The Interaction between vasopressin and modulators of the cardiovascular system

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Citation for published version (APA):

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CHAPTER 3

VASOPRESSIN-INDUCED PRE-SYNAPTIC FACILITATION OF SYMPATHETIC NEUROTRANSMISSION IN THE PITHED RAT.

Journal of Hypertension 2002; 20: 1175-1180
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1. Introduction

The neurohypophyseal peptide hormone arginine vasopressin (AVP) is known to regulate the water reabsorption in the principal cells of the renal collecting duct via the V₂ receptor. AVP is also a potent vasoconstrictor in several vessels through the V₁ receptor. Furthermore AVP appears to mediate vasodilation in some vascular beds through the V₂ receptor. AVP also exerts haemostatic activity, via enhanced platelet aggregation and an increased release of coagulation factors. Moreover AVP regulates the release of adrenocorticotropic hormone (ACTH), through activation of the V₃-pituitary receptor (or V₁b). Elevated plasma AVP levels are a common finding in congestive heart failure and this could play a role in the pathophysiology of this syndrome. Several studies have evaluated a possible role of AVP in essential hypertension. These analyses did not yield conclusive results, but it seems possible that AVP plays a certain role in subgroups of hypertensive subjects. There occurs an important interaction with the sympathetic nerves system in both clinical syndromes. Vasopressin may have indirect effects on the perivascular nerve fibres of the sympathetic nervous system.

The possibility to study the interaction between vasopressin and sympathetic neurons has been improved by the availability of selective, non-peptidergic vasopressin receptor antagonists. The peptidergic AVP antagonists were of limited clinical value because of their short half life, low bioavailability and exclusive parenteral administration. Yamamura et al. were the first to describe a non-peptide AVP antagonist. In the past decade new non-peptide AVP antagonists have been developed, with a better profile, regarding their affinity, efficacy and selectivity for either the V₁-or V₂-receptor.

The present study was designed to investigate whether the potentiation of the sympathetic neurotransmission by vasopressin, already shown in in vitro experiments, could be demonstrated in the in vivo model of the pithed rat. Furthermore it was studied by which receptor subtype AVP could potentiate the sympathetic neurotransmission in vivo. In addition we wanted to evaluate whether this facilitation is pre- or post-synaptically mediated. We used the new non-peptide AVP antagonists SR-49059 (V₁) and SR-121463 B (V₂) as
pharmacological tools for the analysis of the interaction between vasopressin and the sympathetic neurons in the present study.

2. Methods

Experimental procedures
During anaesthesia (hexobarbital, 150 mg/kg, i.p., and after the administration of lidocaine 2%, s.c. in the cervical region) male normotensive Wistar rats of 290-320g (Charles River, France) received a tracheal cannula and were subjected to a pithing procedure. Immediately thereafter they were subjected to artificial respiration with ambient air (60 strokes/min, 200 ml/min) using a positive pressure (Braun Melsungen) pump. The pithing procedure was performed by introducing a partially isolated steel rod through the right orbit and foramen magnum into the spinal canal. Electrical stimulation of the thoracic-lumbar (T5-L4) segments of the spinal cord was performed via the non-coated segment (3.5 cm length) of the pithing rod. The non-coated segment was located 2.5 cm proximally from the tip of the pithing rod. The body temperature of C was maintained by means of a thermostatically controlled heating table. The right jugular vein was catheterised and heparin (1000 IU/kg) was injected via this route. A cannula was introduced into the ipsilateral common carotid artery for arterial blood pressure measurement, by means of a pressure transducer connected to a MacLab data acquisition system (AD Instruments, Castle Hill, Australia). The heart rate was derived on-line from the blood pressure recording. Bilateral vagotomy in the cervical region and bilateral adrenalectomy were performed. The animals were pre-treated with d-tubocurarine (2.5 mg/kg, i.v.) to attenuate muscle contractions during electrical stimulation, propranolol (1 mg/kg i.v.) to rule out β-adrenoceptor-mediated effects, and atropine (2 mg/kg s.c.), to suppress parasympathetic effects, respectively. After an equilibration period of 15 min, irbesartan 30 mg/kg i.v. was administered to prevent the actions of angiotensin II. The infusion of vasopressin 1 pmol/kg/min was initiated 5 min thereafter. The animals were left to recover for 10 minutes. Subsequently, the sympathetic nervous system was locally stimulated, at the level of the T5-L4 segments, at frequencies of 0.125, 0.25, 0.5, 1, 2, 4 Hz (15 seconds per frequency) at 50V with square wave pulses of 2 ms, delivered by a HSE stimulator I. After each period of stimulation at a given frequency, blood pressure was allowed to return to baseline.
We used the vasopressin antagonists SR-49059 (V₁) or SR-121463 B (V₂) and the V₂ agonist desmopressin in order to analyse the possible involvement of V₁ and V₂ receptors. SR49059 10 mg/kg was given orally, at least 60 min. prior to the measurements. SR-49059 is known to inhibit the pressor response to exogenous AVP in conscious normotensive rats for more than 8 hours (at 10 mg/kg p.o.). SR-121463 B 3 mg/kg was given i.v., at least 15 min. prior to the measurements. This dosage is known to induce a marked V₂ mediated diuresis in normally hydrated rats. Desmopressin administered in a dosage lower than 100 pmol/kg/min is known to induce a marked V₂-mediated antidiuretic action.

To evaluate the possible postjunctional effects of vasopressin or its antagonists on the pressor responses to α-adrenoceptor stimulation we applied noradrenaline intravenously in increasing dosages, in combination with the substance under investigation and in the combinations as used in the aforementioned experiments. The pithing procedure and pre-treatment were the same as in the stimulation experiments. The pithed animals were allowed
to equilibrate for at least 15 minutes. Subsequently, increasing doses of noradrenaline 0.03 nmol/kg – 0.3 μmol/kg were administered i.v. in volumes of 0.5 ml/kg. Between doses, blood pressure was allowed to return to baseline. Only the highest doses were injected in a cumulative manner. One complete dose-response curve was constructed in each animal.

Statistical evaluation

The data are expressed as means ± S.E.M. for at least n = 6 animals. The dose/stimulation-response curves for the compounds investigated were analysed by means of a computer program (Graph Pad, Institute for Scientific Informatics, San Diego, CA, U.S.A.). The pD2-value [-log effective concentration (molar) that produce 50% of the maximal inhibitory effect (IC50)], as well as the maximal effect (Emax) were thus obtained from the non-linear regression curve fit analysis for the individual experiments. The statistical significance of the differences was evaluated using a one-way analysis of variance followed by a Tukey post test. In appropriate cases a two-tailed Student's t-test for unpaired data was used. P values <0.05 were considered to indicate significant differences.

Drugs used

Irbesartan, SR49059 and SR-121463 B were a gift of Sanofi (Toulouse, France). The following substances were purchased: Vasopressin and desmopressin (Bachum, Switzerland), (±)-Propranolol HCl (RBI, USA), Atropine Sulfate, d-Tubocurarine Chloride and (-)-Norepinephrine bitartrate from (Sigma, USA). Norepinephrine was dissolved in saline containing L(+)Ascorbic acid [100 μg/ml]. SR-49059 10 mg/kg was administered orally in a 5% arabic gum suspension. All other drugs were dissolved in saline.

3. Results

After an equilibration period of 15 minutes after the pithing procedure the diastolic blood pressure (DBP) was 42.2 ± 1.3 mmHg and the heart rate (HR) was 311.9 ± 3.8 beats/min (n=23). The vasopressin antagonists SR-49059 10 mg/kg (V1) and SR-121463 B 3 mg/kg (V2) did not change the DBP or the HR of the pithed animals. Vasopressin (1 pmol/kg/min to 3 nmol/kg/min) caused
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a dose dependent increase in DBP by 90.8 ± 1.3 mmHg, at a potency of 10.2 ± 0.03 -log mol/kg (Fig. 1). The V₁ antagonist SR-49059 (10 mg/kg), induced a dose-dependent potency shift for vasopressin effects to 9.0 ± 0.03 -log mol/kg (P<0.05), at a comparable maximal DBP change by 96.1 ± 1.0 mmHg. In the presence of the V₂ antagonist SR-121463 B (3 mg/kg), the dose response curve of vasopressin remained unchanged.

Figure 2. The effect of vasopressin (AVP) on the frequency-response curve induced by electrical stimulation of the spinal cord (T5-L4) in the pithed normotensive rat, in the presence of irbesartan 30 mg/kg. The stimulation frequency (in Hz) is shown on the abscissa. The increase in diastolic blood pressure (expressed as mmHg) is shown on the ordinate. Values are expressed as mean ± SEM. * p < 0.05 compared to control. (n=10-13)

Stimulation experiments

The pithed rat is a high-renin model 25-27, in which endogenously generated angiotensin II facilitates neurally mediated increments in vascular resistance 28. Without the administration of the AT₁ receptor-antagonist irbesartan (30 mg/kg), a facilitating effect of AVP on the stimulation-induced rise in DBP was not observed (data not shown). After the administration of the AT₁-antagonist irbesartan (30 mg/kg), the facilitating effect of AVP became apparent. The administration of the AT₁-antagonist irbesartan (30 mg/kg) as such decreased
the baseline blood pressure to a level of 19.6 ± 1.2 mmHg (n=10). The heart rate was uninfluenced by the administration of the AT1-antagonist irbesartan (30 mg/kg).

For this reason all our stimulation and noradrenaline dose response experiments were performed in the presence or absence of the sub-pressure continuous infusion of AVP 1 pmol/kg/min or vehicle, respectively. The heart rate and DBP were not affected by the presence of AVP (1 pmol/kg/min) or by the administration of the various AVP antagonists. In the stimulation experiments the pre- and postsynaptic effects of AVP were evaluated simultaneously, in contrast to the experiments with exogenous noradrenaline,

![Figure 3](image)

**Figure 3.** The effect of vasopressin (AVP) and SR49059 (V₁-antagonist) on the frequency-response curve induced by electrical stimulation of the spinal cord (T5-L4) in the pithed normotensive rat, in the presence of irbesartan 30 mg/kg. The stimulation frequency (in Hz) is shown on the abscissa. The increase in diastolic blood pressure (expressed as mmHg) is shown on the ordinate. Values are expressed as mean ± SEM. * p < 0.05 control vs AVP 1 pmol/kg/min. $ p < 0.05 control vs AVP 1 pmol/kg/min and SR-49059 10 mg/kg. (n=6-13)

where we investigated the post-synaptic effects of vasopressin only. Electrical stimulation of the thoracic-lumbar segments of the spinal cord resulted in a
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frequency-dependent increase in diastolic blood pressure. As depicted in figure 2 the DBP increased at 4 Hz by 63.7 ± 4.5 mmHg (n=10) and this increase was enhanced in the presence of AVP 1 pmol/kg/min to a rise by 78.6 ± 4.2 mmHg (n=10, p<0.05).

This increase in DBP by 23 % could be completely antagonised by the V₁-receptor antagonist SR-49059 10 mg/kg (figure 3). At stimulation frequencies of 0.5 and 1 Hz the DBP increase was inhibited by 43.8% and 37.2%, respectively (p<0.05). In the absence of AVP, SR-49059 did not influence the stimulation-induced increase in blood pressure. In the presence or absence of AVP the V₂-receptor antagonist SR-121463 B 3 mg/kg did not influence the rise in blood pressure (figure 4). Similary the V₂-agonist desmopressin 100 pmol/kg/min did not influence the pressor response to electrical stimulation.

![Figure 4](image_url)

**Figure 4.** The effect of vasopressin (AVP) and SR-121463 B (V₂-antagonist) on the frequency-response curve induced by electrical stimulation of the spinal cord (T5-L4) in the pithed normotensive rat, in the presence of irbesartan 30 mg/kg. The stimulation frequency (in Hz) is shown on the abscissa. The increase in diastolic blood pressure (expressed as mmHg) is shown on the ordinate. Values are expressed as mean ± SEM. (n=6-13)
Exogenous noradrenaline

Again, these experiments were performed in the presence of irbesartan (30 mg/kg). As depicted in figure 5, exogenous noradrenaline provoked a dose dependent DBP increase by $117.6 \pm 0.9$ mmHg at a potency ($pD_2$) of $8.6 \pm 0.03$ -log mol/kg. The dose response curves for the pressor effect of i.v. noradrenaline were the same in the presence or absence of AVP, SR-49059 or SR-121463 B. Accordingly, facilitation of the vasoconstrictor response to noradrenaline did not occur. Neither did a V1- or V2-receptor antagonist influence this response.

**Figure 5.** Effects of vasopressin (AVP) 1 pmol/kg/min on the dose-response curve induced by intravenously administered noradrenaline (NA) in the pithed normotensive rat, in the presence of irbesartan 30 mg/kg. 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. Values are expressed as mean ± SEM. (n=6-8)
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4. Discussion

In the present study, we demonstrated the facilitating effect of exogenous vasopressin on sympathetic neurotransmission in the pithed rat model. This facilitating effect can be modulated by the V₁ antagonist SR-49059, but not by the V₂ antagonist SR-121463 B. Without exogenous vasopressin the V₁ and V₂ antagonists did not influence the increase in DBP after stimulation. In accordance with the results reported by others, 21;23 who used the antagonists in the same dosage leading to obvious V₁ or V₂ mediated effects, our findings indicate that there exists no active vasopressin-mediated tone in the pithed rat. Exogenous vasopressin in the presence of the V₁ antagonist SR-49059 was able to inhibit the DBP increase in the lower frequency range. This effect cannot be explained by the unmasking of an inhibitory V₂ receptor, because in the presence of the V₂ antagonist SR-121463 B vasopressin showed a comparable facilitation of the rise in DBP. Furthermore, desmopressin 100 pmol/kg/min did not influence the DBP increase during stimulation. The vasopressin-induced facilitation and its inhibition by a V₁ antagonist found in our experiments, are in accordance with in vitro experiments by others, 18;29-32 which suggest that the facilitating effect of vasopressin is entirely V₁-receptor dependent.

The facilitating effect of exogenous vasopressin is completely masked by the presence of endogenous angiotensin II in pithed rat. In our experiments the effects of vasopressin could therefore only be demonstrated to occur in the presence of the AT₁ antagonist irbesartan (30 mg/kg). The administration of the AT₁-antagonist irbesartan (30 mg/kg) as such decreased the baseline blood pressure to a level of 20.0 ± 3.1 mmHg. This confirms the active angiotensin tonus in the pithed rat model. Experiments in the pithed rat with exogenous noradrenaline in the presence or absencense of irbesartan showed completely comparable results. 27 This is a strong argument that the acute blood pressure-lowering effect per se, is not responsible for the facilitating effect seen in the presence of vasopressin.

The precise mechanism of the vasopressin-induced fascilitation of sympathetic activity so far remains unknown. An enhanced release of noradrenaline, or a decreased re-uptake of this neurotransmitter can be thought of as
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explanations at the cellular level. There exists no doubt that noradrenaline, through the stimulation of post-synaptic adrenoceptors, is the main neurotransmitter responsible for cardiovascular effects of spinal cord stimulation in the pithed rat model, because the stimulation-induced increase in DBP is very sensitive to blockade by guanethidine or prazosine. To elucidate whether the amplification of the sympathetic neurotransmission by vasopressin was due to pre- or postsynaptic effects, experiments with exogenous noradrenaline were performed (fig. 5). Vasopressin did not influence the increase in DBP as a result of exogenous noradrenaline, and neither did the administration of the vasopressin antagonists under investigation. These findings suggest that the effect of vasopressin on the sympathetic neurotransmission is predominantly caused by pre-synaptic facilitation, in accordance with the findings of Garcia-Villalon et al. Our results also suggest the involvement of a V₁-receptor at the pre-synaptic neuronal level. However, these findings are in contrast with the results of Medina et al., who showed in in vitro experiments with the human vas deferens, mesenteric artery and the saphenous vein, respectively, that vasopressin increased the contractions elicited by exogenously applied noradrenaline and KCl. This would suggest a general postsynaptic modification of the contractile function of the vascular smooth muscle cells. A possible explanation of these differences may be the heterogeneity of responsiveness of vasopressin, depending on the region and species under investigation. A major difference between our study and those published previously by others is the use of the pithed rat model, in which we were able to investigate an intact circulation in contrast to isolated single vessels. To our knowledge our pithed rat experiments offer the first in vivo demonstration of the vasopressin-induced facilitation of sympathetic nervous activity.

In conclusion, the facilitation of sympathetic activity by vasopressin can also be demonstrated in vivo in the pithed rat model. The potentiating effect of vasopressin is likely to be mediated by V₁-receptors at pre-synaptic sites at the sympathetic neurons. In order to demonstrate this facilitation, it is necessary to block the effects of endogenous angiotensin II by means of an AT₁-receptor antagonist.
5. References


