Desmopressin is a Potent Vasorelaxant of Aorta and Pulmonary Artery Isolated from Rabbit and Rat

Roger A. Johns, M.D.*

Hypotension related to the intraoperative use of desmopressin acetate to improve platelet function following cardiopulmonary bypass has recently been reported. To investigate the direct vascular actions of this drug as a potential mechanism of its induced hypotension, cumulative, dose-dependent (3.7 × 10⁻⁹ to 1.2 × 10⁻⁷ M) effects of desmopressin were studied in isolated phenylephrine precontracted rings of rat and rabbit thoracic aorta and rabbit pulmonary artery. Desmopressin was a potent vasodilator of all vessel types studied with significant (P < 0.05) vasodilation beginning at 7.5 × 10⁻⁸ M. Vascular relaxation of all vessels was greater when the vascular endothelium was intact (P < 0.05). Indomethacin potentiated (P < 0.05) vascular relaxation in rat and rabbit aortic rings and partially inhibited (P < 0.05) relaxation in rabbit pulmonary artery rings. Selective antagonists of vasopressin V₁ (d(CH₂)₂Tyr(Me)AVP, 1 × 10⁻⁸ M) and V₂ (d(CH₂)₅[Asp¹Ile⁸,Ile⁹,Ala-NH₂]½ AVP, 1 × 10⁻⁸ M) receptors and of histamine H₁ (diphenhydramine, 1 × 10⁻⁵ M) and H₂ (cimetidine 1 × 10⁻⁵ M) receptors had no effect on desmopressin-induced relaxation of rat aortic rings. Chlorobutanol, the diluent in which desmopressin is supplied, was devoid of vascular effects. To study the effects of desmopressin on vascular cyclic GMP and cyclic AMP concentrations, a cultured bovine aortic smooth muscle—rat vascular smooth muscle coculture model was employed. Desmopressin (1 × 10⁻⁷ and 1 × 10⁻⁶ M) did not significantly alter control values of either cyclic nucleotide. The authors conclude that desmopressin is a potent vasodilator, primarily via a direct action on vascular smooth muscle that is modulated by the endothelium and by dilating and constricting prostanoids and that this vasodilation could account for the hypotension observed with its clinical use. (Key words: Artery: aorta; pulmonary. Cyclic AMP (5',5'-cyclic adenosine monophosphate). Cyclic GMP (5',5'-cyclic guanosine monophosphate). Peptides: desmopressin; dDAVP; 1-desamino-8-D-arginine vasopressin. Endothelium. Indomethacin. Receptors: vasopressin V₁, V₂; histamine H₁, H₂. Sympathetic nervous system: catecholamines; phenylephrine.)

DESMOPRESSIN (1-desamino-8-D-arginine vasopressin; dDAVP) is a synthetic analogue of vasopressin used in the treatment of patients with central diabetes insipidus. More recently it has been used extensively to improve hemostasis and postoperative blood loss in patients with a variety of platelet disorders including the platelet dysfunction observed following cardiopulmonary bypass.

We recently reported the occurrence of severe hypotension following desmopressin administration after cardiopulmonary bypass and have subsequently confirmed these initial observations in a placebo-controlled, randomized double-blind study. Others have reported hypotensive actions of desmopressin in noncardiopulmonary bypass situations.

To further understand the mechanisms by which desmopressin exerts this clinically observed hypotensive effect, we studied the direct actions of desmopressin on isolated vessel segments from rabbit aortic and pulmonary arteries and from rat aorta. Observations were made in the presence and absence of vascular endothelium as well as with inhibitors of cyclooxygenase and inhibitors of vasopressin and histamine receptors. In addition, the effects of desmopressin on vascular cyclic AMP and cyclic GMP concentrations were studied in cocultures of endothelium and vascular smooth muscle cells.

Methods

VASCULAR RING PREPARATION

Male Sprague-Dawley rats (300 g) and New Zealand white rabbits (2 kg) were anesthetized with ketamine (50 mg/kg) and killed by exsanguination in accordance with institutional animal care committee guidelines. Thoracic aortae (rat and rabbit) and first branch pulmonary arteries (rabbit) were gently dissected free, removed, and placed in cold modified Kreb's buffer (NaCl 111 mM, KCl 5 mM, NaH₂PO₄ 1 mM, MgCl₂ 0.5 mM, NaHCO₃ 25 mM, CaCl₂ 2.5 mM, and dextrose 11.1 mM). The vessels were carefully cleaned of fat and extraneous tissue and divided into 2–3 mm ring segments. In some vessels, the endothelium was carefully removed by rotating the ring gently on the tip of a watchmaker's forceps. The rings were suspended in 10 ml water-jacketed tissue baths (37°C) containing modified Kreb's buffer aerated with 95% O₂/5% CO₂ at pH 7.4 and connected to Grass FT-03 force transducers for the measurement of isometric tension. The buffer was changed at 15-min intervals during a 60–90-min equilibration period. Optimal resting tension was determined as that resting tension at which a maximal response to a supramaximal dose of phenylephrine (10⁻⁵ M) was obtained. Active tone was induced in the ring.
segments with an EC₅₀ dose of phenylephrine, and the integrity of the endothelium was documented by observing the vasodilation produced by the addition of methacholine (1 × 10⁻⁶ M). Vascular rings were discarded if methacholine-induced relaxation was less than 30%. The absence of functional endothelium was documented by no response or by slight vasoconstriction in response to the added methacholine. The rings were then washed with modified Kreb’s buffer until stabilized at resting tension.

Vascular Ring Protocols

In all experiments, vascular rings were precontracted with phenylephrine (EC₅₀). Cumulative dose-dependent vascular responses to desmopressin were determined in each vessel type with and without endothelium and in the presence and absence of indomethacin (2.8 × 10⁻⁵ M). Vascular responses in the rat aorta also were studied in the presence of selective antagonists of vasopressin V₁ (d(CH₂)₅Tyr(Me)AVP, 1 × 10⁻⁶ M), vasopressin V₂ (d(CH₂)₅[D-Ile₂, Ile⁴, Ala-NH₂]AVP)²⁵-²⁷ and histamine H₁ (diphenhydramine, 1 × 10⁻⁵ M) and H₂ (cimetidine, 1 × 10⁻⁵ M) receptors. The dose-dependent vascular effects of chlorobutanol, the diluent in which desmopressin is supplied, also were investigated.

Preparation and Use of Cocultures

Bovine aortic endothelial cells were isolated as previously described.²⁷ Purity of the cultures was determined by observing uptake of fluorescent labelled acetylated low density lipoprotein and by demonstrating a single-messenger RNA (northern blots) for actin in endothelium, which migrated separately on gel electrophoresis from the two actin-messenger RNAs for confluent cultures of vascular smooth muscle.²⁷²⁸ The endothelial cells were subsequently placed into microcarrier culture by seeding 2 × 10⁻⁷ endothelial cells onto 0.6 g of Cytodex microcarrier beads in 200 ml of M199 medium containing 20% fetal calf serum in a 2-l roller bottle. After a 2-h incubation period with occasional stirring to allow the cells to attach to the beads, the bottles were rolled at 1 RPM. The medium was changed every third day and the bottles gassed with 95% O₂/5% CO₂. Rat aortic smooth muscle was isolated and maintained as previously described.²⁸

In preparation for experiments, endothelial cells on microcarrier beads were washed with serum-free medium and placed into culture wells (1 cm²) of confluent vascular smooth muscle. The number of each cell type was calculated to provide a 1:1 ratio of endothelial to smooth muscle cells in each coculture. The two cell types in coculture were then allowed to incubate for 3-4 h before experimentation. Cocultures were stimulated with desmopressin 1 × 10⁻⁷ M, desmopressin 1 × 10⁻⁸ M, or control buffer for 60 s following which the medium was aspirated off and 1 ml of 0.1 N HCl was added to the coculture to extract cyclic nucleotides. As positive controls for cyclic GMP, the cocultures were stimulated with the endothelium-dependent dilators bradykinin (1 × 10⁻⁶ M), A23187 (1 × 10⁻⁶ M), and ATP (1 × 10⁻⁴ M) and the endothelium independent vasodilator sodium nitroprusside (1 × 10⁻⁴ M). Isoproterenol (1 × 10⁻⁷ M) served as a positive control for cyclic AMP.

Determination of Cyclic AMP and Cyclic GMP Concentrations

Tissue cyclic AMP and cyclic AMP concentrations were determined by radioimmunoassay of the acetylated HCl extracts by the methods of Harper and Brooker²⁹ using New England Nuclear (Boston, MA) radioimmunoassay kits. Recovery of cyclic GMP and cyclic AMP was greater than 90% in each of the respective assays.

Drugs

Phenylephrine, methacholine, indomethacin, bradykinin, ATP, A23187, isoproterenol, and chlorobutanol were purchased from Sigma Chemical Company (St. Louis, MO). Sodium nitroprusside was purchased from Fisher Scientific (Pittsburg, PA). Desmopressin was purchased from Rorer Pharmaceutical Corporation (Horsham, PA). The selective vasopressin V₁ and V₂ antagonists were kindly provided as a gift from Dr. Maurice Manning of the Medical College of Ohio. All drugs were prepared and diluted in sterile water except for indomethacin which was dissolved in a 150-mM sodium bicarbonate solution (pH 8.8) and readjusted to a pH of 7.4 with 2 N HCl prior to use and A23187 which was prepared as a stock solution in dimethyl sulfoxide (1,000 times greater than final concentration) and diluted into modified Kreb’s buffer immediately prior to use.

Data Analysis

Each relaxation response curve represents data from a minimum of 8-10 animals. With regard to coculture experiments, four replicates were performed for each condition in each of three separate experiments. All data are plotted as mean ± SE. Vascular responses in the presence and absence of endothelium and the presence and absence of indomethacin were compared using two-way analysis of variance. Significant dose-dependent differences for individual dose-response curves were determined using one-way analysis of variance. The different coculture conditions were compared using one-way analysis of variance. Where indicated, Neuman-Keul’s multiple range testing was used for intergroup comparisons. Vascular responses in the presence and absence of specific
receptor inhibitors were compared using a two-tailed unpaired Student's t test. Differences were considered significant at $P < 0.05$.

**Results**

**Vascular Responses with and Without Endothelium**

Desmopressin caused significant ($P < 0.05$) dose-dependent vasodilation in rabbit aorta and pulmonary artery at concentrations ranging from $3.7 \times 10^{-8}$ M to $1.12 \times 10^{-7}$ M and in rat aorta at concentrations ranging from $7.5 \times 10^{-8}$ M to $1.12 \times 10^{-7}$ M (figs. 1–3). While vascular relaxation occurred in the presence and absence of endothelium, the effect was significantly greater in vessels in which the endothelium was intact.

**Effect of Indomethacin on Vascular Responses**

In systemic vessels (rat and rabbit aorta; figs. 1 and 2), indomethacin ($2.8 \times 10^{-6}$ M), an inhibitor of cyclooxygenase metabolism of arachidonic acid to prostaglandins, significantly ($P < 0.05$) potentiated the desmopressin-induced vasodilation of endothelium-intact rings, implying that desmopressin stimulates the release of a constricting prostaglandin in addition to a nonprostaglandin endothelium-derived vasodilator. In contrast, in rabbit pulmonary vessels (fig. 3), indomethacin partially inhibited the endothelium-dependent component of desmopressin-induced vasodilation implying that desmopressin stimu-

**FIG. 2.** Dose-dependent ($P < 0.05$) vascular effects of desmopressin on isolated rat aortic rings in the presence and absence of endothelium (endo+, endo−) and in the presence of indomethacin (indo; $2.8 \times 10^{-6}$ M) at concentrations ranging from $7.5 \times 10^{-8}$ to $1.12 \times 10^{-7}$ M. Values are expressed as mean ± SEM; n = rings from eight to ten animals for each data point. #$P < 0.05$ (endo+ vs endo−). *$P < 0.05$ (endo+ vs endo+ , indo).

lates the release of a dilating prostaglandin from pulmonary vascular rings.

**Effect of Vasopressin and Histamine Receptor Inhibition on Desmopressin-Induced Vasodilation**

Neither the specific vasopressin V$_1$ receptor antagonist (d(CH$_2$)$_5$Tyr(Me)AVP, $1 \times 10^{-6}$ M) nor the specific vasopressin V$_2$ receptor antagonist (d(CH$_2$)$_5$D-Ile$_2$, Ile$_4$, Ala-
NH_{3}\) AVP, \(1 \times 10^{-6}\) M) had a significant effect on dose-dependent desmopressin-induced vasodilation of intact rat thoracic aorta rings (figs. 4 and 5). The combination of histamine H₁ and H₂ receptor antagonists (diphenhydramine \(1 \times 10^{-5}\) M and cimetidine \(1 \times 10^{-5}\) M, respectively; fig. 6) similarly were without effect on dose-dependent desmopressin-induced vascular relaxation of rat aortic rings.

**Vascular Effects of Chlorobutanol**

Chlorobutanol (1,1,1-trichlor-2-methyl-propanol) had no vascular effects on rat or rabbit aorta or rabbit pulmonary artery rings over a wide concentration range (\(5 \times 10^{-4}\) to \(1 \times 10^{1}\) mg/ml).

**Effect of Desmopressin on Cyclic Nucleotide Concentrations in Cocultures**

Concentrations of cyclic GMP and cyclic AMP in desmopressin-stimulated (\(1 \times 10^{-7}\) M and \(1 \times 10^{-8}\) M) cocultures (table I) were not significantly different from control. The positive controls for cyclic GMP (bradykinin, A23187, ATP, and sodium nitroprusside) all caused significant (\(P < 0.05\)) increases in cyclic GMP content. ISOproterenol, the positive control for cyclic AMP, caused increases in cyclic AMP significantly greater than control (\(P < 0.05\)).

**Discussion**

These experiments have investigated the *in vitro* vascular actions of desmopressin on isolated vascular rings from rat and rabbit aorta and rabbit pulmonary artery in an attempt to find insight into the mechanisms of clinically observed hypotension following the use of iv desmopressin. We have found that desmopressin is a potent vasodilator of all of the vessel types studied. It is primarily a direct vasodilator of vascular smooth muscle, although there is an endothelium-dependent component to the observed vasodilation. These vascular effects do not appear to be mediated by either vasopressin V₁ or V₂ receptors or histamine H₁ or H₂ receptors and are not due to chlorobutanol, the diluent in which desmopressin is dissolved.

**Fig. 4.** Effect of the vasopressin V₁ receptor antagonist, d(CH₂)₅Tyr(Me)AVP (\(1 \times 10^{-8}\) M) on dose-dependent vascular responses to desmopressin in phenylephrine precontracted, endothelium-intact rat thoracic aorta rings. Values are expressed as mean \(\pm\) SEM; \(n = \) rings from eight to ten animals for each data point.

**Fig. 5.** Effect of the vasopressin V₂ receptor antagonist, d(CH₂)₅[D-Ille₄,Leu₅,Ala-NH₂] AVP (\(1 \times 10^{-8}\) M) on dose-dependent vascular responses to desmopressin in phenylephrine precontracted, endothelium-intact rat thoracic aorta rings. Values are expressed as mean \(\pm\) SEM; \(n = \) rings from eight to ten animals for each data point.

**Fig. 6.** Effect of the combined histamine H₁ and H₂ receptor antagonists, diphenhydramine (\(1 \times 10^{-8}\) M) and cimetidine (\(1 \times 10^{-8}\) M) on dose-dependent vascular responses to desmopressin in phenylephrine precontracted, endothelium-intact rat thoracic aorta rings. Values are expressed as mean \(\pm\) SEM; \(n = \) rings from eight to ten animals for each data point.
TABLE 1. Effect of Desmopressin on Cyclic Nucleotide
Concentrations in Cocultures

<table>
<thead>
<tr>
<th></th>
<th>Cyclic GMP (pmol/well)</th>
<th>Cyclic AMP (pmol/well)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.778 ± 0.03</td>
<td>1.53 ± 0.21</td>
</tr>
<tr>
<td>Desmopressin,</td>
<td>0.86 ± 0.12</td>
<td>1.38 ± 0.07</td>
</tr>
<tr>
<td>1 × 10⁻⁷ M</td>
<td>0.88 ± 0.14</td>
<td>1.35 ± 0.03</td>
</tr>
<tr>
<td>Desmopressin,</td>
<td>8.07 ± 0.52*</td>
<td>8.07 ± 0.52*</td>
</tr>
<tr>
<td>1 × 10⁻⁸ M</td>
<td></td>
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<tr>
<td>BK, 1 × 10⁻⁷ M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A23187, 1 × 10⁻⁶ M</td>
<td>5.23 ± 0.26*</td>
<td></td>
</tr>
<tr>
<td>ATP, 1 × 10⁻⁴ M</td>
<td>6.65 ± 0.52*</td>
<td>6.65 ± 0.52*</td>
</tr>
<tr>
<td>SNP, 1 × 10⁻⁶ M</td>
<td>7.50 ± 0.14*</td>
<td>7.50 ± 0.14*</td>
</tr>
<tr>
<td>Isoproterenol,</td>
<td>1 × 10⁻⁷ M</td>
<td>4.26 ± 0.37*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

BK = bradykinin. ATP = adenosine triphosphate. SNP = sodium nitroprusside.

* P < 0.01 (compared with control).

The results of our studies using cocultures of vascular endothelial and smooth muscle cells would indicate that the mechanisms of vasodilation induced by desmopressin do not involve cyclic AMP or cyclic GMP.

The potent vasodilating action of desmopressin may well account for the hypotension observed when this drug is administered clinically. The peak plasma concentrations of desmopressin in humans 10 min following an infusion at 0.3 μg·kg⁻¹·20 min⁻¹ is reported to be 891 ± 89 pmol/L.³⁰ The plasma concentration and the concentration seen by the vasculature would be expected to be much higher during the infusion (the time at which hypotensive effects have been observed) and could easily be within range of the 7–10 nmol threshold at which we observed vasodilation in isolated vascular rings.

Desmopressin-induced hypotension was first reported by Derkx et al.³¹ who observed facial flushing, a decrease in diastolic blood pressure and an increase in heart rate with iv infusion of the drug and proposed that the vasodilating properties of desmopressin were due to competitive antagonism of the V₁ receptor-mediated vasoconstricting action of endogenous arginine vasopressin. Such a mechanism would be unlikely given our in vitro results in which desmopressin is a potent vasodilator in the absence of arginine vasopressin and in the presence of a selective V₂ antagonist.

Bichet et al.³⁰ have recently suggested that desmopressin may decrease blood pressure through a direct vascular effect mediated by a V₂ or some other non-V₁ receptor. They observed that carriers of the gene for nephrogenic diabetes insipidus did not exhibit hypotension or change in heart rate with desmopressin infusion, while normal controls and controls with central diabetes insipidus experienced a 10–15% decrease in mean arterial pressure and a 20–25% increase in heart rate. They hypothesized that dDAVP exerts its vasodilating effects through a V₂ receptor and that the receptor or its transducing mechanism (adenylate cyclase-stimulated cyclic AMP) is defective in blood vessels as well as in the kidney tubules of patients with nephrogenic diabetes insipidus. The presence of V₂ receptors in blood vessels, however, is hypothetical³⁰ and the lack of inhibition of vasodilation by a specific V₂ antagonist in the present study does not support V₂-mediated vasodilation by dDAVP.

Vascular vasopressin V₁ receptors are known to mediate the vasoconstriction response of vasopressin,³⁰,²³,²⁶,³³ while V₂ receptors in the renal tubules mediate its anti diuretic effects.²⁴,²⁵,³⁴ Signal transduction by these two receptor types has been extensively studied. The V₁ receptor is linked to the phospholipase C-inositol phosphate system³⁵ while the V₂ receptor leads to activation of adenylate cyclase and an increase in cyclic AMP concentration.³⁵,³⁶,³⁷ We were unable to demonstrate a desmopressin-induced increase in cyclic AMP concentration in our coculture system, providing further evidence that the V₂ receptor is not involved in mediating vasodilation.

Several investigators have studied the effects of desmopressin and vasopressin on prostaglandin production. Greer et al.³⁷ observed that desmopressin stimulated an increase in 6-keto-prostaglandin F₁α (6-keto-PGF₁α), the stable breakdown product of prostaglandin I₂ (PGI₂, prostacyclin), in serum from normal individuals and from patients with Von Willebrand’s Disease. D’Angelo et al.³⁸ however, found no effect of desmopressin on plasma levels of PGI₂ in a similar group of patients. Conflicting results are also found in in vitro studies of prostaglandin release. Belch et al.³⁹ reported increases in PGI₂ but not thromboxane B₂ (TXB₂) in rabbit aortic rings stimulated with desmopressin. In contrast, Barnhart et al.⁴⁰ were unable to demonstrate changes in 6-keto PGF₁α or TXB₂ concentration in the perfusate of human umbilical veins stimulated with desmopressin. Hassid and Williams⁴¹ demonstrated a marked dose-dependent increase in vasopressin-stimulated 6-keto-PGF₁α release from cultured mesenteric arterial vascular smooth muscle which was prevented by a V₁ receptor antagonist. Desmopressin had a relatively small effect on 6-keto-PGF₁α in this preparation.

The present studies support a role for desmopressin-induced prostaglandin release in mediating part of the vascular actions of this drug. Through use of the cyclooxygenase inhibitor indomethacin we have found that the endothelium-dependent component of desmopressin-induced relaxation of rabbit pulmonary artery is dependent on a dilating prostaglandin. In the rabbit aorta, desmopressin-induced vasorelaxation was potentiated by indomethacin providing evidence for the release of cyclooxygenase-derived vasoconstricting prostaglandins. This is consistent with the previously mentioned demonstration...
of PG12 release (a dilator of most blood vessels) from rabbit aortic rings.\(^9\) The aortic preparations also could have produced PG12; but as rabbit and rat aorta do not exhibit a vasoactive response to PG12,\(^4\) the effects of constricting prostaglandins predominated.

The endothelium-dependent component of desmopressin-induced relaxation of these blood vessels does not appear to involve endothelium-derived relaxing factor (EDRF). In pulmonary vessels, the endothelium-dependent component of vasorelaxation was entirely eliminated by indomethacin, an agent that has no effect on EDRF action.\(^43\)\(^44\) In addition, desmopressin exhibited no EDRF activity as measured by cyclic GMP accumulation in our coculture model (table 1). In contrast, the pressor response to arginine vasopressin in the isolated superior mesenteric bed of the rat has been shown to be modulated by the simultaneous release of EDRF.\(^45\)

In conclusion, desmopressin is a potent direct and indirect dilator of isolated vascular rings from rat and rabbit aorta and rabbit pulmonary artery. While its vascular activity is partially modulated by constricting and dilating prostaglandins, the mechanism(s) of its direct dilating effect on vascular smooth muscle is uncertain. The potent vasodilating action of desmopressin may account for the hypotension observed with its clinical use.

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References


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