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Levosimendan and its metabolite OR-1896 elicit K_{ATP} channel-dependent dilation in resistance arteries *in vivo*

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Abstract:
Background: Levosimendan and its long-lived metabolite OR-1896 produce vasodilation in different types of vessels by activating ATP-sensitive (K_{ATP}) and other potassium channels.
Methods: In the present study we applied intravital videomicroscopy to investigate the *in situ* effects of levosimendan and OR-1896 on the diameters of real resistance arterioles (rat cremaster muscle arterioles with diameters of ~ 20 μm).
Results: Levosimendan and OR-1896 induced concentration-dependent (1 nM – 100 μM) dilations to similar extents in these arterioles (maximal dilation from 23 ± 2 to 33 ± 2 μm and from 22 ± 1 to 32 ± 1 μm, respectively). The arteriolar dilations induced by the selective K_{ATP} channel opener pinacidil (1 nM – 10 μM) (maximal dilation from 22 ± 4 μm to 35 ± 3 μm) were diminished in the presence of the selective K_{ATP} channel blocker – glibenclamide (5 μM) (maximal diameter attained: 22 ± 1 μm). Glibenclamide also counteracted the maximal dilations in response to levosimendan or OR-1896 (to 23 ± 3 μm or 22 ± 5 μm, respectively).
Conclusions: In conclusion, this is the first demonstration that levosimendan and OR-1896 elicit arteriolar dilation *in vivo*, *via* activation of K_{ATP} channels in real resistance vessels in the rat.

Key words:
levosimendan, OR-1896, cremaster muscle, vasodilation, K_{ATP} channels, intravital microscopy

Introduction

Levosimendan was developed as a Ca^{2+}-sensitizer drug. Its positive inotropic effect in the heart develops in combination with the vasodilation of peripheral and coronary arteries and of veins [15]. We earlier demonstrated that OR-1896, a long-lived metabolite of levosimendan, is a strong vasodilator in *ex vivo,* cannulated skeletal muscle and coronary arteries of the rat,

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and identified OR-1896 as a probable mediator of the long-term cardiovascular effects of levosimendan [6]. The vasodilations induced by levosimendan or OR-1896 have been found to be mediated by ATP-sensitive potassium (K_{ATP}) channels and possibly other potassium channels (e.g., large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channels and/or voltage-sensitive potassium channels) [6–8, 18, 19]. The relative contributions of the various potassium channels to the drug-induced vasodilation in the above *ex vivo* tested large-diameter preparations did not seem to be uniform, but was rather dependent on vessel type, vessel size and/or species. For example, in isolated porcine epicardial coronary artery, the vasorelaxing mechanism of levosimendan probably involved the activation of voltage-sensitive and BK_{Ca} channels to a significant degree [19]. Similarly, the activation of BK_{Ca} channels appeared essential for OR-1896-induced coronary arterial dilation in the rat [6]. In contrast, the activation of K_{ATP} channels predominated as concerns levosimendan-induced vasodilation in the coronary system of the guinea pig [8], human portal veins [18], human saphenous veins [7], the pulmonary system of the cat [4], the OR-1896 responses in rat skeletal muscle arteries [6]. The studies by Banfor et al. [3] and Segreti et al. [22] indicated that the infusion of rats with levosimendan or OR-1896 resulted in significant reductions in systemic peripheral vascular resistance, suggesting that these drugs exert their vasodilator effects predominantly on resistance-sized vessels. However, the vasomotor effects of levosimendan and OR-1896 on real resistance arteries *in vivo* have not yet been investigated.

Our present aim was therefore, to characterize the microvascular effects of levosimendan and OR-1896 *in vivo*, and hence, to provide evidence as to their vasodilator roles in the microcirculation. The changes in the diameters of third order-cremaster muscle arterioles (diameter: ~ 20 μm) in the presence of increasing concentrations of levosimendan or OR-1896 were monitored by means of intravital videomicroscopy. This allowed a comparative assessment of the vasodilator effects induced by levosimendan and OR-1896 in the microcirculation *in vivo*. Since levosimendan- and OR-1896-induced vasodilations have been shown to be associated with various degrees of K_{ATP} channel activation [7, 19, 28], K_{ATP} channel function modulators were also employed.

Materials and Methods

Animals and chemicals

All experimental procedures were approved by the Institutional Animal Care and Use Committee, in compliance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Experiments were carried out on male Wistar rats (n = 15, 8–10 weeks old, weighing ~ 200 g). The animals were housed under a 12-h light-dark cycle in the animal care facility of the University of Debrecen, and were fed with standard rat chow and drank tap water *ad libitum*. At the end of the experiments the animals were euthanized by an injection of sodium pentobarbital (150 mg/kg).

Levosimendan and OR-1896 were from Orion Pharma, Espoo, Finland. All other salts and chemicals were from Sigma-Aldrich Co. (St. Louis, MO, USA). Levosimendan, OR-1896, pinacidil and glibenclamide were prepared by dissolution in dimethyl sulfoxide (100 mM stock solutions), which were subsequently diluted with ethanol. Solutions were prepared fresh on the day of the experiment.

Intravital microscopy of the rat cremaster muscle

After overnight fasting rats were anesthetized with a subcutaneous injection of sodium pentobarbital (50 mg/kg). A constant level of anesthesia was maintained throughout the experiments by the subcutaneous injection of supplemental doses (20% of the original dose) of the anesthetic agent every 30–45 min. The trachea was cannulated to facilitate respiration. The left cremaster muscle was exposed through a midline scrotal incision, as described previously [7, 14]. An anesthetized rat was placed on a platform, and the cremaster muscle, with nerves and vessels intact, was spread over a heated, transparent pedestal. The whole preparation was then placed on the x–y stage of a microscope (Nikon Eclipse, FN1), at 35°C Krebs buffer was superfused continuously over the muscle at a constant flow rate (2 ml/min). After the surgical procedure, the preparation was allowed to equilibrate for at least 30 min before the start of the experimental protocol. We studied third- and fourth-order cremaster muscle arterioles with internal diameters of ~ 20 μm. Images were collected with a CCD camera and were