Arginine Vasopressin Enhances Sympathetic Constriction Through the V1 Vasopressin Receptor in Human Saphenous Vein

Pascual Medina, PhD; Antonio Acuña, BSc; Juan B. Martínez-León, MD; Eduardo Otero, MD; José M. Vila, PhD; Martín Aldasoro, MD; Salvador Lluch, MD

Background—Arginine vasopressin (AVP) not only acts directly on blood vessels through V1 receptor stimulation but also may modulate adrenergic-mediated responses in animal experiments in vivo and in vitro. The aim of the present study was to investigate whether AVP can contribute to an abnormal adrenergic constrictor response of human saphenous veins.

Methods and Results—Saphenous vein rings were obtained from 32 patients undergoing coronary artery bypass surgery. The vein rings were suspended in organ bath chambers for isometric recording of tension. AVP (3 × 10–9 mol/L) enhanced the contractions elicited by electrical field stimulation at 1, 2, and 4 Hz (by 80%, 70%, and 60%, respectively) and produced a leftward shift of the concentration-response curve to norepinephrine (half-maximal effective concentration decreased from 6.87 × 10–7 to 1.04 × 10–7 mol/L; P < .05). The V1 vasopressin receptor antagonist d(CH2)5:Tyr(Me)AVP (10–6 mol/L) prevented the potentiation evoked by AVP. The selective V1 receptor agonist [Phe5, Orn8]-vasotocin (3 × 10–9 mol/L) induced potentiation of electrical stimulation–evoked responses, which was also inhibited in the presence of the V1 receptor antagonist (10–6 mol/L). In contrast, the V2 receptor agonist desmopressin (10–9 to 10–7 mol/L) did not modify neurogenic responses, and the V2 receptor antagonist [d(CH2)5, D-Ile2, Ile4, Arg8]-vasopressin (10–7 to 10–6 mol/L) did not prevent the potentiation induced by AVP. The dihydropyridine calcium antagonist nifedipine (10–6 mol/L) did not affect the potentiating effect of AVP.

Conclusions—The results suggest that low concentrations of AVP facilitate sympathetic neurotransmission and potentiate constrictor effects of norepinephrine in human saphenous veins. These effects appear to be mediated by V1 receptor stimulation and are independent of calcium entry through dihydropyridine calcium channels. Thus, AVP may contribute to vascular mechanisms involved in acute ischemic syndromes associated with venous grafts, particularly if the sympathetic nervous system is activated. (Circulation. 1998;97:865–870.)

Key Words: veins ■ arginine vasopressin ■ electrical stimulation ■ norepinephrine
human saphenous veins. We also determined whether the modulating effect of AVP on vascular responsiveness depends on activation of $V_1$ or $V_2$ receptors.

**Methods**

Vein segments were taken from portions of human saphenous veins of patients undergoing coronary artery bypass surgery (32 patients; 23 men and 9 women; age range, 52 to 71 years). The study was approved by the ethical committee of our institution, and informed consent was obtained from each patient before the study. During surgical preparation of the saphenous vein, the dilation procedure was avoided. The veins were immediately placed in chilled Krebs-Henseleit solution, and rings 3 mm long were cut for isometric recording of tension.

Two stainless steel L-shaped pins 200 µm in diameter were introduced through the lumen of the vein ring. One pin was fixed to the wall of the organ bath, and the other was connected to a force-displacement transducer (Grass FT03). Changes in isometric force were recorded on a Grass polygraph (model 7). Each vein ring was set up in a 4-ml bath that contained modified Krebs-Henseleit solution of the following millimolar composition: NaCl 115, KCl 4.6, MgCl$_2$ • 6H$_2$O 1.2, CaCl$_2$ 2.5, NaHCO$_3$ 25, glucose 11.1, and disodium EDTA 0.01. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to a pH of 7.3 to 7.4. Temperature was held at 37°C. To establish the resting tension for maximal force development, a series of preliminary experiments was performed on vein rings of similar length and outer diameter that were exposed repeatedly to 100 mmol/L KCl. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was 3g.

The vein rings were allowed to attain a steady level of tension during a 2- to 3-hour accommodation period before testing. Functional integrity of the endothelium was confirmed routinely at the beginning of the experiment by the presence or absence of relaxation induced by acetylcholine ($10^{-6}$ to $10^{-7}$ mol/L) or substance P ($10^{-7}$ mol/L) during contraction obtained with norepinephrine ($10^{-6}$ to $3\times10^{-7}$ mol/L).

Electrical field stimulation was provided by a Grass S88 stimulator via two platinum electrodes positions on each side and parallel to the axis of the vein ring. To assess the nature of the contractile responses and to avoid direct stimulation of the smooth muscle, frequency-response relationships were determined on a group of veins in the presence and absence of $10^{-5}$ mol/L tetrodotoxin following previously described procedures. In summary, the protocol was designed to find the selective $V_1$ receptor agonist [Phe, 2 Orn$^8$]-vasotocin (3 $\times 10^{-8}$ mol/L), or the receptor blocker cocaine ($10^{-6}$ mol/L) plus AVP ($3\times10^{-9}$ mol/L). After 10 to 15 minutes of incubation with the corresponding drug, a second set of stimulations was then performed. In each group of experiments, stimulations without any treatment were run in parallel.

Concentration-response curves for norepinephrine ($10^{-7}$ to $3\times10^{-9}$ mol/L) and KCl (5 to 120 mmol/L) were determined in a cumulative manner. Control (in the absence of AVP) and experimental (in the presence of AVP) data were obtained from separate vascular preparations. Another group of vein rings was incubated with the $V_1$ receptor antagonist (10$^{-6}$ mol/L) plus AVP ($3\times10^{-9}$ mol/L) before exposure to norepinephrine or KCl.

In another group of experiments, electrical field stimulation was carried out under control conditions followed by a second set of stimulations in the presence of nifedipine ($10^{-6}$ mol/L); then a third set of stimulations was performed in the presence of nifedipine plus AVP ($3\times10^{-9}$ mol/L). Frequency-response curves without any treatment were run in parallel. In another group of veins, norepinephrine ($10^{-6}$ to $3\times10^{-7}$ mol/L) or the selective $V_1$ receptor antagonist (10$^{-6}$ to $10^{-5}$ mol/L) or nifedipine ($10^{-5}$ mol/L) or nifedipine plus AVP ($3\times10^{-7}$ mol/L), and the data were compared with those obtained from untreated (control) segments.

**Drugs**

The following drugs were used: tetrodotoxin, nifedipine, prazosin, norepinephrine hydrochloride, acetylcholine chloride, AVP acetate salt, [(1-β-mercapto-β-β-cyclopentamethylenepropionic acid)-2- (O-methyl)-tyrosine, 8-arginine] vasopressin [d(CH$_2$)$_3$Tyr(Me)AVP], deamino-8-arginine vasopressin (desmopressin), substance P acetate salt (Sigma Chemical Co), [Phe,$^2$, Orn$^4$]-vasotocin, [d(CH$_2$)$_3$, D-Ile,$^2$, Ile,$^4$]–vasopressin (Peninsula Laboratories Europe), and cocaine chloride (Abelló). All drugs were dissolved in Krebs-Henseleit solution except nifedipine, which was dissolved initially in ethanol and further diluted in Krebs solution to the proper final concentration. Drugs were added to the organ bath in volumes of <70 µL. Stock solutions of the drugs were freshly prepared every day and kept on ice throughout the experiment.

**Data Analysis**

All values are expressed as mean±SE. Contractions are reported in absolute tension (grams) or as a percentage of response to KCl (100 mmol/L). EC$_{50}$ values (concentrations of agonist producing half-maximal contraction) were determined from individual concentration-response curves by nonlinear regression analysis, and from these values the geometric means were calculated. The EC$_{50}$ values were compared by an unpaired $t$ test and an ANOVA with Scheffé’s test as post hoc test. The number of rings taken from each patient varied from 8 to 16. Concentration-response curves of the tested agonists or frequency-response relationships were performed in the presence and absence of either AVP or the antagonists in rings obtained from the same patient; the responses obtained in each patient were averaged to yield a single value. Therefore, all number (n) values are presented as the number of patients from whom the blood vessel was obtained. For electrical stimulation experiments in which the same veins were stimulated in the absence and presence of AVP, a paired $t$ test was used. Statistical significance was accepted at $P<.05$.

**Results**

**Effects of AVP**

AVP ($10^{-8}$ to $10^{-6}$ mol/L) caused concentration-dependent contractions with an EC$_{50}$ of 1.5$\times10^{-7}$ mol/L, which is similar to values previously reported by us.$^{25}$ The presence of the $V_1$ antagonist d(CH$_2$)$_3$Tyr(Me)AVP in the organ bath displaced the control curve to AVP to the right in a parallel manner (EC$_{50}$ 2.8$\times10^{-7}$ mol/L), but differences in the maximal tensions developed were not significant (23.1±3.3%, n=8, versus 23.3±3.8%, n=5, of response to 100 mmol/L KCl).
The α₁-adrenoceptor blocker prazosin (10⁻⁶ mol/L) did not affect the concentration-response curve to AVP (EC₅₀, 1.1 x 10⁻² mol/L; maximal response, 22.4 ± 3.1% of KCl contraction).

**Effects of Electrical Stimulation**

Electrical stimulation induced frequency-dependent increases in tension in all the experiments that were abolished by tetrodotoxin (10⁻⁶ mol/L) and prazosin (10⁻⁶ mol/L), thus indicating that the effect was due to the release of norepinephrine from perivascular adrenergic nerves acting on α₁-adrenoceptors.

AVP (10⁻¹⁰ to 3 x 10⁻⁹ mol/L) did not change the contractions to electrical stimulation at the frequencies used (1, 2, and 4 Hz). At higher concentrations (10⁻¹⁰ to 3 x 10⁻⁸ mol/L), AVP caused potentiation of the electrically evoked responses (Fig 1A and 1B). The V₁ receptor antagonist d(CH₂)₅Tyr(Me)AVP (10⁻⁶ mol/L) did not change control responses to electrical field stimulation but prevented the amplifying effect of AVP at all the frequencies used (Fig 1C).

The selective V₁ receptor agonist [Phe₂, Orn⁸]-vasotocin induced a potentiation of electrical stimulation–evoked responses of a magnitude similar to that observed in the presence of AVP. This potentiation was also inhibited in the presence of the V₁ receptor antagonist d(CH₂)₅Tyr(Me)AVP (10⁻⁶ mol/L) (Fig 2A).

To determine whether V₂ receptors are involved in the effects of AVP on field electrical stimulation, frequency-response relationships were obtained in the absence and in the presence of the V₂ receptor antagonist [d(CH₂)₅, D-Ile², Ile⁴, Arg⁸]-vasopressin. Fig 2B shows that the potentiation induced by AVP (3 x 10⁻⁸ mol/L) was not affected in the presence of the V₂ receptor antagonist. C, Frequency-response relationship in the absence and presence of cocaine (10⁻⁶ mol/L) or cocaine together with AVP (3 x 10⁻⁹ mol/L). Values are mean ± SEM. *Significant differences (P<.05) from control value.
Effect of AVP on Norepinephrine- and KCl-Induced Contraction

AVP potentiated norepinephrine-induced contractions in a concentration-dependent manner (Fig 3A). The norepinephrine EC$_{50}$ values and maximal responses in the presence and absence of AVP are shown in the Table. The V$_1$ receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP produced a parallel, rightward shift of the potentiating effects of 3×10$^{-7}$ mol/L AVP on the norepinephrine concentration-response curve. At 10$^{-7}$ mol/L, the V$_1$ inhibitor brought the EC$_{50}$ to values similar to those obtained in the norepinephrine control curve. In contrast, AVP (10$^{-7}$ to 3×10$^{-5}$ mol/L) did not affect the concentration-response curve to KCl (10 to 120 mmol/L) (Fig 3B).

AVP and Calcium

The dihydropyridine calcium antagonist nifedipine (10$^{-6}$ mol/L) did not significantly change the contraction induced by AVP and electrical field stimulation (Fig 4A and 4B). The presence of nifedipine diminished maximal responses to norepinephrine, but EC$_{50}$ was not altered (6.6×10$^{-7}$ versus 5.2×10$^{-7}$ mol/L) (Fig 4C). In addition, the enhancement of the contractile responses to electrical field stimulation and norepinephrine by AVP was identical to that observed in the absence of nifedipine (Fig 4B and 4C). However, KCl-induced contractions were significantly reduced in the presence of either nifedipine (10$^{-6}$ mol/L) or nifedipine plus AVP (3×10$^{-3}$ mol/L, hatched bars, n=5). D. Concentration-response curves to KCl in the absence (●, n=7) and in the presence of nifedipine (10$^{-6}$ mol/L, ○, n=7). Values are mean±SEM. *Significant differences from control values, $P<.05$.

### Discussion

The results of this study demonstrate that in the human saphenous vein, low concentrations of AVP enhance the

**Table:** EC$_{50}$ Values and Maximal Contractions to Norepinephrine Alone (Control) and in the Presence of Either AVP or the V$_1$ Receptor Antagonist Together With AVP

<table>
<thead>
<tr>
<th>Norepinephrine</th>
<th>EC$_{50}$, mol/L (95% Confidence Interval)</th>
<th>Maximal Responses, %±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>6.87×10$^{-7}$ (6.5×10$^{-7}$–7.09×10$^{-7}$)</td>
<td>135.2±6.1</td>
</tr>
<tr>
<td>With AVP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3×10$^{-10}$ mol/L (n=4)</td>
<td>2.79×10$^{-7}$ (2.62×10$^{-7}$–2.96×10$^{-7}$)</td>
<td>126.9±9.1</td>
</tr>
<tr>
<td>10$^{-9}$ mol/L (n=4)</td>
<td>2.64×10$^{-7}$ (1.18×10$^{-7}$–4.1×10$^{-7}$)</td>
<td>152.9±4.1</td>
</tr>
<tr>
<td>3×10$^{-9}$ mol/L (n=4)</td>
<td>1.04×10$^{-7}$ (0.53×10$^{-7}$–1.47×10$^{-7}$)</td>
<td>140.3±7.9</td>
</tr>
<tr>
<td>With V$_1$ receptor antagonist</td>
<td>6.08×10$^{-7}$</td>
<td>133.9±6.2</td>
</tr>
<tr>
<td>(10$^{-4}$ mol/L)+AVP (3×10$^{-3}$ mol/L) (n=4)</td>
<td>(5.81×10$^{-7}$–6.19×10$^{-7}$)</td>
<td></td>
</tr>
</tbody>
</table>

AVP indicates arginine vasopressin. Values are mean±SEM. Maximal contraction is expressed as a percentage of response to 100 mmol/L KCl.

* $P<.05$ vs control rings.
contractile effects of electrical field stimulation and norepinephrine. The potentiating effects occur at AVP concentrations substantially lower than those required to produce a clear direct contractile response. In a previous work, we found that contractions of human saphenous veins in response to AVP are relatively low compared with human arteries, indicating that these veins may have a low population or sensitivity of receptor sites for this peptide. However, an interesting finding of the present study is that the low responsiveness of the saphenous vein coincides with a high sensitivity to the modulating effects of AVP on adrenergic-mediated responses. Thus, it appears that the indirect (potentiating) effects of AVP on human saphenous vein may act synergistically with the effects of adrenergic stimulation.

Previous studies have suggested the existence of V₂ receptors in some vascular beds that could mediate vasodilatation. Administration of either V₂ receptor agonists or vasopressin during V₁ receptor blockade increased blood flow in some vascular beds and decreased peripheral resistance in both humans and dogs. In the human saphenous vein, both AVP and the V₂ receptor agonist desmopressin cause relaxation that seems largely dependent on the release of dilating prostaglandins. Therefore, we examined the potential role of V₂ receptor stimulation in the enhancing effects of AVP. The results do not support the intervention of V₂ receptors in these responses. First, the selective V₂ receptor agonist desmopressin did not modify responses to electrical field stimulation. On the other hand, the V₂ receptor antagonist d(CH₂)₅Tyr(Me)AVP and the V₂ receptor agonist desmopressin cause relaxation that may be due to alterations at the receptor level, leading to an increased affinity of norepinephrine for its receptor. This may be a likely explanation because AVP increased the contractions to exogenously applied norepinephrine. Thus, our data are consistent with the suggestion that potentiation of the effects of nerve stimulation by AVP corresponds to a postsynaptic enhancement of the action of norepinephrine.

We also considered the possibility that stimulation of V₁ receptors may facilitate calcium entry through dihydropyridine calcium channels. Our results show that nifedipine did not affect the direct contractions of AVP or prevent the potentiating action of AVP on norepinephrine- and electrical field stimulation–induced contractions. This indicates that influx of extracellular calcium through dihydropyridine-sensitive calcium channels does not importantly contribute to the direct contractile effects of AVP or participate in the potentiating effect of AVP on adrenergic contractions. In line with that interpretation, our results show that AVP did not affect the concentration–response curve to KCl, an agonist that induces contraction by facilitating calcium entry through voltage-dependent calcium channels. Other mechanisms of calcium handling such as an increase in inositol phosphate metabolism and/or increase calcium release from intracellular reservoirs could be involved in the potentiating effects of AVP. In contrast, in human mesenteric arteries, AVP brings about a facilitation of extracellular Ca²⁺ entry by KCl through voltage-dependent calcium channels. The precise explanation for such tissue specificity is not known, but it may be due to differences in distribution and pharmacological properties of V₁ vascular receptors between mesenteric arteries and saphenous veins.

In contrast to the present results, Hilgers et al. did not find any effect of AVP on sympathetic transmission in an in vitro perfused rat hind limb preparation. A common finding in the vascular effects of this peptide is the heterogeneity of responsiveness depending on regional and species differences and may be due to different populations or sensitivity of receptor sites for AVP. In addition, Hilgers et al. used only one concentration of AVP (0.3 nmol/L), which probably was insufficient to modulate neurogenic responses. Because the present findings show that the potentiating effects of AVP are concentration dependent, it is conceivable that differences between the concentrations of AVP used may also account for the discrepant findings of our study and those of Hilgers et al.

Some of the present observations may be of clinical significance. Several reports indicate that large arteries and veins from various animal species may synthesize and store an AVP immunoreactive material that appears identical to authentic AVP, but originates from the neurohypophysis. This raises the possibility that locally released AVP may reach concentrations high enough to act synergistically with the adrenergic neurotransmitter. The concentration of AVP in this study may be lower than those expected to occur in response to hypotension, dehydration, and exercise and in some patients with hypertension or congestive heart failure. Consequently, the amplifying effect of AVP on adrenergic-mediated constriction, shown in the present experiments, may importantly contribute to mechanisms involved in acute ischemic syndromes associated with venous grafts. The human saphenous vein can undergo spasm, which is a clinically relevant problem, immediately after autologous grafts in the arterial circulation or coronary bypass surgery. In view of the specificity and potency of the AVP antagonist d(CH₂)₅Tyr(Me)AVP, it seems
appropriate to consider the use of V1 receptor antagonists in circumstances in which AVP plasma concentrations are raised. Furthermore, provided that V1 receptor blockade is present, AVP induces marked dilatation of previously contracted human arteries and saphenous veins, probably because of the release of vasodilatory prostaglandins from the vessel wall. These findings could explain the reduction of vascular resistance that has been observed after intravenous administration of the V1 antagonist d(CH2)5Tyr(Me)AVP in patients with hypertensive or congestive heart failure in the presence of high plasma AVP levels.34,36

In conclusion, the results of the present study demonstrate that AVP, in addition to its direct vasoconstrictor effect, strongly potentiates the contractions of human saphenous veins to norepinephrine and stimulation of perivascular sympathetic nerves. Both the direct and indirect effects of AVP appear to be mediated by V1 receptor stimulation. The potentiation induced by AVP is not related to activation of dihydropyridine-sensitive calcium channels.

Acknowledgments
This work was supported by the Comisión Interministerial de Ciencia y Tecnología, Ministerio de Sanidad, and Generalitat Valenciana.

References
Arginine Vasopressin Enhances Sympathetic Constriction Through the \( V_1 \) Vasopressin Receptor in Human Saphenous Vein

Pascual Medina, Antonio Acuña, Juan B. Martínez-León, Eduardo Otero, José M. Vila, Martín Aldasoro and Salvador Lluch

_Circulation_. 1998;97:865-870
doi: 10.1161/01.CIR.97.9.865

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/97/9/865

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/