Monkey corpus cavernosum relaxation mediated by NO and other relaxing factor derived from nerves

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Okamura, Tomio, Kazuhide Ayajiki, and Noboru Toda. Monkey corpus cavernosum relaxation mediated by NO and other relaxing factor derived from nerves. Am J Physiol. 274 (Heart Circ. Physiol. 43): H1075–H1081, 1998.—Isolated monkey corpus cavernosum muscle strips contracted with prostaglandin F₂α, and treated with prazosin responded to transmural electrical stimulation with frequency-related relaxations that were abolished by tetrodotoxin. The nitric oxide (NO) synthase inhibitor N⁶-nitro-L-arginine (L-NNA) significantly attenuated but did not abolish the response; L-arginine reversed the inhibition. The neurogenic relaxation was not influenced in the strips treated with atropine or calcitonin gene-related peptide (CGRP)- (8—37), a CGRP receptor antagonist, and those desensitized to vasoactive intestinal polypeptide (VIP) or pituitary adenylate cyclase-activating polypeptide (PACAP). Nerve fibers containing NADPH diaphorase were histochemically demonstrated in cavernous tissues. The relaxant response resistant to NO synthase inhibitor was abolished by high K¹ and tetrabutylammonium but was unaffected by glibenclamide, charybdotoxin, apamin, ouabain, SKF-525a, a cytochrome P-450 inhibitor, and oxyhemoglobin. It is concluded that neurogenic relaxations of monkey corpus cavernosum muscle is associated partly with NO released as a neurotransmitter and that other relaxing factor(s) possibly responsible for K¹ channel opening also participates; however, the type of K¹ channel involved is not determined. Acetylcholine, VIP, CGRP, PACAP, and the Na⁺ pump do not seem to be involved in the neurogenic relaxation.

Nitric oxide synthase inhibitor; potassium channel; penile erection; neurotransmitter

The penile erection is evoked by an elevation of pressure of the corpus cavernosum that is associated with a relaxation of cavernous smooth muscle. Originally relaxant substances, such as acetylcholine and vasoactive intestinal polypeptide (VIP), liberated from nerves were thought to be mediators of the penile erection (17, 25, 36). However, recent studies have provided evidence supporting the hypothesis that nitric oxide (NO) derived from the nerve mainly mediates the muscle relaxation in vitro and the increased intracavernous pressure in vivo in a variety of mammals, including rats (4, 7, 9), rabbits (27), dogs (12, 33, 34), and humans (26). There are species variations in the mechanism of relaxation of the corpus cavernosum. Involvement of vasodilators other than NO, acetylcholine, and VIP is also reported (10).

Our preliminary study suggested that neurogenic relaxation of monkey corpus cavernosum muscle was reduced but not abolished by treatment with NO synthase inhibitors. Therefore, the present study was undertaken to clarify the involvement of nerve-derived NO in the relaxation and to analyze the mechanism of neurogenic relaxation resistant to NO synthesis inhibition in the corpus cavernosum isolated from Japanese monkeys.

Methods

The studies reviewed board at the Shiga University of Medical Sciences approved the use of monkey corpus cavernosum in this study.

Preparation. Male Japanese monkeys (Macaca fuscata), weighing 6–10 kg, were anesthetized with intramuscular injections of ketamine (40 mg/kg) and with intravenous injections of thiopental sodium (20 mg/kg) and killed by bleeding from the carotid arteries. The penis was rapidly removed, and corpora cavernosa were isolated. The tunica albuginea was removed, and two strips (~3 × 5 × 10 mm) from each individual were obtained. The specimens were vertically fixed between hooks in a muscle bath (20-ml capacity) containing the nutrient solution, which was aerated with a mixture of 95% O2-5% CO2 and maintained at 37 ± 0.3°C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer. The resting tension was adjusted to 0.25 g, which is optimal for inducing the maximal contraction. Constituents of the solution were as follows (in mM): 120 NaCl, 5.4 KCl, 2.2 CaCl₂, 1.0 MgCl₂, 25.0 NaHCO₃, and 5.6 dextrose. The pH of the solution was 7.36–7.42. Before the start of experiments, all of the strips were allowed to equilibrate in the bathing media for 60–90 min, during which time the medium was replaced every 10–15 min.

The strips were placed between stimulating electrodes. The gaps between the strip and the electrodes were wide enough to allow undisturbed contraction and relaxation and yet sufficiently narrow to effectively stimulate intramural nerve terminals. A train of 0.2-ms square pulses of supramaximal intensity were transmurally applied at frequencies of 2, 5, and 20 Hz for periods of 100, 40, and 10 s, respectively, or in other series at 0.2, 0.5, 2, and 5 Hz for 40 s. The stimulus pulses were delivered every 10 min by an electronic stimulator (Nihon-Kohden Kogyo, Tokyo, Japan).

Functional study. Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Nihon-Kohden Kogyo). The contractile response to 30 mM K⁺ was obtained, and then the strips were washed three times with fresh media and equilibrated for 30–40 min. The strips were partially contracted with prostaglandin (PG) F₂α (1–7 µM); the contractions were in a range between 30 and 45% of the contraction induced by K⁺ (30 mM). Transmural electrical stimulation at 5 Hz was applied repeatedly at intervals of 10 min until steady responses were obtained, and then blocking agents were applied. In some preparations, in which the stimulation-induced relaxation was stabilized in the presence of 0.1 mM N⁶-nitro-L-arginine (L-NNA), the bathing fluid was replaced by the solution in which LiCl was substituted for NaCl, and L-NNA (0.1 mM) was included; the muscle tone was then adjusted to the same level as that before the change of the solution. At the end of each series of experiment, papaverine (0.1 mM) was added to attain the maximal relaxation; relaxations induced by electrical stimulation and vasodilator agents relative to those induced by papaverine were presented.

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Histochemistry. The corpus cavernosum was rapidly removed and fixed in ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 0.3% glutaraldehyde and 4% paraformaldehyde for 10 min, then postfixed overnight in 0.1 M PBS with 4% paraformaldehyde followed by cryoprotection in 15% sucrose. The fixed blocks were cut into thin sections (20 µm thick) in a cryostat (−18°C) (Cryotom, Nakagawa Selsakusho, Tokyo, J apan) and mounted onto gelatin-chromalin-coated glass slides. The sections were then rinsed in 0.1 M PBS. NADPH diaphorase staining was performed by incubating glass-mounted sections with 0.1 M PBS, pH 8.0, containing 1 mM NADPH (Kohjin, Tokyo, J apan), 2 mM nitroblue tetrazolium (Sigma Chemical, St. Louis, MO), and 0.3% Triton X-100 at 37°C. The period of incubation (range 15–30 min) was determined by staining intensity. The reaction was terminated by washing the sections in 0.1 M PBS containing 0.3% Triton X-100. Histochemical control experiments, in which NADPH was excluded from the reaction mixture, gave no positive staining.

Statistics and drugs used. The results shown in the text, Figs. 1–7, and Table 1 are expressed as means ± SE. Statistical analyses were made using the Student's paired and unpaired t-test and Tukey’s method after one-way analysis of variance. Drugs used were L-NNA, N⁶-nitro-L-arginine (L-NNA), VIP, CGRP-(8–37), pituitary adenylate cyclase-activating polypeptide (PACAP)-27, charybdotoxin (Peptide Institute, Minoh, J apan), N⁶-nitro-L-arginine methyl ester (L-NAME; RBI Research Biochemicals, Natick, MA), L- and D-arginine, tetrabutylammonium (TBA), methylene blue triazolium, glibenclamide, 4-aminopyridine (Sigma Chemical) in distilled water.

RESULTS

Neurogenic response as affected by L-NNA. In a preliminary study on strips of the monkey corpus cavernosum partially contracted with PGF₂α, transmural electrical stimulation at 5 Hz produced a relaxation of 50.6 ± 12.5% (n = 5 from separate monkeys) that was potentiated to 74.2 ± 4.0% (n = 5, 33.2 ± 11.9% increase, P < 0.05, paired t-test) by treatment with 10 μM prazosin. Therefore, mechanisms of neurogenic relaxation were analyzed in the strips treated with prazosin. The electrical stimulation elicited frequency-related relaxations that were abolished by 0.3 μM tetrodotoxin. Mean values of the relaxation induced at 2, 5, and 20 Hz for 100, 40, and 10 s, respectively, were 54.0 ± 3.3% (n = 7), 75.3 ± 3.5% (n = 7), and 67.4 ± 4.1% (n = 5), respectively, relative to those elicited by 0.1 mM papaverine. Reproducible responses were obtained with 5-Hz stimulation; thus, unless otherwise mentioned, the analysis of mechanisms underlying the response was carried out in the strips stimulated at this frequency.

Relaxations induced by 5-Hz stimulation were not influenced by treatment with 0.1 μM atropine (n = 4) but were attenuated by 10 μM L-NNA as shown in Fig. 1. The inhibition was reversed by the addition of L-arginine (1 mM). Quantitative data with 5-Hz stimulation are summarized in Fig. 2. L-NNA (10 μM) did not alter the neurogenic relaxation, and L-arginine (1 mM) failed to restore the response depressed by L-NNA. Prolonged electrical stimulation at 1 Hz elicited a sustained relaxation that was not affected by atropine. During the nerve stimulation, L-NNA (10 μM) produced a contraction, and L-arginine (1 mM) abolished the response (Fig. 3). Tetrodotoxin abolished the stimulation-induced relaxation. Similar results were also obtained in two additional strips from separate monkeys.

Exogenously applied NO (10 μM) produced relaxations of 32.0 ± 6.7% (n = 5), which were not influenced by L-NNA.

L-NNA in a concentration of 10 μM did not abolish but partially inhibited the response to electrical nerve stimulation (from 70.7 ± 5.4 to 23.4 ± 2.7%, n = 9, 63.3 ± 5.2% inhibition; Fig. 2). Additional inhibition was not obtained by increasing the concentration to 0.1 mM (from 75.6 ± 3.7 to 26.3 ± 5.7%, n = 7, 65.4 ± 3.5% inhibition). The addition of L-NAME (0.1 mM) to L-NNA (0.1 mM)-treated strips (n = 4) did not further inhibit the response. The incomplete inhibition by 0.1 mM L-NNA was also seen in the responses to nerve stimulation at frequencies <5 Hz for 40 s (Fig. 4).

Fig. 1. Typical tracing of relaxant response to transmural electrical stimulation (TES; 2, 5, and 20 Hz) of a monkey corpus cavernosum strip before and after treatment with N⁶-nitro-L-arginine (L-NNA; 10 μM), L-arginine (L-Arg; 1 mM), and tetrodotoxin (TTX, 0.3 μM). The strip was partially contracted with 1 μM prostaglandin (PG) F₂α, and treated with 10 μM prazosin. Papaverine (PA; 0.1 mM) produced the maximal relaxation.
in the responses at 0.2, 0.5, 2, and 5 Hz averaged 77.1 ± 6.3, 80.4 ± 3.0, 52.5 ± 6.2, and 51.8 ± 5.7%, respectively; the values at 0.2 and 0.5 Hz were significantly different from those at 2 and 5 Hz (P < 0.05). Despite a marked inhibition of the response to the low frequencies of stimulation, significant relaxations were seen in the presence of L-NNA. However, tetrodotoxin applied at the end of each series abolished the stimulation-induced relaxation. Finally, the mechanism underlying the L-NNA-resistant neurogenic relaxation was analyzed at 5 Hz under treatment with 0.1 mM L-NNA.

Neurogenic relaxation resistant to NO synthase inhibition. The response seen in L-NNA-treated strips was not affected by treatment with CGRP-(8—37) (0.1 µM) (24.0 ± 4.3 vs. 26.3 ± 3.5%, n = 4) or in the strips desensitizied to VIP by repeated applications (10 nM VIP, 4–5 times) of the peptide (29.2 ± 4.6 and 28.3 ± 4.3%, n = 4). The concentration of CGRP-(8—37) is sufficient to significantly inhibit the response to CGRP (35). PACAP (0.1 µM) produced a relaxation averaging 15.2 ± 1.7% (n = 5). Repeated applications (2–3 times) abolished the responsiveness to this peptide of the strips, in which the stimulation-induced relaxation (36.4 ± 5.4%, n = 5) was not different from that before the addition of PACAP (37.8 ± 6.4%). Treatment with oxyhemoglobin (16 µM, n = 5) and methylene blue (10 µM, n = 3) did not inhibit the neurogenic response.

Fig. 2. Modifications by NG-nitro-L-arginine (L-NNA; 10 µM), L-NNA (10 µM), L-NNA + D-arginine (+D-Arg; 1 mM), L-NNA + L-Arg (+L-Arg; 1 mM), and TTX (0.3 µM) of relaxation induced by TES (5 Hz) of monkey corpus cavernosum strips contracted with PGF2α and treated with prazosin (10 µM). Abscissa represents stimulation-induced relaxations relative to those caused by 0.1 mM PA. aP < 0.01: significantly different from control (C); bP < 0.01: significantly different from value with L-NNA; cP < 0.01: significantly different from value with L-NNA + L-Arg; dP < 0.01: significantly different from L-NNA; eP < 0.05: significantly different from value with L-NNA + D-Arg (Tukey's method). Nos. in parentheses indicate no. of strips from separate monkeys. Vertical bars, SEs.

Fig. 3. Typical tracing of response to TES (1 Hz) for 80 s at beginning and −120 min from on to off, as affected by atropine (0.1 µM), L-NNA (10 µM), L-Arg (10 mM), and TTX (0.3 µM) in a monkey corpus cavernosum strip contracted with 1.3 µM PGF2α and 10 µM prazosin. PA (0.1 mM) produced the maximal relaxation.

Fig. 4. Modifications by L-NNA (0.1 mM) and TTX (0.3 µM) of response to TES at low frequency range (0.2–5 Hz) of monkey corpus cavernosum strips contracted with PGF2α and treated with prazosin (10 µM). Abscissa represents stimulation-induced relaxations relative to those induced by 0.1 mM PA. aP < 0.01, bP < 0.01, cP < 0.05: significantly different from value with TTX (Tukey's method). n, No. of strips from separate monkeys. Vertical bars, SEs.
The neurogenic response in the strips treated with 0.1 mM L-NNA was not significantly reduced by 1 mM TBA but was abolished at 3 mM (Fig. 5). In addition, applications of 20 mM K_1 abolished the relaxation (Fig. 6), which was restored by replacement of one-half of the bathing media with normal fluid containing 0.1 mM L-NNA. Complete recovery of the response was observed by repeating the same procedure to reduce the applied K_1 concentration to one-fourth. The results are quantitatively compared in Fig. 7. Treatment with other compounds that are recognized to interfere with the transmembrane passage of K_1 through channels did not significantly inhibit the response to nerve stimulation.

**Fig. 5.** Modifications by L-NNA (0.1 mM) and tetrabutylammonium (TBA; 1 and 3 mM) of relaxant response to TES (5 Hz) of monkey corpus cavernosum strips contracted with PGF_2α and treated with prazosin (10 µM). Abscissa represents stimulation-induced relaxations relative to those induced by 0.1 mM PA. a P < 0.05: significantly different from control (C); b P < 0.01: significantly different from value with L-NNA; c P < 0.05: significantly different from value with 1 mM TBA (Tukey's method). Nos. in parentheses indicate no. of strips from separate monkeys. Vertical bars, SEs.

**Fig. 6.** Typical tracing of relaxation induced by TES (5 Hz) of a monkey corpus cavernosum strip before and after L-NNA (0.1 mM), TBA (1 mM), excess K_1, depletion of K_1 with control media containing 0.1 mM L-NNA (same procedure was done twice), and tetrodotoxin (TTX, 0.3 µM). The strip was partially contracted with PGF_2α (1.2 µM) and prazosin (10 µM); a at top right and bottom left are same response. PA (0.1 mM) produced maximal relaxation.

**Fig. 7.** Modifications by L-NNA (0.1 mM), excess K_1 (20 mM), and K_1 depletion by L-NNA-containing solution; depleted to one-half (K 1/2) and one-fourth K_1 strength (K 1/4) of response to TES (5 Hz) of monkey corpus cavernosum strips contracted with PGF_2α. Abscissa denotes stimulation-induced relaxations relative to those caused by 0.1 mM PA. a P < 0.01; significantly different from control (C); b P < 0.01: significantly different from value with L-NNA; c P < 0.01: significantly different from value with K; d P < 0.05: significantly different from value with K 1/2 (Tukey's method). Nos. in parentheses indicate no. of strips from separate monkeys. Vertical bars, SEs.
stimulation. The data with charybdoxtin, apamin, 4-aminopyridine, glibenclamide, and Ba^{2+} are shown in Table 1. Levromakalim, a K⁺ channel opener, at 0.1 and 1 µM produced relaxations of cavernous strips of 20.2 ± 4.5 and 76.6 ± 6.6% (n = 5), respectively.

Under treatment with 0.1 mM L-NNNA, relaxations induced by electrical stimulation were not attenuated in the cavernous strips exposed for 30 min to 1 µM ouabain (38.9 ± 5.4 vs. 41.6 ± 5.9%, n = 7). Substitution of isotonic LiCl for NaCl did not significantly alter the stimulation-induced response during the observation period of 60 min; mean values were 31.8 ± 5.5% (n = 6) in control media and 36.2 ± 5.2% 30 min after exposure to the experimental solution.

According to Hecker et al. (13), the response to endothelium-derived relaxing factor resistant to NO synthase inhibitors is significantly attenuated by a cytochrome P-450 inhibitor, SKF-525a, suggesting the involvement of epoxide. Treatment with this inhibitor (0.1 mM) did not reduce the response to nerve stimulation (Table 1).

Histochemical study. In the trabecular meshwork of a corpus cavernosum strip, abundant nerve fibers and bundles containing NADPH diaphorase were demonstrated histochemically (Fig. 8). Similar results were also obtained in the strips from two additional monkeys.

**DISCUSSION**

The present study revealed that corpus cavernosum muscle strips obtained from the Japanese monkey responded to electrical nerve stimulation with a frequency-related relaxation that was significantly inhibited by treatment with high concentrations (10 and 100 µM) of an NO synthase inhibitor, L-NNNA, but not by D-NNNA. The inhibition was reversed by L- but not D-arginine. Exogenously applied NO relaxed the cavernous muscle. NO synthesized from L-arginine in nerve terminals appears to partially mediate the relaxation of monkey corpus cavernosum participating in the penile erection. Histochemical demonstration of nerve fibers containing NADPH diaphorase in monkey cavernous trabecular meshworks (present study) and NO synthase in dog corpora cavernosa (12) supports the hypothesis of nitroxidergic innervation (31). Atropine did not alter the neurogenic response, suggesting that the release of endothelium-derived NO by a mediation of muscarinic receptor stimulation by acetylcholine (2) derived from nerves is not involved in the response. VIP, the other candidate of neurotransmitter (17), and CGRP, a vasodilator neurotransmitter (1, 18), are unlikely to mediate the relaxation because the strips made tachyphylactic to VIP and those treated with a sufficient concentration of the CGRP receptor antagonist CGRP-(8—37) (35) did not inhibit the response to nerve stimulation. PACAP is demonstrated to be colocalized with VIP in nerve structures in human cavernous tissues and to be effective in relaxing isolated cavernous strips (14), as seen in the monkey strips in the present study. However, the relaxation in the monkey tissue was not so evident, and the response to nerve stimulation was not reduced in the strips made unresponsive to the peptide, suggesting that PACAP used here is not involved in the neurogenic response. Therefore, it is unlikely that VIP, CGRP, and PACAP are neurotransmitters responsible for cavernous muscle relaxation.

Continuous nerve stimulation at a low frequency (1 Hz, Fig. 3) produced a sustained, intense relaxation that might reflect a prolonged penile erection under effective and nondeteriorating neural influences in vivo. The relaxation was interrupted by an inhibition of NO synthesis and a blockade of nerve action potentials.

The relaxation induced by transmural electrical stimulation was abolished by tetrodotoxin but inhibited only partially by treatment with L-NNNA. Raising the concentration of the inhibitor from 10 to 100 µM did not produce additional inhibition. Cohen et al. (5) have demonstrated that the endothelium-dependent relaxation and hyperpolarization and the NO release induced by acetylcholine in the rabbit carotid artery are not abolished even by combined treatment with high concentrations of L-NNNA and L-NAME, suggesting the NO release is resistant to NO synthase inhibitors. However, this does not seem to be the case in our preparation, because the response in L-NNNA (100 µM)-

**Table 1. Effects of charybdoxtin, apamin, 4-aminopyridine, glibenclamide, Ba^{2+}, and SKF-525a on relaxant response to transmural electrical stimulation at 5 Hz**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Control</th>
<th>Experimental</th>
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<tbody>
<tr>
<td>Charybdoxtin (0.1 µM)</td>
<td>7</td>
<td>29.4 ± 4.9</td>
<td>31.1 ± 4.8</td>
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<tr>
<td>Apamin (1 µM)</td>
<td>7</td>
<td>24.6 ± 5.2</td>
<td>24.0 ± 5.4</td>
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<tr>
<td>Charybdoxtin (0.1 µM) + apamin (1 µM)</td>
<td>4</td>
<td>27.3 ± 6.1</td>
<td>36.9 ± 5.9</td>
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<tr>
<td>4-Aminopyridine (1 mM)</td>
<td>4</td>
<td>31.9 ± 1.8</td>
<td>34.9 ± 7.8</td>
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<tr>
<td>Glibenclamide (1 µM)</td>
<td>8</td>
<td>30.4 ± 5.3</td>
<td>32.1 ± 5.7</td>
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<tr>
<td>Ba^{2+} (1 mM)</td>
<td>4</td>
<td>26.8 ± 6.9</td>
<td>30.0 ± 6.7</td>
</tr>
<tr>
<td>SKF-525a (0.1 mM)</td>
<td>4</td>
<td>24.3 ± 6.2</td>
<td>20.9 ± 5.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of strips from separate monkeys. Neurogenic relaxation percentages are relative to those induced by 0.1 mM papaverine.
treated strips was not influenced by oxyhemoglobin, a NO scavenger, or methylene blue in concentrations sufficient to abolish the response to neurogenic and endothelial NO in monkey (30, 32) and canine blood vessels (29). Therefore, the inhibitory nerve is postulated to liberate relaxant substance(s) other than NO. In isolated rabbit corpus cavernosum strips, the electrical stimulation-induced relaxation at high frequencies is not abolished by L-NNA (30 μM), oxyhemoglobin (10 μM), and methylene blue (10 μM) (16). In isolated canine corpus cavernosum, the neurogenic relaxation is totally abolished by L-NNA, suggesting that the response is mediated exclusively by NO produced from L-arginine (12). Possibility of discriminating the release of different neurotransmitters from nerves by changing stimulation frequencies has been described (20). This may be the case in the material used here because the responses to low frequencies of stimulation (0.2 and 0.5 Hz) were more susceptible to L-NNA than those to high frequencies (2 and 5 Hz) (77–80 vs. 52–53% inhibition obtained from the same 5 strips, Fig. 4), although the relaxation resistant to the NO synthase inhibitor was still observed even when the nerve was stimulated at low frequencies.

The stimulation-induced relaxation resistant to a high concentration of L-NNA was abolished by raising the external concentration of K+ (145 mM), which did not abolish the relaxation induced by NO or PGI2 (data not shown). Lowering the external K+ concentration reversed the response. According to Saito et al. (27), relaxations induced by electrical field stimulation of rabbit corpus cavernosum strips are potentiated by increasing the external K+ concentrations. High concentrations of TBA also abolished the neurogenic response. Therefore, substance(s) opening K+ channels in smooth muscle cell membranes may be involved in the response of the monkey strips. Levromakalim, an ATP-activated K+ channel opener, relaxed the strips. However, glibenclamide, an ATP-activated K+ channel inhibitor, and charybotoxin and apamin, Ca2+-activated K+ channel inhibitors, did not significantly alter the response resistant to L-NNA. Ba2+ and 4-aminopyridine in concentrations used were also ineffective. Because SKF-525a, a cytochrome P-450 inhibitor, depressed the endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxation in canine, bovine, and porcine coronary arteries that was resistant to NO synthase inhibitors (13, 23), the authors suggested the involvement of arachidonic acid metabolite synthesized by cytochrome P-450, presumably epoxide. However, this inhibitor in the same concentration failed to inhibit the response to nerve stimulation. There are numerous papers demonstrating that the hyperpolarization and relaxation via EDHF are ascribed by opening of certain types of K+ channels in subprimate mammals (3, 15, 19, 21, 22, 37), on the basis of findings with so-called selective K+ channel inhibitors. However, in the present study, the type of K+ channels responsible for non-NO relaxing factor(s) could not be specified.

A possible role of Na+–K+–adenosinetriphosphatase in relaxations of human corpus cavernosum muscle is suggested (11). Relaxations and membrane hyperpolarization in vascular smooth muscle are reportedly mediated by the Na+–K+ pump (6, 24), the activity being reduced by ouabain and replacement of extracellular Na+ with Li+. In our preparations treated with high concentrations of L-NNA, the relaxant response to nerve stimulation was not influenced by ouabain or with substitution of LiCl for NaCl. Involvement of a neuronal substance that activates this enzyme would therefore be excluded. The present study revealed for the first time a neurogenic relaxation that was mediated not only by NO but also by other substances in monkey corpus cavernosum smooth muscle, although a non-NO mechanism of neurogenic relaxation has been reported in nonvascular smooth muscle (28). Relaxations by this substance may be due to opening of K+ channels, the types of which were unidentified. Whether this type of channel is present specifically in primate corpus cavernosum or is seen also in other vascular smooth muscle from a variety of mammals remains to be ascertained. Involvement of acetylcholine, VIP, CGRP, PACAP, and the Na+ pump in the response is excluded.

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