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Opposite modulation by tachykinin (NK_1) and CGRP receptors of sympathetic control of mouse vas deferens motility

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Abstract

Electrical field stimulation of isolated mouse vas deferens elicited sympathetic twitch whose amplitude was transiently enhanced by the selective tachykinin NK_1 receptor agonist, [Sar^9,Met(O_2)^{11}]substance P (0.3–30 nM), but not by selective NK_2 and NK_3 receptor agonists. Potentiation by [Sar^9,Met(O_2)^{11}]substance P was antagonized by (±)-CP 96,345 [(2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine] (IC_{50} = 0.1 μM). On the other hand, electrical field stimulation-induced contractions were inhibited by calcitonin gene-related peptide, CGRP (0.1–30 nM), and this action was reduced by its antagonist, human CGRP-(8–37) (3 μM). [Sar^9,Met(O_2)^{11}]substance P (3 nM) did not affect either high-K^+ or noradrenaline-induced contraction, while CGRP (3 nM) significantly reduced the noradrenaline-induced motor response. Capsaicin (1 μM) inhibited sympathetic twitches, and this effect was partially antagonized by human CGRP-(8–37). In the presence of this antagonist, capsaicin induced a short-living and (±)-CP 96,345-sensitive twitch enhancement. These data suggest that the sympathetic control of mouse vas deferens motility can be modulated in an opposite manner by tachykinin NK_1 (prejunctionally located) and by CGRP (pre- and/or postjunctionally located) receptors.

Keywords: Sensory nerve; Neurogenic inflammation; Capsaicin; (Prejunctional); (Postjunctional)

1. Introduction

Tachykinins can prejunctionally affect the neural (cholinergic, sympathetic or non-adrenergic, non-cholinergic) motor control of visceral smooth muscle (Hall et al., 1989; Tousignant et al., 1987; Patacchini et al., 1989; Barnes, 1992). Such an effect has been described in several preparations and species, such as guinea-pig vas deferens (Hall and Morton, 1991), trachea (Hall et al., 1989) and ileum (Featherstone et al., 1986), the rat vas deferens (Tousignant et al., 1987; Patacchini et al., 1989) and the rabbit airway smooth muscle (Tanaka and Grunstein, 1986; Chacko et al., 1993).

The mechanisms underlying these prejunctional modulatory effects of tachykinins are not unique and, depending on the species and types of nerves, the involvement of different tachykinin receptors (NK_1 and/or NK_2) has been suggested. For example, in guinea-pig vas deferens (Hall and Morton, 1991), ileum (Featherstone et al., 1986) and airways (Watson et al., 1993), stimulation of tachykinin NK_1 receptors can fully account for the enhancement of electrically induced motor responses. On the other hand, in rat vas deferens, the tachykinin-induced enhancement of sympathetic twitches has been attributed to activation of NK_2 receptors (Patacchini et al., 1989), while in the rabbit airways (Belvisi et al., 1994) activation of both NK_1 and NK_2 receptors potentiates cholinergically mediated bronchospasm. In the present study, we have further investigated the mechanism of the excitatory prejunctional actions of tachykinins in the mouse vas deferens since: (a) mouse vas deferens is a preparation characterized by an almost pure sympathetic control of its motor activity (Jones and Spriggs, 1975); (b) this tissue is innervated by capsaicin-sensitive substance P-like immunoreactive nerves (Brodin and Nilsson, 1981); (c) mouse vas deferens has been classically used as a preparation to study the prejunctional modulation

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of neurotransmission by other neuromodulators such as opiates (Hughes et al., 1975), neuropeptide Y (Stjärne et al., 1986) or purines (Kurz et al., 1993).

In the present study, the effect of synthetic selective agonists (NK_1, NK_2 and NK_3) and antagonists (NK_1) for tachykinin receptors on the amplitude of sympathetic twitches was studied. Besides, since tachykinins and calcitonin gene-related peptide (CGRP) are usually co-stored and simultaneously released from capsaicin-sensitive primary afferents (Lundberg et al., 1985; Sundler et al., 1985; Maggi, 1991; Hua and Yaksh, 1992), with possible opposite functional effects (Hua and Lundberg, 1986) it appeared worthwhile also to study the effect of capsaicin and CGRP (along with its proposed antagonist human CGRP-(8–37) on electrically induced sympathetic twitches in mouse vas deferens. Evidence was obtained indicating an opposite functional modulation by tachykinins (excitation through tachykinin NK_1 receptors) and CGRP (inhibition) of the sympathetic control of mouse vas deferens motility.

2. Materials and methods

2.1. Mouse isolated vas deferens

Swiss Nossan male mice (25–45 g), were killed by cervical dislocation. Both vas deferens were removed and placed in Krebs-Henderson (Mg-free) solution with the following composition (mM): NaCl 119, KCl 4.7, KH_2PO_4 0.92, $NaHCO_3$ 26.2, glucose 11, $CaCl_2$ 2.54. The mouse vas deferens was mounted vertically in a 5 ml organ bath containing Krebs-Henderson solution gassed with carbogen [O_2 (95%) and CO_2 (5%)], maintained at 37° C. A resting load of 0.5 g was applied to preparations which were allowed to equilibrate for 60 min. Changes in the motor activity were recorded by means of an isometric strain gauge connected to a polygraph. Electrical field stimulation (8 Hz, 60 V, 0.5 ms, trains of 2 s every 30 s) was performed by means of two platinum electrodes placed at the top and at the bottom of the organ bath connected to a Grass stimulator.

Concentration-response curves to CGRP and neuropeptide Y were obtained in a cumulative manner, each concentration being added when the effects of the preceding one had reached a steady state. Preliminary experiments indicated that similar effects could be obtained adding these agonists as single concentrations.

Concentration-response curves to NK_1, NK_2 or NK_3 selective receptor agonists, namely [Sar^9,$Met(O_2)^{11}$]-substance P, [β-Ala^8]neurokinin A-(4–10) or [$MePhe^7$]neurokinin B were obtained in a non-cumulative manner, the interval period between two concentrations being 25 min.

Administration of [Sar^9,$Met(O_2)^{11}$]substance P (3 nM) at a 60 min interval, in the same preparation, produced reproducible enhancements of the electrical field stimulation-induced contractile response. The NK_1 antagonists (±)-CP-96,345 (0.05, 0.1, 0.3 or 1 μM) were added to the organ bath 15 minutes before the second administration of [Sar^9,$Met(O_2)^{11}$]substance P. The effect of a single antagonist concentration was studied in each preparation. The inhibitory effect of CGRP (3 nM) and neuropeptide Y (30 nM) on the electrical field stimulation-induced contractile response, was reproducible at 60 min intervals and the antagonist, human CGRP-(8–37) (3 μM), was injected 15 min before the second administration. In a further set of experiments the action of [Sar^9,$Met(O_2)^{11}$]substance P (3 nM), CGRP (3 nM), phentolamine (0.1 μM) or tetrodotoxin (0.3 μM) was assessed on noradrenaline (3 μM)-induced phasic contraction and on hypertonic high-K+ (80 mM)-induced tonic contraction of mouse vas deferens. Drugs were added 15 min before noradrenaline or when the tonic response to high-K^+ medium was stabilized. Preliminary experiments indicated that two consecutive administrations of noradrenaline, at a 60 min interval, resulted in reproducible contractions.

2.2. Chemical denervation

Some experiments were perfomed in vas deferens excised from capsaicin (50 mg/kg, s.c., 4 days before) pretreated mice (Santicioli et al., 1985). Sympathetic denervation was obtained according to the method of Jones and Spriggs (1975) by administering 6-hydroxydopamine (1 mmol/kg i.v.) 24 h before killing.

2.3. Data analysis

All data are means ± S.E.M. Statistical analysis of the data was performed by means of Student's *t*-test for paired or unpaired data when applicable. The EC_{50} and their 95% confidence limits were calculated according to Tallarida and Murray (1981).

2.4. Drugs

Drugs used were: *l*-noradrenaline (Fluka AG, Chem. Fabrik CH-9470 Buchs), atropine, phentolamine, hexamethonium, tetrodotoxin, 6-hydroxydopamine (Sigma, St. Louis, USA); [Sar^9,$Met(O_2)^{11}$]substance P, [$MePhe^7$]neurokinin B, calcitonin gene-related peptide (CGRP), human CGRP-(8–37), neuropeptide Y (Peninsula Laboratories Europe). (±)-CP96,345 [(2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine] (Snider et al., 1991). GR82,334 [D-Pro^9[Spiro-*y*-lactam]Leu^{10}, Trp^{11}]physalaemin-(1–11) from Neosystem, Strasbourg,

France. SR48,968 ((*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) was provided by Dr Le Fur, Sanofi, Montpellier, France. [β-Ala^8]neurokinin A-(4–10) was synthesized at the Chemistry Department of Menarini Pharmaceuticals, Florence (Italy).

3. Results

3.1. General

Electrical field stimulation (1.5–10 Hz, 60 V, 0.5 ms, trains of 2 s every 30 s) of the mouse vas deferens produced frequency-related contractions; the maximal effect (873 ± 95 mg) ($n = 6$) was obtained at 10 Hz. For subsequent experiments, the submaximal frequency of 8 Hz was chosen. These twitches were unaffected by hexamethonium (10 μM), abolished by tetrodotoxin (0.3 μM) and partially (60 ± 4%) inhibited by phentolamine (1 μM)($n = 5 - 6$). Electrical field stimulation-induced contractions were absent in preparations taken from 6-hydroxydopamine systemically pretreated mice ($n = 4$). At rest, the mouse vas deferens responded to exogenous noradrenaline with a rapid contraction which reached a maximum usually within 5 s. Noradrenaline produced a concentration-related contraction in the range 0.1–100 μM; its EC_{50} (±95% c.l.) being 4 (3–5) μM ($n = 9$). The mean maximal tension developed in response to noradrenaline (100 μM) was 851 ± 70 mg ($n = 6$) and this response was unaffected by tetrodotoxin (0.3 μM, $n = 4$) and abolished by phentolamine (0.1 μM, $n = 5$), indicating that noradrenaline's effect involves activation of α-adrenoceptors located directly on the muscle. The administration of KCl (80 mM hypertonic) elicited a rapid phasic contraction (2.100 ± 70 mg, $n = 15$) followed by a tonic component (600 ± 30 mg, $n = 22$). When the KCl-induced tonus was stable (within 25–30 min), administration of tetrodotoxin (0.3–0.6 μM) or phentolamine (0.1–10 μM) was ineffective ($n = 5$ for each).

3.2. Effect of tachykinin receptor agonists and antagonists on electrical field stimulation-induced contraction of the mouse vas deferens

Administration of a synthetic selective agonist of the NK_1 tachykinin receptor, i.e. [Sar^9,Met(O$_2$)^{11}]substance P (Drapeau et al., 1987), produced a prompt (within 20–30 s), transient (5–10 min) and concentration-related (0.3–30 nM) increase in the amplitude of electrical field stimulation-induced contraction (see Fig. 1 left panel). The maximal increase was 191 ± 32%, ($n = 5$), and its EC_{50} (±95% c.l.) was 6 (4–11) nM ($n = 9$). In this range of concentrations (0.1–30 nM) [Sar^9,Met(O$_2$)^{11}]substance P did not affect the baseline tension of mouse vas deferens. Enhancement by [Sar^9,Met(O$_2$)^{11}]substance P (3 nM) of electrical field stimulation-induced mouse vas deferens contraction was unaffected by hexamethonium (10 μM, $n = 4$) or atropine (1 μM, $n = 4$) (data not shown).

On the other hand, the selective NK_2 and NK_3 receptor agonists, [βAla^8]neurokinin A-(4–10) (Rovero et al., 1989; Parlani et al., 1991) and [MePhe^7]neurokinin B (Drapeau et al., 1987) respectively, elicited enhancing effects only at concentrations above 0.3 μM (Fig. 1 left panel). At the highest concentration tested (1 μM), their maximal effect did not exceed 23 ± 4% for [MePhe^7]neurokinin B and 38 ± 5% for [βAla^8]neurokinin A-(4–10) of the maximal response to [Sar^9,Met(O$_2$)^{11}]substance P (30 nM) and this marginal potentiating effect was significantly reduced in preparations preincubated with the tachykinin NK_1 receptor antagonist GR 82,334 (0.3 μM, $n = 4$) (data not shown). The enhancement by [Sar^9,Met(O$_2$)^{11}]substance P (3 nM) of the electrical field stimulation-in-

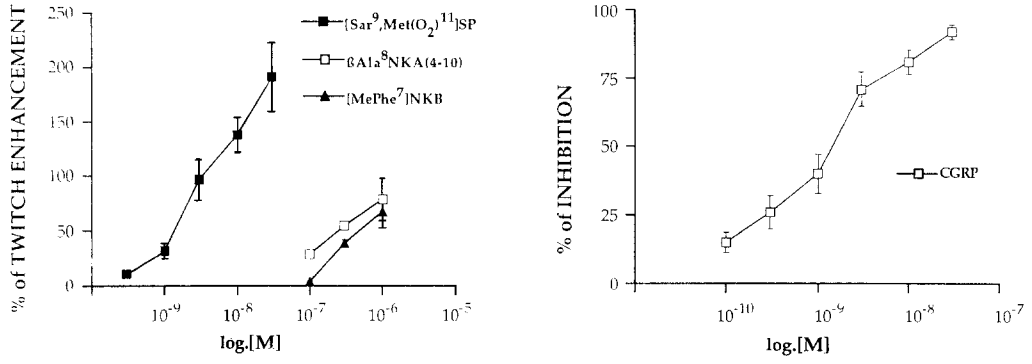

Fig. 1. Concentration-response curves for the enhancement of the electrical field stimulation-induced contractions of the mouse vas deferens produced by NK_1 ([Sar^9,Met(O$_2$)^{11}]substance P), NK_2 ([β-Ala^8]neurokinin A-(4–10)) or NK_3 ([MePhe^7]neurokinin B) selective agonists (left panel). Concentration-response curves were obtained in a non-cumulative manner. Concentration-response curves for the inhibition produced by CGRP on the amplitude of electrical field stimulation-induced contraction of mouse vas deferens (right panel). Concentration-response curves were obtained in a cumulative manner. Each value represents the means ± S.E.M. of six experiments.

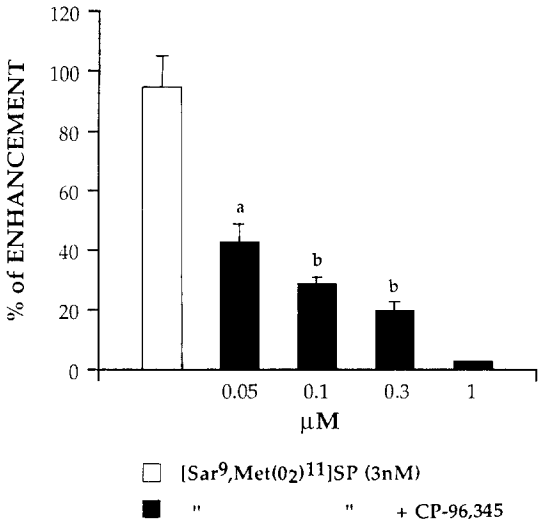

Fig. 2. Antagonism by preincubation with the NK_1 antagonist, (±)-CP-96,345, of [Sar^9,$Met(O_2)^{11}$]substance P (3 nM) induced potentiation of the electrical field stimulation-induced mouse vas deferens contractile response. Each value represents the means ± S.E.M. of six experiments. $^aP < 0.05$ and $^bP < 0.01$ vs. [Sar^9,$Met(O_2)^{11}$]substance P alone.

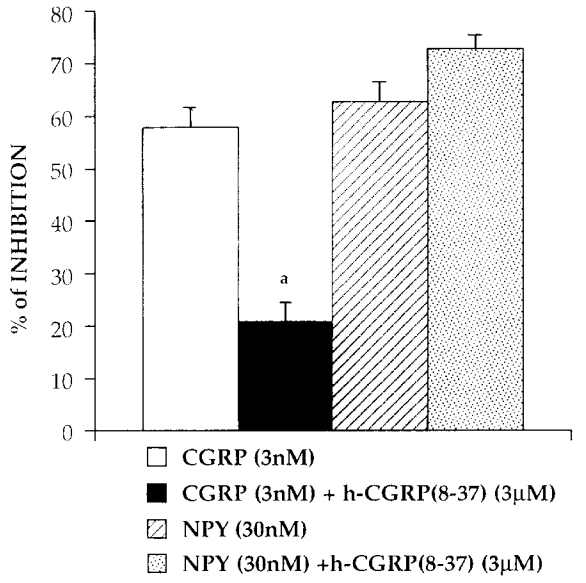

Fig. 3. Antagonism by human CGRP-(8–37) of the inhibitory effect of CGRP or neuropeptide Y on the amplitude of electrical field stimulation-induced contraction of mouse vas deferens. The values represent the means ± S.E.M.of six experiments. $^aP < 0.05$ vs. CGRP (3 nM) alone.

duced twitches was antagonized in a concentration-dependent manner by a non-peptide NK_1 selective receptor antagonist, (±)-CP 96,345 (Hagan et al., 1991), in the range 0.05–1 μM (Fig. 2). Its IC_{50} (±95% c.l.) was 0.1 (0.05–0.2) μM. Moreover, the enhancement produced by [Sar^9,$Met(O_2)^{11}$]substance P (3 nM), was significantly reduced (by 81 ± 10%, $n = 4$) in presence of the peptide NK_1 receptor antagonist, GR 82,334 (0.3 μM), while it was unaffected by previous administration of the NK_2 receptor antagonist, SR 48,968 (30 nM, $n = 4$).

3.3. Effect of CGRP on electrical field stimulation-induced contraction of the mouse vas deferens

The administration of CGRP produced a concentration-dependent (0.1–30 nM) inhibition of electrical field stimulation-induced contraction of mouse vas deferens (see Fig. 1 right panel) its IC_{50} (±95% c.l.) being 1 nM (0.5–3) ($n = 5$). At the maximal concentration used (30 nM), the inhibition was 92 ± 3%. The inhibitory effect of CGRP (3 nM) reached its maximum within a few minutes and remained fairly constant for at least 10–15 min. Administration of neuropeptide Y similarly reduced electrical field stimulation-induced contractions of mouse vas deferens in a concentration-dependent manner (0.01–1 μM) with an IC_{50} (±95% c.l.) of 0.7 (0.5–1) μM ($n = 8$) and a maximal inhibitory effect of 96 ± 1%. The inhibitory effect of CGRP (3 nM), but not of neuropeptide Y (30 nM), was significantly blocked by human CGRP-(8–37) (3 μM) (Fig. 3), that has been proposed as a competitive and selective CGRP receptor antagonist (Chiba et al., 1989). The addition of human CGRP-(8–37) (3 μM) alone itself consistently induced a slight enhancement (190 ± 21 mg, $n = 19$) of electrical field stimulation-induced twitches.

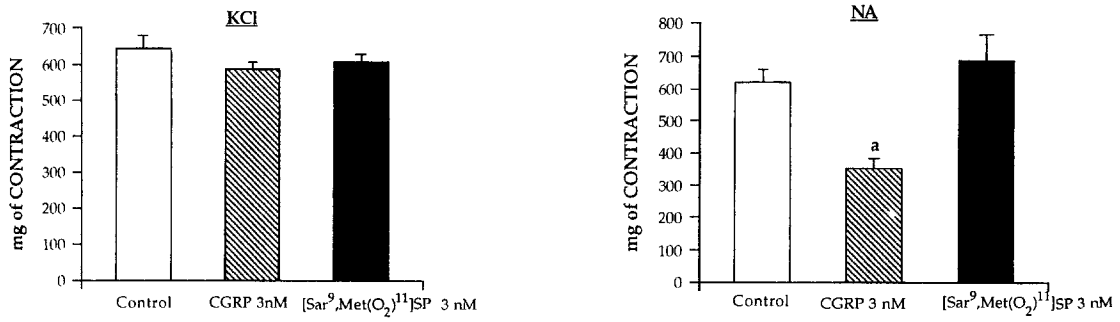

Fig. 4. Effect of CGRP or [Sar^9,$Met(O_2)^{11}$]substance P on high-K^+ (left panel) or noradrenaline (right panel) induced contraction in the mouse vas deferens. The values represent the means ± S.E.M. of six experiments. $^aP < 0.001$.

3.4. Effect of Sar^9,Met(O$_2$)^{11}]substance P and CGRP on the contractile responses to KCl (80 mM) and noradrenaline (3 μM)

To assess whether the effect of [Sar^9,Met(O$_2$)^{11}]substance P and CGRP on electrical field stimulation-induced sympathetic twitches might be pre- and/or postjunctional we performed an additional set of experiments to evaluate their activity (at a concentration of 3 nM which was submaximally effective on twitches) on tonic contraction caused by a depolarizing medium (KCl, 80 mM) and on phasic motor responses elicited by exogenous noradrenaline (3 μM). As shown in Fig. 4, [Sar^9,Met(O$_2$)^{11}]substance P (3 nM) had no effect on these postjunctional responses ($n = 6$). On the other hand, CGRP (3 nM) slightly inhibited noradrenaline (3 μM); but not KCl-induced tonic contraction ($n = 6$ for each). The inhibitory action of CGRP (3 nM) was significantly greater on electrical field stimulation-induced twitches than against noradrenaline-induced contraction (being 71 ± 6% and 38 ± 4% respectively, $P < 0.01$).

3.5. Effect of capsaicin on electrical field stimulation-induced contraction of the mouse vas deferens

Capsaicin (1 μM) reduced the amplitude of electrical field stimulation-induced twitches by 73 ± 5% (n = 10). The capsaicin inhibitory effect reached its maximum within 1–2 min and then contractile tone slowly recovered to 80–100% of its original value within 8–15 min. A second administration of capsaicin within 1–3 h from the first one, was ineffective, indicating complete desensitization ($n = 4$). Capsaicin (1 μM) did not affect nerve-mediated contractions of preparations taken from capsaicin systemically desensitized mice (50 mg/kg s.c. 4 days before). On the other hand, in mouse vas deferens taken from systemically capsaicin-desensitized mice, both CGRP (3 nM) or isoprenaline (1 μM) (data not shown) had a prompt and marked inhibitory effect similar to those observed in control preparations ($n = 5$).

Table 1
Excitatory or inhibitory effect of capsaicin (1 μM) on electrical field stimulation-induced contractions of the isolated mouse vas dererens

	Enhancement (mg)	Inhibition (mg)
Controls	n.p.	260 ± 22
hCGRP(8–37) (10 μM)	130 ± 19	174 ± 12 [a]
CP-96,345 1 μM + hCGRP(8–37) (10 μM)	n.p.	160 ± 6 [b]

Each value is mean ± S.E. of five experiments. [a] $P < 0.02$ and [b] $P < 0.01$ vs. controls. n.p.: not present.

In the presence of the CGRP receptor antagonist, human CGRP-(8–37) (10 μM), the administration of capsaicin (1 μM) resulted in a biphasic response; i.e. an initial and transient enhancement of twitches (130 ± 19 mg, $n = 5$) followed by inhibition of their amplitude and this inhibition was significantly lower than in controls, being 174 ± 12 mg ($n = 5$) and 260 ± 22 mg ($n = 5$), respectively ($P < 0.01$; Fig. 5). In these experimental conditions the capsaicin-induced transient enhancement of twitch amplitude was blunted by the NK$_1$ antagonist, (±)-CP 96,345 (1 μM) (see Table 1 and Fig. 5).

4. Discussion

Mouse vas deferens is a visceral preparation characterized by an almost pure sympathetic excitatory motor input to its smooth muscle bundles (Jones and Spriggs, 1975; Yamauchi and Burnstock, 1969; Sjöstrand, 1965).

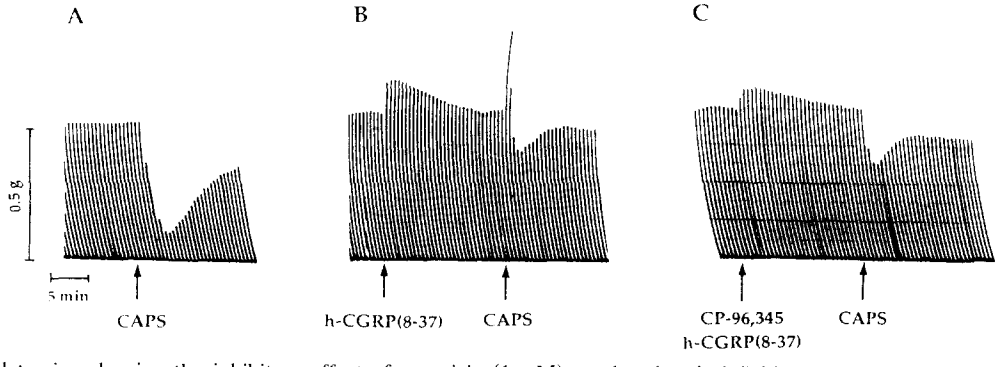

Fig. 5. (A) Typical tracing showing the inhibitory effect of capsaicin (1 μM) on the electrical field stimulation-induced mouse vas deferens contractions under control conditions. (B) In the presence of the CGRP antagonist, human CGRP-(8–37) (10 μM), administration of capsaicin produced a short-lasting excitation followed by an inhibitory effect whose intensity was significantly reduced as compared to controls. (C) When capsaicin (1 μM) was added in preparations preincubated (15 min before) with human CGRP-(8–37) (10 μM) and (±)-CP-96,345 (1 μM), the transient excitatory effect disappeared.

We have confirmed that, in this preparation, electrical field stimulation elicited contractile twitches that are neurogenic (blocked by tetrodotoxin), postganglionic (unaffected by hexamethonium), partially mediated by activation of α-adrenoceptors (reduced by the α-adrenoceptor blocker phentolamine) and that disappeared following 6-hydroxydopamine in vivo sympathectomy. In the present study, we have gained evidence that this sympathetic motor response can be modulated in an opposite manner (activation through tachykinin NK_1 receptors and inhibition through CGRP receptors) by sensory neuropeptides, possibly released from capsaicin-sensitive sensory nerves, such as those described by Brodin and Nilsson (1981) as being present in this preparation.

Tachykinins exert their biological effect through the stimulation of at least three kinds of receptors, namely NK_1, NK_2 and NK_3 (Regoli et al., 1988). Enhancement of the amplitude of motor responses induced in the mouse vas deferens by stimulation of adrenergic nerves is attributable to stimulation of NK_1 receptor since [Sar^9,$Met(O_2)^{11}$]substance P, one of the most selective NK_1 receptor agonists known to date (Drapeau et al., 1987), is active at the threshold concentration of 0.3 nM. In addition, the two selective agonists for NK_2 and NK_3 receptors, ([β-Ala^8]neurokinin A-(4–10) and [$MePhe^7$]neurokinin B, respectively) (Rovero et al., 1989; Parlani et al., 1991; Drapeau et al., 1987) were virtually ineffective up to 0.3 μM concentration. Furthermore, the excitatory action of [Sar^9,$Met(O_2)^{11}$]substance P can be blocked by the non-peptide NK_1 receptor antagonist, (±)-CP 96,345 (Snider et al., 1991). In view of the lack of effect of the selective NK_1 ([Sar^9,$Met(O_2)^{11}$]substance P) receptor agonist on motor responses elicited by high-K^+ medium or exogenous noradrenaline, it might be proposed that these tachykinin NK_1 receptors could be located prejunctionally. Indeed, excitatory prejunctional tachykinin NK_1 receptors have been already postulated in other visceral preparations such as guinea-pig vas deferens, ileum and airways (Hall and Morton, 1991; Featherstone et al., 1986; Watson et al., 1993). There has also been demonstrated an effective increase in the output of the excitatory motor neurotransmitter in the guinea-pig ileum (Featherstone et al., 1986). CGRP inhibits in a concentration-dependent manner the sympathetic control of mouse vas deferens motility.

This effect is significantly blocked by its proposed selective receptor antagonist, human CGRP-(8–37) (Chiba et al., 1989; Dennis et al., 1990; Maggi et al., 1991). On the other hand, human CGRP-(8–37) was unable to modify the prejunctional (Stjärne et al., 1986; Donoso et al., 1988) inhibitory effect of neuropeptide Y (30 nM) thus confirming its selectivity. Pharmacological evidence for an inhibitory role of CGRP in other visceral smooth muscle preparations has already been presented (Ellis and Burnstock, 1989; Maggi et al., 1988; Maggi and Giuliani, 1991; Sann et al., 1992; Kawasaki et al., 1990; Han et al., 1990). It is interesting to note that human CGRP-(8–37) per se induced a slight but consistent increase in the amplitude of electrical field stimulation-induced twitches, suggesting a tonic release of CGRP under basal conditions. Although a prejunctional location of CGRP receptors seems plausible, the observation that CGRP also reduces (although at a lower extent than twitches) the amplitude of exogenous noradrenaline (but not high-K^+)-induced contraction, could suggest a contribution of postjunctional CGRP receptors to its overall inhibitory effects on electrical field stimulation-induced twitches. At this stage, we can rule out neither this hypothesis nor the possibility that the response to exogenous noradrenaline might partially be linked to excitation of the sympathetic nerves and therefore subject to prejunctional inhibitory effects of CGRP. Appropriate experiments measuring noradrenaline release are necessary to substantiate this proposal.

The administration of the sensory neuron activating agent, capsaicin (Holzer, 1988; Maggi and Meli, 1988), produces inhibition of sympathetic twitches in mouse vas deferens. This effect is specific since: (a) it is absent in mouse vas deferens taken from systemically capsaicin-desensitized animals; (b) a second administration of capsaicin in the same preparation, was ineffective, indicating acute desensitization; (c) this inhibitory response is antagonized by the CGRP antagonist. Interestingly enough, when CGRP receptors are, at least partially, blocked by human CGRP-(8–37), capsaicin also elicits a marked, albeit transient, enhancement of electrical field stimulation-induced twitches. This enhancing effect is totally sensitive to blockade by the tachykinin NK_1 receptor antagonist, (±)-CP 96,345. As a whole, these findings suggest that the activation of the efferent function of capsaicin-sensitive sensory nerves leads to the co-release of various sensory neuropeptides with excitatory (tachykinins) and inhibitory (CGRP) effects on sympathetic function. Other examples of functional antagonism of sensory neuropeptides have been described (Hua and Lundberg, 1986; Maggi et al., 1987a,b; Sann et al., 1992). The observation that, under basal conditions, capsaicin induced exclusively an inhibitory action could indicate a preferential activity of CGRP vs. tachykinins, possibly due to a higher amount of mediator released or to a lower rate of metabolizing or both. It is interesting to note that human CGRP-(8–37) exerts a stronger antagonistic effect against exogenous CGRP than capsaicin. Heterogeneity of CGRP receptors has been proposed (Quirion et al., 1995) possibly linked to different anatomical locations (Parlani et al., 1993). Further studies are necessary to investigate whether or not

the receptor activated by exogenously added CGRP could be pharmacologically different from those activated by endogenously released CGRP.

In conclusion we have presented evidence that the sympathetic control of mouse vas deferens motility can be transiently enhanced or long-lastingly inhibited by sensory neuropeptides. These modulatory actions are respectively mediated by tachykinin NK_1 (prejunctionally located) and by CGRP (pre- and/or postjunctionally located) receptors. The mouse vas deferens could represent a preparation of choice for studying, under physiological and pathological conditions, the prejunctional activity of sensory neuropeptides, their functional antagonism and the possible existence of receptor subtypes.

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