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Effects of endothelial impairment by saponin on the responses to vasodilators and nitrergic nerve stimulation in isolated canine corpus cavernosum

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1 Responsiveness to EDRF-releasing substances and inhibitory nerve stimulation of canine isolated penile corpus cavernosum with and without saponin treatment were investigated.

2 Histological studies demonstrated that saponin did not detach endothelial cells from underlying tissues, but induced degenerative changes in the endothelial cells selectively.

3 In the cavernous strips contracted with phenylephrine, addition of acetylcholine, sodium nitroprusside, ATP and Ca^{2+} ionophore A23187 induced relaxations, but substance P and bradykinin did not change the muscle tone.

4 Acetylcholine-induced relaxation was significantly attenuated but not abolished by N^G-nitro-L-arginine (L-NOARG). L-arginine restored the response inhibited by L-NOARG. The L-NOARG resistant relaxation was not influenced by 1H[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one (ODQ) but was suppressed in the strips contracted with K^+. Treatment with saponin abolished the relaxation elicited by acetylcholine and A23187 but did not influence the response to nitroprusside and ATP. The ATP-induced relaxation was attenuated by aminophylline.

5 Transmural electrical stimulation at 2–20 Hz produced endothelium-independent relaxations which were abolished by tetrodotoxin and L-NOARG but unaffected by treatment with saponin. In saponin-treated cavernous strips, the neurogenic relaxation was not affected by acetylcholine, physostigmine, atropine and vasoactive intestinal peptide (VIP) but was abolished by ODQ.

6 It is concluded that acetylcholine-induced relaxations are endothelium-dependent and mediated partly by NO and also by other substances from the endothelium. The endothelium-independent relaxation to ATP is likely to be mediated by P_1 purinoceptors. The function of nitrergic nerve does not seem to be prejunctionally modulated by acetylcholine and VIP.

Keywords: Corpus cavernosum; nitric oxide; acetylcholine; saponin; endothelium-dependent relaxation; nitrergic function; transmural electrical stimulation; ATP

Abbreviations: ATP, adenosine triphosphate; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; L-NOARG, N^G-nitro-L-arginine; NO, nitric oxide; ODQ, 1H[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; VIP, vasoactive intestinal peptide

Introduction

Although cholinergic and vasoactive intestinal peptide (VIP)-releasing nerves have been considered to be involved in provoking penile erection associated with an increase in intracavernous pressure (Willis *et al*., 1981; Andersson *et al*., 1984; Saenz de Tejada *et al*., 1988; Kim *et al*., 1995), accumulated data from recent *in vitro* and *in vivo* studies strongly suggest that nitric oxide (NO) synthesized from L-arginine mainly mediates the relaxation of penile corpus cavernosum muscle and the intracavernous pressor response to nerve stimulation in a variety of mammals (Burnett *et al*., 1992; Rajfer *et al*., 1992; Pickard *et al*., 1993; Hayashida *et al*., 1996; Ayajiki *et al*., 1997). NO is considered to be liberated from the endothelium in corpora cavernosa in response to EDRF-releasing substances, such as acetylcholine (Ignarro *et al*., 1990; Saenz de Tejada *et al*., 1988; Azadzoi *et al*., 1992), which induces relaxation of isolated cavernous strips. The presence of muscarinic receptors in human corpus cavernosum and in endothelial cells from this tissue has been demonstrated (Traish *et al*., 1990). However, little information is available concerning relaxing factors liberated from the endothelium in response to chemical stimuli.

Neurogenic relaxation of cavernous muscle strips is now recognized to be mediated by NO, and the involvement of endothelium-derived NO in the response has been excluded (Kim *et al*., 1991). Histochemical studies have demonstrated dense networks of neurons containing NO synthase (Burnett *et al*., 1993; Hayashida *et al*., 1996), cholinesterase (Shirai *et al*., 1972; Benson *et al*., 1980) and VIP (Gu *et al*., 1983; Hayashida *et al*., 1996) in the corpus cavernosum. Electrical stimulation of these neurons is expected to liberate NO, acetylcholine and VIP. Therefore, synthesis or/and release of neurotransmitter from nitrergic nerves that play a major role in increasing the intracavernous pressure may be modulated by neurogenic acetylcholine and VIP, as was seen in monkey and bovine cerebral arteries in response to endogenous acetylcholine (Toda & Ayajiki, 1990; Toda *et al*., 1997a).

The present study was undertaken to determine the actions and mechanisms of action of substances that liberate relaxing factors from the vascular endothelium, including acetylcholine, bradykinin, substance P, ATP and Ca^{2+} ionophore A23187 (Angus & Cocks, 1989), on strips of the canine corpus

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cavernosum and to clarify whether or not neurogenic relaxations of cavernous strips were influenced by histological impairment of endothelial cells and by endogenous and exogenous acetylcholine and VIP.

Methods

Preparation

The Animal Care and Use Committee at Shiga University of Medical Science approved the use of dog penises in this study. Male mongrel dogs, weighing 7–13 kg, were anaesthetized with intravenous injections of sodium thiopental (50 mg kg^{-1}) and killed by bleeding from the carotid arteries. The penis was rapidly removed, and corpus cavernosum was isolated. The tunica albuginea was removed, and two or three strips (about $1 \times 2 \times 10$ mm) were obtained. The specimens were fixed vertically between hooks in a muscle bath of 20 ml capacity containing the nutrient solution, which was aerated with a mixture of 95% O_2 and 5% CO_2 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 (Nihon-Kohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 0.7 g, which is optimal for inducing the maximal contraction. Constituents of the solution were as follows (mM): NaCl 120, KCl 5.4, $CaCl_2$ 2.2, $MgCl_2$ 1.0, $NaHCO_3$ 25.0 and dextrose 5.6. The pH of the solution was 7.36–7.43. 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 fluids were replaced every 10–15 min.

Some of the strips were placed between stimulating electrodes. The gaps between the strip and the electrodes were wide enough to allow undisturbed mechanical responses and yet sufficiently narrow to stimulate intramural nerve terminals effectively. A train of 0.2 ms square pulses of supramaximal intensity were transmurally applied at 2, 5 and 20 Hz for 100, 40 and 10 s, respectively. In order to evaluate the effects of tested drugs on the responses elicited by electrical stimulation, 5 Hz was selected, because submaximal and reproducible responses could be obtained. The stimulus pulses were delivered by an electronic stimulator (Nihon-Kohden Kogyo Co, Tokyo, Japan).

Functional study

Isometric contractions and relaxations were displayed on an ink-writing oscillograph. The contractile response to K^+ was obtained at first by adding 30 mM K^+ directly to the medium, and the strips were washed three times with fresh media and equilibrated for 30–40 min. The strips were partially contracted with phenylephrine (0.5–2 μM); the contraction (182 ± 22 mg) was in a range between 30 and 40% of the contraction (495 ± 55 mg) induced by 30 mM K^+. Transmural electrical stimulation was applied repeatedly at intervals of 10 min until steady responses were obtained, and then blocking agents were applied. Acetylcholine, sodium nitroprusside and ATP were applied directly to the bathing media in cumulative concentrations, and the strips were repeatedly washed. At the end of each series of experiment, papaverine (0.1 mM) was applied to attain the maximal relaxation (100% relaxation), and relative values of the response to electrical and chemical stimulation were presented. Some of the strips were exposed for 45 min to saponin (1 mg ml^{-1}) to induce endothelial impairment (Samata *et al*., 1986), and repeatedly rinsed with drug-free media (10 times, every 5 min).

Histological study

Corpus cavernosum strips treated with saponin (1 mg ml^{-1}) or vehicle for 45 min were immersed for 2 h in a fixative containing 4% paraformaldehyde, 0.5% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4) at 4°C and then immersed for 24 h in a post-fixative containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer at 4°C. Tissues were washed for 4 days with several changes of 0.1 M phosphate buffer containing 15% sucrose, embedded in 10% gelatin in 0.1 M phosphate buffer and then cut into 50 μm sections on a vibratome. The sections were osmificated for 1 h with 1% OsO_4 in 0.1 M phosphate buffer at 4°C, dehydrated in graded ethanol, and embedded in epoxy resin. Ultrathin sections were cut and mounted on 200 mesh copper grids, stained with uranyl acetate and Reynold's solution and then observed under electron microscopy (H-7100, Hitachi, Japan).

Statistics and drugs used

The results shown in the text and figures are expressed as mean values ± s.e.mean. Statistical analyses were made using the Student's paired and unpaired *t*-test and the Tukey's test after one-way analysis of variance. Drugs used were N^G-nitro-L-arginine (L-NOARG), vasoactive intestinal peptide (VIP), substance P, bradykinin (Peptide Institute, Minoh, Japan), L-arginine (Nacalai Tesque, Kyoto, Japan), acetylcholine chloride (Daiichi Co., Osaka, Japan), sodium nitroprusside (Merck, Darmstadt, Germany), ATP, aminophylline, indomethacin, physostigmine sulphate, 1-phenylephrine hydrochloride (Sigma Chemical, St. Louis, MO, U.S.A.), atropine sulphate, thiopental (Tanabe, Osaka), bretylium tosylate (Glaxo Wellcome, NC, U.S.A.), tetrodotoxin (Sankyo Co., Tokyo), Ca^{2+} ionophore A23187 (C. H. Boehringer Ingelheim, Elmsford, NY, U.S.A.) and papaverine hydrochloride (Dainippon Co., Osaka). ODQ (1H[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) was a generous gift from Dr S. Moncada. Responses to NO were obtained by adding the $NaNO_2$ solution adjusted at pH 2 (Furchgott, 1988), and the concentrations of $NaNO_2$ in the bathing media were expressed as those of NO.

Results

Effects of acetylcholine and other agonists

In corpus cavernosum strips partially contracted with phenylephrine, acetylcholine (10 nM–10 μM) produced a concentration-related relaxation. Dose-response curves of acetylcholine did not significantly differ in the first, second and 3rd trials; mean values of the relaxation at 100 nM were 25.0 ± 3.0, 20.8 ± 4.3 and 21.3 ± 3.8% ($n = 6$), respectively, and those at 1 μM were 65.2 ± 5.9 and 66.5 ± 6.5 and 66.0 ± 6.1% ($n = 6$), respectively. Therefore, the first dose-response curve was taken as a control, and blocking agents or saponin were applied before the 2nd curve was obtained.

Treatment with L-NOARG (10 μM) significantly attenuated the response, and the addition of L-arginine (1 mM) reversed the inhibition (Figure 1, left). Raising the concentration of L-NOARG to 100 μM produced the additional inhibition in the acetylcholine-induced relaxation (Figure 2), but did not abolish it. In order to test the possibility of the involvement of other factors such as EDHF in the acetylcholine-induced relaxation or for insufficient inhibition by 100 μM L-NOARG

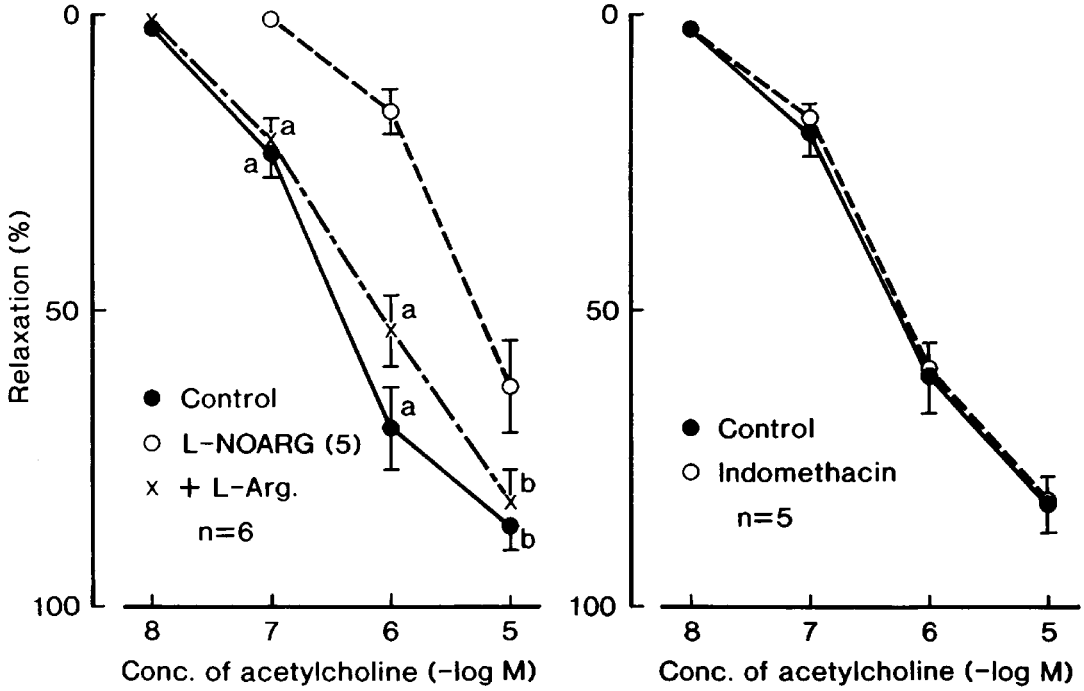

Figure 1 Modifications by L-NOARG (10 μM, 5 in the parenthesis) and L-NOARG plus L-arginine (L-Arg., 1 mM) (left figure) and by indomethacin (1 μM) (right) of the relaxant response to acetylcholine in corpus cavernosum strips contracted with phenylephrine. The ordinate represents relaxations relative to those induced by 0.1 mM papaverine. Significantly different from the value with L-NOARG, $^{a}P<0.01$; $^{b}P<0.05$ (Tukey's test). 'n' denotes the number of strips from separate dogs. Vertical bars represent s.e.mean.

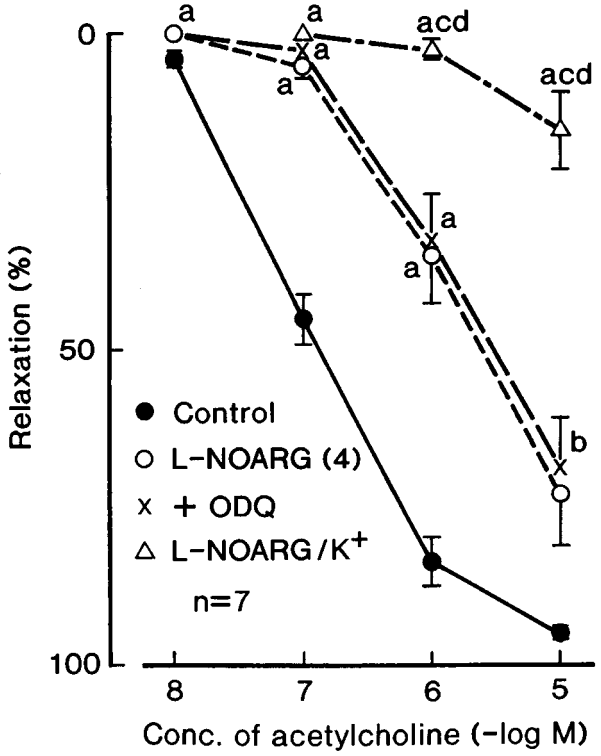

Figure 2 Modifications by L-NOARG (100 μM, 4 in the parenthesis), L-NOARG plus ODQ (1 μM) (+ODQ) and L-NOARG plus K^+ (L-NOARG/K^+) of the relaxant response to acetylcholine in corpus cavernosum strips. The strips were contracted with phenylephrine except for L-NOARG/K^+ in which the preparations were contracted with K^+ (15–20 mM), the concentration being adjusted to match the contraction induced by phenylephrine under control conditions. The ordinate represents relaxations relative to those elicited by 0.1 mM papaverine. Significantly different from control, $^{a}P<0.01$, $^{b}P<0.05$; significantly different from the value with ODQ, $^{c}P<0.01$; significantly different from the value with 100 μM L-NOARG $^{d}P<0.01$ (Tukey's test). 'n' denotes the number of strips from separate dogs. Vertical bars represent s.e.mean.

of the relaxation, the effect of ODQ (1 μM), a soluble guanylate cyclase inhibitor, was examined in the presence of L-NOARG (100 μM). This treatment did not produce further inhibition. However, as shown in Figure 2, the relaxation was markedly inhibited in the L-NOARG (100 μM)-treated strips when contracted with K^+ (15–20 mM). Typical tracings of the response are illustrated in Figure 3. Under the same conditions, the sodium nitroprusside-induced relaxation was not significantly affected ($n=4$). Indomethacin (1 μM) did not alter the acetylcholine-induced relaxation (Figure 1, right), whereas atropine (1 μM) abolished the response at concentrations up to 10 μM ($n=3$).

Substance P (10 nM) and bradykinin (1–100 nM) did not relax the strips ($n=8$), and one out of the eight strips responded to bradykinin with slight contraction.

ATP elicited a dose-related relaxation, which was attenuated by treatment with 20 μM aminophylline; mean values of the response to 0.1, 1 and 10 μM ATP were 4.3 ± 1.7, 23.3 ± 4.3 and $84.7\pm3.5\%$ ($n=6$), respectively, in the control media and those were 0 ($n=6$, $P<0.05$ vs corresponding control; unpaired t-test), 5.7 ± 1.9 ($P<0.01$) and $49.5\pm6.1\%$ ($P<0.001$), respectively, in the aminophylline-containing media.

Modifications by treatment with saponin

Exposure for 45 min to bathing media containing saponin (1 mg ml^{-1}) abolished or markedly attenuated the acetylcholine-induced relaxation but did not alter the dose-response curve of sodium nitroprusside (Figure 4). Relaxations to NO were not influenced by treatment with saponin; mean values of the response at 10 and 100 μM NO were 29.1 ± 2.3 and $72.3\pm8.1\%$ ($n=5$), respectively, in control media, and 31.6 ± 10.5 and $75.4\pm8.9\%$ ($n=5$), respectively, in the strips treated with saponin. Contractions induced by K^+ and phenylephrine were also unaffected by treatment with saponin; mean values of the response to K^+ (30 mM) before and after

saponin treatment were 445±45 and 423±43 mg ($n=4$), respectively, and those to 10 μM phenylephrine before and after the treatment were 133.8±21.6 and 142.5±28.2% relative to those induced by 30 mM K^+, respectively. Relaxations induced by ATP were not influenced by treatment with saponin (Figure 5, left). In contrast, the responses to A23187 (10 and 100 nM) were abolished by the same treatment (Figure 5, right).

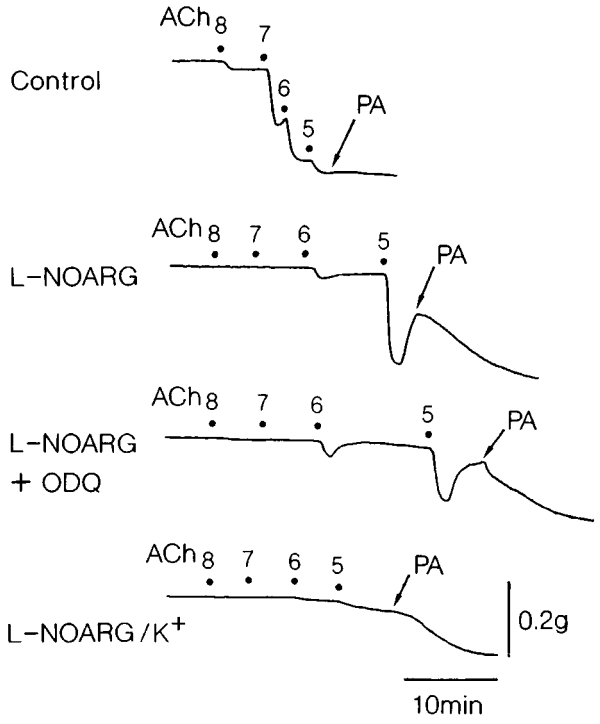

Figure 3 Tracings of the response to acetylcholine (ACh, 10 nM–10 μM) as affected by L-NOARG (100 μM), L-NOARG plus ODQ (1 μM) and L-NOARG plus ODQ (K^+) in a corpus cavernosum strip. The preparation was contracted with phenylephrine in the upper three tracings but with K^+ (15 mM) in the bottom tracing. PA represents 0.1 mM papaverine that produced the maximal relaxation. Numbers indicate the concentrations of the drugs in −log[M].

In order to determine whether NO liberated from the endothelium of copora cavernosa was involved in the relaxation induced by transmural electrical stimulation, the neurogenic response of the cavernous strips treated with bretylium (10 μM) and contracted with phenylephrine was compared before and after treatment with saponin. The frequency-response curve of nerve stimulation was not significantly influenced in the strips treated with saponin; mean values of the response relative to that induced by 0.1 mM papaverine in the control ($n=7$) and the saponin-treated strips ($n=7$) were 43.1±7.0 and 44.6±6.1% at 2 Hz, 59.6±6.1 and 60.6±5.0% at 5 Hz, and 61.3±4.8 and 65.1±3.7%, respectively. The stimulation-induced response was abolished by tetrodotoxin (0.3 μM) in all of the strips and by L-NOARG (10 μM) in three out of three strips tested.

Effects of acetylcholine and VIP on prejunctional receptors in saponin-treated strips

In cavernous strips previously treated with saponin and contracted with phenylephrine, the relaxation elicited by transmural electrical stimulation at 5 Hz was not influenced by acetylcholine (1 and 10 μM), atropine (0.1 μM) and physostigmine (0.1 μM). The mean value of the control response was 58.6±11.2% ($n=5$) relative to that induced by 0.1 mM papaverine, and changes caused by treatment with acetylcholine (1 and 10 μM), atropine (0.1 μM) and physostigmine (0.1 μM) were 1.3±2.7 ($n=5$), −3.8±3.9 ($n=4$), 5.2±3.8 ($n=5$) and 3.4±2.8% ($n=5$), respectively, the differences being statistically insignificant ($P>0.05$). These

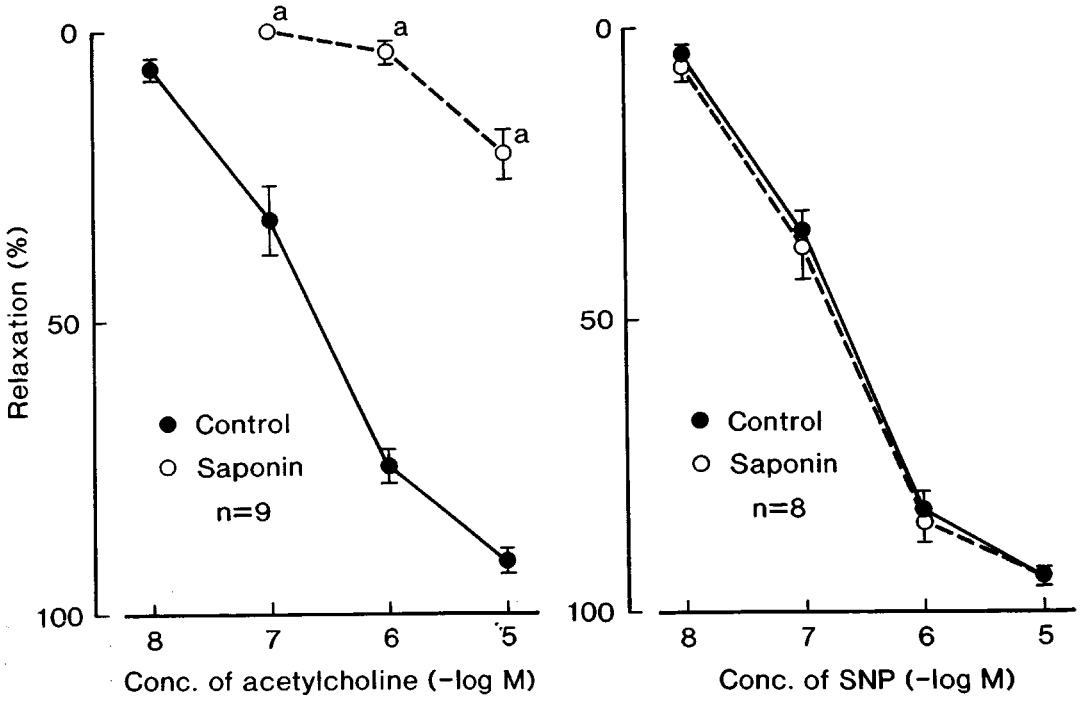

Figure 4 Concentration-response curves of acetylcholine (left figure) and sodium nitroprusside (SNP) (right) in corpus cavernosum strips before (control) and after treatment with saponin. The strips were contracted with phenylephrine. The ordinate represents relaxations relative to those induced by 0.1 mM papaverine. Significantly different from control, $^aP<0.001$ (unpaired t-test). 'n' denotes the number of strips from separate dogs. Vertical bars represent s.e.mean.

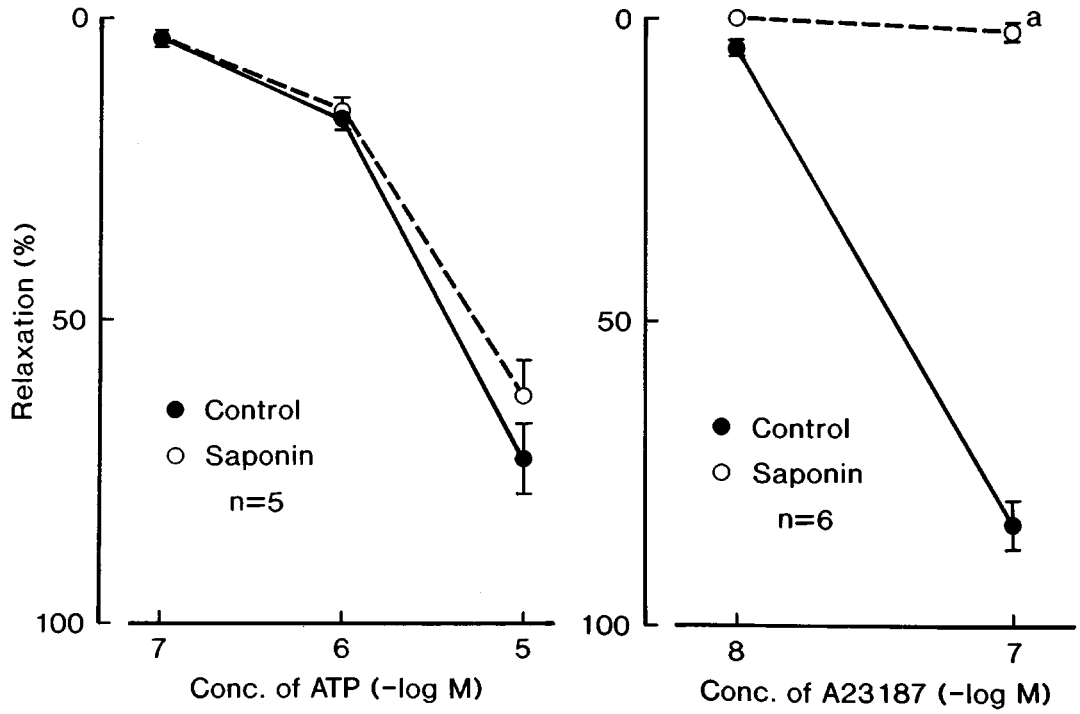

Figure 5 Concentration-response curves of ATP (left figure) and Ca^{2+} ionophore A23187 in corpus cavernosum strips before (control) and after treatment with saponin. The strips were contracted with phenylephrine. Significantly different from control, $^{a}P<0.001$ (unpaired *t*-test). 'n' denotes the number of strips from separate dogs. Vertical bars represent s.e.mean.

concentrations, which were sufficient to stimulate or inhibit prejunctional muscarinic receptors and to possibly inactivate cholinesterase in nitrergic nerve terminals (Toda *et al.*, 1997a,b), did not alter the basal tone of cavernous strips by treatment with saponin. In eight strips without saponin treatment, no significant influence of physostigmine and atropine on the response to nerve stimulation was observed; mean values in control media and those containing these inhibitors were 50.9 ± 8.6, 55.5 ± 8.8 and $55.9\pm6.2\%$, respectively.

VIP (1 and 3 nM) relaxed the strips (14.9 ± 2.5 and $21.0\pm3.8\%$, respectively, $n=8$) but did not significantly alter the response to nerve stimulation; mean values of the response to nerve stimulation before and after treatment with VIP in these two concentrations were 44.6 ± 2.0, 46.1 ± 2.6 and $51.7\pm3.8\%$ ($n=8$), respectively. Additional treatment with 1 μM ODQ abolished the response ($n=5$).

Histological study

Ultrastructural changes in the endothelial cells in the sinus of corpus cavernosa induced by saponin treatment were examined. In the endothelial cells from strips without saponin treatment (control), such organelles as Golgi apparatus, rough endoplasmic reticulum, mitochondria and pinocytotic vesicles are clearly seen in the cytoplasm (Figure 6A). However, in the saponin-treated preparations, the endothelial cells were decreased in size and those organelles seen in controls were hardly visible under electron microscopy (Figure 6B and C). Most characteristically, the cytoplasmic processes were dramatically shrunken by the saponin treatment. They were folded (arrows in Figure 6B), and in severe cases these folds surrounded the perinuclear cytoplasm (arrows in Figure 6C). These results suggest that saponin treatment may induce degeneration of the endothelial cells in corpus cavernosa of penis. However, there was no ultrastructural change in the smooth muscle cells of saponium-treated strips.

Discussion

Canine cavernous strips contracted with phenylephrine responded to acetylcholine, nitroprusside, ATP and Ca^{2+} ionophore A23187 with relaxations but did not relax in response to peptides, such as substance P and bradykinin, which are reportedly strong vasodilators releasing NO from the endothelium in canine arteries (Furchgott, 1983). In contrast, these peptides relax cavernous strips from rabbits (Azadzoi *et al.*, 1992). Treatment of the strips for 45 min with saponin (1 mg ml^{-1}) abolished the relaxant response to acetylcholine and A23187 but failed to alter the response to nitroprusside, NO and ATP. Histological studies demonstrate that saponin did not detach the endothelial cells from underlying tissues, but induced degenerative changes in the endothelial cells, since such organelles as Golgi apparatus, rough endoplasmic reticulum, mitochondria and pinocytotic vesicles clearly seen in the cytoplasm of the strips without saponin treatment were hardly visible under electron microscopy in the saponin-treated strips. In contrast, such a degeneration is not observed in the smooth muscle cells. Therefore, treatment with saponin under the conditions used in this study is considered to selectively impair the endothelial function. Actually, contractions induced by K^+ and phenylephrine were unaffected by treatment with saponin, as were nitroprusside-induced relaxations. Therefore, acetylcholine- and A23187-induced relaxations are considered to be endothelium-dependent as seen in those of a variety of blood vessels from dogs, whereas the response to ATP, an EDRF-releasing substance in canine arteries (Angus & Cocks, 1989), is independent of the endothelium in the corpus cavernosum.

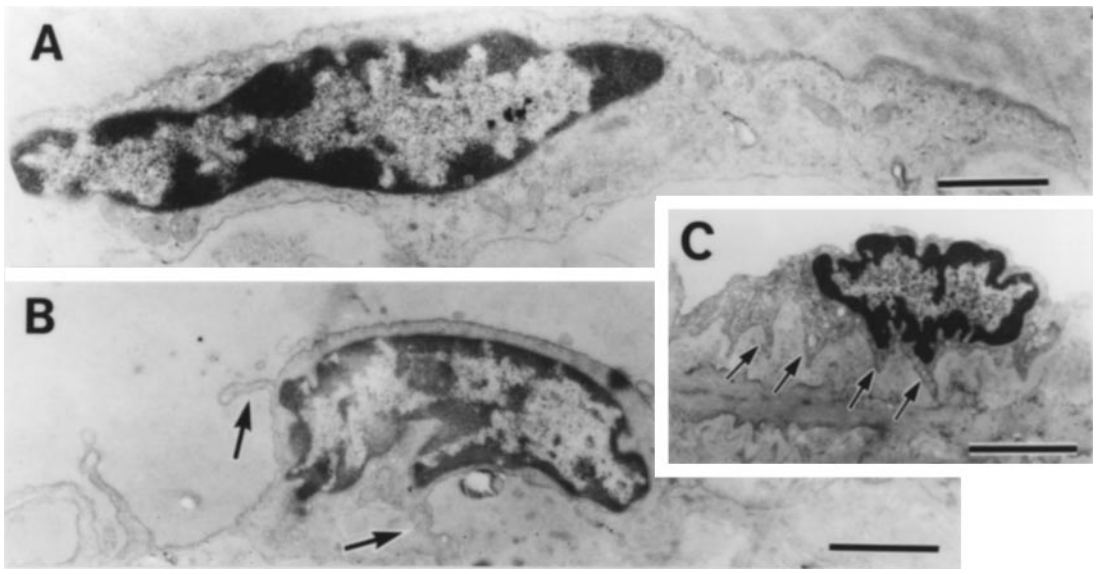

Figure 6 Electron photo micrographs of endothelial cells in the sinus of corpus cavernosa of penis in control (A) and saponin-treated (B and C) strips. Bars = 2 μm. (A) Organelles such as Golgi apparatus, rough endoplasmic reticulum, mitochondria and pinocytotic vesicles are clearly seen in the cytoplasm. (B and C) Cell size is reduced compared to (A) and organelles seen in (A) are hardly seen in the cytoplasm. The cytoplasmic processes elongated laterally in (A) are dramatically shrunken and they are folded around the perinuclear cytoplasm (arrows).

ATP-induced relaxations were attenuated by aminophylline, as those in canine cerebral arteries (Toda *et al*., 1982; Okamura *et al*., 1998), suggesting that the response is mediated by P_1 purinoceptors and production of cyclic AMP (Williams, 1987).

Endothelium-dependent relaxations induced by acetylcholine were significantly attenuated by L-NOARG, and the inhibition was reversed by the addition of L-arginine, suggesting that NO from the endothelium is involved in the response. Similar findings have been reported in isolated rabbit and human cavernous strips (Kim *et al*., 1991). NO synthase binding sites and endothelial NO synthase are present on the endothelium of rat and human corpora cavernosa (Sullivan *et al*., 1996; Seftel *et al*., 1997). However, L-NOARG even in high concentrations and additional treatment with ODQ, an inhibitor of soluble guanylate cyclase (Garthwaite *et al*., 1995), did not abolish the relaxation. The remaining response was abolished in the strips exposed to high K^+ solution. Under the same conditions, the sodium nitroprusside-induced relaxation was not significantly reduced. Therefore, acetylcholine may liberate a substance(s) that opens K^+ channels, possibly resulting in hyperpolarization. The channel subtypes involved could not be analysed in the present study.

Histochemical studies have demonstrated that the corpus cavernosum is innervated by neurons containing NO synthase, cholinesterase and VIP. Release of acetylcholine from cholinergic nerves is detected by measurement of release of [^{3}H]-acetylcholine in human corpus cavernosum strips (Blanco *et al*., 1988). Among the endogenous substances involved, NO is now recognized to play an important role in an elevation of intracavernous pressure and a penile erection (Burnett *et al*., 1992; Holmquist *et al*., 1991; Ayajiki *et al*., 1997). Although the possible involvement of acetylcholine and VIP as neurotransmitters in neurogenic relaxations is excluded (Hayashida *et al*., 1996), functional roles of these substances as neuromodulators have not been determined. The present study revealed that acetylcholine, physostigmine and atropine did not modify the relaxation mediated by NO produced from L-arginine in response to electrical nerve stimulation. Endogenous and exogenous acetylcholine does not seem to modulate functions of nitrergic nerve in canine corpus cavernosum. On the other hand, treatment with acetylcholine and physostigmine inhibited the response to nitrergic nerve stimulation in isolated monkey cerebral and porcine ciliary arteries, whereas atropine potentiated the response (Toda *et al*., 1997b; Toda *et al*., 1998). VIP, although it relaxed the strips, was also without effect on the neurogenic relaxation, suggesting that regulatory roles of endogenous VIP, even if released, are excluded.

Relaxations induced by electrical nerve stimulation and exogenous NO were not influenced by treatment with saponin. Despite the histological (Samata *et al*., 1986) and functional damage (present study) of endothelial cells by saponin, the vasodilator nerve and smooth muscle seem to be functionally unaffected. Further, the possibility that substances released by nerve stimulation elicit relaxation by a mediation of NO derived from the endothelium would be ruled out as reported in rabbit and human corpora cavernosa (Kim *et al*., 1991). The response to electrical stimulation was abolished by L-NOARG (present study; Pickard *et al*., 1993; Hayashida *et al*., 1996) and also by ODQ, despite the fact that acetylcholine-induced relaxations were partially inhibited by L-NOARG and the L-NOARG-resistant response was not susceptible to ODQ. These findings support the hypothesis that the neurogenic relaxation of cavernous smooth muscle is mediated solely by NO synthesized from L-arginine in nerve terminals in dogs. However, the fact that impairment of the endothelial function failed to reduce the response to nerve stimulation may indicate that acetylcholine in concentrations sufficient to liberate significant amounts of endothelium-derived NO is not released from cholinergic nerves.

In summary, acetylcholine liberates NO from the endothelium in canine copora cavernosa but does not act as a neurotransmitter or neuromodulator in inhibitory nerves. Acetylcholine-induced relaxations are likely to be mediated not only by NO but also other substance(s) from the endothelium. Despite the fact that an ability of the endothelium to release EDRF was proved by the use of acetylcholine and Ca^{2+} ionophore, there was no evidence for an endothelium-dependent response to ATP, substance P and

bradykinin. Whether this is due to a lack of purinoceptors and substance P- and bradykinin-receptors in the endothelium of canine cavernous strips remains to be clarified.

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