Pressor and mesenteric arterial hyporesponsiveness to angiotensin II is an early event in haemorrhagic hypotension in anaesthetised rats

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Abstract

Objective: Vascular responsiveness to vasoconstrictors is known to be attenuated in haemorrhagic shock. In this study we assessed the temporal development and the underlying mechanisms of haemorrhage-induced vascular hyporesponsiveness to pressor agents.

Methods: In phenobarbital-anaesthetised rats hypotension was induced by graded haemorrhage (8 ml blood total). Sham-manipulated rats served as controls. Blood flow (BF) was recorded with ultrasonic transit time flow probes.

Results: Following haemorrhage mean arterial pressure (MAP) fell by 25–45 mm Hg and was accompanied by a reduction in mesenteric BF without any alteration of mesenteric vascular conductance (VC). While pressor responses to arginine vasopressin remained unaltered, hyporesponsiveness to phenylephrine (10 nmol kg⁻¹) developed 120–180 min after hypotension had been induced. Pressor and mesenteric constrictor responses to angiotensin II (30 pmol kg⁻¹) became significantly blunted as early as 60 min post haemorrhage. The hypotensive effect of an angiotensin₂ receptor antagonist, telmisartan (1 mg kg⁻¹), was likewise blunted 3 h after haemorrhage. Pretreatment with the cyclooxygenase inhibitor indomethacin (10 mg kg⁻¹) exaggerated the hypotensive reaction to haemorrhage but did not prevent the development of angiotensin II hyporesponsiveness. In contrast, the nitric oxide (NO) synthase inhibitor L-NAME (10 mg kg⁻¹), as investigated 3 h post haemorrhage, restored the systemic pressor responses to angiotensin II and phenylephrine as well as the mesenteric constrictor responses to phenylephrine to normal level and diminished the mesenteric hyporesponsiveness to angiotensin II. Glibenclamide (20 mg kg⁻¹), an inhibitor of ATP-sensitive K⁺ channels given 180 min post haemorrhage, partially reversed haemorrhage-induced hypotension but did not modify angiotensin II hyporesponsiveness. Conclusion: Systemic pressor responsiveness and mesenteric arterial reactivity to endogenous and exogenous angiotensin II is selectively impaired at an early stage of haemorrhagic hypotension. This phenomenon partially involves NO and is not related to ATP-sensitive K⁺ channels.

Keywords: Rat; Haemorrhage; Shock; Hyporeactivity; Angiotensin; Phenylephrine; Vasopressin; Nitric oxide; Prostaglandins; Potassium channels

1. Introduction

Reduced sensitivity to vasoconstrictor agents has been known for a long time to be associated with hypotensive states, as observed in pregnancy [1], portal hypertension [2] or diabetes mellitus [3], and shock, due to endotoxin aemia [4], thermal injury [5] or haemorrhage [6]. Especially after haemorrhage, the ability of the organism to maintain blood pressure and organ perfusion is limited by a progressive loss of responsiveness of the vasculature to the compensatory release of vasoconstrictors, predominantly catecholamines, angiotensin II and [Arg]vasopressin. As a result, blood pressure further declines and becomes refractory to volume substitution (decompensatory phase) which irreversibly leads to death.

The mechanisms involved in peripheral vascular failure are still unclear, but it appears that the metabolic state of...
the tissue is not a primary factor in triggering hyporesponsiveness [7]. The potent vasodilator autacoid nitric oxide (NO) has been identified as a major mediator of vascular hyporesponsiveness to adrenoceptor activation in haemorrhage [8]. However, the impact of NO on other pressor systems such as the renin–angiotensin axis has not yet been investigated. Moreover, it is unknown, whether hyporesponsiveness simultaneously and equally affects several vasopressor systems.

Therefore, it was the aim of the current study to investigate the time course of the development of vascular hyporesponsiveness to various pressor agents, and the underlying mechanisms, in an in vivo model of moderate haemorrhagic hypotension in anaesthetised rats. We recorded mean arterial pressure (MAP) and blood flow through the superior mesenteric artery, which was chosen because it is involved in the regulation of systemic blood pressure and is of crucial importance in the pathophysiology of shock.

2. Methods

2.1. Animal preparation

All experiments of this study were approved by the Animal Care Committee of the Austrian Ministry of Science and Traffic and were performed in agreement with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). Male Sprague-Dawley rats weighing 450–500 g, fed ad libitum, were anaesthetised i.p. with phenobarbital (200 mg kg⁻¹). The rats were placed on a heated table, to maintain rectal temperature at 37°C, and fitted with a tracheal cannula, to facilitate spontaneous respiration. Blood pressure was measured via a cannula in the right carotid artery and recorded with a pressure transducer (ISOTEC; HSE, March-Hugstetten, Germany). A second cannula was placed in the left jugular vein for the i.v. administration of drugs. For blood flow measurements the superior mesenteric artery was exposed by midline laparotomy, isolated over a length of 5 mm, and special care was taken to remove any tissue adherent to the adventitia.

2.2. Haemodynamic measurements

Blood flow, in absolute values of ml min⁻¹, was measured by the ultrasonic transit time shift technique with the use of a small animal flowmeter (model T206; Transonic, Ithaca, NY, USA) and a 1-mm ultrasonic flow probe (model 1RB; Transonic). The BF signal from the flow meter and the BP signal from the amplifier were fed into a personal computer via an analogue–digital converter. MAP, heart rate and mesenteric vascular conductance (VC), in ml min⁻¹ (mm Hg)⁻¹, were calculated on-line by an acquisition–evaluation software programme [9].

2.3. Experimental protocol and study groups

After preparation the rats were allowed to equilibrate until haemodynamic parameters became stable (30–40 min). Haemorrhagic hypotension was induced by slow withdrawal from a side-arm of the carotid cannula of 5 ml, and 30 min later, 3 ml of blood which corresponds to about 25% of the total blood volume of the rats [10]. Sham-manipulated animals were included in all study groups as controls. Animals that died before completion of the experimental protocol were excluded from analysis. The study was subdivided in five parts involving separate groups of rats.

The first part of the study investigated the effect of haemorrhagic hypotension on haemodynamic parameters and responsiveness to angiotensin II (10 pmol kg⁻¹) or phenylephrine (10 nmol kg⁻¹), injected as i.v. bolus in a volume of 0.33 ml kg⁻¹. These doses of the vasoconstrictors were used since they were about half maximally effective. Responsiveness to either angiotensin II or phenylephrine was determined immediately before and after the induction of haemorrhage every 30 min for 150 min.

In the second part dose–response curves were constructed with increasing doses of either angiotensin II (3–300 pmol kg⁻¹), phenylephrine (1–100 nmol kg⁻¹) or [Arg²]vasopressin (1–100 pmol kg⁻¹) starting 3 h after the induction of haemorrhagic hypotension. The intervals between the individual doses of the drugs were usually 10 min by which haemodynamic parameters were allowed to return to pre-injection values.

The third part assessed the involvement of cyclooxygenase products in the haemodynamic alterations due to haemorrhage. To this end the rats were pretreated 30 min before haemorrhage i.p. with vehicle (1 ml kg⁻¹) or indomethacin at a dose (10 mg kg⁻¹) that has been shown to effectively inhibit cyclooxygenase activity [11]. Responsiveness to angiotensin II was determined immediately before and after the induction of haemorrhage every 30 min for 150 min.

The fourth part investigated the role of NO and ATP-sensitive K⁺ channels in altered cardiovascular responsiveness in haemorrhagic hypotension. At 160 min after haemorrhage the rats were injected i.v. with the NO synthase inhibitor N⁵-nitro-L-arginine methyl ester (L-NAME), at a dose (10 mg kg⁻¹) that is maximally effective in increasing MAP [12], or the inactive enantiomer D-NAME (10 mg kg⁻¹). At 3 h post haemorrhage a dose–response curve was recorded with increasing doses of either angiotensin II (3–300 pmol kg⁻¹) or phenylephrine (1–100 nmol kg⁻¹). Similarly, glibenclamide (20 mg kg⁻¹), an inhibitor of ATP-sensitive K⁺ channels, or its vehicle (100 μl dimethyl sulfoxide) were injected i.p. at 150 min post haemorrhage, and responsiveness to angiotensin II (10 pmol kg⁻¹) was recorded immediately.
before and 30 min after glibenclamide/vehicle administration.

In the fifth part of the study the haemodynamic effects of an angiotensin AT₃ receptor antagonist, telmisartan, were recorded during haemorrhagic hypotension. At 3 h after haemorrhage rats received an i.v. bolus of 1 mg kg⁻¹ of telmisartan which has been shown to be the maximally effective dose in decreasing MAP [13].

2.4. Substances

Phenobarbital was dissolved in saline at a concentration of 33 mg ml⁻¹. Stock solutions (1 mM) of angiotensin II, [Arg⁸]vasopressin (Bachem; Bubendorf, Switzerland) and phenylephrine (Sigma; Vienna, Austria) were prepared in distilled water. Further dilutions used for injection were made with saline. N⁵-g-nitro-l-arginine methyl ester (Bachem) was dissolved in saline. Indomethacin (Merck, Sharp & Dohme; München, Germany) was dissolved in 2% Na₂CO₃ at a concentration of 10 mg ml⁻¹. Glibenclamide (RBI; Vienna, Austria) was dissolved in dimethyl sulfoxide (10 mg in 100 μl).

Telmisartan (BIBR 277), 4-[(1,4-dimethyl-2-propyl-[2,6-bi-1H-benzimidazol]-1-y1)methyl]-[1,1-biphenyl]2-

![Graph](image.png)

Fig. 1. Time-dependent effects of haemorrhage on mean arterial pressure (MAP), heart rate, mesenteric blood flow (BF) and mesenteric vascular conductance (VC). Haemorrhage was induced by slow withdrawal of 5 ml, and 30 min later, 3 ml of blood from the carotid artery and is indicated by respective arrows (↓). Numbers of experiments for control and haemorrhaged rats were 62 and 58, respectively. Data are shown as means±SEM. *P<0.05 haemorrhage versus control.
Fig. 2. Time-dependent effects of haemorrhage on (A) mean arterial pressure (MAP), mesenteric vascular conductance (VC) and vascular responsiveness to (B) angiotensin II and (C) phenylephrine. Haemorrhage was induced by slow withdrawal of 5 ml, and 30 min later, 3 ml of blood from the carotid artery and is indicated by respective arrows (↓). A, B: Responses to angiotensin II (10 pmol kg\(^{-1}\)) were investigated in the absence or presence of the cyclooxygenase inhibitor indomethacin (indo). Indomethacin (10 mg kg\(^{-1}\)) or its vehicle (1 ml kg\(^{-1}\)) were injected i.p. 30 min before the induction of haemorrhage. Responses to angiotensin II are expressed relative to the respective pre-injection values. Indomethacin pretreatment had no haemodynamic effects in control rats as compared to vehicle-treated controls (data not shown). Numbers of experiments for controls, vehicle/haemorrhage rats and indomethacin/haemorrhage rats were 11, 7 and 7, respectively. C: Responsiveness to phenylephrine (10 nmol kg\(^{-1}\)) was investigated in the absence of a b indomethacin in six rats per group. Data are shown as means ± SEM. \(P<0.05\) control versus vehicle/haemorrhage, \(P<0.05\) control versus indomethacin/haemorrhage, \(P<0.05\) indomethacin/haemorrhage versus vehicle/haemorrhage.

Table 1
Haemodynamic effects of inhibitors of nitric oxide (NO) synthesis and ATP-sensitive K\(^+\) channels, and an antagonist of AT\(_1\) receptors in control and haemorrhaged rats

<table>
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<th>n</th>
<th>Pre MAP mm Hg</th>
<th>Post MAP mm Hg</th>
<th>Δ MAP mm Hg</th>
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<td>13</td>
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<td>70±7</td>
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<td>160±24</td>
<td>219±45</td>
<td>137±11</td>
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* The NO synthase inhibitor N\(^\text{\textsuperscript{e}}\)-nitro-L-arginine methyl ester (L-NAME, 10 mg kg\(^{-1}\), i.v.), the inhibitor of ATP-sensitive K\(^+\) channels, glibenclamide (20 mg kg\(^{-1}\), i.p.), or the AT\(_1\) receptor antagonist telmisartan (1 mg kg\(^{-1}\)) were injected 160 min after the induction of haemorrhagic hypotension. Mean arterial pressure (MAP) and mesenteric vascular conductance (VC) were recorded immediately before and 20 min (L-NAME and telmisartan) or 30 min (glibenclamide) after the administration of the drugs. Relative changes are expressed as increases above baseline (MAP) or as percent of the preinjection value (VC). The respective vehicles had no effects (data not shown). Data are shown as means±SEM.

* \(P<0.05\) haemorrhage versus control.
tor are not shown since they did not differ from the values described during the time course experiments (Figs. 1 and 2A) and were unchanged during the construction of dose–response curves as compared with immediately after the treatment period (Table 1).

Responses in mean arterial pressure were expressed as Δ mm Hg, while responses in mesenteric vascular conductance in % of baseline since in previous studies we have found that there was no correlation between mean arterial pressure at baseline and pressor responses when calculated as Δ mm Hg whereas a close negative correlation was observed when calculated as % changes. Conversely, % changes did not correlate with basal mesenteric vascular conductance, but a positive correlation was apparent when responses to drugs were expressed as Δ μl min⁻¹ (mm Hg)⁻¹ [9,13].

All data are presented as means±SEM. Time-course experiments were statistically evaluated with the Mann–Whitney U test or in case of more than two treatment groups with Kruskal–Wallis H test. Dose–response curves were compared with two way repeated measures analysis of variance [14]. Two-sided probability values of P <0.05 were regarded as significant.

3. Results

3.1. General

Withdrawal of 5 ml blood from the carotid artery resulted in an immediate fall of MAP from about 110 to 50 mm Hg (data not shown) followed by partial recovery to 70–75 mm Hg after 30 min (Fig. 1). The second haemorrhage of 3 ml of blood further lowered MAP to 30–40 mm Hg (data not shown) after which MAP stabilised at about 75 mm Hg and only slightly declined until the end of the experiments (Fig. 1). Superior mesenteric blood flow closely followed the time course of MAP changes (Fig. 1) with, consequently, no alterations in mesenteric VC (Fig. 1). Heart rate initially decreased after haemorrhage, rapidly recovered and tended to be elevated at 3 h (Fig. 1). All these haemodynamic parameters were constant in control rats throughout the 3-h observation period (Fig. 1). Survival rate at 3 h was about 90% in the control and 70% in the haemorrhaged group.

At baseline (time 0 min) i.v. bolus injections of angiotensin II (10 pmol kg⁻¹) transiently increased MAP by 15–25 mm Hg (Fig. 2B), reduced mesenteric BF to 55–75% (data not shown) and mesenteric VC to 40–60% (Fig. 2B) of the preinjection value (100%). These haemodynamic reactions to angiotensin II (10 pmol kg⁻¹) showed little variability over the next 3 h in control rats (Fig. 2B). In contrast, both systemic pressor and mesenteric vasoconstrictor responses to angiotensin II (10 pmol kg⁻¹) became significantly reduced as early as 1 h after the induction of haemorrhage (Fig. 2B).

The dose of 10 nmol kg⁻¹ phenylephrine, injected i.v. as bolus, was equally effective as 10 pmol kg⁻¹ of angiotensin II in raising MAP and constricting the mesenteric artery (Fig. 2C), however, systemic hyporesponsiveness to the α-adrenoceptor agonist occurred only 2 h post haemorrhage (Fig. 2C). Furthermore, mesenteric constrictor responses were, despite a tendency towards diminution in haemorrhaged rats, similar in both groups during the initial 150 min (Fig. 2C). At 3 h post haemorrhage, however, also mesenteric constrictor responses were found to be significantly blunted (Fig. 3B).

The dose–response curves, which were recorded 3 h after the induction of haemorrhage, revealed that in haemorrhaged rats systemic and mesenteric responsiveness to angiotensin II (3–300 pmol kg⁻¹) and phenylephrine (1–100 nmol kg⁻¹) was blunted over a wide range of test doses with accentuation at the lower doses, while no differences were apparent at the highest doses (Figs. 3A,B). In contrast, only mesenteric constrictor responses, but not systemic pressor responses, to [Arg⁸]vasopressin (1–100 pmol kg⁻¹) were attenuated by haemorrhage (Fig. 3C). Mesenteric hyporeactivity was most pronounced for angiotensin II and least for AVP (Figs. 3A,B,C). A significant correlation between the pressor response to angiotensin II (10 pmol kg⁻¹) and MAP (r=0.56, n=26, P=0.0005; linear regression analysis) was observed at 150 min post haemorrhage whereas phenylephrine (10 nmol kg⁻¹) responses were not significantly correlated to MAP (r=0.29, n=14, not significant). In control rats, the pressor responses to angiotensin II (10 pmol kg⁻¹) and phenylephrine (10 nmol kg⁻¹) did not correlate with MAP (data not shown).

3.2. Effects of cyclooxygenase inhibition

Pretreatment with indomethacin (10 mg kg⁻¹) had no haemodynamic effects in control rats as compared with its vehicle (n=6, data not shown). Indomethacin pretreatment, however, significantly enhanced the initial hypotensive reaction to haemorrhage while the sustained phase of hypotension was not significantly altered (Fig. 2A). In contrast, the initial bradycardia and reduction in mesenteric BF were not modified by indomethacin (n=7, data not shown). The development of angiotensin II hyporeactivity subsequent to haemorrhage was also not prevented by indomethacin pretreatment (Fig. 2B).

3.3. Effects of nitric oxide (NO) synthase inhibition

Administration of L-NAME (10 mg kg⁻¹) 30 min before or shortly (20–40 min) after blood shedding proved lethal in all animals (n=8) within the first 1 h post haemorrhage. L-NAME was, however, well tolerated when given 160 min after haemorrhagic hypotension had been induced.
1-NAME increased MAP and decreased mesenteric VC to a similar extent in hypotensive and control rats (Table 1).

At 20 min after 1-NAME treatment (i.e. 3 h post haemorrhage) responsiveness to angiotensin II or phenylephrine was determined. 1-NAME abolished the difference between hypotensive and control rats in systemic reactivity to either pressor drug over the whole dose–response range (Fig. 4), although it need be mentioned that pressor responses were generally depressed after treatment with 1-NAME. Moreover, 1-NAME restored the mesenteric constrictor responses to phenylephrine to the level seen in control animals (Fig. 4B).

Although mesenteric hyporeactivity to angiotensin II persisted after 1-NAME (Fig. 4A), the difference between control and hypotensive rats was diminished. Since d-NAME (10 mg kg⁻¹) had no appreciable haemodynamic effects and did not alter responsiveness to angiotensin II or phenylephrine neither in control nor haemorrhaged rats (n=4, data not shown), the effect of 1-NAME to improve the vascular reactivity after haemorrhage appears to be specifically related to inhibition of NO synthesis.

### 3.4. Effects of inhibiting ATP-sensitive K⁺ channels

Glibenclamide (20 mg kg⁻¹), given i.p. to control rats, had no significant effect on MAP and mesenteric VC (Table 1), and did not modify vascular responses to angiotensin II (10 pmol kg⁻¹) (n=6, data not shown). In contrast, when injected into rats 150 min after haemorrhage, glibenclamide significantly increased MAP (Table 1) and mesenteric blood flow (n=6, data not shown) without changing mesenteric VC (Table 1). However, glibenclamide was unable to correct the systemic and mesenteric hyporesponsiveness to angiotensin II (10 pmol kg⁻¹) which had developed after haemorrhage (Fig. 5).

### 3.5. Effects of angiotensin AT₁ receptor antagonism

Intravenous injection of telmisartan (1 mg kg⁻¹) immediately caused hypotension and mesenteric vasodilatation as indicated by an increase in mesenteric VC both in control and haemorrhaged rats (Table 1). When given 1 h after haemorrhage had been induced telmisartan (1 mg kg⁻¹) caused death in all animals (n=4) by instantly reducing MAP below 30 mm Hg. At 150 min post haemorrhage, however, all animals (n=8) survived the administration of the AT₁ receptor antagonist. While the mesenteric dilator effect only tended to be attenuated, the hypotensive response to telmisartan was significantly weaker in rats subjected to haemorrhage when compared with control rats (Table 1). Two out of eight shocked animals did not exhibit any haemodynamic response to AT₁ receptor antagonism. The magnitude of the hypoten-
Fig. 4. Effect of N\textsuperscript{G}-nitro-l-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, on the haemorrhage-related vascular hyporesponsiveness to angiotensin II (A) and phenylephrine (B). Responses in mean arterial pressure (MAP) and mesenteric vascular conductance (VC) were recorded 3 h after haemorrhage had been induced by withdrawal of 8 ml of blood from the carotid artery and are expressed relative to the respective pre-injection values. L-NAME (10 mg kg\textsuperscript{-1}) was injected intravenously 20 min before constructing the dose response curves. Numbers of experiments were six in the angiotensin II and seven in the phenylephrine group. Data are shown as means±SEM. \textit{P}<0.05 haemorrhage versus control.

sive reaction to telmisartan was closely related to MAP before the injection of the drug in shocked rats ($r=0.96$, $n=8$, $P=0.0002$; linear regression analysis), but was independent from MAP in control rats ($r=0.47$, $n=8$, not significant).

4. Discussion

In the present study we found that in anaesthetised rats responsiveness to angiotensin II is selectively impaired at an early stage of haemorrhagic hypotension and that this hyporeactivity is in part mediated by nitric oxide, but does not involve prostaglandins or ATP-sensitive K\textsuperscript{+} channels. Our data suggest that hyporeactivity to angiotensin II hampers circulatory adaptation to hypovolaemia and might be an initial event in the development of decompensated shock. It should be noted, though, that the present observations were made in anaesthesia, which is known to alter cardiovascular responsiveness.

Withdrawal of 8 ml of blood resulted in a marked hypotension and transient bradycardia which is a frequent reaction at a blood loss of 20–30% of total blood volume [15]. Mesenteric BF decreased in parallel while mesenteric vasoconstriction did not occur as indicated by unchanged VC. Mesenteric vasoconstriction of an appreciable extent was only observed in animals that died before completion of the experimental protocol and usually began 10–20 min prior to exitus (data not shown). The high survival rate of the animals and the lack of tachycardia and mesenteric vasoconstriction are indices for a moderate and probably reversible nature of the shock model applied here [15,16].

Pressor and mesenteric constrictor responses to angiotensin II were unchanged at 30 min but were blunted at 60 min after the induction of haemorrhage. In contrast, responsiveness to phenylephrine was maintained for at least 2 h, when systemic pressor responses to the \textalpha\textsubscript{-}adrenoceptor agonist decreased. At the end point of our experiments (i.e. 3 h after haemorrhage) systemic pressor responses to angiotensin II and phenylephrine, but not [Arg\textsuperscript{8}]vasopressin, were reduced. Mesenteric hyporesponsiveness to any of these agents was manifest at 3 h post haemorrhage and was most pronounced to angiotensin II while only marginal to [Arg\textsuperscript{8}]vasopressin. To any of these pressor agents hyporeactivity was confined to low and moderate test doses but was absent at the highest doses applied, indicating preserved mechanical coupling of the receptors in smooth muscle. Moreover, the hypotensive reaction to AT\textsubscript{1} receptor antagonism was significantly attenuated in haemorrhaged rats. Telmisartan lowers mean arterial pressure by selectively antagonising angiotensin II on AT\textsubscript{1} receptors [17]. Since the pressor and vasoconstric-
also suggested by a 100% mortality rate after telmisartan administration shortly after bleeding. In contrast, MAP in indomethacin- and vehicle-treated rats did not differ two hour after haemorrhage and telmisartan was well tolerated when given at this time. From these data it might be inferred that angiotensin II plays an important role in the early compensatory phase but consecutively loses its pressor potency, and its supporting role on MAP is taken over by some other vasoactive substances such as vasopressin [21].

Cardiovascular hyporesponsiveness to angiotensin II was, however, not due to the release of vasodilator prostaglandins since its development was not modified by indomethacin pretreatment. Moreover, the lack of effect of indomethacin also suggests that AT_{1} receptor down-regulation or desensitisation by excess angiotensin II was not the underlying mechanism since inhibition of cyclooxygenase is known to prevent the renin/angiotensin response to haemorrhage [18].

The potent vasodilator factor NO has been implicated in the pathogenesis of various shock forms, as induced by bacterial endotoxin, anaphylaxis, thermal injury and also haemorrhage, and has been blamed for causing vasodilatation, hyporesponsiveness to vasoconstrictors and transition to an irreversible stage [22,23]. The role of NO was also confirmed in the current experiments, as inhibition of NO synthesis abolished the difference between haemorrhaged and control rats in the systemic pressor responses to phenylephrine and angiotensin II. It should be, though, also noted that the inhibitor (L-NAME) generally decreased systemic pressor responsiveness, probably by activating counterregulatory reflexes by its hypertensive effect, causing enhanced buffering of pressor responses. Mesenteric constrictor responses, however, were left unaltered by L-NAME in control rats. In contrast, mesenteric responsiveness to phenylephrine, which had become blunted after haemorrhage, was restored to normal by L-NAME, while constrictor responses to angiotensin II remained attenuated even after NO inhibition. These observations confirm earlier reports that hyporesponsiveness to adrenergic agonists after haemorrhage is mediated by NO [8], but also demonstrate that additional, NO-independent factors are involved in hyporeactivity to angiotensin II.

AT_{2} receptors have been proposed to play a role in various cardiovascular disorders, partly via release of vasodilator kinins and NO thereby opposing the action of AT_{1} receptors [24,25]. The involvement of AT_{2} receptors in the current observations, however, appears unlikely, since under normal conditions no or very little AT_{2} receptors are expressed in the adult splanchnic vasculature [26,27] and in a previous study we could not detect a hypotensive or mesenteric dilator effect of angiotensin II after AT_{1} receptor blockade [13]. On the other hand, de novo synthesis and expression of functional AT_{2} receptors is unlikely to occur as quickly as one hour after the

![Fig. 5. Effect of glibenclamide, an inhibitor of ATP sensitive K^+ channels, on the haemorrhage-related vascular hyporesponsiveness to angiotensin II. Responses in mean arterial pressure (MAP) and mesenteric vascular conductance (VC) were recorded (basal) before, (haemorrh) 150 min after haemorrhage had been induced by withdrawal of 8 ml of blood from the carotid artery and (treatment) 30 min after the i.p. injection of vehicle or glibenclamide (20 mg kg^{-1}, i.p.). Responses to angiotensin II (10 pmol kg^{-1}) are expressed relative to the respective pre-injection values. Number of experiments was six in each treatment group. Data are shown as means±SEM.](image-url)
induction of haemorrhage, when hyporesponsiveness to angiotensin II has become manifest.

ATP-sensitive K⁺ channels have recently been shown to be activated in haemorrhagic shock and inhibition of these channels to reverse post-haemorrhagic hypotension [28]. Since openers of ATP-sensitive K⁺ channels inhibit angiotensin II-induced vasoconstriction [29,30], we hypothesised that activation of these channels might underlie the NO-independent hyporeactivity to angiotensin II after haemorrhage. In fact, we observed a pressor effect of glibenclamide, an inhibitor of ATP-sensitive K⁺ channels, in haemorrhaged, but not control, rats. The hyporesponsiveness to angiotensin II, however, was not modified by glibenclamide, suggesting a distinct mechanism for blunted angiotensin II responses.

In summary, the development of systemic pressor hyporesponsiveness and mesenteric arterial hyporeactivity to angiotensin II is an initial event during hypotensive haemorrhage and occurs earlier than with other vasoconstrictors. Hyporesponsiveness to angiotensin II can not be prevented by inhibition of prostaglandin synthesis or reversed by inhibition of ATP-sensitive K⁺ channels. While inhibition of NO formation completely restores responsiveness to adrenoreceptor activation, hyporeactivity to angiotensin II is only partially NO-dependent. We suggest that reduced sensitivity to angiotensin II might be a key event in the shock cascade and, by analogy to inhibition of excess NO formation [31,32], its prevention might have a beneficial effect on clinical outcome.

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References


