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Endogenous angiotensin II and the reflex response to stimulation of cardiopulmonary serotonin $5HT_3$ receptors

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1 Angiotensin (Ang) II modulates cardiovascular baroreflexes; whether or not the peptide influences chemosensitive cardiovascular reflexes is not known. We tested the hypothesis that Ang II modulates the reflex control of sympathetic nerve activity exerted by 5-hydroxytryptamine 3 ($5HT_3$) cardiopulmonary receptors.

2 The $5HT_3$ receptor agonist phenylbiguanide (PBG), infused intravenously for 15 min, elicited a sustained reflex decrease of renal sympathetic nerve activity (RSNA) but only transient (<3 min) changes of arterial blood pressure (BP) and heart rate (HR) in methohexital-anaesthesized rats.

3 Infusion of Ang II at a dose that did not affect baseline BP, HR and RSNA enhanced the PBG-evoked reflex decrease of RSNA ($-54\pm5\%$ in Ang II treated versus $-33\pm6\%$ in control rats after 15 min PBG, $P<0.05$, $n=6$ each) in methohexital-anaesthetized rats.

4 The angiotensin converting enzyme (ACE) inhibitor lisinopril blunted the reflex responses to PBG in anaesthetized as well as conscious animals. The effect of the ACE inhibitor was abolished by concomitant infusion of Ang II.

5 The reflex response to stimulation of cardiopulmonary $5HT_3$ afferents was also impaired by the Ang II type 1 receptor (AT_1) blocker ZD7155 but not by the type 2 (AT_2) blocker PD 123319.

6 Infusion of a volume load to stimulate cardiopulmonary baroreceptors induced a gradual decrease of RSNA which was impaired by exogenous Ang II (RSNA $-26\pm6\%$ in Ang II treated versus $-47\pm6\%$ in control rats after volume load, $P<0.05$, $n=6$ each) but unaffected by ACE inhibition.

7 The reflex control of RSNA by cardiopulmonary $5HT_3$ receptors is enhanced by Ang II *via* AT_1 receptors. Thus, Ang II facilitates a chemosensitive cardiovascular reflex, in contrast to its inhibitory influences on mechanosensitive reflexes.

Keywords: Cardiopulmonary reflex; $5HT_3$ receptors; angiotensin; renal sympathetic nerve; AT_1

Abbreviations: ACE, angiotensin converting enzyme; Ang II, angiotensin II; ANOVA, two-way analysis of variance; AT_1 receptor, type 1 Ang II receptor; AT_2 receptor, type 2 Ang II receptor; BP, arterial blood pressure; HR, heart rate; PBG, phenylbiguanide; RSNA, renal sympathetic nerve activity

Introduction

Angiotensin (Ang) II has been shown to modulate the arterial baroreceptor reflex; the evidence has recently been reviewed (Reid, 1992; Squire & Reid, 1993). The octapeptide blunts baroreflex control of heart rate and sympathetic nerve activity (Guo & Abboud, 1984). The renal sympathetic nerves are important in the control of body fluid (DiBona, 1989). For the control of renal sympathetic nerve activity, low-pressure cardiopulmonary baroreflexes may be even more important than the arterial baroreflexes, as highlighted by the greater involvement of these low-pressure reflexes in states of volume retention (Veelken *et al*., 1994; DiBona & Sawin, 1995). The effect of Ang II on these cardiopulmonary baroreflexes have been less well studied, but the available evidence indicates that Ang II blunts cardiopulmonary mechanoreflexes (Morganti *et al*., 1989; Squire & Reid, 1993).

Chemosensitive cardiopulmonary reflexes can as well influence renal nerve activity. Inhibitory reflex responses (bradycardia and decreased sympathetic nerve activity, often referred to as 'Bezold-Jarisch' reflex), can be elicited by the endogenous autacoid serotonin *via* $5HT_3$ receptors (Veelken *et al*., 1990). In rats, stimulation of cardiopulmonary $5HT_3$ receptors leads to a sustained decrease of renal sympathetic nerve activity, and a transient decrease of heart rate and blood pressure (Veelken *et al*., 1993). The sensitivity of $5HT_3$-sensitive cardiopulmonary reflexes was decreased during the development of hypertension (Veelken *et al*., 1994; Petersen *et al*., 1993). Whether or not the renin-angiotensin system modulates this type of reflex is unknown.

The aim of the present study was to test the hypothesis that circulating Ang II modulates the reflex changes in renal sympathetic nerve activity elicited by 5-HT_3-sensitive cardiopulmonary afferents. These afferent fibres were stimulated by infusing the 5-HT_3 receptor agonist phenylbiguanide (PBG). We either infused exogenous Ang II, or suppressed endogenous Ang II by ACE inhibition and Ang II receptor antagonists.

Methods

Preparation of animals

Male Sprague-Dawley rats, 250–300 g body weight (Charles River Wiga, Sulzfeld, Germany) were kept in a room at $24\pm2^{\circ}C$, 60–80% humidity, and a 23 h light/dark cycle. Rats were fed a normal diet containing 0.2% sodium (C-1320,

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Altromin, Lage, Germany) and were allowed free access to tap water. All procedures performed on animals were done in accordance with the guidelines of the American Physiological Society, and were approved by the local government's ethics committee (Regierung von Mittelfranken, AZ 211-2531.3-19/89). On the day of the experiments, rats were anaesthetized with 30 mg kg^{-1} methohexital sodium intraperitoneally. Two polyethylene catheters were inserted in a femoral vein, and one polyethylene catheter into the femoral artery. The anaesthesia was continued by an intravenous infusion of methohexital sodium (0.6 mg kg^{-1} min^{-1}, dissolved in 20 ml min^{-1} 0.9% NaCl). The catheters were led out at the neck of the rat. For direct recordings of renal sympathetic nerve activity (RSNA), a bipolar electrode (0.2 mm stainless-steel wire, Science Products, Frankfurt, Germany) was placed on the left renal sympathetic nerve by a flank incision as described previously (Veelken *et al*., 1993). The electrode was embedded with a silicone cement (Silgel S4i, Bisico, Bielefeld, Germany), fixed to the trunk muscles by sutures, and led out through the flank incision.

After all sutures were closed, the arterial lines were connected to transducers and a polygraph (Grass Instruments, Quincy, MA, U.S.A.) for recordings of mean arterial blood pressure (BP) and heart rate (HR). One femoral venous line was used for the anaesthesia, and the second venous catheter for all experimental substances (see below). The RSNA signal was amplified (10,000 – 50,000 ×) and filtered (high-pass, 100 Hz; low-pass, 1000 – 3000 Hz) using a Grass P511 bandpass amplifier and a Grass HIP511 high impedance probe (Grass instruments, Quincy, MA, U.S.A.). The amplified and filtered signal was channelled to an oscilloscope HM 512 (Hameg, Frankfurt, Germany) and a Grass AM8 audio amplifier–loudspeaker for visual and auditory evaluation, respectively. The RSNA signal was rectified and integrated using a Grass 7P10 rectifying integrator, and the integrated voltage signals were displayed on the polygraph. The quality of the renal nerve recordings was assessed by a bolus dose of methoxamine hydrochloride to stimulate baroreceptor-mediated decreases of RSNA. At the end of the experiments, 10 mg kg^{-1} of the ganglionic blocking agent trimetapham-camsylate were injected to shut off postsynaptic RSNA. The background activity remaining thereafter was subtracted from the activity recorded throughout the experiment. Changes in RSNA were expressed as per cent changes from the activity during the control period.

Experimental protocols

Experimental protocols were begun 45 min after BP, HR and RSNA had returned to baseline after methoxamine infusion. BP, HR and RSNA were continuously recorded. To elicit the 5HT$_3$-serotonergic sensitive cardiopulmonary reflex, the specific 5HT$_3$ receptor agonist phenylbiguanide (PBG) (Morain *et al*., 1994) was infused for 15 min at a dose of 32 mg min^{-1} (dissolved in saline, infusion volume 8 ml min^{-1}) or 16 mg min^{-1} in one group (see below). We have previously shown that this type of PBG administration leads to transient decreases of BP and HR during the first 3 min of infusion but to sustained decreases of RSNA (Veelken *et al*., 1993). We also showed that this response to PBG was not affected by methohexital sodium anaesthesia (Veelken *et al*., 1993). To test the effects of endogenous and exogenous Ang II on this 5HT$_3$-serotonergic reflex, the following protocols were used.

Effect of exogenous Ang II on the 5HT$_3$-serotonergic cardiopulmonary reflex. Eight rats received saline as a control group. Exogenous Ang II (13 ng min^{-1}) was continuously infused from 15 min before PBG to 10 min after the end of PBG infusion in seven rats. RSNA was not significantly altered by $\Delta 9 \pm 8\%$ after the initiation of the ANG infusion. In these 15 rats, 16 mg min^{-1} PBG was infused which induces an 'intermediate' rather than a maximal decrease of RSNA (Veelken *et al*., 1993). This dose was chosen because it allows the detection of a blunted as well as an accelerated decrease of RSNA during PBG infusion in the presence of Ang II.

Effect of ACE inhibition on the 5HT$_3$-serotonergic cardiopulmonary reflex. Inhibition of ACE was performed to investigate the modulatory influence of endogenous Ang II on the 5HT$_3$-serotonergic reflex. Lisinopril (10 mg kg^{-1}) was given to eight rats as a bolus dose 15 min before start of PBG infusion. Since we expected a blunted RSNA response to PBG infusion during ACE inhibition, we used a high dose of PBG (32 mg min^{-1} (Veelken *et al*., 1993)) to facilitate the detection of an impairment of the PBG-induced RSNA decrease. In these experiments, a bolus dose of Ang I (10 ng) was given before lisinopril and a second time at the end of the experiment to test for the inhibition of ACE. In one further group ($n = 6$), lisinopril was combined with exogenous Ang II infusion as described above to test whether the effects of ACE inhibition were reversible by Ang II. Seven control rats received bolus injections of saline, and infusion of 32 mg min^{-1} PBG. Identical experiments with either saline ($n = 6$) or lisinopril ($n = 7$) were performed in conscious, restrained rats 6 h after the termination of methohexital anaesthesia.

Effect of Ang II receptor blockade on the 5HT$_3$-serotonergic cardiopulmonary reflex. Blockade of Ang II receptors was performed to confirm the results obtained with ACE inhibitors, and to characterize the type of Ang II receptor involved. We employed the specific type 1 Ang II receptor (AT_1) blocker ZD 7155 (120 mg kg^{-1} bolus dose, $n = 6$), and the specific type 2 Ang II receptor (AT_2) blocker PD 123319 (60 mg kg^{-1} bolus dose, $n = 6$). This dose of the type 2 Ang II receptor (AT_2) blocker is considerably higher than the ones used in other experiments with rats to test for acute inhibition of AT_2 receptors (Nishioka *et al*., 1998). Although we could not control for the degree of AT_2 receptor inhibition in our experiments, no effect of PD 123319 on the sensitivity of the serotonergic chemoreflex under investigation would suggest at best a very small role of AT_2 receptors under these circumstances. In the experiments with Ang II receptor blockers, a bolus dose of Ang II (10 ng) was given before administration of the receptor blockers, and a second time at the end of the experiment to test for the blockade of Ang II receptors. Seven control rats received saline. The dose of PBG was 32 mg min^{-1} for all these experiments.

Effect of Ang II and ACE inhibition on volume-sensitive cardiopulmonary reflex responses. For comparison, we also studied the effect of endogenous and exogenous Ang II on the RSNA response to volume-sensitive cardiopulmonary reflexes. Volume expansion was performed by infusing 0.9% NaCl (5% of body weight) over 15 min. We have previously shown that this protocol for volume expansion does not change BP and stimulates only cardiopulmonary afferents but not the arterial baroreceptor reflex (Veelken *et al*., 1993). Angiotensin II (13 ng min^{-1}) was infused continuously beginning from 15 min before start of volume expansion in six rats, lisinopril was given as a bolus dose (10 mg kg^{-1}) in seven further rats, and seven control rats received no treatment.

Angiotensin II measurements in anaesthetized rats. We obtained blood samples (1000 μl) from six rats instrumented with catheters that were anaesthetized and had undergone a sham flank incision. Blood was collected from arterial catheters in tubes containing 50 μl of a mixture of ortho-phenanthroline (26 mmol l^{-1}) and Na-EDTA (125 mmol l^{-1}), chilled, immediately snap frozen on dry ice, and kept at $-26°$. Ang II was measured by radio-immunoassay as described previously [7,241]. The cross reactivity of the Ang II antibody (Celine III) was 1% with Ang I and 100% with the Ang-(2-8) heptapeptide (Ang III), the Ang-(3-8) hexapeptide (Ang IV), and the Ang-(4-8) pentapeptide, respectively. All samples were estimated in duplicate.

Drugs

Angiotensin I and II were purchased from Bachem (Heidelberg, Germany). Lisinopril was generously supplied by Merck, Sharp & Dome (Munich, Germany). The AT$_1$ blocker ZD 7155 was generously supplied by Zeneca (Planckstadt, Germany). ZD 7155 is a recently synthesized compound (Thomas *et al.*, 1992) that specifically blocks AT$_1$ but not AT$_2$ receptors (Oldham *et al.*, 1993; Abdelrahman *et al.*, 1993) and appears to be more potent and longer acting than the prototype AT$_1$ blocker losartan (Junggren *et al.*, 1996). The AT$_2$ blocker PD 123319 (Timmermans *et al.*, 1993) was a generous gift by Dr Th. Unger, Department of Pharmacology, University of Kiel, Kiel, Germany. Trimetapham camsylate was supplied by Hofmann-LaRoche, (Basel, Switzerland). Methohexital sodium was purchased from Eli Lilly (Bad Homburg, Germany). All other substances were obtained from Sigma Chemicals (Munich, Germany). With the exception of the predissolved trimetapham camsylate, all drugs were dissolved in 0.9% NaCl.

Statistical analysis

Two-way analysis of Variance (ANOVA) was used for comparisons between groups. Newman-Keuls tests was employed for *post hoc* testing. A P value <0.05 was considered significant. Statistical analysis was carried out using CSS Statistica software package (StatSoft, Tulsa, OK, U.S.A.). Results are expressed as means $\pm$ s.e.mean.

Results

Effect of exogenous Ang II on the 5HT$_3$-serotonergic cardiopulmonary reflex

Infusion of the specific 5HT$_3$-serotonin-receptor agonist PBG induced transient decreases of BP and HR but a sustained and dose-dependent decrease of RSNA (Figures 1 and 2). Continuous infusion of exogenous Ang II did not alter baseline HR (380 $\pm$ 14 versus 390 $\pm$ 22 beats min^{-1} in controls) and BP (98 $\pm$ 8 versus 92 $\pm$ 6 mmHg in controls) before PBG. Angiotensin II significantly enhanced the decrease of RSNA in response to stimulation of the 5HT$_3$-cardiopulmonary reflex (Figure 1), since 6 min after the beginning of the PBG infusion RSNA decreased to higher degree in ANG II treated animals as compared to the animals receiving a saline bolus instead. In contrast, the transient BP and HR responses to PBG were unaffected by Ang II.

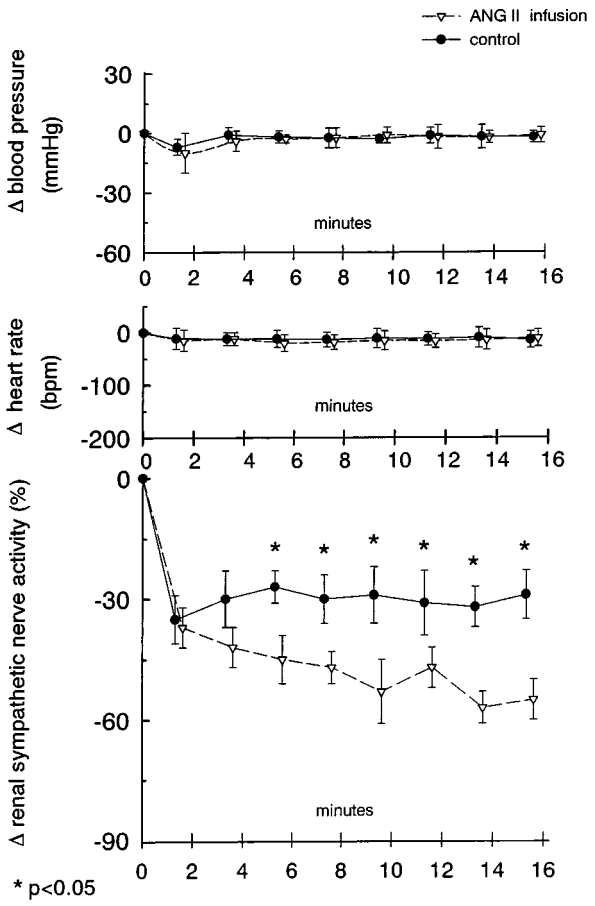

Figure 1 Effect of a 15 min infusion of the 5HT$_3$ receptor agonist phenylbiguanide (16 μg min^{-1}) on blood pressure (mmHg), heart rate beats min^{-1} and renal sympathetic nerve activity (% of baseline) in methohexital-anaesthetized rats. $\triangledown$, animals infused with Ang II (13 ng min^{-1}); ●, control animals (saline infusion). *Significant ($P<0.05$) differences between Ang II infusion and control. All panels indicate changes from baseline values. The baseline values for all parameters were not affected by Ang II treatment.

Effect of ACE inhibition on the 5HT$_3$-serotonergic cardiopulmonary reflex

Lisinopril did not affect baseline BP (95 $\pm$ 6 versus 97 $\pm$ 5 mmHg in controls) and HR (350 $\pm$ 18 versus 340 $\pm$ 12 beats min^{-1} in controls). Some but not all animals showed a transient increase (20–50% of baseline) of RSNA; in all animals, RSNA returned to control values within 5 min or less and remained so until PBG infusion. The effect of ACE inhibition on the response to stimulation of the 5HT$_3$-serotonergic cardiopulmonary reflex by infusion of 32 mg min^{-1} PBG is shown in Figure 2. The transient HR and BP changes were unaffected by lisinopril. The initial phase of the PBG-induced RSNA decrease was also unchanged by lisinopril (Figure 2). However, the sustained phase of the RSNA response to cardiopulmonary 5HT$_3$-receptor stimulation was impaired by ACE inhibition (Figure 2). Infusion of Ang II during ACE inhibition fully prevented the effects of lisinopril; the RSNA response to PBG in lisinopril + Ang II treated rats was not different from the response in control animals (Figure 2). Lisinopril reduced the pressor response to 10 ng Ang I by 66.7% (from 30 $\pm$ 6 to 10 $\pm$ 6 mmHg, $P<0.01$). In conscious rats, lisinopril did not alter baseline BP (109 $\pm$ 12 versus 111 $\pm$ 12 mmHg in controls), HR (420 $\pm$ 32 versus 410 $\pm$ 28

beats min^{-1} in controls), or RSNA. The RSNA response to PBG in conscious rats was impaired by lisinopril in a manner identical to that described in anaesthetized rats (Figure 3).

Effect of Ang II receptor blockade on the 5HT$_3$-serotonergic cardiopulmonary reflex

The Ang II receptor blockers did not affect baseline BP, HR or RSNA. The AT$_1$ blocker ZD 7155 blunted the RSNA response to PBG infusion (Figure 4) in a similar fashion as did the ACE inhibitors. In contrast, the type 2 receptor specific compound PD 123319 did not affect the reflex response to PBG (Figure 4). ZD 7155 reduced the pressor response to exogenous Ang II by 95% (from 60±6 mmHg before ZD 7155 to 3±4 mmHg after ZD 7155, $P<0.05$). Preliminary experiments using a lower dose of ZD 7155 that blocked exogenous Ang II by only 66–75% did not show blunting of the decrease of RSNA in response to PBG ($n=3$, data not shown). PD 123319 did not alter the increase of MAP after Ang II injection (55±5 mmHg before versus 47±4 mmHg after PD 123319, $P>0.1$).

Effect of Ang II and ACE inhibition on volume-sensitive cardiopulmonary reflex

Volume expansion by administration of saline (5% of body weight) over 15 min decreased RSNA, did not change BP and tended to decrease HR (Figure 5). Exogenous Ang II blunted the RSNA response to stimulation of cardiopulmonary mechanosensitive reflex (Figure 5). ACE inhibition with lisinopril did not affect the RSNA response to volume expansion (Figure 5).

Angiotensin II measurements in the plasma of anaesthetized rats

The concentration of Ang II in the plasma of anaesthetized rats was 107±11 fmol ml^{-1}. These values are higher than those measured in conscious rats in our laboratory (Mai *et al*., 1995). However, comparable values are measured in pathophysiological situations like water deprivation, sodium depletion, or renovascular hypertension (Mann *et al*., 1980; Mai *et al*., 1995).

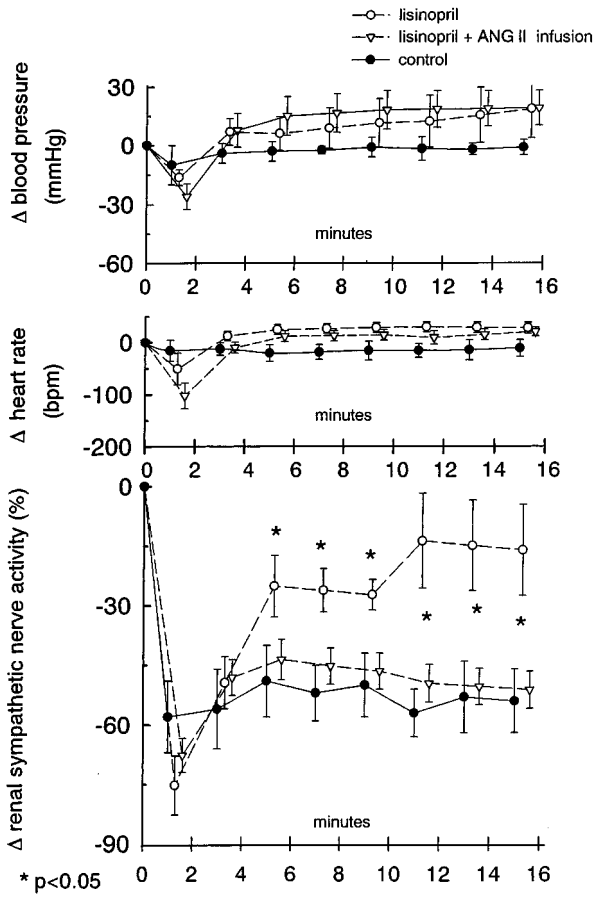

Figure 2 Effect of a 15 min infusion of the 5HT$_3$ receptor agonist phenylbiguanide (32 μg min^{-1}) on blood pressure (mmHg), heart rate beats min^{-1} and renal sympathetic nerve activity (% of baseline) in methohexital-anaesthetized rats. ○, animals pretreated with the ACE inhibitor lisinopril; ▽, animals pretreated with lisinopril and infused with Ang II; ●, control animals (saline infusion). *Significant ($P<0.05$) differences between lisinopril and control. All panels indicate changes from baseline values. The baseline values for all parameters were not affected by lisinopril or Ang II treatment.

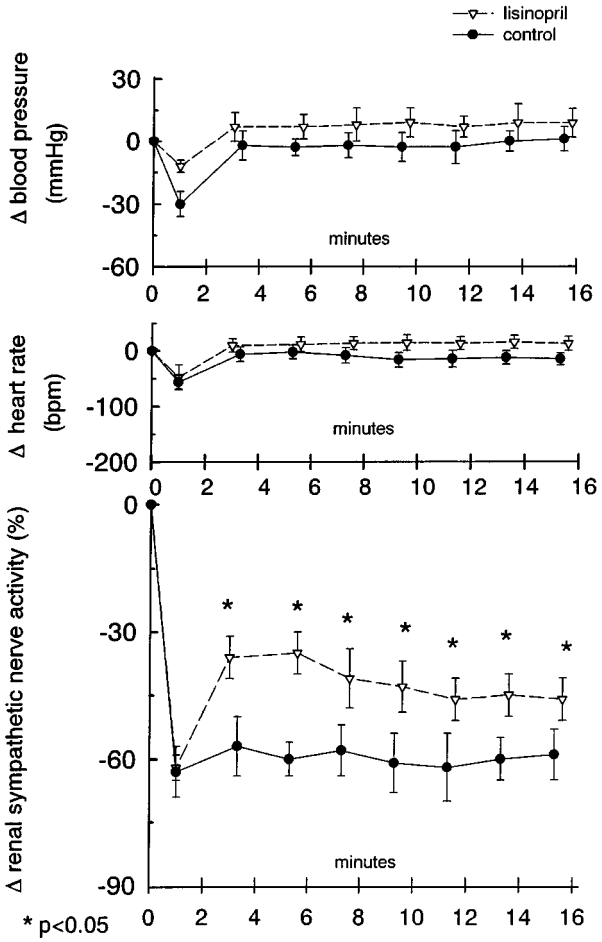

Figure 3 Effect of a 15 min infusion of the 5HT$_3$ receptor agonist phenylbiguanide (32 μg min^{-1}) on blood pressure (mmHg), heart rate beats min^{-1} and renal sympathetic nerve activity (% of baseline) in conscious rats. ▽, animals pretreated with the ACE inhibitor lisinopril; ●, control animals (saline infusion). *Significant ($P<0.05$) differences between treatment and control. All panels indicate changes from baseline values. The baseline values for all parameters were not affected by treatment.

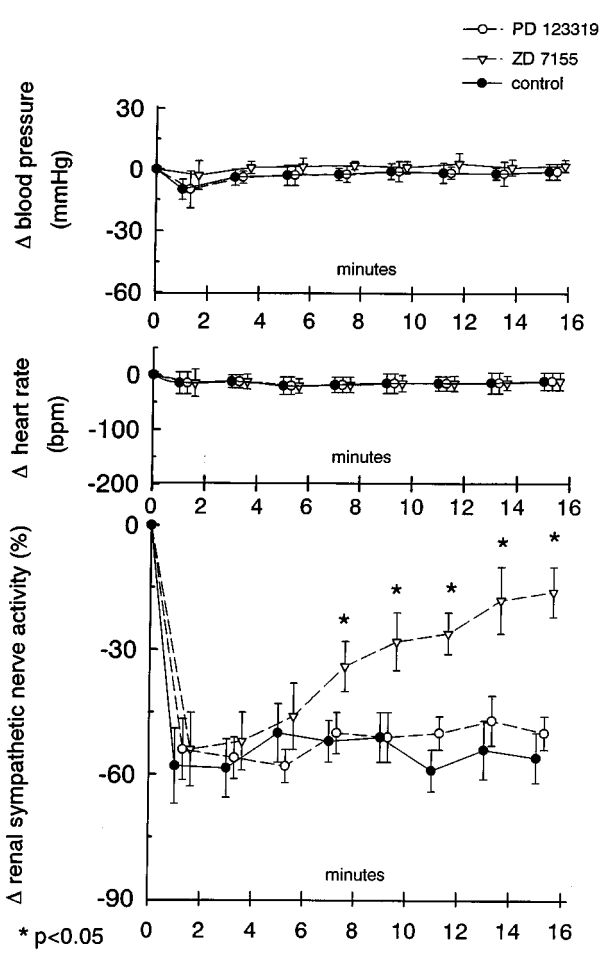

Figure 4 Effect of a 15 min infusion of the 5HT$_3$ receptor agonist phenylbiguanide (32 μg min^{-1}) on blood pressure (mmHg), heart rate beats min^{-1} and renal sympathetic nerve activity (% of baseline) in methohexital-anaesthetized rats. ▽, animals pretreated with the AT$_1$ receptor blocker ZD 7155; ○, animals pretreated with the AT$_2$ receptor blocker PD 123319; ●, control animals (saline infusion). *Significant ($P<0.05$) differences between ZD 7155 and control. All panels indicate changes from baseline values. The baseline values for all parameters were not affected by the receptor blockers.

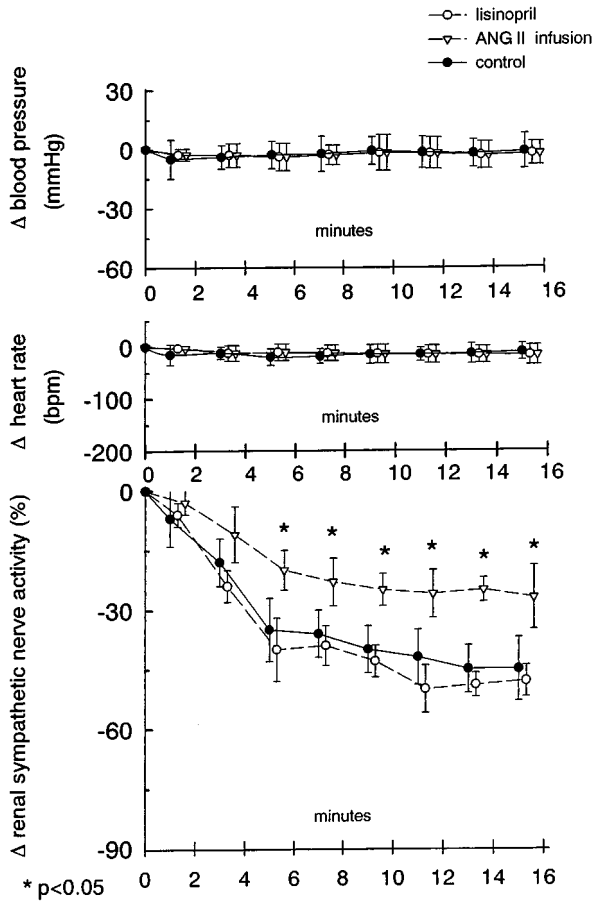

Figure 5 Effect of isotonic volume expansion (saline, 5% of body weight infused over 15 min) on blood pressure (mmHg), heart rate beats min^{-1} and renal sympathetic nerve activity (% of baseline) in methohexital-anaesthetized rats. ○, animals pretreated with the ACE inhibitor lisinopril; ▽, animals infused with Ang II; ●, control animals (saline infusion). *Significant ($P<0.05$) differences between Ang II infusion and control. All panels indicate changes from baseline values. The baseline values for all parameters were not affected by lisinopril or Ang II treatment.

Discussion

Interaction of Ang II with 5-HT$_3$- and mechano-sensitive reflexes

Our data demonstrate for the first time that endogenous Ang II facilitates the reflex control of sympathetic nerve activity by a 5-HT$_3$ sensitive reflex. Infusion of the specific 5-HT$_3$ receptor agonist phenylbiguanide (Veelken *et al.*, 1993; Morain *et al.*, 1994) elicited a sustained decrease of renal nerve activity, which was enhanced by infusion of subpressor doses of Ang II. The ACE inhibitor lisinopril, and the AT1 blocker ZD 7155, impaired the reflex decrease of renal nerve activity. The effects of lisinopril were demonstrated in conscious and anaesthetized rats, and were reversible by concomitant Ang II infusion. Thus, endogenous Ang II facilitated the reflex response of renal sympathetic nerve activity to stimulation of cardiopulmonary 5-HT$_3$ receptors.

The facilitatory action of Ang II on a chemosensitive reflex contrasts sharply with the inhibitory influence of the octapeptide on mechanosensitive reflexes. Evidence for the widely accepted notion that Ang II blunts arterial (high-pressure) and cardiopulmonary (low-pressure) baroreflexes has been reviewed previously (Reid, 1992; Squire & Reid, 1993). The effect of Ang II on low-pressure cardiopulmonary baroreflexes have been less well studied, but the available evidence indicates that Ang II blunts cardiopulmonary mechanoreflexes (Morganti *et al.*, 1989; Squire & Reid, 1993). Our data confirm this notion, since Ang II markedly inhibited the renal sympathoinhibition induced by volume expansion. The lack of effect of ACE inhibition on the response to volume expansion in our study does not necessarily indicate that endogenous Ang II does not modulate the reflex. Rather, the 15 min infusion of saline that we used for volume expansion may by itself suppress the renin-ANG system, thus obscuring any effect of Ang II withdrawal. We choose this infusion protocol for volume expansion because it elicits a reflex response highly similar to the 5-HT$_3$ receptor stimulation and does not involve the arterial baroreceptor reflex (Veelken *et al.*, 1993).

We are aware of only one previous study that investigated the effects of Ang II on a cardiopulmonary chemoreflex: Panzenbeck *et al.* (1988) reported that the cardiodepressor response to veratridine was enhanced by captopril, as opposed to the blunting of the 5-HT$_3$ reflex by ACE inhibition observed in the present study. Besides species differences between dogs

and rats, several other factors may account for this apparent discrepancy. First, Panzenbeck *et al.* (1988) described that the effects of captopril were due to prostaglandin formation, which may be mediated by non-Ang-dependent actions of captopril. In contrast, the present study describes effects that were unequivocally mediated by Ang II. Second, the veratridine used in the previous study is an exogenous poison, and the receptors involved are not well defined. We used PBG to selectively stimulate 5-HT$_3$ receptors (Veelken *et al.*, 1990; Morain *et al.*, 1994) which bind the endogenous autacoid serotonin (Maricq *et al.*, 1991). Finally, we investigated a phase of the 5-HT$_3$ sensitive reflex characterized by sustained renal sympathoinhibition, whereas Panzenbeck *et al.* (1988) studied the initial cardiodepressor response to veratridine. We conclude that our study does not contradict the previous report by Panzenbeck *et al.* (1988) but rather addresses a different reflex response.

Mechanism of the effect of Ang II on the 5-HT$_3$ sensitive reflex

The plasma concentrations of Ang II which are able to enhance the 5HT$_3$ reflex are within the range observed in pathophysiological conditions (Mann *et al.*, 1980; Mai *et al.*, 1995). Blunting of the 5-HT$_3$ sensitive reflex by ACE inhibition was due to blockade of Ang II formation and not to other effects of the drug: Infusion of exogenous Ang II fully reversed, but an Ang II receptor blocker mimicked the effect of ACE inhibition. The effect of Ang II was mediated by AT1 receptors: ZD 7155 blunted the reflex response whereas the AT2 blocker PD 123319 did not. The experiments with exogenous Ang II demonstrate that circulating Ang II facilitates the 5-HT$_3$ cardiopulmonary reflex. However, a high degree of Ang II receptor blockade was necessary to inhibit the facilitatory action of Ang II. ZD 7155 was chosen because the drug inhibited the pressor response to Ang II by $>90\%$ for several hours but did not significantly change the baseline RSNA and hemodynamics, as opposed to other AT1 blockers screened (data not shown).

The facilitation of the 5-HT$_3$ sensitive reflex by endogenous, circulating Ang II is most likely a direct effect on this reflex arc, as opposed to a general effect of the peptide on sympathetic outflow. Since the 5-HT$_3$ mediated sympathoinhibition is not markedly affected by the arterial baroreceptor reflex (Veelken *et al.*, 1993; Verberne & Guyenet, 1992) or volume loading (Veelken *et al.*, 1994; Petersen *et al.*, 1993), any Ang II mediated influence of these mechanoreflexes on the 5-HT$_3$ reflex is less likely, but cannot be fully excluded. However, tonic influences of circulating Ang II on sympathetic outflow would be expected to be stimulatory (Fink *et al.*, 1987) and should blunt rather than facilitate the sympathoinhibition elicited by cardiopulmonary 5-HT$_3$ receptor stimulation. Thus, the afferent limb of the reflex arch is the most likely site of facilitation by ANG II.

Sympathoinhibition by PBG is dependent on peripheral fibres associated with the vagus (Veelken *et al.*, 1993). Vagal 5-HT$_3$ sensitive fibres that mediate renal sympathoinhibition are present both in the heart (Veelken *et al.*, 1990) and lung (Veelken *et al.*, 1997; Lee & Morton, 1995; Leanos *et al.*, 1995) of rats. Ang II might interact locally with these afferent fibres, or with the neuronal cell bodies of those fibres in the nodose ganglion (Bacal & Kunze, 1994; Allen *et al.*, 1988). The local effect of Ang II on arterial baroreflex afferents in the aortic arch is secondary to the vasoconstriction elicited by Ang II (Yang & Andresen, 1990); such a mechanism is not likely to explain the facilitation of the 5-HT$_3$ reflex by Ang II. A more likely site of interaction between Ang II and the 5-HT$_3$ reflex is the nucleus tractus solitarius (NTS), which is influenced by the effects of circulating Ang II *via* afferent connections of the area postrema, that has a high density of Ang II receptors (Lewis *et al.*, 1986; Healy *et al.*, 1989). The majority of Ang II receptors in the NTS is associated with cardiopulmonary vagal rather than arterial baroreceptor afferents (Healy *et al.*, 1989; Lewis *et al.*, 1986). Andresen & Kunze (1994) have found it difficult to reconcile the excitation of NTS neurons by Ang II with the blunting effect of the peptide on mechanoreflexes. A subpopulation of NTS neurons are stimulated only by cardiopulmonary 5-HT$_3$ receptors but not by cardiopulmonary mechanoreceptors (Hines *et al.*, 1994). Interestingly, the facilitation of the 5-HT$_3$ reflex described in our study fits very well to the cellular effects of Ang II in NTS neurons (Andresen & Kunze, 1994), although in our experiments the effect of ANG II on the NTS neurons could have been more indirect by stimulating neural afferents connecting area postrema and the NTS (see above). An interaction of Ang II along the further medullary pathway of the 5-HT$_3$ reflex (Verberne & Guyenet, 1992) appears less likely because the sites involved are not accessible to circulating Ang II, although projections from the area postrema to these pathways (Fink *et al.*, 1987), or to the NTS (Lawler *et al.*, 1989), might play a role (Fink *et al.*, 1987). Additional studies will be necessary to define the precise site of interaction between circulating Ang II and the 5-HT$_3$ reflex. Furthermore, it might be useful in the future to conduct experiments in baroreceptor denervated animals in order to completely isolate the chemoreceptive reflex.

Conclusion

Potential physiologic significance of the facilitation of the 5-HT$_3$ reflex by endogenous Ang II. Changes in the rate of formation of endogenous Ang II could modulate renal sympathoinhibition elicited by the 5-HT$_3$ reflex. In agreement with our present results, we have previously shown that the renal nerve response to stimulation of cardiopulmonary 5-HT$_3$ receptors is blunted in a model of hypertension with suppressed renin-Ang system, DOCA-salt hypertensive rats (Veelken *et al.*, 1994). Chronic NaCl loading alone does not affect the reflex (Veelken *et al.*, 1994; Petersen *et al.*, 1993). Altered renal nerve activity exerts powerful effects on NaCl excretion and body volume homeostasis (DiBona, 1989).

Unfortunately, any further speculation on the physiological significance of the interaction is hampered by the fact that a clear picture of the role of the 5-HT$_3$ reflex itself has not yet emerged (Veelken *et al.*, 1993; 1994). The sustained renal sympathoinhibition investigated in the present study has been described though (Veelken *et al.*, 1993). However, we do not know whether or not any information gathered on the role of the short-term cardiodepressor action of the 5-HT$_3$ reflex (Widdop *et al.*, 1990; Robertson *et al.*, 1985) applies also to the sustained sympathoinhibition elicited by the same receptors. Our observation that neither Ang II nor DOCA-salt hypertension (Veelken *et al.*, 1994) affect the short-term response to PBG suggests that this may not be the case. For now, we are left with the speculation that the mechanism described here may play a role in situations like coronary artery disease, when cardiac serotonin is increased (van den Berg *et al.*, 1989; Robertson *et al.*, 1985). Under these conditions, endogenous Ang II may facilitate renal sympathoinhibition by the 5-HT$_3$ reflex, thus counteracting the volume retention by Ang II, or inhibiting further renin release (DiBona, 1989). Future studies will be necessary to address these hypotheses.

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