Angiotensin II receptor antagonists and sympathetic neurotransmission
Balt, J.C.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 10

Stimulating influence of angiotensin II on sympathetic nerve traffic in the human forearm

Submitted for publication
Chapter 10

Introduction

Numerous studies, mostly in animals, have shown that angiotensin II (Ang II) enhances the influence of the sympathetic nervous system (SNS) at various levels; Ang II enhances sympathetic neuronal activity at the level of the central nervous system, increases ganglionic transmission, facilitates NA release from synaptic nerve terminals, blocks NA-uptake, enhances NA-synthesis and enhances the post-synaptic effects of noradrenaline [1,2].

The facilitating effect Ang II on sympathetic nerve traffic may be of (patho)physiological relevance in diseases in which both the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS) play an (etiologically) important role, such as in hypertension and heart failure. Conversely, part of the clinically beneficial effects of ACE-inhibitors and AT₁-receptor antagonists may therefore be attributed to sympatho-inhibition. ACE-inhibitor therapy and AT₁-blockade have been shown to lower plasma catecholamines in some [3-6] but not all [7-9] studies. Grassi et al. demonstrated that, in patients with congestive heart failure, chronic ACE-inhibition treatment decreases central sympathetic outflow as recorded by microneurography [10]. Using the same technique, these authors found no such an interaction in patients with hypertension [11], in which both the RAS and the SNS are less markedly stimulated than in heart failure.

In the human forearm, Clemson et al. found that intra-arterial Ang II caused an increase of NA spillover [12]. Other authors, however, could not confirm these results [13,14]. In healthy volunteers, Ang II was shown to augment vasoconstriction as elicited by lower body negative pressure (LBNP) [15]. Vice versa, chronic ACE-inhibition had already been demonstrated to diminish the decrements in forearm blood flow caused by LBNP [9].

In the present study, we attempted to develop a model in which the interaction of Ang II with sympathetic neurotransmission could be studied in humans at the peripheral level without stimulating baroreflex afferents and without haemodynamic changes such as they occur during the application of LBNP.

Tyramine provokes the release of noradrenaline from sympathetic nerve terminals, which causes vasoconstriction and thus a decrease in forearm blood flow, mediated by α-adrenoceptor stimulation [16]. Accordingly, we studied the effects of a subpressor dose of Ang II on tyramine-induced responses in forearm blood flow.
Effects of angiotensin II on tyramine-induced vasoconstriction in the human forearm

Methods

Subjects

Ten male healthy non-smoking volunteers (age 28 ± 1 years) participated in the present study. All subjects were normotensive and not obese. A short medical history, physical examination and routine laboratory tests were performed. If no abnormalities were found, subjects were included into the study. Subjects were instructed to refrain from liquorice, drinking alcohol or caffeine-containing beverages at least 12 hours prior to the experiment. Informed consent was obtained and the study protocol was approved by the Medical Ethics Committee of the Academic Medical Center at Amsterdam.

Experimental Conditions

Each experiment was performed in a quiet room at a temperature of 22-23°C, with the subject in the supine position. A one-lead electrocardiogram (ECG) was recorded continuously. After local anesthesia with lidocaine 1%, the brachial artery was cannulated using a XRO Arterial Catheter-Seldinger Technique (Laboratoire Plastimed, Saint-Leu-La-Forêt Cedex, France). The cannula was connected to a Baxter pressure transducer, fixed at heart level. Drugs were infused into the brachial artery using a B. Braun Secura FT Perfusor (B. Braun, Germany). Both arms were instrumented with mercury-in-silastic strain gauges, which were connected to a Hokanson EC-2 plethysmograph (Hokanson Inc., Issaquah, WA, USA) for the measurement of forearm blood flow (FBF). Heart rate (HR) from ECG, intra-arterial blood pressure, and left and right FBF were recorded on a polygraph (Wekagraph Wk-450-R, Depex bv, De Bilt, The Netherlands). Data were analog-to-digital converted (Model DT 2801, Data Translation Inc., Marlborough, MA, USA) and stored on a personal computer. Both upper arms were mounted with pressure cuffs, connected to a Hokanson E-10 rapid cuff inflator. For the measurement of FBF, R-wave triggered cuff inflation (at 40 mmHg) for venous occlusion plethysmography was controlled by the personal computer. FBF was measured 4 times per minute and the mean arterial blood pressure (MAP) was derived from the concomitantly recorded arterial blood pressure. During each infusion experiment the hands were continuously excluded from the circulation by inflating small wrist cuffs to a pressure at least 40 mmHg above systolic blood pressure.

The infusion experiments were started one hour after cannulation of the brachial artery.
Chapter 10

Study protocol

The protocol of the study is summarised in Fig. 1. The experiment started with an infusion of SNP (10 ng/kg/min). Five minutes later, either vehicle (saline 0.9% at 0.3 ml/min) or Ang II (0.1 ng/kg/min at 0.3 ml/min) was infused (see below). Another five minutes later tyramine infusion was started. Tyramine was infused in the dosages 0.25, 0.5, 1.25 and 2.5 μg/kg/min, respectively. Each dose was infused for 5 minutes. The standard infusion rate for vehicle, Ang II, SNP and tyramine was 0.3 ml/min. Tyramine 0.5 and 2.5 μg/kg/min were infused at an infusion-rate of 0.6 ml/kg/min. Before starting any of the infusions, baseline FBF was measured. Two dose-response curves for tyramine effects were constructed in each subject; in five subjects, the first DRC was performed with concomitant infusion of vehicle and the second with simultaneous infusion of Ang II. In another five subjects, this was sequence was reversed; the first DRC to tyramine was constructed during Ang II-infusion, and the second during infusion of vehicle. The interval between the two DRC for the effects of tyramine was one hour.

Drugs

Ang II and tyramine (both from Clinalfa AG, Läuflingen, Switzerland) and sodium-nitroprusside (BUFA bv, Uitgeest, The Netherlands) were dissolved in NaCl 0.9%.

Statistical analysis

Results are presented as means ± SEM. Effects of NaCl, SNP and Ang II on FBF were tested using Student's t-test, or, in case of multiple means, using one-way ANOVA and Dunnett's post test. The effect of Ang II on tyramine induced changes in FBF was assessed using repeated measures ANOVA. A p-value of less than 0.05 was considered to indicate statistically significant differences.
Effects of angiotensin II on tyramine-induced vasoconstriction in the human forearm

**Fig 1.** Schematic representation of the experimental protocol. After 3 minutes of baseline recording, a continuous infusion of sodium nitroprusside (10 ng/kg/min) was started. Five minutes later, a continuous infusion of Ang II 0.1 ng/kg/min or vehicle (NaCl 0.9%) was started. Another five minutes later, a stepwise infusion of tyramine (0.25, 0.5, 1.25 and 2.5 μg/kg/min) was applied. The DRC for the constrictor effects of tyramine was constructed twice in each subject, with an interval of one hour between the DRC’s. In five subjects, the first DRC of tyramine was performed during an infusion of vehicle, the second during infusion of Ang II. In another five subjects, this order was reversed.

**Results**

Baseline forearm blood flow (FBF), expressed as ml/100 ml forearm-volume/minute amounted to 2.8 ± 0.2. Sodium nitroprusside (10 ng/kg/min) increased the FBF to 6.8 ± 0.3 ml/100ml/min (P<0.05 compared to baseline).

After infusion of NaCl 0.9 % (vehicle) or angiotensin II (0.1 ng/kg/min), FBF-values were 6.9 ± 0.5 and 6.8 ± 0.6 ml/100 ml/min, respectively (NS compared with after SNP)

Tyramine (0.25 – 2.5 ng/kg/min), infused together with vehicle (control) caused a dose-dependent decrease in forearm blood flow (fig. 2). Concomitant infusion of Ang II significantly enhanced the responses to tyramine. (p<0.05).

Heart rate, MAP and contralateral FBF remained unchanged by SNP, Ang II or tyramine (table 1). Accordingly, non of the compounds caused any systemic haemodynamic effects.
Table 1. Effects of drugs (sodium nitroprusside, SNP; angiotensin II, Ang II) and the highest dose of tyramine (2.5 μg/kg/min) on heart rate (HR), mean arterial blood pressure (MAP) and forearm blood flow in the non-infused arm (FBFn).

<table>
<thead>
<tr>
<th></th>
<th>baseline</th>
<th>SNP (10 ng/kg/min)</th>
<th>Ang II (0.1 ng/kg/min)</th>
<th>Tyramine (2.5 μg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>82.6 ± 1.7</td>
<td>84.2 ± 2.8</td>
<td>84.2 ± 1.9</td>
<td>85.6 ± 1.8</td>
</tr>
<tr>
<td>HR (BPM)</td>
<td>61.6 ± 1.8</td>
<td>63.0 ± 1.8</td>
<td>62.5 ± 2.7</td>
<td>64.0 ± 1.9</td>
</tr>
<tr>
<td>FBFn</td>
<td>2.9 ± 0.3</td>
<td>2.9 ± 0.6</td>
<td>2.9 ± 0.2</td>
<td>3.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. None of the values were significantly different from baseline (P>0.05, ANOVA and Dunnett’s post-test).

Fig 2. Dose-response curves for the constrictor effects of tyramine administered during co-infusion with vehicle (NaCl 0.9 %) or angiotensin II (0.1 ng/kg/min). Doses of tyramine (expressed as μg/kg/min) are shown on the abscissa. Change in forearm blood flow (FBF) (expressed as % change from baseline [after predilation with SNP]) are shown on the ordinate. Tyramine caused a dose-dependent reduction of forearm blood flow, resulting from vasoconstriction. This effect was significantly enhanced by Ang II, in a dose that did not change FBF itself. * p<0.05, repeated measures ANOVA.
Discussion

In the present study, we demonstrated a facilitatory action of Ang II on tyramine-induced vasoconstriction in the human forearm.

Angiotensin II enhances electrically stimulated NA efflux as well as vasoconstriction in a variety of (animal) models or isolated tissues [1,2]. However, an in vivo setup in humans does not allow such an approach. We chose tyramine, as a pharmacological alternative to electrical stimulation of sympathetic neurons, to avoid great haemodynamic changes such as they may occur during LBNP. We considered that, using tyramine, a peripheral interaction could be studied. Tyramine causes the release of noradrenaline from sympathetic nerve terminals, which via α-adrenoceptors on vascular smooth muscle cells causes vasoconstriction and thus a decrease in forearm blood flow, which is dose-dependent and highly reproducible [16].

The doses of tyramine used in the present study had been shown not to cause any systemic haemodynamic effects [16,17]. In conscious rats as well as in anaesthetized dogs, subpressor infusions of Ang II potentiated the effects of tyramine [18,19]. In normotensive and in hypertensive subjects, systemic subpressor doses of Ang II were reported to enhance the effects of tyramine on mean arterial pressure [20]. However, this effect could not be confirmed by Seidelin et al., who observed no effect of Ang II on responses to tyramine infusion in man [17].

We used a subpressor dose of Ang II that was established in previous experiments with this model [21]. If we had used higher doses, it would not have been possible to distinguish between Ang II-induced vasoconstriction on the one hand, and tyramine-induced vasoconstriction on the other hand. In addition, these findings confirm those of others [15,18,22,23] indicating that facilitation by Ang II occurs in lower concentrations than vasoconstriction caused by this peptide.

The degree of vasoconstriction caused by the two lower doses of tyramine (12 ± 5 and 40 ± 6, expressed as % from baseline) that was susceptible to facilitation was comparable to effects observed by Seidelin et al., who applied LBNP [15]. The same holds true for the degree of facilitation (30 – 115 % increase of vasoconstrictor responses in the presence of Ang II). However, as predicted, in the present investigation, the tyramine-induced vasoconstriction was not accompanied by any systemic effects (table 1).
The design of the present study did not allow to distinguish between a prejunctional or a postjunctional mechanism by which Ang II could act. In the literature, evidence can be found for both a prejunctional as well as a postjunctional mechanism by which Ang II exerts its facilitatory effects [1]. In the pithed rat as well as in the isolated rat mesenteric artery, we found that Ang II enhances sympathetic nerve traffic through pre-junctionally located AT₁-receptors only [22,24]. In the human forearm, both prejunctional [12,15] and postjunctional mechanisms [9,25] have been suggested to play a role. Enhancement of transmitter release by Ang II probably involves a PKC-dependent mechanism [26]. Since tyramine causes the increase of NA in the synaptic cleft via a PKC-independent mechanism, an inhibition of NA-reuptake by the nerve terminal, or postjunctional facilitation are the two possible mechanisms by which Ang II may have caused its facilitatory action in the present study.

In conclusion, these findings demonstrate that in healthy volunteers, a subpressor dose of Ang II enhances tyramine-induced forearm blood flow responses. Accordingly, the present study confirms a facilitatory role of Ang II on sympathetic nerve traffic at the peripheral neuronal level in humans.
Effects of angiotensin II on tyramine-induced vasoconstriction in the human forearm

References


Chapter 10


