The Interaction between vasopressin and modulators of the cardiovascular system

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CHAPTER 4

VASOPRESSIN FACILITATES PRE-SYNAPTIC SYMPATHETIC NERVE ACTIVITY IN HUMANS.

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1. Introduction

Although the role of vasopressin (AVP) in several cardiovascular syndromes such as hypertension and congestive heart failure has not been established in detail, there are strong indications that this nonapeptide is an important modulating factor for vascular tone.\textsuperscript{1,2} The development of new non-peptidergic vasopressin-antagonists has greatly facilitated research concerning the role of AVP in the cardiovascular system.\textsuperscript{3} In addition epidemiological studies have demonstrated higher AVP plasma levels in patients with congestive heart failure, the elevated AVP plasma levels proved to be associated with a deteriorated prognosis.\textsuperscript{4,5} The direct vasoconstrictor effects of AVP are well known, but the concentrations needed for direct vasoconstriction appear to exceed the physiological range.\textsuperscript{6} On the other hand, the vasoconstrictor potency of AVP is increased in humans with an impaired baroreflex-function.\textsuperscript{7} Endogenous AVP augments the cardiopulmonary baroreflex inhibition in the area postrema, resulting in a decreased central sympathetic influence involved in the regulation of vascular tone.\textsuperscript{8} In contrast to the baroreflex-mediated inhibitory effect of AVP on the sympathetic nervous system, AVP appears to evoke opposite effect on the sympathetic nerve endings. A potentiating effect of AVP on the peripheral sympathetic nervous system involved in vasoconstriction has been demonstrated and it is V$_1$-receptor dependent.\textsuperscript{9,10} We have demonstrated previously in an \textit{in vivo} animal model that low concentrations of AVP, without any direct vascular effects, can enhance vasoconstriction induced by peripheral sympathetic nervous system activation.\textsuperscript{11} The direct vasoconstrictor effects of AVP, as well as the possible AVP-mediated facilitation of the peripheral sympathetic nervous system involved in vasoconstriction can be counteracted by the central effects of AVP on the baroreflex. For the evaluation of the interaction between AVP and the peripheral sympathetic nervous system the baroreflex feedback mechanism should be excluded. For this reason we have evaluated this interaction by means of venous occlusion plethysmography in the human forearm.

Accordingly, we investigated whether in humans the facilitatory role of AVP on the peripheral sympathetic nerve terminal can be demonstrated. AVP was administered in low doses devoid of vasoconstrictor activity. Stepwise increasing levels of general sympathetic nervous system activation were provoked by the application of lower body negative pressure (LBNP). We
investigated the effects of sympathetic nervous system activation on the blood flow in the human forearm, in the presence or absence of a local intra-arterial AVP-infusion. The activation of the sympathetic nervous system by LBNP was used to evaluate the combined pre- and post-synaptic effects of AVP on the nerve endings. Accordingly, the influence of AVP (again in a sub-pressor dosage) was investigated on the postsynaptically mediated vasoconstrictor action of norepinephrine (NE), also applied intra-arterially.

2. Methods

Subjects
Eight healthy normotensive volunteers (7 male/1 female, age 32 ± 2.3 years) participated in this study. All volunteers gave informed written consent. The ethical committee of the Academic Medical Centre of the University of Amsterdam approved the protocol. Subjects were instructed to refrain from smoking, drinking alcohol- or caffeine containing beverages for at least 12 hours before the experiment. None of these subjects used any vasoactive medication.

Experimental protocol
Each experiment was performed in the morning in a quiet room at a temperature of 22-24°C. A one-lead electrocardiogram (ECG) was recorded continuously. After local anaesthesia with lidocaine 1%, the brachial artery was cannulated using a XRO Arterial Catheter-Seldinger Technique (Laboratoire Plastimed, Saint-Leu-La-Forêt Cedex, France). The cannula was connected to a Baxter pressure transducer. Drugs were infused into the brachial artery using a Braun Secura FT (B.Braun, Melsungen, Germany) Perfusor. Both arms were instrumented with mercury-in-silastic strain gauges for the measurement of FABF, and with pressure cuffs, connected to a Hokanson EC-2 plethysmograph and to a Hokanson E-10 rapid cuff inflator, respectively (Hokanson Inc., Isaquah, WA, USA). Furthermore we measured heart rate (HR) from the ECG. Data were recorded on a personal computer using an analog-to-digital converter (Model DT 2801, Data Translation Inc., Marlborough, MA, USA). During the measurement of the FABF we used a R-wave-triggered upper-arm cuff inflation (at 40 mmHg) controlled by the personal computer for venous occlusion plethysmography. FABF was
measured during upper arm cuff inflation for 4 heartbeats, at a rate of 4 times per minute. The mean of the 8-10 last measurements of each recording period was used for analysis. The hands of the subjects were continuously excluded from the circulation by inflating small wrist cuffs to a pressure of at least 40 mmHg above systolic blood pressure. The infusion experiments were started at least 60 minutes after cannulation of the brachial artery. Between the various infusion-experiments the wrist cuffs were deflated and sufficient time (at least 30 min.) was allowed for recovery from hand ischemia and to allow FABF to return to baseline levels.

**Figure 1:** Schematic representation of the experimental protocols for the application of lower body negative pressures (LBNP) and norepinephrine (NE), respectively. SNP indicates sodium nitroprusside and AVP, vasopressin.

The protocol of the study is summarised in Fig. 1. The total flow rate of the intra-arterial infusions was kept constant at 0.6 ml/min in the LBNP-experiment and at 1.2 ml/min in the experiment evaluating the effects of exogenous NE. Infusion of sodium nitroprusside (SNP, 10 ng/kg/min) was used to predilate the vascular bed of the forearm in order to quantify more accurately the degree of vasoconstriction. The two series of experiments were carried out in the presence of a continuous infusion of randomly AVP (0.008 ng/kg/min) or NaCl 0.9%, applied randomly. This low dosage of AVP did not cause any vasoconstriction.

High levels of LBNP are known to activate the sympathetic activity and thus cause vasoconstriction. We applied 3 low levels of LBNP of -10, -20 and -30
mmHg for the duration of 5 minutes per pressure step. In a pilot study these levels appeared not to influence the baseline mean arterial blood pressure compared to the blood pressure at -30 mmHg (73.0 ± 4.4 to 75.3 ± 4.3 mmHg, (n=10) p=0.71). An airtight plastic chamber covered the lower part of the subject’s body. The chamber was sealed at the level of the iliac crest. The pressure in the LBNP box was continuously measured by a pressure transducer (Baxter, Utrecht, The Netherlands). During the second protocol NE was intra-arterially infused in three incremental dosages (10, 20, 40 pg/min), again in steps of 5 min each. Due to technical reasons one subject completed only the AVP infusion of the NE protocol.

**Drugs**

[Arg⁸]-vasopressin was obtained from Clinalfa AG (Switzerland). Sodium nitropusside and norepinephrine were purchased from BUFA bv (Netherlands). All substances were dissolved in NaCl 0.9% by our hospital pharmacy.

**Data analysis**

FABF was expressed as ml⁻¹100ml⁻¹min⁻¹ according the method of Whitney. The percentage change in FABF was calculated relative to the baseline values, during the continuous infusion of SNP and AVP or NaCl, prior to the start of the experiment. In the NE-protocol, the variability of blood-flow data was reduced, by using the non-infused arm as a contemporary control. The percentage was calculated as follows:

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\text{% Change in FABF} = 100 \times \frac{[(F/F_{ni})-(F_{bi}/F_{bni})]}{(F_{bi}/F_{bni})},
\]

where \( F_i \) and \( F_{ni} \) are the blood flows in the infused and non-infused arms, respectively, at the time point under investigation. \( F_{bi} \) and \( F_{bni} \) are the blood flows at baseline, during the continuous infusion of AVP or NaCl and SNP.

Repeated measures analysis of variance was used to assess the differences between experiments with vehicle or AVP at the successive levels of LBNP or doses of NE. Student’s t-test was used to examine the effects of the experimental conditions on hemodynamics at individual time points. Results are presented as means ± SEM. A p-value of less than 0.05 was considered to indicate statistically significant differences.
3. Results

Basal blood flow values in the infused and control arm were comparable (4.4 ± 0.3 ml⁻¹100ml⁻¹min⁻¹ vs. 4.6 ± 0.5 ml⁻¹100ml⁻¹min⁻¹; p=0.96). Baseline blood flow remained the same at the beginning of the vehicle- and AVP-LBNP experiments (3.7 ± 0.5 ml⁻¹100ml⁻¹min⁻¹ vs. 4.3 ± 0.6 ml⁻¹100ml⁻¹min⁻¹; p=0.58) and it was also comparable between the vehicle- and AVP-exogenous noradrenaline experiments (5.0 ± 0.5 vs. 5.0 ± 0.9; p=0.96). Continuous SNP (10 ng/kg/min) infusion increased the FABF with respect to baseline (from 4.4 ± 0.3 ml⁻¹100ml⁻¹min⁻¹ to 5.4 ± 0.4 ml⁻¹100ml⁻¹min⁻¹; p=0.004). This increase was not influenced by the continuous co-infusion of vehicle or AVP (0.008 ng/kg/min) (5.2 ± 0.6 ml⁻¹100ml⁻¹min⁻¹ vs. 5.5 ± 0.6 ml⁻¹100ml⁻¹min⁻¹; p=0.26).

![Graph](image)

**Figure 2:** Mean percentage change in forearm blood flow (FABF) during NaCl 0.9 % (□) or AVP (0.008 ng/kg/min) (■) infusions in response to 5-minute applications of subsequent decreases in lower body negative pressure (LBNP). Repeated measure analysis between the combined results of the NaCl- and the AVP-experiment.
Lower Body Negative Pressure
The application of LBNP resulted in a pressure-dependent decrease of FABF by 25.6 ± 4.4%, 29.0 ± 6.1%, and 38.6 ± 6.9% for the LBNP levels of -10, -20 and -30 mmHg, respectively. In the control arm FABF decreased in a comparable manner by 40.2 ± 6.7% at LBNP of -30 mmHg (p=0.88). During the experiment with a continuous infusion of the sub-pressor dosage of AVP (0.008 ng/kg/min), this decrease was enhanced to 38.0 ± 8.6%, 49.3 ± 5.2% and 58.9 ± 6.3% (p=0.014), for the LBNP levels of -10, -20 and -30 mmHg, respectively (Figure 2).

Norepinephrine
NE caused a dose-dependent FABF reduction by 3.1 ± 4.6%, 17.0 ± 4.3% and 23.2 ± 4.9%, at dosages of 10, 20 and 40 pg/min, respectively. In the presence of the continuous infusion of AVP (0.008 ng/kg/min) the FABF showed similar reductions by 9.4 ± 3.3%, 13.3 ± 4.1% and 23.9 ± 6.9% (p=0.91) (Figure 3). The FABF in the control arms was unaffected during the infusion of NE and demonstrated a flow 103.7 ± 4.8% (p=0.45) at the highest dose of NE (40 pg/min).

Figure 3: Mean percentage change in forearm blood flow (FABF) during NaCl 0.9 % (□) or AVP (0.008 ng/kg/min) (■) infusions in response to 5-minute successive incremental infusions of norepinephrine (NE). Repeated measure analysis between the combined results of the NaCl- and the AVP-experiment.
4. Discussion

The main finding of the present investigation is that the vasoconstrictor responses to endogenously released NE are enhanced in the presence of a sub-pressor continuous infusion of AVP (intra-arterially) in the human forearm. Previous studies evaluating the interaction of AVP and the sympathetic nervous system dealt with the possible central regulating action of AVP on the sympathetic nervous system.\(^{14-16}\) In these studies systemically active dosages of AVP were used, which therefore directly modified the baroreflex function and the baseline sympathetic nervous system activity. Previously we have demonstrated in an \textit{in vivo} animal model that there occurs a facilitating effect of a sub-pressor dosage of AVP on the peripheral sympathetic nervous system activation.\(^{11}\) In the present study in humans, we investigated the effects of an intra-arterial infusion of AVP in a dosage that would not relevantly increase the systemic plasma AVP concentration. A moderate decrease in central venous pressure caused by the LBNP does not induce an increase of endogenous AVP levels.\(^{17}\) We assume that this study reflects the activity and influence of the sympathetic nerve terminal in the human forearm. Accordingly the results of the present study will reflect the facilitating action of AVP on the sympathetic nerve endings in the human forearm.

A moderate reduction in lower body pressure as induced in our study results in an activation of the sympathetic nervous system, predominantly although not exclusively, through stimulation of the ‘low-pressure’ cardiopulmonary-baroreflex system. The local intra-arterial infusion of AVP presumably selectively stimulates the sympathetic nerve endings in the forearm. This activation takes place either pre-synaptically by increased NE release or a reduced uptake, or by facilitation at the post-synaptic receptor level. In animal studies data on the pre- or postsynaptic site of AVP-induced facilitation are conflicting. In studies demonstrating a post-synaptic facilitation by AVP, it was most evident in the lower concentration range of exogenous NE.\(^{9,18}\) For that reason we investigated the effects of exogenous NE in the lower dosage range. In the present study in the vascular bed of the human forearm, AVP did not facilitate the effect of exogenous NE, suggesting a selective pre-synaptic site of action. We cannot however, exclude a possible post-synaptic facilitating
effect of AVP at higher dosages of NE. Likewise, we cannot extrapolate these findings to other human vascular beds, because AVP is known to exert heterogeneous vasoactive effects depending on the vascular-bed studied. Furthermore, we cannot rule out a facilitating effect of AVP as a result of a decreased re-uptake of norepinephrine. In vitro studies have demonstrated, that in the presence of the adrenergic re-uptake inhibitor cocaine the potentiating effect of AVP on the sympathetic nervous system is maintained.

It can be imagined that the facilitating effect of AVP on the peripheral sympathetic nervous system is clinically relevant under certain conditions, such as for instance congestive heart failure where both systems are activated. In summary, we have demonstrated that AVP in low concentrations, devoid of direct vascular effects, can facilitate the activity of the peripheral sympathetic nervous system, most likely at the pre-synaptic level.
5. References


AVP in interaction with the SNS in humans


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