Angiotensin II receptor antagonists and sympathetic neurotransmission
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SUMMARY
Summary

Chapter 1

In this introductory chapter, the renin-angiotensin system (RAS) and its interaction with the sympathetic nervous system (SNS) is outlined. The role of the RAS and the SNS in both essential hypertension and heart failure is discussed, as well as the interactions between RAS and SNS under these conditions. Angiotensin II, the main effector of the renin-angiotensin system, plays an important role in the regulation of fluid and electrolyte balance. Many of its effects such as vasoconstriction, the release of aldosterone and cellular growth are mediated by the angiotensin II type 1 receptor (AT₁-receptor). The physiological role of the AT₂-receptor is unclear, but more and more effects such as vasodilation and growth inhibition are being described. Ang II facilitates sympathetic neurotransmission at the level of the central nervous system, the sympathetic ganglia, the adrenals, the sympathetic nerve terminals and also at the level of vascular smooth muscle. All of these effects are mediated by AT₁-receptors.

The involvement of the sympathetic nervous system in hypertension has been demonstrated by means of biochemical, electrophysiological and pharmacological techniques, both in humans and in animal models. The involvement of the (tissue)-RAS in hypertension can be deduced from the fact that blocking the RAS effectively lowers elevated blood pressure.

Various forms of severe congestive heart failure are known to be accompanied by activation of both the renin-angiotensin system and the sympathetic nervous system. In the long term, deleterious effects of these compensatory mechanisms will arise. ACE-inhibitors are widely used as a cornerstone of heart failure therapy and ß-blockers are more and more recognised as beneficial drugs. Although difficult to demonstrate in humans, the interaction between RAS and SNS appears potentially pathophysiologically relevant in both hypertension and heart failure. The interaction between both systems may also play a role in drug treatment of congestive heart failure and essential hypertension.

Chapter 2

The effects of the AT₁-receptor antagonists losartan, irbesartan and telmisartan on angiotensin II-induced facilitation of noradrenergic neurotransmission were investigated in the isolated rat mesenteric artery under isometric conditions. Electrical field stimulation (2, 4 and 8 Hz) caused a frequency-dependent increase of contractile force. At stimulation frequencies of 2, 4 and 8 Hz, Ang II (10 nM) increased the stimulation-induced vasoconstrictor responses by a factor 4.8 ± 0.9,
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2.9 ± 0.7, and 1.3 ± 0.1, respectively (p<0.05 compared to control for all frequencies). The enhancement could be concentration-dependently antagonized by losartan (1 nM – 1 μM), irbesartan (0.1 nM- 0.1 μM) and telmisartan (0.01 nM- 0.01 μM).

At a stimulation-frequency of 2 Hz, the relationship between stimulation-induced vasoconstrictor responses (in presence of Ang II 10 nM) and the concentration of the AT₁-antagonists used, could be described by linear regression. The order of potency concerning sympatho-inhibition was telmisartan>irbesartan>losartan (p<0.05 between linear regression lines). Contractile responses to exogenous noradrenaline were unaltered in the presence of Ang II 10 nM. We conclude that the facilitating effect of Ang II on noradrenergic neurotransmission is mediated by pre-synaptically located AT₁-receptors. Conversely, this facilitating effect can be dose-dependently counteracted by blockade of these receptors.

Chapter 3

The objective of the present study was to quantify the inhibitory effect of the AT₁-receptor blockers losartan, irbesartan and telmisartan and the ACE-inhibitor captopril on sympathetic neurotransmission. In the male, normotensive pithed rat model, we studied the effect of losartan (1, 3, 10 and 30 mg/kg), irbesartan (3, 10, 30 and 60 mg/kg), telmisartan (0.3, 1, 3 and 10 mg/kg) and captopril (1.5, 5, 15 and 45 mg/kg) on electrical stimulation of the thoraco-lumbar spinal cord. To investigate the interaction between postsynaptic AT₁-receptors and α-adrenoceptors, the effects of these compounds on pressor responses to exogenous noradrenaline were studied. Stimulation of the thoracolumbar spinal cord caused a stimulation-frequency dependent rise in DBP that could be dose-dependently reduced by both AT₁-receptor blockade and ACE-inhibition. Interestingly, the highest doses of the AT₁-antagonists caused less than maximal reduction in the rise in DBP. This phenomenon was not observed after ACE-inhibition by captopril. In experiments with exogenous noradrenaline, no effect of AT₁-blockade or ACE-inhibition on α-adrenoceptor mediated blood pressure responses were seen.

We conclude that, in the pithed rat model, the effects of stimulation of the thoraco-lumbar spinal cord on DBP are counteracted by blockade of pre-synaptically located AT₁-receptors. The order of potency concerning sympatho-inhibition is telmisartan>losartan>irbesartan. The finding that all three AT₁ blockers cause less than maximal inhibition in their highest doses, as opposed to captopril, suggests that this is a class effect of the AT₁-antagonists.
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Chapter 4

In the pithed rat model, endogenously generated angiotensin II can enhance sympathetic neurotransmission by acting on AT₁-receptors that are located on sympathetic nerve terminals. In the present study, we wanted to compare the inhibitory potency of candesartan, valsartan, eprosartan and embusartan to block the pre-synaptically located AT₁-receptor on the one hand, and the postsynaptically located AT₁-receptor on the other.

To investigate blockade of presynaptic AT₁-receptors, we studied the effect of AT₁-receptor antagonists on the sequelae of electric stimulation of the thoraco-lumbar sympathetic outflow (0.25-8 Hz). To investigate the interaction between postsynaptic AT₁-blockers and α-adrenoceptors, the effects of these compounds on pressor responses to exogenous noradrenaline were established. To investigate blockade of postsynaptic AT₁-receptors we studied the effect of the AT₁-antagonists on dose-response curves elicited by exogenous Ang II.

The stimulation-induced increase in diastolic blood pressure (DBP) as well as the Ang II elicited DBP response could be dose-dependently reduced by all AT₁-receptor blockers. Interestingly, the highest doses of the AT₁-antagonists caused less than maximal reduction of the stimulation-induced rise in DBP, resulting in a U-shaped dose-response relationship. To compare sympathetic inhibitory potency, the doses which at 2 Hz reduced ΔDBP by 20 mmHg (ED₂₀ values, expressed as -log mol/kg) were calculated; these 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).

To compare the order of potency regarding inhibition of Ang II-induced DBP-increase, we calculated pA₂-values. These 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 regarding the inhibition of direct pressor effects of Ang II was candesartan>valsartan=eprosartan=embusartan (>' signifies p<0.05). In experiments with exogenous noradrenaline, no effect of AT₁-blockade on α-adrenoceptor mediated blood pressure responses was observed.

In conclusion, in the pithed rat, the effects of stimulation of the thoraco-lumbar spinal cord on DBP are partly dependent on endogenously formed angiotensin II. These effects can be counteracted by blockade of presynaptically located AT₁-receptors. No interaction was found between postsynaptically located AT₁-receptors and α-adrenoceptors. The order of potency regarding sympatho-inhibition clearly differs from the order of potency regarding inhibition of
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the direct pressor effects of Ang II. These findings suggest considerable differences in affinity of the various AT₁-blockers for the pre- and postsynaptic AT₁-receptor.

Chapter 5

The effects of the AT₁-receptor antagonists eprosartan and candesartan and the AT₂-receptor antagonist PD123319 on angiotensin II-induced facilitation of noradrenergic neurotransmission were investigated in the isolated rabbit mesenteric artery under isometric conditions. The sympato-inhibitory potency of the AT₁-blockers was compared to their potency concerning inhibition of the direct vasoconstrictor effect of Ang II.

To investigate blockade of the pre-synaptic AT₁ and AT₂-receptors, we studied the effects of Ang II on electrical field stimulation (EFS)-induced contractions in the presence or absence of eprosartan, candesartan or PD 123319. To investigate blockade of the post-synaptic AT₁-receptors, we studied the effects of either eprosartan or candesartan on concentration-response curves of Ang II. In addition, the effect of Ang II on postsynaptic α-adrenoceptor mediated responses was studied using noradrenaline.

EFS (1, 2 and 4 Hz) caused an increase of contractile force. At stimulation frequencies of 1, 2 and 4 Hz, a sub-pressor concentration of Ang II (0.5 nM) increased the stimulation-induced (S-I) vasoconstrictor responses by 2.8 ± 0.5, 2.4 ± 0.4, and 1.6 ± 0.1 of control values, respectively (p<0.05 compared to control for all frequencies). The enhancement could be antagonized by eprosartan (1 nM – 0.1 μM) and candesartan (1 nM – 0.1 μM). The AT₂-antagonist PD123319 (10 nM) did not influence the Ang II induced facilitation of S-I contractions. Contractile responses to exogenous noradrenaline were unaltered in the presence of Ang II 0.5 nM. Ang II (0.3 nM – 0.3 μM) caused a concentration-dependent increase in contractile force, which could be antagonised by eprosartan (pD₂' 8.8 ± 0.19) and candesartan (pD₂' 11.3 ± 0.23).

We conclude that the facilitating effect of Ang II on noradrenergic neurotransmission is mediated by presynaptically located AT₁-receptors, and not by AT₂-receptors. For eprosartan, sympato-inhibition was achieved at concentrations which also block AT₁-receptors on vascular smooth muscle. In contrast, for candesartan, the presynaptic inhibitory concentrations were considerably higher than those required for postsynaptic inhibition.
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Chapter 6

Previously, we demonstrated in the pithed rat model, that inhibition of the facilitatory actions of Ang II appears to be a class effect of the AT₁-receptor antagonists. However, in this model, all AT₁-blockers caused less than maximal inhibition after the highest dose, thus causing a U-shaped dose-response curve with respect to sympatho-inhibition. In the present study, we investigated whether the AT₂-receptor is involved in this ‘upturn’ of the dose-response relationship. Accordingly, we studied the effect of irbesartan (1 – 60 mg/kg) on the sequelae of electric stimulation of the thoraco-lumbar sympathetic outflow in the presence and absence of the AT₂-blocker PD 123319 (0.5 mg/kg + 50 μg/kg/min). Additionally, the effect of the combined (non-selective) AT₁/AT₂-receptor antagonist saralasin (0.001, 0.003, 0.01 or 0.03 mg/kg/min) on stimulation-induced responses was studied. In addition, we measured PRA-levels after administration of the AT₁-receptor antagonist irbesartan in this model.

The stimulation-induced increase in diastolic blood pressure (DBP) could be dose-dependently reduced by irbesartan. The U-shaped dose-response relationship observed with irbesartan, which is illustrative for other AT₁-receptor antagonists in this model, was not observed when PD 123319 was co-administered with irbesartan, nor with the non-selective AT-blocker saralasin. PRA-levels increased from 111.0 ± 17.8 to 198.7 ± 22.2 ng/ml/h after administration of irbesartan. PRA-levels did not differ when measured after the three highest doses of irbesartan.

The present findings indicate a facilitatory role for the AT₂-receptor, which is unmasked by the highest dose of an AT₁-antagonist, in this case irbesartan. Different plasma Ang II-levels are unlikely to have caused the less than maximal inhibition after the highest dose of irbesartan.

Chapter 7

Angiotensin II can enhance sympathetic neurotransmission by acting on AT₁-receptors that are located on sympathetic nerve terminals. We investigated presynaptic blockade by the selective AT₁-receptor antagonist irbesartan in pithed spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto Rats (WKY). We compared the pre-synaptic inhibitory dose with that required for the blockade of AT₁-receptors on vascular smooth muscle in both strains. To investigate blockade of presynaptic AT₁-receptors, we studied the effect of irbesartan on the sequelae of electric stimulation of the thoraco-lumbar sympathetic outflow (0.25-8 Hz). To study the interaction between postsynaptic AT₁-receptors and α-adrenoceptors, the effects of
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Irbesartan on pressor responses to exogenous noradrenaline were established. Additionally, we studied the effect of irbesartan on dose-response curves for the vasoconstriction induced by exogenous angiotensin II.

Pressor responses to electrical stimulation of the thoracolumbar spinal cord, to exogenous Ang II, as well as to noradrenaline were enhanced in SHR compared to WKY. The stimulation-induced rise in DBP could be dose-dependently reduced by irbesartan (0.3-10 mg/kg) in both SHR and WKY. IC₅₀-values (doses which suppress the rise in DBP by 50% compared to control) were 5.60 ± 0.09 and 5.72 ± 0.08 –log mol/kg for SHR and WKY, respectively (p>0.05). In SHR, no effect of irbesartan (3 mg/kg) on pressor responses to exogenous NA was observed. In contrast, in WKY, irbesartan (3 mg/kg) caused a rightward shift of the dose-response curve to exogenous noradrenaline. Irbesartan (0.3-3 mg/kg) caused a depression of Eₘₐₓ-values and a rightward shift of the dose-response curves to exogenous Ang II in a similar fashion in both SHR and WKY. Both in SHR and in WKY, Ang II exerts a facilitatory effect on sympathetic neurotransmission, which is mediated by prejunctional AT₁-receptors in both strains. Irbesartan displays comparable sympatho-inhibitory potency in the normotensive and hypertensive pithed rat preparations. A facilitatory effect via postsynaptically located AT₁-receptors on α-adrenoceptor-mediated responses exists in WKY, but not in SHR. In both strains the required dose to inhibit presynaptic effects is somewhat higher than the dose required to inhibit postsynaptic effects. No differences, therefore, seem to exist between the two strains regarding the affinity of irbesartan for pre- and postjunctional AT₁-receptors, respectively.

Chapter 8

Both in human and in experimental heart failure, the renin-angiotensin system and the sympathetic nervous system are activated. In a previous study we demonstrated a facilitatory action of Ang II in the rabbit mesenteric artery, which was mediated via prejunctionally located AT₁-receptors. Very little is known about the effects of Ang II on sympathetic neurotransmission at the peripheral level in congestive heart failure.

Accordingly, in the isolated mesenteric arteries obtained from rabbits suffering from experimentally induced congestive heart failure (CHF), as well as in age-matched control rabbits we investigated the effect of Ang II on contractions provoked by electrical field stimulation (EFS), in the presence and absence of the AT₁-receptor antagonist eprosartan. Additionally, to investigate a possible post-junctional facilitation, the effects of Ang II on α-adrenoceptor
mediated responses were studied using noradrenaline. Lastly, the vasoconstrictor effects of Ang II were compared between HF-rabbits and controls, by constructing concentration-response curves to Ang II.

In control rabbits, Ang II 0.5 nM caused an enhancement of stimulation-induced responses by a factor $3.2 \pm 0.5, 2.4 \pm 0.3$ and $1.5 \pm 0.08$, at 1, 2 and 4 Hz, respectively ($p<0.05$ at all frequencies compared to vehicle). In rabbits with heart failure, the enhancement by Ang II (0.5 nM) amounted to a factor $2.1 \pm 0.2, 1.7 \pm 0.1$ and $1.2 \pm 0.04$, at 1, 2 and 4 Hz, respectively ($p<0.05$ compared to vehicle at all frequencies). Accordingly, the enhancing effect of Ang II was more pronounced in the control group compared to rabbits with HF ($p<0.05$ at each frequency).

Eprosartan (1 nM – 0.1 µM) could inhibit the facilitatory effects of Ang II in arteries from HF- as well as from control rabbits. Contractile responses to exogenous noradrenaline (3 nM – 0.1 mM) were the same in HF-rabbits and controls, and they were unaltered in the presence of Ang II 0.5 nM. Ang II (0.3 nM – 1 µM) caused a concentration-dependent increase in contractile force, which was the same in HF-rabbits and controls. From these findings we conclude that in rabbits with CHF as well as in control animals, Ang II facilitates the stimulation-induced vasoconstrictor responses via prejunctionally located AT$_1$-receptors. The facilitating effect was decreased in vessels obtained from rabbits with CHF, while responses to exogenous Ang II were unchanged. These findings may be explained by down-regulation or uncoupling of the prejunctional AT$_1$-receptor. The sympathetic inhibitory potency of eprosartan proved unchanged in vessels obtained from rabbits with CHF compared to controls.

Chapter 9

Angiotensin II can enhance responses to electrical field stimulation in various isolated tissues via AT$_1$-receptors, located on sympathetic nerve terminals. Differences in potency exist between AT$_1$-receptor antagonists regarding inhibition of the prejunctional (on sympathetic nerve terminals) and postjunctional AT$_1$-receptors (on vascular smooth muscle), respectively. It was suggested that prejunctional AT$_1$-receptors may be of the AT$_{1h}$-receptor subtype.

Accordingly, we investigated whether inhibition of the AT$_{1h}$-receptor by high concentrations of PD 123319 could suppress the facilitatory actions of Ang II on noradrenergic neurotransmission in the isolated rabbit mesenteric artery. We also investigated whether PD 123319 could influence the (direct) vasoconstrictor responses to Ang II. Ang II (0.5 nM) caused a significant enhancement of responses to EFS by a factor $2.9 \pm 0.3, 2.3 \pm 0.3$ and $1.6 \pm 0.1$, at 1, 2 and 4
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Hz, respectively (p<0.05 compared to vehicle). This enhancement could be inhibited by PD 123319 (1 and 10 μM). The cumulative concentration-response curve for vasoconstriction caused by Ang II was unaffected by PD 123319 (10 μM), the highest concentration of PD 123319 that was applied in the stimulation experiments.

From these findings we conclude that the presynaptic AT_{1}-receptors are of the AT_{1r}-subtype, and postsynaptic AT-receptors are not. Since in other studies vasoconstrictor effects were shown to be inhibited by selective AT_{1}-receptor antagonists, AT_{1r}-receptors on vascular smooth muscle most likely belong to the AT_{1r}-receptor subtype.

Chapter 10

It was the objective of this study to investigate whether in humans, a facilitatory role of Ang II on sympathetic nerve traffic can be demonstrated at the peripheral level. Therefore, using venous occlusion plethysmography, we investigated the effect of Ang II on tyramine induced responses.

10 healthy male subjects (28 ± 1 years) participated in the study. All drugs were infused intrararterially. The protocol consisted of two dose-response curves (DRC) of tyramine (0.25, 0.5, 1.25 and 2.5 ng/kg/min), respectively. Each dose was infused during 5 minutes. Each DRC was performed during infusion of sodium nitroprusside (10 ng/kg/min) for predilation purposes. In five subjects, the first DRC was performed under concomitant infusion of vehicle (0.9 % NaCl), and the second DRC under concomitant infusion of a subpressor dose Ang II (0.1 ng/kg/min). In the five other subjects the first DRC was performed during infusion of Ang II, the second during infusion of vehicle.

Baseline forearm blood flow was 2.8 ± 0.2 ml/100ml/min. Sodium nitroprusside (10 ng/kg/min) increased the FBF to 6.8 ± 0.3 (p<0.05 compared to baseline). After infusion of vehicle or angiotensin II, FBF-values were 6.9 ± 0.5 and 6.8 ± 0.6, respectively (NS compared to after SNP). Tyramine caused a dose dependent decrease in forearm blood flow (12 ± 5, 40 ± 6, 63 ± 3 and 74 ± 3 % for 0.25, 0.5, 1.25 and 2.5 ng/kg/min, respectively). Ang II significantly enhanced the FBF-responses to tyramine (25 ± 6, 51 ± 4, 72 ± 4 and 77 ± 3 %, respectively, p<0.05, repeated measures ANOVA).

We conclude that Ang II can facilitate tyramine induced vasoconstriction in healthy volunteers.
Summary