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Modification of levosimendan-induced suppression of atrial natriuretic peptide secretion in hypertrophied rat atria

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ABSTRACT

This study aimed to determine the effects of levosimendan, a calcium sensitizer, on atrial contractility and atrial natriuretic peptide (ANP) secretion and its modification in hypertrophied atria. Isolated perfused beating rat atria were used from control and isoproterenol-treated rats. Levosimendan and its metabolite OR-1896 caused a positive inotropic effect and suppressed ANP secretion in rat atria. Similar to levosimendan, the selective phosphodiesterase 3 (PDE3) or PDE4 inhibitor also suppressed ANP secretion. Suppression of ANP secretion by 1 μM levosimendan was abolished by PDE3 inhibitor, but reversed by PDE4 inhibitor. Levosimendan-induced suppression of ANP secretion was potentiated by K_{ATP} channel blocker, but blocked by K_{ATP} channel opener. Levosimendan alone did not significantly change cyclic adenosine monophosphate (cAMP) efflux in the perfusate; however, levosimendan combined with PDE4 inhibitor markedly increased this efflux. The stimulation of ANP secretion induced by levosimendan combined with PDE4 inhibitor was blocked by the protein kinase A (PKA) inhibitor. In isoproterenol-treated atria, levosimendan augmented the positive inotropic effect and ANP secretion in response to an increased extracellular calcium concentration ($[Ca^+]_o$). These results suggests that levosimendan suppresses ANP secretion by both inhibiting PDE3 and opening K_{ATP} channels and that levosimendan combined with PDE4 inhibitor stimulates ANP secretion by activating the cAMP–PKA pathway. Modification of the effects of levosimendan on $[Ca^+]_o$-induced positive inotropic effects and ANP secretion in isoproterenol-treated rat atria might be related to a disturbance in calcium metabolism.

1. Introduction

During cardiac excitation–contraction coupling, calcium binds to troponin C, which results in sliding of the thick and thin filaments, muscle contraction, and production of enough force to eject blood from the ventricle into the aorta (Gambardella et al., 2017). Myocardial contractility depends primarily on the levels of intracellular calcium, which is diffused through voltage-gated calcium (Ca^{2+}) channels located on the transverse tubules of the cardiac cells and the intracellular Ca^{2+} release channels located on the sarcoplasmic reticulum (Gambardella et al., 2017). The most important strategy to improve cardiac contractility in patients with acute decompensatory heart failure is to facilitate Ca^{2+} mobilization in response to the activation of the β-adrenergic–cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) signaling pathway (Teerlink et al., 2009). However, changes in Ca^{2+} handling in atrial cells might contribute to initiation and perpetuation of atrial fibrillation but also develop as a result of atrial fibrillation (Greiser et al., 2011; Xie et al., 2015). Due to adverse side effects of Ca^{2+} mobilizers, such as increased oxygen demand and arrhythmias (Udvary et al., 1995; Endoh, 2008), Ca^{2+} sensitizers were developed. Levosimendan ({(R)-([4 -(1, 4, 5, 6-tetrahydro-4- methyl-6-oxo-3-pyridazinyl) phenyl]-hydrazo-o)-propanedinitrile}) is a promising positive inotropic agent that sensitizes myofibrils to Ca^{2+}. Levosimendan improves myocardial contractility (Papp et al., 2012; Endoh, 2002; Boknik et al., 1997) and reduces oxidative stress (Akhtar et al., 2016) without increasing oxygen demand and Ca^{2+} concentration in the myocytes. Experimental studies have indicated that the positive inotropic effect of levosimendan is the result of a combination of Ca^{2+} sensitization and phosphodiesterase 3 (PDE3) inhibition (Endoh, 2008). Based on the promising results of a large-scale study, levosimendan was improved for the short-term treatment of acute decompensatory heart failure (Packer et al., 2013; Tasal et al., 2014); however, more studies are needed to prove its clinical importance in the long-term treatment of congestive heart failure.

PDE3 inhibitors have been known to have detrimental effects on patients suffering from cardiac failure; hence, it is particularly

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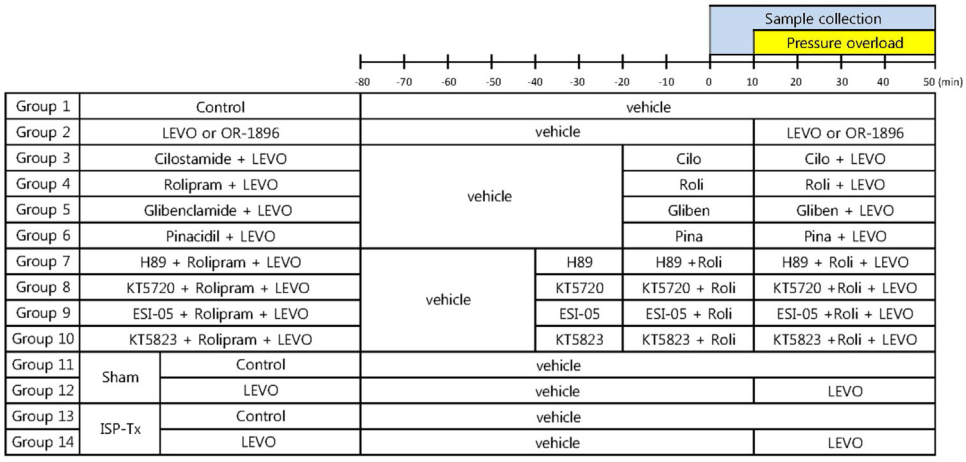

Fig. 1. Experimental protocols.

important to clarify the relative effects of levosimendan on Ca^{2+} sensitization and PDE3 inhibition. Therefore, in the present study, we investigated the effects of levosimendan on atrial natriuretic peptide (ANP) secretion and atrial contractility. ANP is a well-known cardiac hormone that regulates body fluid and blood pressure and protects the cardio-renal system under various diseased conditions (Nishikimi et al., 2006). Calcium is one of the most important factors affecting ANP secretion, because it plays a crucial role in the stimulus-secretion coupling of various hormones, including insulin (Kappel et al., 2013), renin (Fray et al., 1987; Park et al., 1992), and parathormone (Brown, 1991). Because Ca^{2+} closely regulates both the mechanical and endocrine functions of atrial myocytes, it is difficult to separately assess each function to evaluate ANP secretion; therefore, the effect of Ca^{2+} on ANP secretion remains controversial. That is, there is disagreement about whether ANP secretion increases in response to increased intracellular calcium concentration ($[Ca^{2+}]_i$) (Ruskoaho et al., 1985; Page et al., 1991), decreases in response to increased extracellular calcium concentration ($[Ca^{2+}]_o$) (De Bold and De Bold, 1989; Kim et al., 2002), or both (Doubell and Thibault, 1994). Although a Ca^{2+} sensitizer might be acceptable to evaluate the relationship between ANP secretion and Ca^{2+}, there are no reports that have investigated this relationship. The present study aimed to investigate the effects of levosimendan on atrial hemodynamics and ANP secretion and its modification using a control and hypertrophied beating rat atria.

2. Materials and methods

2.1. Animals and chemicals

Male Sprague-Dawley rats, weighing 230–250 g, purchased from Dae Han Bio Link Co. Ltd. (Eumsung, Korea) were housed in a temperature-controlled room with a 12:12-h light-dark cycle. Animals were provided free access to standard laboratory chow (5L79 Purina rat & mouse 18% chow, Charles River Laboratories Inc., Wilmington, MA, USA) and water. All experimental protocols conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996) and were approved by Chonbuk National University Medical School.

Levosimendan, OR-1896, cilostamide, rolipram, H89, KT5720, KT5823, glibenclamide, ANP, and forskolin were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). 4-Methylphenyl-2, 4, 6-trimethylpheylsulfone (ESI-05) was purchased from BIOLOG Life Science Institute (Bremen, Germany). [^{3}H]-inulin was purchased from Amersham Biosciences (Västerbotten, Sweden).

2.2. Preparation of perfused beating rat atria

Isolated perfused beating atria were prepared using a previously described method (Han et al., 2008). In brief, the rats were killed and the hearts were rapidly excised. A cannula was inserted into the left atria and the tissue was ligated using silk sutures. The cannulated atria were kept in an organ chamber perfused with oxygenated HEPES buffered saline at 37 °C and were then paced at 1.2 Hz (duration, 0.4 ms; voltage, 30 V). The intra-atrial pressure was recorded using the ML-820 PowerLab (AD Instruments Pty. Ltd, Bella Vista NSW, Australia) through the pressure transducer (Statham Instruments, Oxnard, CA, USA), and atrial pulse pressure (APP) was obtained from the difference between the systolic and diastolic pressures. The composition of HEPES-buffered saline was as follows (mM): 10 HEPES, 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 glucose, and 0.1% bovine serum albumin (BSA). The pericardial buffer solution containing [^{3}H]-inulin and used for measuring the translocation of extracellular fluid (ECF) was also oxygenated through silicone tube coils inside the organ chamber. The atria were perfused for 80 min to stabilize secretion of ANP and to maintain a steady-state [^{3}H]-inulin level in the extracellular space. The atrial perfusate was collected at 2-min intervals at 4 °C for 10 min while paced at 1.2 Hz. To induce atrial stretch, the height of the outflow catheter was increased from 5.0 to 7.5 cmH_2O by a connecting 2.5-cm-long catheter after a 10-min collection period and the atrial perfusate was collected for 50 min (Han et al., 2008). The loaded volume to the atria during diastole was 736 µl (Oh et al., 2011).

2.3. Experimental protocols for high atrial stretch

Experiments were performed on 14 groups (Fig. 1).

Group 1 was high-stretched control atria (n = 10). After a 10-min control period, atrial perfusate was collected for 40 min with high atrial stretch condition.

Group 2 was high-stretched atria perfused with levosimendan or OR-1896. After a 10-min control period, the atria were perfused with different doses of levosimendan (0.01, 0.1, 1.0, or 10.0 µM; n = 8 for each group) or OR-1896 (0.01, 0.1, 1.0, or 10.0 µM; n = 8 for each group) with high stretch.

Groups 3, 4, 5, and 6 were atria perfused with levosimendan in the presence of PDE3 inhibitor (cilostamide, 0.1 µM), PDE4 inhibitor (rolipram, 1 µM), K_{ATP} channel blocker (glibenclamide, 500 µM) or K_{ATP} channel opener (pinacidil, 10 µM). Atria were perfused with cilostamide, rolipram, glibenclamide, or pinacidil 20 min before sample collection, and then levosimendan (1.0 µM, n = 8 for each group) was perfused into the high-stretched atria.

Groups 7, 8, 9, and 10 were atria perfused with levosimendan and