Angiotensin II receptor antagonists and sympathetic neurotransmission

Balt, J.C.

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GENERAL DISCUSSION AND CONCLUSIONS
General discussion and conclusions

Angiotensin II plays an important role in the homeostasis of blood pressure and the circulating volume. Blocking the various components of the renin-angiotensin system has proven to be very beneficial in the treatment of hypertension and heart failure.

In the present study, in isolated arteries, in the pithed rat, and in the human forearm, we quantified the enhancing effect of angiotensin II on noradrenergic nerve traffic and its sequelae. We investigated the potency of various selective AT₁-receptor antagonists, regarding the inhibition of the presynaptic site. We compared this sympatho-inhibitory effect with the potency regarding inhibition on AT₂-receptors on smooth muscle. In addition, we investigated the facilitatory actions of Ang II in two pathological models, the spontaneously hypertensive rat and a rabbit model of induced heart failure. Lastly, we investigated the role of putative receptor subtype-differences between the prejunctional and postjunctional sites.

In the isolated rat mesenteric artery, Ang II (10 nM), a concentration that generated a small contractile response, caused a significant increase in the contractions evoked by electrical field stimulation. The enhancement was most pronounced at a stimulation frequency of 2 Hz, leading to an almost five-fold increase in contractile force. Ang II (10 nM) did not alter the responses to noradrenaline. Losartan, irbesartan and telmisartan concentration-dependently inhibited the facilitating effect of Ang II, and the order of sympatho-inhibitory potency was telmisartan>irbesartan>losartan. Consequently, the enhancing effect of Ang II on noradrenergic neurotransmission was mediated via prejunctional AT₁-receptors. The three AT₁-receptor antagonists studied could equi-effectively inhibit the prejunctionally located AT₁-receptor, but there were significant differences in their sympatho-inhibitory potency. For telmisartan, this was the first time that a sympatho-inhibitory action was reported.

The pithed rat appears to be a suitable model to investigate the interactions between the RAS and the SNS. Neuronally mediated increments in vascular resistance are known to be facilitated by endogenously formed Ang II. Additionally, the pithing procedure destroys the central nervous system, ruling out central effects. Electrical stimulation of the spinal cord (T5-L4) caused a frequency-dependent rise in diastolic blood pressure. This could be dose-dependently inhibited by losartan, irbesartan and telmisartan, administered intravenously. The order of potency was
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telmisartan > losartan > irbesartan. Thus, compared to the order of potency that we described in the isolated mesenteric artery, the ranking order of losartan and irbesartan was reversed. This may be explained by conversion of losartan into its more active metabolite EXP 3174. Like in the mesenteric artery, AT₁-blockade did not affect the responses to exogenous noradrenaline. Therefore, the facilitatory effect of Ang II appears to be mediated by prejunctional AT₁-receptors. Interestingly, the highest doses of the AT₁-receptor antagonists caused less than maximal inhibition. This phenomenon was not observed with the ACE-inhibitor captopril. Therefore we speculated that, during high dose AT₁-blockade this phenomenon may have been caused by activation of the (unoccupied) AT₂-receptor. We addressed this issue in a separate study (see below).

In the pithed rat model, we quantified the sympatho-inhibitory potency as well as the activity regarding inhibition of the direct vasoconstrictor effect of Ang II, of four selective AT₁-receptor antagonists; valsartan, candesartan, eprosartan and embusartan. Accordingly, we could compare their ability to block the prejunctioonal as well as the postjunctional receptor, respectively. The ranking order regarding sympatho-inhibition (eprosartan > valsartan = candesartan = embusartan) differed from the ranking order regarding post-synaptic inhibition (candesartan > valsartan = eprosartan = embusartan). These findings suggest differences in affinity of the various AT₁-receptor antagonists for prejunctional and postjunctional AT₁-receptors.

We further investigated the potency of eprosartan and candesartan regarding pre- and postjunctional AT₁-receptors in the rabbit isolated mesenteric artery, and evaluated whether the AT₂-receptor is involved in the facilitatory actions of Ang II.

In this model, Ang II (0.5 nM), a subpressor concentration, significantly enhanced electrical field stimulation-induced contractions, but did not alter responses to noradrenaline. The AT₂-receptor antagonist PD 123319 (10 nM) did not affect the facilitatory effect of Ang II. For eprosartan, sympatho-inhibition was achieved at concentrations that also block AT₁-receptors at vascular smooth muscle. In contrast, for candesartan, the presynaptic concentrations were considerably higher than those required for postsynaptic inhibition. Taken together, we can assume that in this model, facilitation of sympathetic neurotransmission is mediated by prejunctionally located AT₁-receptors, and not by AT₂-receptors. Our findings with eprosartan and candesartan suggest differences in AT₁-receptor subtype between the prejunctional and postjunctional AT₁-receptor.
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In the pithed rat model, seven different AT₁-receptor antagonists could, dose-dependently, inhibit the facilitation by endogenously generated Ang II. However, for each of these blockers, we observed less than maximal inhibition after the highest dose. We hypothesized that (an unmasking of) an AT₂-receptor population was involved in this U-shaped dose-response relationship, since this phenomenon was not observed with the ACE-inhibitor captopril. Therefore, we studied the effects of multiple doses of irbesartan on stimulation-induced DBP-responses in the presence and absence of the AT₂-receptor antagonist PD 123319. In addition, we studied the effect of several doses of the non-selective AT-receptor antagonist saralasin on stimulation-induced DBP-responses. In accordance with our hypothesis, we did not observe a U-shaped dose-response relationship when AT₁ and AT₂-receptor blockade was combined. These findings indicate a facilitatory role for the AT₂-receptor, which is unmasked by high doses of the AT₁-receptor antagonists.

The role of the sympathetic nervous system in the development and maintenance of hypertension in the spontaneously hypertensive rat (SHR) is well established. Additionally, the facilitating effect of Ang II on noradrenergic neurotransmission has been shown to be enhanced in SHR. Therefore, we investigated the sympatho-inhibitory actions of irbesartan in the pithed SHR and their normotensive controls, the Wistar Kyoto rat (WKY). In both strains, we compared the doses required for sympatho-inhibition with doses needed for inhibition of the vasoconstrictor effects of Ang II. No differences were observed between the strains, either for pre- nor for postjunctional AT₁-blockade. Therefore, no differences appear to exist regarding the affinity of irbesartan for pre- and postjunctional AT₁-receptors. Interestingly, in contrast to in SHR, irbesartan caused a rightward shift of the dose-response curve to noradrenaline in WKY. Therefore, a facilitatory role of Ang II at the level of vascular smooth muscle appears to exist in the WKY and not in the SHR. In SHR and neither in WKY did we observe a less than maximal inhibition with the highest dose of irbesartan, as we previously found in pithed Wistar rats. Apart from the conclusion that indeed Wistar rats are not appropriate control animals for the SHR, the presynaptic AT₂-receptor does not appear to play a role in either SHR or WKY.

Virtually all forms of severe congestive heart failure are known to be accompanied by activation of the renin-angiotensin system and the sympathetic nervous system. Very little is known about the effects of Ang II on sympathetic neurotransmission at the peripheral level in vessels derived from animals with experimental heart failure. In the mesenteric artery, isolated from rabbits with a chronic volume- and pressure overload type of heart failure, as well as vessels derived from age-
matched control rabbits, we investigated the effects of Ang II on stimulation-induced contractile force. Additionally, we compared (the effect of Ang II on) contractile responses to noradrenaline. In mesenteric arteries from heart failure- and control rabbits, Ang II (0.5 nM) facilitated sympathetic neurotransmission via prejunctionally located AT₁-receptors. The facilitating effect was decreased in vessels derived from heart failure rabbits. In contrast, responses to noradrenaline and angiotensin II were unchanged. The sympatho-inhibitory potency of eprosartan was the same in vessels derived from heart failure rabbits and controls, respectively. These findings may be explained by down-regulation or uncoupling of the prejunctional AT₁-receptor in heart failure.

In the human forearm, we studied the effects of Ang II on tyramine-induced vasoconstriction. Intra-arterially (i.a.) infused tyramine caused a dose-related decrease in forearm blood flow. Angiotensin II (10 ng/kg/min, also i.a.), a dose that did not cause vasoconstriction, significantly enhanced the vasoconstrictor effects of tyramine. From these findings we conclude that in humans, at the peripheral level, Ang II can enhance noradrenergic nerve traffic.

For eprosartan and candesartan, the order of potency regarding inhibition of the prejunctional and postjunctional AT₁-receptor was different in the pithed rat as well as in the isolated rabbit mesenteric artery. In the literature, it had already been suggested that the pre- and postjunctional AT₁-receptors belong to different subtypes. We hypothesised that the prejunctional AT₁-receptor was of the AT₁B-subtype.

PD 123319, an AT₂-receptor antagonist, in high concentrations (>0.5 μM) can inhibit the AT₁B receptor. Therefore, we investigated whether PD 123319 (in high concentrations) could inhibit the facilitatory actions of Ang II (0.5 nM). PD 123319 (1 and 10 μM) could indeed inhibit the facilitatory effects of Ang II, whereas a concentration-dependent increase in contractile force to Ang II (stimulation of the AT₁-receptor on vascular smooth muscle) was unchanged. These findings suggest that the prejunctional AT₁-receptors are of the AT₁B-subtype, and postjunctional AT₁-receptors of the AT₁A-subtype.

In general, we conclude that (subpressor concentrations of) Ang II can facilitate sympathetic nerve traffic. In the animal models, both in vitro and in vivo, this was mediated via prejunctionally located AT₁-receptors. All AT₁-receptor antagonists can inhibit the prejunctional AT₁-receptor. The ranking order regarding inhibition of the prejunctional AT₁-receptor is different from the ranking order regarding inhibition of the AT₁-receptor on vascular smooth muscle, thus
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suggesting differences in receptor subtype. In the isolated rabbit mesenteric artery, we found evidence that the prejunctional AT₁-receptors are of the AT₁₄ subtype, and postjunctional AT₁-receptors are not.

Sympatho-inhibition is a class effect of the AT₁-receptor-antagonists. In conditions in which the sympathetic nervous system plays a pathophysiological role, such as hypertension and congestive heart failure, this property may well be of therapeutic relevance.