Comparison of Neurogenic Contraction and Relaxation in Canine Corpus Cavernosum and Penile Artery and Vein

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ABSTRACT—Functional roles of autonomic efferent nerves were compared in the isolated canine corpus cavernosum, penile artery and penile vein that participate in the penile erection by changing blood distribution. Nicotine produced moderate contraction in the arterial strips, but only a slight or no contraction in the corpus and venous strips. The contraction was suppressed or reversed to a relaxation by prazosin. Under \( \alpha_1 \)-adrenoceptor blockade, relaxations induced by nicotine were in the order of the corpus > artery >> vein. The response was abolished by \( \text{N}^\text{G} \)-nitro-L-arginine (L-NA) and restored by L-arginine. The responses to nicotine and exogenous nitric oxide (NO) were abolished by oxyhemoglobin. The relaxant response to transmural electrical stimulation at 5 Hz was greater in the corpus than venous strips treated with prazosin, and it was abolished by L-NA. Contractions caused by nicotine under treatment with L-NA were greater in the artery than in the vein and corpus. Histochemical studies demonstrated nerve fibers containing NO synthase and tyrosine hydroxylase immunoreactivity in the corpus cavernosum, artery and vein. It is concluded that the canine corpus cavernosum, penile artery and penile vein are innervated by adrenergic, vasoconstrictor and nitroxidergic, vasodilator nerves; neurogenic vasodilatation is predominant in the corpus muscle, whereas neurogenic vasoconstriction predominates in the artery. Such a different functioning of the nerves may be responsible for the penile erection.

Keywords: Nerve-derived nitric oxide, Adrenergic nerve, Corpus cavernosum, Penile vein, Penile erection

Autonomic nerves participate importantly in the control of functions of sexual organs and tissues, including penile erection. Decreased tone of the trabecular smooth muscle of the corpus cavernosum penis and increased pooling of blood in the corpus, possibly responsible for the erection, may be associated with an increase in the inhibitory nerve activity and/or a decrease in the constrictor nerve activity. The contraction of the penile arterial and cavernous muscle is associated with sympathetic nerve activation, and the muscle relaxation is elicited by vasodilator nerve stimulation. Neurotransmission of the vasodilator nerve in the corpus has been considered to be mediated by vasoactive intestinal polypeptide (VIP) (1, 2). Due to a recent finding reported by Bernett et al. (3), considerable attention is directed to nitric oxide (NO) derived from the inhibitory nerve. Studies on isolated tissue (4–7) support the idea that the cavernous muscle relaxation is mediated by neurogenic NO. However, systematic analyses have not been carried out concerning the roles of adrenergic and vasodilator innervation in the corpus cavernosum as well as the penile artery and vein that supply blood to the corpus and remove it from the tissue, respectively.

Accumulated data obtained from in vitro and in vivo studies have provided evidence to support the hypothesis that NO acts as a vasodilator neurotransmitter of non-adrenergic, non-cholinergic nerves in a variety of blood vessels from various mammals (8–12), including the bovine penile artery (13). Therefore, the present study was undertaken to elucidate the functional role of adrenergic and non-adrenergic, non-cholinergic nerves in the corpus cavernosum and the penile artery and vein obtained from dogs, to clarify the mechanism of neurogenic relaxation of smooth muscle in special reference to NO, to quantitatively compare functions of the nerves in these tissues, and to histologically determine NO synthase-containing nerves. Nicotine was used for the stimulation of perivascular nerves, because these tissue preparations of different

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thickness could not be placed between stimulating electrodes in a comparable way, and nicotine produces consistent responses and shares pharmacological actions with electrical nerve stimulation (14, 15). Such a comparison of nerve function may provide new ideas about the mechanisms underlying the penile erection in reference to autonomic innervation.

MATERIALS AND METHODS

Tension recording
The studies review board at our university approved the use of animal blood vessels in this study. Twenty-four male mongrel dogs, weighing 8 to 14 kg, were anesthetized with intravenous injections of sodium pentobarbital (30 mg/kg) and killed by bleeding from the carotid arteries. The penis was rapidly removed, and the corpus cavernosum penis, distal portions of the dorsal penile artery (0.4 to 0.7 mm outside diameter) and the accompanying dorsal penile vein were isolated. The reasons why the dorsal artery was used are as follows: 1) although the deep penile artery may be more appropriate for this study, the size is too small to provide reliable preparations; 2) since the deep artery is tightly bound to the surrounding tissues, it is difficult to isolate; 3) the dorsal artery has anastomoses with the deep artery and contributes to supplying blood to the cavernous tissue. From the corpus, the tunica albuginea was extensively removed, and strips of cavernous tissues of approximately 15 mm in length were prepared. The arteries and veins were cut into helical strips of approximately 15 mm in length. The specimens were vertically fixed between hooks in a muscle bath containing the modified Ringer-Locke solution maintained at 37 ± 0.3°C and aerated with a mixture of 95% O2 and 5% CO2. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo). Resting tensions were adjusted to 0.7 g for the corpus, 1.5 g for the artery and 1.0 g for the vein, which were optimal for inducing the maximal contraction, as determined from the study on the resting tension-nor-epinephrine-induced contraction curve. The composition of the bathing solution was as follows: 120 mM NaCl, 5.4 mM KCl, 2.2 mM CaCl2, 1.0 mM MgCl2, 25.0 mM NaHCO3 and 5.6 mM dextrose. The pH of the solution was 7.36 to 7.42. Before the start of experiments, all of the strips were allowed to equilibrate for 60 to 90 min in the bathing media, during which time the fluid was replaced every 10 to 15 min.

Isometric mechanical responses were displayed on an ink-writing oscillograph. The contractile response to 30 mM K+ was first obtained (mean values: 383 ± 48 mg in the corpus, 1227 ± 158 mg in the artery and 277 ± 35 mg in the vein, n=10 each), and the strips were repeatedly washed with fresh media and equilibrated. Only one strip per dog per individual type of experiment was used. The concentration-response curve for nicotine (10^-3, 10^-4 and 3 x 10^-4 M) was obtained by applying a single concentration in each series to avoid tachyphylaxis. The strips were partially contracted with endothelin-1 (0.5 to 5 x 10^-9 M), because this agent was effective in producing sufficient magnitudes of contraction in the corpus, arterial and venous strips; the contraction was in a range between 30% and 45% of the contraction caused by 30 mM K+.

Except for the determination of the concentration-response curve, 10^-4 M nicotine was used to analyze the mechanism underlying the responses. At the end of each experiment, papaverine (10^-4 M) was added to obtain the maximal relaxation. Relaxations and contractions induced by test stimuli were presented as relative values to the relaxation caused by 10^-4 M papaverine and the contraction caused by 30 mM K+, respectively. Some of the corpus and venous strips were placed between platinum electrodes to transmurally stimulate nerve terminals by the application of electrical square pulses of 0.2-msec duration at 5 and 2 Hz for periods of 40 and 100 sec, respectively, by an electronic stimulator (Nihon Kohden Kogyo Co.) (14). The strips had been exposed for 20 to 30 min to blocking agents, before the responses to agonists or electrical stimulation were obtained. Responses to nicotine and NO were compared in the corpus, artery and vein obtained from the same dogs. In some experiments, responses to nicotine were compared in the arterial strips with and without the endothelium, obtained from the same dogs. The endothelium was removed by gently rubbing the intimal surface with a cotton ball. The endothelial function was verified from the relaxant response to 10^-6 M acetylcholine.

Histology
Isolated dog corpus cavernosum penis, penile artery and penile vein were fixed in ice-cold 0.1 M phosphate-buffered saline (PBS) containing 0.3% glutaraldehyde and 4% paraformaldehyde and then postfixed overnight in PBS with 4% paraformaldehyde, followed by cryoprotection in 15% sucrose. Thin sections (20-μm-thick) were cut on a cryostat (−18°C) and kept in 0.1 M PBS containing 0.3% Triton X-100 at 4°C for 4 days. The specimens were exposed to affinity purified serum against rat cerebellum NO synthase (1:300) in PBS with 0.3% Triton X-100 for 4 days at 4°C. Subsequently, biotinylated goat anti-rabbit immunoglobulin G antibody and avidin-biotinylated peroxidase complex (Vector Lab., Inc., Birlingame, CA, USA) were conjugated to the primary antibody at room temperature for 1 hr each. Immunolabeled peroxidase was visualized by incubation.
at room temperature for 3–5 min with 0.56 mM 3,3'-diaminobenzidine tetrahydrochloride (Dojindo Lab., Kumamoto), 1.3 μM hydrogen peroxide and 10 mM nickel ammonium sulfate. The specimens were mounted onto gelatin/chrome-alum-coated glass slides. After several washes with distilled water, the sections were air-dried and cover-slipped with Entellan (Merck, Darmstadt, Germany). The antiserum used in this study was raised against NO synthase purified from rat cerebellum (16), and it demonstrated immunoreactivity for NO synthase in perivascular nerves of the rat and dog cerebral and peripheral arteries (17, 18). By Western blots, this affinity-purified antiserum reacts specifically with NO synthase in brain extracts from rodents, guinea pigs, dogs and primates. A histochemical control experiment, in which the antiserum against NO synthase was excluded from the reaction mixture, gave no positive staining.

For the tyrosine hydroxylase histochemistry, the sections of the corpus, artery and vein provided as described above were exposed to purified rabbit antiserum raised against bovine adrenal tyrosine hydroxylase (1 : 6,000; Eugene Tech. Int., Inc., Ramsey, NJ, USA) in PBS with

![Diagram of dog corpus cavernosum and penile artery & vein](image)

**Fig. 1.** Tracings of the responses to nicotine (N, 10^-4 M) and NO (10^-7 to 10^-5 M) of canine penile artery, corpus cavernosum and penile vein before and after prazosin (10^-5 M) and those as affected by L-NA (10^-5 M) and l-arginine (L-Arg., 3 x 10^-3 M) under prazosin-treatment. The strips were partially contracted with endothelin-1. PA represents 10^-4 M papaverine, which produced the maximal relaxation.
0.3% Triton X-100 at 4°C for 4 days. Subsequent procedures were the same as those described for NO synthase histochemistry. An immunohistochemical control experiment, in which the antiserum against tyrosine hydroxylase was removed from the reaction mixture, gave no positive staining.

**Statistics and drugs used**

The results shown in the text, tables and figures were expressed as mean values ± S.E. Statistical analyses were made by Student's paired and unpaired t-tests for two groups and Tukey's method after one-way analysis of variance for three or more groups. Drugs used were nicotine, L- and D-arginine, hexamethonium bromide (Nacalai Tesque, Kyoto); atropine sulfate (Tanabe Co., Osaka); indomethacin (Sigma Chemical Co., St. Louis, MO, USA); timolol hydrochloride (Banyu Co., Tokyo); \( N^2 \)-nitro-L-arginine (L-NA), \( N^2 \)-nitro-D-arginine (D-NA), endothelin-1 (human) (Peptide Institute, Minoh); prazosin hydrochloride (Pfizer Co., Tokyo); acetylcholine chloride (Daiichi Co., Tokyo); \( dl \)-norepinephrine hydrochloride and tetrodotoxin (Sankyo Co., Tokyo); and papaverine hydrochloride (Dainippon Co., Osaka). Oxyhemoglobin was prepared from dog hemoglobin (Sigma) by the method described by Martin et al. (19). Responses to NO were obtained by adding the NaNO\(_2\) solution adjusted at pH 2 just before application (20), and the NO concentrations were expressed as concentrations of acidified NaNO\(_2\) in the bathing media.

**RESULTS**

**Tension recording**

The addition of nicotine (10\(^{-5}\) to 3 \times 10\(^{-4}\) M) produced a dose-dependent contraction in penile arterial strips partially contracted with endothelin-1. The response was
consistently obtained with $10^{-4}$ M; therefore, analyses of
the response were carried out by the use of this concen-
tration. Contractions caused by nicotine were compared
in penile arterial, corpus cavernosum and penile venous
strips obtained from separate dogs ($n=10$). Nicotine con-
tracted all of the arterial strips used ($n=10$) and 6 venous
strips out of 10. In 10 cavernous strips, 4 strips responded
to nicotine with a contraction followed by a relaxation
(Fig. 1); in the remaining strips, only slight or moderate
relaxations were induced. Typical recordings of the
response in the artery, corpus and vein from the same
dogs are illustrated in Fig. 1. The contractile responses
were abolished by hexamethonium ($10^{-5}$ M). In order to
compare the actual response to adrenergic nerve stimula-
tion, the preparations were treated with L-NA ($10^{-5}$ M)
that eliminated the response to vasodilator nerve stimula-
tion, as described later. Mean values of the contraction
relative to that caused by 30 mM K$^+$ did not differ in the
corpus and vein, but the value was significantly greater in
the artery (Fig. 2).

Cumulative dose-response curves of norepinephrine
($2 \times 10^{-8}$ to $5 \times 10^{-5}$ M) were obtained in the strips of
corpus cavernosum, penile artery and penile vein (Fig. 3).

Fig. 4. Modifications by L-NA ($10^{-5}$ M) and l-arginine (L-Arg., $3 \times 10^{-3}$ M) of the response to nicotine ($10^{-4}$ M) of the penile
artery, corpus cavernosum and penile vein treated with $10^{-5}$ M prazosin. The $\alpha_1$-adrenoceptor antagonist reversed the
nicotine-induced contraction to a relaxation in 5 out of
7 arterial strips contracted with endothelin-1; in the
remaining 2, the contraction was markedly attenuated
(from 605 and 370 mg to 12 and 30 mg, respectively) by
prazosin, and the depressed contraction was followed by
a moderate relaxation (24% and 48% relative to relaxa-
tion caused by $10^{-4}$ M papaverine). Contractions seen in
the corpus were reversed to relaxations by prazosin
($n=4$), and relaxations were potentiated ($n=1$) or un-
affected ($n=2$) by $\alpha_1$-adrenoceptor blockade. The venous
contractions induced by nicotine were reversed to relaxa-
tions ($n=4$), abolished ($n=1$) or markedly suppressed
($n=1$, from 270 mg to 38 mg) by prazosin. In the
remaining one, no response was induced by nicotine in
the absence and presence of $\alpha_1$-adrenoceptor blockade.
Nicotine-induced relaxations in the artery, corpus and
vein treated with prazosin were $34.8 \pm 5.0\%$ ($n=7$,
P<0.05 vs corpus, Tukey's method), 51.2±6.2% (n=7) and 10.3±3.0% (n=4, P<0.01 vs corpus and artery), respectively (Fig. 4). Raising the concentration of nicotine to 3×10^{-4} M did not increase the relaxation of venous strips, as compared to that caused at 10^{-4} M (n=3).

Relaxations induced by nicotine in prazosin-treated arterial and cavernous strips were not influenced by 10^{-7} M timolol (n=4), 10^{-7} M atropine (n=3) or 10^{-6} M indomethacin (n=3), but were abolished by 10^{-5} M hexamethonium (n=7) or 10^{-5} M oxyhemoglobin (n=3). Endothelium denudation did not inhibit the relaxation in the arterial strips (39.3±4.8% vs 40.6±5.1%, n=4). The relaxations of the arterial, cavernous and venous strips were abolished by treatment with 10^{-5} M L-NA, and the effect was reversed by 3×10^{-3} M L-arginine (Figs. 1 and 4). D-NA and d-arginine were without effect. Exogenously applied NO (10^{-7} to 10^{-5} M) elicited a concentration-related relaxation, which was not influenced by L-NA and L-arginine (Table 1), but was abolished by 10^{-5} M oxyhemoglobin (n=4 each in these tissues). Mean values of the NO (10^{-5} M)-induced relaxation did not significantly differ in the artery, corpus and vein (66.4%, 68.7% and 49.3%, respectively; Table 1).

Transmural electrical stimulation at 2 and 5 Hz caused a frequency-related relaxation in corpus strips treated with prazosin and partially contracted with endothelin-1. The relaxation was not influenced by indometh-
acrin (10^{-6} M), timolol (10^{-7} M), atropine (10^{-7} M) and D-NA (10^{-5} M) but was abolished by L-NA (10^{-3} M). L-Arginine, but not D-arginine, restored the response that was abolished by tetrodotoxin (3 \times 10^{-7} M). Typical tracings of the response are demonstrated in Fig. 5. Similar results were also obtained in 3 additional strips. In prazosin-treated, endothelin-contracted venous strips, transmural electrical stimulation at 5 Hz produced a slight relaxation that was abolished by 10^{-5} M L-NA or 3 \times 10^{-7} M tetrodotoxin (n=4). Mean values of the response to 5 Hz stimulation of venous and cavernous strips were 6.2\pm1.6\% (n=4) and 40.7\pm4.5\% (n=4), respectively, the difference being statistically significant (P<0.001, unpaired t-test).

Fig. 6. NO synthase immunohistochemistry of sections of the penile artery (A), corpus cavernosum (B) and penile vein (C). There are nerve fibers containing NO synthase immunoreactivity in the adventitia and media of the artery and vein and trabecular meshwork of the corpus. Bar=50 \mu m.

Fig. 7. Tyrosine hydroxylase immunohistochemistry of sections of the penile artery (A), corpus cavernosum (B) and penile vein (C). There are nerve fibers containing the enzyme immunoreactivity in the arterial and venous wall and the trabecula of corpus. Bar=50 \mu m.
**Histological study**

In the corpus cavernosum and penile artery and vein, nerve fibers containing NO synthase and tyrosine hydroxylase were immunohistochemically investigated. Figure 6 compares NO synthase immunoreactive nerve fibers. In the artery, there are big bundles and many fine fibers in the adventitia and some fibers also in the outer layer of the media (Fig. 6A). In the corpus, abundant networks of fine nerve fibers are observed in the trabecular meshwork (Fig. 6B). On the other hand, positively-stained fibers are only sparse in the vein (Fig. 6C), compared to A and B. This was also true in the tissues obtained from two additional dogs.

Nerve fibers containing tyrosine hydroxylase immunoreactivity are also present in the tissues used (Fig. 7). The positive fibers tend to be more in the artery (Fig. 7A) than in the corpus (Fig. 7B) and vein (Fig. 7C); however, the density could not quantitatively be compared in these tissues. Similar findings were also obtained in two additional dogs.

**DISCUSSION**

The addition of nicotine produced contractions consistently in isolated canine penile arteries and in many but not all penile veins and corpus cavernosum; these contractions were abolished by hexamethonium and were suppressed or reversed to relaxations by prazosin, a selective α1-adrenoceptor antagonist (21). In addition, vascular responses to nicotine are abolished by bretylium or guanethidine (22, 23) and pretreatment of animals with reserpine (24), and nicotine increases the ³H-overflow from isolated, superfused vasculature previously soaked in ³H-norepinephrine, as does electrical nerve stimulation (22). These findings strongly suggest that norepinephrine liberated from adrenergic nerves in the penile artery, corpus and penile vein in response to nicotine stimulates α1-adrenoceptors and elicits contraction. Since the relaxant response to nerve stimulation mediated by NO was found in these tissues in the present and previous (7) studies, the contractile responses to nicotine were compared under treatment with a NO synthase inhibitor in a dose sufficient to abolish the neurogenic relaxation. The rank order of the contractile response was artery >> corpus = vein. Median effective concentrations of exogenously-applied norepinephrine and maximal contractions caused by the amine did not significantly differ in the corpus, artery and vein, suggesting that the different responsiveness to adrenergic nerve stimulation is associated with the amount of norepinephrine released by stimulation with the same concentration of nicotine. Noradrenergic innervation was immunohistochemically demonstrated in the canine corpus and the penile artery and vein by the use of tyrosine hydroxylase antibody, and the innervation tended to be more evident in the artery.

In the artery, corpus and vein treated with prazosin and partially contracted with endothelin-1, nicotine produced a relaxation that was abolished by hexamethonium and oxyhemoglobin, a NO scavenger (19), but not influenced by atropine, timolol and indomethacin. Involvement of cholinergic, β-adrenergic and PG-related mechanisms would be excluded in the canine tissues; however, neurogenic acetylcholine is postulated to be involved partially in relaxations of the human corpus cavernosum (25). The nicotine-induced relaxation was also abolished by L-NA but not by D-NA, and the depressed response was reversed by l-arginine. Relaxations induced by exogenously applied NO were abolished by oxyhemoglobin but not by L-NA. In addition to nicotine, transmural electrical stimulation elicited relaxations of cavernous and venous strips that were also abolished by L-NA and tetrodotoxin. Bovine penile arterial relaxation to electrical nerve stimulation is also abolished by L-NA (13). Similar findings with electrical nerve stimulation and nicotine were also obtained in canine cerebral and peripheral arteries and temporal veins (8, 9, 11, 13, 26).

The release of nitroxy compounds, including NO, NO₃ and NO₂, from endothelium-denuded cerebral and temporal arteries is increased by transmural electrical stimulation and nicotine (9, 27). These findings led us to speculate that NO is liberated as a neurotransmitter from the inhibitory nerve (nitroxidergic nerve, ref. 28) of the canine penile artery and vein and the corpus cavernosum. In these tissues, we demonstrated nerve fibers and bundles containing NO synthase immunoreactivity. Our functional study indicated that nitroxidergic nerve functions are in the order of corpus > artery >> vein.

In addition to NO and norepinephrine, acetylcholine, VIP, calcitonin gene-related peptide (CGRP) and neuropeptide Y are also considered to be candidates of neurotransmitters in vasodilator and vasoconstrictor nerves (29). However, in the canine tissues used in the present study, the relaxation caused by nerve stimulation was abolished by the NO synthase inhibitor and oxyhemoglobin, suggesting that the induced response is due at least mainly to a mediation of NO but not the other substances. This idea is supported by the findings that the neurogenic response was neither influenced by atropine (present study) nor in the preparations made insensitive to VIP by pretreatment with high concentrations of the peptides, and the relaxation elicited by VIP was not attenuated by L-NA (7). On the other hand, the nicotine-induced contraction was markedly depressed or reversed to a relaxation by treatment with prazosin. Therefore, the involvement of neuropeptide Y in the neurogenic contraction is minimal if any in the tissues used.
Relaxations induced by nicotine and transmural electrical stimulation were markedly greater in the corpus than in the vein (51.2% vs 5.9% with nicotine and 40.7% vs 6.2% with electrical stimulation), whereas NO-induced relaxations did not significantly differ (66.4% vs 44.3%). Slight responsiveness to nicotine of the vein does not seem to derive from an insufficient concentration, because raising the concentration did not increase the response. There tended to be fewer innervation of NO synthase-immunoreactive nerve fibers in the vein than the corpus, as observed histologically, although the difference could not be quantitatively evaluated. It can be concluded that functioning of NO-mediated vasodilator nerves is predominant in the corpus cavernosum over the penile vein.

The present study revealed that the canine corpus cavernosum and the penile artery and vein are innervated by noradrenergic vasoconstrictor and nitrooxidergic vasodilator nerves, but the balance of functional importance in these nerves differs; vasoconstriction is predominant in the artery over the corpus and vein, whereas vasodilatation is more evident in the corpus than the vein. Inflow of blood into the corpus cavernosum is controlled by penile arterial resistance relative to systemic vascular resistance. Even though the inflow was reduced by sympathetic activation, the important factor responsible for penile erection is the increased capacity of the corpus cavernosum to stagnate circulating blood. Nitrooxidergic nerve excitation would increase the cavernous capacity with a minimal effluence because of a paucity of vasoconstrictor nerve fibers. The present study revealed that the increased capacity of the corpus cavernosum is responsible for penile erection, whereas vasodilatation is more evident in the corpus than the vein.

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