Pelvic nerve stimulation-induced pressor responses in corpus cavernosum of anesthetized dogs

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Ayajiki, Kazuhide, Hideshi Hayashida, Tomio Okamura, and Noboru Toda. Pelvic nerve stimulation-induced pressor responses in corpus cavernosum of anesthetized dogs. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2141–H2145, 1997.—To analyze the mechanism of penile erection and pathogenesis of impotence, pressures in the corpus cavernosum in anesthetized dogs were measured. Pelvic nerve stimulation produced pressor responses in a frequency-dependent manner. Intravenous injections of N\textsuperscript{G}-nitro-L-arginine, a nitric oxide (NO) synthase inhibitor, dose dependently attenuated the response, and the mechanism was reversed by intracavernous injection of L-arginine but not of d-arginine. The response was also inhibited by N\textsuperscript{G}-nitro-argininidine methylarginine, an endogenous NO synthase inhibitor, dose dependently attenuated the stimulation-induced response, which was restored by an intracavernous injection of L-arginine. An intravenous injection of hexamethonium abolished the pressor response to nerve stimulation, whereas phentolamine and atropine did not significantly alter the response. These findings suggest that an increase in intracavernous pressure caused by pelvic nerve stimulation in anesthetized dogs is mediated by NO liberated from postganglionic neurons that originate in the ganglion located in the vicinity of corpus cavernosum. nitric oxide; N\textsuperscript{G}-nitro-L-arginine; N\textsuperscript{G},N\textsuperscript{G}-dimethylarginine; intracavernous pressure

NITRIC OXIDE (NO) is hypothesized to be an inhibitory neurotransmitter from findings obtained in isolated rabbit, dog, and human penile corpora cavernosa (6, 7, 10, 12). Histochemical studies have demonstrated the presence of NO synthase-immunoreactive or NADPH diaphorase-positive nerve fibers innervating the corpus cavernosum (1, 2, 6). Electrical stimulation of the pelvic nerve increases intracavernous pressure and produces penile erection in anesthetized rats, rabbits, cats, and dogs (4, 8, 9, 13, 16), and the responses are inhibited by NO synthase inhibitors, suggesting a mediation of NO liberated from pelvic nerves in cavernous smooth muscle relaxation. However, mechanisms underlying the penile erection and its impairment in vivo have not systematically been analyzed.

Recent studies have revealed that N\textsuperscript{G},N\textsuperscript{G}-dimethylarginine [asymmetric dimethylarginine (ADMA)], an endogenous NO synthase inhibitor (11, 14), accumulates in the plasma of patients with chronic renal failure (15). Increased production or decreased elimination of the endogenous inhibitors produced locally or in the circulation may play a role in the genesis of impotence. Therefore, the present study was undertaken to determine the neural connections of the pelvic nerve between the site of electrical stimulation close to the pelvic plexus and corpus cavernosum muscle and to quantitatively evaluate the effects of N\textsuperscript{G}-nitro-L-arginine (L-NNA), an NO synthase inhibitor, applied intravenously or into the corpus cavernosum, compared with those of ADMA applied locally.

MATERIALS AND METHODS

These studies were approved by the Animal Care and Use Committee at Shiga University of Medical Science (1 jpan). Twenty-four male mongrel dogs weighing 9–13 kg were premedicated with an intramuscular injection of ketamine (15 mg/kg) and anesthetized with pentobarbital sodium (20 mg/kg) given intravenously, and stable anesthetic conditions were maintained by additional injections as needed. The animals were intubated to breathe spontaneously. Arterial, systolic and diastolic pressures were monitored with a pressure transducer (MPU05, Toyo Measuring Instruments, Tokyo, J apan) and amplifier (AP641G, Nihon-Koden Kogyo, Tokyo, J apan) via a catheter inserted into the right femoral artery. The heart rate was monitored by a cardiotachometer (AT610G, Nihon-Koden Kogyo). The abdomen was opened through a midline abdominal incision. The left pelvic nerve distal to the pelvic plexus was carefully isolated and placed on a bipolar electrode (Iwashita-Kishimoto Medical Instrument, Kyoto, J apan) connected to an electronic stimulator (Nihon-Koden Kogyo). Two 21-gauge venous needles were placed in the cavity of the left corpus cavernosum (~2.0 mm apart); one was connected to the pressure transducer and recorder, and the other was used for the intracavernous drug injection. Drugs were applied systemically via the right femoral vein or locally into the left corpus cavernosum. The pelvic nerve was stimulated by 1.0-ms electrical square pulses of 10 V at frequencies of 5, 10, and 20 Hz for a period of 10 s at intervals of 7–15 min. After the stabilization of pressor responses to the nerve stimulation at 5, 10, and 20 Hz, phentolamine (1 mg/kg iv), atropine (1 mg/kg iv), hexamethonium (4 mg/kg iv plus a continuous intravenous infusion of 0.1 mg·kg\textsuperscript{-1}·min\textsuperscript{-1}), or L-NNA (0.5, 1.0, and 2.0 mg/kg iv) was applied intravenously to 14 of 24 dogs. The effects of phentolamine and hexamethonium were confirmed by an abolishment of hypertensive action of intravenously injected norepinephrine (3 µg/kg) and a reversal of the reflex decrease in heart rate by norepinephrine to tachycardia, respectively. Twenty minutes after intravenous treatment with L-NNA (2.0 mg/kg), d-arginine (500 mg/kg iv), or L-arginine (500 mg/kg iv), nerve stimulation was commenced. In the remaining dogs, the effects of intracavernously injected L-NNA (0.05 mg/kg), N\textsuperscript{G}-nitro-d-arginine (o-\textsuperscript{NNA}; 0.05 mg/kg), and ADMA (0.25 and 0.5 mg/kg) on the pressor response to pelvic nerve stimulation were examined. In this series of experiments, recovery of the response was determined by intracavernous injections of d- and L-arginine (5 mg/kg for each 0.05 mg/kg of L-NNA and 1.5 mg/kg for each 0.5 mg/kg of ADMA).

The results are expressed as means ± SE. Statistical analyses were made with Student’s paired and unpaired t-tests and Tukey’s method after one-way analysis of variance. Drugs used were L-NNA and o-NNA (Peptide Institute, Minoh, J apan); L-arginine, d-arginine, and hexamethonium
bromide (Nacalai Tesque, Kyoto, Japan); phentolamine mesylate (Novartis, Takarazuka, Japan); atropine sulfate (Tanabe, Osaka, Japan); dl-norepinephrine hydrochloride (Sankei, Tokyo, Japan); and ADMA (Sigma Chemical, St. Louis, MO).

RESULTS

Intracavernous pressure under resting conditions in 24 anesthetized dogs averaged $5.4 \pm 1.2$ cmH$_2$O. Pelvic nerve stimulation at frequencies of 5, 10, and 20 Hz increased the pressure, with a visible penile erection in a frequency-dependent manner. The stimulation-induced pressor response was not affected by intravenous treatment with phentolamine (1 mg/kg; $n = 3$ dogs) or atropine (1 mg/kg; $n = 6$ dogs; Fig. 1, A and B, respectively), whereas hexamethonium (4 mg/kg; $n = 6$ dogs) abolished the response (Fig. 1C). The pressor response was inhibited by intravenous injections of L-NNA (0.5, 1, and 2 mg/kg) in a dose-dependent manner (Fig. 2A). Injections of L-arginine (500 mg/kg iv) restored the neurogenic response, whereas D-arginine was without effect (Fig. 2B). Mean values of the duration of responses at the half-maximal pressure rise due to the nerve stimulations at frequencies of 5, 10, and 20 Hz were $23.1 \pm 2.2$, $32.0 \pm 3.2$, and $59.5 \pm 9.4$ s, respectively. Inhibitions by L-NNA in the magi-
titude and duration of pressor responses were inversely dependent on the stimulation frequencies. Typical recordings of the response before and after the NO synthase inhibition are illustrated in Fig. 3. Changes by the inhibitor of systolic and diastolic blood pressures and heart rate are summarized in Table 1. The increased diastolic blood pressures caused by L-NNA (2 mg/kg) were restored by the additional intravenous injection of L-arginine (500 mg/kg; from 95.7 ± 3.6 to 88.9 ± 3.9 mmHg diastolic blood pressure; \( P \), 0.01 by paired \( t \)-test; \( n \), 7 dogs).

The pressor response to nerve stimulation was inhibited by L-NNA (0.05 mg/kg; 71.6 ± 16.4% inhibition at 5 and 10 Hz; \( P \), 0.05 by paired \( t \)-test; \( n \), 4 dogs) injected into the corpus cavernosum but not by D-NNA (0.05 mg/kg; Fig. 4). The intracavernous injection of L-arginine (5 mg/kg) reversed the inhibition (Fig. 4A). ADMA (0.25 and 0.5 mg/kg by intracavernous injection), an endogenous NO synthase inhibitor (14), dose dependently attenuated the response to nerve stimulation (Fig. 5A). The response was also restored by the addition of L-arginine but not of D-arginine (1.5 mg/kg by intracavernous injection; Fig. 5B). The drugs intracavernously applied did not affect the systemic blood pressure, heart rate, or intracavernous pressure under resting conditions.

**DISCUSSION**

Intravenous injection of L-NNA dose dependently attenuated the pressor responses of the corpus cavernosum in anesthetized dogs, the magnitude of inhibition being inversely related to the stimulation frequency. The responses depressed by L-NNA were restored by the injection of L- but not of D-arginine. Intracavernously applied L-NNA was also effective in attenuating the pressor responses, the inhibitory potency being 10 times that induced by intravenous injection. The attenuation was restored by an additional injection of L-arginine into the corpus cavernosum. Similar results were also obtained in anesthetized dogs in which the corpus cavernosum was treated with intracavernous L-NNA methyl ester (13). Transmural electrical stimulation relaxes strips of the canine corpus cavernosum (6). The relaxation is abolished by L-NNA and restored by L-arginine; therefore, the response is postulated to derive exclusively from NO liberated from inhibitory nerves (6). Atropine failed to significantly reduce the pressor response, suggesting that the release of NO from the endothelium due to activation of muscarinic

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**Table 1. Effects of phenolamine, atropine, hexamethonium, and L-NNA on SBP, DBP, and HR in anesthetized dogs**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Phenolamine (n = 3)</th>
<th>Control</th>
<th>Atropine (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>136.7 ± 6.9</td>
<td>119.0 ± 3.1</td>
<td>132.2 ± 6.8</td>
<td>130.2 ± 7.3</td>
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<tr>
<td>DBP, mmHg</td>
<td>97.5 ± 5.0</td>
<td>86.7 ± 4.1</td>
<td>90.3 ± 4.1</td>
<td>92.5 ± 3.2</td>
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<tr>
<td>HR, beats/min</td>
<td>128.3 ± 9.7</td>
<td>172.0 ± 18.2</td>
<td>160.5 ± 13.7</td>
<td>173.0 ± 13.1</td>
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<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hexamethonium (n = 7)</th>
<th>Control</th>
<th>L-NNA (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>129.4 ± 2.9</td>
<td>81.1 ± 8.2*</td>
<td>135.7 ± 3.9</td>
<td>140.3 ± 4.6</td>
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<tr>
<td>DBP, mmHg</td>
<td>84.1 ± 3.5</td>
<td>53.0 ± 6.3*</td>
<td>85.9 ± 3.3</td>
<td>95.7 ± 3.6*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>131.3 ± 14.7</td>
<td>103.1 ± 8.1</td>
<td>148.7 ± 7.4</td>
<td>122.7 ± 7.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; *n*, no. of dogs. Phenolamine, 1 mg/kg iv; atropine, 1 mg/kg iv; hexamethonium, 4 mg/kg iv; N(G)-nitro-L-arginine (L-NNA), 2 mg/kg iv; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. Data were obtained when responses to drugs were stabilized. Significantly different from control: *\( P < 0.001 \); †\( P < 0.01 \); ‡\( P < 0.05 \) (by paired t-test).
receptors from cholinergic nerves by acetylcholine is not involved. These findings suggest that the pressor response of the corpus cavernosum and the penile erection by pelvic nerve stimulation are mediated by NO synthesized from L-arginine in inhibitory nerve terminals. Involvement of neurogenic vasoactive intestinal peptide in the pressor response to nerve stimulation was not excluded from the study in vivo but has been ruled out in the earlier study with isolated canine corpus cavernosum (6).

Intracavernously injected ADMA also inhibited the neurogenic pressor response, and the inhibitory effect was reversed by L-arginine, as was the case with L-NNA. Endogenous NO synthase inhibitors, when accumulated in the vicinity of cavernous tissues, are expected to suppress the neurally induced penile erection. In patients with chronic renal failure, circulating concentrations of the endogenous inhibitor are raised to ~10 µM (15). The present study revealed that the inhibition by 0.5 mg/kg of intravenous L-NNA of the neurogenic response was comparable to that by 0.05 mg/kg of intracavernous L-NNA, suggesting 10 times more effectiveness with local application. If the intravenously applied drug is supposed to be diluted to 1:100 (blood volume, approximately 1/13 of body weight), an effective dose of ADMA (0.25 mg/kg intracavernously; Fig. 5) applied in the present study would lead to the intracavernous concentration of 25 µg/ml (125 µM), which is one order higher than the plasma concentration attained in renal failure patients. It is intriguing to determine whether ADMA is produced locally in the endothelium of the corpus cavernosum as in human endothelial cells (3).

Pressor responses to pelvic nerve stimulation were not potentiated by α-adrenoceptor blockade with phentolamine. Isolated canine corpus cavernosum strips responded to exogenously applied norepinephrine with a contraction by activation of an α-adrenoceptor, al-

Fig. 4. Modification of pressor response by L-NNA [0.05 mg/kg (L-NNA 0.05) by intracavernous injection] and by L-NNA + L-Arg (5 mg/kg by intracavernous injection; A) or by D-NNA (0.05 mg/kg by intracavernous injection; B) to pelvic nerve stimulation (5, 10, and 20 Hz for 10 s) in corpus cavernosum of anesthetized dogs. In A, dogs were first treated with L-NNA, and then L-Arg was applied. Values are means ± SE; n, no. of dogs.

Fig. 5. Modification of pressor response by N\textsuperscript{G},N\textsuperscript{G}-dimethylarginine (ADMA; 0.25 and 0.5 mg/kg (ADMA 0.25 and ADMA 0.5, respectively) by intracavernous injection; A) and by ADMA (0.5 mg/kg) + L-Arg or D-Arg (1.5 mg/kg by intracavernous injection; B) to pelvic nerve stimulation (5, 10, and 20 Hz for 10 s) in corpus cavernosum of anesthetized dogs. In B, dogs were first treated with ADMA, and then D-Arg or L-Arg was applied. Values are means ± SE; n, no. of dogs. Significantly different from control; \(a^p < 0.01, b^p < 0.05\) (by Tukey’s method).
though the magnitude is less than that of the response of penile arteries (5). Therefore, electrical stimulation of the pelvic nerve under the experimental conditions used may not release norepinephrine from adrenergic nerves in the amount sufficient to interfere with the pressor response to nitroxidergic nerve stimulation. Possible mediation by muscarinic-receptor activation of the response would be ruled out from the ineffectiveness of atropine in the neurogenic pressor response (Fig. 1). On the other hand, hexamethonium abolished the response, suggesting the neurons electrically stimulated to be preganglionic. According to our preliminary histochemical studies with dog materials (unpublished data) and those with human materials by Burnett et al. (2), there are nerve cells and bundles containing NO synthase immunoreactivity or NADPH diaphorase in the neighboring tissues of the corpus cavernosum. These findings suggest that electrical stimulation applied to pelvic nerves invokes action potentials in preganglionic fibers, and synaptic information via nicotinic receptors is transferred to postganglionic nitroxidergic neurons innervating the corpus cavernosum.

Elevation of the intracavernous pressure and penile erection in anesthetized dogs would be mediated by NO liberated from inhibitory nerves. It appears that the nerve originates in the ganglion located in the vicinity of the corpus cavernosum and that the pelvic plexus sends nerve fibers to this ganglion. Although the possibility of local accumulation of endogenous NO synthase inhibitors in the corpus cavernosum is not ruled out, the raised concentration in the plasma of renal failure patients so far reported could not be estimated to be high enough to impair the neurogenic penile erection.

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REFERENCES


