Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 8226–8231, July 1997
Pharmacology

Nitrergic control of peripheral sympathetic responses in the human corpus cavernosum: A comparison with other species

(noradrenergic/anococcygeus muscle)

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ABSTRACT **Noradrenergic contractions induced by electrical field stimulation (EFS) of the rabbit anococcygeus muscle and the human and rabbit corpus cavernosum did not occur until termination of stimulation, even when EFS was applied for long periods (10 min). After treatment with a nitric oxide synthase inhibitor, a scavenger of NO, or a specific inhibitor of the soluble guanylate cyclase, EFS-induced contraction began as soon as stimulation commenced and its magnitude and duration were increased. In the presence of a cGMP-phosphodiesterase inhibitor, the lag period between the end of EFS and the onset of contraction was longer, and the response was smaller. Even when the concentration of endogenous noradrenaline was increased with cocaine, the contraction still did not occur during EFS and the lag period was unchanged, although the response was enhanced. When tissue tone was elevated, relaxation occurred during EFS followed by a contraction. After blockade of neuronal noradrenaline release with guanethidine, contractions of the tissues to increasing concentrations of exogenous noradrenaline were significantly reduced by EFS, an effect that was reversible by inhibition of NO synthase. In contrast, in the rat and mouse anococcygeus muscles contraction began immediately with EFS, and nitrergic stimulation by EFS did not affect the responses elicited by high concentrations of exogenous noradrenaline. These results suggest that the human and rabbit genitourinary organs have a powerful nitrergic innervation that does not merely modulate, but actually controls, the sympathetic responses. Our observations may increase understanding of the balance between nitrergic and sympathetic systems in humans, disruption of which may contribute to certain pathological conditions.**

The excitatory innervation of the anococcygeus muscle (1) and corpus cavernosum (2) has been known for many years to be noradrenergic. It was later found that blockade of the sympathetic nerves and elevation of tone in the tissues revealed an inhibitory, relaxant response, the mediator of which could not be identified at that time (3, 4). This relaxant response, and many similar responses in the genitourinary system, gut, bronchi, and blood vessels, was attributed to nonadrenergic, noncholinergic (NANC) innervation in the autonomic nervous system (for review, see ref. 5).

The discovery in the 1980s of the biological actions of nitric oxide (6, 7) suggested that this might be the inhibitory mediator released from NANC nerves (8). This has now been widely accepted because relaxations induced by stimulation of NANC nerves are prevented by inhibitors of NO synthase, by a scavenger of NO, oxyhemoglobin (5), and by a specific inhibitor of the soluble guanylate cyclase (9). Moreover, calcium/calmodulin-dependent constitutive NO synthase has been identified in NANC-innervated tissues (5), and NO has been shown to be released during nerve stimulation (10, 11). NO-mediated transmission by NANC nerves is now known as nitrergic transmission (12).

Interaction between the noradrenergic and nitrergic innervation in the anococcygeus muscle was suspected from early studies (3). More recently, it was shown that the contractions induced in this tissue by electrical field stimulation (EFS) are enhanced by inhibitors of the NO synthase (13).

While characterizing nitrergic neurotransmission in the rabbit anococcygeus muscle and corpus cavernosum, we recently made the unexpected observation that excitatory noradrenergic contractions did not occur at all during the period of EFS but always happened as an aftereffect. Inhibition of NO synthesis, however, resulted in the contraction occurring during the period of stimulation (11). This suggested to us that the nitrergic system was not acting as a mere modulator of sympathetic transmission but that the latter was actually under nitrergic control. We have now investigated this interaction in more detail and extended it to the human corpus cavernosum, which exhibits similar behavior.

MATERIALS AND METHODS

Rabbit Anococcygeus Muscle and Rabbit Corpus Cavernosum. Male New Zealand rabbits (3.0–3.5 kg) were sacrificed by an overdose of pentobarbitone (Euthesate, Willows Francis Veterinary, United Kingdom) injected into the marginal vein of the ear after local anaesthesia (Xylocaine Gel 2%, Astra Pharmaceuticals, United Kingdom). The abdominal aorta was exposed, and the distal part was perfused with 200 ml of modified Krebs' solution (136.9 mM NaCl/2.7 mM KCl/1.8 mM $CaCl_2$/0.6 mM $MgSO_4$/11.9 mM $NaHCO_3$/0.5 mM KH_2PO_4/11.5 mM glucose, and gassed with 5% CO_2 in O_2 at pH 7.4–7.6). The corpus cavernosum (6 × 15 mm) and bilateral anococcygeus muscles (3 × 10 mm) were excised. The corpus cavernosum was cut longitudinally to obtain two identical strips (4 × 10 mm).

Rat and Mouse Anococcygeus Muscle. Adult male rats (200–250 g) and adult male mice (25–35 g) were sacrificed by a blow on the head. Bilateral anococcygeus muscles (3 × 8 mm for rat and 1 × 4 mm for mouse) were isolated.

Human Corpus Cavernosum. Whole corpus cavernosum was obtained from patients (ages 26, 29, 35, and 42; mean age: 33 ± 4.1; $n = 4$) undergoing penectomy for sex change in Sussex Nuffield Hospital, Brighton, United Kingdom. All patients gave written informed consent to the study, which was approved by the East Unit Research Ethics Committee of the Mid Sussex National Health Service Hospital, United Kingdom. The tissues were transported from the hospital to the

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Abbreviations: EFS, electrical field stimulation; NANC, nonadrenergic, noncholinergic; L-NOARG, N^G-nitro-L-arginine; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one.

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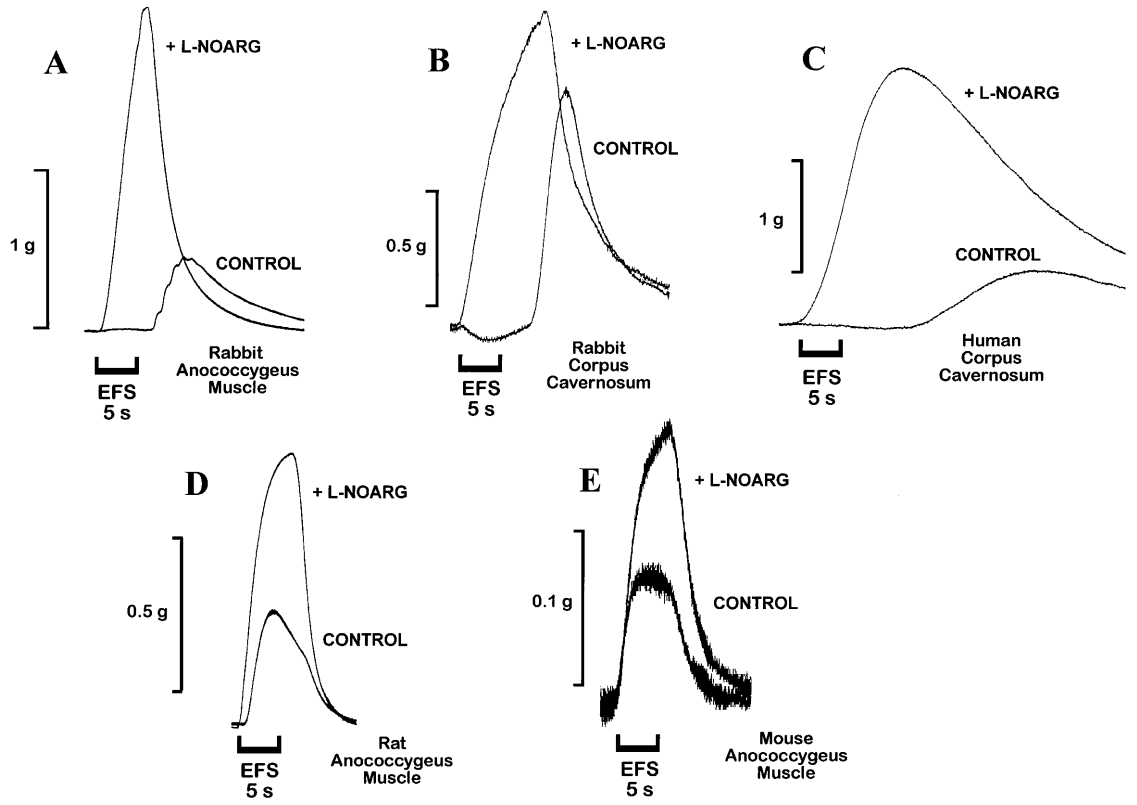

FIG. 1. Mechanical responses obtained from the rabbit anococcygeus muscle (*A*), rabbit corpus cavernosum (*B*), human corpus cavernosum (*C*), rat anococcygeus muscle (*D*), and mouse anococcygeus muscle (*E*) to EFS (30–50 V, 0.3-ms pulse duration, 5 Hz, for 5 s) in the absence and presence of L-NOARG (100 μM) as indicated. The mechanograms are original recordings of the responses and are representative of all experiments in each series ($n = 4$ for each).

laboratories in ice-cold modified Krebs' solution in less than 3 hr. On arrival, they were transferred into modified Krebs' solution at room temperature and cleaned of adherent tissue and blood. Four to eight strips (3 × 13 mm) were cut from the middle part of each corpus cavernosum.

Mounting the Tissues and Recording the Mechanical Responses. Each preparation was cleaned of adherent tissues and mounted horizontally between two ring electrodes (4 mm diameter) located at a distance of 10 mm and 26 mm from the outlet of a 1-ml plastic double-jacketed tissue chamber (37°C). The chambers were perfused with modified Krebs' solution at a constant flow of 1.0 ml/min by means of peristaltic pumps (Miniplus 2, Gilson). One end of the preparation was tied to a Grass FT 03C force-displacement transducer connected to a Linearcorder WR 3101 (Graphtec, Tokyo, Japan) for registration of isometric changes in tension. The preparations were stretched until they reached approximately the *in situ* length (0.2–0.4 g in rabbit and rat anococcygeus muscle; 0.4–1 g in rabbit corpus cavernosum; 0.1–0.3 g in mouse anococcygeus, and 0.5–1 g in human corpus cavernosum) and allowed to equilibrate for 90 min. The preparations were stimulated electrically for 5 s-10 min with trains of rectangular pulses of 50 V, 0.3-ms pulse duration and frequencies ranging from 0.2 to 40 Hz, delivered by Grass S88 stimulators. Previous studies in the rabbit tissues (11) have shown that 5 Hz is the optimal frequency for the nitrergic response in these preparations, and this was used unless otherwise stated. In some experiments, the recorder was run at a speed of 50 mm/min to record the mechanical responses in more detail. The mechanical responses were also recorded on a computer by a specialized data acquisition system (Axon Instruments, Foster City, CA).

Drugs were applied either to the medium reservoir or directly into the chamber at a rate of 50 μl in 30 s with a Hamilton syringe (Hamilton). Scopolamine (10 μM) was added to the perfusion medium to eliminate cholinergic neurotransmission. It caused enhancement of contractile responses in rabbit anococcygeus muscle and rabbit corpus cavernosum, as has been shown previously (11), and experiments were carried out after the preparations had stabilized for least 30 min after its administration. In experiments in which the tone of the tissue was increased with histamine (0.5 μM), the tissues were allowed to stabilize at the elevated tone for 20 min. The concentration-response studies with N^G-nitro-L-arginine (L-NOARG), oxyhemoglobin, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), zaprinast, and cocaine were performed by cumulative addition of the drug into the medium reservoir, and the tissues were allowed to incubate with each concentration for at least 30 min. In experiments in which contractions were induced with exogenous noradrenaline in the rabbit and rat anococcygeus muscles and human corpus cavernosum, guanethidine (10 μM) was added into the medium reservoir to block nerve-induced release of noradrenaline, and the tissues were allowed to incubate for at least 30 min with guanethidine.

Chemicals. Cocaine hydrochloride, guanethidine monosulfate, histamine hydrochloride, noradrenaline hydrochloride, L-NOARG, scopolamine hydrochloride, and zaprinast were all from Sigma. Oxyhemoglobin was obtained from Wellcome, and ODQ (code NNC07–9008) was obtained from Novo-Nordisk, Copenhagen.

Statistical Analysis and Presentation of the Results. The effect of drugs on EFS-induced contraction was expressed as the percentage of the height of contraction of the same tissue under control conditions. The area under the curve was measured for the contractions induced by exogenous noradrenaline. Results are expressed as mean ± standard error of the mean. Statistical analysis was performed using Student's *t* test. A probability value (*P*) less than 0.05 was considered as

statistically significant. *n* denotes the number of experiments using rabbit, rat, and mouse tissues. Due to low availability of the material, in experiments with human tissues *n* denotes the number of strips obtained from four patients.

RESULTS

Experiments with Rabbit Anococcygeus Muscle, and Human and Rabbit Corpus Cavernosum. *Characterization of EFS-induced contractions.* EFS (50 V, 0.3-ms pulse duration, 5 Hz, for 5 s to 3 min, every 120 s) elicited reproducible contractions of the tissues. In all three tissues, the contraction was never observed during the period of EFS but only occurred as an aftereffect with a lag period (time between the end of the EFS and beginning of the contraction) of 1–5 s, (2.11 ± 0.11 s for rabbit anococcygeus, 1.04 ± 0.04 s for rabbit corpus cavernosum, 5.83 ± 0.28 s for human corpus cavernosum; n = 4–10, Fig. 1). When the EFS was applied for 10 min, the contraction started only after termination of the stimulation (not shown). After treatment with the inhibitor of NO synthase, L-NOARG (100 μM), the contractile response started on initiation of EFS and was greater in magnitude and longer in duration (Fig. 1). L-NOARG (1–500 μM) caused concentration-dependent enhancement of EFS-induced contractions in all three tissues. After treatment with 300 μM L-NOARG, the contractions were 530.0 ± 150.0%, 361.0 ± 94.0%, and 190.1 ± 18.8% of the control in the rabbit anococcygeus muscle, human and rabbit corpus cavernosum, respectively (n = 4 for each). The EC_{50} values of L-NOARG for enhancement of the magnitude of the contractions of the three tissues were 50.0 ± 19.5 μM, 46.3 ± 17.4 μM, and 119.5 ± 20.8 μM (n = 4 for each), respectively. L-NOARG (300 μM) did not affect the basal tone of the tissues (not shown).

Effect of oxyhemoglobin and ODQ on EFS-induced contractions. In the presence of oxyhemoglobin (15 μM) the contractile response started at the initiation of the period of electrical stimulation and was greater in magnitude than in its absence (190.8 ± 1.3% and 130.0 ± 8.7% of control in the rabbit anococcygeus muscle and rabbit corpus cavernosum, respectively, n = 4 for each). Oxyhemoglobin (1–15 μM) enhanced EFS-induced contractions in a concentration-dependent manner, with EC_{50} values of 5.9 ± 0.9 μM and 9.9 ± 1.3 μM for rabbit anococcygeus muscle and corpus cavernosum, respectively (n = 4 for each).

ODQ (1 μM) also led in all three tissues to an early onset of the contractile response, which was also greater in magnitude (Fig. 2). The effect of ODQ (0.001–10 μM) was concentration-dependent; its EC_{50} values were 0.09 ± 0.01 μM, 1.9 ± 0.6 μM, and 0.89 ± 0.4 μM (n = 4 for each) for the rabbit anococcygeus muscle and human and rabbit corpus cavernosum, respectively. In the presence of 1 μM ODQ, EFS-induced contractions were 301.5 ± 82.9%, 199.0 ± 35.9%, and 124.3 ± 4.3% of control in the rabbit anococcygeus muscle and human and rabbit corpus cavernosum, respectively (n = 4 for each).

Effect of zaprinast on EFS-induced contractions. After treatment of the tissues with the cGMP phosphodiesterase inhibitor zaprinast (0.01–10 μM), the lag period was longer than in control tissues (486.0 ± 78.6% of control in rabbit anococcygeus muscle after 3 μM zaprinast). The magnitude of contraction was smaller and its duration was shorter in the presence of zaprinast (magnitude 20.5 ± 3.7%, 35.7 ± 4.3%, and 47.1 ± 6.6% of control in rabbit anococcygeus muscle and human and rabbit corpus cavernosum, respectively in the presence of 3 μM zaprinast, n = 4 for each; Fig. 2). The inhibitory effect of zaprinast on EFS-induced contractions was concentration-dependent; its IC_{50} values for rabbit anococcy-

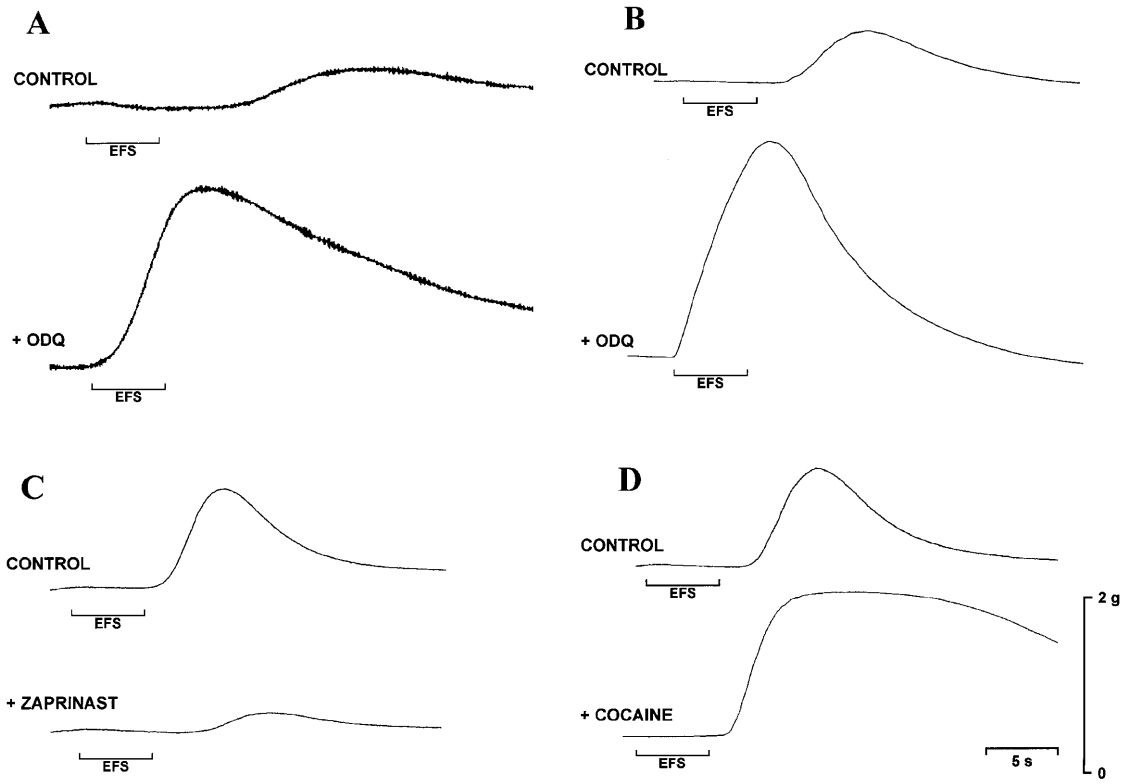

FIG. 2. (*A*) Mechanical responses obtained from the human corpus cavernosum to EFS in the absence (CONTROL) and presence (+ODQ) of 3 μM ODQ (n = 4). (*B*) Responses obtained from the rabbit anococcygeus muscle to EFS in the absence (CONTROL) and presence (+ODQ) of 1 μM ODQ (n = 6). (*C*) Responses obtained from the rabbit anococcygeus muscle to EFS in the absence (CONTROL) and presence (+ZAPRINAST) of 3 μM zaprinast (n = 6). (*D*) Responses obtained from the rabbit anococcygeus muscle to EFS in the absence (CONTROL) and presence (+COCAINE) of 1 μM cocaine (n = 6). EFS (50 V, 0.3-ms pulse duration, 5 Hz, for 5 s) is indicated by the horizontal bar. The mechanograms are original recordings of the responses and are representative of all experiments in each series.

geus muscle, and human and rabbit corpus cavernosum were 1.0 ± 0.2 μM, 1.88 ± 0.21 μM, and 4.5 ± 1.7 μM (n = 4–6 for each), respectively.

Effect of an inhibitor of noradrenaline uptake on EFS-induced contractions of the rabbit anococcygeus muscle. In the presence of cocaine (1 μM), the lag period was not changed (1.98 ± 0.32 s; $P > 0.05$ vs control; n = 4) in the rabbit anococcygeus muscle but the magnitude and duration of contraction were increased (Fig. 2). This effect of cocaine on EFS-induced contractions was concentration-dependent (EC_{50}= 0.6 ± 0.1 μM; n = 6). In the presence of 1 μM cocaine, the contraction was 211.0 ± 27.1% of the control (n = 6).

Effect of EFS on the tissues with an elevated tone. When the tone of the rabbit anococcygeus muscle or human corpus cavernosum was increased with histamine (0.5 μM), relaxation of the tissue occurred during EFS (50 V, 5 Hz, 0.3 ms pulse duration, for 5 s-10 min), followed by a contraction on termination of the stimulus (Fig. 3). When the period of EFS was increased to 10 min a relaxation response was observed that lasted during the entire stimulation period and was followed by a contractile response, which occurred only after termination of the stimulus (Fig. 3). In the presence of L-NOARG (100 μM), the tone of the tissue was elevated, the relaxation was abolished, and the contractile response started at the onset of stimulation and was greater in magnitude and longer in duration (Fig. 3).

When the rabbit anococcygeus muscle was stimulated with frequencies that are optimum for noradrenergic responses in this preparation (10–25 Hz), a relaxation response was always observed during EFS. This was followed by a contraction after cessation of the stimulation (not shown; n = 4).

The effect of EFS on noradrenaline-induced contractions in guanethidine-treated rabbit anococcygeus muscle and human corpus cavernosum. After treatment with guanethidine (10 μM), infusion of exogenous noradrenaline (0.03 μM–300 μM) induced a concentration-dependent contraction of the tissues. When the tissues were stimulated with EFS (50 V, 5 Hz, 0.3-ms pulse duration) during these contractions, the magnitude and duration of contractions were decreased significantly, and this effect of EFS was completely reversed by treatment with L-NOARG (300 μM; Figs. 4 and 5). EC_{50} values for noradrenaline-induced contractions in control conditions, during EFS and during EFS after L-NOARG treatment are given in Table 1.

Experiments with Rat and Mouse Anococcygeus Muscles. EFS (30–50 V, 1–10 Hz, 0.3-ms pulse duration, for 5–20 s, every 120 s) of the rat and mouse anococcygeus muscles produced a contraction that started immediately with the stimulation (Fig. 1). Treatment of the tissues with L-NOARG (100 μM) enhanced the magnitude of contraction (Fig. 1).

In the presence of guanethidine, administration of exogenous noradrenaline (0.1–100 μM) caused a concentration-dependent contraction of the rat anococcygeus muscle. The magnitude of contractions to 0.1 and 1 μM noradrenaline was significantly decreased when EFS was applied at the same time as noradrenaline infusion (Table 2). However, the responses to the higher concentrations (10–100 μM) of noradrenaline were not affected by EFS (Tables 1 and 2). The effect of EFS on the

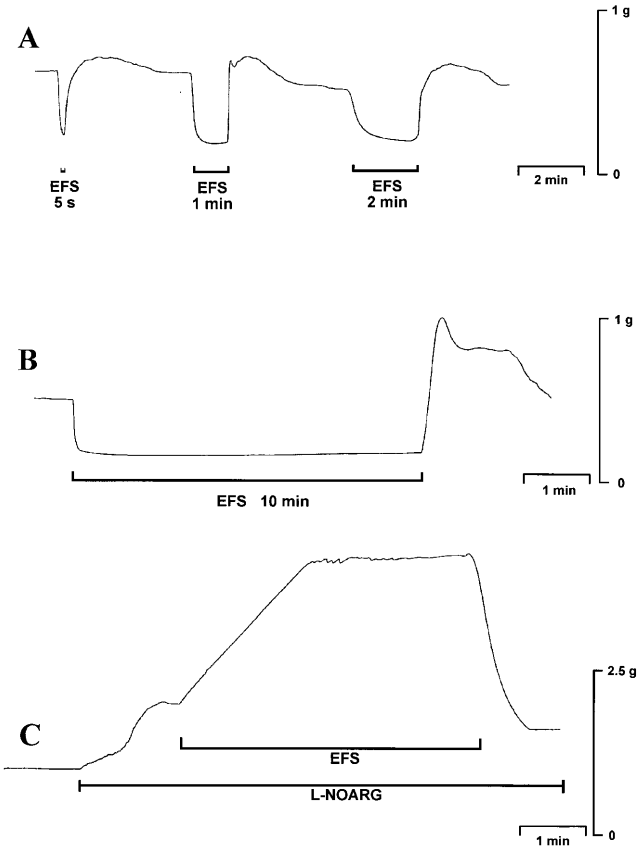

FIG. 3. Biphasic responses of the rabbit anococcygeus muscle elicited by EFS (50 V, 0.3-ms pulse duration, 5 Hz) for 5 s, 1 min, 2 min (A), and 10 min (B) after treatment with histamine (0.5 μM) in control conditions and for 10 min in the presence (C) of L-NOARG (100 μM). EFS and L-NOARG are indicated by separate horizontal bars. The mechanogram is an original recording of the responses and is representative of all experiments in this series (n = 8).

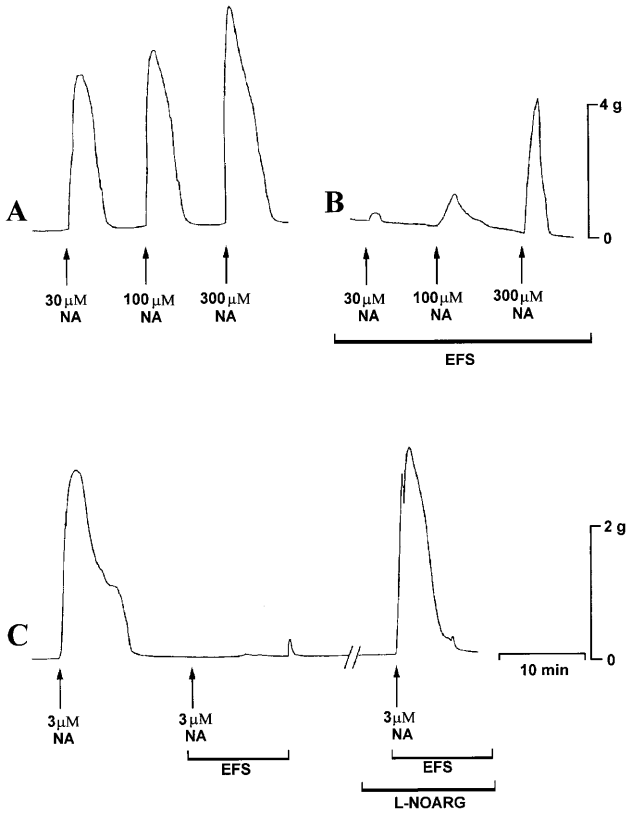

FIG. 4. Concentration-dependent contraction of the rabbit anococcygeus muscle induced by noradrenaline infusion into the perfusion fluid in control conditions (A) and in the presence of EFS (B). (C) The contraction to a single concentration of noradrenaline alone, with EFS, and with EFS and L-NOARG (300 μM). Noradrenaline infusion is shown by arrows. EFS and L-NOARG are shown by separate horizontal bars. The mechanogram is an original recording of the responses of one preparation and is representative of all experiments in this series (n = 4).

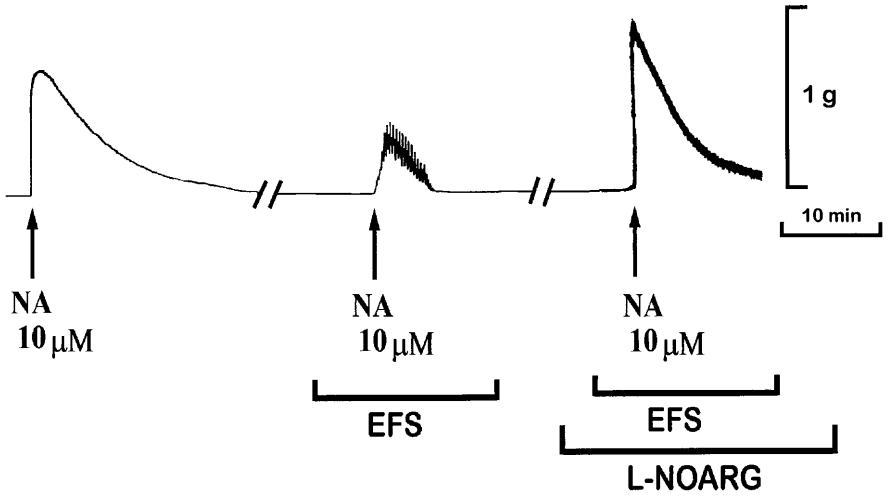

FIG. 5. Contraction of the human corpus cavernosum induced by infusion of noradrenaline (10 μM) into the perfusion fluid under control conditions, in the presence of EFS, and in the presence of EFS and L-NOARG (300 μM) as indicated. Noradrenaline infusion is shown by arrows. EFS and L-NOARG are shown by separate horizontal bars. The mechanogram is an original recording of the responses of one preparation and is representative of all experiments in this series ($n = 4$).

contractions induced with 0.1 and 1 μM of noradrenaline was reversed after treatment of the tissues with L-NOARG (300 μM; Table 2). EC_{50} values for the rat anococcygeus muscle under control conditions, during EFS, and during EFS in the presence of L-NOARG are given in Table 1.

DISCUSSION

EFS of the rabbit anococcygeus muscle and human and rabbit corpus cavernosum did not induce any response during the stimulation period (up to 10 min) but led to a contraction that began 0.1–0.3 s after termination of the stimulation. Administration of L-NOARG or a specific inhibitor of the soluble guanylate cyclase, ODQ (9, 14) or a scavenger of NO, oxyhaemoglobin resulted in the EFS-induced contraction beginning immediately after initiation of the stimulus and increased the magnitude and duration of the contraction. In contrast, in the presence of zaprinast, a specific inhibitor of cGMP-phosphodiesterase (15), the lag period between stimulation and contraction was increased, and the contraction was smaller in magnitude and shorter in duration. Thus, when nitrergic transmission was blocked prejunctionally by L-NOARG, at the nerve junction by oxyhemoglobin, or postjunctionally by ODQ, the noradrenergic response occurred during EFS, with no lag period and was increased. Conversely, when the nitrergic transmission was enhanced postjunctionally by inhibiting the breakdown of cGMP with zaprinast, the contractile response was delayed and reduced. These results indicate that the lack of contractile response during the stimulation period is due to release of NO (11), which also modulates the magnitude and the duration of the eventual contraction (13).

When the tone of the tissue was elevated with histamine, a relaxation was observed during the period of stimulation, followed by a contraction, which again started only after termination of the stimulus. Experiments in which the stimulation period was increased from the usual 5 s to 1, 5, or 10 min showed that relaxation was always observed during stimulation and that contraction never occurred before termination of EFS. Treatment of these preparations with L-NOARG resulted in abolition of the relaxation and in a contraction that began immediately after initiation of the EFS. These experiments not only revealed the biological action of NO during the period of stimulation but also showed that during EFS there is a co-release of NO and noradrenaline. In this situation the net biological response is always nitrergic rather than noradrenergic in nature, suggesting that noradrenergic transmission is not just modulated but is actually controlled by the neuronal release of NO.

To investigate the effectiveness of the nitrergic control of noradrenergic responses we used cocaine, which increases the concentration of available noradrenaline at the nerve junction (16). Cocaine enhanced, in a concentration-dependent manner, the contractions of the rabbit anococcygeus muscle, but even at its highest effective concentration it did not enable the contraction to occur before termination of the stimulus. This result, which suggests that NO is able to counteract the response of even the increased concentrations of noradrenaline achieved at the junction after cocaine, led us to investigate the effect of neurally induced release of NO on the responses

Table 1. EC_{50} values for noradrenaline-induced contractions

	EC_{50}, μM		
Conditions	Rabbit anococcygeus muscle	Human corpus cavernosum	Rat anococcygeus muscle
Control	1.8 ± 0.3	56.1 ± 6.2	19.4 ± 1.9
During EFS	>300*	>300*	20.8 ± 1.2
During EFS +L-NOARG	1.7 ± 0.3	48.3 ± 2.7	19.6 ± 1.0

EC_{50} values for noradrenaline-induced contractions under control conditions, during EFS, and during EFS after L-NOARG (300 μM) treatment in tissues treated with guanethidine (10 μM).
*$P < 0.001$ vs. control value; $n = 4$ for each. Where values are >300 μM, the EC_{50} was considered to be 300 μM for statistical comparison.

Table 2. The magnitude of contractions elicited by exogenous noradrenaline in the rat anococcygeus muscle

Noradrenaline, μM	Control	During EFS	During EFS +L-NOARG
0.1	1.4 ± 0.2	0.7 ± 0.2*	1.8 ± 0.3
1	9.3 ± 1.8	4.2 ± 0.8*	8.8 ± 1.7
10	35.9 ± 2.8	32.5 ± 2.7	38.3 ± 3.9
100	94.8 ± 3.4	90.3 ± 3.3	99.3 ± 0.7

The magnitude of contractions (shown as % of maximum contraction) elicited by exogenous noradrenaline (0.1–100 μM) in the rat anococcygeus muscle treated with guanethidine (10 μM) under control conditions, during EFS, and during EFS following L-NOARG (300 μM).
*$P < 0.05$ vs. control value; $n = 8$.

to exogenous noradrenaline. After blockade of neuronal noradrenaline release with guanethidine, the rabbit anococcygeus muscle and human corpus cavernosum were contracted with pharmacological concentrations of exogenous noradrenaline. Under these conditions, electrical stimulation of the tissue resulted in a significant depression of the elicited contractions, which could be reversed by treatment with L-NOARG. These experiments clearly show that neuronal NO has a potent down-regulatory effect even on very high pharmacological concentrations of the sympathetic transmitter.

In the rat and mouse anococcygeus muscle, nitrergic transmission has a modulatory action on the noradrenergic system, because inhibitors of the NO synthase are known to enhance EFS-induced contractions. However, we found that in these species nitrergic transmission was not as effective as in the rabbit and human tissues against noradrenergic transmission, because the noradrenergic contractile response occurred during EFS without a lag period and, in addition, nitrergic stimulation of the tissues did not affect contractile responses elicited by high concentrations of exogenous noradrenaline. This discrepancy between human/rabbit and mouse/rat may be due to the fact that rabbit tissues have less noradrenergic innervation than rat, as has been shown previously (17), and the same may be the case in the human tissues. Whichever the explanation, our results show that the rabbit resembles the human more than the rat or mouse in relation to the interaction between nitrergic and noradrenergic systems.

The way in which nitrergic transmission controls noradrenergic responses seems to be through a postjunctional physiological antagonism between the two mediators, because EFS in human and rabbit preparations treated with guanethidine is effective in reducing the responses to exogenous noradrenaline. In addition, EFS-induced contractions are enhanced by ODQ. Moreover, noradrenaline release is not affected by NO synthase inhibitors in the rabbit (unpublished observations) and rat anococcygeus muscle (18–20). The mechanisms involved in this postjunctional interaction certainly deserve further investigation. In addition, it is worth investigating whether an interaction between the two systems at a prejunctional level also occurs, as has been suggested in other preparations (21).

When the tone of the rabbit anococcygeus muscle was raised with histamine, L-NOARG caused a further increase in tone (see Fig. 3). This finding shows that there is a basal release of NO. In our previous study (11), we showed that the raised basal tone of the tissue could be further elevated by treatment with L-NOARG and that the basal release of NO was also inhibited by *N*-iminoethyl-L-ornithine and tetrodotoxin. This was further supported by the findings of a reduction in basal cGMP concentrations and a further elevation of the elevated tone by ODQ (9). Thus NO is released constantly from the nerve endings and maintains a sustained relaxant tone in the smooth muscle. This is in agreement with the suggestion that smooth muscles innervated by nitrergic nerves have a constant low tone as a result of continuous release of NO (22, 23), akin to that maintained in the vasculature by NO released from the vascular endothelium (24, 25) and from perivascular nitrergic nerves (26). The determinants of this continuous basal release of NO and its interaction with noradrenergic responses also remain to be studied.

We therefore conclude that in some organs in which sympathetic innervation is accompanied by nitrergic innervation, the latter dominates and the net biological response during EFS is nitrergic in nature. We have recently shown that a similar nitrergic control occurs in the opossum lower esophageal sphincter in which excitatory innervation is parasympathetic (unpublished work). Therefore nitrergic control of excitatory innervation may be a widespread phenomenon not only restricted to the sympathetic system. If it also occurs *in vivo*, our observations will be significant for the understanding of neuronal control of gastrointestinal, airway, genitourinary, and vascular systems. Of particular interest is the regulation of blood pressure, where evidence suggests that, at least in some vascular beds, NO released from nitrergic nerves, in addition to that from the vascular endothelium, plays a regulatory role (27). Our findings may be relevant to the understanding of some pathophysiological conditions such as diabetic impotence (28, 29) and hypertension (30, 31) in which an impairment in the L-arginine:NO pathway is accompanied by an enhanced noradrenergic response.

We are grateful to Dr. M. Royle from Sussex Nuffield Hospital for supplying the human tissue. We thank Ms. Annie Higgs for her help in preparing this manuscript. Some of these results were presented at the Winter Meeting of The British Pharmacological Society, Brighton, United Kingdom, December 18–20, 1996.

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