Effects of arginine vasopressin and 1-desamino-8-D-arginine vasopressin on forearm vasculature of healthy subjects and patients with a \( V_2 \) receptor defect

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**Objectives:** To assess which vasopressin receptor subtype mediates the vasodilation occurring in response to arginine vasopressin and 1-desamino-8-D-arginine vasopressin and whether nitric oxide is involved in these effects.

**Materials and methods:** Vasoactive effects of arginine vasopressin and DD-arginine vasopressin on forearm vasculature were studied in healthy subjects and in patients with congenital nephrogenic diabetes insipidus with a vasopressin type 2 \( (V_2) \) receptor gene defect. Venous occlusion plethysmography was used to assess the forearm blood flow responses to the infusion of arginine vasopressin and its analogue into the brachial artery, in the presence and the absence of the nitric oxide synthase inhibitor \( L-\text{NG-} \text{monomethyl-arginine (L-NMMA)} \).

**Results:** In healthy subjects \((n=10)\), DD-arginine vasopressin \((0.1, 1, 10\), or \(5, 10\), and \(20\) ng/min per dl) induced a dose-related increase in forearm blood flow, but did not affect forearm blood flow in the patients with nephrogenic diabetes insipidus \((n=3)\). In two healthy subjects, seven increasing doses of arginine vasopressin \((0.25-12\) ng/min per dl) induced an initial decrease in forearm blood flow and then a gradual increase. In one of the patients, the same arginine vasopressin doses produced a persistent decrease in forearm blood flow. In the healthy subjects, infusion of L-NMMA reduced forearm blood flow significantly \((n=10)\). Subsequent administration of DD-arginine vasopressin during L-NMMA infusion produced a slight reduction in the forearm blood flow increase compared with DD-arginine vasopressin alone, but this was significant only for the absolute forearm blood flow increase induced by 10 ng/min per dl in all subjects. Infusion of arginine vasopressin in the presence of L-NMMA did not increase forearm blood flow significantly.

**Conclusions:** In human forearm vasculature, extrarenal \( V_2 \) receptors mediate the vasodilation induced by DD-arginine vasopressin or high doses of arginine vasopressin, whereas these receptors are not necessary for arginine vasopressin-induced vasoconstriction. The DD-arginine vasopressin-induced vasodilation seems to be mediated predominantly by a mechanism other than endothelial nitric oxide release, whereas arginine vasopressin-induced vasodilation seems to involve nitric oxide release only.


**Keywords:** Arginine vasopressin, 1-desamino-8-D-arginine vasopressin, \( V_1 \) receptor, \( V_2 \) receptor, vasoconstriction, vasodilation, nephrogenic diabetes insipidus, nitric oxide, \( L-\text{NG-} \text{monomethyl-arginine }

**Introduction**

The neurohypophyseal hormone arginine vasopressin, which is also referred to as the antidiuretic hormone, owes its two names to its main functions: vasoconstriction and antidiuresis. Vasopressin type 1 \( (V_1) \) receptors on vascular smooth muscle cells mediate the vasoconstrictive action, whereas vasopressin type 2 \( (V_2) \)
receptors are involved in the antidiuretic effect exerted in renal collecting duct cells. A less well-known effect of arginine vasopressin is vasodilation, which has been found to occur in response to high local concentrations of the hormone [1–5]. Attempts to assess the type of receptor mediating this vasodilatory action have yielded contradictory results, with discrepancies between in vivo and in vitro studies, among different species and among vascular beds [6–8]. In vivo studies using V1 receptor antagonists have indicated that the vasodilation occurring in response to high doses of arginine vasopressin is mediated by V2 receptors [1,3,9–13], an observation which is in accord with the vasodilatory effect of the V2 receptor agonist 1-desamino-8-d-arginine vasopressin [1]. Furthermore, recent investigations have suggested a role for endothelium-derived nitric oxide in the vasodilator response to arginine vasopressin [4,14–17].

In the present study, we assessed in human forearm vasculature which vasopressin receptor subtype mediates arginine vasopressin- and DD-arginine vasopressin-induced vasodilation by comparing the responses of healthy subjects with those of patients with a proven V2 receptor defect and whether nitric oxide is involved in arginine vasopressin- and DD-arginine vasopressin-induced vasodilation.

Materials and methods

Subjects

The study protocol was approved by the local ethical committee of the University Hospital Nijmegen. All subjects gave written informed consent.

Fifteen healthy male volunteers (aged 19–31 years) and three male patients with congenital nephrogenic diabetes insipidus (aged 21–30 years) participated in the study. All of the nephrogenic diabetes insipidus patients had had polyuria and polydipsia since early childhood and showed no antidiuretic response to DD-arginine vasopressin. In the three patients, DNA sequencing analysis of the V2 receptor gene had been performed as described previously [18]. In all of the patients, point mutations had been detected in this gene, resulting in amino acid substitutions in two of them (phenylalanine 44 to leucine and arginine 202 to cysteine, respectively) and introduction of a premature stop codon in the third patient (arginine 337 to stop codon).

A physical examination and electrocardiography revealed no abnormalities in any of the participants. The experiments were performed after overnight fasting by the subjects. During the 24 h preceding the experiment, they did not smoke or drink beverages containing caffeine or alcohol. In the 2 weeks preceding the study none of the healthy volunteers had taken any medication. One of the diabetes patients complied with a request to stop taking hydrochlorothiazide, amiloride and indomethacin 36 h before the start of the experiment, and one patient, who was taking hydrochlorothiazide and amiloride, continued using the medication. The third patient was not taking any medication.

Study design

The brachial artery of the left arm was cannulated. The experiments started after an equilibration period of 30 min. Forearm blood flow of both arms was measured by venous occlusion mercury-in-silastic strain gauge plethysmography. Simultaneously, circulation of the hand was arrested by inflating a cuff around the wrist. The forearm blood flow values obtained during the last 2 min of each infusion were used for further analysis. Blood pressure and heart rate were recorded intra-arterially (Hewlett-Packard GmbH, Böblingen, Germany).

Protocols were designed to assess the vascular response of DD-arginine vasopressin and arginine vasopressin in healthy subjects; the vascular response to DD-arginine vasopressin and arginine vasopressin in nephrogenic diabetes insipidus patients with a V2 receptor defect; and the effect of L-NG-monomethyl-arginine (L-NMMA) on DD-arginine vasopressin- and arginine vasopressin-induced vasodilation. One healthy subject and one nephrogenic diabetes insipidus patient participated in both the DD-arginine vasopressin and the arginine vasopressin experiment.

Drugs

Desmopressin-acetatehydrate (1-desamino-8-d)-arginine vasopressin, Minrin; Ferring, Malmö, Sweden; 0.4 μg/l) was diluted with 0.9% sodium chloride. Doses of either 0.1, 1 and 10 or 5, 10 and 20 ng/min per dl forearm volume were infused during subsequent 5-min periods. Arginine vasopressin (Pitressin; Parke-Davis, Berlin, Germany; 0.05 g/l) was diluted with 0.9% sodium chloride. Doses of 0.25, 0.5, 1, 2, 4, 8 and 12 ng/min per dl were administered during subsequent 5-min periods. L-NG-monomethyl-arginine was dissolved in 0.9% sodium chloride and administered at a dose of 0.1 mg/min per dl. An L-NMMA infusion was started 5 min before and maintained during the second infusion of DD-arginine vasopressin or arginine vasopressin. All drugs were infused intra-arterially by an automatic syringe infusion pump at a constant total infusion rate of 50 μl/min per dl.

Statistical analysis

Paired student’s t-tests were used to evaluate the effect of L-NMMA on baseline forearm blood flow and, by comparison of changes in forearm blood flow from baseline, the effect of L-NMMA on DD-arginine vasopressin-induced vasodilation.
Results

Effects of DD-arginine vasopressin and arginine vasopressin in healthy subjects

Infusion of DD-arginine vasopressin at 0.1, 1 and 10 ng/min per dl had no significant effect at the lowest dose but an increase in forearm blood flow occurred at the two higher doses (from 1.6±0.5 during saline infusion to 7.9±2.5 ml/min per dl at the highest dose; n=3). Doses of DD-arginine vasopressin at 5, 10 and 20 ng/min per dl administered to seven healthy subjects produced a dose-related increase in forearm blood flow (Table 1; Fig. 1).

Table 1. Forearm blood flow response of healthy subjects to intra-arterial infusion of different doses of 1-desamino-8-D-arginine vasopressin (n = 7).

<table>
<thead>
<tr>
<th>Dose (ng/min per dl)</th>
<th>Forearm blood flow (ml/min per dl)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Before L-NMMA</td>
<td>2.3±0.7</td>
</tr>
<tr>
<td>During L-NMMA</td>
<td>1.7±0.3</td>
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Percentage forearm blood flow

<table>
<thead>
<tr>
<th>Dose (ng/min per dl)</th>
<th>Forearm blood flow (ml/min per dl)</th>
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<td></td>
<td>Before L-NMMA</td>
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Values are expressed as means±SEM. L-NMMA, administration of L-NG-monomethyl-arginine.

Fig. 1. Forearm blood flow response to 1-desamino-8-D-arginine vasopressin (DDAVP) in seven healthy subjects (■—■) and three patients with nephrogenic diabetes insipidus with a V2 receptor defect (●—●). Values are expressed as means±SEM.

The administration of increasing doses of arginine vasopressin (0.25–12.0 ng/min per dl) resulted in a slight initial decrease in forearm blood flow, from 1.4±0.4 to 1.1±0.4 ml/min per dl, and then a gradual increase to 3.1±0.5 ml/min per dl at the highest dose (n=2, Fig. 2).

Fig. 2. Forearm blood flow response to arginine vasopressin (AVP) in two healthy subjects (■—■) and a patient with nephrogenic diabetes insipidus with a V2 receptor defect (●—●). Values are expressed as means±SEM.

Effects of DD-arginine vasopressin and arginine vasopressin in patients with nephrogenic diabetes insipidus

The administration of DD-arginine vasopressin at 5, 10 and 20 ng/ml per dl in three patients with nephrogenic diabetes insipidus did not affect forearm blood flow (Fig. 1). Infusion of the arginine vasopressin dose scheme into one of the patients resulted in a decrease in forearm blood flow from 1.2 ml/min per dl during saline infusion to 0.4 ml/min per dl at the highest arginine vasopressin dose (Fig. 2).

Effects of L-NMMA on DD-arginine vasopressin- and arginine vasopressin-induced vasodilation in healthy subjects

During the 5-min period of L-NMMA infusion preceding the second DD-arginine vasopressin infusion, forearm blood flow decreased significantly from 2.3±0.5 to 1.6±0.2 ml/min per dl (n=10, P=0.05). Subsequent administration of DD-arginine vasopressin at 0.1, 1 and 10 ng/min per dl (n=3) and 5, 10 and 20 ng/min per dl (n=7) during L-NMMA infusion revealed a partial reduction in the forearm blood flow increase induced by DD-arginine vasopressin, which reached statistical significance for the 10-ng dose administered to all 10 subjects (5.7±1.0 versus 4.2±0.8 ml/min per dl, n=10, P<0.01, Fig. 3). The forearm blood flow increase at the 5 - and 20-ng doses was not significantly reduced during L-NMMA. The forearm blood flow increase as a percentage of the baseline value was not significant for any of the doses. The increase in forearm blood flow induced by a second infusion of DD-arginine vasopressin at 5, 10 and 20 ng/min per dl without concomitant...
L-NMMA (n=4) did not differ significantly from the increase during the first infusion.

Administration of arginine vasopressin at 0.5, 4 and 12 ng/min per dl during an infusion of L-NMMA produced no significant increase in forearm blood flow (0.8 ± 0.1 at baseline versus 0.7 ± 0.1, 1.0 ± 0.09 and 1.2 ± 0.2 ml/min per dl, respectively).

None of the studies showed significant changes in blood pressure, heart rate or forearm blood flow in the contralateral arm.

**Discussion**

Studies assessing the vasodilatory mechanism of arginine vasopressin and its analogue DD-arginine vasopressin have yielded conflicting data. *In vitro* experiments in canine cerebral vessels and rat pulmonary artery and aorta suggested involvement of V₁ receptors [15,16,19]. Similarly, in human *in vivo* studies, arginine vasopressin-induced vasodilation of cerebral and mesenterial arteries was found to be independent of V₂ receptor stimulation [19,20]. However, most *in vivo* studies provide evidence that V₂ receptors are involved in arginine vasopressin- and DD-arginine vasopressin-induced vasodilation [1,3,5,9—13]. Furthermore, it has been postulated that the vasodilation and associated release of coagulation and fibrinolytic factors occurring in response to arginine vasopressin and DD-arginine vasopressin administration is neither mediated by V₁ nor by V₂ receptors because studies in rat aortae and in conscious dogs have revealed an absence of inhibition both by V₁ and by V₂ antagonists [22,23]. The most plausible reason for this confusion seems to be the variability in the pharmacological profile of agonists and/or antagonists that has been suggested to exist among species, between *in vitro* and *in vivo* studies and among different vascular beds [6—8].

**Human *in vivo* studies** assessing the effect of high systemic doses of arginine vasopressin are scarce because of the risk of severe vasoconstriction. However, it is clear that no substantial increase in blood pressure occurs despite maintenance of high plasma arginine vasopressin levels [24]. The vascular effects of V₂ receptor antagonists have only recently been examined in a human *in vivo* study, which appeared to confirm that in the human forearm V₂ receptors are involved in the vasodilatory effect of arginine vasopressin [5]. However, these conclusions need to be considered with caution, because most V₂ receptor antagonists show partial V₁ receptor antagonism and their putative specificity has been based mainly on their aquaretic potencies in animals [6—8]. However, studies in patients with X-linked nephrogenic diabetes insipidus have provided convincing evidence for involvement of the V₂ receptor in the vasodilatory actions of DD-arginine vasopressin [25]. As a result of mutations in the V₂ receptor gene, patients with nephrogenic diabetes insipidus lack the renal antidiuretic response to DD-arginine vasopressin [26]. In addition, these patients show no vasodilatory, coagulation or fibrinolytic responses to DD-arginine vasopressin, indicating that these extrarenal effects are normally mediated either by an extrarenal V₂ receptor encoded by the same gene or by the renal V₂ receptor.

In the present study we circumvented the restrictions imposed by the side effects of high systemic doses of arginine vasopressin and DD-arginine vasopressin and the problems caused by lack of specificity of V₂ receptor antagonists, by studying the vascular effects of arginine vasopressin and DD-arginine vasopressin in the human forearm both of healthy subjects and of patients with a proven V₂ receptor gene defect. The absence of vasodilation during administration of DD-arginine vasopressin and high doses of arginine vasopressin in nephrogenic diabetes insipidus patients proves that both effects are mediated by the receptor encoded by the V₂ receptor gene, excluding the occurrence of non-specific vasodilatory effects or actions mediated by other receptors, for instance the V₁ or oxytocin receptor. In addition, it shows that the high selectivity of DD-arginine vasopressin for the renal V₂ receptor also applies to the extrarenal V₂ receptor. Moreover, the present study unequivocally proves that the absence of extrarenal response to DD-arginine vasopressin in patients with X-linked nephrogenic diabetes insipidus is a direct consequence of an extrarenal V₂ receptor defect and is neither caused nor influenced by their renal V₂ receptor defect.
As in studies seeking the receptor involved in arginine vasopressin- and DD-arginine vasopressin-induced vasodilation, attempts to determine which vasodilatory substance mediates this action have produced varying results. In vitro studies in canine cerebral vessels and rat sartorii have suggested that nitric oxide causes these effects [14-16]. However, Russ and colleagues [14,27] observed only partial inhibition of arginine vasopressin-induced vasodilation by high doses of nitric oxide synthase inhibitors in rat lungs and provided evidence against involvement of prostaglandins and hyperpolarization of vascular smooth muscle. Moreover, in their in vitro study of human cerebral and mesenteric arteries, Martinez et al. [20,21] found that release of nitric oxide in these vessels accounts neither for arginine vasopressin- nor for DD-arginine vasopressin-induced vasodilation.

It has been shown that the counter-regulation observed in healthy humans after systemic administration of high doses of arginine vasopressin is abolished by indomethacin, suggesting that prostaglandins are involved in its vasodilatory effect [28]. In contrast, studies of arginine vasopressin-induced vasodilation in the human forearm by Suzuki et al. [2] suggested that prostaglandins are not involved while experiments by Tagawa et al. [4] strongly implied the involvement of nitric oxide. Hasumuma et al. [29] found that vasodilation after systemic DD-arginine vasopressin infusion was not influenced by indomethacin or propranolol administration; however, they did observe an increase in urinary 6-keto prostaglandin F1α. We observed that the L-NMMA-induced reduction in DD-arginine vasopressin response, if any, was rather small, whereas the current data show, in accord with the findings by Tagawa et al. [4], that L-NMMA inhibited arginine vasopressin-induced vasodilation almost completely.

How can these observations be reconciled with the conclusion that arginine vasopressin and DD-arginine vasopressin both cause vasodilation by stimulating the V2 receptor? Since DD-arginine vasopressin elicited a threefold stronger increase in forearm blood flow than arginine vasopressin did, the present L-NMMA dose of 0.1 mg/min per dl might not have been sufficient to compete with the quantity of nitric oxide released by DD-arginine vasopressin, whereas it is adequate in the case of arginine vasopressin. However, even with DD-arginine vasopressin at 5 mg/min per dl, the inhibition was not complete and did not clearly decrease with higher doses of DD-arginine vasopressin.

A more plausible explanation is based on the fact that arginine vasopressin interacts with both V1 and V2 receptors. The V1 receptor pathway could, for instance, attenuate an L-NMMA-insensitive vasodilatory component of the V2 receptor pathway. Another possibility is that arginine vasopressin, because of a possible lower availability or affinity for the V2 receptor, initiates only the nitric oxide-dependent mechanisms, whereas DD-arginine vasopressin is capable of inducing an additional L-NMMA-insensitive pathway [30]. Insight into these mechanisms might be increased by the discovery of the exact location of the extrarenal V2 receptor. Although physiological evidence has now been obtained that these receptors exist in the human forearm, radioligand binding studies have not been able to confirm their presence in endothelial or vascular smooth muscle cells [31]. However, blood cells, especially monocytes, are a likely location [32,33]. Furthermore, although the present study shows that extrarenal V2 receptors are the product of the same gene as renal V2 receptors, the possibility cannot be excluded that, as a result of alternative splicing, there is some heterogeneity among receptors encoded by the V2 receptor gene, as recently shown to be the case in the kidney [34]. Further research awaits the detection of extrarenal gene expression of this gene at the messenger RNA or protein level.

The present study proves that extrarenal V2 receptors in the human forearm mediate the vasodilation induced by DD-arginine vasopressin and high doses of arginine vasopressin, whereas these receptors are not necessary for arginine vasopressin-induced vasodilatation. Secondly, DD-arginine vasopressin-induced vasodilation seems to be mediated predominantly by a mechanism other than endothelial nitric oxide release, whereas arginine vasopressin-induced vasodilation seems to involve nitric oxide release only. Thus, despite the fact that the same receptor is involved, it appears that the mechanisms underlying arginine vasopressin- and DD-arginine vasopressin-induced vasodilation are not completely identical.

References


