

# Vasopressin V<sub>1A</sub> receptors mediate the increase in gastric mucosal oxygenation during hypercapnia

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## Abstract

Hypercapnia (HC) improves systemic oxygen delivery (DO<sub>2</sub>) and microvascular hemoglobin oxygenation of the mucosa (μHbO<sub>2</sub>). Simultaneously, HC increases plasma levels of vasopressin. Although vasopressin is generally regarded a potent vasoconstrictor particularly in the splanchnic region, its effects on splanchnic microcirculation during HC is unclear. The aim of this study was to evaluate the role of endogenous vasopressin on gastric mucosal oxygenation and hemodynamic variables during physiological (normocapnia) and hypercapnic conditions. Five dogs were repeatedly anesthetized to study the effect of vasopressin V<sub>1A</sub> receptor blockade ([Pmp<sup>1</sup>, Tyr(Me)<sup>2</sup>]-Arg<sup>8</sup>-Vasopressin, 35 μg/kg) on hemodynamic variables and μHbO<sub>2</sub> during normocapnia or HC (end-tidal CO<sub>2</sub> 70 mmHg). In a control group, animals were subjected to HC alone. μHbO<sub>2</sub> was measured by reflectance spectrophotometry, systemic DO<sub>2</sub> was calculated from intermittent blood gas analysis, and cardiac output was measured by transpulmonary thermodilution. Data are presented as mean ± s.e.m. for n=5 animals. During HC alone, DO<sub>2</sub> increased from 12 ± 1 to 16 ± 1 ml/kg per min and μHbO<sub>2</sub> from 70 ± 4 to 80 ± 2%. By contrast, additional vasopressin V<sub>1A</sub> receptor blockade abolished the increase in μHbO<sub>2</sub> (80 ± 2 vs 69 ± 2%) without altering the increase in DO<sub>2</sub> (16 ± 1 vs 19 ± 2 ml/kg per min). Vasopressin V<sub>1A</sub> receptor blockade (VB) during normocapnia neither affected DO<sub>2</sub> (13 ± 1 vs 14 ± 1 ml/kg per min) nor μHbO<sub>2</sub> (75 ± 3 vs 71 ± 5%). Vasopressin V<sub>1A</sub> receptor blockade abolished the increase in μHbO<sub>2</sub> during HC independent of DO<sub>2</sub>. Thus, in contrast to its generally vasoconstrictive properties, the vasopressin V<sub>1A</sub> receptors seem to mediate the increase in gastric microcirculatory mucosal oxygenation induced by acute HC.

## Key Words

- ▶ hypercapnia
- ▶ vasopressin
- ▶ microcirculation
- ▶ gastrointestinal oxygenation

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## Introduction

The gastrointestinal tract is a complex organ system with multiple functions. Apart from being responsible for nutrient absorption, it is an important metabolic and immunological system, functioning as an effective barrier against endotoxin and bacteria from the intestinal lumen. To maintain this barrier, adequate perfusion and oxygenation of the mucosa are vital (Trzeciak *et al.* 2008).

However, during severe illness, the blood flow is redistributed to preserve perfusion of more vital organs (i.e. heart and brain) and thus splanchnic oxygenation is impaired early (Jakob & Takala 2000, Schwartges *et al.* 2010). An impaired mucosal barrier function resulting from insufficient microcirculatory oxygen supply has been shown to enable translocation of bacteria and bacterial

toxins into portal venous and local lymphatic circulation (Deitch *et al.* 2004) and to mediate an inflammatory response syndrome (Chen *et al.* 2003). Therefore, adequate splanchnic perfusion and in particular oxygenation of the gastrointestinal mucosa are considered crucial for the prevention and therapy of critical illness (Trzeciak *et al.* 2008). Thus, growing effort is made to develop strategies to improve splanchnic mucosal oxygenation.

In this context, we have recently shown that hypercapnia (HC) improves systemic oxygen delivery (DO<sub>2</sub>) and microvascular oxygenation of the gastric mucosa not only during physiological conditions (Schwartzes *et al.* 2008) but also during hemorrhagic shock (Schwartzes *et al.* 2010). Apart from these effects on macro- and microcirculation, HC exerts multiple other changes in homeostasis, like an increase in vasopressin plasma level (Philbin *et al.* 1970, Forsling & Rees 1975, Farber *et al.* 1982, Rose *et al.* 1984) with subsequent binding to the vasopressin V<sub>1A</sub> receptors that could mediate or modulate the effects on microcirculation. Vasopressin is regarded as a potent vasoconstrictor particularly in the splanchnic region and reduces systemic DO<sub>2</sub>, cardiac output, and jejunal mucosal oxygen partial pressure (Knotzer *et al.* 2005). Thus, the positive effects on microcirculatory oxygenation induced by acute HC might be partially counteracted by an increase in vasopressin levels.

However, the effects of vasopressin on the splanchnic microcirculation were recently controversially discussed (Asfar *et al.* 2006). The expected negative effects of vasopressin could not be observed in a septic animal model (Knotzer *et al.* 2006) and even an improvement of gut microcirculatory flow is possible (Asfar *et al.* 2003). Furthermore, the effect seems to depend on flow. In animals with high systemic flow *per se*, gut microcirculatory flow was even increased additionally due to terlipressin (Asfar *et al.* 2003). By contrast, during low flow conditions, terlipressin infusion increased mortality (Asfar *et al.* 2003). However, despite its generally vasoconstrictive properties, vasopressin and its V<sub>1A</sub> receptor exert vasodilation as well (Evora *et al.* 1993). Little is known about the effect of exogenous vasopressin on regional oxygenation. The dosage applied seems to be an important factor, though studies are rare (Asfar *et al.* 2006). But most studies focused on macro- and microcirculatory hemodynamic variables (Asfar *et al.* 2008). Thus, the impact of endogenous vasopressin and its receptor on splanchnic oxygenation remains unclear, particularly during non-physiological situations like acute HC. The aim of our study was to evaluate the role of endogenous vasopressin during normo- and HC on gastric mucosal oxygenation in healthy animals.

## Materials and methods

### Animals

The data were derived from repetitive experiments on five dogs (female foxhounds, weighing 28 ± 1 kg) treated in accordance with NIH guidelines for animal care and performed with approval of the local animal care and use committee.

Before the experiments, 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 randomized order and served as its own control. Experiments were performed at least 3 weeks apart to prevent carryover effects. In each experiment, the dogs received an i.v. catheter that was removed at the end of the experiment. The experiments were performed under general anesthesia. General anesthesia was induced via the i.v. catheter (application of 4 mg/kg propofol) and maintained with inhalation of sevoflurane (end-tidal concentration 3.0%, 1.5 minimum alveolar concentration (MAC) in dogs). The animals were mechanically ventilated after endotracheal intubation (semi-closed circle ventilation, inspiratory oxygen concentration F<sub>i</sub>O<sub>2</sub> = 0.3, tidal volume = 12.5 ml/kg, a normal tidal volume for dogs) with the respiratory frequency adjusted to achieve normocapnia (end-expiratory concentration of CO<sub>2</sub> etCO<sub>2</sub> = 35 mmHg), verified by continuous capnography (Capnomac Ultima, Datex Instrumentarium, Helsinki, Finland). After induction of anesthesia and during the experiments, the dogs were placed on their right side covered with warming blankets to maintain body temperature within the physiological range for dogs (37–38.5 °C, continuous arterial measurement). Throughout the experiments, no additional fluid replacement was administered to avoid volume effects that could influence tissue perfusion and oxygenation. However, after each blood withdrawal, normal saline was infused three times the sampling volume to maintain blood volume.

### Measurements

**Gastric mucosal oxygenation** Microvascular oxygen saturation (μHbO<sub>2</sub>) of the gastric mucosa was continuously assessed by tissue reflectance spectrophotometry (O2C, LEA Medizintechnik, Gießen, Germany), as detailed previously (Frank *et al.* 1989). Briefly, after induction of anesthesia, the flexible light-guide probe is introduced into the stomach non-traumatically via an orogastric

silicone tube. White light (450–1000 nm) is transmitted to the tissue of interest, in this case the gastric mucosa, via the micro-light guide and the reflected light is then analyzed *in vivo*. The wavelength-dependent absorption of the applied white light can be used to calculate the percentage of oxygenated hemoglobin (Kuchenreuther *et al.* 1996).

As light is totally absorbed in vessels with a diameter >100 µm, only microvascular oxygenation of nutritive vessels of the mucosa is measured. The biggest fraction of the blood volume is stored in venous vessels; therefore, mainly postcapillary oxygenation is measured that represents the critical partial pressure of oxygen (pO<sub>2</sub>) for ischemia.

The flexible light-guide probe introduced into the stomach is positioned facing the greater curvature (Scheeren *et al.* 2002), a site demonstrated to represent the microvascular oxygenation of other gastric and upper intestinal mucosa regions (Temmesfeld-Wollbrück *et al.* 1998). Online evaluation of the signal quality throughout the experiments allows verification of the correct position of the probe tip. The µHbO<sub>2</sub> values reported are the means of the last 5 min (150 spectra, 2 s each) of the respective intervention under steady state conditions. This method allows detection of splanchnic ischemia with similar precision as laser flowmetry (Leung *et al.* 1987), intra-vital 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 allows the determination of mucosal oxygenation in the absence of surgical stress. This is particularly desirable with respect to the marked alterations surgical stress exerts on splanchnic circulation (Gelman 1976). 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).

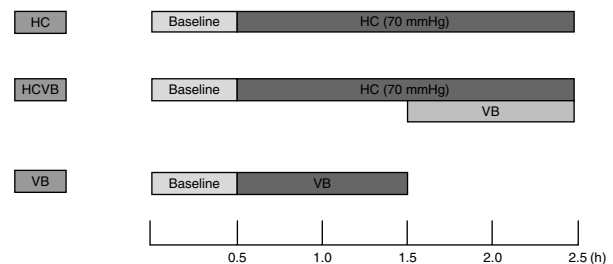
**Systemic hemodynamics and oxygenation** For continuous measurement of mean arterial pressure, a catheter (PiCCO 4.2 non-US, PULSION Medical Systems, Munich, Germany) was inserted via the left carotid artery into the aorta. This catheter was connected to a pressure transducer (MAP, Gould-Statham pressure transducers P23ID, Elk Grove, IL, USA) and mean arterial pressure was continuously recorded. Via this catheter, blood samples were withdrawn intermittently for measurement of blood gas tensions (arterial CO<sub>2</sub> tension, arterial oxygen

tension, and arterial oxygen saturation) and acid-base-related variables like pH and bicarbonate (Rapidlab 860, Bayer AG). Blood lactate concentrations could only be obtained in 13 of 15 experiments. Arterial oxygen content (CaO<sub>2</sub>) and systemic DO<sub>2</sub> (DO<sub>2</sub>=CaO<sub>2</sub>×cardiac output) were calculated. Cardiac output was determined via transpulmonary thermodilution (PiCCO 4.2) at the end of each intervention, at least every 30 min, as described previously (von Spiegel *et al.* 1996). Briefly, cold saline was injected intravenously. The temperature of injected saline was measured at the injection site (PiCCO 4.2). The subsequent change in blood temperature measured at the site of the arterial catheter depends on cardiac output that was calculated with the Stewart–Hamilton equation.

Heart rate (HR) 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.

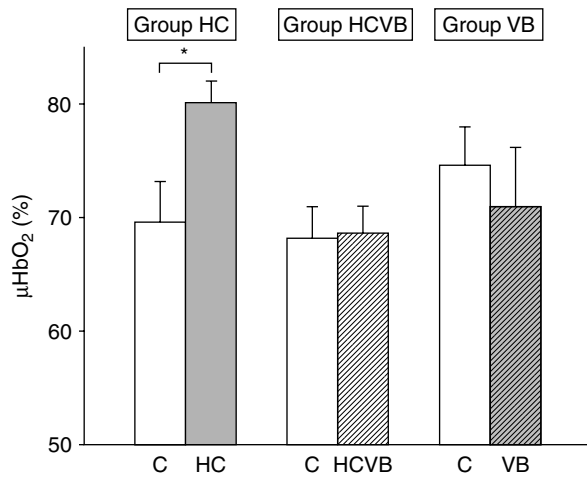
### Vasopressin receptor blockade

Vasopressin receptor blockade was induced by i.v. infusion of 35 µg/kg [Pmp<sup>1</sup>,Tyr(Me)<sup>2</sup>]-Arg<sup>8</sup>-Vasopressin (Peptide Institute, Inc., 4-1-2 Ina, Minoh-shi Osaka, 562-8686, Japan), a selective V<sub>1A</sub> receptor antagonist (Howl & Wheatley 1995), as single dose. Complete receptor blockade was confirmed by administration of vasopressin (250 mU, [Arg<sup>8</sup>]-Vasopressin, Sigma–Aldrich) at the end of each experiment. This dose had no effect on hemodynamic variables at the end of the observation period but increased mean arterial pressure by about 20 mmHg without preceding vasopressin receptor blockade.



**Figure 1**

Experimental protocol: hypercapnia *per se* (HC), HC with subsequent vasopressin receptor blockade (HCVB), and vasopressin receptor blockade (VB) during normocapnia in dogs.



**Figure 2**

Microvascular hemoglobin oxygenation. Effects on microvascular hemoglobin oxygenation ( $\mu\text{HbO}_2$ ) during hypercapnia (HC), HC with subsequent vasopressin receptor blockade (HCVB), and vasopressin receptor blockade during normocapnia (VB) with corresponding baseline values (C) in dogs. Data are presented as absolute values for  $n=5$  dogs, mean  $\pm$  s.e.m., \* $P<0.05$ .

### Vasopressin plasma level

Vasopressin plasma levels during HC were determined only exemplarily in single experiments because increase in vasopressin plasma levels during HC has been observed before (Philbin *et al.* 1970, Forsling & Rees 1975, Farber *et al.* 1982, Rose *et al.* 1984). Blood samples were collected in EDTA-containing tubes, immediately centrifuged, and stored at  $-20^\circ\text{C}$  for later analysis by an external laboratory (Labor Limbach, Heidelberg, Germany). Vasopressin plasma levels during vasopressin receptor blockade are not of informative value and were not determined.

### Experimental program

Following the instrumentation, 30 min were allowed to establish steady state conditions and baseline values were recorded before the animals were randomized to the respective protocol (Fig. 1).

**Hypercapnia (HC)** As control group, effects of HC on  $\mu\text{HbO}_2$  were tested without vasopressin receptor blockade. End-tidal  $\text{CO}_2$  was continuously increased from 35 to 70 mmHg over 30 min via reduction in respiratory frequency as described previously (Schwartzges *et al.* 2008). HC was maintained for 2 h.

### Vasopressin receptor blockade during hypercapnia (HCVB)

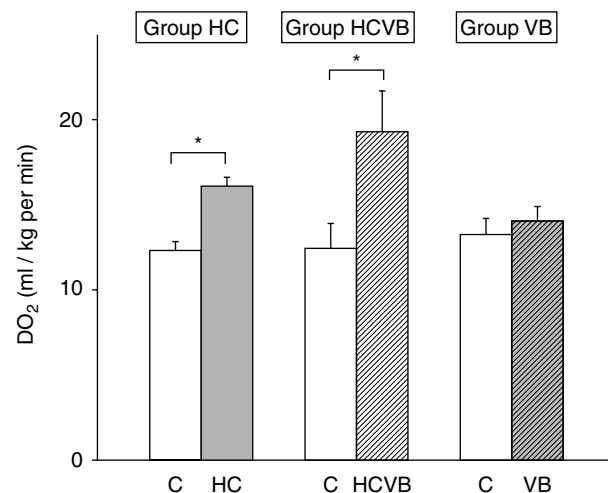
To analyze the effects of HC during vasopressin  $V_{1A}$  receptor blockade (HCVB), HC was induced as in control experiments. After 60 min of HC, vasopressin  $V_{1A}$  receptor blockade was initiated as described earlier with an additional observation period of 60 min.

### Vasopressin receptor blockade (VB)

Effects of vasopressin  $V_{1A}$  receptor blockade alone were tested during normocapnia. After baseline conditions, vasopressin  $V_{1A}$  receptor blockade was initiated as described earlier with an additional observation period of 60 min. At the end of each intervention, blood samples were obtained for blood gas analysis.

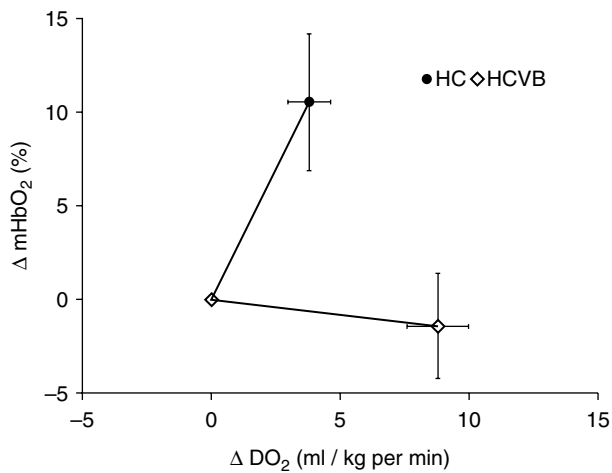
### Statistical analysis

Data for analysis were obtained during the last 5 min of each intervention under steady state conditions. Normal data distribution was assessed in Q-Q plots (IBM SPSS Statistics, International Business Machine Corp.). All data are presented as absolute values of mean ( $\pm$ s.e.m.) for  $n=5$  animals. Differences within the groups and between the groups were tested using an ANOVA for repeated measurements (ANOVA) and a Fisher's PLSD test as *post hoc* test (StatView V4.1, SAS Institute Inc., Cary, NC, USA);  $P<0.05$  was considered significant.



**Figure 3**

Systemic oxygen delivery ( $\text{DO}_2$ ). Effects on systemic  $\text{DO}_2$  during hypercapnia (HC), HC with subsequent vasopressin receptor blockade (HCVB), or vasopressin receptor blockade during normocapnia (VB) with corresponding baseline values (C) in dogs. Data are presented as absolute values for  $n=5$  dogs, mean  $\pm$  s.e.m., \* $P<0.05$ .



**Figure 4**

Relationship between mucosal oxygenation and systemic oxygen transport. Relationship between regional mucosal oxygenation ( $\mu\text{HbO}_2$ ) and systemic oxygen transport ( $\text{DO}_2$ ) under hypercapnia (HC, group HC) and HC with subsequent vasopressin receptor blockade (HCVB, group HCVB) in dogs. To compensate for individual baseline differences, the respective effects were zero-referenced in this figure.

## Results

HC increased  $\mu\text{HbO}_2$  from  $70 \pm 4$  to  $80 \pm 2\%$  after 2 h of HC (Fig. 2). Subsequent vasopressin  $V_{1A}$  receptor blockade (HCVB) abolished the HC-induced increase in  $\mu\text{HbO}_2$  ( $80 \pm 2$  vs  $69 \pm 2\%$ ). By contrast, vasopressin  $V_{1A}$  receptor blockade (VB) during normocapnia had no effect on  $\mu\text{HbO}_2$  ( $75 \pm 3$  vs  $71 \pm 5\%$ ).

During HC, systemic  $\text{DO}_2$  increased from  $12 \pm 1$  to  $16 \pm 1$  ml/kg per min similar to the increase in  $\mu\text{HbO}_2$ . This increase mainly resulted from increased cardiac output during HC (from  $86 \pm 6$  to  $111 \pm 8$  ml/kg per min). However, while the increase in  $\mu\text{HbO}_2$  was abolished during additional vasopressin  $V_{1A}$  receptor blockade,  $\text{DO}_2$  increased both during HC and vasopressin  $V_{1A}$  receptor blockade (Fig. 3). The relationship between  $\mu\text{HbO}_2$  and  $\text{DO}_2$  is shown in Fig. 4. HC with intact vasopressin system increases  $\mu\text{HbO}_2$  and  $\text{DO}_2$  in parallel, while during vasopressin  $V_{1A}$  receptor blockade HC selectively increased  $\text{DO}_2$ . Cardiac output increased both during HC and vasopressin  $V_{1A}$  receptor blockade (from  $80 \pm 8$  to  $118 \pm 10$  ml/kg per min). The increase in cardiac output both under HC and HC with vasopressin  $V_{1A}$  receptor blockade is related to a similar increase in stroke volume (from  $23 \pm 2$  and  $22 \pm 2$  to  $29 \pm 3$  and  $29 \pm 3$  ml respectively) without differences between both groups. The additional increase in CO and  $\text{DO}_2$  in group HCVB could

be attributed to the changes in HR, which did not change during HC ( $104 \pm 4$  to  $109 \pm 5$  per min) but significantly increased during additional vasopressin  $V_{1A}$  receptor blockade ( $104 \pm 3$  to  $113 \pm 4$  per min).

By contrast, during vasopressin  $V_{1A}$  receptor blockade under physiological conditions,  $\text{DO}_2$  remained unchanged according to cardiac output, which did not change either. Likewise, neither HR nor stroke volume changed.

Systemic vascular resistance (SVR) decreased in the HC and HCVB groups; however, SVR tended to be lower in the HCVB group, while vasopressin  $V_{1A}$  receptor blockade under normocapnic conditions did not change SVR.

Arterial  $\text{CO}_2$  tension ( $P_a\text{CO}_2$ ) was increased in the hypercapnic groups and pH was accordingly reduced. This was not influenced by vasopressin  $V_{1A}$  receptor blockade. Blood lactate concentrations remained almost unchanged in all groups. Vasopressin  $V_{1A}$  receptor blockade under physiological conditions did not affect blood gas tensions. Further hemodynamic variables are shown in Table 1 and blood gas tensions in Table 2.

## Vasopressin plasma levels

In two representative pilot experiments, vasopressin plasma levels increased from 17 ng/ml in one animal

**Table 1** Microvascular oxygenation and hemodynamic variables in dogs. Effects on systemic vascular resistance (SVR), cardiac output (CO), stroke volume (SV), mean arterial pressure (MAP), and heart rate (HR) during vasopressin blockade during normocapnia (VB) and during hypercapnia (HCVB) as well as hypercapnia *per se* (HC); data presented as absolute values,  $n=5$ , mean  $\pm$  s.e.m.

Variable	Group	Baseline	Endpoint ( $\pm$ HC $\pm$ VB)
SVR (mmHg/l per min)	HC	$26 \pm 2$	$23 \pm 2^*$
	HCVB	$29 \pm 3$	$22 \pm 2^*$
	VB	$29 \pm 2$	$27 \pm 1$
CO (ml/kg per min)	HC	$86 \pm 6$	$111 \pm 8^*$
	HCVB	$80 \pm 8$	$118 \pm 10^*$
	VB	$81 \pm 6$	$84 \pm 4$
SV (ml)	HC	$23 \pm 2$	$29 \pm 3^*$
	HCVB	$22 \pm 2$	$29 \pm 3^*$
	VB	$21 \pm 2$	$22 \pm 1$
MAP (mmHg)	HC	$62 \pm 2$	$69 \pm 3^*$
	HCVB	$61 \pm 2$	$70 \pm 2^*$
	VB	$64 \pm 1$	$63 \pm 1$
HR (per min)	HC	$104 \pm 4$	$109 \pm 5$
	HCVB	$104 \pm 3$	$113 \pm 4^*$
	VB	$108 \pm 2$	$110 \pm 4$

\* $P < 0.05$  vs baseline.

**Table 2** Blood gas tensions, pH, bicarbonate, hemoglobin, lactate, and potassium in dogs. Effects of vasopressin receptor blockade on arterial oxygen saturation ( $S_aO_2$ ), arterial  $CO_2$  tension ( $P_aCO_2$ ), arterial oxygen tension ( $P_aO_2$ ), pH,  $HCO_3^-$ , hemoglobin (Hb), lactate, and potassium ( $K^+$ ) during normocapnia (VB) and during hypercapnia (HCVB) as well as hypercapnia *per se* (HC); data presented as absolute values,  $n=5$ , mean  $\pm$  s.e.m.

Variable	Group	Baseline	Endpoint (HC $\pm$ VB)
$S_aO_2$ (%)	HC	98 $\pm$ 1	95 $\pm$ 1*
	HCVB	99 $\pm$ 1	96 $\pm$ 1*
	VB	99 $\pm$ 1	99 $\pm$ 1
$P_aCO_2$ (mmHg)	HC	39 $\pm$ 1	67 $\pm$ 1*
	HCVB	39 $\pm$ 1	66 $\pm$ 1*
	VB	38 $\pm$ 1	39 $\pm$ 1
$P_aO_2$ (mmHg)	HC	154 $\pm$ 5	119 $\pm$ 6*
	HCVB	153 $\pm$ 4	120 $\pm$ 3*
	VB	153 $\pm$ 3	155 $\pm$ 4
pH	HC	7.33 $\pm$ 0.01	7.15 $\pm$ 0.01*
	HCVB	7.37 $\pm$ 0.01 <sup>†</sup>	7.18 $\pm$ 0.01* <sup>†</sup>
	VB	7.4 $\pm$ 0.01	7.4 $\pm$ 0.01
$HCO_3^-$ (mmol/l)	HC	20 $\pm$ 1	23 $\pm$ 1*
	HCVB	22 $\pm$ 1 <sup>†</sup>	24 $\pm$ 1* <sup>†</sup>
	VB	23 $\pm$ 1	23 $\pm$ 1
Hb (g/100 ml)	HC	11 $\pm$ 1	11 $\pm$ 1*
	HCVB	12 $\pm$ 1	12 $\pm$ 1*
	VB	12 $\pm$ 1	12 $\pm$ 1
Lactate (mmol/l)	HC	2.1 $\pm$ 0.3	1.2 $\pm$ 0.1*
	HCVB	1.9 $\pm$ 0.2	0.8 $\pm$ 0.1* <sup>†</sup>
	VB	1.6 $\pm$ 0.4	1.4 $\pm$ 0.3
$K^+$ (mmol/l)	HC	4.0 $\pm$ 0.1	4.3 $\pm$ 0.1*
	HCVB	3.9 $\pm$ 0.1	4.4 $\pm$ 0.1*
	VB	3.9 $\pm$ 0.1	4.1 $\pm$ 0.1*

\* $P < 0.05$  vs baseline, <sup>†</sup> $P < 0.05$  vs HC group at the same time.

and 13 ng/ml in another one under baseline conditions to 33 and 22 ng/ml after 30 min of HC and was almost doubled after 60 min of HC (34 and 24 ng/ml).

## Discussion

### Interpretation of the results

The main results from our experiments are that i) vasopressin  $V_{1A}$  receptor blockade abolished the increase in gastric mucosal oxygenation during HC. ii)  $DO_2$  increased during vasopressin  $V_{1A}$  receptor blockade under HC without effect on  $\mu HbO_2$ . iii) Vasopressin  $V_{1A}$  receptor blockade did not alter systemic or regional microcirculation during normocapnic (physiological) conditions.

The results lead to the following conclusions that will be discussed in detail.

1. Vasopressin  $V_{1A}$  receptors mediate hypercapnic increases in gastric mucosal oxygenation.
2. HC modulates  $\mu HbO_2$  mainly independently of systemic  $DO_2$ .
3. Vasopressin  $V_{1A}$  receptors are not involved in regulation of gastrointestinal microvascular oxygenation under physiological conditions.

The vasopressin  $V_{1A}$  receptors are responsible for the increase in  $\mu HbO_2$  during HC. This effect might be related to an increase in endogenous vasopressin. In our study, vasopressin plasma levels seemed to increase during HC as measured exemplarily only in two representative experiments without statistical analysis because increase in vasopressin plasma levels during HC is already well known (Philbin *et al.* 1970, Forsling & Rees 1975, Farber *et al.* 1982, Rose *et al.* 1984). Although the relation between increased vasopressin plasma levels and the effect of the  $V_{1A}$  receptors might indicate an effect of vasopressin, we cannot exclude the effect of other possible ligands, e.g. oxytocin, on the receptor. However, vasopressin has a 30-fold higher affinity to the receptor than oxytocin (Holmes *et al.* 2003), indicating a rather minor role of oxytocin. Additionally, one study suggests that vasodilatory effects of the  $V_1$  receptors are linked to binding of vasopressin (Evora *et al.* 1993).

This supports the hypothesis that the increase in gastric mucosal oxygenation via the  $V_{1A}$  receptors might be mediated by an increase in vasopressin, although the application of exogenous vasopressin exerts rather negative effects on splanchnic perfusion. However, vasopressin is known to have vasodilatory properties as well (Holmes *et al.* 2003).

Accordingly, many studies have recently shown that effects of vasopressin are not confined to vasoconstriction in the splanchnic region solely and our study contributes another novel fact to the unexpected new understanding of vasopressin and its receptor (Asfar *et al.* 2006). While vasopressin is known to jeopardize splanchnic circulation (Knotzer *et al.* 2005), this effect is abolished in the same model during endotoxemia (Knotzer *et al.* 2006). Another study using terlipressin has shown even increased hepatic arterial blood flow with unaffected splanchnic  $DO_2$  (Asfar *et al.* 2005). On the other hand, cardiac output decreased in several studies (Knotzer *et al.* 2005). This was not the case in our study under physiological conditions, probably because we did not apply vasopressin exogenously. Another explanation might be that physiological plasma levels are too low to exert those effects. During HC – and hence higher vasopressin plasma levels – receptor

blockade increased CO in our model concordant with the mentioned studies. Thus, once activated, release of vasopressin exerts substantial effects on cardiac output and splanchnic circulation. Therefore, those effects seem to be concentration dependent and vasopressin plasma levels under physiological conditions do not exert substantial effects on cardiac output and splanchnic circulation. Concentration-dependent effects of vasopressin have been observed in human beings as well (Russell 2011). Additionally, effects of vasopressin differ between organs and therefore different splanchnic organs have to be regarded separately (Asfar *et al.* 2006).

Nevertheless, vasopressin  $V_{1A}$  receptor blockade might increase vasopressin binding to the other vasopressin receptors. But other receptors are either not involved in regulation of vascular tone or – in case of endothelial oxytocin receptors and  $V_2$  receptors – mediate vasodilation (Holmes *et al.* 2004). Increased binding to other receptors would thus rather increase  $\mu\text{HbO}_2$  during HC instead of abolishing this effect.

The effect of the vasopressin  $V_{1A}$  receptor on gastric oxygenation during HC seems to be confined to regional hemodynamic variables as vasopressin receptor blockade during HC solely affects  $\mu\text{HbO}_2$  and does not reduce any of the other parameters like cardiac output or systemic oxygen transport that could have been an alternative reason for changes in  $\mu\text{HbO}_2$ .

An alternative explanation for the increased  $\mu\text{HbO}_2$  might be an increased shunting of oxygen to the venular compartment, which represents the biggest fraction of  $\mu\text{HbO}_2$ . However, in this case, lactate levels might increase as an indicator of insufficient oxygen supply, which could not be observed (Table 2). Thus, the reason for these unexpected results might rather be a vasopressin receptor-mediated redistribution of cardiac output (Asfar *et al.* 2008) in favor of gastric mucosa during HC. However, this remains speculative as we do not have any direct evidence. During HC and vasopressin  $V_{1A}$  receptor blockade, the increased systemic  $\text{DO}_2$  does not seem to reach the gastric mucosal microcirculation (Fig. 4). Redistribution of cardiac output might be due to different vasopressin receptor expression, which can be decreased, e.g. in models of sepsis. Whether vasopressin receptor expression is decreased during HC as well is so far unknown.

Each dog underwent each protocol in a randomized order and hence served as its own control. Therefore, interindividual differences can be eliminated. This approach allows use of a small group size of  $n=5$  animals.

Thus, the results from this study extend our knowledge from previous studies. Vasopressin is increased

during epidural anesthesia to maintain blood pressure (Picker *et al.* 2001). Additionally, it is increased during anesthesia with high doses (3 MAC) of sevoflurane (Picker *et al.* 2004). With regard to our results, the role of vasopressin and its receptor seems to be of minor importance under physiological conditions with low vasopressin plasma levels, but it is necessary, e.g. to maintain blood pressure, under a variety of pathological conditions. According to our results, HC is one of those pathological conditions with major importance of vasopressin that exerts so far unknown properties.

### Clinical impact

Our results indicate one new possible mechanism of hypercapnic improvement of microcirculatory oxygenation and demonstrate for the first time a new role of the  $V_{1A}$  receptors in regulating splanchnic oxygenation apart from vasoconstriction. This is of particular interest as permissive HC is widely used in the intensive care unit especially in critically ill patients, e.g. sepsis-associated acute respiratory distress syndrome (ARDS) and exogenous vasopressin remains a possible therapeutic option for severe hemodynamic instability. Thus, it is important to know about the interaction of HC with vasopressin and about the effects on splanchnic oxygenation, which is vital for these patients in particular. However, especially in these patients, vasopressin maintains systemic blood pressure at the expense of splanchnic microcirculation, which is normally impaired due to vasopressin (Boerma *et al.* 2005). Still, despite clinical trials, there is no definite answer to whether vasopressin affects mortality.

The effect of HC on  $\mu\text{HbO}_2$  can still be observed during hemorrhagic shock (Schwartzges *et al.* 2010), a situation that leads to extended release of vasopressin with subsequent binding to the vasopressin  $V_{1A}$  receptors (Fujisawa *et al.* 1994). Whether this extended release of vasopressin is involved in increasing  $\mu\text{HbO}_2$  during hemorrhage has to be studied yet but might lead to further interesting results of clinical importance.

Summarizing recently published studies, it still seems difficult to draw a definite conclusion on the effects of vasopressin on splanchnic hemodynamics (Asfar *et al.* 2008). While many studies analyzed effects of exogenous vasopressin with diverse results, our study helps to understand physiological effects of vasopressin  $V_{1A}$  receptors on splanchnic hemodynamics during physiological and hypercapnic conditions. In addition, most studies focused on macro- and microcirculatory hemodynamic variables, while only some studies addressed

local oxygenation (Asfar *et al.* 2008). Thus, our results provide first evidence of the vasopressin  $V_{1A}$  receptors to increase regional oxygenation during HC.

## Conclusions

Vasopressin  $V_{1A}$  receptor blockade during HC suppressed the HC-induced increase in  $\mu\text{HbO}_2$ . Thus, in contrast to its generally vasoconstrictive properties, the vasopressin  $V_{1A}$  receptors seem to mediate hypercapnic increase in gastric mucosal oxygenation. This increase in  $\mu\text{HbO}_2$  during HC is independent of systemic oxygen transport.

## 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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