Angiotensin II receptor antagonists and sympathetic neurotransmission
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CHAPTER 4

Inhibition of both facilitation of sympathetic neurotransmission and Ang II-induced pressor effects in the pithed rat; A comparison between valsartan, candesartan, eprosartan and embusartan

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Introduction

Numerous studies have demonstrated that angiotensin II (Ang II) may enhance the influence of the sympathetic nervous system (SNS) at various levels. Ang II has been shown to enhance sympathetic neuronal activity centrally, to enhance ganglionic transmission, to facilitate NA release from sympathetic nerve terminals, block NA-uptake, enhance NA-synthesis, and to enhance the post-synaptic effects of noradrenaline. Although most of the evidence is derived from animal experiments, in sometimes non-physiological concentrations of Ang II, it seems possible that part of the potent vasoconstrictor effect of angiotensin II is brought about by the synergistic interaction between angiotensin II and various components of the SNS and its receptors [1-4]. Conversely, it can be imagined that part of the vasodilator effects of the AT₁-receptor blockers is caused by direct or indirect suppression of the sympathetic nervous system. This interaction may be clinically relevant, taking into account the important role of SNS-activity in the genesis and maintenance of hypertension [5-7] and even more so in congestive heart failure [8-10].

The pithed rat is a model which appears to be suitable to study interactions between angiotensin II and the peripheral nervous system. Both plasma renin activity and plasma angiotensin II levels are elevated [11,12]. Endogenously generated angiotensin II facilitates neuronally mediated increments in vascular resistance [13]. Additionally, in this model, noradrenaline release was shown to be modulated by endogenously released angiotensin II [14]. In the propranolol-treated pithed rat, potential central nervous system effects [15] and indirect cardiac effects [16] of Ang II are known to be suppressed or even abolished. In this model, the increase in blood pressure caused by stimulation of the sympathetic nervous system can be attenuated in normotensive animals by both ACE-inhibition [13,17,18] and by AT₁-receptor blockade [19-23].

ACE-inhibitors and the more recently developed AT₁-receptor antagonists are widely used in the treatment of hypertension and heart failure. Moreover, they are sophisticated tools in the analysis of the renin-angiotensin system and its various activities. Several new, non-peptidergic AT₁-selective receptor blockers are now available. A few studies have recently been published in which the potency of these drugs with respect to the attenuation of the facilitation by Ang II of sympathetic neurotransmission was compared [20,21,24-26]. From the studies cited above, it seems clear that the Ang II-induced sympathetic facilitation is mediated by the AT₁-receptor and that all AT₁-antagonists can attenuate this facilitation. However, differences in potency have been
Prejunctional and postjunctional AT₁-receptor blockade

described between the various AT₁-antagonists regarding inhibition of the presynaptic site. It now appears to be of interest to make quantitative comparisons with respect to the pre- and postsynaptic activities of the various AT₁-blockers under carefully standardised conditions.

Accordingly, it was the objective of the present study to quantitatively compare the sympatho-inhibitory properties of the selective AT₁-receptor blockers valsartan, candesartan, eprosartan and embusartan with their inhibitory activity on the direct vasoconstrictor effect of exogenous Ang II in the pithed rat model. To investigate blockade of pre-synaptic AT₁-receptors we studied the effect of AT₁-blockade on the sequelae of electric stimulation of the thoracolumbar sympathetic outflow. To investigate the role of postsynaptic AT₁-receptor blockade we studied the effect of the AT₁-antagonists on dose-response curves elicited by exogenous Ang II. In addition, the effect of AT₁-blockade on post-synaptic α-adrenoceptor mediated responses was investigated by means of exogenously administered noradrenaline (NA). In the present study, multiple doses of each compound were used, enabling us to quantitatively compare both sympatho-inhibitory and direct vasopressor-inhibiting potencies on the basis of dose-response relationships.
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Methods

Male, normotensive Wistar rats (270-320g) which were kept on a normal rat chow diet and had water ad libitum, were used throughout. The pithed rat model, as previously described [21] was used. In short, rats were anaesthetised, pithed and artificially respirated. The pithing rod was insulated except for a 3.5 cm area, 2.5 cm from the tip in order to allow the selective stimulation of thoracolumbar sympathetic segments. The left carotid artery was cannulated, heparin 150 IU/kg was administered and blood pressure was monitored continuously. The heart rate was derived from this signal. The left internal jugular vein was cannulated for the administration of drugs. Bilateral vagotomy and bilateral adrenalectomy were performed. Rats were treated with propranolol 1 mg/kg i.v. to rule out β-adrenoceptor-mediated effects, tubocurarine 2.5 mg/kg i.v. to prevent muscle contraction during electrical stimulation and atropine 2 mg/kg i.p. to inhibit parasympathetic effects. Animals were left to recover for 15 minutes. Subsequently, either saline, or one of the four AT₁-blockers was administered intravenously in various concentrations. Groups consisted of 6 animals, unless stated otherwise. Only one dose of a drug was used in each animal.

Stimulation experiments

Fifteen minutes after drug administration, the sympathetic nervous system was stimulated at 0.25, 0.5, 1, 2, 4 and 8 Hz (15 seconds per frequency) at 50V with square wave pulses of 2 ms delivered by a HSE stimulator I at the level of the T5-L4 segments. After each period of stimulation at a given frequency, blood pressure was allowed to return to baseline. The following dose ranges of the AT₁-blockers were studied: valsartan 0.3, 1, 3 and 10 mg/kg, candesartan 0.3, 1, 3 and 10 mg/kg, eprosartan 0.1, 0.2, 0.3 and 1 mg/kg and embusartan 0.3, 1, 0.3 and 10 mg/kg, respectively. All doses were administered in a volume of 1 ml/kg.

Noradrenaline experiments

In order to investigate the effect of the various compounds on postjunctional vasopressor responses to α-adrenoceptor stimulation we applied noradrenaline intravenously in increasing dosages. Pithing and pretreatment were performed as in the stimulation experiments. Either saline or valsartan (3 mg/kg), candesartan (3 mg/kg), eprosartan (0.3 mg/kg) or embusartan (3 mg/kg) were administered, in a volume of 1 ml/kg. Another 15 minutes later, 0.5 ml/kg saline was injected i.v. in each animal as a volume challenge. Subsequently, intravenous administration
of increasing doses of noradrenaline 0.03 nmol/kg - 0.3 μmol/kg was started, injected in volumes of 0.5 ml/kg.

**Angiotensin II experiments**

In order to investigate the effect of the various compounds on postjunctional vasopressor responses to AT₁-receptor stimulation we applied Ang II intravenously in increasing dosages. Pithing and pretreatment were performed as in the stimulation experiments. Either saline or valsartan (0.03, 0.1 and 0.3 mg/kg), candesartan (0.003, 0.01, 0.03 mg/kg), eprosartan (0.03, 0.1 and 0.3 mg/kg) or embusartan (0.03, 0.1, 0.3 mg/kg) were administered, in a volume of 1 ml/kg. After 15 minutes, 0.5 ml/kg saline was injected i.v. in each animal as a volume challenge. Subsequently, intravenous administration of increasing doses of Ang II 3 pmol/kg - 0.3 μmol/kg was started, injected in volumes of 0.5 ml/kg.

**Drugs**

Angiotensin II (Bachem, Bubendorf, Switzerland) was dissolved in saline. Valsartan (Novartis Pharma AG, Basel, Switzerland), candesartan (AstraZeneca, Södertälje, Sweden), eprosartan (Solvay, Hannover, Germany) and embusartan (Bayer, Wuppertal, Germany) were dissolved in NaOH 1 M. Using HCl 0.01 M, the pH of the solution was lowered to a value of 8 and saline was added. (±)-propranolol HCl (RBI, Natick, USA), atropine Sulfate (Sigma, St. Louis, USA) and d-tubocurarine chloride (Sigma, St. Louis, USA) were dissolved in saline. (-)-noradrenaline bitartrate (Sigma, St. Louis, USA) was dissolved in saline containing L(+) ascorbic acid (Merck, Darmstadt, Germany) 100 μg/ml.

**Statistical analysis**

All data are expressed as means ± SEM of the number of observations. Comparison of means was performed using Student's t-test. For comparison of the sympatho-inhibitory potency between drugs, linear regression was performed. Subsequently, the ED₅₀-value was determined for each individual compound (ED₅₀ = dose which at 2 Hz reduced ΔDBP by 20 mmHg). For comparison of inhibitory potency concerning inhibition of direct pressor effects of Ang II, pA₂-values were calculated using Schild regression. The ED₅₀ values and the pA₂ values of the four AT₁-antagonists were compared using analysis of variance. A p value < 0.05 was considered to indicate statistical significance.
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Results

Baseline diastolic blood pressure (DBP) and heart rate (HR) (after pithing, treatment with propranolol, tubocurarine and atropine) amounted to 46.0 ± 1.7 mmHg and 310.9 ± 5.5 beats per minute (BPM), respectively (N = 18). Administration of the AT₁-blocking drugs caused a significant, dose dependent reduction in blood pressure (see table 1). All compounds studied caused very similar maximal hypotensive effects (p>0.05). Heart rate was unaffected by AT₁-receptor blockade (table 2).

Stimulation experiments

Stimulation of the thoraco-lumbar spinal cord (T5-L4) caused an increase in diastolic blood pressure and heart rate. The magnitude of the effects was dependent on the frequency of the electrical stimulation. The increase in HR amounted to 23.7 ± 3.7 BPM at 8 Hz in the control group. No effect of AT₁-blockade on the stimulation-induced increase in HR was observed. (data not shown). The increase in DBP could be dose-dependently and significantly attenuated by the four AT₁-receptor blockers, in the lower three concentrations used (fig. 1A-D). This attenuation was more pronounced at the lower frequencies of spinal cord stimulation (0.25-2 Hz). Interestingly, the highest doses of all three AT₁-receptor antagonists did not further attenuate the rise in DBP. Conversely, the rise in DBP was stronger than observed after administration of the lower dose, resulting in a U-shaped dose-response relationship. The strongest effect of AT₁-receptor blockade was observed at a stimulation frequency of 2 Hz. In fig. 2, the rise in DBP observed at 2 Hz is plotted against the lower doses used of the three compounds studied (doses expressed as log mol/kg). The degree of maximal attenuation was similar for all AT₁-blockers used in this study (ΔDBP at a stimulation frequency of 2 Hz was 63.2 ± 7.6, 49.7 ± 3.3, 58.3 ± 8.0 and 52.7 ± 5.5, for valsartan, candesartan, eprosartan and embusartan, respectively, p>0.05). A linear correlation was observed between their doses and ΔDBP (fig. 2). ED₅₀ values, expressed as -log mol/kg were 5.50 ± 0.12, 5.77 ± 0.10, 6.32 ± 0.12 and 5.62 ± 0.13, for valsartan, candesartan, eprosartan and embusartan, respectively. The order of potency, therefore, was eprosartan>valsartan=candesartan=embusartan ('>' signifies p<0.05).
**Table 1.** Baseline diastolic blood pressure (mmHg) in the pithed normotensive rat after administration of saline (control) or a single dose of either valsartan, candesartan, eprosartan or embusartan, respectively.

<table>
<thead>
<tr>
<th>Dose (mg/kg i.v.)</th>
<th>Diastolic Blood Pressure (mmHg)</th>
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<tbody>
<tr>
<td></td>
<td>saline</td>
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<tr>
<td>0.003</td>
<td>46.0 ± 1.7</td>
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<tr>
<td>0.01</td>
<td>46.0 ± 1.7</td>
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<tr>
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<td>46.0 ± 1.7</td>
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<tr>
<td>10</td>
<td>46.0 ± 1.7</td>
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Values are expressed as means ± SEM. Doses are expressed as mg/kg i.v. Pooled data from groups before spinal cord stimulation, noradrenaline or Ang II administration. N = 6-18 animals per group. * p<0.05 compared to control.

**Table 2.** Baseline heart rate in the pithed normotensive rat after administration of saline (control) or a single dose of either valsartan, candesartan, eprosartan or embusartan, respectively.

<table>
<thead>
<tr>
<th>Dose (mg/kg i.v.)</th>
<th>Heart Rate (BPM)</th>
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<tr>
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<td>saline</td>
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<td>0.003</td>
<td>310.9 ± 5.5</td>
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<td>310.9 ± 5.5</td>
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<td>10</td>
<td>310.9 ± 5.5</td>
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</table>

Values are expressed as means ± SEM. Pooled data from groups before spinal cord stimulation, noradrenaline or Ang II administration. N = 6-18 animals per group.
**Fig. 1.** Effect of AT<sub>1</sub>-receptor blockade on the frequency-response curve induced by electrical stimulation of the spinal cord (T5-L4) in the pithed normotensive rat. The stimulation frequency (in Hz) is shown on the abcissa. The increase in diastolic blood pressure (expressed as mmHg) is shown on the ordinate.

A: valsartan (0.1, 0.3, 1, 3 and 10 mg/kg). B: candesartan (0.3, 1, 3 and 10 mg/kg). C: eprosartan (0.1, 0.2, 0.3 and 1 mg/kg). D: embusartan (0.3, 1, 3 and 10 mg/kg). Values are expressed as means ± SEM. *P<0.05 compared to control. N = 6 in each group.
Prejunctural and postjunctural AT_1-receptor blockade

Fig. 2. Effects of either valsartan, candesartan, eprosartan or embusartan on the diastolic blood pressure increase observed at a stimulation frequency of 2 Hz. Doses of each compound are shown on the abscissa and expressed as log mol/kg. The increase in diastolic blood pressure (expressed as mmHg) is shown on the ordinate. The three lower doses of each compound and linear regression lines are shown. The order of potency with respect to sympato-inhibition was epro > val = cande = embu (comparison of doses which at 2 Hz reduced ΔDBP by 20 mmHg (ED_{50} for values see text), ‘>’ signifies p<0.05).

Exogenous noradrenaline

In these experiments, exogenous NA caused a dose-dependent increase in DBP (EC_{50} 8.52 ± 0.09 -log mol/kg, E_{max} 120.2 ± 4.7 mmHg) (fig. 3A-D). None of the AT_1-antagonists, at doses which caused maximal reduction of DBP-responses to stimulation, altered the BP-response to the administration of exogenous noradrenaline.
Fig. 3. Effects of AT₁-receptor blockade on the dose-response curve induced by intravenously administered noradrenaline in the pithed normotensive rat. The noradrenaline doses (expressed as log mol/kg) are shown on the abscissa. The increase in diastolic blood pressure (expressed as mmHg) is shown on the ordinate.

A: valsartan (3 mg/kg). B: candesartan (3 mg/kg) C: eprosartan (0.3 mg/kg) D: embusartan (3 mg/kg). Values are expressed as means ± SEM. N = 6 in each group.
Exogenous Angiotensin II

Exogenously administered Ang II caused a dose-dependent increase in DBP (pEC$_{50}$ 9.80 ± 0.06 log mol/kg). Treatment with the AT$_1$-antagonists caused a parallel rightward-shift of the Ang II – dose-response curve. (fig. 4A-D). A small reduction of $E_{\text{max}}$ (of approximately 10%) was observed for all compounds used, except eprosartan. pA$_2$-values, 7.20 ± 0.17, 8.01 ± 0.01, 7.20 ± 0.03 and 7.25 ± 0.16, for valsartan, candesartan, eprosartan and embusartan, respectively. Accordingly, the order of potency regarding the inhibition of direct pressor effects of Ang II was candesartan > valsartan = eprosartan = embusartan (>' signifies p<0.05).

At the highest doses, an increase in HR was observed as well ($E_{\text{max}}$ 32.7 ± 6.0 BPM). This increase was reduced by all AT$_1$- receptor antagonists (data not shown).

We calculated the ratio between the ED$_{50}$-values (obtained from stimulation experiments) and A$_2$-values (obtained from Ang II experiments). A$_2$-values were calculated by taking the exponential of the pA$_2$-values. The ratios are shown in fig. 5. For eprosartan, doses needed for inhibition of stimulation-induced DBP-responses are close to those required for inhibition of direct effects of Ang II on DBP-responses. Conversely, for candesartan, these respective doses differ considerably.
Fig. 4. Effects of AT₁-receptor blockade on the dose-response curve induced by intravenously administered Ang II in the pithed normotensive rat. The Ang II doses (expressed as log mol/kg) are shown on the abissa. The increase in diastolic blood pressure (expressed as mmHg) is shown on the ordinate.

A: valsartan (0.03, 0.1 and 0.3 mg/kg). B: candesartan (0.003, 0.01 and 0.03 mg/kg)

C: eprosartan (0.03, 0.1 and 0.3 mg/kg) D: embusartan (0.03, 0.1 and 0.3 mg/kg).

Values are expressed as means ± SEM. N = 6 in each group.
Prejunctional and postjunctional AT₁-receptor blockade

Discussion

In the present study, the sympatho-inhibitory properties of the selective AT₁-receptor blockers valsartan, candesartan, eprosartan and embusartan were compared with the inhibition of the direct vasoconstrictor effect of exogenous Ang II in the pithed rat model.

In the pithed rat model, neuronally mediated increments in vascular resistance are known to be facilitated by endogenously formed Ang II [13]. Presynaptically located AT₁-receptors are probably responsible for this facilitation. Blockade of these receptors may be therapeutically relevant, especially when one considers the role of the sympathetic nervous system in the aetiology and treatment of both hypertension and heart failure. For several of the AT₁-antagonists, sympatho-inhibitory properties have been demonstrated [19-26]. Differences in potency seem to exist regarding inhibition of the prejunctional AT₁-receptor. However, it is unclear whether the order of potency regarding sympatho-inhibition is comparable to the order of potency regarding inhibition of direct vasopressor effects of Ang II. In the present study, multiple doses of each compound were used, enabling us to quantitatively compare both sympatho-inhibitory and direct vasopressor-inhibiting potencies on the basis of dose-response relationships. As shown in table 1, all AT₁-receptor antagonists significantly lowered baseline blood pressure. However, heart rate was unaffected (table 2). Apparently, in this model, endogenously generated Ang II does not modulate HR. Indeed, effects of Ang II on HR were shown to be mediated by β-adrenoeceptors in this model [16]. In the present study, such effects were prevented by administration of propranolol. The decrease in blood pressure was comparable to that described in other studies in which either AT₁-blockers or ACE-inhibitors were used. There was no difference in maximal BP-lowering effect between the four compounds (p>0.05).

Stimulation experiments

In our investigation, as in other studies, electrical stimulation of the thoracolumbar spinal cord caused stimulation-frequency dependent increases in diastolic blood pressure and heart rate. In the present study, the increase in HR was very small compared to that in other studies [13,19] which is explained by the pretreatment with propranolol in the present study. We found no significant differences in ΔHR between the control group and in the animals subjected to AT₁-receptor blockade. These findings are in accordance with Kaufman & Vollmer [13] and Wong et al. [19], who also concluded that the stimulation-induced increase in HR was unaffected by blockade of the RAS. The increase in DBP could be dose-dependently attenuated by AT₁-blockade (fig. 1A-D). At the lower frequencies (0.25-2 Hz), the attenuation was more
pronounced, indicating a more important facilitating role of Ang II in the lower frequency range. In other studies in the pithed rat model, stimulation frequencies of 1-5 Hz have been shown to be most sensitive to facilitation [13, 14, 17, 19]. At the higher frequencies, NA release seems to be maximal and less sensitive to facilitation by Ang II.

In order to compare the potency of the compounds used, effects observed at 2 Hz were plotted separately (fig. 2). The degree of maximal attenuation was similar for all AT$_1$-blockers used in this study. A linear correlation was observed between the doses administered and ΔDBP. ED$_{50}$ values differed significantly between eprosartan on the one hand and the other three AT$_1$-blockers on the other (p<0.05). Accordingly, on a molar base, the order of potency regarding inhibition of the facilitatory effect of Ang II in stimulation-induced increase of DBP-response is eprosartan > candesartan = valsartan = embusartan. These findings are in global agreement with the findings reported by Ohlstein et al. [20], who reported that eprosartan 0.3 mg/kg, but not valsartan 0.3 mg/kg inhibited pressor responses to spinal cord stimulation in the pithed rat. We extended their findings regarding valsartan, by showing that higher doses of valsartan (1-10 mg/kg) do indeed cause sympatho-inhibition. In isolated rat atria, valsartan, losartan, eprosartan and irbesartan had already been shown by Shetty et al. to inhibit prejunctional actions of Ang II [25]. However, in contrast with our findings, in those experiments valsartan was more potent than eprosartan. Differences in the fraction of AT$_1$-antagonist unbound to plasma-protein in our model cannot explain the differences between our in vivo findings and their and their in vitro findings. Protein-binding of eprosartan and valsartan are 98% and 95%, respectively [27], resulting in a higher free fraction of valsartan compared to eprosartan.

A similar finding as reported earlier by our group for losartan, irbesartan and telmisartan [21] was obtained for the highest doses of the AT$_1$-receptor blockers used in the present study. These doses caused less than maximal suppression (see fig. 1A-D). The fact that these dosages still caused a significant fall in baseline blood pressure implies that AT$_1$-blockade was indeed achieved. The consistency of this observation for all AT$_1$-blockers suggests that it is a class effect. Earlier, we reported that supramaximal doses of captopril do not show this phenomenon [21]. Additionally, Cox et al. reported that losartan can in fact increase stimulation-induced contractions and noradrenaline release in vitro [28]. The synergistic actions of losartan and Ang II could be abolished by PD123319, indicating a role for the AT$_2$-receptor.
Prejunctional and postjunctional AT₁-receptor blockade

Noradrenaline experiments

To confirm that the attenuation of sympathetic neurotransmission by AT₁-blockade was due to presynaptic effects only, as reported by us earlier, experiments using exogenous noradrenaline were performed (fig. 3A-D). The doses of AT₁-blocker which caused the greatest reduction of DBP-rise in the stimulation experiments were used. As to be expected, exogenous NA caused dose dependent increases in DBP. AT₁-blockade by eprosartan (0.3 mg/kg), candesartan (3 mg/kg), valsartan (3 mg/kg), and embusartan (3 mg/kg) did not affect DBP-responses to exogenous NA. After AT₁-blockade, Eₘₐₓ values were slightly, although not significantly increased in all groups. This probably reflects the differences in baseline blood pressure between control-experiments and those after AT₁-blockade. Because the DBP after the highest doses of NA are very similar in each group (around 150 mmHg), the maximal ΔDBP tends to be slightly higher after AT₁-blockade. Since no difference was observed in the responses to exogenous noradrenaline between control experiments and those after AT₁-blockade, the sensitivity of the postsynaptic α-receptors appears not to be affected by angiotensin II in our experimental model. This confirms our earlier experiments with AT₁-blockade in this model [21].

Angiotensin II experiments

Ang II (iv) caused a dose-dependent rise in DBP. Treatment by AT₁-blockade caused a parallel rightward-shift of the Ang II DRC (fig. 4A-D). The Eₘₐₓ was only slightly decreased by valsartan, candesartan and embusartan and not at all by eprosartan. Therefore, at doses used in our experiments, all AT₁-blockers acted as competitive antagonists on the direct effects of Ang II on DBP. Thus, it was possible to calculate pA₂-values for all blockers investigated. pA₂-values were 7.20 ± 0.17, 8.01 ± 0.01, 7.20 ± 0.03 and 7.25 ± 0.16, for valsartan, candesartan, eprosartan and embusartan, respectively. Accordingly, the order of potency was candesartan > valsartan = eprosartan = embusartan. In rabbit aorta rings, candesartan is a non-competitive antagonist [29], valsartan displays mixed antagonism [30], and both eprosartan and embusartan are competitive antagonists, while embusartan’s metabolite, M-1 shows a depression of Eₘₐₓ [31,32]. That candesartan displays competitive antagonism in our model and non-competitive antagonism in vitro is difficult to explain. However, differences in type of antagonism between different models have been described earlier. Losartan, which is a competitive antagonist in the rabbit aorta [33], displays mixed antagonism in the rabbit renal artery [34] and non-competitive antagonism in the human forearm [35].
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Exogenous Ang II, in the highest dose-range, caused an increase in HR of 32.7 ± 6.0 BPM. The positive chronotropic effect of Ang II was shown to be mediated via the ganglionic neurotransmission through β-receptors [16]. In our model, this was inhibited by 1 mg/kg propranolol (iv). The rise in HR could be completely blocked by the highest doses of the AT$_1$-receptor antagonists. The positive chronotropic effect, therefore, was mediated through the AT$_1$-receptor.

We quantitatively compared the potency between the four AT$_1$-receptor antagonists in our model both at the pre- and postsynaptic level. This enabled us to address the issue of relative affinity of the AT$_1$-blockers at the two sites. Firstly, the order of potency concerning sympatho-inhibition differs from the order of potency regarding inhibition of the direct effect of Ang II on the vasculature. This may be due to differences in affinity of the AT$_1$-blockers for the presynaptic AT$_1$-receptor on the one hand and the postsynaptic AT$_1$-receptor on the other. Additionally, it is noteworthy that the doses needed for sympatho-inhibition are higher than the doses needed for inhibition of direct pressor effects of Ang II. Especially if one considers that for facilitation of sympathetic neurotransmission we relied on endogenous Ang II and for direct pressor effects we added exogenous Ang II. Interestingly, the ratios between ED$_{20}$-values (as a measure of presynaptic inhibitory dose) and A$_2$-values (calculated by taking the exponential of the pA$_2$-values, a measure of the postsynaptic inhibitory dose) differ considerably between the various antagonists (fig. 5). For eprosartan, sympatholytic doses and postsynaptic inhibitory doses differ far less than for candesartan. However, comparison between the two models must be made with utmost care. Altogether, our findings imply that perhaps presynaptic AT$_1$-receptors are of a different subtype than postsynaptic receptors. In rodents, but not in humans, AT$_{1A}$ and AT$_{1B}$ receptor subtypes have been described. Differences in subtype between pre- and postsynaptic AT-receptors have been suggested earlier by Guimarães et al. [36]. In the dog isolated mesenteric artery, these authors saw no effect of losartan on Ang II - induced facilitation in doses which clearly inhibited the post-synaptic receptor. However, because no binding studies of AT-receptors on sympathetic nerve terminals are available, this hypothesis remains speculative.
Prejunctional and postjunctional AT₁-receptor blockade

Fig. 5. Ratio between the ED₃₀-values (obtained from stimulation experiments; reflect sympatho-inhibitory potency) and A₂-values (obtained from Ang II experiments; reflect inhibitory potency regarding direct effects of Ang II on the vasculature). ED₃₀: dose which at 2 Hz reduced ΔDBP by 20 mmHg. A₂-values were calculated by taking the exponential of the pA₂-values. Note the substantial differences between the AT₁-blockers studied. The presynaptic effect related with impairment of NA release occurs at relatively low doses for eprosartan when compared with postsynaptic inhibition of vasoconstriction by Ang II. In contrast, there is a substantial difference between these two dosages for candesartan.

In conclusion, our findings once more confirm that facilitation by Ang II of stimulation induced DBP-response is mediated by presynaptically located AT₁-receptors. The order of potency regarding sympatho-inhibition by AT₁-blockers (eprosartan>valsartan=candesartan=embusartan) differs from the order of potency regarding inhibition of the direct pressor effect of Ang II (candesartan>eprosartan>valsartan=embusartan). These findings suggest differences in affinity of the AT₁-blockers for the pre- and postsynaptic AT₁-receptor. Sympatho-inhibitory properties of AT₁-receptor antagonists are particularly interesting when one considers the role of the sympathetic nervous system in hypertension and heart failure, two conditions in which AT₁-blockers are increasingly being used.
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References


Prejunctional and postjunctional AT₁-receptor blockade


