Hypercapnia counteracts captopril-induced depression of gastric mucosal oxygenation

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Abstract

Hypercapnia (HC) increases systemic oxygen delivery (DO_2) and gastric mucosal oxygenation. However, it activates the renin–angiotensin–aldosterone system (RAAS), which conversely reduces mesenteric perfusion. The aims of this study were to evaluate the effect of RAAS inhibition during normocapnia and HC on oral and gastric mucosal oxygenation (μHbO_2) and to assess the effect of blood pressure under these circumstances. Five dogs were repeatedly anesthetized to study the effects of ACE inhibition (ACE-I; 5 mg/kg captopril, followed by 0.25 mg/kg per h) on μHbO_2 (reflectance spectrophotometry) and hemodynamic variables during normocapnia (end-tidal $\text{CO}_2 = 35$ mmHg) and HC (end-expiratory carbon dioxide (etCO_2) = 70 mmHg). In the control group, the dogs were subjected to HC alone. To exclude the effects of reduced blood pressure, in one group, blood pressure was maintained at baseline values via titrated phenylephrine (PHE) infusion during HC and additional captopril infusion. ACE-I strongly reduced gastric μHbO_2 from 72 ± 2 to $65 \pm 2\%$ and mean arterial pressure (MAP) from 64 ± 2 to 48 ± 4 mmHg, while DO_2 remained unchanged. This effect was counteracted in the presence of HC, which increased gastric μHbO_2 from 73 ± 3 to $79 \pm 6\%$ and DO_2 from 15 ± 2 to 22 ± 4 ml/kg per min during ACE-I without differences during HC alone. However, MAP decreased similar to that observed during ACE-I alone from 66 ± 3 to 47 ± 5 mmHg, while left ventricular contractility (dP_{max}) increased from 492 ± 63 to 758 ± 119 mmHg/s. Titrated infusion of PHE had no additional effects on μHbO_2 . In summary, our data suggest that RAAS inhibition reduces gastric mucosal oxygenation in healthy dogs. HC not only abolishes this effect, but also increases μHbO_2 , DO_2 , and dP_{max} . The increase in μHbO_2 during ACE-I under HC is in accordance with our results independent of blood pressure.

Key Words

- hypercapnia
- renin–angiotensin–aldosterone system
- microcirculation
- gastrointestinal oxygenation

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Introduction

Hypercapnia (HC) occurs frequently during lung-protective ventilation strategies where it is rather tolerated than intended ('permissive HC'). However, HC exerts positive effects on systemic perfusion and oxygenation such as an increase in cardiac output (CO) and systemic

oxygen delivery (DO_2). Additionally, it protects microvascular oxygenation of the gastric mucosa during physiological conditions (Schwartges *et al.* 2008) and hemorrhagic shock (Schwartges *et al.* 2010). In this context, we have recently shown that the increase in gastric mucosal

oxygenation during otherwise physiological conditions is mediated via vasopressin V1A receptors (Vollmer *et al.* 2013) and might be due to increased levels of vasopressin during HC (Philbin *et al.* 1970). However, HC interacts with other hormone systems as well.

In particular, HC enhances renin secretion and thus leads to the activation of the renin–angiotensin–aldosterone system (RAAS; Kurz & Zehr 1978). Conversely, renin via angiotensin reduces especially mesenteric perfusion (McNeill *et al.* 1977, Bulkley *et al.* 1985, Bailey *et al.* 1986, Reilly *et al.* 1997) due to high affinity and quantity of the angiotensin receptor in the mesenteric region (Gunther *et al.* 1980) and increased regional angiotensin production (Bailey *et al.* 1986). The inhibition of the RAAS prior to hemorrhagic shock improves intestinal blood flow and reduces mortality (Aneman *et al.* 2000). Thus, the positive effects on microcirculatory oxygenation induced by acute HC might be partially counteracted by the activation of the RAAS (Kurz & Zehr 1978).

However, blood flow to the stomach is – in contrast to that to other splanchnic organs – not generally influenced by RAAS inhibition (Cullen *et al.* 1994). Additionally, RAAS inhibition reduces blood pressure, which might negatively affect gastric microcirculation. Concerning oxygenation of the gastric mucosa, the influence of RAAS inhibition is unclear so far.

The aims of this study were to evaluate the effect of RAAS inhibition with captopril during normocapnia and HC on oral and gastric mucosal oxygenation and assess the role of blood pressure under these circumstances. We expected either an additional increase in gastric oxygenation during RAAS inhibition due to the inhibition of a vasoconstrictor in the splanchnic region or a decrease in gastric oxygenation due to a reduction in blood pressure. Mucosal oxygenation is of particular interest as the gastrointestinal mucosa functions as an effective barrier against bacteria and an insufficient microcirculatory oxygen supply leads to an impaired mucosal barrier function. Subsequently, this enables the translocation of bacteria and bacterial toxins into portal venous and local lymphatic circulation (Deitch *et al.* 2004) and mediates an inflammatory response syndrome (Chen *et al.* 2003). Particularly, alterations in the oral microcirculation are an independent predictor of organ failure and associated with death (Maciel *et al.* 2004, Sakr *et al.* 2004).

The role of RAAS activation and RAAS inhibition during HC with special regard to blood pressure is of clinical importance for mainly two reasons. First, permissive HC is widely used in intensive care units during lung-protective ventilation, e.g. in patients with

acute respiratory distress syndrome. Second, the inhibition of the RAAS with angiotensin-converting enzyme inhibition is very common in patients for therapy of arterial hypertension and heart failure. Thus, it is not only important to know about the effects of HC and the RAAS on gastrointestinal mucosal oxygenation, but also essential to know about the interaction and possible counter-acting effects of both therapies with special regard to side effects, e.g. reduction in blood pressure, that again exert effects on mucosal oxygenation.

Taken together, the aim of this study was to assess the influence of RAAS inhibition on gastric and oral mucosal oxygenation during otherwise physiological conditions. Additionally, we evaluated the effect of RAAS inhibition during acute HC on microcirculation and systemic hemodynamic variables. Furthermore, we analyzed the effect of changes in blood pressure during RAAS inhibition.

Materials and methods

Animals

The data were derived from repetitive experiments on five dogs (female foxhounds, weighing 28 ± 1 kg) treated in accordance with the NIH guidelines for animal care. Experiments were performed with the approval of the Local Animal Care and Use Committee (North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection, Recklinghausen, Germany; ref. 50.05-230-74/05). The same group of five dogs used in this study was used in another study published previously (Vollmer *et al.* 2013). Each dog underwent each protocol in a randomized order and served as its own control. Based on the assumption that the physiological reaction on a given stimulus is almost identical in the same animal in repetitive experiments, usually observed inter-individual differences are reduced, allowing smaller sample sizes. A higher number of animals would allow us to detect even smallest significant differences; however, smallest differences will not be clinically relevant. The *a priori* power analysis (G*Power Version 3.1.7, University of Duesseldorf, Duesseldorf, Germany) with five dogs at a given $\alpha \leq 0.05$ (two tailed) and an expected difference in μHbO_2 of at least 10% (e.g. 70 vs 80%) with an expected s.d. of 5–6% per group (based on previous studies) suggested a power of 81.5%.

Prior to the experiments, access to food was withheld for 12 h with water *ad libitum* to ensure complete gastric depletion and to avoid changes in perfusion and

oxygenation due to digestive activity. Each dog underwent each experimental protocol in a randomized order and served as its own control (except group phenylephrine (PHE), in which measurements were carried out after completing the measurements in the other groups as described below). The experiments were performed at least 3 weeks apart to prevent carryover effects. The experiments were performed under general anesthesia (induction of anesthesia with 4 mg/kg propofol, maintenance with sevoflurane, end-tidal concentration of 3.0%, and 1.5 minimum alveolar concentration (MAC) in the dogs (Kazama & Ikeda 1988)). The dogs were mechanically ventilated after endotracheal intubation ($F_{iO_2}=0.3$ and $VT=12.5$ ml/kg, a normal tidal volume for the dogs (Dyson 2012)) with the respiratory frequency adjusted to achieve normocapnia (end-expiratory carbon dioxide ($etCO_2$)=35 mmHg), verified by continuous capnography (Capnomac Ultima, Datex Instrumentarium, Helsinki, Finland). During baseline conditions, the dogs were placed on their right side and covered with warming blankets to maintain body temperature at 37.5 °C (continuous arterial measurement). Throughout the experiments, no additional fluid replacement was carried out to avoid volume effects that could influence tissue perfusion and oxygenation. However, after each blood sample withdrawal, normal saline was infused three times the sampling volume to maintain blood volume.

Measurements

Mucosal oxygenation Microvascular oxygen saturation (μHbO_2) of the oral and gastric mucosa was continuously assessed by tissue reflectance spectrophotometry (O2C, LEA Medizintechnik, Gießen, Germany), as detailed previously (Frank *et al.* 1989).

White light (450–1000 nm) was transmitted into the tissue of interest via a micro-lightguide and the reflected light was then analyzed. The wavelength-dependent absorption of the applied white light can be used to calculate the percentage of oxygenated hemoglobin (Hb; Kuchenreuther *et al.* 1996).

Since light is totally absorbed in vessels with a diameter >100 μm (Gandjbakhche *et al.* 1999), only microvascular oxygenation of nutritive vessels of the mucosa was measured. The highest fraction of blood volume is stored in venous vessels; therefore, mainly postcapillary oxygenation was measured, which represents the critical partial pressure of oxygen (pO_2) for ischemia (Siegemund *et al.* 1999).

The flexible lightguide probe was placed in the mouth facing the buccal side of the oral mucosa, while another one was introduced into the stomach via an orogastric silicone tube and positioned facing the greater curvature (Scheeren *et al.* 2002). Both sites represent the microcirculation of other gastrointestinal mucosal regions (Temmesfeld-Wollbrück *et al.* 1998, Verdant *et al.* 2009). Online evaluation of the signal quality throughout the experiments allowed for the verification of the correct position of the probe tip. The μHbO_2 values reported are the means of the last 5 min (150 spectra, 2 s each) of the respective intervention period under steady-state conditions. This method allows for the detection of splanchnic ischemia with a precision similar to that of laser flowmetry (Leung *et al.* 1987), intravital microscopy (Bellamy *et al.* 1997), or hydrogen gas clearance (Machens *et al.* 1995). The non-traumatic instrumentation and in particular non-traumatic access to the gastric mucosa allow for the determination of mucosal oxygenation in the absence of surgical stress. This is particularly desirable with respect to the marked alterations that surgical stress exerts in splanchnic circulation (Mythen *et al.* 1993). In this situation, reflectance spectrophotometry reliably detects even clinically asymptomatic reductions in mucosal oxygenation (Fournell *et al.* 2003) and highly correlates with the morphologic severity and extent of gastric mucosal tissue injury (Sato *et al.* 1986).

Systemic hemodynamics and oxygenation The aorta was catheterized via the left carotid artery for the continuous measurement of mean arterial pressure (MAP; Gould-Statham pressure transducers P23ID, Elk Grove, IL, USA) and intermittent withdrawal of blood samples for the measurement of carbon dioxide partial pressure (pCO_2), pO_2 , pH, bicarbonate (HCO_3^-), Hb, and lactate (Rapidlab 860, Bayer AG). Oxygen saturation (S_aO_2) was calculated for dog blood from pO_2 and adjusted to pH and temperature (Rossing & Cain 1966). Arterial oxygen content (CaO_2) and systemic DO_2 ($DO_2=CaO_2 \times CO$) were calculated subsequently. CO was determined via transpulmonary thermodilution (PiCCO 4.2 non-US, PULSION Medical Systems, Munich, Germany) and the velocity of pressure increase $dP_{max} = \text{maximum } dP/dt$ as an indicator of left ventricular contractility via pulse contour analysis (PiCCO 4.2) at the end of each intervention, at least every 30 min, as described previously (von Spiegel *et al.* 1996).

Heart rate was continuously measured by electrocardiography (Powerlab, ADInstruments, Castle Hill, NSW, Australia). All hemodynamic and respiratory

variables were recorded on a personal computer after analog to digital conversion (Powerlab, ADInstruments) for later analysis.

Experimental program

The parameters described above were measured in all the experimental groups. Following the instrumentation instructions, 30 min were allowed to establish steady-state conditions and baseline values were recorded before the dogs were randomized to the respective protocol (Fig. 1). Groups that included HC were pretreated with HC for 1 h (end-tidal carbon dioxide was continuously increased over 30 min from 35 to 70 mmHg via reduction of respiratory frequency as described previously (Schwartzes *et al.* 2008)).

ACE inhibition The effects of ACE inhibition (ACE-I) alone were tested during normocapnia. After baseline conditions, ACE-I was initiated with an additional observation period of 60 min. ACE-I was induced by the administration of 5 mg/kg ACE inhibitor captopril (Sigma–Aldrich), followed by infusion of 0.25 mg/kg per h until the end of the experiments. Complete receptor blockade was confirmed by the administration of 20 ng/kg angiotensin I (Sigma–Aldrich) at the end of each experiment. This dose had no effect on hemodynamic variables in dogs with preceding ACE-I at the end of the observation period, but increased the MAP by about 25 mmHg in dogs without preceding ACE-I.

Figure 1

Experimental protocol: hypercapnia (HC), HC with ACE inhibition (HC/ACE-I), HC with phenylephrine during ACE-I (HC/ACE-I + PHE), ACE-I, and PHE infusion.

RAAS inhibition during HC (HC/ACE-I) To analyze the effects of ACE-I during HC, HC was maintained after hypercapnic pretreatment, while ACE-I was initiated as described above with an additional observation period of 60 min.

RAAS inhibition during HC with PHE (HC/ACE-I + PHE) To analyze the effects of HC during ACE-I independent of changes in blood pressure, after hypercapnic pretreatment, HC was maintained and ACE-I was initiated as described above. During ACE-I, blood pressure was maintained by continuous infusion of PHE ((R)-(–)-PHE hydrochloride, Sigma–Aldrich). Blood pressure was recorded continuously, and infusion rate was constantly adjusted if necessary.

Hypercapnia The effects of HC alone on μHbO_2 were tested without RAAS inhibition. After pretreatment, HC was maintained for an additional hour. The results of this control group have been published already as those of the control group (Vollmer *et al.* 2013) because we are forced by law to minimize animal experiments. However, this group was randomized in this series.

Effect of PHE To distinguish the effects mediated solely by PHE, this group was measured after completing the measurements in the first four groups. After baseline conditions, PHE was infused at the individual rate recorded in the same dog in the preceding protocol HC/ACE-I + PHE.

Blood samples were obtained for blood gas analysis every 30 min.

Statistical analysis

Data for analysis were obtained during the last 5 min of baseline and intervention periods under steady-state conditions. All data are presented as absolute values of mean $\pm$ S.E.M. for five dogs. Differences within the groups and between the groups were tested using a Wilcoxon's signed-rank test (StatView V4.1, SAS Institute, Inc., Cary, NC, USA); $P < 0.05$ was considered significant.

Results

ACE-I strongly reduced gastric μHbO_2 , which was completely reversed by HC without differences being observed during HC alone. This effect of HC was independent of blood pressure.

ACE inhibition

In detail, ACE-I alone reduced gastric μHbO_2 from 72 ± 2 to $65 \pm 2\%$ (Fig. 2), although systemic DO_2 remained unchanged (13 ± 1 vs 13 ± 1 ml/kg per min; Fig. 3). However, MAP strongly decreased from 64 ± 2 to 48 ± 4 mmHg (Fig. 4).

HC and HC during ACE-I (HC/ACE-I)

In contrast, during HC and additional ACE-I, DO_2 increased from 15 ± 2 to 22 ± 4 ml/kg per min and gastric μHbO_2 increased from 73 ± 3 to $79 \pm 6\%$, while oral μHbO_2 remained unchanged. This effect was similar to that observed during HC alone, which increased gastric μHbO_2 from 70 ± 4 to $80 \pm 2\%$ and systemic DO_2 from 12 ± 1 to 16 ± 1 ml/kg per min (Fig. 3) without any significant effect on oral μHbO_2 . The increase in gastric μHbO_2 during HC with additional ACE-I was observed despite a severe decrease in MAP from 66 ± 3 to 47 ± 5 mmHg. However, left ventricular contractility (dP_{max}) increased from 492 ± 63 to 758 ± 119 mmHg/s. In contrast, HC alone increased the MAP from 62 ± 2 to 69 ± 3 mmHg with no effect on dP_{max} .

Effect of additional PHE (HC/ACE-I + PHE)

When PHE was titrated to maintain blood pressure during HC and additional ACE-I, gastric μHbO_2 was increased similar to that observed during HC and HC with additional ACE-I (from 68 ± 3 to $79 \pm 2\%$). However, DO_2 was doubled from 13 ± 1 to 26 ± 2 ml/kg per min and was significantly higher than that observed during HC with ACE-I without PHE infusion. This change in systemic oxygen transport did not affect regional oxygenation (Figs 2, 3 and 4). Further hemodynamic variables are given in Table 1.

Figure 2

Effects of hypercapnia (HC), HC with ACE inhibition (HC/ACE-I), HC with phenylephrine during ACE-I (HC/ACE-I + PHE), ACE-I, and PHE infusion on gastric mucosal microvascular hemoglobin oxygen saturation (μHbO_2). Filled symbols, hypercapnic pretreated groups. Data are presented as mean $\pm$ s.e.m. for $n=5$ dogs; * $P<0.05$ vs HC/ACE-I for group ACE-I.

Figure 3

Effects of hypercapnia (HC), HC with ACE inhibition (HC/ACE-I), HC with phenylephrine during ACE-I (HC/ACE-I + PHE), ACE-I, and PHE infusion on systemic oxygen delivery (DO_2). Filled symbols, hypercapnic pretreated groups. Data are presented as mean $\pm$ s.e.m. for $n=5$ dogs; * $P<0.05$ vs HC/ACE-I for groups HC/ACE-I + PHE and ACE-I.

Influence of CO and oxygen content

The increase in systemic DO_2 during HC is related to not increased arterial oxygen content but rather to CO, which increased from 86 ± 6 to 111 ± 8 ml/kg per min. Increase in CO was similar during HC with ACE-I. CO tended to be higher during additional PHE infusion, though not significantly, but might explain the higher DO_2 compared with that during HC alone.

In contrast to the increase in μHbO_2 in all the three groups during HC, arterial oxygen partial pressure was reduced during HC from 154 ± 5 to 119 ± 6 mmHg, during HC with additional ACE-I from 161 ± 3 to 127 ± 3 mmHg, and during additional PHE infusion from 158 ± 5 to 132 ± 4 mmHg, while arterial oxygen partial pressure did not change during ACE-I without HC (153 ± 8 vs 155 ± 7 mmHg). Thus, mucosal oxygenation increased despite reduced arterial oxygen content.

Plasma lactate levels

Plasma lactate levels decreased during HC independent of additional ACE-I and independent of blood pressure. In contrast, during ACE-I without HC, plasma lactate levels did not change significantly. Further metabolic and respiratory variables are given in Table 2.

Discussion

Interpretation of the results

Our data suggest that ACE-I reduces gastric but not oral mucosal oxygenation in healthy dogs. HC during additional ACE-I seems to counteract the decrease in

Figure 4

Effects of hypercapnia (HC), HC with ACE inhibition (HC/ACE-I), HC with phenylephrine during ACE-I (HC/ACE-I+PHE), ACE-I, and PHE infusion on mean arterial pressure (MAP). Filled symbols, hypercapnic pretreated groups. Data are presented as mean $\pm$ s.e.m. for $n=5$ dogs; * $P<0.05$ vs HC/ACE-I for groups HC and HC/ACE-I+PHE.

gastric μHbO_2 and even to increase gastric μHbO_2 , DO_2 , and left ventricular contractility. The effect of HC on gastric μHbO_2 during additional ACE-I is independent of reduced blood pressure, which affects μHbO_2 only during normocapnia. The restrictions and implications of these findings will be discussed in detail below.

The results are partially unexpected. First, ACE-I reduced gastric μHbO_2 . The RAAS is known to reduce mesenteric perfusion (McNeill *et al.* 1977, Bulkley *et al.* 1985, Bailey *et al.* 1986, Reilly *et al.* 1997), and inhibition of the RAAS during captopril therapy increased blood flow in the superior mesenteric artery (Ray-Chaudhuri *et al.* 1993). Similar effects are observed during long-term treatment (Rozsa & Sonkodi 1995) and in critically ill trauma patients (Kincaid *et al.* 1998). Hence, one might expect RAAS inhibition to increase gastric μHbO_2 . The contradictory reduction in μHbO_2 might be related to the severe decrease in MAP reducing blood flow to the splanchnic region. Still, blood pressure was equally reduced during additional HC without negative effects on μHbO_2 . Hence, our divergent results are not related to blood pressure. However, angiotensin II antagonism does not alter splanchnic perfusion under all circumstances (Strohmenger *et al.* 1998), and captopril increased blood flow in another study selectively to the small intestine but not to the stomach (Cullen *et al.* 1994). This might be related to the blood supply in the gastric region that does not depend on the superior mesenteric artery. Thus, our results indicate that gastric oxygenation and perfusion might not benefit from RAAS inhibition. On the contrary, gastric oxygenation even decreased during captopril infusion, which is not attributed to short-term effects such as a decrease in blood pressure. This effect is of major

importance as RAAS inhibition with captopril is a widely used therapy for hypertension and heart failure. Especially, the critically ill patients might benefit from increased gastric oxygenation. However, our reported results are based on experiments in five healthy dogs. Whether the

Table 1 Hemodynamic variables of the experimental groups.

Data are presented as absolute values during baseline conditions and the respective intervention (at the end of the experiment), mean $\pm$ s.e.m., $n=5$

Variable	Group	Baseline	Intervention (1 h)
Gastric μHbO_2 (%)	HC	70 $\pm$ 4	80 $\pm$ 2*
	HC/ACE-I	73 $\pm$ 3	79 $\pm$ 6*
	HC/ACE-I+PHE	68 $\pm$ 3	79 $\pm$ 2*
	ACE-I	72 $\pm$ 2	65 $\pm$ 2* [†]
	PHE	68 $\pm$ 4	73 $\pm$ 3
Oral μHbO_2 (%)	HC	72 $\pm$ 2	81 $\pm$ 3
	HC/ACE-I	78 $\pm$ 2	74 $\pm$ 4
	HC/ACE-I+PHE	77 $\pm$ 2	75 $\pm$ 2 [†]
	ACE-I	79 $\pm$ 2	77 $\pm$ 4
	PHE	76 $\pm$ 2	60 $\pm$ 4*
DO_2 (ml/kg per min)	HC	12 $\pm$ 1	16 $\pm$ 1*
	HC/ACE-I	15 $\pm$ 2	22 $\pm$ 4*
	HC/ACE-I+PHE	13 $\pm$ 1	26 $\pm$ 2* [†]
	ACE-I	13 $\pm$ 1	13 $\pm$ 1 [†]
	PHE	14 $\pm$ 3	17 $\pm$ 2*
SVR (mmHg/l per min)	HC	26 $\pm$ 2	23 $\pm$ 2
	HC/ACE-I	28 $\pm$ 3	14 $\pm$ 1* [†]
	HC/ACE-I+PHE	28 $\pm$ 3	21 $\pm$ 3* [†]
	ACE-I	29 $\pm$ 3	21 $\pm$ 1* [†]
	PHE	30 $\pm$ 2	54 $\pm$ 4*
CO (ml/kg per min)	HC	86 $\pm$ 6	111 $\pm$ 8*
	HC/ACE-I	87 $\pm$ 10	125 $\pm$ 19*
	HC/ACE-I+PHE	84 $\pm$ 9	126 $\pm$ 16*
	ACE-I	83 $\pm$ 9	83 $\pm$ 8 [†]
	PHE	80 $\pm$ 10	75 $\pm$ 9
SV (ml)	HC	23 $\pm$ 2	29 $\pm$ 3*
	HC/ACE-I	22 $\pm$ 2	31 $\pm$ 4*
	HC/ACE-I+PHE	22 $\pm$ 2	33 $\pm$ 4* [†]
	ACE-I	21 $\pm$ 2	21 $\pm$ 2 [†]
	PHE	20 $\pm$ 2	23 $\pm$ 1*
MAP (mmHg)	HC	62 $\pm$ 2	69 $\pm$ 3*
	HC/ACE-I	66 $\pm$ 3	47 $\pm$ 5* [†]
	HC/ACE-I+PHE	62 $\pm$ 1	70 $\pm$ 1* [†]
	ACE-I	64 $\pm$ 2	48 $\pm$ 4*
	PHE	65 $\pm$ 2	111 $\pm$ 9*
HR (min)	HC	104 $\pm$ 4	109 $\pm$ 5
	HC/ACE-I	111 $\pm$ 5	113 $\pm$ 7
	HC/ACE-I+PHE	106 $\pm$ 4	105 $\pm$ 4
	ACE-I	110 $\pm$ 7	111 $\pm$ 7
	PHE	111 $\pm$ 5	93 $\pm$ 6*
dP_{max} (mmHg/min)	HC	470 $\pm$ 57	542 $\pm$ 46
	HC/ACE-I	492 $\pm$ 63	758 $\pm$ 119*
	HC/ACE-I+PHE	468 $\pm$ 36	736 $\pm$ 43* [†]
	ACE-I	450 $\pm$ 53	513 $\pm$ 45
	PHE	442 $\pm$ 30	468 $\pm$ 30

μHbO_2 , gastric and oral mucosal hemoglobin oxygenation; DO_2 , systemic oxygen transport; SVR, systemic vascular resistance; CO, cardiac output; SV, stroke volume; MAP, mean arterial pressure; HR, heart rate; dP_{max} , left ventricular contractility. * $P<0.05$ vs baseline, [†] $P<0.05$ vs HC for groups HC/ACE-I and HC/ACE-I+PHE, and [‡] $P<0.05$ vs HC/ACE-I for groups HC/ACE-I+PHE and ACE-I.

Table 2 Metabolic and respiratory variables of the experimental groups. Data are presented as absolute values during baseline conditions and the respective intervention (at the end of the experiment), mean $\pm$ S.E.M., $n=5$

Variable	Group	Baseline	Intervention (1 h)
S _a O ₂ (%)	HC	98 $\pm$ 1	95 $\pm$ 1*
	HC/ACE-I	99 $\pm$ 1	96 $\pm$ 1*
	HC/ACE-I + PHE	99 $\pm$ 1	96 $\pm$ 1*,†
	ACE-I	98 $\pm$ 1	98 $\pm$ 1†
	PHE	99 $\pm$ 1	98 $\pm$ 1
pCO ₂ (mmHg)	HC	39 $\pm$ 1	67 $\pm$ 1*
	HC/ACE-I	38 $\pm$ 1	67 $\pm$ 2*
	HC/ACE-I + PHE	38 $\pm$ 1	64 $\pm$ 1*
	ACE-I	38 $\pm$ 1	38 $\pm$ 1†
	PHE	38 $\pm$ 1	36 $\pm$ 1
pO ₂ (mmHg)	HC	154 $\pm$ 5	119 $\pm$ 6*
	HC/ACE-I	158 $\pm$ 5	125 $\pm$ 5*
	HC/ACE-I + PHE	158 $\pm$ 5	132 $\pm$ 4*,†
	ACE-I	153 $\pm$ 8	155 $\pm$ 7†
	PHE	149 $\pm$ 1	147 $\pm$ 1
pH	HC	7.33 $\pm$ 0.01	7.15 $\pm$ 0.01*
	HC/ACE-I	7.37 $\pm$ 0.01	7.17 $\pm$ 0.01*
	HC/ACE-I + PHE	7.36 $\pm$ 0.01	7.17 $\pm$ 0.01*
	ACE-I	7.35 $\pm$ 0.01	7.35 $\pm$ 0.01*,†
	PHE	7.38 $\pm$ 0.02	7.37 $\pm$ 0.01
HCO ₃ ⁻ (mmol/l)	HC	20 $\pm$ 1	23 $\pm$ 1*
	HC/ACE-I	21 $\pm$ 1	24 $\pm$ 1*
	HC/ACE-I + PHE	21 $\pm$ 1	23 $\pm$ 1*
	ACE-I	21 $\pm$ 1	21 $\pm$ 1†
	PHE	22 $\pm$ 1	21 $\pm$ 1
Hb (g/100 ml)	HC	11 $\pm$ 1	11 $\pm$ 1*
	HC/ACE-I	12 $\pm$ 1	13 $\pm$ 1
	HC/ACE-I + PHE	12 $\pm$ 1	16 $\pm$ 1*,†,‡
	ACE-I	12 $\pm$ 1	12 $\pm$ 1
	PHE	12 $\pm$ 1	17 $\pm$ 1*
Lactate (mmol/l)	HC	2.1 $\pm$ 0.3	1.2 $\pm$ 0.1
	HC/ACE-I	1.9 $\pm$ 0.1	1.0 $\pm$ 0.1
	HC/ACE-I + PHE	2.7 $\pm$ 0.1	1.3 $\pm$ 0.1*
	ACE-I	1.9 $\pm$ 0.1	2.0 $\pm$ 0.2
	PHE	2.1 $\pm$ 0.3	2.1 $\pm$ 0.3

S_aO₂, systemic oxygen saturation; pCO₂, carbon dioxide partial pressure; pO₂, oxygen partial pressure; HCO₃⁻, bicarbonate; pH; Hb, hemoglobin; and lactate. * $P < 0.05$ vs baseline, † $P < 0.05$ vs HC for groups HC/ACE-I and HC/ACE-I + PHE, and ‡ $P < 0.05$ vs HC/ACE-I for groups HC/ACE-I + PHE and ACE-I.

results can be transferred into the clinical setting on critically ill patients has to be confirmed in larger clinical trials. Additionally, our results do not indicate the effects of long-term treatment where adaptive mechanisms might occur and where the RAAS is only partially inhibited. However, the effects of captopril seem similar during long-term treatment (Rozsa & Sonkodi 1995).

Still, in critically ill patients, permissive HC could be tested as a preventive option to compensate for the decrease in oxygenation during additional captopril infusion despite the decrease in blood pressure. In healthy

dogs, μ HbO₂ even increased during HC under ACE-I comparable to the increase during HC alone. In addition, CO and accordingly systemic oxygen transport increased. Thus, HC was able to increase μ HbO₂, despite reduced blood pressure due to captopril. Additionally, HC with additional ACE-I increased left ventricular contractility, while neither HC nor ACE-I alone affected contractility. The increase in dP_{max} is not related to the reduction in systemic vascular resistance, which is reduced during ACE-I alone as well without an effect on dP_{max}.

The observation of increased μ HbO₂ during HC in our previous studies (Schwartzes *et al.* 2008, 2010) was confirmed in the present study. However, as one possibility, we expected HC to activate the RAAS and thus to partially antagonize its positive effects on μ HbO₂. If this were the case, captopril would further increase μ HbO₂ during HC. This could not be observed as additional captopril infusion during HC did not change μ HbO₂ compared with that during HC alone. One explanation might be the above-mentioned effect of angiotensin mainly in the superior mesenteric artery, but angiotensin exerts effects on the gastric mucosa as well. This could be observed during angiotensin I application prior to captopril infusion in pilot experiments. However, another explanation might be the acute reduction in blood pressure during captopril infusion under HC comparable to that during captopril infusion alone, which even reduced μ HbO₂. However, the maintenance of blood pressure with PHE did not change the effects of HC and captopril on μ HbO₂, although PHE increased oxygen delivery under physiological as well as under hypercapnic conditions. One further explanation might be that HC alone had already increased μ HbO₂ to 80%. As μ HbO₂ represents mainly the postcapillary oxygenation, a further increase might not be possible.

The most likely explanation for our unexpected results of decreased μ HbO₂ during RAAS inhibition compared with that in other studies (McNeill *et al.* 1977, Bulkley *et al.* 1985, Bailey *et al.* 1986, Reilly *et al.* 1997) might be related to our approach in assessing oxygenation rather than to perfusion. The measurement of perfusion does not indicate oxygen supply necessary for the gastrointestinal mucosa. Additionally, angiotensin might be necessary for the redistribution of blood in favor of the gastric mucosa during physiological conditions. An explanation for the redistribution of blood might be the unique configuration of parallel and series-coupled circuits. Initially, mesenteric blood flow is divided into two parallel circuits, one serving the muscularis and the other serving the submucosa/mucosa (Ceppa *et al.* 2003). Angiotensin-mediated

vasoconstriction of peripheral arteries might reduce blood flow to the muscularis and thus maintain blood flow to the submucosa and mucosa. Therefore, angiotensin might be necessary for maintaining μHbO_2 under physiological conditions, while higher angiotensin levels during pathological conditions reduce splanchnic flow and μHbO_2 . This theory is supported by the observation that oral μHbO_2 does not change during ACE-I or additional HC and thus seems to be independent of angiotensin-mediated redistribution.

We have to critically discuss that enzyme or receptor inhibition might result in an increased activation of other enzymes or receptors with adverse side effects. In this case, ACE-I leads to reduced aldosterone production, which, however, rather exerts a long-term effect on water retention, which did not influence our observation period. Additionally, inactivation of bradykinin is inhibited as well with subsequent endothelium-dependent vasodilation via the B_2 receptor, which would partially explain the severe reduction in blood pressure. Without the conversion of angiotensin I to angiotensin II, the substrate angiotensin I might accumulate; however, it is not vasoactive. We did not choose an AT_1 receptor antagonist to selectively block the vascular effect of angiotensin II. AT_1 receptor antagonism leads to increased binding to the AT_2 receptor with possible adverse side effects. Additionally, our main intention was to analyze the effect of the total RAAS on gastric oxygenation during HC, especially since captopril is widely used, particularly in critically ill patients with hypertension or heart failure.

In the repeated-measurements design, treatment cross-over could be a confounding variable. However, the used drugs have a half-life of 1.9 h (captopril) and 2–3 h (PHE), assuring complete elimination after 8–12 h. The experiments were performed at least 3 weeks apart, making the carryover effects unlikely. This is confirmed by the absence of any significant differences concerning macrocirculatory and microcirculatory variables during baseline conditions.

In summary, captopril infusion reduces gastric oxygenation in healthy dogs, despite improved perfusion in the splanchnic region in other studies. We demonstrated that the acute, negative effects of captopril were completely reversed in the presence of HC. During permissive HC, additional therapy with captopril does not seem to affect the increase in gastric oxygenation. However, the combination of HC and captopril increases left ventricular contractility. Nevertheless, the results obtained from dogs have to be tested in the clinical setting in critically ill patients in the future.

Conclusions

Our data suggest that captopril reduces gastric mucosal oxygenation in healthy dogs. In this situation, HC seems to not only abolish this effect but also increase gastric oxygenation, systemic DO_2 , and left ventricular contractility. According to our data, this effect is independent of blood pressure.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Aneman A, Svensson M, Broome M, Biber B, Petterson A & Fandriks L 2000 Specific angiotensin II receptor blockage improves intestinal perfusion during graded hypovolemia in pigs. *Critical Care Medicine* **28** 818–823. (doi:10.1097/00003246-200003000-00034)
- Bailey RW, Bulkley GB, Hamilton SR, Morris JB & Smith GW 1986 Pathogenesis of nonocclusive ischemic colitis. *Annals of Surgery* **203** 590–599. (doi:10.1097/0000658-198606000-00002)
- Bellamy MC, Mullane D, O'Beirne HA, Young Y, Pollard SG & Lodge JP 1997 Dopexamine and microcirculatory flow in transplanted small bowel: the Leeds experience. *Transplantation Proceedings* **29** 1847–1849. (doi:10.1016/S0041-1345(97)00093-6)
- Bulkley GB, Kvietys PR, Parks DA, Perry MA & Granger DN 1985 Relationship of blood flow and oxygen consumption to ischemic injury in the canine small intestine. *Gastroenterology* **89** 852–857.
- Ceppa EP, Fuh KC & Bulkley GB 2003 Mesenteric hemodynamic response to circulatory shock. *Current Opinion in Critical Care* **9** 127–132. (doi:10.1097/00075198-200304000-00008)
- Chen LW, Egan L, Li ZW, Greten FR, Kagnoff MF & Karin M 2003 The two faces of IKK and NF- κ B inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia–reperfusion. *Nature Medicine* **9** 575–581. (doi:10.1038/nm849)
- Cullen JJ, Ephgrave KS, Broadhurst KA & Booth B 1994 Captopril decreases stress ulceration without affecting gastric perfusion during canine hemorrhagic shock. *Journal of Trauma* **37** 43–49. (doi:10.1097/00005373-199407000-00009)
- Deitch EA, Forsythe R, Anjaria D, Livingston DH, Lu Q, Xu DZ & Redl H 2004 The role of lymph factors in lung injury, bone marrow suppression, and endothelial cell dysfunction in a primate model of trauma–hemorrhagic shock. *Shock* **22** 221–228. (doi:10.1097/01.shk.0000133592.55400.83)
- Dyson DH 2012 Positive pressure ventilation during anesthesia in dogs: assessment of surface area derived tidal volume. *Canadian Veterinary Journal* **53** 63–66.
- Fournell A, Schwarte LA, Kindgen-Milles D, Muller E & Scheeren TW 2003 Assessment of microvascular oxygen saturation in gastric mucosa in volunteers breathing continuous positive airway pressure. *Critical Care Medicine* **31** 1705–1710. (doi:10.1097/01.CCM.0000063281.47070.53)
- Frank KH, Kessler M, Appelbaum K & Dummiller W 1989 The Erlangen micro-lightguide spectrophotometer EMPHO I. *Physics in Medicine and Biology* **34** 1883–1900. (doi:10.1088/0031-9155/34/12/011)

- Gandjbakhche AH, Bonner RF, Arai AE & Balaban RS 1999 Visible-light photon migration through myocardium *in vivo*. *American Journal of Physiology* **277** H698–H704.
- Gunther S, Gimbrone MA Jr & Alexander RW 1980 Identification and characterization of the high affinity vascular angiotensin II receptor in rat mesenteric artery. *Circulation Research* **47** 278–286. (doi:10.1161/01.RES.47.2.278)
- Kazama T & Ikeda K 1988 Comparison of MAC and the rate of rise of alveolar concentration of sevoflurane with halothane and isoflurane in the dog. *Anesthesiology* **68** 435–437. (doi:10.1097/00000542-198803000-00020)
- Kincaid EH, Miller PR, Meredith JW & Chang MC 1998 Enalaprilat improves gut perfusion in critically injured patients. *Shock* **9** 79–83. (doi:10.1097/00024382-199802000-00001)
- Kuchenreuther S, Adler J, Schutz W, Eichelbronner O & Georgieff M 1996 The Erlanger microlightguide photometer: a new concept for monitoring intracapillary oxygen supply of tissue – first results and a review of the physiological basis. *Journal of Clinical Monitoring* **12** 211–224. (doi:10.1007/BF00857642)
- Kurz KD & Zehr JE 1978 Mechanisms of enhanced renin secretion during CO₂ retention in dogs. *American Journal of Physiology* **234** H573–H581.
- Leung FW, Morishita T, Livingston EH, Reedy T & Guth PH 1987 Reflectance spectrophotometry for the assessment of gastroduodenal mucosal perfusion. *American Journal of Physiology* **252** G797–G804.
- Machens HG, Pallua N, Mailaender P, Pasel J, Frank KH, Reimer R & Berger A 1995 Measurements of tissue blood flow by the hydrogen clearance technique (HCT): a comparative study including laser Doppler flowmetry (LDF) and the Erlangen micro-lightguide spectrophotometer (EMPHO). *Microsurgery* **16** 808–817. (doi:10.1002/micr.1920161208)
- Maciel AT, Creteur J & Vincent JL 2004 Tissue capnometry: does the answer lie under the tongue? *Intensive Care Medicine* **30** 2157–2165. (doi:10.1007/s00134-004-2416-0)
- McNeill JR, Wilcox WC & Pang CC 1977 Vasopressin and angiotensin: reciprocal mechanisms controlling mesenteric conductance. *American Journal of Physiology* **232** H260–H266.
- Mythen MG, Purdy G, Mackie IJ, McNally T, Webb AR & Machin SJ 1993 Postoperative multiple organ dysfunction syndrome associated with gut mucosal hypoperfusion, increased neutrophil degranulation and C1-esterase inhibitor depletion. *British Journal of Anaesthesia* **71** 858–863. (doi:10.1093/bja/71.6.858)
- Philbin DM, Baratz RA & Patterson RW 1970 The effect of carbon dioxide on plasma antidiuretic hormone levels during intermittent positive-pressure breathing. *Anesthesiology* **33** 345–349. (doi:10.1097/00000542-197009000-00016)
- Ray-Chaudhuri K, Thomaidis T, Maule S, Watson L, Lowe S & Mathias CJ 1993 The effect of captopril on the superior mesenteric artery and portal venous blood flow in normal man. *British Journal of Clinical Pharmacology* **35** 517–524. (doi:10.1111/j.1365-2125.1993.tb04178.x)
- Reilly PM, Toung TJ, Miyachi M, Schiller HJ & Bulkley GB 1997 Hemodynamics of pancreatic ischemia in cardiogenic shock in pigs. *Gastroenterology* **113** 938–945. (doi:10.1016/S0016-5085(97)70190-2)
- Rossing RG & Cain SM 1966 A nomogram relating pO₂, pH, temperature, and hemoglobin saturation in the dog. *Journal of Applied Physiology* **21** 195–201.
- Rozsa Z & Sonkodi S 1995 The effect of long-term oral captopril treatment on mesenteric blood flow in spontaneously hypertensive rats. *Pharmacological Research* **32** 21–25. (doi:10.1016/S1043-6618(95)80004-2)
- Sakr Y, Dubois MJ, De Backer D, Creteur J & Vincent JL 2004 Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Critical Care Medicine* **32** 1825–1831. (doi:10.1097/01.CCM.0000138558.16257.3F)
- Sato N, Kawano S, Kamada T & Takeda M 1986 Hemodynamics of the gastric mucosa and gastric ulceration in rats and in patients with gastric ulcer. *Digestive Diseases and Sciences* **31** 35S–41S. (doi:10.1007/BF01309321)
- Scheeren TW, Schwarte LA, Loer SA, Picker O & Fournell A 2002 Dopexamine but not dopamine increases gastric mucosal oxygenation during mechanical ventilation in dogs. *Critical Care Medicine* **30** 881–887. (doi:10.1097/00003246-200204000-00028)
- Schwartzes I, Schwarte LA, Fournell A, Scheeren TW & Picker O 2008 Hypercapnia induces a concentration-dependent increase in gastric mucosal oxygenation in dogs. *Intensive Care Medicine* **34** 1898–1906. (doi:10.1007/s00134-008-1183-8)
- Schwartzes I, Picker O, Beck C, Scheeren TW & Schwarte LA 2010 Hypercapnic acidosis preserves gastric mucosal microvascular oxygen saturation in a canine model of hemorrhage. *Shock* **34** 636–642. (doi:10.1097/SHK.0b013e3181e68422)
- Siegemund M, van Bommel J & Ince C 1999 Assessment of regional tissue oxygenation. *Intensive Care Medicine* **25** 1044–1060. (doi:10.1007/s001340051011)
- von Spiegel T, Wietasch G, Bursch J & Hoeft A 1996 Cardiac output determination with transpulmonary thermodilution. An alternative to pulmonary catheterization? *Der Anaesthetist* **45** 1045–1050. (doi:10.1007/s001010050338)
- Strohmenger HU, Lindner KH, Wienen W & Radermacher P 1998 Effects of an angiotensin II antagonist on organ perfusion during the post-resuscitation phase in pigs. *Critical Care* **2** 49–55. (doi:10.1186/cc125)
- Temmesfeld-Wollbruck B, Szalay A, Mayer K, Olschewski H, Seeger W & Grimminger F 1998 Abnormalities of gastric mucosal oxygenation in septic shock: partial responsiveness to dopexamine. *American Journal of Respiratory and Critical Care Medicine* **157** 1586–1592. (doi:10.1164/ajrccm.157.5.9710017)
- Verdant CL, De Backer D, Bruhn A, Clausi CM, Su F, Wang Z, Rodriguez H, Pries AR & Vincent JL 2009 Evaluation of sublingual and gut mucosal microcirculation in sepsis: a quantitative analysis. *Critical Care Medicine* **37** 2875–2881. (doi:10.1097/CCM.0b013e3181b029c1)
- Vollmer C, Schwartzes I, Naber S, Beck C, Bauer I & Picker O 2013 Vasopressin V1A receptors mediate the increase in gastric mucosal oxygenation during hypercapnia. *Journal of Endocrinology* **217** 59–67. (doi:10.1530/JOE-12-0526)

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