

# Effect of hypotensive hypovolemia and thoracic epidural anesthesia on plasma pro-atrial natriuretic peptide to indicate deviations in central blood volume in pigs: a blinded, randomized controlled trial

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**Purpose:** Changes in plasma pro-atrial natriuretic peptide (proANP) may indicate deviations in the central blood volume (CBV). We evaluated the plasma proANP response to hypotensive hypovolemia under the influence of thoracic epidural anesthesia (TEA) in pigs. We hypothesized that plasma proANP would decrease in response to hypotensive hypovolemia and that TEA would aggravate the proANP response, reflecting a further decrease in CBV.

**Design:** Randomized, blinded, controlled trial.

**Setting:** A university-affiliated experimental facility.

**Participants:** Twenty pigs randomized to administration of saline (placebo) or bupivacaine with morphine (TEA) in the epidural space at Th8-Th10.

**Interventions:** Relative hypovolemia was established by an inflatable Foley catheter positioned in the inferior caval vein just below the heart (caval obstruction), and hemorrhage-induced hypovolemia was by withdrawal of blood from the femoral artery, both aiming at a mean arterial pressure (MAP) of 50–60 mmHg. Hemodynamic variables and plasma proANP were determined before and after the interventions.

**Results:** Caval obstruction and withdrawal of blood reduced MAP to 50–60 mmHg. Accordingly, cardiac output, central venous pressure, and mixed venous oxygen saturation decreased ( $p < 0.05$ ). Yet, plasma proANP was stable after both caval obstruction (TEA: 72 [63–78] to 80 pmol/L [72–85],  $p = 0.09$  and placebo: 64 [58–76] to 69 pmol/L [57–81],  $p = 0.06$ ) and withdrawal of blood (TEA: 74 [73–83] to 79 pmol/L [77–87],  $p = 0.07$  and placebo: 64 [56–77] to 67 pmol/L [58–78],  $p = 0.15$ ).

**Conclusion:** Plasma proANP was stable in response to relative and hemorrhage-induced hypovolemia to a MAP of 50–60 mmHg, and the response was independent of TEA. The findings suggest that alterations in plasma proANP do not follow deviations in CBV during hypotensive hypovolemia in pigs.

**Keywords:** atrial natriuretic factor, hemorrhage, hypovolemia, epidural anesthesia, pigs, hypotension

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## Introduction

Maintenance of the central blood volume (CBV) during surgery by fluid and vasopressors is important for compensating vasodilation induced by both general and regional anesthesia and for a potential blood loss.<sup>1,2</sup> Yet, evaluation of CBV is

challenging because monitoring of hemodynamic variables such as blood pressure and heart rate (HR) is not a reliable determinant of the circulating blood volume in response to fluid administration.<sup>3,4</sup>

Atrial natriuretic peptide (ANP) is produced by the atrial myocytes, and the main stimulus for its release is atrial wall distention<sup>5,6</sup> eliciting diuresis and vasorelaxation of importance for blood volume regulation.<sup>7,8</sup> Accordingly, alteration in plasma ANP reflects changes in CBV as determined by scintigraphy and thoracic electrical impedance.<sup>9–12</sup> Thus, plasma ANP increases in response to blood volume expansion<sup>13</sup> or exercise<sup>14</sup> and, conversely, decreases when preload to the heart is reduced by head-up tilt<sup>12</sup> and lower body negative pressure.<sup>15</sup> Furthermore, perioperative monitoring of plasma ANP may be feasible to evaluate CBV during surgery. Plasma proANP, a stable variant of ANP, has been applied to evaluate perioperative fluid balance during esophagectomy<sup>16</sup> and cystectomy,<sup>17</sup> and an inverse relation to the perioperative blood loss is reported.<sup>17</sup>

This study evaluated the plasma proANP response to the reduction in CBV in pigs. To evaluate both relative and hemorrhage-induced hypovolemia, caval obstruction of venous flow was induced, or withdrawal of blood was carried out. The pigs were randomized to thoracic epidural anesthesia (TEA) or epidural injection of saline (placebo) applied during the interventions. It was considered that by the TEA-induced block of sympathetic influence on vascular tone, the ability to preserve CBV by vasoconstriction during relative or hemorrhage-induced hypovolemia would be affected.<sup>2,18</sup> We hypothesized that plasma proANP would decrease in response to both caval obstruction of venous blood to the heart as to hemorrhage and that the reduction in plasma proANP would be aggravated by TEA.

## Materials and methods

### Study design

The study is a blinded, randomized, controlled trial aiming at evaluating the impact of hypotensive hypovolemia on plasma proANP during the influence of TEA/placebo as a primary end point with hemodynamic variables as secondary end points. The study was approved by the local Animal Experiments Inspectorate (protocol number 2014-15-0201-00385 approved on October 30, 2014) in accordance with the national and the European Union legislation. The manuscript adheres to the applicable ARRIVE guidelines.

## Randomization

The pigs were allocated to TEA or placebo (www.random.org) with numbered envelopes padded by non-transparent paper. A veterinarian was responsible for the envelopes and attested adherence to the randomization protocol and initiated dispensation of TEA/placebo while the principal investigator was blinded to the administration during the study.

## Instrumentation of animals

Twenty female pigs (Danish Landrace/Yorkshire, 10–12 weeks of age) were housed in pairs and acclimatized under standardized room temperature, humidity, and light–dark cycles for two weeks with feeding provided until 12 hRS before anesthesia.

In the morning of the experiment, the pigs were sedated by intramuscular benzodiazepine and tiletamine (5 mg/kg, Zoletil Vet., Virbac, Kolding, DK) and anesthetized by intravenous propofol (15 mg/kg/hR) and fentanyl (5 µg/kg/hR). The body was kept warm by foil blankets and ventilation was maintained by an endotracheal tube and adjusted to an end-tidal carbon dioxide tension of 4.5–6.5 kPa and oxygen tension of 11–15 kPa by 25–45% oxygen. The CBV was supported by lactated Ringer's solution (5 mL/kg/hR) administered continuously during the experiment. A catheter (Epidural Minipack System 1, Smiths Medical, Hranice, CZ) was placed at the 8th–9th or 9th–10th thoracic intervertebral space and its position verified by x-ray after injection of 2 mL contrast. A pulmonary artery catheter was inserted via the right external jugular vein for determination of cardiac output (CO) by thermol dilution (Vigilance Monitor, Edwards Life Sciences, Irvine, CA, USA). Catheters were placed in the femoral arteries, the right for monitoring blood pressure and the left for withdrawal of blood. To reduce venous flow to the heart (relative hypovolemia), hypotension was induced by caval vein obstruction. The left femoral vein was identified by dissection, and a Foley catheter (8 Fr x 60 cm, MILA international, Florence, KY, USA) advanced to the right atrium through an introducer (12 Fr, Peel-Away® Introducer, Cook Medical, Bloomington, IN, USA). The catheter was inflated and withdrawn until there was an abrupt decrease in systolic blood pressure (SBP >20 mmHg), and the tip of the catheter was then considered positioned at the inlet of the right atrium. Next, the balloon was deflated and withdrawn 2.5 cm for a position just below the heart without obstructing the hepatic vein.

Hemodynamic variables were recorded by Powerlab 16/35 (AD Instruments, Dunedin, NZ) including CO, mean arterial pressure (MAP), HR, and central venous pressure (CVP). Systemic vascular resistance (SVR) was:  $SVR = \frac{80 \times (MAP - CVP)}{CO}$ . Also, arterial blood samples for plasma proANP were obtained.

Hemodynamic variables were measured and blood for determination of plasma proANP withdrawn before and after the interventions. Also, a blood sample for mixed venous oxygen saturation (SvO<sub>2</sub>) was obtained before and after withdrawal of blood reflecting that a volume deficit by approximately 100 mL corresponds to a decrease in SvO<sub>2</sub> by 1% in adult humans.<sup>1</sup> The blood volume of the pigs was estimated as 8% of body weight.<sup>19</sup>

## TEA dosage

To rule out spinal or intravascular placement of the epidural catheter injection of 2 mL lidocaine/adrenaline was administered (20 mg/5 µg/mL, SAD, Amgros I/S, DK). Then, TEA was established by a bolus of 2 mL bupivacaine (5 mg/mL, SAD) followed by infusion (2 mL/hR) of bupivacaine/morphine (2.5 mg/50 µg/mL, SAD) through the remaining procedure. The placebo group was administered saline (0.9%) in the epidural catheter in similar amounts as in the TEA group.

## Experimental procedure

The animals were placed in a supine position during the experimental procedure and baseline recordings were obtained prior to activation of TEA. After epidural injections of local anesthesia or placebo, cardiovascular variables and blood samples were obtained after 15 and 30 mins. The balloon of the Foley catheter was then inflated to obstruct venous flow to the heart aiming at a 50–60 mmHg MAP with evaluation after 5 and 15 mins. After the balloon was deflated and MAP normalized, the measurements were repeated after 5 and 15 mins. Lastly, withdrawal of blood until a MAP of 50–60 mmHg was carried out and measurements were repeated after 5 and 15 mins. After the study, the animals were euthanized by intravenous pentobarbital (at least 100 mg/kg) while anesthetized.

## Plasma proANP

Plasma proANP (amino-acids 53–90), rather than ANP (amino acids 98–128), was determined because ANP has a short half-life in plasma.<sup>20–22</sup> Also, plasma proANP may be a more precise plasma marker because little proteolytic

degradation is assumed to occur at the mid-region of the prohormone,<sup>22</sup> and it is released in equimolar amounts as ANP.<sup>23</sup> Arterial blood samples for plasma proANP were obtained in EDTA tubes and centrifuged at 3,000 rpm at 4°C for 10 mins and stored at –80°C until analysis. Plasma proANP was determined using an automated method from Thermo-Fisher (the Kryptor Plus platform), and the sandwich immunoassay was validated with excellent performance.<sup>20,24</sup>

## Statistical analysis

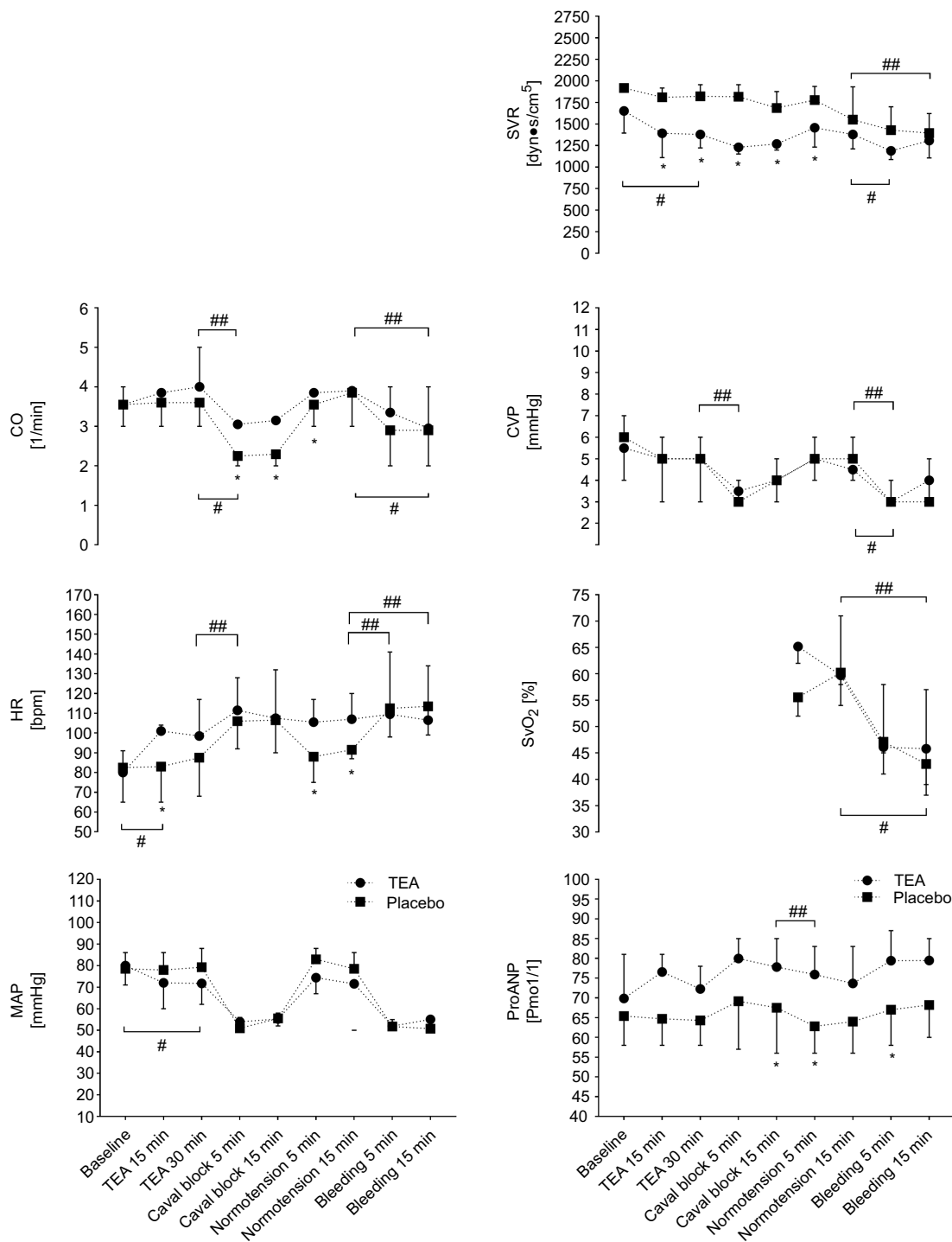
Statistical analysis was by SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA) and graphs were constructed by GraphPad Prism Software (Version 7.0, San Diego, CA, USA). Data were tested for normality using the Shapiro–Wilk test, and due to a non-normal distribution, non-parametric statistic was chosen. For repeated measurements within groups, Friedman’s test with Bonferroni correction was applied (primary and secondary outcomes). To test for differences between groups at a single time point, Mann–Whitney’s *U*-test was applied for continuous variables and the  $\chi^2$ -test or Fisher’s exact test for dichotomous variables. A two-sided *p*-value ≤0.05 was considered statistically significant. The hemodynamic variables are presented as medians with interquartile range and represent recordings over 30 s. A decrease in plasma ANP by at least 24% (SD 1.6 pmol/L) is reported during hypotensive hypovolemia in man,<sup>12</sup> and a power calculation (power: 0.8 and  $\alpha$ -level: 0.05) predicted that eight pigs were required to detect a difference in any of the groups. To account for possible exclusion, 20 pigs were studied.

## Results

For two animals, the epidural catheter was not placed correctly as evaluated by x-ray. Thus, the study included eight pigs provided TEA, and 10 pigs were administered saline in the epidural space. The body weight was similar (41 (39–43) [TEA] vs 42 kg (41–43) [placebo], *p*=0.24), and hemodynamic variables and plasma proANP were also not significantly different between the groups at baseline (Figure 1).

## TEA

After administration of saline to the epidural space (placebo group), no hemodynamic changes were observed. However, activation of TEA increased HR (*p*=0.03) at a stable CO (*p*=0.5), while MAP (*p*=0.01) decreased and thus SVR was reduced (*p*<0.01) (Figure 1).



**Figure 1** Plasma pro-atrial natriuretic peptide (proANP) and hemodynamic variables during induced hypotension in pigs by caval blockade and withdrawal of blood, respectively. Values are medians with interquartile range. Change during the marked period in the thoracic epidural anesthesia (TEA), #, and the placebo-groups, ## by Friedman’s test,  $p < 0.05$ . \*Significant difference between groups at a single time point by Mann–Whitney’s *U*-test,  $p < 0.05$ . Error bars are shown in one direction only to enhance visualization.

## Hypotension

As intended, MAP was reduced to 50–60 mmHg both following caval obstruction of venous flow and in response to withdrawal of blood in both the TEA and placebo

groups (Figure 1; Table 1). There was no significant difference in fluid administration between the two groups of pigs ( $p = 0.10$ ) and urine output ( $p = 0.10$ ) was similar. However, more blood was needed to be drawn in the

**Table 1** Changes in hemodynamic variables and plasma pro-atrial natriuretic peptide following induced hypotension in pigs by caval blockade and withdrawal of blood, respectively

	30 mins after TEA activation		Caval block		Normotension		Hemorrhage	
	5 mins	15 mins	5 mins	15 mins	5 mins	15 mins	5 mins	15 mins
<b>MAP</b>								
Placebo	79 (71–88)	56 (50–58)	51 (50–56)	56 (50–58)	83 (74–88)	79 (73–86)	52 (49–55)	51 (50–54)
TEA	72 (62–78)	55 (52–59)	54 (52–55)	55 (52–59)	74 (67–89)	72 (50–55)	52 (50–55)	55 (54–57)
<b>HR</b>								
Placebo	88 (68–101)	107 (90–129)	106 (62–119) <sup>a</sup>	107 (90–129)	88 (75–102)	92 (87–97)	113 (98–124) <sup>c</sup>	114 (99–122) <sup>c</sup>
TEA	99 (93–117)	108 (89–132)	112 (93–128)	108 (89–132)	106 (93–117) <sup>a*</sup>	107 (94–120) <sup>a*</sup>	110 (90–141)	107 (88–134)
<b>CO</b>								
Placebo	3.6 (3.1–3.8)	2.3 (2.2–2.8) <sup>a</sup>	2.3 (2.0–2.6) <sup>a</sup>	2.3 (2.2–2.8) <sup>a</sup>	3.6 (3.2–3.9)	3.9 (3.1–4.4)	2.9 (2.3–3.2)	2.9 (2.3–3.2) <sup>c</sup>
TEA	4.0 (3.5–4.5)	3.2 (2.7–3.3) <sup>a,b*</sup>	3.1 (2.5–3.4) <sup>a,b*</sup>	3.2 (2.7–3.3) <sup>a,b*</sup>	3.9 (3.7–4.3) <sup>a*</sup>	3.9 (3.9–4.2)	3.4 (2.8–3.5)	3.0 (2.9–3.7) <sup>c</sup>
<b>SVR</b>								
Placebo	1820 (1527–1955)	1685 (1630–1875)	1816 (1554–1956)	1685 (1630–1875)	1778 (1635–1935)	1551 (1355–1931)	1429 (1235–1700)	1395 (1277–1621) <sup>c</sup>
TEA	1377 (1222–1507) <sup>a*</sup>	1268 (1197–1644) <sup>a*</sup>	1230 (1151–1615) <sup>a*</sup>	1268 (1197–1644) <sup>a*</sup>	1456 (1232–1615) <sup>a*</sup>	1378 (1210–1531)	1187 (1087–1445) <sup>c</sup>	1307 (1105–1564)
<b>CVP</b>								
Placebo	5 (4–6)	4 (3–5)	3 (3–4) <sup>a</sup>	4 (3–5)	5 (4–6)	5 (4–6)	3 (2–4) <sup>c</sup>	3 (2–5)
TEA	5 (3–5)	4 (3–6)	4 (3–6)	4 (3–6)	5 (4–7)	5 (4–7)	3 (3–5) <sup>c</sup>	4 (3–6)
<b>ProANP</b>								
Placebo	64 (58–76)	68 (56–78)	69 (57–81)	68 (56–78)	63 (56–77) <sup>b</sup>	64 (56–77)	67 (58–78)	68 (60–79)
TEA	72 (63–78)	78 (73–85) <sup>a*</sup>	80 (72–85)	78 (73–85) <sup>a*</sup>	76 (72–83) <sup>a*</sup>	74 (73–83)	79 (77–87) <sup>a*</sup>	79 (73–85)

**Notes:** Values are medians with interquartile range. <sup>a</sup>Different from “30 mins after TEA activation” within groups,  $p < 0.05$ . <sup>b</sup>Different from “caval block” within groups,  $p < 0.05$ . <sup>c</sup>Different from “normotension” within groups,  $p < 0.05$ . <sup>\*</sup>Different between groups,  $p < 0.05$ .  $P$ -values by Friedman’s test.

**Abbreviations:** MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; SVR, systemic vascular resistance; CVP, central venous pressure; TEA, thoracic epidural anesthesia; ProANP, pro-atrial natriuretic peptide.

**Table 2** Fluid input and output

Fluid input and output	TEA	Placebo	p-value
Electrolytes, mL	1600 (1475–1700)	1500 (1400–1525)	0.10
Urine output, mL	300 (250–350)	400 (288–500)	0.10
Withdrawal of blood, mL	275 (230–365)	602 (423–740)	0.03

**Notes:** Fluid input and output for pigs provided with thoracic epidural anesthesia (TEA) or saline (placebo) in the epidural space. Values are medians with interquartile range. P-values by Mann–Whitney's U- test.

placebo group to attain the targeted MAP (602 [423–740] [placebo] vs 275 mL [230–365] [TEA],  $p=0.03$ ) (Table 2) corresponding to 18% (11–22) and 8% (7–12) of the estimated blood volume, respectively.

In the placebo group, obstruction of caval flow reduced CO and CVP (both  $p<0.01$ ) while HR increased ( $p=0.04$ ) with a stable SVR ( $p=0.37$ ) (Figure 1; Table 1). With TEA, CO decreased similarly, however, to a lesser extent than in the placebo group (–19% vs –38%,  $p<0.01$ ), while CVP was unchanged ( $p=0.81$ ). Also, SVR did not decrease further in response to caval obstruction ( $p=0.88$ ) but remained reduced after activation of TEA, ie SVR was lower than in the placebo group ( $p<0.01$ ). Likewise, HR remained elevated and did not change further ( $p=0.33$ ). Deflation of the caval balloon normalized the hemodynamic variables in both groups, yet, HR remained elevated in the TEA group and was, therefore, higher than in the placebo group ( $p=0.03$ ).

In response to the withdrawal of blood CO ( $p<0.01$ ), CVP ( $p<0.01$ ) and SVR ( $p=0.01$ ) decreased in the placebo group while HR increased ( $p=0.02$ ). With TEA, CO ( $p=0.02$ ) decreased similarly to the placebo group (–19% vs –21%,  $p=0.15$ ) and also CVP was reduced ( $p=0.03$ ). SVR decreased further ( $p=0.04$ ), and HR was unchanged and not different from the value obtained in the placebo group ( $p=0.90$ ). In both groups, SvO<sub>2</sub> decreased during withdrawal of blood (–13%,  $p=0.04$  [placebo], –16%,  $p=0.02$  [TEA]).

## Plasma proANP

In the placebo group, plasma proANP tended to increase during caval obstruction of venous flow (64 [58–76] to 69 pmol/L [57–81],  $p=0.06$ ) and decreased toward baseline when the caval balloon was deflated and MAP normalized ( $p=0.01$ ) (Figure 1; Table 1). After the withdrawal of blood, plasma proANP did not change in the placebo group (64 [56–77] to 67 pmol/L [58–78],  $p=0.15$ ). With TEA, plasma proANP was stable during both caval obstruction (72 [63–78] to 80 pmol/L [72–85],  $p=0.09$ )

and withdrawal of blood (74 [73–83] to 79 pmol/L [77–87],  $p=0.07$ ). Yet, plasma proANP was elevated in the TEA group compared with the placebo group 15 mins after caval blockade ( $p=0.03$ ), when blood pressure was re-established after deflation of the caval balloon ( $p=0.03$ ) and during withdrawal of blood ( $p=0.03$ ).

## Discussion

Considering the apparent relation between plasma ANP and CBV,<sup>9,11,12</sup> a decrease in plasma proANP was expected when venous flow to the heart was limited by caval obstruction or hemorrhage, and even more so under the influence of TEA. Yet, plasma proANP was stable in the TEA group in response to both episodes of hypotensive hypovolemia and even tended to increase in the placebo group during caval obstruction.

The hemodynamic response to activation of TEA in pigs (a decrease in SVR and MAP) is consistent with reports in humans<sup>2,25</sup> and was absent in the placebo group. Also, in these pigs, SVR remained lower than in the placebo group during caval obstruction and after normotension was re-established illustrating that TEA elicited depression of vasoconstrictor drive. The higher plasma proANP in the TEA group as compared with the placebo group may have added to this vasodilatory response due to the vasorelaxant effect of the hormone.<sup>26</sup> Likewise, to reach the target MAP during withdrawal of blood, a smaller amount was needed to be withdrawn in the TEA group compared with the placebo group (about 8% vs 18% of the estimated blood volume). The difference in the amount of blood needed to reach the targeted MAP likely represents a loss of vasomotor tone to splanchnic vessels due to inhibition of sympathetic influence and therefore impaired cardiovascular tolerance to compensate for a blood loss by vasoconstriction.<sup>27,28</sup>

A decrease by 20% in CO was observed in the TEA group following caval obstruction as was also the case during withdrawal of blood along with a decrease in CVP and SvO<sub>2</sub> supporting that cardiac preload was

affected.<sup>29</sup> Yet, plasma proANP was unchanged, and higher plasma levels were observed during caval blockade and withdrawal of blood as compared with the placebo group. Similar hemodynamic and proANP responses as measured in the TEA group was established in the placebo group during hypotensive hypovolemia. However, despite CO was more reduced (by 38%) as compared with the TEA group (by 20%) during caval obstruction, plasma proANP tended to increase. Thus, despite cardiac performance being impaired in response to hypotensive hypovolemia as indicated by its filling pressure (CVP), output (CO), and systemic oxygenation (SvO<sub>2</sub>), plasma proANP was stable with or without the addition of TEA. Thus, the results indicate that secretion of ANP does not depend only on deviations in CBV.

The plasma ANP response to hemorrhage is reported by others with varying results. During prolonged exsanguination of anesthetized rats, N-terminal-proANP increased at a MAP ≤50 mmHg, while it decreased during non-hypotensive hypovolemia (MAP > 100 mmHg).<sup>30</sup> Similarly, plasma ANP is reported to increase in pigs exposed to hemorrhage to a MAP of about 50 mmHg.<sup>31</sup> On the other hand, plasma ANP

decreased or remained unchanged in rabbits,<sup>32</sup> sheep,<sup>33,34</sup> dogs,<sup>35</sup> and rats<sup>36,37</sup> when bled to a MAP between 62 and 75 mmHg (Table 3). Also, in healthy humans, plasma ANP was unchanged following the withdrawal of up to a 1,000 mL of blood at a stable systolic blood pressure.<sup>38</sup> Taken together, it seems that hypotensive hypovolemia to a moderate extent reduces or maintains plasma ANP and proANP, but that hypotensive hypovolemia to a marked degree provokes a “paradoxical” increase.

Discrepancies in results could relate to the use of different animal species and the experimental setup as the duration and the extent of the blood withdrawal vary. Also, these findings may at least in part be explained by altered plasma clearance of ANP during hypotension.<sup>39</sup> ANP is primarily eliminated in the pulmonary, splanchnic/hepatic, and renal circulations.<sup>40</sup> Clearance of ANP is reduced when perfusion pressure to these organs is compromised, and elevated plasma ANP is observed in patients with liver cirrhosis and renal failure.<sup>41</sup> Accordingly, plasma clearance of proANP may have been affected by impaired peripheral perfusion when MAP was reduced to 50–60 mmHg.

**Table 3** Reported changes in plasma ANP and blood pressure in response to withdrawal of blood

Study	Species	Intervention	Change in MAP/SBP during the intervention	Measured ANP fragment	Change in plasma ANP during the intervention
Garcia et al <sup>37</sup>	Rat	Blood withdrawal of 10% decrements	110–70 mmHg (SBP)	iANP	13–8 pmol/L <sup>a</sup>
Hodsman et al <sup>38</sup>	Healthy humans	Withdrawal of 500–1,000 mL blood	Not specified	α-hANP	Not specified, but “no change” reported
Leskinen et al <sup>30</sup>	Rat	Blood withdrawal of 1.5 mL decrements	Anesthetized rats: 100–35 mmHg (MAP) Conscious rats: 120–100 mmHg (MAP)	NT-ProANP	Anesthetized rats: 500–700 pmol/L <sup>a</sup> Conscious rats: 1,500–1,000 pmol/L <sup>a</sup>
Shackford et al <sup>31</sup>	Pig	Withdrawal of 40% of the blood volume	106–53 mmHg (MAP)	iANP	1,138–1,505 pmol/mL* <sup>a</sup>
Courneya et al <sup>32</sup>	Rabbit	Blood withdrawal of 10% decrements	107–75 mmHg (MAP)	iANP	220–184 pmol/L* <sup>a</sup>
Yoshida et al <sup>36</sup>	Rat	Blood withdrawal up to 2% of body weight	118–62 mmHg (MAP)	α-hANP	786–518 pmol/L* <sup>a</sup>
Yates et al <sup>33</sup>	Sheep	Blood withdrawal of 25% of blood volume	72–62 mmHg (MAP)	ANP (99–126)	176–140 pmol/L* <sup>b</sup>
Levy et al <sup>35</sup>	Dog	Blood withdrawal of 30% of blood volume	125–78 mmHg (SBP)	iANP	147–125 pmol/L* <sup>b</sup>
Cameron et al <sup>34</sup>	Sheep	Blood withdrawal of 15 mL/kg	88–70 mmHg (MAP)	iANP	800–810 pmol/L <sup>b</sup>

**Notes:** <sup>a</sup>p<0.05, <sup>b</sup>p>0.05, \*converted values from pg/mL.

**Abbreviations:** MAP, mean arterial pressure; SBP, systolic blood pressure; ANP, atrial natriuretic peptide; iANP, immunoreactive ANP; α-hANP, alpha-human ANP; NT-ProANP, N-terminal proANP.

Alternative explanations for a stable plasma proANP in response to hypotensive central hypovolemia should be considered. We speculate that a marked reduction in CBV could imply that the walls of the atrium meet and release ANP or that ANP is released by deformation of the atrial wall. An evaluation of the atrium by, eg, MRI could reveal the deformation of the atrial walls during hypotensive central hypovolemia. In both patients with congestive heart failure and healthy humans, plasma ANP correlates to MRI-determined right atrial volume ( $r=0.91$ ,  $p<0.001$ ) but the association has not been examined during hypotensive hypovolemia.<sup>6</sup>

The strengths of the study include the randomized blinded design and that a correct position of the thoracic epidural catheter was verified by x-ray. Thus, two animals were excluded due to incorrect placement of the catheter. A determination of plasma brain-natriuretic peptide (BNP) was not included for evaluating CBV because BNP seems, in contrast to ANP,<sup>9,12,42</sup> to be a poor marker of acute changes in CBV.<sup>43</sup> On the other hand, BNP is a sensitive marker for predicting heart failure<sup>44</sup> and cardiac events after non-cardiac surgery.<sup>45</sup>

## Conclusion

Plasma proANP was stable in response to relative or hemorrhage-induced hypovolemia to a MAP of 50–60 mmHg, and the response was independent of TEA activation. Thus, plasma proANP does not follow deviations in CBV in pigs during hypotensive hypovolemia.

## Ethics approval and informed consent

The study was approved by the local Animal Experiments Inspectorate (protocol number 2014-15-0201-00385 approved on October 30, 2014) in accordance with the national and the European Union legislation.

## Data sharing statement

The data are available from the corresponding author on reasonable request.

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## Disclosure

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