Pre- and postsynaptic studies concerning the interaction between the renin angiotensin system and the sympathetic nervous system
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GENERAL DISCUSSION AND CONCLUSIONS
General discussion and conclusions

In recent years a vast body of research has addressed the interaction between the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS). These cardiovascular control systems are known to be extensively involved in hypertension and heart failure. After numerous functional studies concerning the RAS-SNS interaction by our group we pursued these investigations by means of a modified biochemical spillover technique. Important tools for these investigations are the now available angiotensin AT₁-receptor antagonists (sartans).

In the present study, we developed a variant of the classical superfusion technique, which involves the use of a radiolabeled tracer (e.g. \(^{3}H\)) inserted into the neurotransmitter molecule, to quantify the activities of the peripheral sympathetic nervous system. Accordingly, we quantified the enhancing effect of angiotensin II on sympathetic neurotransmission and its sequelae. At the presynaptic level of the sympathetic nerve terminal we investigated the inhibitory potency of various selective angiotensin AT₁-receptor antagonists. We compared this sympatho-inhibitory potency with the inhibitory potency regarding postjunctional angiotensin AT₁ receptors, located on the vascular smooth muscle. We additionally addressed the issue of receptor subtype-differences between the prejunctional and postjunctional sites. Furthermore, we investigated whether angiotensin II could enhance sympathetic nerve traffic in a pathological model (a rabbit model of induced heart failure), and whether this process could be modulated by a selective AT₁-receptor antagonist. At both the level of the peripheral sympathetic nervous system and the vasculature we studied the influence of maturation of young animals on angiotensin II-mediated responses and AT₁-receptor blockade. Lastly, we investigated the validity of our developed model to modulate sympathetic nerve traffic using drugs which do not primarily influence the RAS, that is different types of calcium antagonists.

In the isolated rabbit thoracic aorta electrical field stimulation (EFS; 2 Hz, 3 ms, 150 mA) caused a significant increase of tritium-label release by approximately a factor 4. The fractional release of noradrenaline during two consecutive periods of stimulation remained unaltered. The EFS-evoked release could be (nearly) abolished by the selective sodium-channel blocker tetrodotoxin in addition to the N-type calcium channel antagonist ω-conotoxin GVIA. The postganglionic sympathetic neuron blocker guanethidine decreased the EFS-evoked sympathetic outflow to approximately fifty percent. These results thus confirm that in our modified model the applied EFS-procedure selectively evoked neuronal release of neurotransmitter by activation of
sympathetic neurons. Angiotensin II clearly and concentration-dependently enhanced the stimulation-evoked \[^3\text{H}\text{-noradrenaline release. Our modified experimental approach appears to be suitable to study presynaptic influences on sympathetic transmission in the rabbit thoracic aorta.}

We further investigated the influence of angiotensin II on stimulation-induced sympathetic nerve traffic. In the isolated rabbit thoracic aorta angiotensin II caused a ‘bell-shaped’ concentration-dependent enhancement of EFS-evoked noradrenaline release, with a maximum of approximately 100%. This phenomenon may be explained by attributing the attenuation of angiotensin II-enhanced sympathetic neurotransmission to concomitant release of prostaglandins. The AT\(_1\)-receptor blockers losartan, telmisartan and irbesartan concentration-dependently and equieffectively inhibited the facilitating effect of angiotensin II. The facilitating effect of angiotensin II on the sequelae of neuronal stimulation appears to be mediated by prejunctionally located AT\(_1\)-receptors.

To determine the inhibitory profile of two angiotensin AT\(_1\) receptor antagonists at the level of the prejunctional and postjunctional AT\(_1\)-receptor we assessed the inhibitory potency of the selective angiotensin AT\(_1\) receptor antagonists, eprosartan and candesartan. Angiotensin II caused a 2-fold increase of the stimulation-evoked sympathetic outflow, which was concentration-dependently inhibited by both eprosartan and candesartan. At the level of the vasculature angiotensin II caused a concentration-dependent increase in contractile force that was counteracted in a competitive manner by eprosartan, whereas candesartan displayed non-competitive antagonism. Candesartan proved a more potent antagonist than eprosartan at both the prejunctional and postjunctional angiotensin AT\(_1\)-receptor. For eprosartan, however, vascular inhibitory concentrations were 10-fold lower than sympatho-inhibitory concentrations, whereas for candesartan inhibitory concentrations at both sites were similar. Our findings suggest differences in affinity for the pre- and postjunctional AT\(_1\)-receptor or may be explained by angiotensin AT\(_1\)-receptor subtype differences.

We found that for eprosartan differences in inhibitory potency exist regarding the AT\(_1\)-receptor at the prejunctional site compared to the postjunctional site, respectively. We hypothesized, as was suggested in the literature, that the prejunctional AT\(_1\)-receptor might belong to the pharmacological AT\(_{1B}\)-receptor subtype. We investigated whether PD123319 (a low angiotensin AT\(_2\)-receptor antagonist, that in high concentrations is known to block the AT\(_{1B}\)-receptor) could
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suppress angiotensin II-augmented noradrenergic transmission in addition to its influence on angiotensin II-mediated vasoconstriction. In both the isolated rabbit thoracic aorta and the mesenteric artery PD123319 attenuated the angiotensin II-enhanced sympathetic nerve traffic, whereas the constrictor response to angiotensin II remained unaffected by addition of PD123319. From these findings we conclude that the prejunctional AT₁-receptor belong to the AT₂ receptor subtype, whereas the postjunctional AT₁-receptor consists of the AT₃ receptor subtype.

Recently, it was demonstrated that inhibition of angiotensin II-mediated facilitation in the pithed rat (in vivo) by the selective AT₁-receptor antagonist irbesartan resulted in a U-shaped dose response curve, which was not observed when PD123319, in a concentration that selectively blocks the AT₂-receptor, was co-administered. Hence, the irbesartan-mediated “upstroke” might be explained by the involvement of the AT₂-receptor after AT₁-blockade with high-dose irbesartan. We further studied this issue in vitro. In the rat isolated vena cava inferior angiotensin II caused a concentration-dependent enhancement of EFS-evoked noradrenaline release by maximally 60%. PD123319, in a concentration known to block the AT₂-receptor did not influence angiotensin II-enhanced sympathetic nerve traffic. Irbesartan concentration-dependently attenuated the angiotensin II-augmented transmitter release, whereas no U-shaped concentration-response relationship for irbesartan was observed. Co-administration of PD123319 with irbesartan proved unable to influence angiotensin II-mediated facilitation differently compared to irbesartan alone. The experimental observations indicate that the AT₂-receptor is not involved in angiotensin II-mediated enhancement of sympathetic nerve traffic in the present in vitro study, as was evidenced previously.

Congestive heart failure is associated by activation of the renin angiotensin system (RAS) and the sympathetic nervous system (SNS). Both systems are known to interact and to potentiate each others activities. In isolated thoracic aorta preparations, obtained from rabbits suffering from experimentally induced congestive heart failure (CHF) and from age-matched control animals, angiotensin II caused a concentration-dependent increase of the stimulation-evoked sympathetic nerve traffic. Facilitation of sympathetic neurotransmission was less significant in CHF-preparations. Eprosartan concentration-dependently attenuated the angiotensin II-enhanced sympathetic outflow in both CHF- and control preparations, thus confirming that angiotensin II evoked facilitation is mediated by the prejunctional AT₁-receptor in both control and CHF-preparations. Additionally, the sympatho-inhibitory potency of eprosartan was similar in both
groups. Angiotensin II concentration-dependently increased the contractile force in control preparations, which could be attenuated by eprosartan. In CHF-preparations no angiotensin II-mediated contraction was observed. Moreover, potassium- and noradrenaline-induced responses were attenuated in CHF-preparations compared to responses observed in control preparations. The decreased facilitation of SNS effects by angiotensin II may be explained by downregulation or desensitisation of the neuronal AT₁-receptor. Additionally, the aortic contractile capacity in CHF-preparations appears to be decreased, probably as a result of heart failure-associated neuroendocrine and functional changes.

We additionally investigated the influence of maturation (young versus adult) on the angiotensin II-mediated facilitation of sympathetic nerve traffic (prejunctional AT₁-receptor) and on the angiotensin II-mediated vasoconstriction (postjunctional AT₁-receptor). Moreover, the inhibitory effect of eprosartan on angiotensin II-mediated responses at both sites was studied. Angiotensin II concentration-dependently and equi-effectively enhanced the EFS-evoked noradrenaline release in both groups, although a difference in potency was observed (young > adult). Eprosartan attenuated the angiotensin II-mediated responses in both groups. Angiotensin II caused a similar increase in contractile force with a difference in potency (young > adult). Surprisingly, eprosartan inhibited the angiotensin II-mediated contractions in a competitive manner in young adolescents, whereas a mixed form of antagonism, in the same concentration range, was observed in young adult rabbits. The responses at both the pre- and postjunctional site may be explained by a maturation-dependent AT₁-receptor desensitisation or expression. This additionally may offer a possible explanation for the different types of antagonism induced by eprosartan in young compared to adult rabbits.

Lastly, the validity of our developed model to study sympathetic nerve traffic was tested by using fully different cardiovascular drugs, such as various types of voltage dependent calcium channel (VDCC) antagonists. Sympathetic neurotransmission is extensively dependent on calcium influx via N-type calcium-channels located on sympathetic nerve terminals. We compared the claimed sympatholytic effect of the 1,4-dihydropyridine compound cilnidipine with other voltage-dependent calcium channel (VDCC) antagonists. In the isolated rabbit thoracic aorta preparation cilnidipine as well as the combined N/L-type calcium channel blocker mibebradil significantly attenuated the response to EFS in a noradrenaline spillover model, which may be explained by partial N-type channel blockade. The 1,4-dihydropyridines nifedipine and amlodipine did not influence the evoked noradrenaline release. The stimulation-induced constrictor response
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(prejunctional effect) in the rat isolated tail artery could be attenuated by cilnidipine, whereas it did not influence the constrictor response to noradrenaline (postjunctional effect). These findings advocate an N-type channel blocking effect of cilnidipine in this preparation as well. In the pithed rat model, however, the responses to electrical stimulation of the cardio-accelerator nerves (C7–Th1, prejunctional effect) were equally attenuated as responses to noradrenaline (postjunctional effect) by the various calcium channel antagonists. We conclude that the presumed sympatholytic properties of cilnidipine could be demonstrated in vascular tissue but not in cardiac tissue and that our model appears to be suitable to study the activities of the sympathetic nervous system.

In general, we demonstrated by means of different agents, such as angiotensin II and various voltage dependent calcium antagonists, that our modified experimental approach appears to be suitable to study presynaptic influences on sympathetic transmission. In our model angiotensin II can clearly facilitate sympathetic nerve traffic. In the animal in vitro model this enhancement was mediated via prejunctionally located AT₁-receptors. In addition, all selective AT₁-receptor antagonists can inhibit the prejunctional AT₁-receptor, although with differences in sympatho-inhibitory potency. Consequently, sympatho-inhibition appears to be class effect of the AT₁-receptor antagonists. Regarding the difference in inhibitory potency displayed by eprosartan and PD123319 at the prejunctional compared to the postsynaptic AT₁-receptor, we suggest the existence of pharmacologically different AT₁-receptor subtypes. This concept is supported by the observation that angiotensin II-mediated facilitation occurs at concentrations lower than those required to induce vasoconstriction. We evidenced that the prejunctional AT₁-receptors belongs to the AT_{1B}-subtype, whereas postjunctional AT₁-receptors do not. It appears that facilitation of the SNS by angiotensin II in congestive heart failure is attenuated. This phenomenon may be explained by downregulation or desensitisation of the presynaptic AT₁-receptor. Functionally, the peripheral vascular contractile capacity appears to be decreased in heart failure, probably as a result of neuroendocrine changes. Maturation appears to influence the responses to angiotensin II based on maturation dependent AT₁-receptor expression.

It is concluded that the interaction between the RAS and the SNS appears to be highly relevant, both for pathophysiologival and therapeutic reasons.