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The effects of levosimendan and glibenclamide on circulatory and metabolic variables in a canine model of acute hypoxia

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Abstract Purpose: To study the effects of pretreatment with levosimendan (LEVO, a Ca^{2+} -sensitizer and $\text{K}_{\text{ATP}}^{+}$ channel opener) and/or the $\text{K}_{\text{ATP}}^{+}$ channel antagonist glibenclamide (GLIB) on systemic hemodynamics, metabolism, and regional gastromucosal oxygenation during hypoxic hypoxemia. **Methods:** Chronically instrumented, healthy dogs (24–32 kg, $n = 6$ per group, randomized cross-over design) were repeatedly sedated, mechanically ventilated ($\text{FiO}_2 \sim 0.3$) and subjected to the following interventions: no pretreatment, LEVO pretreatment, GLIB pretreatment, or combined LEVO + GLIB pretreatment, each followed by hypoxic hypoxemia ($\text{FiO}_2 \sim 0.1$). We measured cardiac output (CO, ultrasonic flow probes), oxygen consumption (VO_2 , indirect calorimetry), and gastromucosal microvascular hemoglobin oxygenation (μHbO_2 , spectrophotometry). **Statistics:** data are presented as mean \pm SEM and compared by one-way ANOVA (direct drug effects within group) and two-way ANOVA (between all hypoxic conditions) both with Bonferroni corrections; $p < 0.05$. **Results:** In LEVO-pretreated hypoxemia, CO was significantly higher compared to unpretreated hypoxemia. The

increased CO was neither associated with an increased VO_2 nor with markers of aggravated anaerobiosis (pH, BE, lactate). In addition, LEVO pretreatment did not further compromise gastromucosal μHbO_2 in hypoxemia. After combined LEVO + GLIB pretreatment, systemic effects of GLIB were apparent, however, CO was significantly higher than during unpretreated and GLIB-pretreated hypoxemia, but equal to LEVO-pretreated hypoxemia, indicating that GLIB did not prevent the increased CO in LEVO-pretreated hypoxia. **Conclusions:** LEVO pretreatment resulted in improved systemic circulation (CO) during hypoxemia without fueling systemic VO_2 , without aggravating systemic anaerobiosis markers, and without further compromising microvascular gastromucosal oxygenation. Thus, LEVO pretreatment may be an option to support the systemic circulation during hypoxia.

Keywords Levosimendan · Hypoxia · Glibenclamide · Microcirculation · Oxygen · Metabolism

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Introduction

Hypoxemia complicates critical care settings, ranging from progressed lung disease to certain medical interventions [1–3]. Clearly, reversing hypoxemia is pivotal [4], but adapting pharmacological interventions to support the subject during impending or actual hypoxemia also appears beneficial. Systemic hypoxemia triggers multiple patho-mechanisms [5] also affecting the cardiovascular system: among others, at the cardiac level hypoxia depresses cardiac function by myofilament Ca^{2+} -desensitization [6, 7], and at the vascular level hypoxia triggers activation of K_{ATP}^+ channels [8], facilitating blood flow to tissues otherwise at risk for hypoperfusion by intense vasoconstriction.

In this context, the inodilator levosimendan (LEVO) appears to be an attractive option to support the cardiovascular system at risk in (impending) hypoxemia since it acts both as cardiac myofilament Ca^{2+} -sensitizer and K_{ATP}^+ channel opener, thus reversing hypoxia-induced cardiodepression and supporting regional vasodilation. This appears to be particularly of interest in hypoxic settings, since LEVO putatively increases cardiac output (CO) partly independent of an additional increase in oxygen consumption (VO_2) [9].

Hypoxia, like other cardiovascular stressors, triggers impairment of splanchnic oxygenation, likely through a combination of abnormal mesenteric perfusion [10–12] and hypoxemia. However, particularly the gastrointestinal mucosa is susceptible to hypoxia, and mucosal hypoxia appears to trigger the development of critical illness [13]. Thus, interventions intended to improve systemic (hemodynamic) variables during hypoxia should not further compromise regional mucosal circulation or oxygenation [14]. Therefore, in addition to systemic variables, we measured the effects on regional gastromucosal oxygenation.

Clinically, LEVO is primarily indicated for the treatment of acute and chronic ischemic cardiac failure, with promising clinical data available [15, 16]. Moreover, LEVO is also increasingly advocated to treat nonischemic cardiac failure, including septic or toxic cardiodepression [17–23]. The beneficial effects of LEVO, in addition to positive inotropic effects, may be caused by vasodilation [24]. Mechanistically, vasodilation by LEVO is mediated by activation of K_{ATP}^+ channels, which also appear involved in the vasodilation triggered by hypoxia [8, 25].

These K_{ATP}^+ channels, activated by LEVO or hypoxia, are pharmacologically blocked by glibenclamide (GLIB) [26–30]. In this context, GLIB pretreatment has previously been published as a research tool to study the contribution of GLIB-sensitive K_{ATP}^+ channels to the effects of LEVO and other cardiovascular challenges [26–30]. Thus, to elucidate the contribution of K_{ATP}^+ channels in the present study, we tested if K_{ATP}^+ channel blockade

by GLIB pretreatment would alter the (circulatory) condition during hypoxia, both with and without a LEVO pretreatment.

GLIB, based on the same mechanism, i.e., antagonizing K_{ATP}^+ channel-dependent vasodilation, is of growing interest in intensive care medicine [31]. In this context, GLIB is studied as nonadrenergic vasopressor in various shock-related vasodilatory states, caused by excessive K_{ATP}^+ channel activation. Examples for this therapeutic concept are septic or hemorrhagic vasodilation [32, 33]. In this regard, GLIB increases systemic vascular resistance and thus arterial blood pressure [32].

In the present study, the combination of LEVO (to increase cardiac contractility) and GLIB (to increase systemic vascular resistance) could achieve the putatively beneficial combination of antagonizing both possible cardiodepressant and vasodilatory effects of systemic hypoxia.

Materials and methods

The data derive from repetitive experiments on healthy dogs (foxhounds, $n = 6$, 24–32 kg) treated in accordance with the National Institutes of Health guidelines for animal care and with approval of the District Governmental Animal Investigation Committee.

For the continuous measurement of cardiac output (CO), ultrasonic flow transducers (S-series, Transonic, Ithaca, NY, USA) were chronically implanted around the pulmonary artery [34]. Before the experiments, food was withheld for 12 h. The experiments were performed under sedation with sevoflurane (end-tidal concentration 3.0 vol%, corresponding to ~ 1.25 MAC in dogs [35]) and neuromuscular block (rocuronium 0.6 mg kg^{-1} , followed by 1.0 mg $\text{kg}^{-1} \text{h}^{-1}$). Mechanical ventilation (FiO_2 0.3; tidal volume 12.5 mL kg^{-1} in dogs [36], respiratory rate $\sim 20 \text{ min}^{-1}$) was adjusted to maintain normocapnia as verified by continuous capnography (end-tidal CO_2 35 torr) and arterial blood gas analysis. The dogs were covered with warming blankets to maintain body temperature ($\sim 37.5^\circ\text{C}$, rectal thermoprobe).

Measurements

Systemic oxygen consumption

Systemic oxygen consumption (VO_2) was measured continuously by indirect calorimetry (Deltatrac-II metabolic monitor, Datex, Helsinki, Finland) and thus was methodologically independent from the determination of CO and DO_2 [9].

Systemic hemodynamics and oxygenation

We continuously measured heart rate (HR, electrocardiogram), mean arterial (aortic) pressure (MAP; P23ID, Elk Grove, CA, USA), central venous pressure (P23ID; right atrial catheter tip position confirmed by fluoroscopy), and ultrasound-derived CO (flowmeter T106, Transonic). At the end of each intervention, we performed transpulmonary thermodilution (PiCCO monitor with thermistor-tipped carotid catheter PV-2014L, Pulsion, Munich, Germany; measurements performed according to the manufacturer; with ~ 0.4 mL kg⁻¹ iced saline) to determine the following: global end diastolic volume (GEDV), derived intrathoracic blood volume (ITBV), and extravascular lung water (EVLW). The PiCCO system also provided the pulse-contour-derived stroke volume variation (SVV) and the estimate of cardiac contractility $+dP/dt_{\max}$. This system is validated to measure EVLW in dogs [37] and has also been applied in other canine studies [38].

Intermittently we measured arterial blood gas tensions (PaO₂, PaCO₂) and acid/base-related variables [pH, base excess (BE); ABL-700, Radiometer, Copenhagen, Denmark; no buffer solutions were used]. Additionally, we determined arterial serum metabolites (glucose, lactate) and electrolytes ([K⁺], [Ca²⁺], [Na⁺], [Cl⁻]). According to standard formulas, we calculated systemic vascular resistance (SVR), arterial oxygen content (CaO₂), and systemic oxygen transport (DO₂).

Gastromucosal oxygenation

Gastromucosal oxygenation was continuously assessed by reflectance spectrophotometry [9, 39]. Briefly, light (502–628 nm) is transmitted to the tissue via a micro light guide, and the reflected light is analyzed for the percentage of oxygenated microvascular hemoglobin (μHbO_2). The flexible probe (diameter 2.0 mm) was introduced into the stomach via an orogastric silicone tube (14 Charrière). During the experiments, correct probe position was confirmed by online evaluation of the signal quality (software version 2.0).

Experimental program

After induction (propofol 4 mg kg⁻¹ i.v.) and endotracheal intubation, all catheters were inserted and 30 min was allowed to establish steady-state conditions. Blood was sampled for baseline analysis, and thereafter a randomization was performed to allocate the dogs to the experimental groups.

Hypoxia episodes

The hypoxic episodes were induced by switching FiO₂ from 0.30 to 0.11 [40], as continuously measured (Capnomac ultima, Datex Instrumentarium, Helsinki, Finland). Each hypoxic episode was maintained for 15 min to allow steady states of the measured variables to be achieved.

Drugs

The agents LEVO and GLIB were infused as follows by a pressure-limited infusion pump via the central venous catheter.

LEVO

After baseline measurements, LEVO (Simdax, Abbott, Wiesbaden, Germany) was administered with an initial loading dose of 20.0 $\mu\text{g kg}^{-1}$ over 15 min, followed by continuous infusion of 0.25 $\mu\text{g kg}^{-1} \text{min}^{-1}$ for the remaining experimental period, a dosing regimen adapted from a similar canine model [9].

GLIB

GLIB (G0639, Sigma, Frankfurt, Germany), as K_{ATP}⁺ channel antagonist, was infused at a dose of 0.2 mg kg⁻¹ over 10 min, and 30 min were allowed for stabilization. This dose was derived from previous canine studies addressing the contribution of K_{ATP}⁺ channels to the effects of LEVO and other cardiovascular modulators [26–30].

Experimental protocols

Group I

This protocol served to study the effects of hypoxia per se and to study the associated effects of GLIB pretreatment. After steady-state baseline measurements, a first hypoxic challenge was performed. After 60 min recovery from this hypoxia (measurements after 30 and 60 min recovery), GLIB was infused. Following this GLIB pretreatment, a second hypoxic episode was induced.

Group II

This protocol served to study the effects of LEVO pretreatment on hypoxia and further to study the role of GLIB pretreatment in this regard. After a baseline period,

LEVO pretreatment was administered, and thereafter a first hypoxic episode was induced. After 60 min recovery from this hypoxia (measurements after 30 and 60 min recovery), with LEVO continuously infused, GLIB pretreatment was administered. Thereafter, with the combined LEVO + GLIB pretreatment, a second hypoxic challenge was induced.

Statistical analysis

Statistical analysis was performed using Prism software (version 5.0, GraphPad, La Jolla, CA, USA). Data are presented as absolute values of mean \pm SEM for $n = 6$ animals per group in the manuscript and tables. Direct drug effects (before onset of hypoxemia) were described by one-way ANOVA for repeated measurements, corrected for multiple comparisons (Bonferroni). Differences during hypoxemia were tested both between groups (i.e., I and II) and between the hypoxia conditions (i.e., without and with GLIB pretreatment) using a two-way ANOVA, corrected for multiple comparisons (Bonferroni). Significance was assumed at $p < 0.05$.

Results

Hypoxia model

Hypoxia was induced by reducing FiO_2 from 0.3 to 0.11, with maintenance of end-tidal CO_2 (35 torr) and end-tidal sevoflurane (3.0 vol%) concentrations. This was achieved for all four hypoxic conditions without significant inter- or intragroup differences (Table 1).

Hypoxia in untreated animals

In our hypoxia model, reduction of FiO_2 from 0.3 to 0.11 in untreated animals decreased PaO_2 from 133 ± 9 to 39 ± 1 Torr and thereby SaO_2 from 98 ± 0 to $68 \pm 1\%$. Together with an unchanged Hb (stable at ~ 12.5 g dL^{-1} , data not shown) this decreased CaO_2 from 17.3 ± 0.8 to 11.7 ± 0.6 mL dL^{-1} (Table 1). At the regional level, gastromucosal μHbO_2 decreased from 56 ± 3 to $32 \pm 4\%$ (Fig. 1). DO_2 decreased from 14 ± 1 to 9 ± 1 mL $\text{kg}^{-1} \text{min}^{-1}$, since CaO_2 decreased without compensatory increase in CO. However, systemic VO_2 was preserved at ~ 3.5 mL $\text{kg}^{-1} \text{min}^{-1}$ (Table 2). Mechanical ventilation adjusted to maintain etCO_2 at 35 Torr maintained PaCO_2 at 35–38 Torr. Acid/base-related variables remained stable, i.e., pH at 7.35–7.37 and HCO_3^- at ~ 20 mmol L^{-1} . Glucose and lactate (Table 1) and all measured electrolytes remained close to baseline (data not shown).

Hypoxia in GLIB-pretreated animals

GLIB pretreatment, used to block GLIB-sensitive K_{ATP}^+ channels, significantly increased SVR and significantly affected some blood-derived variables, e.g., it decreased arterial glucose concentration (Tables 1, 2).

Hypoxia after GLIB pretreatment resulted in a similar decreased CaO_2 (11.1 ± 0.5 mL dL^{-1}), compared to untreated animals (11.7 ± 0.6 mL dL^{-1}). Also, no statistical differences were observed in ventilation- and acid/base-derived parameters (PaCO_2 , pH, and BE) and the O_2 -related variables μHbO_2 , DO_2 , and VO_2 between these two hypoxic conditions. However, in GLIB-pretreated hypoxia, arterial lactate was significantly lower (~ 1.2 mmol L^{-1}) compared to untreated hypoxia (~ 2.6 mmol L^{-1}). Systemic hemodynamics (i.e., MAP and CO) did not differ between these two hypoxic episodes. Arterial electrolytes did not differ, and arterial glucose was significantly lower in GLIB-pretreated hypoxia (~ 80 mg dL^{-1}) compared to untreated hypoxia (~ 110 mg dL^{-1}) (Table 1).

Hypoxia in LEVO-pretreated animals

LEVO pretreatment at the macrocirculatory level significantly increased SV, CO, $+dP/dt_{\text{max}}$, and DO_2 and decreased SVR, without significant changes in HR. At the microcirculatory level, LEVO pretreatment significantly increased μHbO_2 at a stable systemic SaO_2 .

Hypoxia after LEVO pretreatment resulted in a similar decrease in CaO_2 to 10.3 ± 0.5 mL dL^{-1} , compared to untreated animals. In LEVO-pretreated hypoxia, CO was significantly higher compared to untreated hypoxia, i.e., ~ 100 versus ~ 80 mL $\text{kg}^{-1} \text{min}^{-1}$ (Figs. 2, 3). This was not paralleled by systemic VO_2 , which was comparable to untreated animals at ~ 3.5 mL $\text{kg}^{-1} \text{min}^{-1}$. Also gastromucosal μHbO_2 equally decreased in both untreated and LEVO-pretreated animals to $\sim 30\%$ (Fig. 1). Systemic vascular resistance was significantly lower in LEVO-pretreated hypoxia, compared to untreated hypoxia.

Hypoxia in LEVO- plus GLIB-pretreated animals

Administration of GLIB during LEVO infusion induced significant effects, e.g., GLIB significantly decreased serum glucose from 103 ± 4 to 87 ± 3 mg dL^{-1} (Table 1).

Hypoxic ventilation under combined pretreatment with LEVO and GLIB resulted in a PaO_2 of 32 Torr. During hypoxia with LEVO + GLIB pretreatment, CaO_2 tended to be lower compared to untreated hypoxia (9.4 ± 0.9 vs. 11.7 ± 0.6 mL dL^{-1}), however, without statistical significance. CO was significantly higher than during hypoxia in solely GLIB-pretreated animals and did

Table 1 Hypoxia model

Variable	Condition	Baseline	LEVO	Hypoxia-1	Recovery-30	Recovery-60	GLIB	Hypoxia-2	Recovery-30
FiO ₂ (%)	Group I	29.0 ± 0.0	(n.d.)	10.9 ± 0.1 ^a	29.0 ± 0.0	29.0 ± 0.0	29.0 ± 0.0	11.3 ± 0.1 ^a	29.1 ± 0.0
	Group II	29.3 ± 0.2	29.1 ± 0.3	10.6 ± 0.3 ^a	29.3 ± 0.2	29.2 ± 0.1	29.0 ± 0.1	10.7 ± 0.3 ^a	29.0 ± 0.1
CaO ₂ (mL dL ⁻¹)	Group I	17.3 ± 0.8	(n.d.)	11.7 ± 0.6 ^a	16.8 ± 0.8	16.4 ± 0.8	16.6 ± 1.0	11.1 ± 0.5 ^a	16.9 ± 0.8
	Group II	17.1 ± 0.5	16.9 ± 0.5*	10.3 ± 0.8 ^a	16.7 ± 0.5	16.8 ± 0.5	16.8 ± 0.5	9.4 ± 0.7 ^a	17.1 ± 0.7
PaO ₂ (torr)	Group I	133 ± 9	(n.d.)	39 ± 1 ^{b,c}	133 ± 8	137 ± 8	135 ± 8	36 ± 1 ^b	128 ± 8
	Group II	136 ± 8	135 ± 8	34 ± 1 ^{b,c}	129 ± 8	136 ± 8	134 ± 8	32 ± 2 ^b	125 ± 9
SaO ₂ (%)	Group I	98 ± 0	(n.d.)	68 ± 1 ^b	98 ± 0	98 ± 0	98 ± 0	64 ± 2 ^b	97 ± 0
	Group II	98 ± 0	98 ± 0	60 ± 3 ^b	97 ± 0	98 ± 0	98 ± 0 ^{\$}	54 ± 4 ^b	97 ± 0
PaCO ₂ (torr)	Group I	38 ± 1	(n.d.)	35 ± 1 ^a	38 ± 1	38 ± 1	37 ± 1	36 ± 1 ^a	38 ± 1
	Group II	37 ± 1	38 ± 1*	37 ± 1 ^a	39 ± 1	38 ± 1	38 ± 1	37 ± 1 ^a	38 ± 1
pH	Group I	7.35 ± 0.01	(n.d.)	7.37 ± 0.01 ^a	7.34 ± 0.01	7.36 ± 0.01	7.36 ± 0.01	7.38 ± 0.01 ^a	7.35 ± 0.01
	Group II	7.36 ± 0.01	7.36 ± 0.01	7.37 ± 0.01 ^a	7.34 ± 0.01	7.35 ± 0.01	7.35 ± 0.01	7.36 ± 0.01 ^a	7.33 ± 0.01
HCO ₃ ⁻ (mmol L ⁻¹)	Group I	20.2 ± 0.6	(n.d.)	19.8 ± 0.5 ^a	20.0 ± 0.5	20.6 ± 0.5	20.6 ± 0.5	20.6 ± 0.5 ^a	20.5 ± 0.6
	Group II	20.3 ± 0.4	20.8 ± 0.3	20.6 ± 0.3 ^a	20.5 ± 0.5	20.6 ± 0.5	20.6 ± 0.5	20.2 ± 0.4 ^a	19.6 ± 0.5
Lactate (mmol L ⁻¹)	Group I	2.3 ± 0.1	(n.d.)	2.6 ± 0.0 ^b	2.5 ± 0.2	2.2 ± 0.2	1.2 ± 0.1 ^{\$}	1.2 ± 0.1 ^b	0.9 ± 0.1
	Group II	1.8 ± 0.1	2.0 ± 0.1	2.3 ± 0.1 ^b	2.1 ± 0.1	2.0 ± 0.2	1.1 ± 0.1 ^{\$}	1.1 ± 0.1 ^b	0.8 ± 0.1
Glucose (mg dL ⁻¹)	Group I	100 ± 2	(n.d.)	106 ± 2 ^b	99 ± 3	98 ± 3	84 ± 2 ^{\$}	78 ± 3 ^b	52 ± 2
	Group II	94 ± 5	106 ± 3	114 ± 3 ^b	103 ± 3	103 ± 4	87 ± 3 ^{\$}	82 ± 6 ^b	51 ± 8

Values are mean ± SEM for $n = 6$ per group. There was no statistical difference in FiO₂ and resulting CaO₂ across the four hypoxic conditions

Recovery-30 and recovery-60 are measurement points at 30 and 60 min of normoxic recovery, FiO₂ inspiratory oxygen fraction, CaO₂ arterial oxygen content, PaO₂ arterial oxygen partial pressure, SaO₂ arterial oxygen saturation, PaCO₂ arterial carbon dioxide partial pressure, pH arterial pH, HCO₃⁻ arterial bicarbonate concentration, lactate arterial lactate concentration, glucose arterial glucose concentration

* Significant drug effects of LEVO and \$ GLIB

^a No difference between hypoxia episodes

^b Significant difference within one group's hypoxia episodes

^c Significant difference between the two groups' hypoxia episodes

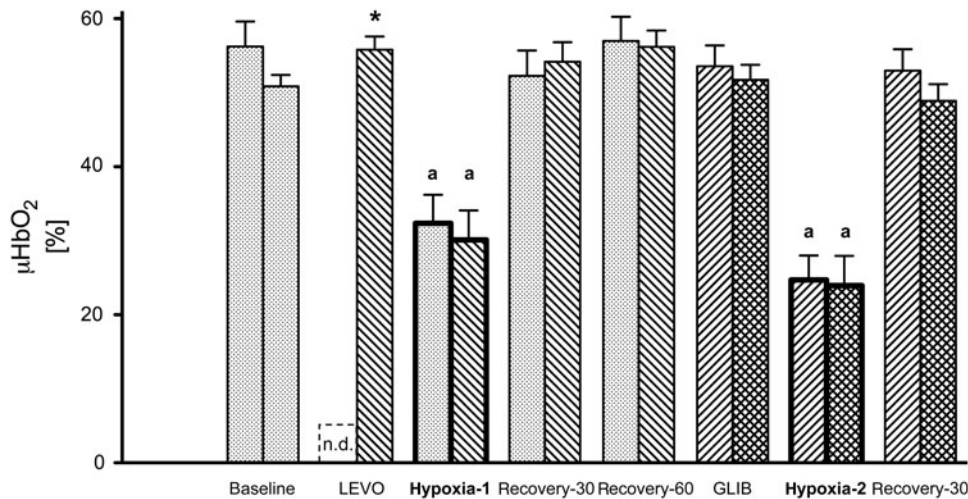


Fig. 1 Gastric mucosal microcirculatory hemoglobin oxygenation (μHbO_2). Gastromucosal μHbO_2 during normoxia ($\text{FiO}_2 \sim 0.3$, thin outlined bars) and the two hypoxia episodes ($\text{FiO}_2 \sim 0.1$, bold outlined bars) of the two experimental groups I (left bar per condition) and II (right bar per condition). Levosimendan was not given in group I, thus condition LEVO in group I is not determined (n.d.). Filling patterns: gray no pretreatment, downward stripes levosimendan (LEVO) pretreatment, upward stripes glibenclamide

(GLIB) pretreatment, hatches levosimendan + glibenclamide pretreatment. Recovery-30 and recovery-60 are measurement points at 30 and 60 min of normoxic recovery. Mean \pm SEM for $n = 6$ per group. ^aNo difference between hypoxia episodes, ^bsignificant difference within one group's hypoxia episodes, ^csignificant difference between the two groups' hypoxia episodes, * significant drug effects of LEVO and ^s GLIB

Table 2 Systemic hemodynamic variables and systemic oxygen metabolism

Variable	Condition	Baseline	LEVO	Hypoxia-1	Recovery-30	Recovery-60	GLIB	Hypoxia-2	Recovery-30
SV (mL)	Group I	18 \pm 1	(n.d.)	17 \pm 1 ^b	18 \pm 1	18 \pm 1	17 \pm 1	15 \pm 1 ^b	17 \pm 1
	Group II	20 \pm 1	22 \pm 1*	21 \pm 1 ^b	22 \pm 1	22 \pm 1	21 \pm 1	21 \pm 1 ^b	20 \pm 1
HR (bpm)	Group I	116 \pm 3	(n.d.)	121 \pm 2 ^a	114 \pm 3	113 \pm 3	112 \pm 2	117 \pm 2 ^a	112 \pm 2
	Group II	111 \pm 6	117 \pm 6	126 \pm 4 ^a	116 \pm 5	117 \pm 5	116 \pm 4	124 \pm 4 ^a	119 \pm 4
MAP (mmHg)	Group I	62 \pm 3	(n.d.)	66 \pm 4 ^a	63 \pm 2	62 \pm 2	65 \pm 2	62 \pm 4 ^a	63 \pm 1
	Group II	61 \pm 1	62 \pm 1	67 \pm 4 ^a	64 \pm 2	62 \pm 1	61 \pm 2	65 \pm 6 ^a	61 \pm 2
SVR (dyn s cm ⁻⁵)	Group I	2,468 \pm 235	(n.d.)	2,647 \pm 165 ^c	2,502 \pm 131	2,403 \pm 127	2,792 \pm 224 ^s	2,765 \pm 118 ^c	2,720 \pm 113
	Group II	2,295 \pm 127	1,943 \pm 87*	2,039 \pm 162 ^c	2,024 \pm 98	1,969 \pm 84	2,006 \pm 78	2,014 \pm 178 ^c	2,071 \pm 93
VO ₂ (mL kg ⁻¹ min ⁻¹)	Group I	3.5 \pm 0.1	(n.d.)	3.4 \pm 0.1 ^a	3.7 \pm 0.1	3.7 \pm 0.1	4.1 \pm 0.1 ^s	3.6 \pm 0.1 ^a	4.2 \pm 0.1
	Group II	3.3 \pm 0.2	3.7 \pm 0.2	3.3 \pm 0.3 ^a	3.5 \pm 0.1	3.6 \pm 0.1	3.8 \pm 0.1	3.5 \pm 0.2 ^a	3.5 \pm 0.2

Values are mean \pm SEM for $n = 6$ per group

Recovery-30 and recovery-60 are measurement points at 30 and 60 min of normoxic recovery, HR heart rate, MAP mean arterial pressure, SVR systemic vascular resistance, VO₂ systemic oxygen consumption

* Significant drug effects of LEVO and ^s GLIB

^a No difference between hypoxia episodes

^b Significant difference within one group's hypoxia episodes

^c Significant difference between the two groups' hypoxia episodes

not differ from solely LEVO-pretreated animals (Fig. 2). SVR was significantly lower in LEVO + GLIB-pretreated hypoxia compared to GLIB-pretreated hypoxia. Systemic VO₂ was comparable to all other hypoxic conditions at ~ 3.5 mL kg⁻¹ min⁻¹. Glucose, as expected, significantly decreased with GLIB.

Thermodilution-derived variables

Transpulmonary thermodilution (PiCCO) revealed no significant differences in the estimated blood volumes

ITBV (~ 20 mL kg⁻¹) and GEDV (~ 16 mL kg⁻¹) or estimated EWLTV (~ 9 mL kg⁻¹) between the four hypoxemia conditions (data not shown).

Discussion

For the present study we developed a model of moderate hypoxic hypoxemia, adapting reported canine hypoxia models [40, 41]. Repetitive experiments were performed

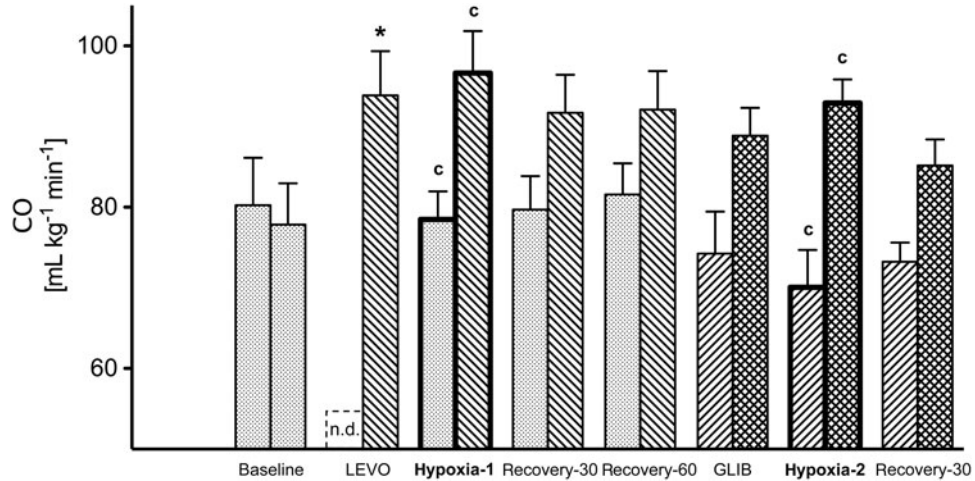


Fig. 2 Cardiac output. Cardiac output during normoxia ($\text{FiO}_2 \sim 0.3$, *thin outlined bars*) and the two hypoxia episodes ($\text{FiO}_2 \sim 0.1$, *bold outlined bars*) of the two experimental groups I (*left bar per condition*) and II (*right bar per condition*). Levosimendan was not given in group I, thus condition LEVO in group I is not determined (n.d.). Filling patterns: *gray* no pretreatment, *downward stripes* levosimendan (LEVO) pretreatment, *upward stripes*

glibenclamide (GLIB) pretreatment, hatches levosimendan + glibenclamide pretreatment. *Recovery-30* and *recovery-60* are measurement points at 30 and 60 min of normoxic recovery. Mean \pm SEM for $n = 6$ per group. ^aNo difference between hypoxia episodes, ^bsignificant difference within one group's hypoxia episodes, ^csignificant difference between the two groups' hypoxia episodes, *significant drug effects of LEVO and ^sGLIB

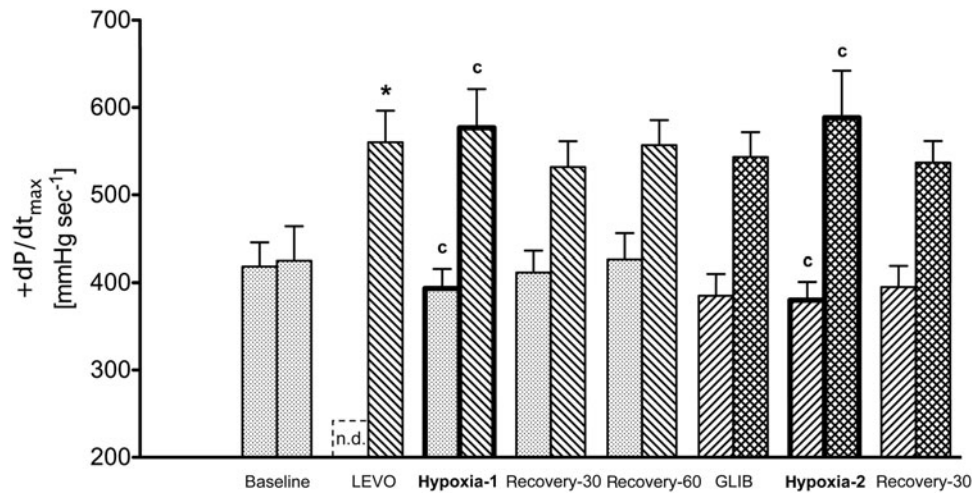


Fig. 3 Pressure-curve-derived contractility, $+dP/dt_{\max}$. Resulting $+dP/dt_{\max}$ during normoxia ($\text{FiO}_2 \sim 0.3$, *thin outlined bars*) and the two hypoxia episodes ($\text{FiO}_2 \sim 0.1$, *bold outlined bars*) of the two experimental groups I (*left bar per condition*) and II (*right bar per condition*). Levosimendan was not given in group I, thus condition LEVO in group I is not determined (n.d.). Filling patterns: *gray* no pretreatment, *downward stripes* levosimendan (LEVO) pretreatment, *upward stripes* glibenclamide (GLIB)

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in a randomized mode on healthy, chronically instrumented dogs with intervals of ≥ 2 weeks to exclude carryover effects and to minimize interindividual differences. Furthermore, the use of chronically instrumented animals avoided acute, surgical instrumentation and thus

confounders such as endocrine stress responses to surgery. In addition, our model allowed us to study the animals during sedation with sevoflurane and rocuronium alone, i.e., without need for analgesics or other confounding drugs [42].

In this study, two subsequent episodes of hypoxia were induced per group. While the first hypoxic episode served to elucidate the effect of LEVO pretreatment, the second hypoxic episode served to elucidate the role of K_{ATP}^+ channels (by subsequently adding GLIB pretreatment, reported to block GLIB-sensitive K_{ATP}^+ channels [26–30]). Between the two hypoxic episodes, an extended normoxic period was interposed to allow complete recovery of measured variables. In addition, to exclude the possibility that the first hypoxic episode was affecting the second with respect to measured variables, pilot experiments were performed, comparing a first with a second hypoxic period without adding any medication (i.e., no GLIB) in between. These pilot experiments demonstrated no differences between the first and second hypoxic episode, with respect to the measured variables.

Gastromucosal μHbO_2 was continuously measured by reflectance spectrophotometry [43], with the light guide nontraumatically introduced via an orogastric tube [9, 39]. Reflectance spectrophotometry allows determination of microcirculatory oxygen availability [44], and gastric endoluminal reflectance spectroscopy has been reported predominantly to measure capillary hemoglobin oxygenation of the mucosa [45].

Both hypoxia and the administered drugs may affect pulmonary endothelial permeability. Thus, we assessed EVLW by the PiCCO system, a method validated in anaesthetized dogs [37]. The EVLW data do not support pulmonary edema as cause of the small but significant differences in PaO_2 between distinct hypoxia conditions (i.e., slightly lower with LEVO and/or GLIB), rendering an altered pulmonary shunt, as also reported for other vasoactive agents [46], as the most likely explanation.

LEVO appears to be a promising candidate agent to support the cardiocirculatory system during hypoxemia, particularly because circulatory stimulation by LEVO is reportedly independent of metabolic stimulation [9]. Our data support this concept, demonstrating that LEVO pretreatment resulted in improved systemic circulation (CO) also during hypoxemia without fueling aerobic (VO_2) metabolism. Thus, the pattern of increased CO at stable VO_2 was present also during hypoxemia in LEVO-pretreated subjects and, in turn, other factors triggered by hypoxemia, e.g., changed pharmacology [47], did not alter this pattern. Measured key modulators of vascular tone (PaCO_2 , pH, temperature, arterial $[\text{K}^+]$ and $[\text{Ca}^{2+}]$) did not differ between the hypoxemic episodes, supporting that LEVO-specific mechanisms cause the differences in cardiovascular response.

The present study was designed to suggest a concept to improve the condition of the subject before an anticipated hypoxemia evolves; however, the majority of clinical hypoxemia episodes occur unexpectedly. Therefore, future studies will have to demonstrate if our findings are confirmed in the clinically more likely

setting, i.e., with LEVO administered in a therapeutic rather than prophylactic approach [24, 48].

Increased CO during hypoxemia after LEVO pretreatment, compared to unpretreated hypoxemia, was associated with an increased $+dP/dt_{\text{max}}$, compatible with positive inotropic effects of LEVO by myocardial Ca^{2+} sensitizing. LEVO, besides acting as a cardiac Ca^{2+} sensitizer, also activates K_{ATP}^+ channels. Physiologically, various K_{ATP}^+ channel isoforms exist, classified by their respective sulfonylurea receptors (SUR) in pancreatic β -cells (SUR-1), and striated (SUR-2A) and smooth muscle cells (SUR-2B). Vascular smooth muscle K_{ATP}^+ channels link regional metabolism to perfusion, since intracellular ATP depletion (e.g., during hypoxia) triggers these channels, leading to hyperpolarization and thus vasorelaxation [8]. However, excessive activation of these K_{ATP}^+ channels is involved in multiple pathological vasodilatory states, e.g., septic shock [8, 25]. Thus, whether pharmacological activation of K_{ATP}^+ channels under pathological conditions by LEVO is beneficial might also depend on the individual vasodilatory state. Pharmacologically, K_{ATP}^+ channels are antagonized by sulfonylurea derivatives, with GLIB (glyburide) reported to unspecifically block all receptor isoforms, SUR-1, SUR-2A, and SUR-2B. GLIB has been reported to be effective as a K_{ATP}^+ channel antagonist in dogs before, in similar dosages as applied in the present study [26–30]. Activity of GLIB in our model is suggested by the hypoglycemic response after GLIB pretreatment (SUR-1 effect), with arterial glucose decreased from baseline values of ~ 100 to ~ 50 mg dL^{-1} (end of experiments). In addition, GLIB pretreatment significantly increased SVR, suggesting (partial) blockade of vascular K_{ATP}^+ channels.

Thus, although GLIB pretreatment significantly increased SVR in this model, the demonstrated marked hypoglycemic effect of GLIB may limit its clinical use.

Combined GLIB + LEVO pretreatment resulted in significantly higher CO and $+dP/dt_{\text{max}}$ than GLIB pretreatment alone during hypoxemia, compatible with the notion that mechanisms other than K_{ATP}^+ channel opening contribute to LEVO effects, also during hypoxemia. Besides Ca^{2+} (re-)sensitization, LEVO's action as a phosphodiesterase (PDE) inhibitor could also contribute. In this regard, other PDE-III inhibitors have demonstrated positive systemic and regional gastrointestinal mucosal effects during hypoxemia [14].

Despite significant systemic differences, regional gastromucosal oxygenation did not differ between LEVO-pretreated and unpretreated hypoxemia, which decreased equally to $\sim 30\%$. Regional regulation of mucosal perfusion during hypoxemia could overrule the effects of LEVO, despite an increased CO and reduced SVR in LEVO-pretreated hypoxia. A possible mechanism is that local hypoxia activates vasodilatory K_{ATP}^+ channels to such extents that LEVO can not confer significant additional vasodilation. Indeed after GLIB pretreatment, administered to block these vasodilatory K_{ATP}^+ channels

and thus to induce vasoconstriction, we observed clear trends towards lower μHbO_2 both in LEVO-pretreated (from 32 ± 4 to $24 \pm 4\%$) and unpretreated hypoxemia (from 30 ± 4 to $25 \pm 3\%$). Interestingly, also in both groups arterial lactate concentrations were significantly lower after GLIB pretreatment. Since arterial pH and the other acid/base-related variables remained close to baseline under all hypoxemia conditions, we doubt that these significant differences in lactate concentration are caused by different perfusion states, rendering direct metabolic effects of GLIB more likely to contribute.

LEVO is clinically indicated for the treatment of chronic or acute cardiac failure, with promising results [15, 16], and indications have already extended to septic and toxic cardiac depression [17–23]. If our experimental data

apply to the clinical setting, LEVO pretreatment may also be an option to support the cardiovascular system during hypoxemia, without fueling VO_2 , without aggravating systemic anaerobiosis markers, and without further compromising the regional gastromucosal oxygenation. Further studies will have to extend these experimental results to other hypoxia models and ultimately to the clinical setting.

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