*Am J Physiol Lung Cell Mol Physiol* 313: L950–L956, 2017.
First published August 24, 2017; doi:10.1152/ajplung.00213.2017.

**RESEARCH ARTICLE**

# Levosimendan prevents bronchoconstriction and adverse respiratory tissue mechanical changes in rabbits

**Barna Babik,[1] Adam L. Balogh,[1,2] Roberta Sudy,[1,2] Orsolya Ivankovitsne-Kiss,[2] Gergely H. Fodor,[2] and Ferenc Petak[2]**

[1]*Department of Anesthesiology and Intensive Therapy, University of Szeged, Szeged, Hungary; and* [2]*Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary*

Submitted 16 May 2017; accepted in final form 15 August 2017

**Babik B, Balogh AL, Sudy R, Ivankovitsne-Kiss O, Fodor GH, Petak F.** Levosimendan prevents bronchoconstriction and adverse respiratory tissue mechanical changes in rabbits. *Am J Physiol Lung Cell Mol Physiol* 313: L950–L956, 2017. First published August 24, 2017; doi:10.1152/ajplung.00213.2017.—Levosimendan has a calcium-sensitizing effect in the myocardium and opens ATP-sensitive potassium channels ($K_{ATP}$) in vascular smooth muscle. Because airway smooth muscle also expresses $K_{ATP}$, we characterized the protective potential of levosimendan against increased airway and respiratory tissue resistances. Animals were administered levosimendan alone (*group L*), levosimendan after pretreatment with a $K_{ATP}$ channel blocker (glibenclamide, *group LG*), glibenclamide only (*group G*), or solvent alone (dextrose, *group C*). Airway resistance ($R_{aw}$), tissue damping, and elastance were determined by forced oscillations under baseline conditions and following provocation tests with intravenous methacholine (MCh). Cardiac output (CO) was assessed by transpulmonary thermodilution. The same sequence of measurements was then repeated during intravenous infusion of levosimendan in *groups L* and *LG* or glucose in *groups G* and *C*. Sham treatments in *groups C* and *G* had no effect on lung responsiveness. However, levosimendan treatment in *group L* elevated CO and inhibited the MCh-induced airway responses [$R_{aw}$ changes of 87.8 ± 83% (SD) vs. 24.4 ± 16% at 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ MCh, $P < 0.001$], and in G (35.2 ± 12.7 vs. 25.2 ± 12.9%, $P < 0.05$). The preventive affect of levosimendan against lung constriction vanished in the LG group. Levosimendan exerts a $K_{ATP}$-mediated potential to prevent bronchoconstriction and may prohibit adverse lung peripheral changes both in the small bronchi and the pulmonary parenchyma. The identification of a further pleiotropic property of levosimendan that is related to the pulmonary system is of particular importance for patients with decreased cardiorespiratory reserves for which simultaneous circulatory support is complemented with prevention of adverse respiratory events.

bronchodilator agents; bronchoconstriction; cardiovascular drugs; cardiac inotropism; respiratory mechanics

LEVOSIMENDAN IS AN inodilator recommended for the treatment of acute (38) and chronic (29) heart failure. Its positive inotropic effect is attributed to $Ca^{2+}$ sensitization, which is achieved by the stabilization of the $Ca^{2+}$-bound conformation of cardiac troponin C (18). Levosimendan provides a well-established advantage: its use does not increase intracellular cAMP while elevating intracellular $Ca^{2+}$ (11). Thus, myocardial contractility is facilitated without an increase in oxygen demand and without provoking malignant tachyarrhythmias (11). These processes are responsible for the elevations in cardiac output (CO) and improved myocardial oxygen balance (3, 10, 21), without compromising mean arterial pressure (MAP) (44).

Another key feature of levosimendan is the opening of ATP-sensitive potassium ($K_{ATP}$) channels in smooth muscle cells (31, 37). The activation of $K_{ATP}$ channels induces an efflux of K ions that results in decreased membrane potential and, consequently, an inhibition of L-type calcium channels, which leads to the relaxation of smooth muscle cells. This mechanism has been demonstrated in arterial (16) and venous (32) vascular smooth muscle cells (VSMC). Although $K_{ATP}$ channels are also expressed by airway smooth muscle cells (ASMC) (6), there is an essential lack of knowledge about the effects of levosimendan on the respiratory system.

To address this lack, we sought to determine the potential of levosimendan to prevent the constrictor response of the respiratory system by measuring airway mechanics and viscoelastic properties of respiratory tissues in an animal model relevant to a clinical scenario. To learn the underlying mechanism, we also investigated the role of $K_{ATP}$ channels in the bronchoprotective effects of levosimendan. We hypothesize that levosimendan is able to inhibit constriction at the level of contractile elements embedded in both the airways and lung parenchyma, and the $K_{ATP}$ channels mediate this effect. Glibenclamide was applied to verify the involvement of the $K_{ATP}$ channels in the pulmonary effect of levosimendan, since glibenclamide selectively inhibits $K_{ATP}$ channel activity without affecting contractility of the myocardium.

## MATERIALS AND METHODS

This study was approved by the Experimental Ethics Committee of the University of Szeged, Szeged, Hungary, on the 7th of December 2012 (no. I-74-50/2012), and authorized by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary (no. XIV/152/2013, Chairperson Cs. Farle), on the 9th of January 2013. The procedures were performed according to the guidelines of the Scientific Committee of Animal Experimentation of the Hungarian Academy of Sciences [updated Law and Regulations on Animal Protection: 40/2013 (II. 14.) Government of Hungary], following the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes, and reported in compliance with the ARRIVE guidelines.

*Animal preparation.* We performed experiments on male New Zealand White rabbits ($n = 27$, weighing 2.0–2.5 kg). The rabbits

Address for reprint requests and other correspondence: F. Peták, Department of Medical Physics and Informatics, University of Szeged, 9 Korányi fasor, H-6720, Szeged, Hungary (e-mail: petak.ferenc@med.u-szeged.hu).

were sedated by an intramuscular injection of xylazine (5 mg/kg, CP-xylazine; CP-Pharma, Burgdorf, Germany), and an intravenous line was secured to the ear. Anesthesia induction was then performed by an intravenous injection of pentobarbital sodium (30 mg/kg; Sigma-Aldrich, Budapest, Hungary), and the rabbit was placed in a supine position on a heating pad to maintain body temperature in the 38 ± 0.5°C range. After endotracheal intubation by tracheostomy was achieved, the rabbits were connected to a small animal ventilator (model 683; Harvard Apparatus, South Natick, MA) and ventilated with room air (ventilation frequency 40–50 $min^{-1}$, tidal volume 8 ml/kg). A femoral artery and femoral and jugular veins were catheterized for drug delivery, blood pressure monitoring, and cardiac output assessment. Anesthesia and neuromuscular blockade were maintained by repeated (every 30 min) intravenous boluses of pentobarbital sodium (5 mg/kg) and pipecuronium (0.3 mg/kg, Arduan; Gedeon Richter, Budapest, Hungary), respectively.

*Cardiovascular monitoring.* Systemic hemodynamic parameters were monitored by using a transpulmonary arterial thermodilution system (PiCCO; PULSION Medical Systems, Feldkirchen, Germany). A 3-ml thermal indicator bolus (<8°C) was injected in the right atrium via the central venous catheter inserted in the jugular vein. A data acquisition and analysis system (BIOPAC, Goleta, CA) was used to record continuous arterial pressure and heart rate (HR) signals. The MAP was estimated as the diastolic blood pressure plus a third of the pulse pressure.

*Measurement of respiratory mechanics.* A T piece with two collapsible segments was connected to the tracheal tube. One end was attached to the ventilator and the other to a loudspeaker-in-box system of a forced oscillatory measurement apparatus. Mechanical ventilation was halted for 8-s periods at end expiration, and a computer-generated small-amplitude (less than ±1 $cmH_2O$) pseudo-random signal containing 23 sinusoidal components in the interval of 0.5–20.75 Hz was delivered by the loudspeaker and introduced into the lungs. The resulting gas flow (V′) was measured with a differential pressure transducer (model 33NA002D; IC Sensors, Milpitas, CA). Tracheal pressure was measured with an identical pressure transducer relative to the atmosphere (P). The P and V′ signals were low-pass filtered at 25 Hz and sampled with a data acquisition board (NI USB-6211; National Instruments, Austin, TX) at a rate of 256 Hz. The input impedance spectra of the respiratory system ($Z_{rs}$) were calculated by using a fast-Fourier transformation with 4-s time windows and 95% overlap ($Z_{rs}$ = P/V′). The input impedance of the tracheal tube and the connections were also measured and subtracted from the $Z_{rs}$ spectra.

The mechanical properties of the airways and the respiratory tissues were determined using model fitting by minimizing the weighted difference of the measured and the modeled spectra. The well-validated model (27) contained frequency-independent airway resistance ($R_{aw}$) and inertance ($I_{aw}$) connected in series to a constant-phase tissue compartment incorporating damping (G) and elastance (H) parameters (19). $R_{aw}$ represents primarily the resistance of the conducting airways; $I_{aw}$ is related to the mass of gas in the airways, and its decrease indicates enhanced ventilation heterogeneities (35). Parameters G and H indicate the resistive (i.e., damping or energy loss) and elastic (i.e., stiffness) characteristics, respectively, of the respiratory tissues, including the lung parenchyma and chest wall.

*Experimental protocol.* In the first control stage of the protocol, four successive $Z_{rs}$ recordings were collected from all of the rabbits to establish the baseline (Fig. 1). Provocation tests with increasing doses (0.5, 1, 2, and 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$) of intravenous methacholine (MCh) were performed on all of the animals. When a stable level of constriction had been established, four $Z_{rs}$ data epochs were collected during each MCh infusion rate. A 15-min recovery period was allowed for the normalization of the respiratory and systemic hemodynamic parameters, and a second set of baseline parameters was measured. The rabbits were then randomly assigned to one of four groups as follows: control (*group C*, $n = 9$), levosimendan (*group L*, $n = 10$), glibenclamide (*group G*, $n = 6$), and levosimendan + glibenclamide (*group LG*, $n = 8$). Animals in *group C* received the solvent used for levosimendan (5% dextrose) in the same volumes as for other groups for matching intervals. Rabbits in *group G* also received 5% dextrose and an intraperitoneal injection of the $K_{ATP}$

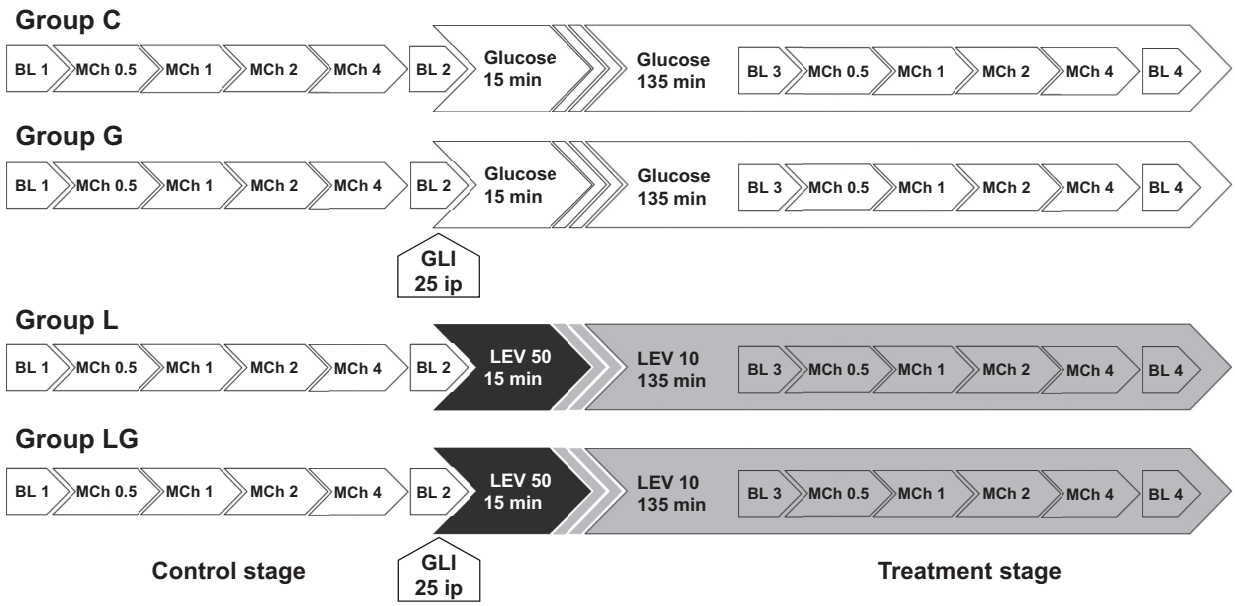


Fig. 1. Scheme of the experimental protocol. In the control phase, oscillatory measurements were performed at baseline (BL1) and for each step of the methacholine (MCh) provocation with increasing doses (0.5, 1, 2, and 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$). After completing the MCh infusions (15 min), a second set of baseline measurements was performed (BL2). Subsequently, glucose was infused in control (*group C*) and glibenclamide (*G*) groups at 5 $ml \cdot kg^{-1} \cdot h^{-1}$ for 15 min and again at 1 $ml \cdot kg^{-1} \cdot h^{-1}$ for 135 min. Rabbits in *group G* received an ip injection of 25 mg glibenclamide (GLI). Levosimendan (LEV) dissolved in glucose was administered to *group L* in matching volumes (50 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 15 min initially and then 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 135 min). In addition to the levosimendan infusion, rabbits of *group LG* received an intraperitoneal injection of 25 mg glibenclamide. Provocations with MCh were then repeated in the same manner with the maintained infusion of the corresponding agents (glucose or levosimendan) in all four groups.

Table 1. *Baseline characteristics for protocol groups*

| | *Group C* | *Group G* | *Group L* | *Group LG* | *P* |
|---|---|---|---|---|---|
| Body wt, g | 2,314 ± 300 | 2,660 ± 321 | 2,417 ± 208 | 2,489 ± 175 | 0.1 |
| $R_{aw}$, $cmH_2O \cdot s \cdot l^{-1}$ | 13.0 ± 3.3 | 11.1 ± 2.7 | 12.1 ± 1.9 | 14.8 ± 5.7 | 0.3 |
| $I_{aw}$, $cmH_2O \cdot s^2 \cdot ml^{-1}$ | 43.4 ± 36 | 41.7 ± 15.4 | 33.1 ± 13.0 | 57.0 ± 16.4 | 0.2 |
| G, $cmH_2O/l$ | 141 ± 16.5 | 140 ± 24.0 | 130 ± 21.2 | 159 ± 31.1 | 0.1 |
| H, $cmH_2O/l$ | 631 ± 75 | 632 ± 171 | 660 ± 101 | 667 ± 212 | 0.9 |

Values are means ± SD. *Group C*, control group that received solvent alone; *group G*, group that received glibenclamide; *group L*, group that received levosimendan; *group LG*, group that received levosimendan + glibenclamide; $R_{aw}$, airway resistance; $I_{aw}$, airway inertance; G, respiratory tissue damping; H, respiratory tissue elastance.

channel blocker glibenclamide (25 mg/kg) (20) (Sigma-Aldrich, St. Louis, MO) dissolved in dimethyl sulfoxide. Levosimendan (SIMDAX; Orion, Espoo, Finland) was infused in doses of 50 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 15 min and then 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 135 min in the animals assigned to *group L*. In the rabbits in *group LG*, an intraperitoneal injection of glibenclamide was applied before an identical levosimendan treatment. In the treatment stage of the protocol, the MCh provocation test was repeated in the same way as in the previous stage. CO was assessed under the initial baseline conditions and at 15 and 150 min after initiating the treatments. Ensemble-averaged $Z_{rs}$ spectra obtained during each experimental stage were used to establish airway and respiratory tissue mechanical parameters. The MCh doses eliciting a 25% increase in $R_{aw}$ relative to baseline ($PD_{25}$) were determined by fitting linear models to the individual dose-response curves; $R^2 > 0.8$ was obtained for these linear models for each rabbit.

*Statistical analysis.* The scatter of the values of the measured variables is presented as SD of the mean. The baseline characteristics of the rabbits in the protocol groups were compared by using one-way ANOVA tests on ranks. Three-way repeated-measures ANOVA was used to assess the effects of group allocation (C, L, or LG), protocol stage (control or treatment), and MCh dose (0.5, 1, 2, or 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the changes in the respiratory mechanical parameters. Two-way ANOVA with repeated measures was used to compare the hemodynamic indexes in the protocol groups at different time points (0, 15, or 150 min after commencing each treatment), and the $PD_{25}$ values obtained in the two stages. Post hoc analyses were performed using the Holm–Sidak multiple-comparisons procedure. The association between changes in CO and MAP was assessed by a Pearson product-moment correlation test. Sample size was estimated for MCh-induced change in $R_{aw}$ as the primary outcome variable with an expected 25% difference in the lung responsiveness, a power of 0.8, and two-sided α-error of 0.05. The estimation resulted in a required sample size of five for each group, based on the MCh-induced changes in $R_{aw}$ in our earlier study (13). The statistical tests were performed within the *R* core package with the *lme4* (4) and *lsmeans* (25) packages and SigmaPlot 13 (version 13; Systat Software, Chicago, IL). The statistical tests were performed with a significance level of $P < 0.05$, and all *P* values were two sided.

## RESULTS

There was no significant difference among the protocol groups in the body mass or in the respiratory mechanical parameter values obtained under the baseline conditions (Table 1).

*Hemodynamic effects.* In Fig. 2, we have plotted the systemic hemodynamic parameter values under the baseline conditions and at 15 and 135 min following each treatment, according to the allocation of the animals to the protocol groups. No significant differences were observed in any of the systemic hemodynamic parameter values between the protocol groups under the baseline conditions. Rabbits in *groups C* and *G* exhibited no statistically significant changes in CO and MAP; however, slight statistically significant increases were observed in HR ($P < 0.001$) only. In contrast, the similar rises in HR ($P < 0.001$) were associated with significant elevations in CO ($P < 0.001$ for both *groups L* and *LG*) and MAP ($P < 0.001$ and 0.003 for *groups L* and *LG*, respectively) in both groups of animals receiving levosimendan treatment. At the end of the protocol (150 min), CO values were significantly higher in *group L* than that in the other three protocol groups ($P < 0.05$).

The relationship between the relative changes in CO and MAP 150 min after initiating the treatment is depicted in Fig. 3 for each rabbit in the four protocol groups. There was a statistically significant correlation between the percentage changes of these systemic hemodynamic variables ($r = 0.71$, $P < 0.0001$).

*Respiratory mechanics.* Figure 4 shows the alterations in the MCh-induced responses in the airway ($R_{aw}$ and $I_{aw}$) and respiratory tissue mechanical parameters (G and H) at two stages of the experiment. No statistically significant difference in airway or tissue constrictor responses was detected during the control phase of the protocol. In the rabbits of *groups C* and *G*, no difference was observed in the lung responses provoked

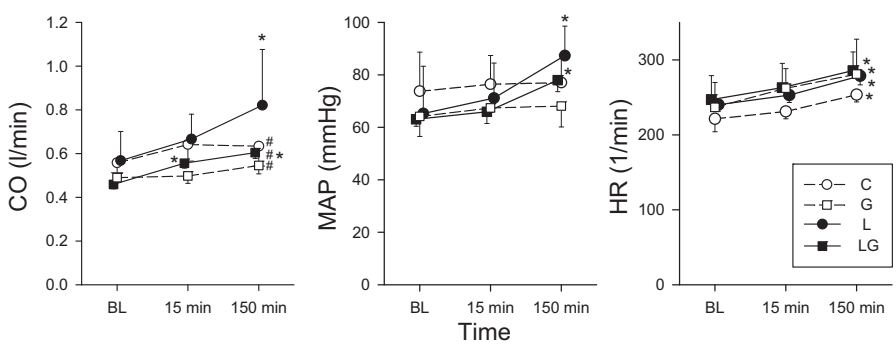


Fig. 2. Cardiac output (CO), mean arterial pressure (MAP), and heart rate (HR) in *groups C* (white circles), *G* (white squares), *L* (black circles), and *LG* (black squares) under baseline conditions (BL) and at 15 and 150 min after commencement of treatment. Error bars represent SE. $P < 0.05$ vs. baseline within a group (*) and vs. *group L* within a time point (#).

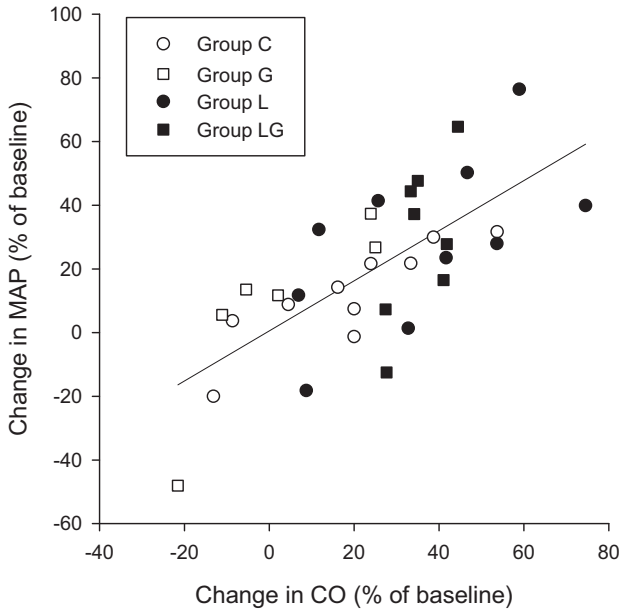


Fig. 3. Relationship between changes in CO and MAP induced by levosimendan in each rabbit in *groups C* (white circles), *G* (white squares), *L* (black circles), and *LG* (black squares) groups. Line denotes linear regression curve ($r = 0.71$, $P < 0.0001$).

by MCh at the two stages of the protocol, demonstrating the reproducibility of the dose-response curves in these vehicle-treated animals for both the presence (*group G*) and the absence (*group C*) of glibenclamide treatment. In contrast, levosimendan treatment in the animals of *group L* resulted in significantly smaller responses in $R_{aw}$ and H at the three highest MCh doses ($P < 0.03$ and $P < 0.05$, respectively), and in G at the two highest MCh infusion rates ($P < 0.05$). The MCh-induced decreases in $I_{aw}$ were reversed by levosimendan treatment in the rabbits of *group L* ($P < 0.05$). The inhibition of lung responsiveness by levosimendan vanished in the glibenclamide-treated rabbits (*group LG*), i.e., the lung responsiveness to MCh did not differ before or after a combined glibenclamide-levosimendan treatment.

Figure 5 depicts the $PD_{25}$ values for $R_{aw}$ determined from the MCh dose-response curves for the three protocol groups. During the first phase of the protocol (when no treatment was applied), no significant differences were seen among the protocol groups. Levosimendan infusion led to a marked increase in $PD_{25}$ in *group L* ($P < 0.0001$), whereas no significant changes were observed in the C, G, and LG groups.

## DISCUSSION

We used an in vivo animal model with clinical relevance to characterize the effects of levosimendan on the respiratory system. Our results revealed that the known circulatory effect of levosimendan is associated with its marked protective potential against bronchospasm and increases in the respiratory tissue viscoelastic parameters induced by the exogenous administration of a cholinergic constrictor agonist. The blockade of $K_{ATP}$ channels significantly reversed the ability of levosimendan to inhibit the constrictive response of the lung, which indicated the primary role of the $K_{ATP}$ channels expressed by contractile cells in the pulmonary system.

*Systemic circulatory effects of levosimendan.* In agreement with the findings of previous experimental (41) and clinical (14) investigations, elevations in CO were observed in the present study following sustained levosimendan treatments. This finding can be attributed to the positive inotropic effects of levosimendan and the decreased afterload. However, the CO was higher in *group L* than in *group LG* at 150 min. This circulatory effect of glibenclamide may be explained by the diminished vascular effect of levosimendan, leading to higher afterload. The lack of a significant difference in MAP between *groups L* and *LG* may support this explanation, since the smaller elevations in CO associated with an increased afterload result in MAP increases in *group LG*.

The effect of levosimendan on systemic blood pressure is governed by the opposing contributions of the increased CO that gives rise to the MAP as well as by the relaxation of the systemic VSMC that results in a decrease in MAP. Although the dominance of the VSMC relaxation was demonstrated in earlier clinical investigations (30) and in experimental studies in rats (41) and dogs (2), these factors seem to have comparable effects in rabbits (9, 23); alternatively, the increase in CO dominated (3, 21). Our data revealed a significant correlation

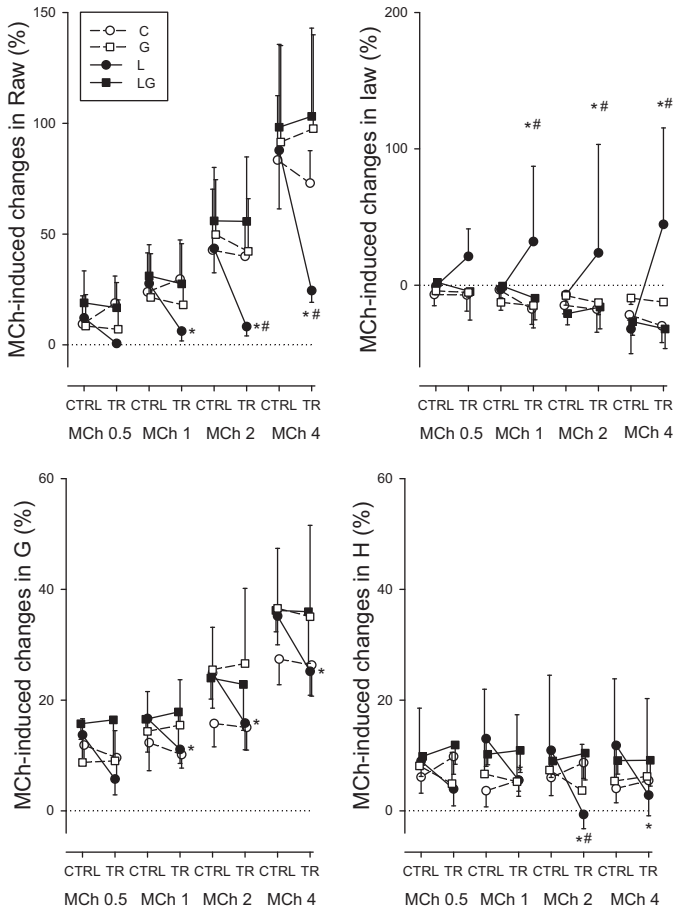


Fig. 4. Changes in airway resistance ($R_{aw}$), airway inertance ($I_{aw}$), respiratory tissue damping (G), and tissue elastance (H) to MCh provocation with increasing doses (0.5, 1, 2, and 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in *groups C* (white circles), *G* (white squares), *L* (black circles), and *LG* (black squares) groups at the control (CTRL) and treatment (TR) stages. Error bars represent SE. $P < 0.05$ vs. the control stage within a group (*) and vs. *group C* within a MCh dose in the same phase (#).

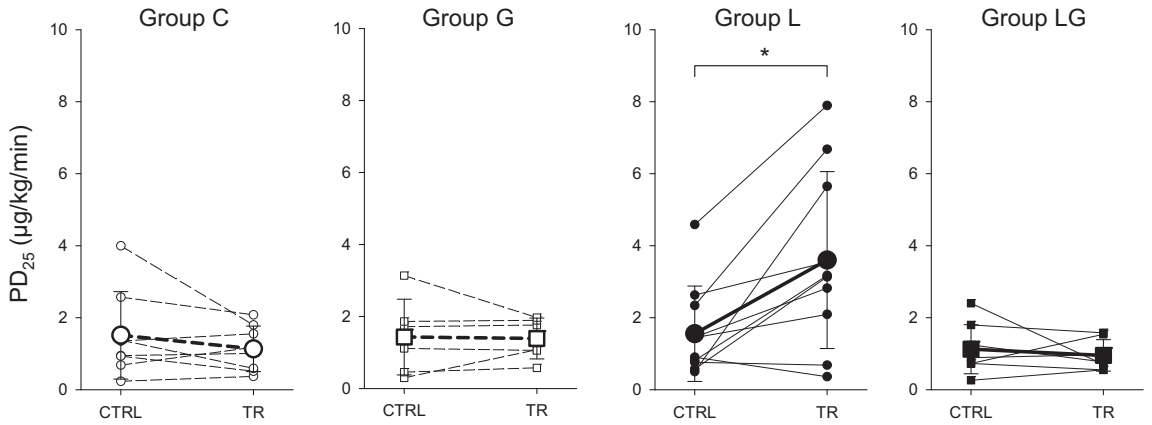


Fig. 5. Provocative dose leading to a 25% increase in $R_{aw}$ ($PD_{25}$) in each rabbit of *groups C* (white circles), *G* (white squares), *L* (black circles), and *LG* (black squares) groups in the control and treatment stages. Symbols with thick lines represent means ± SD. $*P < 0.05$.

between the changes in CO and MAP (Fig. 3), suggesting that the increases in CO are responsible for the elevations in MAP, rather than alterations in the systemic vascular smooth muscle tone. We also observed slight elevations in HR uniformly in all groups by the end of the experiments. This elevation may be attributed to the gradual development of mild hypovolemia, since intravenous administration of the different drugs and saline (for PiCCO readings) may not have been sufficient to account for the diuresis of the rabbits and the fluid loss resulting from excessive salivation during the MCh challenges. The mild tachycardia was not likely to bias the main findings for the respiratory mechanics.

*Bronchial effects of levosimendan.* An important finding of the present study is the strong ability of levosimendan to protect against MCh-induced bronchospasm. The bronchoprotective potential demonstrated in the present study is in accordance with earlier results obtained with isolated guinea pig tracheal rings (12). In the guinea pig study, however, levosimendan was added to an organ bath containing a tracheal ring after establishing bronchospasm. Thus, the experimental approach allowed only the assessment of the ability of this inodilator to acutely reverse an existing ASMC contraction. In addition, the use of isolated tracheal rings as an experimental model does not allow an overall picture of the entire bronchial tree, and the lack of systemic effects precludes a comprehensive overview of the cardiopulmonary effects of levosimendan at an organ level. Furthermore, adding levosimendan to an organ bath does not represent a clinical situation either in dose or administration regimen.

The current results significantly extend our knowledge of the bronchial effects of levosimendan. Because the onset time of levosimendan is relatively long (22), the study protocol was designed to characterize the preventive rather than the acute treatment potential of levosimendan. Such an approach may have clinical relevance in the perioperative care of patients with heart failure, which is often associated with an elevated bronchial tone (7). Lung mechanics may further deteriorate perioperatively as a consequence of airway manipulation and tracheal suctioning (33), fluid shifts (42), blood transfusion (15), lung tissue damage (46), or systemic hypothermia (1). Likewise, cardiac surgery with cardiopulmonary bypass is known to trigger perioperative bronchial exacerbation (1), with particularly severe cardiopulmonary consequences in patients with airway hyperresponsiveness. All of these adverse respiratory complications elevate work of breathing that might necessitate prolonged mechanical ventilation.

The MCh-induced responses in $R_{aw}$ were dramatically inhibited by levosimendan infusion. Because $R_{aw}$ primarily reflects the flow resistance of the central conducting airways (5), the ability of levosimendan to prevent proximal airway constriction can be anticipated. MCh also induced decreases in $I_{aw}$, suggesting the enhancement of ventilation heterogeneities in the lung periphery (35). Because levosimendan eliminated these changes, it can be inferred that levosimendan might also prevent the constriction of small peripheral airways.

The bronchoprotection effect of levosimendan vanished with the prior administration of glibenclamide, demonstrating that the relaxation of the ASMC by levosimendan was mediated by $K_{ATP}$ channels. This finding suggests that other mechanisms attributed to levosimendan in the relaxation of VSMC, such as the inhibition of the phosphodiesterase III pathway (31), do not play a role in the ASMC response in this species. Thus, the mechanism responsible for the bronchoprotective potential of levosimendan resembles that of volatile anesthetic agents that have also been shown to induce bronchodilation by opening $K_{ATP}$ channels (8, 47).

*Effects of levosimendan on the viscoelastic parameters of respiratory tissues.* We observed another important benefit of levosimendan related to its ability to prevent MCh-induced elevations in the viscoelastic parameters of the respiratory tissues. Levosimendan has been shown to alleviate the development of lung edema (39). Thus, the significant inhibition of the MCh-induced elevations in G and the decreases in H may have been attributed to intrinsic changes in tissue viscoelasticity because of altered fluid content of the respiratory tissues. Moreover, indirect mechanisms associated with prevention of lung volume loss by inhibiting peripheral airway closures might have also contributed to the observed effects of levosimendan on the G and H responses. Interestingly, the ability of levosimendan to prevent MCh-induced elevations in respiratory tissue damping and stiffness is more pronounced than that of the volatile anesthetic agents, where opening the $K_{ATP}$ channels has little (24) or no effect on this lung compartment (17, 28). This feature of levosimendan confirms its potent beneficial action on the lung periphery, as outlined above.

*Limitations.* One limitation of our results related to the experimental protocol warrants discussion. To avoid prolonged mechanical ventilation that could potentially interfere with the other interventions, a somewhat shorter administration period of clinically relevant concentrations of levosimendan was adopted in the present study than is applied in routine clinical settings (30, 36). This administration was in accordance with previous protocols applied in rabbits (48) and reproduced the well-established circulatory effects of levosimendan. Hence, our approach was appropriate to investigate the pulmonary effects of this agent.

Another possible limitation of our impedance data is related to the measurement of the total respiratory system, including pulmonary and chest wall compartments (43). Because rabbits were under complete muscle relaxation during the entire protocol, the possible effects of levosimendan on the diaphragm and/or on the chest wall muscles (10, 40, 45) were completely absent. Therefore, the changes in the tissue mechanical parameters were of pulmonary origin. However, the magnitude of this change was underestimated by the amount proportional to the contribution of the chest wall to the total respiratory tissue parameters, which is ~35–45% in smaller rodents (26, 34).

*Summary and conclusions.* The results of the present study demonstrate the strong ability of levosimendan to protect against MCh-induced changes in respiratory system mechanics. The measurement of mechanical parameters reflecting the entire bronchial tree revealed that levosimendan prevents central airway constriction and might prohibit the enhancement of ventilation heterogeneities in peripheral airways. Moreover, the beneficial effects of levosimendan on the lung periphery were also reflected in its ability to prevent elevations in respiratory tissue damping and stiffness. This protective effect of levosimendan was caused by opening $K_{ATP}$ channels, as demonstrated by the fact that the effect was completely eliminated by a prior administration of the $K_{ATP}$ channel blocker, glibenclamide. Levosimendan elevates CO and improves myocardial oxygen balance combined with a strengthened diaphragm function (10). The association of these well-known circulatory effects with the currently demonstrated beneficial changes in respiratory mechanics might decrease the oxygen demand of spontaneous breathing. This multimodal cardiorespiratory benefit has a potential of shortening the time of invasive ventilation following its application.

## GRANTS

This research was supported by a Hungarian Basic Research Council Grant (OTKA-NKFIH K115253) and GINOP-2.3.2-15-2016-00006. G. H. Fodor received a research grant from the NTP-EFÖ-P-15 project funded by the Human Capacities Grant Management Office and the Hungarian Ministry of Human Capacities.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

B.B., A.L.B., G.H.F., and F.P. conceived and designed research; B.B., A.L.B., G.H.F., and F.P. analyzed data; B.B., A.L.B., R.S., O.I.-K., G.H.F., and F.P. interpreted results of experiments; B.B., A.L.B., G.H.F., and F.P. drafted manuscript; B.B., A.L.B., R.S., O.I.-K., G.H.F., and F.P. edited and revised manuscript; B.B., A.L.B., R.S., O.I.-K., G.H.F., and F.P. approved final version of manuscript; A.L.B., R.S., O.I.-K., G.H.F., and F.P. performed experiments; F.P. prepared figures.

## REFERENCES

1. **Apostolakis EE, Koletsis EN, Baikoussis NG, Siminelakis SN, Papadopoulos GS.** Strategies to prevent intraoperative lung injury during cardiopulmonary bypass. *J Cardiothorac Surg* 5: 1, 2010. doi:10.1186/1749-8090-5-1.
2. **Banfor PN, Preusser LC, Campbell TJ, Marsh KC, Polakowski JS, Reinhart GA, Cox BF, Fryer RM.** Comparative effects of levosimendan, OR-1896, OR-1855, dobutamine, and milrinone on vascular resistance, indexes of cardiac function, and $O_2$ consumption in dogs. *Am J Physiol Heart Circ Physiol* 294: H238–H248, 2008. doi:10.1152/ajpheart.01181.2007.
3. **Barraud D, Faivre V, Damy T, Welschbillig S, Gayat E, Heymes C, Payen D, Shah AM, Mebazaa A.** Levosimendan restores both systolic and diastolic cardiac performance in lipopolysaccharide-treated rabbits: comparison with dobutamine and milrinone. *Crit Care Med* 35: 1376–1382, 2007. doi:10.1097/01.CCM.0000261889.18102.84.
4. **Bates D, Mächler M, Bolker B, Walker S.** Fitting linear mixed-effects models using lme4. *J Stat Softw* 67: 1–48, 2015. doi:10.18637/jss.v067.i01.
5. **Bayat S, Strengell S, Porra L, Janosi TZ, Petak F, Suhonen H, Suortti P, Hantos Z, Sovijärvi AR, Habre W.** Methacholine and ovalbumin challenges assessed by forced oscillations and synchrotron lung imaging. *Am J Respir Crit Care Med* 180: 296–303, 2009. doi:10.1164/rccm.200808-1211OC.
6. **Black JL, Barnes PJ.** Potassium channels and airway function: new therapeutic prospects. *Thorax* 45: 213–218, 1990. doi:10.1136/thx.45.3.213.
7. **Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, Menezes AM, Sullivan SD, Lee TA, Weiss KB, Jensen RL, Marks GB, Gulsvik A, Nizankowska-Mogilnicka E, Group BCR; BOLD Collaborative Research Group.** International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. *Lancet* 370: 741–750, 2007. doi:10.1016/S0140-6736(07)61377-4.
8. **Chen X, Yamakage M, Namiki A.** Inhibitory effects of volatile anesthetics on K+ and Cl- channel currents in porcine tracheal and bronchial smooth muscle. *Anesthesiology* 96: 458–466, 2002. doi:10.1097/00000542-200202000-00035.
9. **Das B, Sarkar C.** Pharmacological preconditioning by levosimendan is mediated by inducible nitric oxide synthase and mitochondrial KATP channel activation in the in vivo anesthetized rabbit heart model. *Vascul Pharmacol* 47: 248–256, 2007. doi:10.1016/j.vph.2007.06.008.
10. **Doorduin J, Sinderby CA, Beck J, Stegeman DF, van Hees HWH, van der Hoeven JG, Heunks LMA.** The calcium sensitizer levosimendan improves human diaphragm function. *Am J Respir Crit Care Med* 185: 90–95, 2012. doi:10.1164/rccm.201107-1268OC.
11. **Edes I, Kiss E, Kitada Y, Powers FM, Papp JG, Kranias EG, Solaro RJ.** Effects of Levosimendan, a cardiotonic agent targeted to troponin C, on cardiac function and on phosphorylation and Ca2+ sensitivity of cardiac myofibrils and sarcoplasmic reticulum in guinea pig heart. *Circ Res* 77: 107–113, 1995. doi:10.1161/01.RES.77.1.107.
12. **Eksert B, Usta C.** Role of potassium channels in the relaxant effect of levosimendan in guinea pig tracheal preparations. *Pharmacol Rep* 61: 275–280, 2009. doi:10.1016/S1734-1140(09)70032-5.
13. **Filep Á, Fodor GH, Kun-Szabó F, Tiszlavicz L, Rázga Z, Bozsó G, Bozóki Z, Szabó G, Peták F.** Exposure to urban PM1 in rats: development of bronchial inflammation and airway hyperresponsiveness. *Respir Res* 17: 26, 2016. doi:10.1186/s12931-016-0332-9.
14. **Follath F, Cleland JGF, Just H, Papp JGY, Scholz H, Peuhkurinen K, Harjola VP, Mitrovic V, Abdalla M, Sandell EP, Lehtonen L; Steering Committee and Investigators of the Levosimendan Infusion versus Dobutamine (LIDO) Study.** Efficacy and safety of intravenous levosimendan compared with dobutamine in severe low-output heart failure (the LIDO study): a randomised double-blind trial. *Lancet* 360: 196–202, 2002. doi:10.1016/S0140-6736(02)09455-2.
15. **Gajic O, Gropper MA, Hubmayr RD.** Pulmonary edema after transfusion: how to differentiate transfusion-associated circulatory overload from transfusion-related acute lung injury. *Crit Care Med Suppl* 34: S109–S113, 2006. doi:10.1097/01.CCM.0000214311.56231.23.
16. **Gödény I, Pollesello P, Edes I, Papp Z, Bagi Z.** Levosimendan and its metabolite OR-1896 elicit KATP channel-dependent dilation in resistance arteries in vivo. *Pharmacol Rep* 65: 1304–1310, 2013. doi:10.1016/S1734-1140(13)71488-9.

17. **Habre W, Peták F, Sly PD, Hantos Z, Morel DR.** Protective effects of volatile agents against methacholine-induced bronchoconstriction in rats. *Anesthesiology* 94: 348–353, 2001. doi:10.1097/00000542-200102000-00026.
18. **Haikala H, Linden IB.** Mechanisms of action of calcium-sensitizing drugs. *J Cardiovasc Pharmacol* 26, *Suppl* 1: S10–S19, 1995. doi:10.1097/00005344-199506261-00003.
19. **Hantos Z, Daróczy B, Suki B, Nagy S, Fredberg JJ.** Input impedance and peripheral inhomogeneity of dog lungs. *J Appl Physiol (1985)* 72: 168–178, 1992.
20. **Ichinose M, Barnes PJ.** A potassium channel activator modulates both excitatory noncholinergic and cholinergic neurotransmission in guinea pig airways. *J Pharmacol Exp Ther* 252: 1207–1212, 1990.
21. **Katircioglu SF, Seren M, Parlar AI, Turan NN, Manavbasi Y, Aydog G, Cicekcioglu F, Tutun U, Ulus AT.** Levosimendan effect on spinal cord ischemia-reperfusion injury following aortic clamping. *J Card Surg* 23: 44–48, 2008. doi:10.1111/j.1540-8191.2007.00486.x.
22. **Kivikko M, Antila S, Eha J, Lehtonen L, Pentikäinen PJ.** Pharmacokinetics of levosimendan and its metabolites during and after a 24-hour continuous infusion in patients with severe heart failure. *Int J Clin Pharmacol Ther* 40: 465–471, 2002. doi:10.5414/CPP40465.
23. **Lafci B, Yasa H, Ilhan G, Ortac R, Yilik L, Kestelli M, Goktogan T, Gurbuz A.** Protection of the spinal cord from ischemia: comparative effects of levosimendan and iloprost. *Eur Surg Res* 41: 1–7, 2008. doi:10.1159/000121394.
24. **Lele E, Petak F, Carnesecchi S, Virag K, Argiroffo CB, Habre W.** The protective effects of volatile anesthestics against the bronchoconstriction induced by an allergic reaction in sensitized rabbit pups. *Anesth Analg* 116: 1257–1264, 2013. doi:10.1213/ANE.0b013e31828e5ccf.
25. **Lenth RV.** Least-squares means: The R package lsmeans. *J Stat Softw* 69: 1–33, 2016. doi:10.18637/jss.v069.i01.
26. **Loring SH, Pecchiari M, Della Valle P, Monaco A, Gentile G, D'Angelo E.** Maintaining end-expiratory transpulmonary pressure prevents worsening of ventilator-induced lung injury caused by chest wall constriction in surfactant-depleted rats. *Crit Care Med* 38: 2358–2364, 2010. doi:10.1097/CCM.0b013e3181fa02b8.
27. **Lutchen KR, Hantos Z, Peták F, Adamicza A, Suki B.** Airway inhomogeneities contribute to apparent lung tissue mechanics during constriction. *J Appl Physiol (1985)* 80: 1841–1849, 1996.
28. **Myers CF, Fontao F, Jánosi TZ, Boda K, Peták F, Habre W.** Sevoflurane and desflurane protect cholinergic-induced bronchoconstriction of hyperreactive airways in rabbits. *Can J Anaesth* 58: 1007–1015, 2011. doi:10.1007/s12630-011-9578-3.
29. **Nieminen MS, Altenberger J, Ben-Gal T, Böhmer A, Comin-Colet J, Dickstein K, Edes I, Fedele F, Fonseca C, García-González MJ, Giannakoulas G, Iakobishvili Z, Jääskeläinen P, Karavidas A, Kettner J, Kivikko M, Lund LH, Matskeplishvili ST, Metra M, Morandi F, Oliva F, Parkhomenko A, Parissis J, Pollesello P, Pölzl G, Schwinger RH, Segovia J, Seidel M, Vrtovec B, Wikström G.** Repetitive use of levosimendan for treatment of chronic advanced heart failure: clinical evidence, practical considerations, and perspectives: an expert panel consensus. *Int J Cardiol* 174: 360–367, 2014. doi:10.1016/j.ijcard.2014.04.111.
30. **Packer M, Colucci W, Fisher L, Massie BM, Teerlink JR, Young J, Padley RJ, Thakkar R, Delgado-Herrera L, Salon J, Garratt C, Huang B, Sarapohja T, Group RHFS; REVIVE Heart Failure Study Group.** Effect of levosimendan on the short-term clinical course of patients with acutely decompensated heart failure. *JACC Heart Fail* 1: 103–111, 2013. doi:10.1016/j.jchf.2012.12.004.
31. **Papp Z, Édes I, Fruhwald S, De Hert SG, Salmenperä M, Leppikangas H, Mebazaa A, Landoni G, Grossini E, Caimmi P, Morelli A, Guarracino F, Schwinger RH, Meyer S, Algotsson L, Wikström BG, Jörgensen K, Filippatos G, Parissis JT, González MJ, Parkhomenko A, Yilmaz MB, Kivikko M, Pollesello P, Follath F.** Levosimendan: molecular mechanisms and clinical implications: consensus of experts on the mechanisms of action of levosimendan. *Int J Cardiol* 159: 82–87, 2012. doi:10.1016/j.ijcard.2011.07.022.
32. **Paticrica J, Hõhn J, Petri A, Balogh A, Papp JG.** Comparison of the vasorelaxing effect of cromakalim and the new inodilator, levosimendan, in human isolated portal vein. *J Pharm Pharmacol* 52: 213–217, 2000. doi:10.1211/0022357001773715.
33. **Pedersen CM, Rosendahl-Nielsen M, Hjermind J, Egerod I.** Endotracheal suctioning of the adult intubated patient–what is the evidence? *Intensive Crit Care Nurs* 25: 21–30, 2009. doi:10.1016/j.iccn.2008.05.004.
34. **Peták F, Hall GL, Sly PD.** Repeated measurements of airway and parenchymal mechanics in rats by using low-frequency oscillations. *J Appl Physiol (1985)* 84: 1680–1686, 1998.
35. **Peták F, Hantos Z, Adamicza A, Asztalos T, Sly PD.** Methacholine-induced bronchoconstriction in rats: effects of intravenous vs. aerosol delivery. *J Appl Physiol (1985)* 82: 1479–1487, 1997.
36. **Pierrakos C, Velissaris D, Franchi F, Muzzi L, Karanikolas M, Scolletta S.** Levosimendan in critical illness: a literature review. *J Clin Med Res* 6: 75–85, 2014..
37. **Pollesello P, Papp Z, Papp JG.** Calcium sensitizers: what have we learned over the last 25 years? *Int J Cardiol* 203: 543–548, 2016. doi:10.1016/j.ijcard.2015.10.240.
38. **Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; Authors/Task Force Members.** 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 37: 2129–2200, 2016. doi:10.1093/eurheartj/ehw128.
39. **Rieg AD, Rossaint R, Verjans E, Maihöfer NA, Uhlig S, Martin C.** Levosimendan relaxes pulmonary arteries and veins in precision-cut lung slices - the role of KATP-channels, cAMP and cGMP. *PLoS One* 8: e66195, 2013. doi:10.1371/journal.pone.0066195.
40. **Schellekens W-JM, van Hees HW, Linkels M, Dekhuijzen PN, Scheffer GJ, van der Hoeven JG, Heunks LM.** Levosimendan affects oxidative and inflammatory pathways in the diaphragm of ventilated endotoxemic mice. *Crit Care* 19: 69, 2015. doi:10.1186/s13054-015-0798-8.
41. **Segreti JA, Marsh KC, Polakowski JS, Fryer RM.** Evoked changes in cardiovascular function in rats by infusion of levosimendan, OR-1896 [(R)-N-(4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)acetamide], OR-1855 [(R)-6-(4-aminophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one], dobutamine, and milrinone: comparative effects on peripheral resistance, cardiac output, dP/dt, pulse rate, and blood pressure. *J Pharmacol Exp Ther* 325: 331–340, 2008. doi:10.1124/jpet.107.132530.
42. **Silva JM Jr, de Oliveira AM, Nogueira FA, Vianna PM, Pereira Filho MC, Dias LF, Maia VP, Neucamp CS, Amendola CP, Carmona MJ, Malbouisson LM.** The effect of excess fluid balance on the mortality rate of surgical patients: a multicenter prospective study. *Crit Care* 17: R288, 2013. doi:10.1186/cc13151.
43. **Sullivan KJ, Durand M, Ye TH, Chang HK.** Evaluation of lung mechanics in rabbits using short duration flow pulses. *Am Rev Respir Dis* 140: 17–24, 1989. doi:10.1164/ajrccm/140.1.17.
44. **Tritapepe L, De Santis V, Vitale D, Guarracino F, Pellegrini F, Pietropaoli P, Singer M.** Levosimendan pre-treatment improves outcomes in patients undergoing coronary artery bypass graft surgery. *Br J Anaesth* 102: 198–204, 2009. doi:10.1093/bja/aen367.
45. **van Hees HWH, Dekhuijzen PNR, Heunks LM.** Levosimendan enhances force generation of diaphragm muscle from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 179: 41–47, 2009. doi:10.1164/rccm.200805-732OC.
46. **Wang T, Gross C, Desai AA, Zemskov E, Wu X, Garcia AN, Jacobson JR, Yuan JX, Garcia JG, Black SM.** Endothelial cell signaling and ventilator-induced lung injury: molecular mechanisms, genomic analyses, and therapeutic targets. *Am J Physiol Lung Cell Mol Physiol* 312: L452–L476, 2017. doi:10.1152/ajplung.00231.2016.
47. **Yamakage M, Chen X, Kimura A, Iwasaki S, Namiki A.** The repolarizing effects of volatile anesthetics on porcine tracheal and bronchial smooth muscle cells. *Anesth Analg* 94: 84–88, 2002.
48. **Yasa H, Yakut N, Emrecan B, Ergunes K, Ortac R, Karahan N, Ozbek C, Gurbuz A.** Protective effects of levosimendan and iloprost on lung injury induced by limb ischemia-reperfusion: a rabbit model. *J Surg Res* 147: 138–142, 2008. doi:10.1016/j.jss.2007.08.002.