins (Pataricza *et al*., 2000). In these studies, the levosimendan-induced vasodilation was related to interactions with both K_{ATP} and BK_{Ca} channels (Hohn *et al*., 2004). Interestingly, GLI did not affect the action of levosimendan in endothelium-denuded, isolated porcine epicardial coronary arteries precontracted with potassium chloride. By contrast, TEA, employed as a nonselective inhibitor of K^+ channels, decreased the coronary artery-relaxing effect of levosimendan. Furthermore, IBTX, a specific blocker of BK_{Ca} channels, caused a significant reduction in levosimendan-induced relaxation (Pataricza *et al*., 2003). These results indicated that, in the porcine coronary artery, the vasorelaxing mechanism of levosimendan primarily involves the activation of BK_{Ca} channels (Pataricza *et al*., 2003).

Accordingly, in this study, an attempt was made to employ K^+ channel function modulators to allow the characterization of the mechanism of OR-1896-induced arteriolar vasodilation. This study has revealed that more than 50% of the OR-1896-induced vasodilation could be prevented by nonselective blocker of K^+ channel, TEA at maximal OR-1896 concentra-

tion, independently of the nature of the microvessels. Moreover, the concentration dependence of the K^+ channel-independent component of OR-1896-induced vasodilation (i.e. that obtained after K^+ channel inhibition) at therapeutically relevant drug concentrations (1–10 nM) was relatively small. The data reported in the present study imply OR-1896-mediated interactions with K_{ATP} and BK_{Ca} channels, and indicate that the OR-1896-induced opening of these channels contributes to its vasodilator action. Careful comparison of the dose dependences obtained in the absence and presence of different K^+ channel blockers suggests that a considerable proportion of the OR-1896-induced vasodilation is a consequence of K_{ATP} channel activation, and argues against the contribution of BK_{Ca} channels at all applied OR-1896 concentrations in gracilis muscle arterioles. In comparison, activation of the BK_{Ca} channels seems to dominate in the OR-1896-induced vasodilation in the arterioles of the coronary system and a limited contribution of K_{ATP} channel openings to OR-1896-evoked vasodilation cannot be excluded at submicromolar OR-1896 concentrations.

Our finding that OR-1896 distinctly activates K^+ channels in the two observed vascular beds is seemingly discrepant, as presumably both vascular tissues express K_{ATP} and BK_{Ca} channels. However, the relative contribution of different K^+ channels may vary depending on the vessel type as well as the vessel size (Michelakis *et al*., 1997; Sun *et al*., 2004). In addition, one can speculate that a tissue-specific expression of different K^+ channel subunits may result in a variable sensitivity to pharmacological agents. For example, the K_{ATP} channels often contain different types of pore-forming (Kir6.1 and Kir6.2) and drug-targeting sulfonylurea receptor (SUR) subunits leading to the well-known tissue-specific differences of K_{ATP} channel inhibition (Ashcroft & Gribble, 1999). Further studies are needed in order to elucidate the existence of pharmacologically distinct K^+ channels mediating OR-1896-induced arteriolar responses in different vascular beds.

In summary, our present data suggest for the first time that, in addition to Ca^{2+} sensitization in cardiac cells, the levosimendan metabolite OR-1896 acts on a distinct molecular target in arterioles, that is, it activates K^+ channels leading to substantial vasodilation. It can be assumed that OR-1896 induces dilation in coronary and skeletal muscle arterioles through the activation of K_{ATP} and BK_{Ca} channels. Although the relative participations of different K^+ channel types in OR-1896-induced vasodilation may vary with as yet unknown mechanisms, we suggest a role for OR-1896 as a potential mediator of the long-term cardiovascular effects of levosimendan.

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