The interaction between vasopressin and modulators of the cardiovascular system
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GENERAL DISCUSSION AND CONCLUSIONS
General discussion and conclusions

The neuro hormone vasopressin (AVP), which appears to display a relevant role in certain cardiovascular and renal diseases, is known to interact with a few important modulators of the cardiovascular system. In the present study we therefore investigated three of such modulators which may interact with the vascular function of the neurohormone AVP. The first modulator evaluated was the peripheral sympathetic nervous system (SNS). The interaction with AVP was investigated in the isolated mesenteric artery; furthermore we determined whether the potentiating role of AVP on the SNS could be demonstrated in the intact circulation of the pithed rat and in the forearm circulation of humans. We investigated the receptor selectivity, and the pre- and post-synaptic sites of action. This type of investigation has been greatly facilitated by the introduction of non-peptidergic vasopressin receptor antagonists, which are relatively selective for the V1- or V2-receptor, respectively. Before we focused on the second modulator we performed a methodological study for isometric recordings of vessels in a standard organ bath set-up. In this study we investigated the interaction of precontracting levels and measured vasodilation, induced by well-known vasodilating compounds. The second modulator of our interest was the endothelium. To study the role of endothelium dependent factors we used the renal artery of the rabbit to assess the interaction with AVP. Lastly we investigated the extracellular signal-regulated kinase (MAPK\textsuperscript{erk}) subfamily of the mitogen-activated protein kinase pathway, with respect to its possible interaction with AVP-induced vasoconstriction, using the isolated rat aorta.

The characteristics of the V-receptors involved in the effects of the various agonists/antagonists were studied in the isolated mesenteric artery of the rat. We applied electrical field stimulation to activate sympathetic nerve endings. A sub-pressor concentration AVP facilitated the contractile response of the vessel segments induced by sympathetic nerve activation. The V\textsubscript{1}-antagonist SR-49059 displayed AVP-inhibitory activity on both the direct vasoconstrictor effects and on the potentiating effects on the stimulated SNS. The selective V\textsubscript{2}-agonist desmopressin showed no direct vasoconstrictor or vasodilator effects, nor any effect on the sequelae of sympathetic nervous activity. SR-121463 B, a selective V\textsubscript{2}-antagonist, proved less active. The post-synaptic
effects were evaluated by studying the vasoconstrictor action of noradrenaline. AVP proved to facilitate the selective post-synaptic stimulation by noradrenaline. Taken together these results indicate that AVP facilitates the sympathetic nerve activity via the V₁-receptor, at least partly via the postsynaptic receptors. It cannot be decided to which extent pre-synaptic V₁-receptors may be involved.

Subsequently we investigated whether the potentiation of the sympathetic neurotransmission by AVP, already shown in vitro, could be demonstrated in the in vivo model of the pithed rat. Initially we failed to demonstrate the facilitation of the peripheral sympathetic nervous activities by AVP. Since in this model the facilitation was already at an optimal level due to the high circulating levels of Angiotensin II. However, after blockade of the AT₁-receptors with irbesartan, it became possible to investigate the potentiating effect of a sub-pressor dosage of AVP on the effects of the peripheral SNS. This facilitating effect was V₁-receptor mediated, as proven by means of the selective V₁ and V₂-antagonists SR-49059 and SR-121463 B, respectively, and the V₂-agonist desmopressin. In this experimental model with a relatively intact circulation, a sub-pressor dose of AVP proved unable to influence the pressor effect of noradrenaline. From these findings we conclude that a sub-pressor dosage of AVP can facilitate the peripheral sympathetic nervous effect, by pre-synaptically located V₁-receptors, whereas AVP does not attenuate postsynaptic effects.

A third study was conducted to evaluate the interaction of AVP and the peripheral SNS in humans. We used the model of forearm venous occlusion plethysmography in combination with general SNS activation induced by the application of lower body negative pressure (LBNP). Forearm blood flow decreased after initiation of the LBNP, and this effect could be potentiated by the presence of a continuous infusion with a sub-pressor dosage of AVP. The vasoconstrictor effect of noradrenaline mediated by postsynaptic α₁-adrenoceptors proved uninfluenced by a continuous infusion of sub-pressor dosage of AVP. Consequently we conclude that AVP facilitates in the human forearm the actions of peripheral sympathetic nervous activity, via pre-synaptic V-receptors.
In vascular pharmacology the organ bath set-up for isometric recordings is a well-known method for the evaluation of vasodilator properties of new compounds. Before the vascular characteristics of a certain vasodilator can be evaluated, it is necessary to pre-contrace the vessel segment. Therefore we aimed to determine to what extent the vasodilator properties are dependent of the mechanism of precontraction and the level of precontraction. These results illustrate the importance when comparing different vasodilators, the levels of precontraction should be comparable and preferably at a sub-maximal level.

We investigated in the isolated rabbit renal artery the interaction between AVP and the endothelium, an important modulator of vascular tone. AVP-mediated vasoconstriction was increased in the presence of the NO-synthase inhibitor L-NNA, whereas endothelium removal did not influence AVP-mediated vasoconstriction, when compared with endothelium intact segments. These results are in contrast with the data derived from phenylephrine-induced vasoconstriction, which showed an increase of contractile force in endothelium-denuded vascular segments. By means of SR 49059 and SR 121463 was demonstrated that the AVP-mediated vasoconstriction was V₁-receptor dependent. These data suggest a V₁-receptor mediated release of an endothelium-dependent contractile factor, which proved uninfluenced by indomethacin (a cyclooxygenase inhibitor), meclofenamic acid (a cyclooxygenase and lipoxygenase inhibitor), or bosentan (an endothelin antagonist).

Activation of several G-protein-coupled receptors can result in a mitogen-activated protein kinase\textsuperscript{erk1/2} (MAPK\textsuperscript{erk1/2})-dependent vasoconstriction. Although in cultured cells the AVP-induced MAPK\textsuperscript{erk1/2}-activation has been demonstrated to be involved in mitogenic and hypertrophic effects, a direct link with vasoconstriction has not been established so far. For this reason we investigated the effects of PD 98059 and U 0126, two MAPK\textsuperscript{erk1/2} kinase inhibitors, on the AVP-induced vasoconstriction and also on MAPK\textsuperscript{erk1/2} phosphorylation. Western blot analyses revealed that AVP stimulated the MAPK\textsuperscript{erk1/2} phosphorylation in differentiated rat aortae segments, and PD 98059 or U 0126 dose dependently prevented this effect. In the same concentration range these MAPK\textsuperscript{erk1/2} kinase inhibitors inhibited AVP-induced vasoconstriction. These data indicate that the MAPK\textsuperscript{erk1/2} pathway is involved in AVP-mediated vasoconstriction.
The following general conclusions can be drawn from the studies presented in this thesis.

1) AVP is able to facilitate the peripheral sympathetic neurotransmission via the stimulation of V₁-receptor subtypes. Pre- and postsynaptic sites seem to be involved in this effect.

2) AVP is able to release endothelial factors which are either vasodilators, like NO, or vasoconstrictors. The latter are at least partly responsible for the overall vasoconstrictive effect of AVP. Although several candidates have been eliminated, the nature of the constricting endothelial factor remains unknown.

3) The MAPKerk1/2 pathway has been identified to be part of the signal transduction which mediates the mechanical responses of vascular tissue to AVP.
General discussion and conclusions