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# Endothelin-1 affects capsaicin-evoked release of neuropeptides from rat vas deferens

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**Abstract**

Capsaicin-sensitive neurones release a number of neuropeptides, such as substance P, neurokinin A, somatostatin and calcitonin gene-related peptide (CGRP), which exert a number of effects on smooth muscle tissues. Endothelin-1 was thought to potentiate the capsaicin-evoked release of neuropeptides from sensory neurones of the rat. We have investigated the neuromodulatory effects of endothelin-1 on capsaicin-induced release of neurotransmitters from rat vas deferens. Capsaicin and human α calcitonin gene-related peptide (human αCGRP) reduced the rat vas deferens twitch responses induced by electrical field stimulation. Human β calcitonin gene-related peptide-(8–37) [human βCGRP-(8–37)] (1 μM), a selective αCGRP receptor antagonist, antagonized the inhibitory effects of both drugs. Endothelin-1 concentration dependently evoked an increase in basal tone of the musculature and potentiated the amplitude of the electrically stimulated responses, blocking inhibitory effects of capsaicin but not of human αCGRP. Moreover, endothelin-1 did not markedly change the inhibitory effects of papaverine (0.1–100 μM) or isoprenaline (1 nM–100 μM) on responses to electrical field stimulation. FR 139317 [(*N*,*N*-hexamethylene) carbamoyl-Leu-D-Trp(*N*-Me)-D-2-Pya], a selective endothelin $ET_A$ receptor antagonist, administered 30 min before endothelin-1 restored the capsaicin effects whereas BQ 788 [Dmpc-γ-MeLeu-D-Trp-(1-methoxycarbonyl)-D-Nle], a selective endothelin $ET_B$ receptor antagonist, was completely ineffective. The endothelin-1-induced block of the capsaicin effect was resistant to tetrodotoxin (1 μM) and 30-min pre-treatment with MEN 10.627 (cyclo[(Met-Asp-Trp-Phe-Dap-Leu) cyclo (2β–5β)]), a selective tachykinin $NK_2$ receptor antagonist, did not abolish the endothelin-1 effect on the inhibitory response to capsaicin. These results suggest that endothelin-1 selectively inhibits the capsaicin-induced release of neurotransmitters from rat vas deferens and these effects are mediated via endothelin $ET_A$ receptors but not by tachykinin release. 

*Keywords:* Endothelin-1; Vas deferens, rat; Capsaicin; Sensory neurone; Neuropeptide release

## 1. Introduction

Peptides of the endothelin family exert potent contractile activity on various vascular (Yanagisawa et al., 1988) and non-vascular (Filep et al., 1991) smooth muscles. Endothelins are also believed to modulate neuroeffector transmission, through interaction at specific endothelin receptors distributed in peripheral tissues (Power et al., 1989) and in the central nervous system (Jones et al., 1989; D'Amico et al., 1995). It has been demonstrated that, in rat vas deferens, endothelin-1 affects not only muscle tone but also the twitch-like contractile responses induced by electrical stimulation (Wiklund et al., 1990). It is well known that the electrically induced responses of isolated smooth muscle preparations are due to the release of neurotransmitters through efferent nerves. In particular, the co-release of noradrenaline and ATP is responsible for electrical field stimulation-induced contractions in rat vas deferens (Major et al., 1989) and endothelin-1 potentiates the postjunctional effects of ATP acting at $P_{2x}$-purinoceptors. Moreover, it has been demonstrated that in vitro capsaicin reduces the twitch response induced in rat vas deferens by transmural nerve stimulation through the release of endogenous calcitonin gene-related peptide (CGRP) from primary sensory neurones (Saito et al., 1987). Capsaicin, which is the pungent ingredient in a wide

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variety of red peppers of the genus *Capsicum*, selectively stimulates thin afferent neurones i.e., C- and probably Aδ-neurones (Holzer, 1991). These sub-populations of nociceptive nerve fibres, excited by capsaicin or other stimuli, transmit information to the central nervous system, and are capable of releasing peptide mediators from peripheral or central endings as well; such an activity is known as the 'local effector function' of these neurones. Because of its specific action, capsaicin has been widely used as a pharmacological tool to investigate the 'efferent' function of primary afferent neurones (Maggi and Meli, 1988). Substance P, somatostatin, neurokinin A and CGRP are among the identified peptides released by capsaicin from central and peripheral endings of afferent neurones (Holzer, 1988). Recently, endothelin-1 has been thought to increase the capsaicin-evoked peptide release in cultures of rat sensory neurones (Dymshitz and Vasko, 1994). Therefore, the aim of the present investigation was to evaluate the neuromodulatory effect of endothelin-1 on capsaicin-induced release of neurotransmitters from sensory neurones of rat vas deferens.

## 2. Materials and methods

### 2.1. Isolated preparations

Male Wistar rats (280–330 g) were decapitated and the vasa deferentia rapidly removed and bisected transversely into epididymal and prostatic segments. The latter preparations were suspended vertically under 1-g tension in 10-ml organ baths, with built-in platinum stimulating electrodes (0.45 mm thick), containing warm (37°C), oxygenated (5% $CO_2$ in $O_2$) Krebs solution (pH 7.4) of the following composition (mM): NaCl 118, KCl 4.7, $KH_2PO_4$ 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11. Organ baths were siliconised to prevent peptide adsorption to their walls.

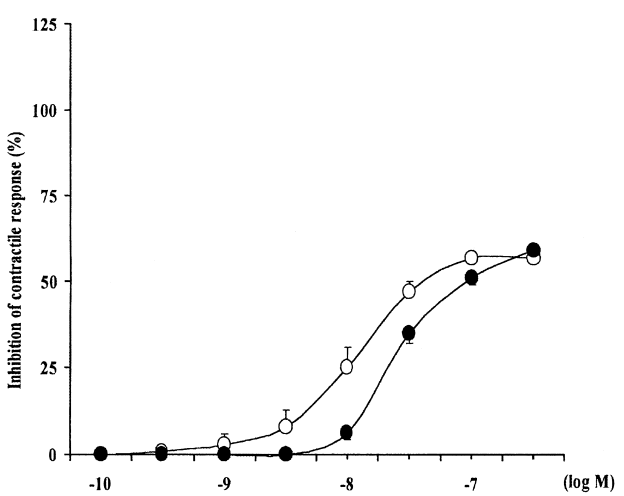


Fig. 1. Concentration–response curves of rat vas deferens for capsaicin (0.1 to 100 nM) alone [(– ○ –) $n = 20$] or in the presence of human βCGRP-(8–37) (1 μM) [(-- ● –) $n = 5$]. Percent inhibition of contractile response induced by electrical field stimulation. Data are expressed as means and vertical lines show S.E.M.

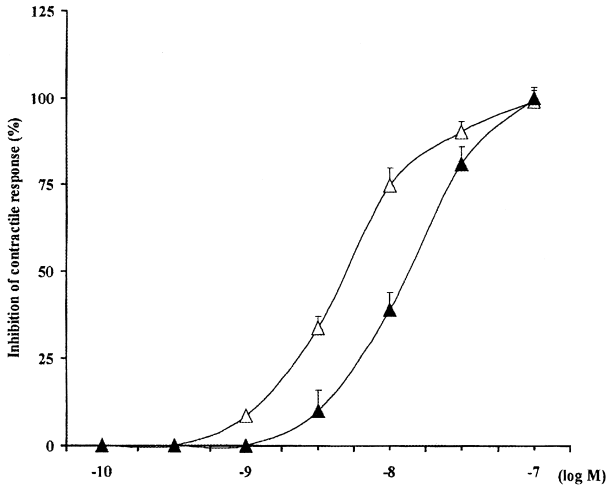


Fig. 2. Concentration–response curves of rat vas deferens for human αCGRP (0.1 to 100 nM) alone [(-Δ-) $n = 15$] or in the presence of human βCGRP-(8–37) (1 μM) [(-▲-) $n = 8$]. Percent inhibition of contractile response induced by electrical field stimulation. Data are expressed as means and vertical lines show S.E.M.

### 2.2. Mechanical activity

Both the pharmacologically induced changes of smooth muscle tone, and electrically stimulated contractile responses were studied. Mechanical activity was recorded with a force-displacement transducer (NARCO F60) connected to a physiograph (LINSEIS 2046).

### 2.3. Electrical field stimulation

Electrical field stimulation (Paton, 1955; Paton and Vizi, 1969; Ventura and Pennefather, 1994) was applied from two parallel platinum electrodes. Square-wave single pulses of 1 ms duration, 60 V submaximal voltage (dial setting value) at 0.1 Hz were applied with a dual impedance stimulator (Hugo Sachs Elektronik 215/II). Tetrodotoxin (1 μM) completely abolished the contractile response to electrical stimulation. To elicit contractions of the rat vas deferens through direct stimulation of the smooth muscles but not through the excitation of nerves, two pulses (50-ms interval) with 33 ms duration and an intensity of 60 V were applied every 10 s in the presence of 1 μM tetrodotoxin according to the modified method of Goto et al. (1987).

### 2.4. Experimental protocol

After the equilibration period (90 min), the tissues were electrically stimulated and cumulative concentration–response curves to capsaicin (0.1 nM to 0.3 μM) and human

αCGRP (0.1–100 μM) were obtained (one concentration–response curve per tissue). Human βCGRP-(8–37), a human αCGRP receptor antagonist (Longmore et al., 1994) (1 μM), was added to the bath 15 min before the administration of both capsaicin and human αCGRP. Human βCGRP-(8–37) 3 μM behaved as an agonist, therefore we used it only at 1 μM concentration. In studies with endothelin-1, the tissues were challenged with single concentrations of this peptide (10, 30 and 100 nM) administered consecutively. Capsaicin activity was determined in the absence and presence of endothelin-1 in order to evaluate the influence of endothelin-1 on capsaicin-evoked effects. When endothelin receptor antagonists were used, the tissues were incubated for 30 min with FR 139317 [(*N*,*N*-hexamethylene) carbamoyl-Leu-D-Trp(*N*-Me)-D-2-Pya], a selective endothelin $ET_A$ receptor antagonist (Battistini et al., 1994) (0.1–10 μM), or BQ 788 [Dmpc-γ-MeLeu-D-Trp-(1-methoxycarbonyl)-D-Nle], a selective endothelin $ET_B$ receptor antagonist (Ishikawa et al., 1994) (0.1–10 μM), prior to the addition of single concentrations of endothelin-1 and the concentration–response curve to capsaicin. In further experiments, preparations were preincubated in the organ bath for 30 min with the cyclooxygenase inhibitor, indomethacin (10 μM), or the tachykinin $NK_2$ selective antagonist, MEN 10.627 (cyclo[(Met-Asp-Trp-Phe-Dap-Leu) cyclo (2β–5β)]) (Patacchini et al., 1994), to evaluate the involvement of arachidonate products and neurokinins on the endothelin-1 effects. Concentration–response curves for papaverine (0.1 to 100 μM) and isoprenaline (1 nM to 100 μM in the presence of phenoxybenzamine 3 nM to avoid interference of $\alpha_1$-adrenoceptor-mediated effects with $\beta_2$-adrenoceptor-mediated activity) were made in the presence and absence of endothelin-1. Additionally, concentration–response curves for capsaicin were made in presence and absence of endothelin-1 (10, 30 and 100 nM) with tetrodotoxin (1 μM)-treated tissues.

### 2.5. Drugs

Endothelin-1, human β calcitonin gene-related peptide-(8–37), human α calcitonin gene-related peptide, BQ 788

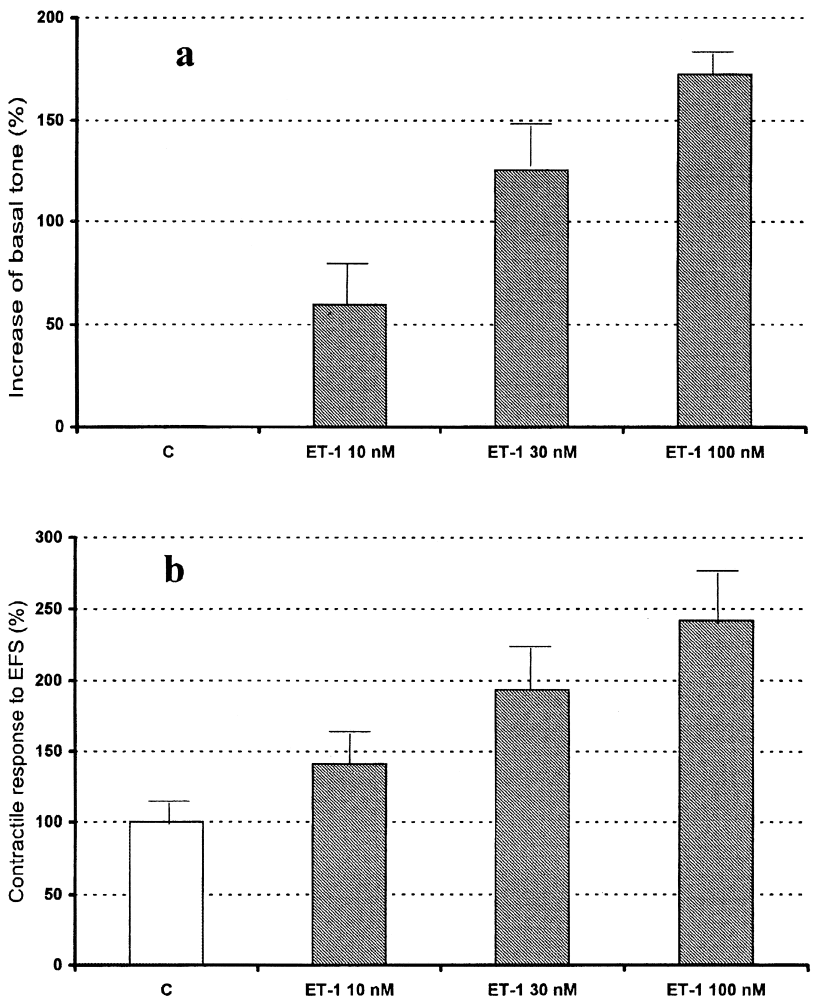


Fig. 3. Effect of endothelin-1 on electrical field stimulation of rat vas deferens; (a) increase of smooth muscle basal tone; (b) amplitude increase of contractile response after electrical field stimulation. Data are expressed as % compared of control values. Vertical lines show S.E.M. ($n = 20$).

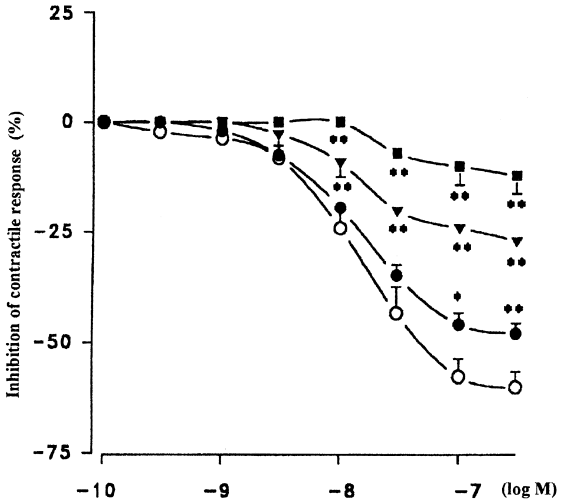


Fig. 4. Concentration–response curves of rat vas deferens for capsaicin (0.1 to 100 nM) alone (– ○ –) and in the presence of endothelin-1 [10 nM (– ● –); 30 nM (-▼-); 100 nM (– ■ –)] on electrical field stimulation-induced twitch response. Percent inhibition of contractile response induced by electrical field stimulation. Data are expressed as means and vertical lines show S.E.M. ($n = 4$ to 6 animals for each curve). $^{*}P < 0.05$; $^{**}P < 0.01$.

[Dmpc-γ-MeLeu-D-Trp-(1-methoxycarbonyl)-D-Nle] and FR 139317 [(*N*,*N*-hexamethylene) carbamoyl-Leu-D-Trp(*N*-Me)-D-2-Pya] were supplied by Neosystem Lab (Strasbourg, France). Capsaicin, phenoxybenzamine hydrochloride, indomethacin, papaverine hydrochloride, isoprenaline hydrochloride, MEN 10.627 (cyclo[(Met-Asp-Trp-Phe-Dap-Leu) cyclo (2β–5β)]) and tetrodotoxin were purchased from Research Biochemicals International, Natick, MA, USA. All peptides and drugs available as hydrochloride or sodium salts were dissolved in distilled water. Stock solutions of capsaicin and phenoxybenzamine were prepared using 95% ethanol.

### *2.6. Data analysis*

Results are means ± S.E.M. Agonist potency was calculated on the basis of data from individual preparations and is expressed as $EC_{50}$ (i.e., agonist concentration needed to produce 50% of the maximal response). $P < 0.05$ was taken to reflect a significant difference (paired and unpaired Student's *t*-test).

## 3. Results

### *3.1. Effects of capsaicin and endothelin-1, administered separately, on electrically stimulated preparations of the rat vas deferens*

Electrical field stimulation with single pulses (1 ms, 60 V, 0.1 Hz), elicited twitch-like contractile responses of the

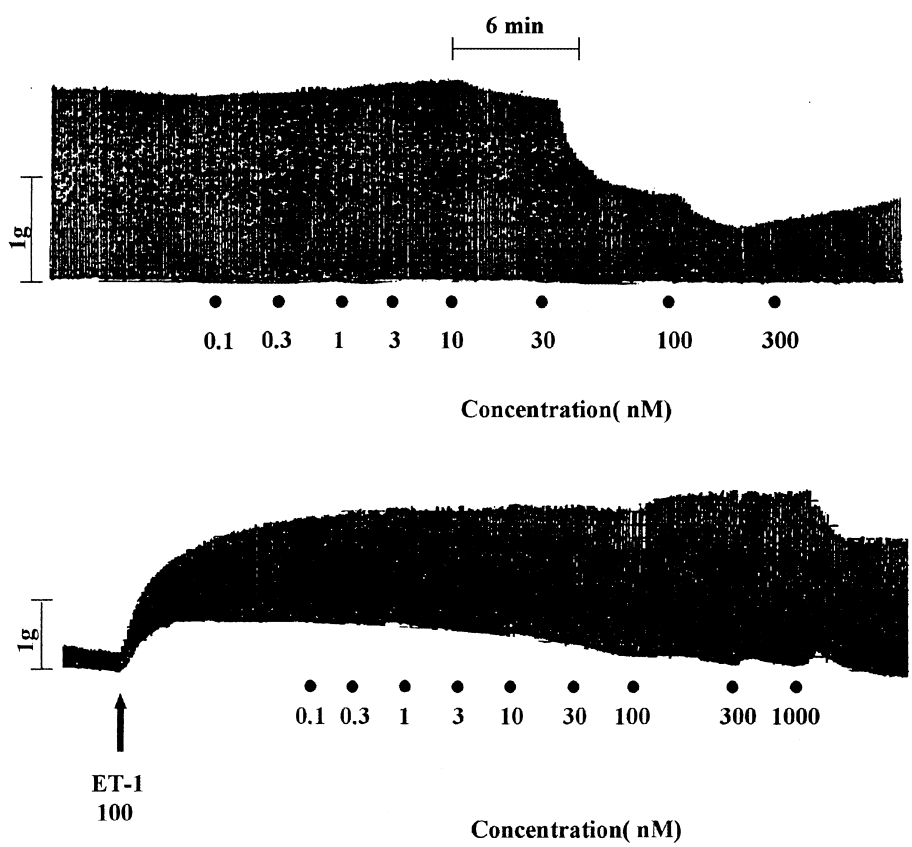


Fig. 5. Typical tracings illustrating the concentration-related responses to capsaicin alone (upper panel) and in the presence of endothelin-1 (ET-1) 100 nM (lower panel) in rat vas deferens.

prostatic portion of the isolated rat vas deferens. Neither the amplitude of electrically stimulated contractions nor the tone of the preparations was greatly changed during the stimulation in the control experiments. Both capsaicin (0.1–300 nM) and human αCGRP (0.1–100 nM) added cumulatively, reduced the amplitude of electrical field stimulation-induced twitches, with maximal inhibition at 100 nM (57 ± 3.9% and 100%, respectively) (Figs. 1 and 2). The inhibitory effect of both drugs reached its maximum within 1–2 min and the contractile tone slowly returned to 80–100% of its original value within 8–15 min. Human βCGRP-(8–37) (1 μM) produced a rightward displacement of the concentration–response curves to human αCGRP and capsaicin without depression of the maximal response obtainable (Figs. 1 and 2). From the dose-ratios obtained in the presence and absence of human βCGRP-(8–37), apparent $pK_B$ values of 5.93 and 5.21 were calculated for human βCGRP-(8–37) against human αCGRP and capsaicin, respectively (Figs. 1 and 2).

Endothelin-1, (10, 30 and 100 nM), elicited a concentration-dependent increase in both muscle tone and in the amplitude of electrical field stimulation-induced twitch responses (Fig. 3). At lower concentrations (10 and 30 nM) the tonic effects of endothelin-1 decreased gradually while the amplitude increase of electrically stimulated contractions persisted. At 100 nM, both tonic and amplitude endothelin-1 effects continued during the observation period (36 to 40 min).

### *3.2. Effects of capsaicin, after administration of endothelin-1 and other drugs, on electrically stimulated preparations of rat vas deferens*

Endothelin-1, concentration dependently and significantly, blocked the capsaicin inhibitory effect on electrical field stimulation-induced contractile responses (Fig. 4). Its effects became evident at 30 nM and were maximal at 100 nM (Fig. 5). Incubation (30 min) with indomethacin

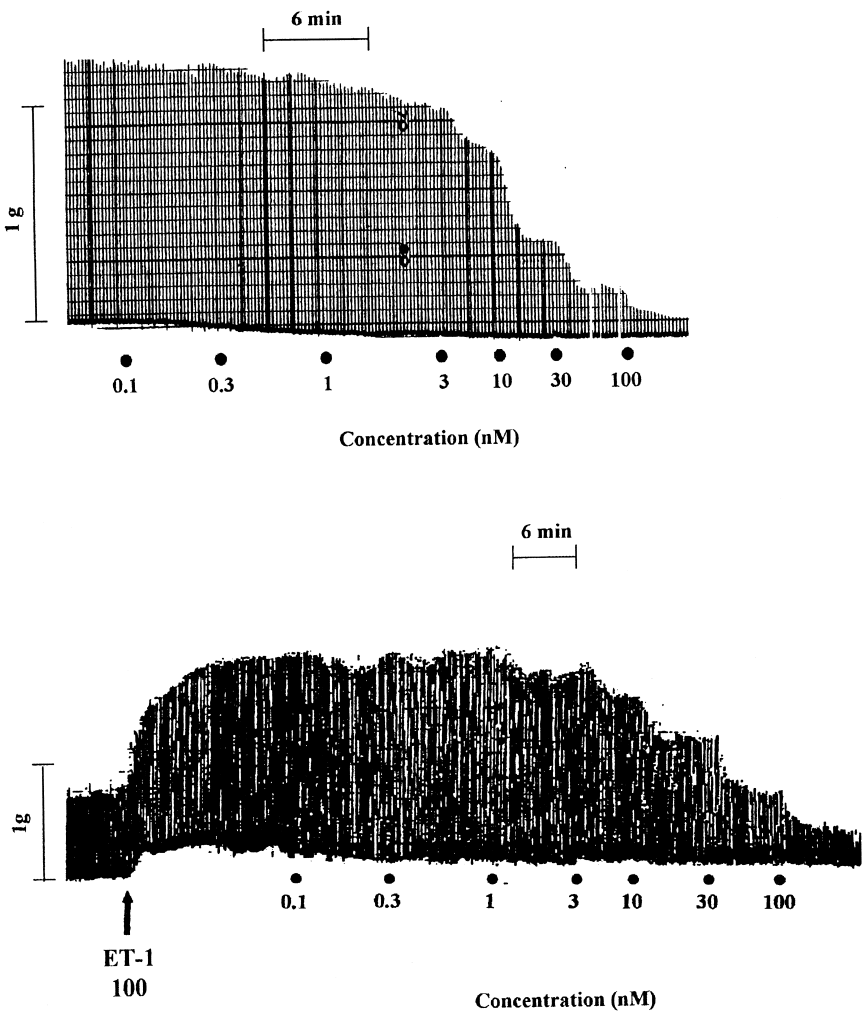


Fig. 6. Typical tracings illustrating the concentration-related responses to human αCGRP alone (upper panel) and in the presence of endothelin-1 (ET-1) 100 nM (lower panel) in rat vas deferens.

(10 μM) did not modify the endothelin-1-dependent block of capsaicin inhibitory effects on electrical field stimulation-induced contractions (data not shown). On the other hand, endothelin-1 (10, 30 and 100 nM) did not produce any change of the inhibitory effects evoked by human αCGRP added cumulatively (0.1–100 nM) (Fig. 6).

FR 139317 [(*N*,*N*-hexamethylene) carbamoyl-Leu-D-Trp(*N*-Me)-D-2-Pya] (0.1 to 10 μM), a selective endothelin $ET_A$ receptor antagonist, added 30 min before 30 nM endothelin-1, was able to restore the capsaicin inhibitory effects only at 10 μM concentration (Fig. 7). When 100 nM endothelin-1 was applied, FR 139317 [(*N*,*N*-hexamethylene) carbamoyl-Leu-D-Trp(*N*-Me)-D-2-Pya] at the highest concentration utilised (10 μM), partially restored the capsaicin effect (Fig. 7). BQ 788 [Dmpc-γ-MeLeu-D-Trp-(1-methoxycarbonyl)-D-Nle] (0.1 to 10 μM), a selective endothelin $ET_B$ receptor antagonist, was completely ineffective (data not shown). In order to find if the endothelin-1 activity was dependent on neurokinin A release from sensory neurones, MEN 10.627 (cyclo[(Met-Asp-Trp-Phe-Dap-Leu) cyclo (2β–5β)]) (0.1 to 10 μM), a selective tachykinin $NK_2$ receptor antagonist, was employed, given that tachykinin $NK_2$ receptors seem to be the main, if not the sole, mediators of the response to tachykinins in the rat vas deferens (Tousignant et al., 1987). However, its application for 30 min before endothelin-1 did not affect the endothelin-1 interference with capsaicin actions (data not shown).

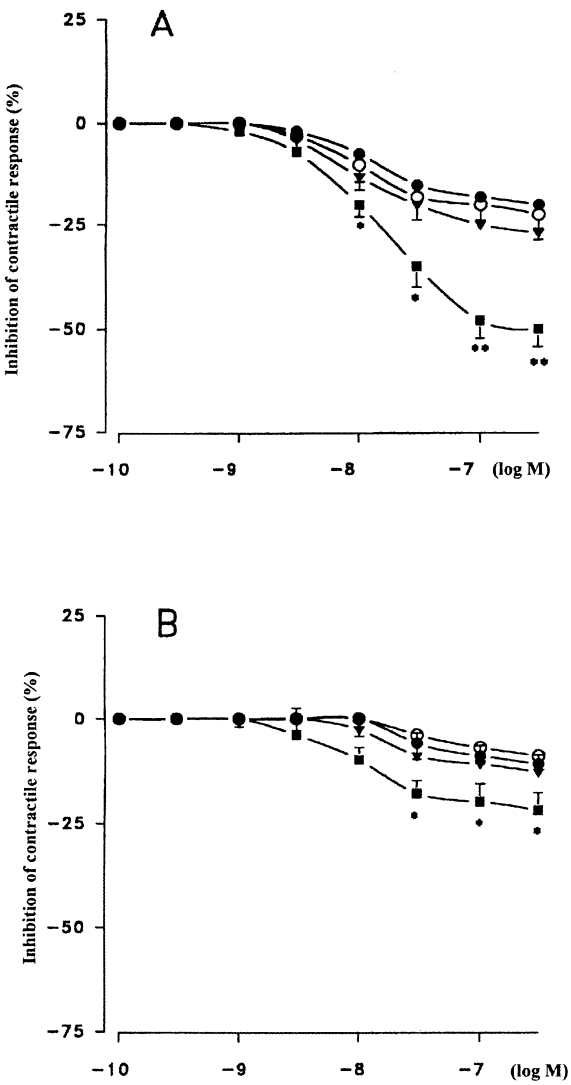


Fig. 7. Concentration–response curves of rat vas deferens for capsaicin in the presence of endothelin-1 (A: 30 nM; B: 100 nM) alone (– ○ –) or after previous incubation with FR 139317 [0.1 μM (– ● –); 1 μM (-▼-); 10 μM (– ■ –)] on electrical field stimulation-induced twitch response. Data are expressed as means and vertical lines show S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$.

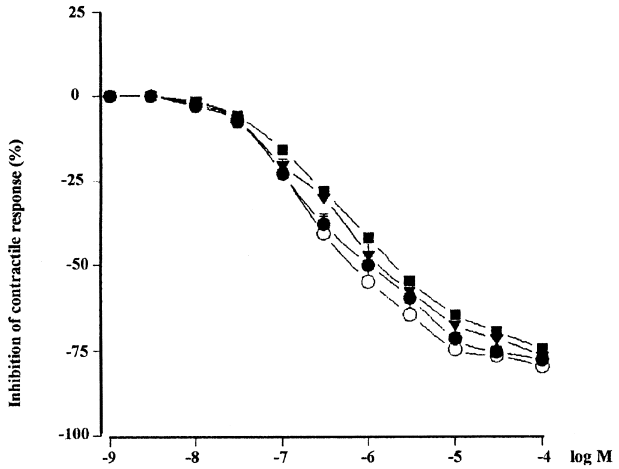


Fig. 8. Concentration–response curves of rat vas deferens for isoprenaline (0.1 to 100 μM) alone (– ○ –) or in the presence of endothelin-1 [10 nM (– ● –); 30 nM (-▼-); 100 nM (– ■ –)], on electrical field stimulation-induced twitch response. Data are expressed as means and vertical lines show S.E.M. ($n = 4$ to 6 animals for each curve).

### *3.3. Effects of isoprenaline and papaverine on electrically stimulated preparations of rat vas deferens in the presence and in the absence of endothelin-1*

Papaverine (0.1 to 100 μM) produced a concentration-related inhibition of the contractile response induced by electrical field stimulation, which was maximal at 100 μM (64 ± 7%). Isoprenaline (1 nM to 100 μM), in the presence of phenoxybenzamine (3 nM), necessary to prevent any $\alpha_1$-adrenoceptor activity, also reduced the twitches induced by electrical field stimulation, with the maximal inhibition at 10 μM (75 ± 6,2%). These effects were not appreciably modified by endothelin-1 added 30 min before either isoprenaline or papaverine (Figs. 8 and 9).

### *3.4. Effects of capsaicin on tetrodotoxin (1 μM) denervated rat vas deferens in the presence and absence of endothelin-1*

In the presence of tetrodotoxin, stimulation of the tissue with a longer electrical current caused a contraction as-

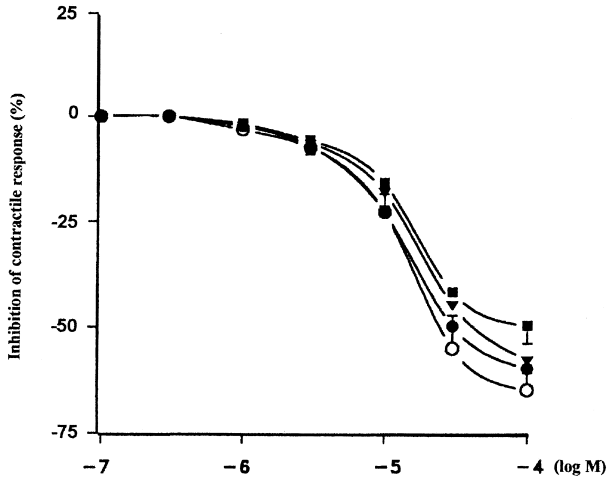


Fig. 9. Concentration–response curves of rat vas deferens for papaverine (1 nM to 100 μM) alone (– ○ –), or in the presence of endothelin-1 [10 nM (– ● –); 30 nM (- ▼ -); 100 nM (– ■ –)], on electrical field stimulation-induced twitch response. Data are expressed as means and vertical lines show S.E.M. ($n = 4$ to 6 animals for each curve).

sumed to be induced by direct stimulation of the smooth muscle cells. Capsaicin concentration dependently inhibited the response.

Endothelin-1 (10, 30 and 100 nM), while it did not evoke any potentiation of contractions induced by smooth muscle electrical stimulation, was able to suppress the capsaicin effect, the 100 nM concentration being the most efficacious (Fig. 10).

## 4. Discussion

In the present study, capsaicin and human αCGRP, in vitro, reduced the electrical field stimulation-induced twitch responses of the rat vas deferens, and endothelin-1 significantly inhibited capsaicin but not the human αCGRP effects. It has been demonstrated that capsaicin exerts its inhibitory effect through the release of endogenous CGRP, a peptide with 37 amino acid residues (Saito et al., 1987), from the sensory neurones of the rat vas deferens. Our findings support this conclusion, given that human βCGRP-(8–37), which acts as a competitive antagonist at αCGRP receptors (Longmore et al., 1994), blocked both capsaicin- and human αCGRP-evoked inhibition of electrical field stimulation-induced contractions of rat vas deferens.

Wiklund et al. (1989a) reported that endothelin-1 exerts stimulatory or inhibitory actions on neuroeffector transmission in a variety of smooth muscle preparations. Indeed, it decreases the nerve-induced release of noradrenaline in guinea pig femoral and pulmonary artery as well as the release of acetylcholine in guinea pig ileum, while it also has a postjunctional stimulant effect in guinea pig pul-

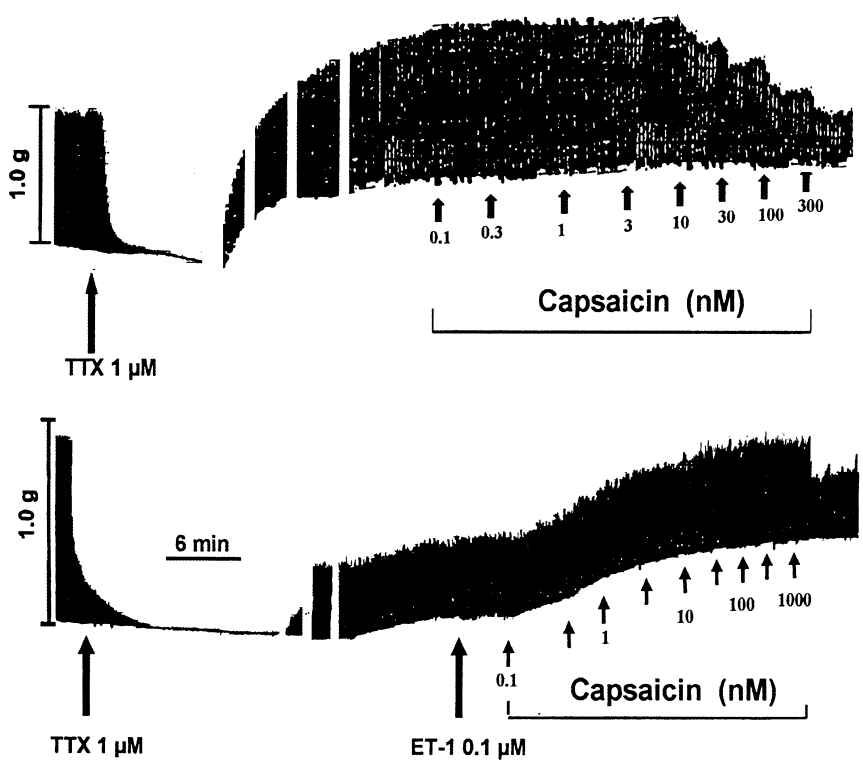


Fig. 10. Typical tracings showing the blocking effect of tetrodotoxin (TTX) (1 μM) on the electrical field stimulation-induced rat vas deferens twitch-like contractions. After tissue exposure to the toxin, electrical field stimulation was switched from single pulse with 1 ms duration to two pulses with 33 ms duration, in order to directly stimulate smooth muscle fibers. Under these conditions, concentration–response curves were obtained with capsaicin alone (upper panel) and in the presence of endothelin-1 (ET-1 0.1 μM).

monary artery and ileum (Wiklund et al., 1989a,b). Endothelin-1 may be involved in the modulation of neurotransmitter/neuropeptide release (Dymshitz and Vasko, 1994); it enhances capsaicin-induced neuropeptide release in cultures of rat sensory neurones (Dymshitz and Vasko, 1994). As reported by Lau et al. (1995), our investigations showed that administration of endothelin-1 concentration dependently enhanced the electrically evoked contractile responses and increased the smooth muscle tone of the electrically stimulated prostatic portion of rat vas deferens, with little or no effect on unstimulated tissue. Our finding was that endothelin-1 concentration dependently blocked the capsaicin inhibitory effect on electrical field stimulation in rat vas deferens. Therefore, we suggest a negative neuromodulatory effect of endothelin-1 on capsaicin-induced release of neuropeptides from sensory neurones in this smooth muscle preparation.

Even though endothelin-1 has been demonstrated to potentiate CGRP release from capsaicin-stimulated cultured rat sensory neurones (Dymshitz and Vasko, 1994), the exact mechanism by which it acts is not known and whether endothelin-1 interacts directly with these nerve fibres is not clear. Nevertheless, endothelin-1-induced prejunctional inhibition of neuropeptide release could be hypothesized to occur in rat vas deferens afferent neurones, as what happens in efferent ones, in agreement with Wiklund et al. (1990). This inhibition may, in turn, negatively influence the capsaicin effect. A possible endothelin-1 interaction at a CGRP postjunctional site of action could be excluded however, given that, in our experiments, endothelin-1 did not modify the human αCGRP-induced inhibition of electrical field stimulation-elicited contractions of rat vas deferens.

We did not investigate which molecular mechanism is involved in the interaction between endothelin-1 and the capsaicin effect. We suggest that one of the processes involved could depend on endothelin-1-induced large conductance $K^+$ channel activation (Kanyicska et al., 1997), leading to capsaicin-sensitive sensory neurone hyporesponsiveness.

The endothelin-1-induced block of the capsaicin effect was antagonised by FR 139317 [(*N*,*N*-hexamethylene) carbamoyl-Leu-D-Trp(*N*-Me)-D-2-Pya], a selective endothelin $ET_A$ receptor antagonist (Battistini et al., 1994), only at 10 μM concentration. Conversely, BQ 788 [Dmpc-γ-MeLeu-D-Trp-(1-methoxycarbonyl)-D-Nle], an agent reported to be a selective endothelin $ET_B$ receptor antagonist (Ishikawa et al., 1994), was completely ineffective, suggesting endothelin $ET_A$-like receptor predominance in rat vas deferens. Moreover, in our experiments MEN 10.627 (cyclo[(Met-Asp-Trp-Phe-Dap-Leu) cyclo (2β–5β)]), a selective tachykinin $NK_2$ receptor antagonist (Patacchini et al., 1994), did not abolish the endothelin-1 effect on capsaicin-induced inhibition of contractile responses of electrically stimulated rat vas deferens. These findings suggest that endothelin-1 does not exert its effect via tachykinin release. Such a conclusion agrees with Saito et al. (1987) who showed that neither substance P-like nor neurokinin-like immunoreactivity was present in the rat vas deferens. In agreement with Wiklund et al. (1990), pretreatment with indomethacin, an inhibitor of cyclooxygenases, also did not modify the endothelin-1 concentration-dependent block of capsaicin inhibitory effects, suggesting that metabolites of arachidonic acid are not critically involved in the mechanism of action of endothelin-1. In addition, in rat vas deferens preparations, the relaxant effects of isoprenaline, an agonist of postjunctional β-adrenoceptors (Lotti et al., 1980; Krstew et al., 1982), and papaverine, a phosphodiesterase activity inhibitor (Taddei et al., 1991), were not greatly changed in the presence of endothelin-1.

These results seem to suggest that endothelin-1 effects in rat vas deferens are due to a specific action on the capsaicin-induced release of neurotransmitters. Together with the data cited, our results suggest that CGRP may be considered the principal neurotransmitter released by thin afferent neurones in rat vas deferens (Tan et al., 1994) and, thus, be the major effector of capsaicin-induced inhibition. Furthermore, in the presence of tetrodotoxin, capsaicin inhibited the contraction induced by direct stimulation of rat vas deferens smooth muscle cells. Endothelin-1 antagonised the capsaicin effect, demonstrating that there was no interference attributable to a neuroeffector system. In conclusion, our results, together with complementary evidence, demonstrated that (1) endothelin-1 significantly reduces the inhibitory effect of capsaicin on electrical field stimulation-induced contractile responses of rat vas deferens; (2) these effects are mediated via the activation of endothelin $ET_A$ receptors; (3) tachykinin and prostanoid release was not involved; (4) endothelin-1 can modulate neuroeffector transmission in rat vas deferens through an inhibitory prejunctional mechanism. However, it remains uncertain which mechanisms are really involved in the alteration of the capsaicin effect in the presence of endothelin-1. Therefore, further investigations are required to clarify these mechanisms and their pharmacological significance.

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