Role of Nitric Oxide in Desmopressin-Induced Vasodilation of Microperfused Rabbit Afferent Arterioles

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We have previously reported that desmopressin (dDAVP) increased the lumen diameter of norepinephrine (NE)-constricted isolated microperfused rabbit afferent arterioles. In this study, we examined the role of nitric oxide in dDAVP-induced vasodilation of afferent arterioles. We microdissected a superficial afferent arteriole from the kidney of a New Zealand white rabbit. Each afferent arteriole was cannulated with a pipette system and microperfused in vitro at 60 mmHg. dDAVP increased the lumen diameter of NE-preconstricted rabbit afferent arterioles dose-dependently. dDAVP-induced vasodilation was abolished by pretreatment with NG-nitro-L-arginine (L-NNA, 10⁻⁴M) (L-NNA + NE, 6.7±1.1 μm; L-NNA + NE + dDAVP, 7.3±1.4 μm, n=8). dDAVP increased the lumen diameter of NE-preconstricted afferent arterioles pretreated with L-NNA and L-arginine (10⁻²M) (L-NNA + L-arginine + NE, 6.1±1.1 μm; L-NNA + L-arginine + NE + dDAVP, 8.7±0.9 μm*; * p < 0.05, n=6). Aspirin-DL-lysine (10⁻⁴M) did not influence dDAVP-induced afferent arteriolar vasodilation (aspirin + NE, 6.4±0.8 μm; aspirin + NE + dDAVP, 9.6±1.3 μm*; * p < 0.05, n=5). These results suggest that nitric oxide may be responsible for dDAVP-induced afferent arteriolar vasodilation. (Hypertens Res 1997; 20: 29-34)

Key Words: microperfused afferent arteriole, desmopressin, nitric oxide, NG-nitro-L-arginine, aspirin-DL-lysine

Desmopressin (dDAVP, a V2 agonist) is used for the treatment of diabetes insipidus, and one of its major side effects is hypotension (1-3). Intravenous infusion of dDAVP decreased mean blood pressure in healthy volunteers (4), and dDAVP produced a significant decrease in diastolic blood pressure in patients undergoing coronary artery bypass grafting (5). Intracarotid administration of dDAVP increased cerebral blood flow in anesthetized rats (6). dDAVP decreased total peripheral resistance in conscious dogs (7). The intraarterial infusion of dDAVP into the brachial artery increased forearm blood flow in humans (8). These studies suggest that dDAVP-induced vasodilation may cause hypotension. Recently, we have demonstrated that dDAVP increased the lumen diameter of norepinephrine (NE)-constricted microperfused rabbit afferent arterioles (9). However, the mechanisms responsible for the dDAVP-mediated afferent arteriolar vasodilatory effects remain to be clarified.

We have also reported that arginine-vasopressin (AVP) V2 receptor stimulation induced renal vasodilation via nitric oxide production in dogs anesthetized with pentobarbital (10). Infusion of exogenous AVP at subpressor concentrations induced renal vasodilation in anesthetized rats, and this vasodilation was completely abolished by inhibiting nitric oxide synthesis (11). The infusion of NG-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, prevented the decrease in total peripheral resistance induced by injection of dDAVP in conscious chronically instrumented dogs (12). These findings suggest that nitric oxide production may cause dDAVP-induced vasodilation. In this study, we examined the role of nitric oxide in dDAVP-induced vasodilation of microperfused rabbit afferent arterioles.

Materials and Methods

Isolation and Microperfusion of the Rabbit Afferent Arteriole

We used a method similar to that described previously (9, 13, 14). Male New Zealand white rabbits (1.5-2.0 kg), maintained on standard rabbit chow, were anesthetized with intravenous sodium pentobarbital (25 mg/kg) and given an intravenous injection of heparin (500 U, Green Cross, Osaka, Japan). The kidney was exposed through a retroperitoneal flank incision, and the renal pedicle was clamped and cut. The kidney was quickly removed and placed in ice-cold modified Krebs-Ringer solution (pH 7.4), containing 105 mM NaCl, 5
mM KCl, 25 mM NaHCO₃, 2.3 mM Na₂HPO₄, 10 mM Na acetate, 1 mM MgSO₄, 2 mM CaCl₂, 8.3 mM glucose, 5 mM alanine, 0.01 mM EDTA, and 10 mM HEPES. Then, the kidney was sliced along the corticomedullary axis. A thin slice was transferred to a dish containing chilled modified Krebs-Ringer solution and microdissected under a stereoscopic microscope (SZH, Olympus, Tokyo, Japan) using thin steel needles and a sharpened forceps (No. 5, Dumont, Switzerland) at 4°C. The superficial afferent arteriole was dissected free from the surrounding tissues, and all tubular fragments were removed. Great care was taken to avoid touching the vessels and exerting longitudinal or transverse tension on them. An afferent arteriole with its glomerulus was severed from the interlobular artery by cutting it with a disposable 27-gauge injection needle (TOP, Tokyo, Japan). The final preparation was transferred with a micropipette to a temperature-regulated chamber (ITM, San Antonio, TX, USA) mounted on the stage of an inverted microscope with Hoffman modulation (Diaphot, Nikon, Tokyo, Japan). The volume of the chamber was 1 ml. For drainage, fresh bath medium was supplied to the bottom right side of the chamber at 0.5 ml/min, and the medium was gently aspirated from the top of the left side of the chamber. The bath medium was identical to the dissection solution and was bubbled with 95% O₂ and 5% CO₂. During the experiment, water-saturated gas (90% O₂ and 10% CO₂) was gently blown over the surface of the bath to maintain the pH at 7.4.

The afferent arteriole was cannulated with a pipette system (Fig. 1). The method used for cannulating the afferent arteriole into the micropipette system was similar to that reported by Osgood et al. (15) and by us (9). One end of the afferent arteriole was drawn into the holding pipette, which had a constriction (internal diameter, about 20 μm). The tip of the perfusion pipette (outer diameter, about 12 μm) was advanced into the lumen of the afferent arteriole. A stronger vacuum was then applied to the holding pipette to pull the afferent arteriole further toward the constriction in the holding pipette and thereby seal it between the two pipettes. Microperfusion of the afferent arteriole was carried out by the method described by Ito et al. (16, 17) and by us (9). The perfusate was oxygenated with modified Krebs-Ringer, containing 5% bovine serum albumin. The pressure pipette (outer diameter, approximately 3 μm), which was filled with NaCl solution containing 5% FD&C green and 4% KCl, was then advanced into the afferent arteriole through the opening of the perfusion pipette. The intraluminal pressure was measured by Landis’ technique (15) using this pressure pipette. The pressure at which neither the colored solution flowed into the afferent arteriole nor the intraluminal fluid flowed into the pressure pipette was regarded as being equal to the pressure in the afferent arteriole. After the completion of cannulation, the intraluminal pressure was set at 60 mmHg and maintained throughout the experiment. The intraluminal pressure was continuously monitored with a pressure transducer and monitor (Digic VPC, Valcom, Tokyo, Japan). Major leaks of fluid could be seen because of the different refractile properties of the perfusate and bath solution. If the intraluminal pressure was not maintained at a constant level, the experiment was discarded. Microdissection and cannulation of the afferent arteriole were completed within 90 min. The temperature of the bath was gradually increased to 37°C and monitored during the experiment (ESCS, Omron, Tokyo, Japan). The oxygenated bath medium was supplied at 0.5 ml/min, and vasoactive substances were added to the bath medium. A 30-min equilibration period was allowed before each experiment. The image of the afferent arteriole during the experiment was recorded with a video system consisting of a CCD camera and control unit (CCD-10, Olympus, Tokyo, Japan), a monitor (NV-0930Z, Mitsubishi, Tokyo, Japan), and a video recorder (TIMELAPSE BR-9000, JVC, Tokyo, Japan). The effect of dDAVP was evaluated on the basis of the change in the lumen diameter of the microperfused afferent arteriole. The lumen diameter of the afferent arteriole was measured directly on the video monitor screen.

The viability of the vessel was assessed by the response to 10⁻⁵ M nor-epinephrine (NE).
Experimental Protocols

Effect of dDAVP on the lumen diameter of nor-epinephrine (NE)-constricted afferent arterioles

Following a 30-min period of equilibration, we added NE to the bath medium, and afferent arterioles were preconstricted to approximately 50% of the basal diameter. After we confirmed that the lumen diameter was stable, we added dDAVP to the bath medium in increasing concentrations. The continuous bath exchange was stopped, and the bath medium was rapidly exchanged for the medium containing the dDAVP. The bath exchange was resumed with medium containing the same concentration of dDAVP, and the arteriole was observed for 5 min. Every 5 min, the concentration of dDAVP was increased by two orders of magnitude, up to $10^{-6}$M. The lumen diameter of the afferent arteriole was measured 2 to 3 min after the addition of dDAVP.

Effect of NG-nitro-L-arginine (L-NNA, $10^{-4}$M) or L-NNA plus L-arginine ($10^{-2}$M) on the dDAVP ($10^{-8}$M)-induced afferent arteriolar vasodilation

Following a 30-min period of equilibration, L-NNA or L-NNA plus L-arginine was applied to the bath. After 20-min pretreatment with L-NNA or L-NNA plus L-arginine, we added NE to the bath medium. After preconstriction with NE, dDAVP ($10^{-8}$M) was added to the bath medium, and changes in the lumen diameter were observed.

Drugs

Desmopressin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). NG-nitro-L-arginine and L-arginine were purchased from the Peptide Institute (Osaka, Japan). Bovine albumin fraction V was purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). Norepinephrine was kindly supplied by Sankyo (Tokyo, Japan) and aspirin DL-lysine was supplied by Green Cross (Osaka, Japan).

Data Analysis

Values are expressed as means ± SEM. Statistical analyses were performed with Student’s paired t-tests for the two groups. The data were analyzed by one-way analysis of variance, followed by a least significance test for more than three groups. A $p$ value of less than 0.05 was considered to indicate statistical significance.

Results

Effect of dDAVP on the lumen diameter of nor-epinephrine (NE)-constricted afferent arterioles

The basal lumen diameter of micropерfused afferent arterioles was $13.6 ± 0.4 \mu m$ ($n = 8$). dDAVP increased the lumen diameter of NE-constricted afferent arterioles dose-dependently (Fig. 2, 3).

Effect of L-NNA ($10^{-4}$M) or L-NNA plus L-arginine ($10^{-2}$M) on the dDAVP ($10^{-8}$M)-induced afferent arteriolar vasodilation

The rabbit received an intravenous injection of aspirin DL-lysine (50 mg/kg) after anesthesia. Twenty minutes after cyclooxygenase inhibitor injection, a kidney was removed, and an afferent arteriole was dissected. After cannulation of the afferent arteriole, a bath medium containing aspirin DL-lysine ($10^{-4}$M) was continuously exchanged for 1 h. After preconstriction with NE, dDAVP ($10^{-4}$M) was added to the bath medium, and changes in the lumen diameter were observed.

Table 1. Effect of Aspirin DL-lysine (10^{-4}M) on Desmopressin (dDAVP)-Induced Vasodilation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lumen diameter of afferent arterioles ((\mu m))</th>
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<tbody>
<tr>
<td>Aspirin + norepinephrine (NE)</td>
<td>6.4 ± 0.8 (\mu m)</td>
</tr>
<tr>
<td>Aspirin + NE + dDAVP (10^{-8}M)</td>
<td>9.6 ± 1.3 (\mu m)*</td>
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Mean ± SEM. * \(p<0.05\), n=5.

Fig. 3. Dose-response effects of desmopressin (dDAVP) on the lumen diameter of norepinephrine (NE)-preconstricted afferent arterioles (n = 8). * \(p < 0.05\), compared with the lumen diameter of NE-preconstricted AAs.

Fig. 4. Left panel: Effect of desmopressin (dDAVP, 10^{-8}M) on the lumen diameter of afferent arterioles pretreated with N\(^\text{G}\)-nitro-L-arginine (L-NNA, 10^{-4}M) and norepinephrine (NE). n=8. Right panel: Effect of desmopressin (dDAVP, 10^{-8}M) on the lumen diameter of afferent arterioles pretreated with L-NNA (10^{-4}M) plus L-arginine (10^{-2}M) and norepinephrine (NE). n=6, * \(p<0.05\).

Several lines of evidence have recently suggested that the vasodilatory mechanism of AVP V2 receptor stimulation involves the nitric oxide production pathway. We have already reported that L-NNA, a nitric oxide synthase inhibitor, attenuated V2-receptor-mediated renal vasodilation in dogs anesthetized with pentobarbital (10). It has been shown that N\(^\text{G}\)-monomethyl-L-arginine, a blocker of nitric oxide synthase, inhibited AVP-induced vasodilation in the human forearm (18). It has also been reported that the hemodynamic effects of dDAVP were blunted by the systemic administration of N\(^\text{G}\)-nitro-L-arginine methyl ester, a blocker of nitric oxide synthase, in conscious dogs (12). Thus, it appears that dDAVP, a V2 receptor agonist, may act to liberate nitric oxide. However, the role of nitric oxide in dDAVP-induced vasodilation at the resistance vessel level has not been studied. We have already reported that dDAVP increased the lumen diameter of NE-constricted rabbit afferent arterioles, but the mechanisms responsible for the dDAVP-induced afferent arteriolar vasodilatory effects remain to be clarified (9). In this study, our results suggest that nitric oxide may be responsible for dDAVP-induced afferent arteriolar vasodilatory effects.

At least three types of nitric oxide synthase (NOS), endothelial NOS, neuronal NOS, and macrophage NOS, have been identified (19). In the kidney,...
ney, nitric oxide may be synthesized not only in the vascular endothelium but also in other cells, such as macula densa cells (20), vascular smooth muscle (21), peripheral nerves (22), mesangium (23), and macrophages (24). Our acute experiment suggests that dDAVP may stimulate constitutive NOS. Bachmann et al. (25) reported that endothelial NOS was identified in macula densa cells and identified in the afferent endothelium and neuronal structures. These results suggest that nitric oxide is responsible for the renal dilator action of AVP (10, I1). Beck et al. (31) and Craven et al. (32) reported that the administration of dDAVP to rats increased urinary excretion of PGE2. dDAVP also increased urinary PGE2 excretion in humans (33). However, Liard et al. (34) reported that intravenous injection of the V2 receptor agonist 4-valine-8-D-arginine vasopressin (VDAVP) decreased mean arterial pressure and total peripheral resistance and that indomethacin did not affect the hemodynamic response to VDAVP. Indomethacin did not influence the dDAVP-induced decrease in blood pressure in healthy volunteers (4). Indomethacin also did not affect the dDAVP-induced relaxation in preconstricted rat aorta strips (35). In our isolated resistance vessel preparation, we showed that dDAVP increased the lumen diameter of NE-preconstricted afferent arterioles and that dDAVP also increased the lumen diameter of NE-preconstricted afferent arterioles pretreated with aspirin dl-lysine, a cyclooxygenase inhibitor. This means that vasodilatory prostaglandins are not responsible for dDAVP-induced vasodilation, at least in in vitro microperfused afferent arterioles.

In summary, dDAVP increased the lumen diameter of NE-preconstricted rabbit afferent arterioles dose-dependently. dDAVP did not change the lumen diameter of NE-constricted afferent arterioles pretreated with NO3-nitro-l-arginine. dDAVP increased the lumen diameter of NE-constricted afferent arterioles pretreated with NO3-nitro-l-arginine and l-arginine. Aspirin-dl-lysine did not influence dDAVP-induced afferent arteriolar vasodilation. These results suggest that nitric oxide may be responsible for dDAVP-induced afferent arteriolar vasodilation.

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