Vasodilatory Effect of Arginine Vasopressin Is Mediated by Nitric Oxide in Human Forearm Vessels

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Abstract

Arginine vasopressin (AVP) causes biphasic changes in vascular resistance in human forearms; vasoconstriction at lower doses and vasodilation at higher doses. Vasoconstriction is mediated by the V1 receptor. However, the mechanism of AVP-induced vasodilation is not known. We investigated whether AVP-induced vasodilation is mediated by nitric oxide (NO) in human forearms by examining the effects of L-arginine (a precursor of NO) and N^G-monomethyl-L-arginine (L-NMMA, a blocker of NO synthase) on AVP-induced vasodilation. AVP was infused intraarterially at doses of 0.05, 0.1, 0.2, 0.5, and 1.0 ng/kg per min ($n = 8$). The lower doses of AVP (≤ 0.1 ng/kg per min) increased, whereas the higher doses of AVP (≥ 0.5 ng/kg per min) decreased forearm vascular resistance (FVR) ($P < 0.01$). Intraarterially infused L-arginine at 10 mg/min did not alter arterial pressure, baseline FVR, or heart rate. L-arginine did not alter the magnitude of AVP-induced vasoconstriction at the lower doses, but L-arginine augmented the magnitude of AVP-induced vasodilation at doses of 0.2 ($P < 0.05$), 0.5 ($P < 0.01$), and 1.0 ($P < 0.05$) ng/kg per min. In another group ($n = 6$), intraarterially infused L-NMMA (4 μmol/min for 5 min) increased baseline FVR without systemic effects, and inhibited acetylcholine-induced vasodilation ($P < 0.01$). L-NMMA at this dose inhibited AVP-induced vasodilation ($P < 0.01$) but did not affect vasoconstriction. L-arginine reversed the inhibitory effect of L-NMMA. Our results suggest that the vasodilatory effect of AVP may be mediated by NO in human forearms. (*J. Clin. Invest.* 1993. 92:1483–1490.) Key words: L-arginine • endothelium-derived relaxing factor • N^G-monomethyl-L-arginine • plethysmography • human study

Introduction

It has been believed for long time that arginine vasopressin (AVP)[1] is a potent vasoconstrictor (1, 2). However, recent studies in humans have demonstrated that the effects of AVP on forearm resistance arteries are biphasic (3, 4). Intraarterially infused AVP at lower doses causes vasoconstriction, and AVP at higher doses causes vasodilation (3, 4). AVP-induced vasoconstriction is mediated by the V1 receptor because it is inhibited by a specific V1 receptor antagonist (4, 5). However, the mechanism of AVP-induced vasodilation is not known.

Recently, it has been well accepted that the endothelium plays an important role in control of vascular tone. Nitric oxide (NO) is produced in the process of conversion of a semi-essential amino acid, L-arginine, to L-citrulline by NO synthase in endothelial cells (6–9). NO is one of endothelium-derived relaxing factors. NO is a potent and direct activator of soluble guanylate cyclase and increases 3′,5′ monophosphate (cGMP) (10–12), which causes vasodilation. Acetylcholine-induced vasodilation in the human forearm is mediated by NO since the vasodilation is inhibited by N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of NO as well, and augmented by supplementation of L-arginine (13, 14).

In vitro, it has been shown in several experiments that AVP causes vasodilation (15–18), that the vasodilation is inhibited by pretreatment with L-NMMA, and that pretreatment with L-arginine prevented the inhibitory effects of L-NMMA (18). These results suggest that vasodilation by AVP is probably mediated by NO in animals. It is possible that AVP-induced vasodilation in humans also is mediated by NO. However, this possibility has not been examined.

In this study, we investigated the possibility that AVP-induced vasodilation is mediated by NO in human forearm vessels. First, we infused small amounts of AVP intraarterially into the brachial artery while measuring forearm blood flow (FBF). AVP caused vasoconstriction at lower doses and caused vasodilation at higher doses. Second, we determined whether supplementation of L-arginine augmented AVP-induced vasodilation. Third, we determined whether L-NMMA inhibited AVP-induced vasodilation. Fourth, we determined whether L-arginine reversed the inhibitory effects of L-NMMA.

Methods

General procedure. Subjects were all young, healthy male students (21–28 yr old) at our university who volunteered for the study. The protocol was explained, and informed written consent was obtained from each subject. The study was approved by Ethical Committee for Human Study in our institution. The study was done with subjects in a supine position and in an air-conditioned room with room temperature of ~ 25–26°C. Under local anesthesia with 2% procaine, the left brachial artery was cannulated with a 20-gauge intravascular over the needle poly(tetrafluoroethlene) catheter (Quick-Cath; Travenol Laboratories, Inc., Baxter Healthcare Corp., Deerfield, IL) for drug infusion. The catheter was connected by a three-way stopcock to a pressure transducer (Viggo-Spectramed, Oxnard, CA) for direct measurement of arterial pressure. The arterial line was kept open by infusing heparinized saline (0.1 ml/min) when no drug was being administered. In some subjects, a vein in the antecubital region of the ispilateral arm was

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1. *Abbreviations used in this paper:* AVP, arginine vasopressin; FBF, forearm blood flow; FVR, forearm vascular resistance; L-NMMA, N^G-monomethyl-L-arginine; MBP, mean blood pressure; NO, nitric oxide.

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cannulated with the same cannula as used for the artery to obtain blood samples for measuring plasma levels of AVP and L-arginine. Heart rate was obtained by counting pulse rate for a few minutes on arterial pressure recordings.

Measurements of forearm blood flow. Forearm blood flow was measured by using a mercury in silastic strain gauge plethysmograph with a venous occlusion technique (19, 20). The strain gauge was placed ~ 5 cm below the antecubital crease. Forearm blood flow (milliliters per minute per 100 ml forearm) was calculated from the rate of increase in forearm volume, while venous return from the forearm was prevented by inflating the cuff on the upper arm. The pressure in the venous occlusion or congesting cuff on the upper arm was 40 mmHg. Circulation to the hand was arrested by inflating a cuff around the wrist. The wrist cuff was inflated before the determination of forearm blood flow and continuously throughout the measurements. Forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic pressure plus one third of the pulse pressure in millimeters of mercury) by the forearm blood flow. These values are expressed as units throughout this report. An average of four flow measurements made 15-s intervals, which were calculated by two authors independently, was used for later analysis.

Forearm vascular responses to drugs. After the placement of cannulae and a strain gauge plethysmograph, ≥ 15 min were allowed for subjects to become accustomed to the study condition before the experiments were begun.

Four experimental protocols were performed. In the first protocol, we determined responses of forearm blood flow to intraarterially infused AVP at graded doses before and after L-arginine or D-arginine (n = 8). First, we examined forearm responses of forearm blood flow to intraarterially infused AVP at 0.05, 0.1, 0.2, 0.5, and 1.0 ng/kg per min for 2 min at each dose. Forearm blood flow and arterial pressure were continuously monitored and recorded. At least 15 min after the final dose of AVP, when forearm blood flow had returned to the baseline level, intraarterial infusion of D-arginine was started and continuously infused at a dose of 10 mg/min. The baseline measurements of forearm blood flow, arterial pressure, and heart rate were obtained 15 min after infusion of D-arginine. While infusion of D-arginine at 10 mg/min, AVP was infused intraarterially in the same way as before D-arginine. Forearm hemodynamic measurements were repeated as described above. After recovery, infusion of L-arginine was similar performed described as above. Forearm hemodynamic measurements were repeated as described above.

In another group of subjects (n = 6), the second protocol was performed. Because AVP at higher doses caused vasodilation in the first protocol, we examined whether pretreatment with L-NMMA blocked the vasodilator effects of AVP. We examined responses of forearm blood flow to intraarterially infused acetylcholine at 4 and 12 μg/min or AVP at 0.1, 0.5, and 1.5 ng/kg per min for 2 min at each dose. Series of infusions of acetylcholine and those of AVP were alternated. At least 15 min later, when forearm blood flow had returned to the baseline value, L-NMMA was infused at 4 μmol/min for 5 min. Then acetylcholine or AVP was infused immediately after stopping L-NMMA in the same way as before infusion of L-NMMA. Forearm blood flow and arterial pressure were continuously monitored and recorded during infusion of drugs.

The third protocol was performed in five subjects after the second protocol was finished. In this protocol, we determined whether the effects of L-NMMA were reversed with L-arginine. After finishing the second protocol and the parameters had returned to the baseline values, we determined responses of forearm blood flow to intraarterially infused AVP at 1.5 ng/kg per min for 2 min. After the parameters had returned to the baseline values, L-NMMA was infused at 4 μmol/min for 5 min. Then AVP was immediately infused in the same way as before infusion of L-NMMA. After ≥ 15 min, when the forearm blood flow had returned to the baseline value, L-NMMA (4 μmol/min) and L-arginine (8 mg/min) were simultaneously infused for 5 min, and then AVP was infused in the same way as before infusion of L-NMMA. Forearm blood flow and arterial pressure were continuously monitored and recorded during infusion of drugs.

The fourth protocol was performed in the third group of subjects (n = 5). In this protocol, we determined whether responses to AVP were altered over a period of 2 h. First, we examined responses of forearm blood flow to intraarterially infused AVP at 0.05, 0.1, 0.2, 0.5, and 1.0 ng/kg per min for 2 min at each dose. Forearm blood flow and arterial pressure were continuously monitored and recorded. Within 2 hs, the same protocol was repeated three times.

The total duration of the experiment in a subject did not exceed 2 h and 30 min. The infusion volume did not exceed 0.6 ml/min. We had confirmed that this volume of infusion itself did not alter forearm blood flow (19, 20). The last 1 min of measurements of forearm blood flow during infusion of each dose of the drug was used for later analysis.

Measurements of plasma arginine vasopressin and L-arginine. In seven subjects, 5 ml blood was drawn for measurements of AVP during the control period and during infusion of AVP at the dose of 1.0 ng/kg per min. Blood was sampled into a tube containing EDTA-2K (1 mg/ml). In seven subjects, 5 ml blood was drawn for measurements of L-arginine during control period and during infusion of L-arginine at dose of 10 mg/min. Blood samples were centrifuged immediately and stored in a freezer at −20°C. Plasma AVP and L-arginine were measured at a commercially available laboratory (SRL, Tokyo, Japan). Plasma AVP was measured in duplicate by RIA kits obtained from Mitsubishi Petrochemical Co. Ltd. (Tokyo, Japan), after Sep-Pak C18 extraction of plasma as previously described (21, 22). The crossreactivity of antiserum with 8-lysine vasopressin was 0.04%; it was 0.19% with 1-deamino-8-D-arginine vasopressin and < 0.01% with oxytosin and 8-arginine vasotocin. The sensitivity of the assay was 0.06 fg/ml. The recovery of AVP ranged from 79.3–99.4% at AVP concentrations of 0.50–8.00 pg/ml plasma; the results were corrected for recovery. The intra- and interassay coefficients of variation were 7.8% and 7.4%, respectively, for AVP concentrations of 1.15 and 1.50 pg/ml. Plasma L-arginine was measured by high performance liquid chromatography. The analyses were performed in a high performance analyzer (System 6300; Beckman Instruments, Inc., Fullerton, CA) equipped with a data integrator (model 7000; Systems Instruments Corporation of America, Santa, CA) (23). For a calibration standard mixture of 1.0 and 2.5 nmol/50 μl, the average CV in the measured areas was 1.4% and 1.6%, respectively.

Preparation of drugs. Synthetic AVP (20 pressor U/ml) (Pitressin, arginine vasopressin; Parke-Davis, Inc., Morris Plains, NJ) was dissolved in physiological saline immediately before use. For the infusion of L-arginine, commercially available L-arginine solution (0.1 g of L-arginine/ml, Morishita Pharmaceutical, Osaka, Japan) was used. D-Arginine was obtained from Sigma Chemical Co. (St. Louis, MO) and prepared similarly to L-arginine at the pharmacological section in our hospital. L-NMMA was obtained from Clinalfa AG (Bahnhofstrasse, Switzerland). Because acetylcholine is unstable in solution, 100 mg acetylcholine (Daiichi Pharmaceutical, Tokyo, Japan) was lyophilized and stored in a vial (0.4 mg acetylcholine per vial). It was dissolved in physiological saline (10 ml) immediately before use.

Statistical analysis. The hemodynamic values during infusions of AVP, acetylcholine, L-arginine, D-arginine, and L-NMMA were compared by one-way ANOVA for repeated measures to test treatment effects. The hemodynamic responses to AVP before and during infusion of L- or D-arginine at 10 mg/min were compared by two-way ANOVA. The hemodynamic responses to AVP or acetylcholine before and after infusion of L-NMMA at 4 μmol/min were compared by two-way ANOVA. The hemodynamic responses to AVP at 1.5 ng/kg per min before, after L-NMMA at 4 μmol/min, or L-NMMA plus L-arginine at 8 mg/min were compared by one-way ANOVA. The three dose-response curves to AVP in the fourth protocol were compared by two-way ANOVA. When significantly different by one-way or two-way ANOVA, they were compared by paired or unpaired t test to determine the location of difference. All values are expressed as means±SEM and $P < 0.05$ was to be statistically significant.

Table I. Forearm Vascular Responses to Intraarterial Infusion of Arginine Vasopressin before and after L-Arginine (n = 8)

	Control	AVP 0.05	AVP 0.1	AVP 0.2	AVP 0.5	AVP 1.0	*P* by two-way ANOVA
		ng/kg per min	*ng/kg per min*	*ng/kg per min*	*ng/kg per min*	*ng/kg per min*	
MBP (mmHg)							
Before L-A	91±3	90±4	90±4	90±4	91±3	93±4	NS
After L-A	89±4	90±4	90±4	90±4	91±4	93±4	
Heart rate (bpm)							
Before L-A	62±3	61±3	60±3	60±4	59±4	55±3	NS
After L-A	60±3	60±3	59±3	61±3	57±4	58±4	
FBF (ml/min per 100 ml)							
Before L-A	3.5±0.3	2.8±0.3	2.4±0.2	3.4±0.3	4.2±0.4	4.5±0.4	$P < 0.01$
After L-A	3.2±0.4	2.3±0.2	2.5±0.3	3.8±0.4	4.9±0.7	5.7±0.8	
FVR (U)							
Before L-A	26.4±1.7	33.5±2.5	38.6±3.0	27.7±2.3	22.8±1.7	21.2±1.1	$P < 0.01$
After L-A	29.4±3.0	41.2±4.0	39.6±5.6	25.5±2.9	20.6±2.5	17.9±1.9	
Percent FVR							
Before L-A	100	127±4	147±6	106±7	88±5	82±5	$P < 0.01$
After L-A	100	144±10	132±10	87±8	72±8	65±9	

Control, during infusion of saline; L-A, L-arginine; MBP, mean blood pressure. Values are mean±SEM.

Results

Responses to intraarterial infusion of arginine vasopressin (Table I). Intraarterial infusion of AVP ($n = 8$) caused biphasic changes in forearm blood flow. AVP decreased forearm blood flow at lower doses (0.05 and 0.1 ng/kg per min) and decreased forearm blood flow at higher doses (≥ 0.5 ng/kg per min) ($P < 0.01$) (Fig. 1). Arterial pressure or heart rate was not altered during infusion of AVP (Table I). Intraarterial infusion of AVP at doses of 1.0 ng/kg per min ($n = 7$) increased the plasma AVP from 3.8±0.8 to 1,173.8±196.6 pg/ml in the venous effluents of the ispilateral arm of AVP infusion ($P < 0.01$).

Effects of L-arginine (Table I). Intraarterial infusion of L-arginine at doses of 10 mg/min ($n = 7$) increased the plasma L-arginine from 17±3 to 602±65 μg/ml in the venous effluents of the ispilateral arm. Arterial pressure, heart rate, forearm blood flow, or forearm vascular resistance were not altered during infusion of L-arginine at 10 mg/min (Table I).

After infusion of L-arginine, AVP caused biphasic changes in forearm blood flow as before L-arginine ($P < 0.01$) (Fig. 1) (Table I). Simultaneous infusion of L-arginine ($n = 8$) did not alter vasoconstrictor responses to intraarterial AVP at 0.05 and 0.1 ng/kg per min, but it augmented vasodilator responses at 0.2 ($P < 0.05$), 0.5 ($P < 0.01$), and 1.0 ng/kg per min ($P < 0.05$) (Fig. 1). Infusion of D-arginine (10 mg/min) did not alter baseline forearm hemodynamics (Table II) nor altered responses to graded infusions of AVP (Fig. 2).

Effect of L-NMMA (Tables III and IV). Intraarterial infusion of acetylcholine ($n = 6$) at doses of 4 and 12 μg/min increased forearm blood flow ($P < 0.01$) and decreased fore-

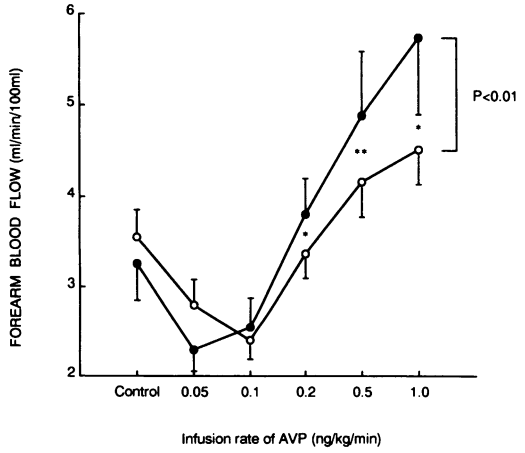

Figure 1. Line graph shows responses of FBF to intraarterial AVP during simultaneous infusion of saline (○) or L-arginine at 10 mg/min (●) ($n = 8$). *Indicates $P < 0.05$ and ** indicates $P < 0.01$.

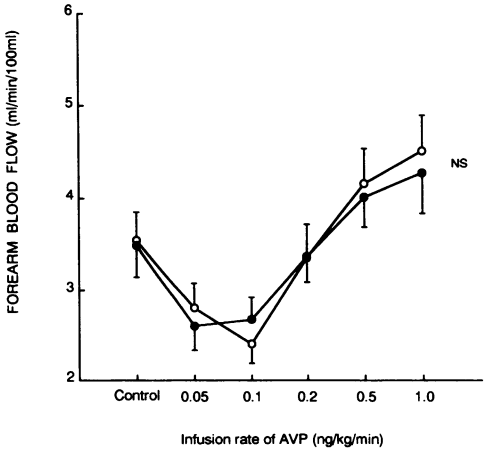

Figure 2. Line graph shows responses of FBF to AVP during simultaneous infusions of saline (○) or D-arginine at 10 mg/min (●) ($n = 8$).

Table II. Baseline Hemodynamics during Intraarterial Infusion of D-Arginine (n = 8)

	Control	D-arginine	P by one-way ANOVA
		10 mg/min	
MBP (mmHg)	91±3	88±4	NS
Heart rate (bpm)	62±3	59±3	NS
FBF (ml/min per 100 ml)	3.5±0.3	3.5±0.4	NS
FVR (U)	26.4±1.7	26.6±2.5	NS

Control, during infusion of saline. Values are mean ± SEM.

arm vascular resistance ($P < 0.01$) dose dependently (Fig. 3). Arterial pressure or heart rate was not altered during infusion of acetylcholine (Table III). Intraarterial infusion of L-NMMA ($n = 6$) at 4 μmol/min for 5 min decreased baseline forearm blood flow ($P < 0.01$) and increased forearm vascular resistance ($P < 0.01$) without changes in arterial pressure or heart rate (Table III). L-NMMA (4 μmol/min for 5 min) partially but significantly blocked vasodilator responses to acetylcholine at doses of 4 ($P < 0.01$) and 12 μg/min ($P < 0.01$) (Fig. 3).

Intraarterial infusion of AVP ($n = 6$) caused biphasic changes in forearm blood flow before L-NMMA (Fig. 4). Arterial pressure or heart rate was not altered during infusion of AVP (Table IV). Intraarterial infusion of L-NMMA ($n = 6$) at 4 μmol/min for 5 min decreased baseline forearm blood flow ($P < 0.01$) and increased forearm vascular resistance ($P < 0.01$) without changes in arterial pressure or heart rate (Table IV). L-NMMA (4 μmol/min for 5 min) significantly blocked vasodilator responses to AVP at doses of 0.5 ($P < 0.01$) and 1.5 ng/kg per min ($P < 0.01$) but did not affect vasoconstrictor responses to AVP at 0.1 ng/kg per min (Fig. 4).

Simultaneous infusions of L-arginine (8 mg/min for 5 minutes) and L-NMMA reversed the inhibitory effects of L-NMMA on AVP-induced vasodilation ($P < 0.05$) (Fig. 5).

Control experiment (Table V). We carried out three vasopressin blood flow studies and obtained dose-response curves over a period of 2 h in the same subject without other drug treatment ($n = 5$). The three dose-response curves were similar (Fig. 6) (Table V).

Discussion

The major findings of this study were that in young healthy subjects, intraarterial infusion of L-arginine augmented AVP-induced vasodilation, that intraarterial infusion of L-NMMA blocked AVP-induced vasodilation, and that L-arginine reversed the inhibitory effects of L-NMMA. Thus our results suggest that AVP-induced vasodilation in the human forearm may be mediated by NO.

AVP-induced vasodilation. AVP is a potent vasoconstrictor in vitro. However, AVP does not produce the expected rise in blood pressure when given intravenously to intact animals (24, 25) or humans (26). Several mechanisms are considered to account for the discrepancy of the effects of AVP between in vivo and in vitro studies. These include withdrawal of the sympathetic tone because of several mechanisms (27, 28), negative inotropic and chronotropic action (29), and direct vasodilation (30). Previously, our research group and Hirsch et al. have shown that intraarterial infusion of AVP causes vasodilation in forearm vessels in humans (3, 4). In the present study, we also demonstrated the vasodilating effect of AVP.

In human forearms, AVP causes vasodilation only at high plasma concentrations (3). Although we measured the plasma level only at an infusion rate of 1.0 ng/kg per min, which increased the plasma level from 4 to 1,174 pg/ml, the results in our previous study indicated that AVP appeared to cause vasodilation at plasma levels higher than 100–300 pg/ml (3). The vasodilating effects of intraarterially infused AVP is not caused by systemic effects since Hirsch and our research group have shown in previous studies that systemic concentration of AVP at which AVP causes vasodilation was low at 20 pg/ml, and that the contralateral forearm blood flow was not altered (3, 4). Furthermore, arterial pressure or heart rate was not altered during infusion of AVP in the present and our previous studies (3).

AVP-induced vasodilation may be mediated by the V2 receptor. Hirsch et al. and our research group have demonstrated that the vasodilating effect of intraarterially infused AVP was potentiated after a specific intravenous or oral V1 receptor antagonist (4, 5). Furthermore, Hirsch et al. demonstrated that intraarterial infusion of a V2 agonist increased forearm blood flow and decreased forearm vascular resistance. Thus, AVP may cause vasodilation via the V2 receptor. However, other possibilities remain to be clarified.

Is AVP-induced vasodilation mediated by NO? In two different ways, we examined the possibility that AVP-induced vasodilation is mediated by NO; we determined whether supplementation of L-arginine augmented AVP-induced vasodilation and also determined whether L-NMMA inhibited AVP-induced vasodilation.

Table III. Forearm Vascular Responses to Intraarterial Infusion of Acetylcholine before and after N^G-Monomethyl-L-Arginine (n = 6)

	Control	ACh	ACh	P by two-way ANOVA
		4 μmol/min	*12 μmol/min*	
MBP (mmHg)				
Before L-NMMA	89±3	88±3	88±3	NS
After L-NMMA	89±4	89±3	88±3	
Heart rate (bpm)				
Before L-NMMA	63±4	61±4	61±5	NS
After L-NMMA	60±4	62±5	59±4	
FBF (ml/min per 100 ml)				
Before L-NMMA	4.9±1.0	12.7±3.1	29.0±6.5	$P < 0.01$
After L-NMMA	3.0±0.3	5.0±1.6	15.1±4.0	
FVR (U)				
Before L-NMMA	21.7±4.1	9.0±2.3	3.8±0.9	$P < 0.01$
After L-NMMA	30.7±3.2	23.9±5.0	7.7±2.0	
Percent FVR				
Before L-NMMA	100	45±9	21±7	$P < 0.05$
After L-NMMA	100	80±15	26±7	

Control, during infusion of saline; ACh, acetylcholine. Values are mean±SEM.

Table IV. Forearm Vascular Responses to Intraarterial Infusion of Arginine Vasopressin before and after N^G-Monomethyl-L-Arginine (n = 6)

	Control	AVP	AVP	AVP	*P* by two-way ANOVA
		0.1 ng/kg per min	*0.5 ng/kg per min*	*1.5 ng/kg per min*	
MBP (mmHg)					
Before L-NMMA	90±3	89±4	91±4	92±3	NS
After L-NMMA	91±3	90±3	91±3	93±3	
Heart rate (bpm)					
Before L-NMMA	60±4	60±4	59±4	57±5	NS
After L-NMMA	60±4	59±4	59±4	61±6	
FBF (ml/min per 100 ml)					
Before L-NMMA	4.6±1.0	2.6±0.3	5.5±0.5	8.2±1.1	$P < 0.01$
After L-NMMA	3.0±0.3	2.2±0.3	2.5±0.2	3.3±0.4	
FVR (U)					
Before L-NMMA	23.4±4.2	35.5±3.6	17.1±1.8	12.1±1.6	$P < 0.01$
After L-NMMA	30.9±2.8	43.3±6.3	37.7±3.2	30.1±3.7	
Percent FVR					
Before L-NMMA	100	168±25	82±13	57±9	$P < 0.01$
After L-NMMA	100	143±23	148±19	126±15	

Control, during infusion of saline. Values are mean±SEM.

It is well known that L-arginine is a substrate of NO (6–9). Although L-arginine itself does not dilate normal vessels in the in vitro and in vivo animal studies, it has been shown that intravenous infusion of L-arginine decreases blood pressure in healthy humans (31, 32). Recently, we demonstrated in healthy young subjects that intraarterial infusion of L-arginine dilated forearm blood vessels dose-dependently and augmented acetylcholine-induced vasodilation (14). The effects of L-arginine were not nonspecific because D-arginine (isomer of L-arginine) did not increase forearm blood flow nor augment acetylcholine-induced vasodilation (14). The systemic effects of L-arginine were excluded for the following reasons; absence of changes in forearm blood flow of the contralateral forearm and low systemic concentration of L-arginine at high infusion rates (14). Furthermore, vasodilation in response to sodium nitroprusside (endothelium-independent vasodilator) was not altered by supplementation of L-arginine (14). Thus, our results may suggest that supplementation of L-arginine facilitate the production of NO in human forearms.

In the same line of thinking, we examined possibility in this study that supplementation of L-arginine would augment AVP-induced vasodilation if AVP-induced vasodilation is mediated by NO. Intraarterial infusion of L-arginine at 10 mg/min did not affect baseline forearm vascular resistance (FVR) or AVP-induced vasoconstriction, but augmented AVP-induced vasodilation. D-Arginine did not affect AVP-induced va-

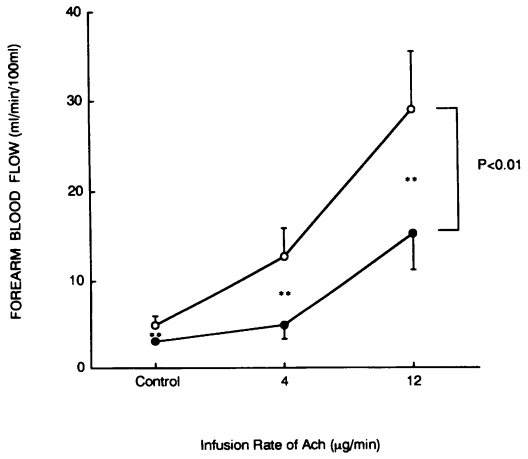

Figure 3. Line graph shows responses of FBF to acetylcholine before (○) and after (●) infusion of L-NMMA ($n = 6$). ** Indicates $P < 0.01$.

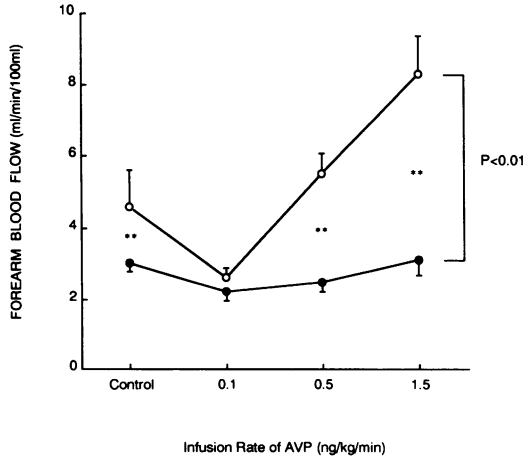

Figure 4. Line graph shows responses of FBF to AVP before (○) and after (●) infusion of L-NMMA ($n = 6$). ** Indicates $P < 0.01$.

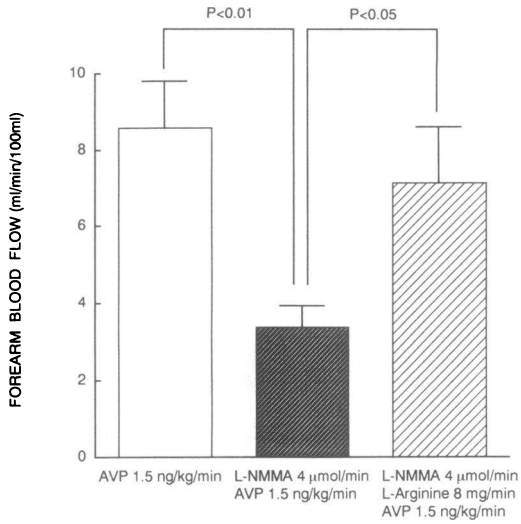

Figure 5. Bar graphs show responses FBF to AVP at doses of 1.5 ng/kg per min before and after infusion of L-NMMA without or with L-arginine ($n = 5$).

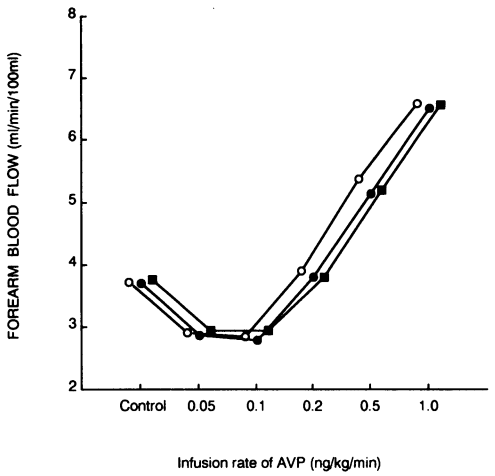

Figure 6. Line graph shows responses of FBF to intraarterial AVP at control (○), 1 h after the start of the experiment (●), or 2 h after the start of the experiment (■) ($n = 5$).

soconstriction and vasodilation. These results suggest that AVP-induced vasodilation may be mediated by NO. To explore this possibility in a decisive way, we examined effects of L-NMMA (a specific blocker of the NO synthase) on AVP-induced vasodilation in the second protocol.

L-NMMA is an analogue of L-arginine and a blocker of the formation of NO from L-arginine in blood vessels (33, 34). L-NMMA that we used in this study was commercially available and purchased. To examine the potency of this compound in humans, we examined effects of this compound on acetylcholine-induced vasodilation in the human forearm. In this study, intraarterial infusion of L-NMMA at 4 μmol/min decreased baseline FVR significantly and completely inhibited vasodilation induced by intraarterial infusion of acetylcholine at 4 μg/min. Our results are compatible with those by Vallance et al. (13). They showed that intraarterial infusion of L-NMMA at 4 μmol/min for 5 min completely abolished vasodilation induced by intraarterial infusion of acetylcholine at 30 nmol/min (about 5.5 μg/min). Thus, L-NMMA that we used in this study possessed a high potency to inhibit NO synthesis. In both studies by our research group and Vallance et al., the inhibition of vasodilation in response to acetylcholine at high

Table V. Forearm Vascular Responses to Intraarterial Infusion of Arginine Vasopressin over a Period of 2 h (n = 5)

	Control	AVP	AVP	AVP	AVP	AVP	*P* by two-way ANOVA
		0.05 ng/kg per min	*0.1 ng/kg per min*	*0.2 ng/kg per min*	*0.5 ng/kg per min*	*1.0 ng/kg per min*	
MBP (mmHg)							
Control	92±2	91±2	91±2	92±2	93±2	93±3	
1 h	92±2	91±2	91±2	92±2	92±2	93±2	NS
2 h	92±2	91±2	92±2	92±2	93±2	94±2	
Heart rate (bpm)							
Control	62±5	59±4	60±4	60±5	60±4	59±4	
1 h	61±5	59±4	59±4	59±3	58±4	58±4	NS
2 h	60±5	60±4	64±4	61±3	61±3	63±4	
FBF (ml/min per 100 ml)							
Control	3.7±0.7	2.9±0.5	2.8±0.6	3.9±1.0	5.4±1.2	6.6±2.0	
1 h	3.7±0.6	2.9±0.4	2.8±0.5	3.8±0.8	5.1±1.3	6.5±1.9	NS
2 h	3.7±0.7	2.9±0.5	2.9±0.5	3.8±0.9	5.1±1.2	6.5±1.9	
FVR (U)							
Control	29.3±7.7	37.5±10.5	39.8±13.0	29.9±8.9	20.3±4.7	17.6±3.7	
1 h	27.5±5.3	34.8±6.6	37.3±8.6	29.2±8.0	21.9±5.1	17.3±3.4	NS
2 h	28.7±6.7	35.6±7.7	36.6±8.8	30.4±8.6	21.3±4.5	17.5±3.5	

Control, during infusion of saline. Values are mean±SEM.

doses was partial (13). Several possibilities are considered. Since acetylcholine dilates blood vessels by three different mechanisms; a release of NO from the endothelium (35–37), prejunctional inhibition of adrenergic neurotransmission (38, 39), and a release of prostacyclin from blood vessels (36, 37), the partial inhibition of vasodilation induced by high doses of acetylcholine may not be caused by inadequate potency of this compound. It is possible that the acetylcholine-induced vasodilation might have been completely inhibited if we had used a larger dose of L-NMMA for longer periods.

Intraarterial infusion of L-NMMA did not affect AVP-induced vasoconstriction, but completely abolished AVP-induced vasodilation (Fig. 4). These results strongly suggest that AVP-induced vasodilation in the human forearm is mediated by NO. We further tested possibility by determining the effects of simultaneous infusions of L-arginine and L-NMMA on AVP-induced vasodilation. Simultaneous infusion of L-arginine reversed the inhibitory effects of L-NMMA on AVP-induced vasodilation (Fig. 5). Taken together, AVP-induced vasodilation in the human forearm is most likely mediated by NO converted from L-arginine.

Although we did multiple AVP infusions, we do not think that one dose response curve affected subsequent dose-response curves because we performed control experiments in which three dose-response curves to AVP were obtained and similar.

Physiological and pathophysiological implications. The vasodilatory response to AVP is only at high concentrations. Thus, physiological meanings may not be clear. Nonetheless, our finding may be important to consider the role of endothelium in control of vascular tone. In animals, it has been shown that the AVP receptor is present in the endothelium and AVP releases NO to cause vasodilation. Our findings clearly demonstrated that AVP release NO and causes vasodilation in humans, suggesting a presence of AVP receptor in the endothelium.

AVP causes vasodilation at higher concentrations > 300 pg/ml. This high level of plasma AVP concentration is not reached in the normal or pathological state, such as hypertension or heart failure. However, plasma AVP concentration would increase > 300 pg/ml when massive hemorrhage occurs, in which severe vasoconstriction supervenes. Released AVP at high concentrations into circulation may counteract severe vasoconstriction in the case of massive hemorrhage.

Finally, the V2 receptor may mediate production of NO in the human forearm and cause vasodilation. We did not explore this possibility in the present study.

In summary, we demonstrated in young healthy humans that intraarterial infusion of L-arginine augmented AVP-induced vasodilatation, that intraarterial infusion of L-NMMA blocked AVP-induced vasodilatation, and that L-arginine reversed the inhibitory effects of L-NMMA. Our results suggest that AVP causes vasodilatation via production of NO from L-arginine in forearm vessels of humans.

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