EXPERIMENTAL

Mats Laesser
Emma Spak
Sara Ewert
Anders Aneman
Lars Fandriks

Candesartan improves survival following severe hypovolemia in pigs; a role for the angiotensin II type 2 receptor?

M. Laesser · E. Spak · S. Ewert ·
L. Fandriks (✉)
Department of Gastrosurgical Research,
Institute of Surgical Sciences,
Sahlgrenska Academy,
University of Gothenburg,
Box 75038, 40036 Gothenburg, Sweden
e-mail: lars.fandriks@gastro.gu.se
Tel.: +46-31-3424123

A. Aneman
Department of Anesthesiology
and Intensive Care,
Institute of Surgical Sciences,
Sahlgrenska Academy,
University of of Gothenburg,
40036 Gothenburg, Sweden

Abstract *Objective:* To investigate the involvement of intestinal angiotensin II type 2 receptors in the outcome of acute severe hypovolemia as well as systemic and regional mesenteric hemodynamics and intestinal mucosal functions in anesthetized pigs. *Design and setting:* Prospective, interventional animal study in a university research laboratory. *Subjects:* 53 landrace pigs, 28–35 kg. *Interventions:* 30+30% or 20+20% hemorrhage of estimated total blood volume followed by retransfusion performed in untreated controls, in animals treated with the angiotensin II type 1 receptor blocker candesartan or with a combination of candesartan and the angiotensin II type 2 receptor blocker PD123319. *Measurements and results:* Following 30+30% hemorrhage the candesartan-treated animals attained a significantly higher survival rate than controls and animals treated with PD123319 in combination with candesartan. Less pronounced hemorrhage (20+20%) resulted in no mortality and functional variables were assessed. A significantly higher output of jejunal intraluminal nitric oxide occurred during hypovolemia in the candesartan treated group than in controls and animals that received PD123319 in combination with candesartan. Jejunal transmucosal potential difference was significantly better preserved after retransfusion in candesartan-treated animals than in controls. Expression of angiotensin II type 2 receptors in intestinal tissue was significantly higher in animals surviving the 30+30% hemorrhage than in nonsurvivors. *Conclusions:* Lethal circulatory failure is possibly influenced by use of angiotensin receptor ligands, and activation of intestinal angiotensin II type 2 receptors may play a significant role in improving the outcome of severe hypovolemia.

Keywords Renin-angiotensin system · Gastrointestinal · Hemorrhage · Angiotensin II receptors · Candesartan · PD123319

Introduction

It has been proposed that gut hypoperfusion and suppressed intestinal mucosal barrier function contribute to the pathophysiology leading to multiple organ dysfunction and failure following circulatory collapse [1]. Intestinal dysoxia impairs the mucosal barrier function in turn allowing mucosal penetration of luminal microbes and toxins that activate systemic inflammatory cascades that distort function and structural integrity of distant organs [2]. Several vasoregulatory systems have been suggested to mediate trauma-induced gut hypoperfusion, and among these the renin-angiotensin system appears to play a key role [3, 4, 5]. Angiotensin II, which is the primary vasoactive component of the renin-angiotensin system, binds to two membrane receptors: the angiotensin II type 1 (AT1) receptor and the angiotensin II type 2 (AT2) receptor. The AT1 receptor mediates the vast majority of

"classical" physiological effects related to angiotensin II, for example, vasoconstriction and Na$^+$ retention. Both receptors are of the seven-transmembrane domain type, but unlike the AT1 receptor the AT2 receptor is not coupled to any of the classical, well established, second messengers, such as cAMP or inositol phosphates, and its coupling to a G protein, reported by several authors, is controversial [6]. Expression of the AT2 receptor varies considerably depending on the cell types and experimental conditions used. Also, the AT2 postreceptor pathways for intercellular signaling are not completely settled but appear to involve formation of bradykinin, nitric oxide (NO), prostaglandins, and possibly cytokins [6]. In porcine models of severe hypovolemic and septic shock selective AT1 receptor antagonists have been shown to alleviate mesenteric hypoperfusion and maintain short-term survival [7, 8, 9]. The better preserved mesenteric perfusion is thus believed to result from reduced vasoconstriction due to AT1 receptor blockade. However, recent data suggest that improved circulation following AT1 receptor blockade is due to circulating angiotensin II activating the unopposed AT2 receptor that mediates vasodilatation [10, 11, 12]. The present study investigated whether intestinal AT2 receptors are involved in the outcome of acute severe hypovolemia in anesthetized pigs. Experiments were designed for expression analysis of angiotensin II receptors. Survival, systemic and regional mesenteric hemodynamics, and intestinal mucosal functions (represented by mucosal perfusion, transmucosal electrical potential difference and NO formation) were investigated subsequent to pharmacological interference using the AT1 receptor antagonist candesartan, alone or in combination with the AT2 receptor antagonist PD123319.

Materials and methods

The study was approved by the Ethics Committee of Experiments on Animals, Gothenburg, Sweden, based on the Swedish regulations corresponding to the National Institutes of Health guidelines for use of experimental animals.

Anesthesia and surgical procedures and measurements

Landrace pigs of both sexes weighing 28–35 kg (mean 30.8 kg) were fasted for 24 h with free access to water and a sugar-salt solution. Anesthesia was induced with ketamin (Ketalar, Parke-Davis, Stockholm, Sweden 15 ml/kg bodyweight) and azaperon (Stresnil, Jansen-Cilag, Vienna, Austria; 80 mg) intramuscularly, after which a bolus injection of α-chloralose (Kebo Lab, Spanga, Sweden; 100 mg/kg) was given intravenously. Anesthesia was maintained with a continuous intravenous infusion of α-chloralose (25–50 mg/kg·per hour). A continuous infusion of Ringer's solution was given at an hourly rate of 20 ml/kg during instrumentation via laparotomy and 10 ml/kg during the rest of the protocol. The animals were tracheotomized and mechanically ventilated (Servo 900 ventilator, Siemens Elema, Sweden) with 30% oxygen in air to normocapnia. Catheters were positioned to monitor systemic and

central blood pressures and cardiac output (CO). After midline laparotomy a 16-mm ultrasonic flowmetry probe (QPV; Transonic Systems, Ithaca, N.Y., USA) was placed around the portal vein. Continuous measurements of mean arterial blood pressure (MAP; DPT6003 pressure transducers, Peter von Berg, Medizintechnik, Eglharting, Germany), portal venous flow (QPV), and intermittent measurements of CO by thermodilution were performed on a digital monitoring system (AS/3 monitoring system, Datex-Engstrom Division, Helsinki, Finland).

In one series of experiments (20+20% blood loss) the portal vein was catheterized to sample blood to assess blood-gas, acid-base status, and lactate levels (Radiometer ABL 700; Radiometer Medical, Copenhagen, Denmark).

An intraluminal laser-Doppler probe (0.25 mm fiber separation; Perimed, Järfälla, Sweden) was inserted 20 cm into the lumen via a small jejunal incision approx. 80 cm below the Treitz ligament. The laser-Doppler catheter was connected to a Periflux 4001 base unit (780 nm laser light wavelength, 12 kHz band width, 0.2 s time constant; Perimed), and the signal was recorded (LabView 4.1, National Instruments, Austin, Tex., USA) by use of a Macintosh computer. The laser-Doppler signal was averaged over 2 min to compensate for possible movement derived artifacts. Results are given as a percentage of individual baseline.

To measure transmucosal potential difference one sodium chloride electrolyte bridge connected to a calomel electrode was inserted in the jejunum 15 cm below the Treitz ligament, and another bridge was placed in the bloodstream via the femoral vein. The two bridges were connected to an amplifier and functioned as a high-impedance voltmeter. During baseline conditions jejunal transmucosal electrical potential difference averaged −1.3 mV (lumen-negative; range −5 to 2 mV, n=18; no significant intergroup differences). Results are for sake of clarity given as change from individual baseline ($\triangle$PD) in absolute values.

A tonometer (Tonometrics, Datex-Engstrom Division) was positioned in the jejunum 60 cm below the Treitz ligament. Room air (5 ml) was insufflated into the tonometer balloon, over 15 min this is estimated to give a greater than 90% equilibration with surrounding tissues [12]. NO was then analyzed based on chemiluminescence (CLD 77 AM, Eco Physics, Dürnten, Switzerland). Jejunal intraluminal NO output varied considerably between animals (mean 3349 ppm; range 6–25,000 ppm; n=18, no significant intergroup difference) and was analyzed in relation to individual baseline as previously described [13, 14].

Study design

The experimental protocol is depicted in Fig. 1. Animals were randomized to either no treatment (controls), treatment with candesartan (bolus of 25 μg/kg followed by continuous intravenous infusion of 15 μg/kg·per hour; CV-11974, a kind gift from Dr. P. Morsing, Astra Zeneca R&D Mölndal, Sweden) or a combination of candesartan (dose as above) and PD123319 (bolus of 300 μg/kg followed by 30 μg/kg·per hr intravenously; Sigma-Aldrich, Stockholm, Sweden). The doses of candesartan and PD123319 used in this protocol have previously demonstrated blockade of the AT1 receptor and AT2 receptor, respectively [7, 14]. Total blood volume (ml) was estimated as: $(179 \times bw^{-0.27}) \times bw$ (bw = bodyweight) [15]. Blood was withdrawn (20+20% or 30+30% of estimated total blood volume) in two steps 30 min after onset of treatment, each over 15 min. Measurements were conducted after 30 min of stabilization. The removed heparinized (5000 IE, Heparin Leo, Leo Pharma) blood was retransfused during 15 min (see Fig. 1). After final recordings the animals were killed during deepened anesthesia using potassium chloride.

In an additional series of experiments mortality associated with severe (30+30%) hypovolemia was analyzed in a larger untreated control group in which the jejunal expression of AT1 and AT2
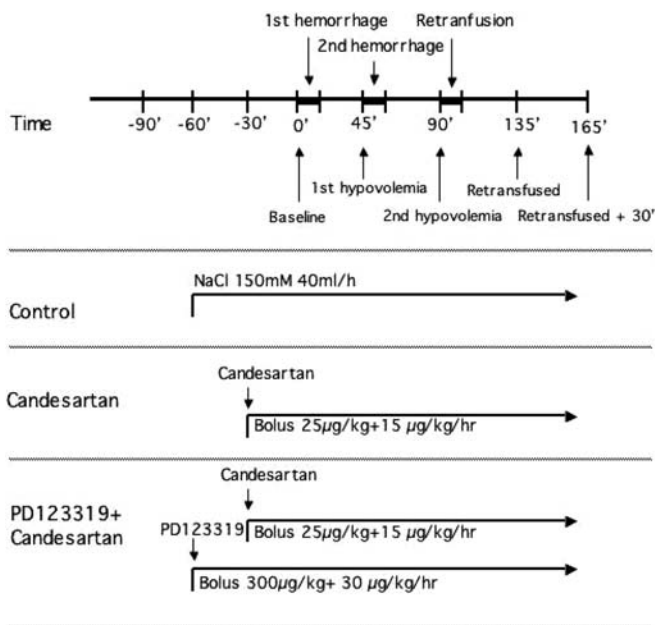
Fig. 1 Experimental protocol showing the principle of the two-step induction of hypovolemia; time points for recordings and type of pharmacological treatment

receptors was evaluated (n=14). These animals were instrumented as above but no intestinal variables were recorded.

Western blot AT1 and AT2 receptors

Full-wall specimens were obtained from jejunal incisions made for intraluminal instrumentation. The tissue was snap frozen in liquid nitrogen and subsequently stored at −70°C until use. Specimens were homogenized on ice (Polytron, Kinematica, Switzerland) in buffer A (10% glycerol, 20 mM Tris-HCl pH 7.3, 100 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, 2 mM EDTA, 2 mM EGTA, 10 mM sodium orthovanadate, 10 µg/ml leupeptin, and 10 µg/ml aprotinin) [16]. Centrifugation was performed at 30,000 g for 30 min at 4°C. The pellets were resuspended in buffer B (1% NP-40 Sigma-Aldrich in buffer A) and subsequently stirred at 4°C for 1 h before centrifugation at 30,000 g for 30 min at 4°C. The supernatants were then analyzed for protein content according to Bradford [17]. Electrophoresis was run on 10% Bis-Tris using N-2-morpholino propane sulfonic acid running buffer (Invitrogen, Sweden). Whole-cell lysates of KNRK (for AT2 receptors) and PC-12 (for AT1 receptors; Santa Cruz, Calif., USA) were used as positive controls; a prestained molecular weight standard was also used. Polyvinyldifluoride membranes were incubated with polyclonal specific antibodies of rabbit origin directed at the AT1 receptor (N10, Santa Cruz) and AT2 receptor (H143, Santa Cruz). Followed by an alkaline phosphatase conjugated goat anti-rabbit antibody and CDP-Star substrate to identify immunoreactive proteins by chemiluminescence. Images were captured by an LAS-100 cooled charge-coupled device camera (Fujifilm, Tokyo, Japan). Data were assessed as optical density per microgram of protein.

Table 1 Hemodynamic effects of 30+30% blood loss (*MAP* mean arterial pressure, *CO* cardiac output, *QPV* portal venous flow)

	MAP (mmHg)	CO (l/min)	QPV (ml/min)
Controls			
Baseline (n=7)	105±15	3.9±0.7	1328±276
45 min hypovolemia 30% (n=7)	84±16	2.5±0.4	737±172
90 min hypovolemia 60% (n=3)	27±12	1.2±0.6	230±143
Candesartan			
Baseline (n=7)	97±12	4.2±0.9	1077±390
45 min hypovolemia 30% (n=7)	79±18	2.8±0.3	826±97 *
90 min hypovolemia 60% (n=7)	44±18	1.7±0.2	424±97
Candesartan+PD123319			
Baseline (n=7)	110±15	3.5±0.9	945±277
45 min hypovolemia 30% (n=7)	96±18	2.3±0.5	638±201
90 min hypovolemia 60% (n=2)	26±23	1.4±0.2	130±89

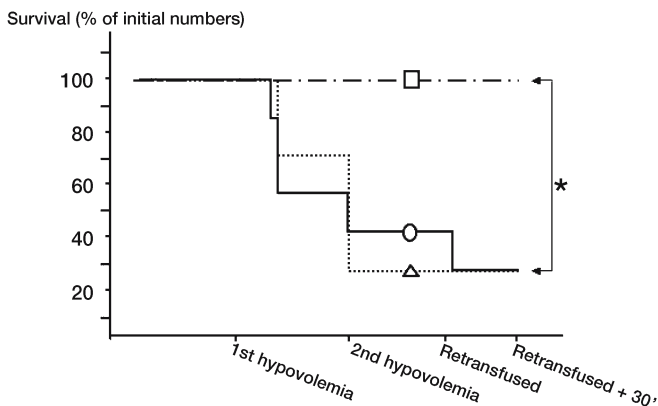*$p \leq 0.05$ vs. control

Statistical analysis

Analysis of variance was used to determine intergroup differences, and the significance of detected differences was analyzed using Bonferroni's post-hoc test. Survival was analyzed using Fisher's exact test, and western blot results were analyzed by the Mann-Whitney U test (Statview 4.5, SAS Institute, Cary, N.C., USA). Statistical significance was set at $p < 0.05$.

Results

Severe hypovolemia
(30+30% of estimated total blood volume)

Acute infusion of neither candesartan alone nor the combination of candesartan and PD123319 induced any significant changes from predrug values 30 min after administration (data not shown). It follows that baseline hemodynamics in presence of treatment were within physiological limits, and no significant differences were found between the treatment groups with regard to MAP, CO, or QPV (Table 1). Following the 30% hemorrhage MAP and CO decreased approx. 20% and 35%, respectively (Table 1). These changes did not differ between treatment groups. Also QPV decreased markedly upon blood loss (Table 1). However, when QPV was related to baseline, the candesartan-treated animals decreased significantly less (group mean −15%) than both the untreated controls (group mean −45%) and the group treated with candesartan and PD123319 in combination (group mean −32%).

Additional withdrawal of 30% of the estimated total blood volume elicited acute irreversible circulatory failure in four of the seven animals in the control group and

Survival (% of initial numbers)

Fig. 2 Effects of 30+30%blood loss in controls (*circle*), candesartan treated animals (*square*), and PD123319 plus candesartan treated animals (*triangle*). *$p \leq 0.05$ candesartan treated group vs. controls and candesartan treated group vs. PD123319+candesartan treated group (Fisher's exact test; $n=7$ in each group at start of protocol)

five of seven animals in the candesartan and PD123319 treated group. All of the candesartan-treated animals survived the protocol including retransfusion whereas only two of seven survived in the two treatments arm (Fig. 2).

Moderate hypovolemia
(20+20% of estimated total blood volume)

At baseline no significant intergroup differences were found regarding MAP, CO, or QPV (Table 2). After each of the two subsequent sets of 20% hemorrhage MAP was lower in the groups treated with candesartan with or without PD123319, whereas CO and QPV decreased

similarly in all three groups. Retransfusion restored CO and QPV without significant intergroup differences, whereas animals treated with candesartan or with PD123319 and candesartan in combination failed to restore MAP to baseline levels (Table 2). All animals survived the hemorrhage and retransfusion procedure. The first 20% blood loss decreased jejunal laser-Doppler flowmetry (LDF) to 85–86% of baseline. The additional 20% blood withdrawal resulted in an LDF signal at 65–67% of baseline independently of treatment. The mucosal LDF signal returned to baseline following the reperfusion without any significant intergroup differences (data not shown in figure).

Following the second hemorrhage (total blood-withdrawal 40%) intraluminal NO increased significantly in the candesartan-treated group compared to the untreated controls and animals treated with PD123319 and candesartan in combination. The increased NO production returned to baseline after retransfusion (Fig. 3). The jejunal lumen transmucosal potential difference did not change significantly from baseline during hypovolemia in any of the treatment groups. However, following retransfusion the transmucosal potential difference in the controls moved significantly in the positive direction (i.e., from lumen negativity towards zero) whereas it was maintained rather constant in the candesartan-treated animals independent on presence of PD123319 (Fig. 4).

Starting from baseline levels within physiological limits, portal venous oxygen saturation (pv-sO$_2$) and portal venous standard base excess (pv-SBE) decreased in all three groups with a concomitant moderate increase in portal venous lactate (pv-Lac) during hypovolemia. Retransfusion resulted in a complete restoration of pv-sO$_2$ and normalization of pv-Lac, whereas pv-SBE failed to regain prehemorrhagic levels. No intergroup differences were found (Table 2).

Table 2 Hemodynamic effects and portal blood status following 20+20% blood loss, $n=6$ in each group (*MAP* mean arterial preassure, *CO* cardiac output, *QPV* portal venous flow, *pv-sO$_2$* portal venous oxygen saturation, *pv-SBE* portal venous standard base excess, *pv-Lac* portal venous lactate)

|	MAP (mmHg)	CO (l/min)	QPV (ml/min)	Pv-sO$_2$ (%)	Pv-SBE (mmol/l)	Pv-Lac (mmol/l)
Controls ($n=6$)						
Baseline	96±13	4.2±1.0	1118±212	84.5±5.3	0.5±3.7	1.4±0.3
45 min hypovolemia 20%	70±13	3.2±0.8	866±249	77.8±5.4	0.9±2.5	1.9±0.4
90 min hypovolemia 40%	48±13	2.5±0.9	674±242	62.8±6.1	−1.3±2.2	2.6±0.6
135 min retransfused	85±17	4.3±1.2	1342±193	88.5±5.3	−0.1±1.3	1.9±0.5
165 min retransfused	76±22	4.2±1.4	1185±174	87.9±2.6	−0.3±4.2	1.8±0.6
Candesartan ($n=6$)						
Baseline	78±12	4.4±0.3	1042±227	77.5±11.1	1.3±4.1	1.4±0.3
45 min hypovolemia 20%	50±13*	3.2±0.5	786±165	69.5±7.9	1.1±3.6	1.8±0.7
90 min hypovolemia 40%	33±7*	2.1±0.3	554±76	58.2±12.9	−0.8±3.5	3.0±1.6
135 min retransfused	58±17*	3.8±0.5	1265±268	81.2±8.2	−0.3±3.7	2.2±1.2
165 min retransfused	52±15*	3.4±0.7	1079±253	82.2±9.8	−0.8±3.9	2.0±1.0
Candesartan+PD123319 ($n=7$)						
Baseline	80±18	3.8±0.4	1049±266	82.4±6.5	2.8±2.7	1.9±0.4
45 min hypovolemia 20%	44±10*	2.8±0.4	760±190	71.3±3.3	1.7±2.4	2.2±0.5
90 min hypovolemia 40%	28±5*	2.0±0.5	536±191	55.8±7.3	−1.6±2.6	3.7±1.1
135 min retransfused	46±14*	3.7±1.2	1178±401	78.3±7.6	−0.3±2.8	2.9±1.2
165 min retransfused	40±13*	3.3±1.3	879±331	79.8±5.6	0.1±3.4	2.5±1.1

*$p \leq 0.05$ vs. control
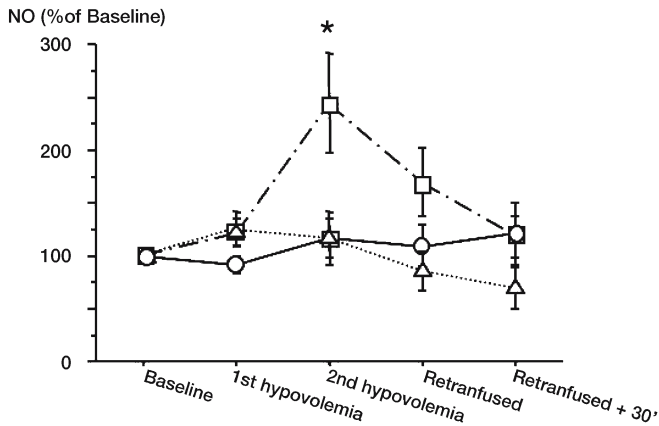
Fig. 3 Mucosal nitric oxide output as percentage of baseline. Effects of 20+20% blood loss in controls (*circles*), candesartan treated animals (*squares*), and PD123319 in combination with candesartan treated animals (*triangles*); means ±SE. *$p \leq 0.05$ candesartan treated group vs. controls and candesartan treated group vs. PD123319+candesartan treated group (analysis of variance, Bonferroni's post-hoc test; $n=6$ in each group)
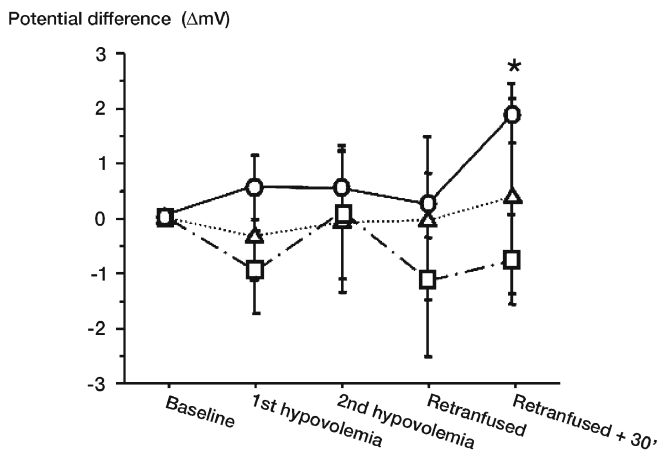
Fig. 4 Jejunal transmucosal potential difference as change from baseline. Effects of 20+20% blood loss in controls (*circles*), candesartan treated animals (*squares*), and PD123319 plus candesartan treated animals (*triangles*); means ±SE. *$p \leq 0.05$ candesartan treated group vs. control group (analysis of variance, Bonferroni's post-hoc test; $n=6$ in each group)

Expression of AT1 and AT2 receptors

In the additional series of control experiments with severe hypovolemia 6 of 14 animals survived the retransfusion, thus a similar proportion as in the untreated control group described above. Both AT1 and AT2 receptors were expressed in the jejunum at baseline in all animals, confirming previous results [14]. A significantly higher baseline expression of AT2 receptors was observed in the animals that survived the bleeding-retransfusion procedure than those that died during hypovolemia (Fig. 5).
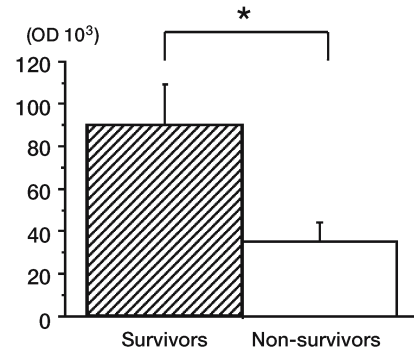
Fig. 5 AT2 receptor expression in jejunal biopsyspecimens at baseline, displayed as optical density (*OD*). Results split into two groups, survivors $n=6$ (*stripes*) and nonsurvivors $n=8$ (*clear*) of the 245-min protocol. The survivors have a significantly higher baseline level of AT2 receptors than the nonsurvivors; means ±SE. *$p \leq 0.05$ (Mann-Whitney *U* test)

Discussion

In the present study acute withdrawal and retransfusion of 30+30% of the estimated total blood volume resulted in circulatory failure and marked mortality in the control group. Interestingly, animals treated with the selective AT1 receptor antagonist candesartan all survived both the hemorrhage and retransfusion procedure. It is well known that angiotensin II increases dramatically in response to severe blood loss and particularly so in pigs [18]. The improved survival in presence of candesartan suggests that AT1 receptor activation may be deleterious in critical circulatory conditions. However, when the AT1 receptors are selectively blocked the AT2 receptors become the main target for angiotensin II. Several cardiovascular effects obtained by pharmacological blockade of AT1 receptors have been ascribed to activation of the unopposed AT2 receptors [6, 10]. In addition, AT1 receptor blockade enhances angiotensin II formation [19] that, as a consequence, reinforces AT2 receptor activation. The present study shows that mortality in the group given candesartan together with the AT2 receptor antagonist PD123319 was similar to controls. Apparently PD123319 blocked the improved survival obtained by AT1 receptor blockade. It follows that the AT2 receptors appear to play a major role for the improved survival observed in presence of candesartan. This conclusion is further supported by the finding of a higher baseline level of AT2 receptors in intestinal tissue from individuals surviving the severe hypovolemia.

Despite the limited size of this study the candesartan-associated effect on survival is striking. Although type II errors cannot be excluded, it can be noted that among all the hemodynamic variables monitored, i.e., arterial pressure, CO, mesenteric and mucosal perfusion, only one differed significantly between the treatment arms. Confirming previous results [7, 8], it was observed that the

mesenteric perfusion was better preserved in the candesartan-treated group after the first 30% blood loss. Although this effect was sensitive to PD123319, it can hardly explain the AT2-associated effect on survival, but it indicates that the gastrointestinal tract can be an area of interest for further research. As a complete functional evaluation was impossible due to the low number of survivors following severe (30+30%) hypovolemia, the study was repeated with a more moderate hypovolemia (20+20% of estimated total blood volume). The study design was based on the assumption that the 20+20% is subthreshold to mortality but still a stimulus strong enough to induce (dys)functional alterations preceding the deleterious 30+30% hemorrhage. Arterial pressure was somewhat lower in the presence of candesartan during the 20+20% blood loss as well as following retransfusion, but other hemodynamic recordings were similar independent of treatment. The major findings at this level of hypovolemia (20+20% blood loss) were an increased intestinal lumen NO output and, subsequent to retransfusion, a maintained transmucosal potential difference in the candesartan-treated group. The hypovolemia induced increased NO output in presence of candesartan was sensitive to PD123319, suggesting an AT2 receptor dependent mediation.

Previous studies have shown activation of AT2 receptors to mediate vasorelaxation and NO formation in various tissues including the porcine jejunal mucosa [14, 20]. NO has dual functions: in high ("pathophysiological") concentration and particularly in presence of reactive oxygen species NO becomes cytotoxic. On the other hand, in the low ("physiological") concentration range as assessed in the present study, it is involved in signal transmission and promotes tissue protection, for example, by stabilizing inflammation, the latter not the least in the gut [21, 22]. By acting on epithelial electrolyte transport and intercellular permeability NO can significantly influence intestinal mucosal functions in a way that contributes to improved barrier functions [22].

Intestinal mucosal NO formation has been shown to decrease in situations with reduced mesenteric perfusion, such as hypovolemia and cardiac tamponade [23, 24]. Since oxygen is required for the enzymatic degradation of L-arginine to citrulline and NO, local dysoxia has been suggested to cause of the reduction in NO formation. However, in the present study NO formation did not decrease despite a confirmed reduction in intestinal mucosal blood flow in response to hemorrhage. On the other hand, only moderate effects on mesenteric lactate levels were observed, indicating that pronounced ischemia was not induced by 20+20% blood loss. Thus the maintained NO formation during hypovolemia in the present study was probably related to a relatively well-maintained mucosal oxygenation. Apparently the animals in the present study endured hypovolemia better than animals in previous studies [24]. The reason(s) may be related to genetic predisposition but also to the fact that a new animal research facility was employed with improved animal health in terms of nutrition and electrolyte status at the time of the experiments.

Transmucosal potential difference is a complex variable depending on the viability of the epithelium, particularly its ability to transport ions and its paracellular electrical conductivity (both energy-demanding processes). The transmucosal potential difference in the jejunum is low, usually in the order of 3–7 mV (lumen negative). In the present study the potential difference was reduced in control animals following retransfusion, indicating a compromised mucosal function [25]. The candesartan-treated group maintained the potential difference during the hypovolemia and retransfusion, indicating a mucosa-protective effect. This candesartan-related effect was not sensitive to PD123319, suggesting that the suppressed potential difference observed at retransfusion is mediated solely via activation of the AT1 receptor, not involving AT2 receptors.

Taken together, AT2 receptor activation may contribute to the maintained mesenteric perfusion and increased mucosal NO output during hypovolemia seen in presence of candesartan. Both effects are barrier promoting, and the data thus support the hypothesis that AT2 receptor stimulation, for example, by preventing intestinal luminal microbes and toxins to penetrate into tissue, reduces the risk for induction of systemic inflammatory responses [21]. However, such effects should be viewed as long term and can hardly explain the improved survival observed over a short period in the candesartan-treated group of the present study. Additional mechanisms are probably involved, and one such factor was recently reported by Newton et al. [26]. These authors showed that microvascular hydraulic permeability is regulated by angiotensin receptors. Microvessel permeability is lowered by AT2 receptor activation or AT1 receptor blockade, and increased by AT2 receptor blockade or AT1 receptor activation Although still speculative, a bleeding-induced increase in angiotensin II could via AT1 receptors increase microvascular permeability and shift intravascular volume to the extravascular space. If this happens in an already critical hypovolemic situation, even marginal additional reductions in intravascular volume may be lethal. Blockade of AT1 and activation of AT2 receptors (if present) would thus counteract such a deleterious event. It could thus be hypothesized that angiotensin II regulated microvessel permeability could be one mechanism of action explaining the presently reported pharmacological effects on hypovolemia induced acute mortality. Furthermore, pigs with pronounced baseline expression of jejunal AT2 receptors survived the bleeding procedure, suggesting that the gastrointestinal tract is of vital particular importance. Indeed, mesenteric organs have a marked vascular capacity, but additional studies are clearly needed

to directly address angiotensin II receptor expression and actions also in other vascular beds and tissues.

In conclusion, the present study indicates that lethal circulatory failure can be pharmacologically influenced by the use of angiotensin receptor ligands, and that activation of intestinal angiotensin II type 2 receptors may play a significant role for improving the outcome of severe hypovolemia.

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