Anesthesiology 2004; 100:885–93

Hemorrhage during Isoflurane–Nitrous Oxide Anesthesia

Effects of Endothelin-A or Angiotensin II Receptor Blockade or Both

Claudia Höhne, M.D., Pia Vogler,* Ilka Frerking, M.D.,* Roland C. E. Francis, M.D.,* Erik R. Swenson, Ph.D., Prof.,† Gabriele Kaczmarczyk, M.D., Prof.,‡ Willehad Boemke, M.D., Ph.D.§*

Background: **The objective of this study was to determine whether endothelin-A receptor blockade (ET_AB) impairs hemodynamic and hormonal regulation compared with controls and angiotensin II receptor blockade (AT_1B) during hypotensive hemorrhage in dogs under isoflurane–nitrous oxide anesthesia.**

Methods: **Six dogs were studied in four protocols: (1) control experiments (controls); (2) ET_A blockade using ABT-627 (ET_AB); (3) AT_1 blockade using losartan (AT_1B); and (4) combined AT_1B and ET_AB ($AT_1B + ET_AB$). After a 30-min awake period, isoflurane–nitrous oxide anesthesia was established (1.3 minimum anesthetic concentration). After 60 min of anesthesia, 20 ml blood/kg body weight was withdrawn within 5 min, and the dogs were observed for another hour. Thereafter, the blood was retransfused, and the dogs were observed for a final hour.**

Results: Anesthesia: **Cardiac output decreased in all protocols, whereas mean arterial pressure decreased more in AT_1B and $AT_1B + ET_AB$ than in controls and ET_AB. *Hemorrhage:* After 60 min, cardiac output had decreased less in controls than in all other protocols. Mean arterial pressure decreased more during ET_AB than in controls, but most severely during AT_1B and $AT_1B + ET_AB$. Angiotensin II increased further only in controls and ET_AB, whereas vasopressin and catecholamines increased similarly in all protocols. *Retransfusion:* Mean arterial pressure remained below controls in all protocols but was lowest when the AT_1 receptor was blocked. Cardiac output fully recovered in all but the ET_AB protocol.**

Conclusions: **ET_AB impairs long-term hemodynamic regulation after hemorrhage and retransfusion during anesthesia despite an activation of vasoconstrictive hormones. This suggests that endothelins have a role in long-term cardiovascular regulation. AT_1B impairs both short- and long-term blood pressure regulation during anesthesia and after hemorrhage.**

HEMODYNAMIC regulation after acute hemorrhage depends greatly on an intact interaction of the sympathetic nervous system and vasoactive hormones such as epinephrine and norepinephrine, vasopressin, endothelins, and angiotensin II. An important effect of these hormones is to limit a hemorrhage-related decrease in mean arterial pressure (MAP) and cardiac output (CO), to defend the perfusion of vital organs and tissues.[1] If hemorrhage occurs during inhalation anesthesia, the situation may become worse because inhalation anesthesia *per se* is frequently associated with some degree of hypotension owing to decreased vascular smooth muscle tone, impaired myocardial contractility, and blunted autonomic nervous system baroreflex responses.[2] The situation may become even more problematic when two of the more powerful vasoconstrictor hormones known to date, endothelin 1 and angiotensin II, become ineffective in the face of therapeutic receptor blockade.

Angiotensin II binds to two different receptor subtypes, AT_1 and AT_2. Besides stimulation of aldosterone and vasopressin release, angiotensin II dose-dependently increases total peripheral resistance and thus MAP.[3] To date, antagonists of the AT_1 receptor, such as losartan, have become standard therapy in the treatment of hypertension and congestive heart failure.

Endothelins mediate their vasoconstrictive effects *via* ET_A and ET_B receptor subtypes located on vascular smooth muscle cells, while a vasodilatory effect is mediated through stimulation of ET_B receptors on vascular endothelial cells.[4] It was shown that selective ET_A receptor antagonists can decrease MAP in humans,[5] and there are randomized, clinical trials in progress investigating the effects of endothelin receptor antagonists on improvement and survival in patients with chronic heart failure and pulmonary hypertension.

In a previous study of our laboratory that investigated the effects of selective ET_A receptor inhibition on conscious, nonsedated dogs after acute hemorrhage, it was found that ET_A inhibition is associated with striking increases in angiotensin II, norepinephrine, and vasopressin plasma concentrations,[6] without impairing hemodynamic regulation more than in controls.

The aim of the current study was to explore to what extent endothelin-A receptor inhibition impairs hemodynamic regulation and endogenous compensatory mechanisms during hypotensive hemorrhage in dogs undergoing isoflurane–nitrous oxide anesthesia. The results were compared with controls and a protocol with angiotensin II receptor inhibition. Our goal was to gain more insight into the physiologic reactions to hypovolemic shock during anesthesia in subjects in which the ET_A or AT_1 receptors or both are blocked. This may provide some basic information that in the future might help us to more rationally select exogenous vasopressor hormones for situations of hypovolemic shock in individuals

* Staff Anesthesiologist, ‡ Senior Staff Anesthesiologist and Professor of Applied Physiology, § Senior Staff Anesthesiologist, Clinic of Anesthesiology and Intensive Care Medicine, Campus Virchow-Klinikum, Charité, Humboldt-University of Berlin. † Professor of Medicine, Department of Medicine, University of Washington, Seattle, Washington.

Received from the Clinic of Anesthesiology and Intensive Care Medicine, Campus Virchow-Klinikum, Charité, Humboldt-University of Berlin, Berlin, Germany. Submitted for publication July 9, 2003. Accepted for publication October 23, 2003. Supported by grant No. Bo 1755/1 from the Deutsche Forschungsgemeinschaft, Bonn, Germany (to Dr. Boemke). Losartan was supplied by Merck Research Laboratories, Rahway, New Jersey. ABT-627 was donated by Abbott Laboratories, North Chicago, Illinois. Presented in part at the Euroanaesthesia Meeting, Glasgow, Scotland/United Kingdom, May 31–June 3, 2003. Data in this article are part of the doctoral thesis of Pia Vogler, Medical Student, Humboldt-University of Berlin, Charité, Campus Virchow, Berlin, Germany.

Address reprint requests to Dr. Höhne: Klinik für Anästhesiologie und operative Intensivmedizin, Charité, Campus Virchow-Klinikum, Medizinische Fakultät der Humboldt-Universität zu Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany. Address electronic mail to: claudia.hoehne@charite.de.

with antagonized ET_A or AT_1 receptors or both when surgical treatment or volume resuscitation are not readily available or successful.

Materials and Methods

Permission to perform the experiments was obtained from the Governmental Animal Protection Committee (AZ G0424/99, Berlin, Germany) and conforms with the *Guidelines on the Care and Use of Laboratory Animals*.[7]

Animals, Maintenance, and Diets

A total of 24 experiments were performed in six purebred female Beagle dogs (body weight, 13 ± 0.5 kg), with four experiments in each dog. The dogs were kept under highly standardized conditions and received a standardized dietary regimen as described previously.[8]

Eight days before an experiment, 100 ml of each dog's own blood was collected *via* puncture of a foreleg vein and stored in a blood bag at 4°C (Biopack®; Biotrans, Dreieich, Germany). The blood served to replace the blood withdrawn for analysis during the experiments. The interval between experiments in the same dog was at least 14 days.

Experimental Protocols

Each of the six dogs underwent four protocols in randomized order:

1. control experiments (controls)
2. ET_A receptor blockade (ET_AB)
3. angiotensin II receptor blockade (AT_1B)
4. angiotensin II plus ET_A receptor blockade ($AT_1B + ET_AB$)

Protocol 1 (Controls). After a baseline period of 30 min in the awake dogs (baseline awake), anesthesia was induced, and the dogs were observed for 60 min during isoflurane–nitrous oxide anesthesia (baseline anesthesia; for details, see Procedures during the Experiments). Thereafter, the dogs had 20 ml blood/kg body weight withdrawn (equal to 25% of the total blood volume) within 5 min and were studied for another 60 min (hemorrhage), after which time the shed blood—which had meanwhile been stored in a blood bag—was rapidly retransfused within 5 min. After retransfusion, the dogs were observed for a final 60-min period (retransfusion).

Protocol 2 (ET_AB). General procedures were the same as in protocol 1. In addition, a selective ET_A receptor blocker (ABT-627; Abbott Laboratories, North Chicago, IL) was administered (intravenous bolus of 1 mg/kg body weight 30 min before the start of the experiment, followed by a continuous infusion of 0.01 $\mu g \cdot kg\ body\ weight^{-1} \cdot min^{-1}$). This ABT-627 infusion regimen has also been established in our laboratory and has been used in other experiments.[6]

Protocol 3 (AT_1B). General procedures were the same as in protocol 1. In addition, a selective AT_1 receptor blocker (losartan; Merck Research Laboratories, Rahway, NJ) was continuously infused (0.1 $\mu g \cdot kg\ body\ weight^{-1} \cdot min^{-1}$). The infusion was started 30 min before the baseline awake period. The losartan infusion regimen has been established in our laboratory and has been applied in other experiments.[8] Adequate AT_1 receptor inhibition was tested at the end of the experiment by finding no blood pressure increase after an intravenous bolus injection of 1 μg angiotensin II.

Protocol 4 ($AT_1B + ET_AB$). General procedures were the same as in protocol 1, but this time, AT_1 plus ET_A receptor blockers were coinfused in the amounts and concentrations as described in protocols 2 and 3.

Procedures during the Experiments

Preparation of the dogs started at 7:30 AM. Body weight and temperature were recorded. The urinary bladder was catheterized with a self-retaining Foley catheter. A foreleg vein was punctured, and an infusion of creatinine was started (priming dose, 1.4 g for 30 min; maintenance infusion, 4.7 mg/min) for assessment of glomerular filtration rate (GFR; exogenous creatinine clearance).[9] After local anesthesia (1% lidocaine; Braun, Melsungen, Germany), an arterial line (20 gauge, No. 4235-8; Ohmeda, Erlangen, Germany) was advanced into the abdominal aorta *via* the femoral artery. Also after local anesthesia, a percutaneous sheath (Arrow-Flex, No. SI-09600; Arrow, Erding, Germany) was inserted into the right and left external jugular veins: one for the hemorrhage procedure and one for the insertion of a pulmonary artery catheter (5 French, No. 132F5; Baxter, Unterschleissheim, Germany). Thereafter, the conscious dogs were given approximately 30 min to adjust to the experimental situation, followed by a 30-min observation period (baseline awake). Thereafter, anesthesia was induced with 10 mg/kg intravenous propofol (Disoprivane® 1% lipid emulsion; AstraZeneca GmbH, Wedel, Germany). After induction, the dogs were intubated with a cuffed endotracheal tube (36 gauge) and mechanically ventilated (Servo Ventilator 900D; Siemens Company, Lund, Sweden). The respiratory rate was set to 12 breaths/min, the positive end-expiratory pressure was set to 5 mmHg, and the tidal volume was adjusted to keep the arterial carbon dioxide tension between 35 and 40 mmHg. Inhalation anesthesia was adjusted to maintain an end-tidal isoflurane concentration of 1.2 vol% in a 30% oxygen–70% nitrous oxide mixture. This is equal to a minimum anesthetic concentration (MAC) of 1.3 in dogs. This results because 1 MAC is 1.28 vol% for isoflurane and 188 vol% for nitrous oxide in dogs. The two MAC values are additive.[10]

Heart rate, MAP, and central venous pressure were measured continuously, and data were stored on a computer. CO was determined by using the thermodilution

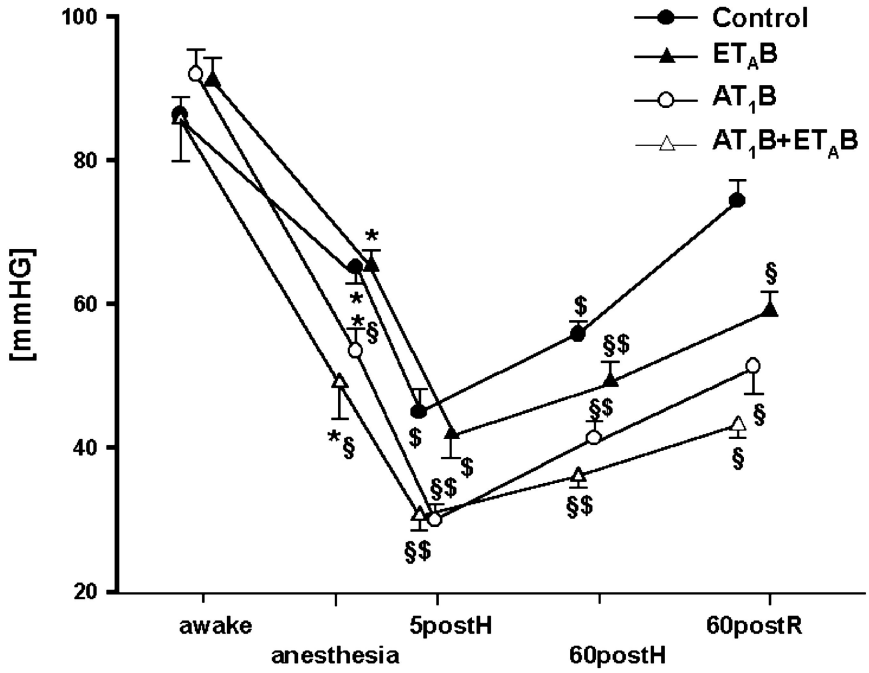

Fig. 1. Mean arterial pressure in awake and anesthetized dogs, 5 min (5postH) and 60 min after hemorrhage (60postH) and 60 min after retransfusion (60postR). Values are presented as mean ± SEM; n = 6. * $P < 0.05$ *versus* awake. § $P < 0.05$ *versus* control. $ $P < 0.05$ *versus* anesthesia. AT_1B = angiotensin II receptor blockade; $AT_1B + ET_AB$ = combined receptor blockade; ET_AB = endothelin-A receptor blockade.

technique (5-ml injection volume at 5–10°C, Vigilance; Baxter Edwards Critical Care, Unterschleissheim, Germany). Five consecutive measurements were performed. The highest and lowest values were rejected. The mean CO was calculated from the three remaining determinations and used for calculation of systemic vascular resistance by the standard formula.

Blood samples were taken to determine plasma hormones (see Measurement of Plasma Values), creatinine, arterial and mixed venous blood gases, actual bicarbonate, and base excess at the following time points: the end of the awake period, 60 min after anesthesia, 5 and 60 min after hemorrhage, and 60 min after retransfusion. The blood withdrawn for analysis was immediately replaced with an equal amount of each dog's own blood precollected 1 week before the experiment.

At the end of each period, urine flow and creatinine excretion were measured after complete evacuation of the urinary bladder. Exogenous creatinine clearance was calculated by the standard formula to assess GFR.

Measurement of Plasma Values

Plasma creatinine concentration was determined with a creatinine analyzer (modified Jaffé reaction; Beckman Instruments, Brea, CA), blood gas analyses were performed with the ABL 505 analyzer (Radiometer, Copenhagen, Denmark).

Blood samples for hormone measurements were placed into precooled Na–EDTA vials and centrifuged at 4°C immediately. The separated plasma was stored at −20°C until analysis. The hormones were determined by commercially available kits: plasma renin activity (New England Nuclear, North Billerica, MA), angiotensin II concentration (Eurodiagnostika, Arnhem, The Netherlands), plasma aldosterone concentration (Biomedica, ALDO CTK-2R; Sorin Company, Saluggia, Italy), vasopressin (Vasopressin-RIA; Biermann GmbH, Bad Nauheim, Germany), plasma endothelin (Endo; Biomedica, Vienna, Austria). Epinephrine and norepinephrine were determined with high-performance liquid chromatography (Chromosystems, München, Germany).

Statistical Analysis

All values are presented as mean ± SEM (n = 6). For intragroup comparison (time course), a general linear model of analysis of variance for repeated measures was applied (SPSS 10, Chicago, IL). Intergroup comparison was performed using the Student *t* test. The level of significance for the error of first order was adjusted according to the Holm procedure.[11] Statistical significance was assumed at $P < 0.05$.

Results

Hemodynamics

In controls, MAP decreased from 86 ± 3 in the awake state to 65 ± 2 mmHg during anesthesia. Five minutes after hemorrhage, MAP decreased to 45 ± 3 mmHg ($P < 0.05$), and it increased spontaneously to 56 ± 2 mmHg within the next 60 min. Retransfusion of the shed blood increased MAP to 74 ± 3 mmHg. In the ET_AB protocol, the decrease in MAP during anesthesia was comparable with controls, but it was more severe 60 min after hemorrhage (49 ± 3 mmHg; $P < 0.05$). After retransfusion, the increase in MAP was less than in controls (59 ± 3 mmHg; $P < 0.05$; fig. 1). In the AT_1B protocol, MAP decreased to 54 ± 3 mmHg during anesthesia and de-

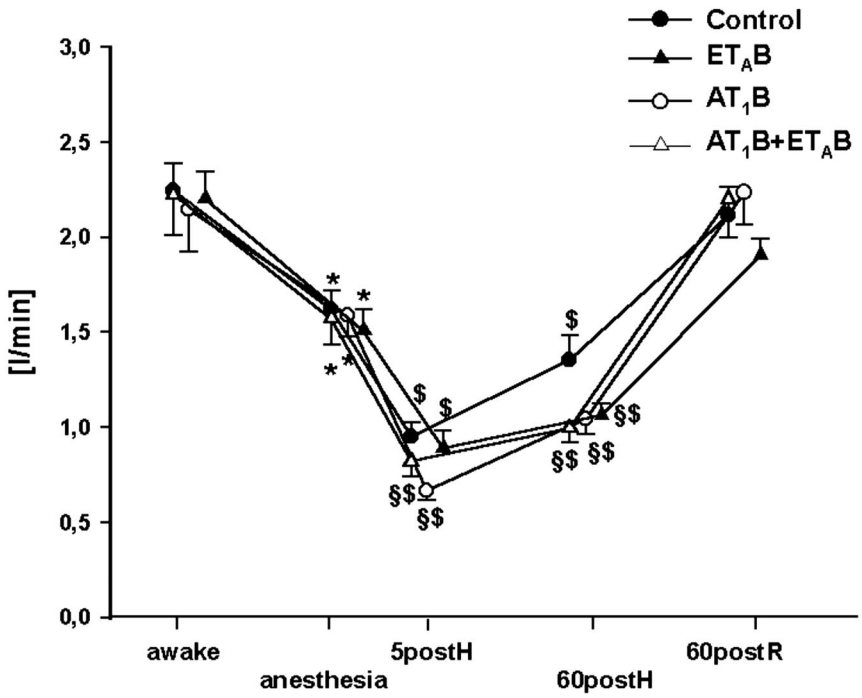

Fig. 2. Cardiac output in awake and anesthetized dogs, 5 min (5postH) and 60 min after hemorrhage (60postH) and 60 min after retransfusion (60postR). Values are presented as mean ± SEM; n = 6. * $P < 0.05$ *versus* awake. § $P < 0.05$ *versus* control. $ $P < 0.05$ *versus* anesthesia. AT_1B = angiotensin II receptor blockade; $AT_1B + ET_AB$ = combined receptor blockade; ET_AB = endothelin-A receptor blockade.

creased to 41 ± 2 mmHg 60 min after hemorrhage. Sixty minutes after retransfusion, MAP reached only 51 ± 4 mmHg ($P < 0.05$; fig. 1). In the $AT_1B + ET_AB$ protocol, the decrease in MAP was most severe, being 49 ± 5 mmHg during anesthesia, 36 ± 2 mmHg 60 min after hemorrhage, and only 43 ± 2 mmHg 60 min after retransfusion ($P < 0.05$; fig. 1).

Cardiac output in the awake state was 2.1–2.2 l/min in all protocols and decreased to 1.5–1.6 l/min during anesthesia ($P < 0.05$; fig. 2). After hemorrhage, CO decreased less in controls (1.3 ± 0.1 l/min) than in the AT_1B, ET_AB, and $AT_1B + ET_AB$ protocols (1.0–1.1 l/min; $P < 0.05$; fig. 2). Sixty minutes after retransfusion, CO increased, less in the ET_AB than in the other protocols (fig. 2).

Heart rate in the awake state was similar in all protocols (88–93 beats/min) and did not change during anesthesia (table 1). Heart rate increased by approximately 10 beats/min 60 min after hemorrhage in all protocols and increased further after retransfusion ($P < 0.05$; table 1).

Central venous pressure was similar in all protocols. Only 5 min after hemorrhage, the central venous pressure was decreased ($P < 0.05$), whereas it had returned to prehemorrhage values 60 min after hemorrhage (table 1).

Table 1. Hemodynamic Parameters in Control, ET_AB, AT_1B, and $AT_1B + ET_AB$

	Awake	Anesthesia	5 min after Hemorrhage	60 min after Hemorrhage	60 min after Retransfusion
Heart rate, beats/min					
Control	88 ± 3	98 ± 3	102 ± 4	110 ± 3	120 ± 8†
ET_AB	92 ± 6	100 ± 3	110 ± 3	112 ± 3	124 ± 3†
AT_1B	90 ± 5	98 ± 4	93 ± 3	105 ± 3	134 ± 5†
$AT_1B + ET_AB$	93 ± 4	97 ± 7	98 ± 4	106 ± 4	129 ± 7†
Central venous pressure, cm H_2O					
Control	2 ± 0.2	2 ± 0.2	0 ± 0†	1 ± 0.5	2 ± 0.5
ET_AB	2 ± 0.5	3 ± 0.8	1 ± 0.5†	2 ± 0.5	3 ± 0.3
AT_1B	1 ± 0.6	2 ± 0.2	0 ± 0†	2 ± 0.7	2 ± 0.2
$AT_1B + ET_AB$	2 ± 0.6	3 ± 0.7	1 ± 0.7†	3 ± 0.9	4 ± 0.8
Systemic vascular resistance, $dyn \cdot s^{-1} \cdot cm^{-5}$					
Control	3,025 ± 222	3,148 ± 121	3,787 ± 253†	3,592 ± 190	2,813 ± 169
ET_AB	3,221 ± 270	3,373 ± 280	3,660 ± 243	3,515 ± 122	2,349 ± 167†
AT_1B	3,653 ± 215	2,568 ± 212*	3,351 ± 163†	2,919 ± 226	1,649 ± 163†
$AT_1B + ET_AB$	3,077 ± 255	2,355 ± 206*	2,830 ± 254†	2,746 ± 219	1,524 ± 173†

Values are presented as mean ± SEM; n = 6. Values were measured during 30 min awake, 60 min isoflurane–nitrous oxide anesthesia, 5 min and 60 min after hemorrhage, and 60 min after retransfusion.

* $P < 0.05$ *vs.* awake. † $P < 0.05$ *vs.* anesthesia.

AT_1B = angiotensin II receptor blockade; ET_AB = endothelin-A receptor blockade.

Table 2. Plasma Hormones in Control, ET_AB, AT_1B, and $AT_1B + ET_AB$

	Awake	Anesthesia	5 min after Hemorrhage	60 min after Hemorrhage	60 min after Retransfusion
Plasma renin activity, ngAngII · $ml^{-1} \cdot h^{-1}$					
Control	3.4 ± 0.6	24.9 ± 2.9*	—	46.1 ± 4.6†	33.3 ± 5.6
ET_AB	5.8 ± 1.3	32.3 ± 4.6*	—	77.1 ± 9.2†‡	66.9 ± 9.4†‡
AT_1B	13.9 ± 3.5‡	82.3 ± 16.3*‡	—	71.5 ± 12.8‡	64.3 ± 11.2‡
$AT_1B + ET_AB$	24.2 ± 5.4‡	85.4 ± 13.2*‡	—	65.1 ± 5.3‡	52.9 ± 6.6†
Angiotensin II, pg/ml					
Control	8 ± 1	51 ± 5*	102 ± 27†	126 ± 17†	79 ± 17
ET_AB	9 ± 1	98 ± 14*	151 ± 27†	179 ± 39†	127 ± 35
AT_1B	26 ± 6	231 ± 48*‡	287 ± 65‡	273 ± 65‡	152 ± 36‡
$AT_1B + ET_AB$	41 ± 10‡	244 ± 32*‡	251 ± 67‡	225 ± 43‡	173 ± 38‡
Aldosterone, pg/ml					
Control	78 ± 15	672 ± 52*	—	1,234 ± 78†	923 ± 119†
ET_AB	83 ± 13	776 ± 93*	—	1,178 ± 39†	978 ± 62†
AT_1B	42 ± 8	283 ± 45*	—	879 ± 106†‡	455 ± 109†‡
$AT_1B + ET_AB$	39 ± 5	368 ± 67*	—	897 ± 77†‡	718 ± 164†
Epinephrine, pg/ml					
Control	73 ± 19	66 ± 31	827 ± 203†	956 ± 155†	93 ± 29
ET_AB	102 ± 34	92 ± 37	1,212 ± 251†	901 ± 160†	205 ± 57†‡
AT_1B	157 ± 45	35 ± 16	972 ± 222†	1,296 ± 187†	323 ± 57†‡
$AT_1B + ET_AB$	138 ± 41	72 ± 31	780 ± 257†	1,288 ± 119†	369 ± 79†‡
Norepinephrine, pg/ml					
Control	166 ± 6	131 ± 21	224 ± 67	263 ± 36†	137 ± 20
ET_AB	143 ± 22	117 ± 18	266 ± 66†	278 ± 64†	165 ± 46
AT_1B	151 ± 34	97 ± 33	222 ± 96	396 ± 90†	244 ± 53
$AT_1B + ET_AB$	149 ± 17	78 ± 18	127 ± 32	268 ± 27†	190 ± 20
Vasopressin, pg/ml					
Control	0.5 ± 0.1	21 ± 15*	162 ± 29†	91 ± 5†	8 ± 6
ET_AB	0.6 ± 0.02	17 ± 3*	280 ± 62†	154 ± 42†	20 ± 6
AT_1B	0.5 ± 0.1	14 ± 5*	182 ± 30†	223 ± 91†	30 ± 10
$AT_1B + ET_AB$	0.6 ± 0.01	27 ± 8*	213 ± 59†	170 ± 21†	29 ± 6
Endothelin 1, pg/ml					
Control	1.0 ± 0.1	1.1 ± 0.1	—	1.5 ± 0.2†	1.4 ± 0.1†
ET_AB	1.4 ± 0.1‡	1.5 ± 0.1‡	—	3.2 ± 0.3†‡	2.7 ± 0.4†‡
AT_1B	0.9 ± 0.1	1.0 ± 0.1	—	1.7 ± 0.2†	1.8 ± 0.1†
$AT_1B + ET_AB$	1.6 ± 0.1‡	1.9 ± 0.2‡	—	2.9 ± 0.4‡	3.1 ± 0.4‡

Values are presented as mean ± SEM; n = 6. Values were measured after 30 min awake, after 60 min of isoflurane–nitrous oxide anesthesia, 5 and 60 min after hemorrhage, and 60 min after retransfusion.

* $P < 0.05$ *vs.* awake. † $P < 0.05$ *vs.* anesthesia. ‡ $P < 0.05$ *vs.* control.

Aldosterone = plasma aldosterone concentration; AngII = plasma angiotensin II concentration; AT_1B = angiotensin II receptor blockade; endothelin 1 = plasma endothelin 1 concentration; epinephrine = plasma epinephrine concentration; ET_AB = endothelin-A receptor blockade; norepinephrine = plasma norepinephrine concentration; vasopressin = plasma vasopressin concentration.

Systemic vascular resistance in controls and in the ET_AB protocol was always greater during anesthesia and after hemorrhage and retransfusion than in the AT_1B and $AT_1B + ET_AB$ protocols ($P < 0.05$; table 1).

Plasma Hormones and Plasma Values

Plasma norepinephrine concentrations were similar in all protocols during the awake state and during baseline isoflurane–nitrous oxide anesthesia. Norepinephrine increased after hemorrhage and decreased after retransfusion in all protocols ($P < 0.05$; table 2).

Plasma epinephrine concentrations were comparable in all protocols during the awake state (70–157 pg/ml) and did not change during the baseline anesthesia period. After hemorrhage, epinephrine concentrations increased to 827–1,296 pg/ml (table 2) and decreased in all protocols after retransfusion ($P < 0.05$), remaining above the levels observed during anesthesia alone in all protocols but controls.

Plasma renin activity in the awake state was higher in AT_1B and $AT_1B + ET_AB$ protocols than in controls ($P < 0.05$; table 2). During anesthesia, plasma renin activity increased in all protocols ($P < 0.05$). After hemorrhage, plasma renin activity increased further only in controls and in the ET_AB protocol ($P < 0.05$; table 2).

Plasma angiotensin II concentrations were higher in AT_1B and $AT_1B + ET_AB$ protocols than in controls and the ET_AB protocol in the awake state (table 2). Angiotensin II increased during anesthesia, especially in the AT_1B and $AT_1B + ET_AB$ protocols ($P < 0.05$; table 2). Angiotensin II increased after hemorrhage in controls and the ET_AB protocol ($P < 0.05$; table 2) but not in the AT_1B and $AT_1B + ET_AB$ protocols (table 2). After retransfusion, angiotensin II decreased slightly in all protocols.

Table 3. Arterial and Mixed Venous Blood Gases, Arterial and Mixed Venous Plasma pH, and Base Excess in Control, ET_AB, AT_1B, and $AT_1B + ET_AB$

	Awake	Anesthesia	60 min after Hemorrhage	60 min after Retransfusion
Pa_{O_2}, mmHg				
Control	95 ± 1	183 ± 4*	175 ± 2	178 ± 3
ET_AB	97 ± 2	182 ± 6*	172 ± 7	186 ± 5
AT_1B	97 ± 1	187 ± 2*	183 ± 7	179 ± 4
$AT_1B + ET_AB$	98 ± 2	182 ± 8*	180 ± 8	185 ± 9
Pa_{CO_2}, mmHg				
Control	37 ± 1	35 ± 1	34 ± 2	32 ± 1
ET_AB	37 ± 1	35 ± 2	36 ± 2	34 ± 2
AT_1B	36 ± 1	34 ± 2	34 ± 2	32 ± 2
$AT_1B + ET_AB$	37 ± 1	35 ± 2	37 ± 1	34 ± 1
Pv_{O_2}, mmHg				
Control	44 ± 1	47 ± 1	38 ± 1†	46 ± 1
ET_AB	47 ± 1	48 ± 2	37 ± 2†	46 ± 1
AT_1B	45 ± 1	46 ± 2	33 ± 3†	47 ± 1
$AT_1B + ET_AB$	46 ± 2	43 ± 3	34 ± 2†	54 ± 5
Pv_{CO_2}, mmHg				
Control	41 ± 2	41 ± 1	46 ± 2†	36 ± 1†
ET_AB	42 ± 1	40 ± 2	46 ± 2†	41 ± 2
AT_1B	40 ± 1	40 ± 2	46 ± 2†	36 ± 2†
$AT_1B + ET_AB$	42 ± 1	43 ± 2	49 ± 1†	41 ± 1
pH_a				
Control	7.39 ± 0.01	7.39 ± 0.01	7.35 ± 0.01†	7.42 ± 0.01†
ET_AB	7.37 ± 0.01	7.37 ± 0.02	7.31 ± 0.02†	7.39 ± 0.02†
AT_1B	7.40 ± 0.01	7.39 ± 0.01	7.34 ± 0.02†	7.39 ± 0.03
$AT_1B + ET_AB$	7.40 ± 0.01	7.37 ± 0.01	7.30 ± 0.01†	7.35 ± 0.02
pH_v				
Control	7.37 ± 0.01	7.34 ± 0.01*	7.28 ± 0.01†	7.39 ± 0.01†
ET_AB	7.36 ± 0.01	7.33 ± 0.01*	7.25 ± 0.01†	7.34 ± 0.01
AT_1B	7.38 ± 0.01	7.34 ± 0.01*	7.26 ± 0.01†	7.36 ± 0.01
$AT_1B + ET_AB$	7.37 ± 0.01	7.31 ± 0.01*	7.25 ± 0.01†	7.31 ± 0.01
BE_a, mM				
Control	−2.2 ± 0.7	−4.2 ± 0.3*	−6.8 ± 0.7†	−3.1 ± 0.5
ET_AB	−2.9 ± 0.7	−4.8 ± 0.6*	−7.1 ± 0.4†	−4.2 ± 0.3
AT_1B	−2.4 ± 0.6	−4.4 ± 0.7*	−8.4 ± 1.1†	−5.1 ± 1.0
$AT_1B + ET_AB$	−1.8 ± 0.8	−4.7 ± 0.9*	−7.1 ± 0.6†	−6.3 ± 0.9

Values are presented as mean ± SE; n = 6. Values were measured after 30 min awake, after 60 min of isoflurane–nitrous oxide anesthesia, 60 min after hemorrhage, and 60 min after retransfusion.

* $P < 0.05$ *vs.* awake. † $P < 0.05$ *vs.* anesthesia.

AT_1B = angiotensin II receptor blockade; BE_a = arterial base excess; ET_AB = endothelin-A receptor blockade; Pa_{CO_2} = arterial carbon dioxide tension; Pa_{O_2} = arterial oxygen tension; pH_a = arterial pH; pH_v = mixed venous pH; Pv_{CO_2} = mixed venous carbon dioxide tension; Pv_{O_2} = mixed venous oxygen tension.

Plasma aldosterone concentrations were lower in AT_1B and $AT_1B + ET_AB$ protocols during the awake state. Aldosterone increased during anesthesia and 60 min after hemorrhage in all protocols ($P < 0.05$; table 2) and decreased slightly after blood retransfusion (table 2).

Vasopressin plasma concentrations were low during the awake state (0.5–0.6 pg/ml) and increased during anesthesia (14–27 pg/ml) and after hemorrhage ($P < 0.05$), being lowest in controls (162 ± 29 pg/ml) and highest in ET_AB (280 ± 62 pg/ml) (table 2). After retransfusion, vasopressin returned almost to baseline levels ($P < 0.05$; table 2).

Plasma endothelin 1 concentrations were higher in ET_AB and $AT_1B + ET_AB$ protocols compared with control and AT_1B protocols ($P < 0.05$; table 2). After hemorrhage, the plasma endothelin 1 concentration increased in all protocols ($P < 0.05$; table 2). Even 60 min after blood retransfusion, the endothelin 1 concentration remained increased in the range of values found after hemorrhage.

Arterial oxygen tension increased after induction of anesthesia (fraction of inspired oxygen = 0.3; $P < 0.05$) but did not change throughout the experiments in all protocols. Arterial carbon dioxide tensions were similar in all protocols and did not change during the experiments. Mixed venous oxygen tension decreased during hemorrhage in all protocols and increased to anesthesia baseline levels after retransfusion ($P < 0.05$; table 3). Mixed venous carbon dioxide tension increased after hemorrhage in all protocols ($P < 0.05$) and decreased to, or even below, anesthesia baseline levels after retransfusion.

Plasma osmolarity was not different between awake (299–300 mOsM) and anesthetized dogs (298–300 mOsM). After hemorrhage, however, plasma osmolarity

Table 4. Renal Excretion Parameters in Control, ET_AB, AT_1B, and $AT_1B + ET_AB$

	Awake	Anesthesia	60 min after Hemorrhage	60 min after Retransfusion
Urine flow, $\mu l \cdot min^{-1} \cdot kg^{-1}$				
Control	25 ± 5	6 ± 2*	1 ± 1	25 ± 7†
ET_AB	38 ± 11	4 ± 1*	1 ± 1	18 ± 4†
AT_1B	98 ± 24	6 ± 3*	0 ± 0	6 ± 4
$AT_1B + ET_AB$	69 ± 16	2 ± 1*	0 ± 0	0.2 ± 0.02
GFR, $ml \cdot min^{-1} \cdot kg^{-1}$				
Control	4.4 ± 0.5	2.2 ± 0.5*	1.1 ± 0.6	3.3 ± 0.3
ET_AB	3.9 ± 0.4	2.1 ± 0.5*	0.6 ± 0.3†	2.8 ± 0.7
AT_1B	4.2 ± 0.3	0.6 ± 0.3‡	0.1 ± 0.05	0.4 ± 0.3‡
$AT_1B + ET_AB$	3.8 ± 0.3	0.5 ± 0.2‡	0.1 ± 0.1	0.2 ± 0.2‡

Values are presented as mean ± SEM; n = 6. Values were measured after 30 min awake, after 60 min of isoflurane–nitrous oxide anesthesia, 60 min after hemorrhage, and 60 min after retransfusion.

* $P < 0.05$ *vs.* awake. † $P < 0.05$ *vs.* anesthesia. ‡ $P < 0.05$ *vs.* control.

AT_1B = angiotensin II receptor blockade; ET_AB = endothelin-A receptor blockade; GFR = glomerular filtration rate.

decreased in all protocols (294–296 mOsM; $P < 0.05$) and remained in this range also after retransfusion.

Urine Flow and Glomerular Filtration Rate

Urine flow ranged between 25 and 98 $\mu l \cdot min^{-1} \cdot kg^{-1}$ in the awake state, being greatest in the protocols in which losartan had been used. Urine flow decreased during anesthesia ($P < 0.05$; table 4) and was almost zero during hemorrhage. After retransfusion, urine flow increased above baseline anesthesia levels in control and ET_AB dogs ($P < 0.05$; table 4), whereas in AT_1B and $AT_1B + ET_AB$ dogs, it returned to baseline anesthesia levels.

Glomerular filtration rates were similar in all protocols during the awake state. During anesthesia, the decrease in GFR was most pronounced in the AT_1B and $AT_1B + ET_AB$ protocols ($P < 0.05$; table 4). After hemorrhage, GFR was close to zero in the AT_1B and $AT_1B + ET_AB$ protocols ($P < 0.05$; table 4). After retransfusion, GFR increased above baseline anesthesia values in ET_AB and controls, whereas it remained low in AT_1B and $AT_1B + ET_AB$ dogs (table 4).

Discussion

The current study investigated whether ET_A receptor antagonism impairs the integrated cardiovascular, hormonal, and renal responses to acute hemorrhage in dogs anesthetized with isoflurane–nitrous oxide, compared with controls and with selective AT_1 antagonism. The anesthetic gas mixture consisted of isoflurane (1.2 vol% end-tidal concentration) in oxygen–nitrous oxide (30/70 vol%), which is equal to approximately 1.3 MAC in dogs.[10] Thus, the following results were obtained during moderately deep anesthesia.

In the following discussion, the effects of isoflurane–nitrous oxide anesthesia on hemodynamics and hormones in controls, ET_A, and AT_1 receptor–inhibited dogs will be addressed first, followed by the discussion of the effects of hemorrhage and, finally, of the blood retransfusion.

Isoflurane–Nitrous Oxide Anesthesia: Hemodynamic and Hormonal Effects during ET_A and AT_1 Receptor Inhibition

In our control experiments, isoflurane–nitrous oxide anesthesia decreased CO by 28% and mean arterial blood pressure by 25% (figs. 1 and 2), leaving systemic vascular resistance almost unchanged (table 1). This is in line with previously published data in dogs.[12] Heart rate increased (table 1) because of the vagolytic effect of isoflurane.[13] In ET_A-inhibited dogs, MAP and CO were not different from controls, during the awake state as well as during isoflurane–nitrous oxide anesthesia alone (figs. 1 and 2). This supports the notion that ET_1 is not substantially involved in the short-term blood pressure and CO regulation in normotensive dogs.[6,14] In contrast, AT_1 receptor inhibition caused a greater decrease in MAP (−41%) during isoflurane–nitrous oxide anesthesia than in controls (−25%). This emphasizes the importance of angiotensin II in short-term blood pressure regulation.

After induction of isoflurane–nitrous oxide anesthesia, activation of the renin–angiotensin–aldosterone system and an increase in vasopressin concentrations were unanimous findings in all our protocols (table 2). The activation of the renin–angiotensin–aldosterone system is most likely due to the decrease in MAP and thus renal perfusion pressure during isoflurane–nitrous oxide anesthesia, activating the renal baroreceptor mechanism.[15] Stimulation of renal sympathetic nerves may also be involved.[16] The increase in vasopressin secretion during isoflurane–nitrous oxide anesthesia is probably also due to the decrease in MAP,[17] because other factors that might stimulate vasopressin release, such as plasma osmolarity[18] and acid–base status[19] (table 3), remained unaffected by isoflurane–nitrous oxide anesthesia *per se* in all protocols.

Hemorrhage during Isoflurane–Nitrous Oxide Anesthesia: Hemodynamic and Hormonal Effects of ET_A and AT_1 Receptor Inhibition

Acute withdrawal of approximately 25% of blood volume decreased MAP and CO (figs. 1 and 2) and increased systemic vascular resistance in both controls and ET_A-inhibited dogs (5 min after hemorrhage; table 1). However, the increase in endogenous vasopressors (vasopressin, angiotensin II, epinephrine) was more pronounced during ET_A receptor inhibition compared with controls (table 2). A direct, stimulating effect of endothelins on vasopressin release is possible[20] but seems unlikely in our experiments because the ET_1 plasma concentration increased only slightly after hemorrhage. It is possible that ET_A inhibition induced an additive vasodilative effect *via* ET_B receptors[21]—in addition to that caused by isoflurane anesthesia *per se*, which was counteracted by the more pronounced increase of vasopressors after hemorrhage. During AT_1 receptor inhibition, the decrease in MAP after hemorrhage was greater than in controls or ET_A receptor–inhibited dogs, although there was no additive effect of simultaneous ET_A receptor inhibition (fig. 1), whereas the decrease in CO, the decrease in central venous pressure, and the increase in heart rate (table 1) after hemorrhage were comparable with controls. All endogenous compensatory mechanisms, such as the increases in plasma epinephrine, norepinephrine, vasopressin, aldosterone, sympathetic traffic were insufficient to return MAP into the range of controls. Therefore, other than in ET_A receptor–antagonized individuals, the anesthesiologist must be prepared to quickly and continuously replace blood loss or to exogenously support the endogenous compensatory vasoregulatory mechanisms or both in AT_1 receptor–inhibited individuals.

Mean arterial pressure and CO partly recovered within 60 min after blood loss in controls. This recovery was paralleled by slow increases of plasma norepinephrine, endothelin, and aldosterone concentrations ("late" hormonal regulation; table 2), and was accompanied by an increase in heart rate. Unlike controls, spontaneous recovery of MAP and CO 60 min after blood loss was markedly blunted in ET_A-antagonized dogs (figs. 1 and 2), suggesting that ET_A receptor inhibition impairs long-term rather than short-term hemodynamic regulation.

Blood Replacement during Isoflurane–Nitrous Oxide Anesthesia: Hemodynamic and Hormonal Effects of ET_A and AT_1 Receptor Inhibition

After retransfusion of the shed blood, the concentrations of all vasoconstrictive hormones decreased and MAP returned to baseline anesthesia levels in controls but not with AT_1 and ET_A receptor inhibition (fig. 1). The relative increase in CO was even greater than for MAP, returning CO almost to awake levels in all protocols. The increase in CO can mainly be attributed to the further increase in heart rate after blood retransfusion (fig. 2 and table 1). This increase in heart rate probably ensued from the swift (5-min) blood retransfusion into a constricted vascular system. The phenomenon of hypervolemia-induced tachycardia was first described by Bainbridge[22] (Bainbridge reflex) in anesthetized dogs. The Bainbridge reflex increases CO in response to an increased venous return to the heart, thereby reducing the elevation of cardiac preload. After retransfusion, the increase in CO may also serve to overcome the slight metabolic acidosis that developed during the hemorrhage period (table 3). In ET_A-inhibited dogs, blood retransfusion did restore CO but not MAP to prehemorrhage values (figs. 1 and 2). This indicates that endothelins are essential in the long-term regulation of MAP and for MAP restoration after blood retransfusion in anesthetized dogs (fig. 2). However, compared with ET_A-inhibited dogs, blood pressure regulation was much more compromised during both AT_1 inhibition protocols, suggesting the importance of angiotensin II in both short- and long-term responses to hypovolemic shock. The smaller decrease in epinephrine concentrations after blood retransfusion in both AT_1 inhibition protocols (table 2) may indicate continued endogenous sympathetic support.

Renal Function

Anesthesia reduced urine flow markedly in all protocols (table 4), probably because of a decrease in MAP combined with the stimulation of water- and sodium-retaining hormones (angiotensin II, vasopressin, aldosterone). GFR decreased in all protocols during anesthesia, but the decrease was most severe with AT_1 receptor inhibition (table 4). This can probably be explained by the decrease of MAP and renal perfusion pressure below the threshold pressure for renin release of approximately 70–80 mmHg in AT_1-antagonized dogs. Below this pressure, the kidney starts to release renin that induces angiotensin II production, which normally constricts the efferent arteriole of the glomerulus to prevent a decrease in GFR. AT_1 receptor inhibition impairs this response because it impairs the angiotensin II–mediated constriction of the efferent glomerular arteriole,[23] leading to GFR values close to zero after hemorrhage (table 4). Urinary excretion after hemorrhage almost ceased in all protocols as a result of the renal vasoconstriction during hypovolemic hypotension mediated by reflex sympathetic excitation, the increase in circulating catecholamines,[24] the increase in sodium- and water-retaining hormones (table 2), and the decrease in GFR (table 4).

After retransfusion, urinary excretion rate and GFR increased to awake levels in controls and ET_A-antagonized dogs, whereas in both AT_1 inhibition protocols, renal function remained compromised, possibly because MAP, and thus renal perfusion pressure, only increased

to 40–50 mmHg even after blood retransfusion in these protocols.

In conclusion, endothelin-A receptor inhibition impairs long-term blood pressure regulation after hemorrhage and retransfusion during isoflurane–nitrous oxide anesthesia despite an impressive activation of vasoconstrictive hormones such as vasopressin, angiotensin II, and epinephrine and activation of the sympathetic nervous system during hemorrhage. This indicates that endothelins seem to have a place especially in long-term (hours range) cardiovascular regulation. In contrast, angiotensin II (AT_1) receptor inhibition impairs both short and long-term blood pressure regulation after hypovolemic hemorrhage to a greater extent than during ET_A inhibition. Moreover, renal function after hemorrhage and after retransfusion was by far more compromised by AT_1 than by ET_A inhibition.

The authors thank Rainer Mohnhaupt (Engineer, Clinic of Anesthesiology and Intensive Care Medicine, Charité, Campus Virchow, Berlin, Germany) for statistical advice, and Daniela Bayerl and Birgit Brandt (Medical Laboratory Assistants, Experimental Anesthesia, Charité, Campus Virchow) for expert technical assistance during the experiments.

References

1. Fukusaki M, Maekawa T, Kobayashi I, Hara T, Sumikawa K: Catecholamine and renin-angiotensin response during controlled hypotension induced by prostaglandin E_1 combined with hemodilution during isoflurane anesthesia. J Clin Anesth 1997; 9:321–7

2. Ullman J: Influence of neurohumoral blockade on heart rate and blood pressure responses to hemorrhage in isoflurane anaesthetized rats. Acta Physiol Scand 2000; 169:189–94

3. Ardaillou R: Angiotensin II receptors. J Am Soc Nephrol 1999; 10:30S–9S

4. Clozel M, Gray GA, Breu VL: The endothelin ETB receptor mediates both vasodilation and vasoconstriction in vivo. Biochem Biophys Res Commun 1992; 186:867–73

5. Verhaar MC, Grahn AY, van Weerdt AWM, Honing MLH, Morrison PJ, Yang YP, Padley RJ, Rabelink TJ: Pharmacokinetics and pharmacodynamic effects of ABT-627, an oral ETA selective endothelin antagonist, in humans. Br J Clin Pharmacol 2000; 49:562–73

6. Höhne C, Francis RCE, Kaczmarczyk G, Boemke W: Haemodynamic and hormonal changes during haemorrhage in conscious dogs treated with an endothelin-A receptor antagonist. Clin Sci 2002; 103:336S–9S

7. Committee on Care and Use of Laboratory Animals: Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resource, National Research Council, Department of Health and Human Services, Washington, D.C., National Academy of Science, 1996

8. Krebs MO, Kröhn T, Boemke W, Mohnhaupt R, Kaczmarczyk G: Renal and hemodynamic effects of losartan in conscious dogs during controlled mechanical ventilation. Am J Physiol 1999; 276:F425–32

9. Heller J, Kramer HJ, Maly J, Cervenka L, Horacek V: Effect of intrarenal infusion of angiotensin-(1-7) in the dog. Kidney Blood Press Res 2000; 23:89–94

10. Cullen D: Anesthetic depth and MAC, Anesthesia. Edited by Miller R. New York, Churchill Livingstone, 1986, pp 553–80

11. Schaffer JP: Modified sequentially rejective multiple test procedures. J Am Stat Assoc 1986; 81:826–31

12. Deryck YLJ, Brimioulle S, Maggiorini M, de Canniere D, Naeije R: Systemic vascular effects of isoflurane versus propofol anesthesia in dogs. Anesth Analg 1996; 83:958–64

13. Picker O, Scheeren TWL, Arndt JO: Inhalation anesthetics increase heart rate by decreasing cardiac vagal activity in dogs. Br J Anaesth 2001; 87:748–54

14. Teerlink JR, Carteaux JP, Sprecher U, Löffler BM, Clozel M, Clozel JP: Role of endogenous endothelin in normal hemodynamic status of anesthetized dogs. Am J Physiol 1995; 268:H432–40

15. Ullman J: Vasopressin and angiotensin II in blood pressure control during isoflurane anesthesia in rats. Acta Anaesthesiol Scand 1999; 43:860–5

16. Davis JO, Freeman RH: Mechanisms regulating renin release. Phys Rev 1976; 56:1–56

17. Wang BC, Flora-Ginter G, Leadley RJ, Goetz KL: Ventricular receptors stimulate vasopressin release during hemorrhage. Am J Physiol 1988; 254: R204–11

18. Bourque CW, Oliet SHR: Osmoreceptors in central nervous system. Annu Rev Physiol 1997; 59:601–19

19. Bestle MH, Olsen NV, Poulsen TD, Roach R, Fogh-Andersen N, Bie P: Prolonged hypobaric hypoxemia attenuates vasopressin secretion and renal response to osmostimulation in men. J Appl Physiol 2002; 92:1911–22

20. Nakamoto H, Suzuki H, Murakami M, Kageyama Y, Naitoh M, Sakamaki Y, Ohishi A, Saruta T: Different effects of low and high doses of endothelin on haemodynamics and hormones in the normotensive conscious dog. J Hypertens 1991; 9:337–44

21. Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, Webb DJ: Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. Circulation 1998; 97:752–6

22. Bainbridge FA: The influence of venous filling upon the rate of the heart. J Physiol 1915; 50:65–84

23. Hall JE: Regulation of glomerular filtration rate and sodium excretion by angiotensin II. Fed Proc 1986; 45:1431–7

24. Koyama S, Fujita T, Shibamoto T, Matsuda Y, Hayashi T Jr, Saeki Y, Kawamato M, Yamaguchi Y: Relative contribution of renal nerve and adrenal gland to renal vascular tone during prolonged canine hemorrhagic hypotension. Circ Shock 1993; 39:269–74