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An immunohistochemical and pharmacological study of tachykinins in the rat and guinea-pig prostate glands

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Abstract

This study investigated the presence and effects of tachykinin peptides within the rat and guinea-pig prostate glands. Immunohistochemical studies demonstrated the presence of substance P and neurokinin A immunoreactive nerve fibres, sparsely distributed throughout the prostatic fibromuscular stroma in both species. In organ bath experiments, nerve terminals within rat and guinea-pig prostatic tissues were electrically field stimulated (60 V, 0.5 ms, 10 Hz, 20 pulses every 50 s). In rat preparations, the exogenous application of substance P, neurokinin A and the tachykinin NK_3 receptor agonist senktide (1 nM–1 μM) had no effect on contractile responses. In contrast, substance P and neurokinin A (1 nM–3 μM) concentration-dependently enhanced electrically-evoked contractile responses in the guinea-pig prostate. Senktide was without effect. The potentiation of electrical field stimulation-induced contractions by substance P and neurokinin A in the guinea-pig prostate was competitively antagonized by ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl]ethyl}-4-phenyl-1-azonia-bicyclo[2.2.2]octane, chloride) (SR 140333) at 10 nM, a tachykinin NK_1 receptor antagonist. The tachykinin NK_2 receptor antagonist (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide (SR 48968) was without effect at 10 nM, suggesting that neuromodulation of electrically-evoked contractions in the guinea-pig prostate occurs through activation of a tachykinin NK_1 receptor subtype.

Keywords: Tachykinin; Prostate; (Rat); (Guinea-pig); Neuromodulation; Innervation

1. Introduction

Tachykinins are a group of neuropeptides that are widely distributed throughout the body, interacting with receptors to elicit a variety of biological responses. One such response is the motor control of visceral smooth muscle in various species, namely the gastro-intestinal tract, bladder and vas deferens (Hall et al., 1990; Maggi, 1995a; Parlani et al., 1995). The three most investigated members of the tachykinin family are substance P, neurokinin A and neurokinin B. Substance P and neurokinin A, in particular, appear to be the predominant mediators in peripheral sensory function. All are structurally related containing a common carboxy terminal sequence, as Phe-X-Gly-Leu-Met-NH_2 (Chang et al., 1971).

Endogenous tachykinins are able to activate each of the three known tachykinin receptors in high concentrations, although at lower concentrations they display high affinity preferentially. Substance P is tachykinin NK_1 receptor preferring, neurokinin A is tachykinin NK_2 receptor preferring while neurokinin B is the preferential ligand of tachykinin NK_3 receptors (for reviews see Henry, 1986; Watling and Guard, 1992; Maggi, 1995b).

Primary afferent sensory neurones terminate at neuroeffector junctions of the prostate gland along with short noradrenergic and cholinergic nerve fibres (McVary et al., 1998), the latter two regulating prostatic growth, secretion and contractility. There is little information on the role of the sensory nervous system in prostatic regulation, although histochemical studies have demonstrated the presence of substance P-containing varicose fibres, sparsely associated with the prostatic stroma, submucosa and epithelium of the secretory acini (Gu et al., 1983; Lamano Carvalho et al., 1986; Chapple et al., 1991; Dhami and Mitchell, 1994; Jen and Dixon, 1995; Tainio, 1995).

In contrast to other tissues in the male urogenital tract, there has been a lack of investigation on the effects of

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tachykinins in the prostate gland. Palea et al. (1996) attempted to identify the nature of tachykinin receptor subtypes in the human prostate gland based on the rank order of agonist potencies and the use of selective tachykinin receptor antagonists. They suggested that a population of tachykinin NK_2 receptors was mediating the tachykinin-induced contractility.

The main aims of this study were to confirm whether tachykinins are present within nerve fibres innervating the rat and guinea-pig prostate gland and also to identify the functional role of these neuropeptides in neurotransmission. Tachykinin receptor classification was undertaken using potent and selective non-peptide antagonists synthesized by Sanofi Recherche (Emonds-Alt et al., 1992, 1993).

2. Materials and methods

2.1. Animals

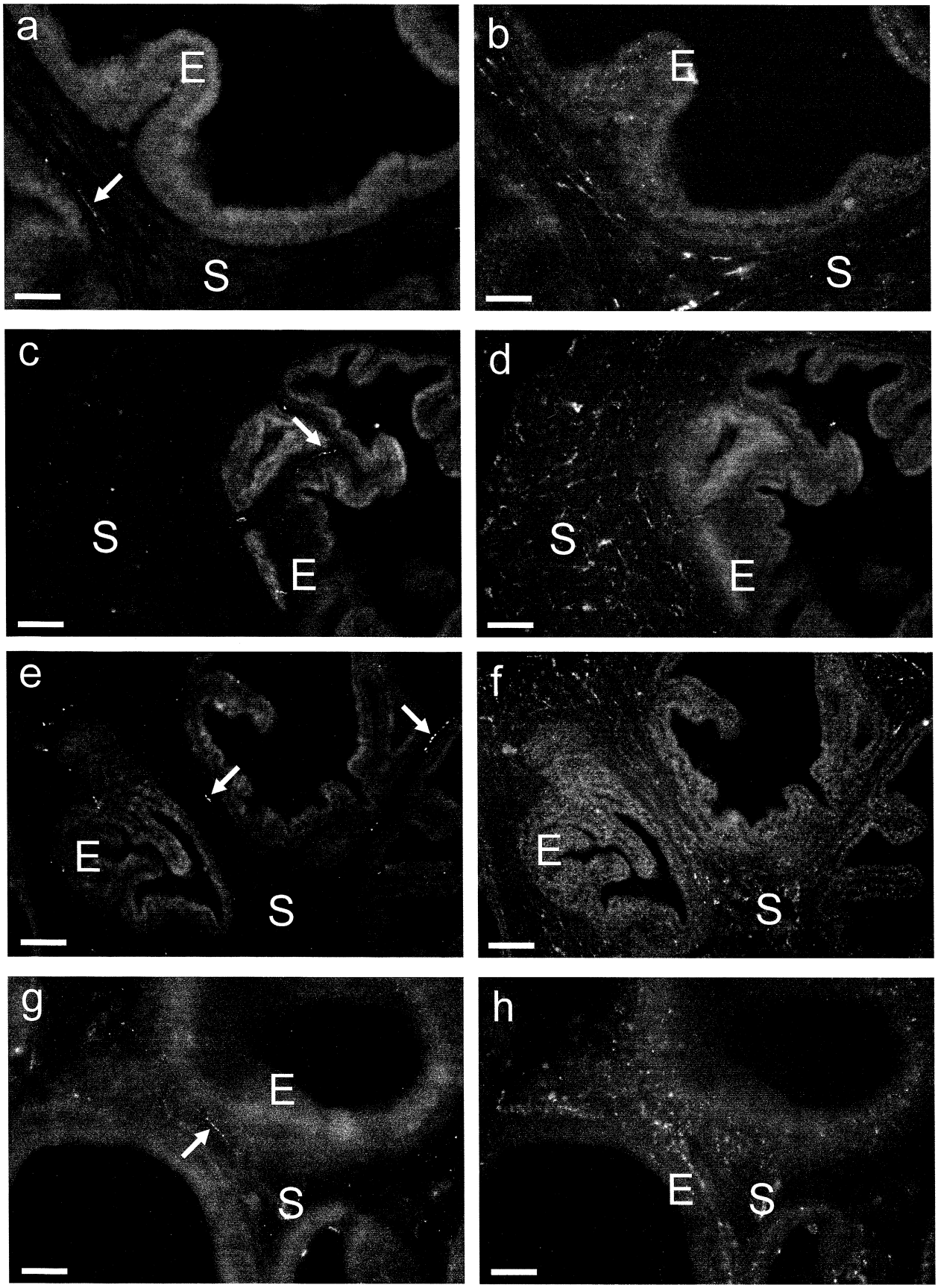
Mature male Sprague–Dawley rats (250–500 g) and Tricolor Monash strain guinea-pigs (500–1000 g) were housed at 22°C with a photoperiod of 12 h light and 12 h dark. Rats had free access to rodent chow and water ad libitum. Guinea-pigs had free access to water and standard guinea-pig pellets with ascorbic acid ad libitum. Guinea-pigs were killed by cervical dislocation and exsanguination; rats were killed by stunning and exsanguination. The entire prostate gland (dorsal and ventral lobes) was then dissected from these animals. Prior ethical approval for these experiments was obtained from the Standing Committee of Ethics in Animal Experimentation of Monash University (No. 97/009) and conformed to the guidelines laid down by the National Health and Medical Research Council of Australia.

2.2. Immunohistochemistry

Prostates from five rats and five guinea-pigs were fixed in phosphate-buffered saline containing 4% paraformaldehyde for 2 h. Tissues were then washed in phosphate buffered-saline containing 7% sucrose and 0.01% sodium azide four times for 10 min each time and stored in this solution for 48 h at 4°C. Tissues were embedded in Tissue-Tek (OCT embedding compound), then snap frozen in liquid nitrogen and maintained at −80°C. Twelve-micrometer sections were cut using a cryostat at −21°C and thawed onto gelatin coated slides, prior to incubation with a rabbit polyclonal antibody for substance P (Serotec) or neurokinin A (Phoenix) for 18–20 h at room temperature. Antibodies were diluted (1:1000) in an antibody diluting medium containing 0.1% w/v sodium azide, 0.01% w/v bovine serum albumin, 0.1% w/v lysine and 0.1% v/v Triton in phosphate buffered-saline. For control sections, the primary antibody was omitted. Tissue sections were washed in saline four times for 10 min each time before a 1 h incubation with fluoroscein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulin (Vector) at room temperature. This secondary antibody was raised in goat and diluted in the same antibody diluting medium (1:150). Tissues were washed in saline four times every 10 min, then mounted in ''Vectashield'' (Vector), coverslipped and examined under an Olympus fluorescence photomicroscope.

Since nerve mediated smooth muscle responses of the rat and guinea-pig prostate are predominantly mediated by noradrenaline, the relationship of substance P and neurokinin A immunopositive nerve fibres to tyrosine hydroxylase immunopositive nerve fibres was also investigated. In these studies, fixed frozen slide mounted sections of rat and guinea-pig prostate were simultaneously incubated with either a rabbit polyclonal antibody for substance P (Serotec) (1:1000) or neurokinin A (Phoenix) (1:1000) and a mouse monoclonal antibody for tyrosine hydroxylase (Boehringer Mannheim) (1:100) for 18–20 h at room temperature. Tissue sections were washed in saline four times for 10 min each time before a 1 h incubation with fluoroscein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulin (Vector) (1:300) and Texas Red-conjugated anti-mouse immunoglobulin (Vector) (1:500) at room temperature. These secondary antibodies were raised in goat and horse, respectively. Tissues were washed in saline four times every 10 min, then mounted in ''Vectashield'' (Vector), coverslipped and examined under an Olympus fluorescence photomicroscope. In order to prevent cross reaction with endogenous immunoglobulins in double labelling experiments, primary and secondary antibody incubations were done in antibody diluting medium containing 2% normal serum from the same species as the tissue under study.

Fig. 1. Representative photomicrographs showing cross sections of guinea-pig prostate double immunostained with substance P (a) and tyrosine hydroxylase (b) or neurokinin A (c) and tyrosine hydroxylase (d). Rat prostatic sections likewise double immunostained with substance P (e) and tyrosine hydroxylase (f) or neurokinin A (g) and tyrosine hydroxylase (h). Nerve fibres immunoreactive for tachykinins are indicated by arrows. Cross sections of guinea-pig prostate show sparse immunostaining to tachykinins of nerve fibres in the smooth muscle stroma (S) and epithelial lining of the secretory acini (E). Tyrosine hydroxylase immunostaining was found only in the stroma (S) and in different nerve fibres to those positively immunostained for either tachykinin. A similar distribution of immunostained nerve fibres was seen in the rat prostate which has a much lower content of stromal smooth muscle and a sparser distribution of tachykinin immunopositive nerve fibres. Scale Bar = 100 μm (a,b,c,d); 50 μm (e,f,g,h).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2.3. Isolated organ bath studies

2.3.1. Experimental procedure

In the rat, the left and right prostates from each animal were dissected to provide two preparations, while in the guinea-pig, four preparations were obtained by further separating the prostates into ventral and dorsal lobes. Tissues were mounted vertically in 5 or 10 ml organ baths containing a modified Krebs–Henseleit solution (nM: NaCl 118.1, KCl 4.69, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7, $MgSO_4$ 0.5, $CaCl_2$ 2.5), maintained at 37°C and bubbled with 5% CO_2 in O_2. Prostatic preparations were placed under a resting force of 0.5–0.6 g and equilibrated for 30 min. A further 30 min equilibration was allowed during which intramural nerve terminals were electrically field stimulated with trains of 20 pulses at 10 Hz, 60 V, 0.5 ms pulse duration every 50 s. Unresponsive tissues were occasionally stimulated at 20 Hz. Responses of the rat and guinea-pig prostate evoked by these stimulation parameters are tetrodotoxin-sensitive and are markedly attenuated in the presence of guanethidine or prazosin, confirming their neural origin (Lau et al., 1998). Electrical field stimulation was performed by means of two parallel platinum electrodes incorporated in the tissue holder and connected to a *Grass S88* stimulator. Smooth muscle contractions were recorded isometrically via a *Grass FTO3*-transducer connected to a MacLab data acquisition system.

When using rat prostate, concentrations of substance P, neurokinin A and senktide (1 nM–1 μM) were added sequentially. Each concentration remained in contact with the tissue for 3–4 min then was removed by several bath medium changes. Tissues were left for 15 min between addition of each concentration. Increasing concentrations of substance P or neurokinin A were applied to each tissue (30 min apart) in the absence and presence of thiorphan (10 μM) to prevent degradation of peptides by endopeptidase (EC 3.4.24.11) and/or angiotensin converting enzyme (EC 3.4.15.1) (Patacchini et al., 1989). Senktide, a tachykinin NK_3 receptor selective agonist (Wormser et al., 1986) is resistant to enzyme cleavage. Thiorphan was pre-incubated with the tissue for 15 min.

In the guinea-pig prostate, cumulative log concentration–response curves were constructed to substance P, neurokinin A and senktide (1 nM–3 μM). Curves (30 min apart) to substance P or neurokinin A were constructed in the absence and presence of thiorphan (10 μM) to block neutral endopeptidase and angiotensin converting enzyme (Patacchini et al., 1989) or the neutral endopeptidase inhibitor, *N*-[*N*-[1-(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenyl-alanyl-(*S*)-isoserine (SCH 39370, 10 μM) (Sybertz et al., 1989; Fisher and Pennefather, 1997). SCH 39370 was pre-incubated with the tissue for 30 min.

In the guinea-pig prostate, curves to substance P and neurokinin A were also constructed in the absence and presence of a single antagonist concentration of either SR 140333 (tachykinin NK_1 receptor selective, Emonds-Alt et al., 1993) or SR 48968 (tachykinin NK_2 receptor selective, Emonds-Alt et al., 1992). The selectivity of these antagonists for tachykinin receptors as well as any interactions with endothelin or cholinergic mechanisms were examined by constructing cumulative concentration–response curves to endothelin-1 (1–100 nM) and carbachol (100 nM–1 mM) in the absence and presence of the same single antagonist concentration. The SR compounds were pre-incubated for 2 h at a concentration of 10 nM (Emonds-Alt et al., 1992, 1993). Time/vehicle (0.001% ethanol) control experiments were carried out on parallel preparations.

In order to investigate whether the tachykinins had any postjunctional inhibitory effects, in some experiments the trains of field stimulation were omitted and discrete concentration–response curves to exogenous noradrenaline (0.1 μM–1 mM) were applied in the absence and presence of a single approximate EC_{50} concentration of substance P (100 nM). In these experiments, substance P was added to the organ bath 10 min before the application of each concentration of noradrenaline.

In order to further investigate whether cholinergic mechanisms are involved in mediating or modulating tachykinin induced responses of prostatic smooth muscle, log concentration–response curves to substance P and neurokinin A were constructed in the absence and presence of the muscarinic receptor antagonist, atropine (0.1 μM). Atropine was preincubated for a 30-min period.

2.3.2. Data analysis

All data are expressed as mean ± S.E.M. ''*n*'' represents the number of experimental animals used. Tachykinin-induced responses were expressed as a percentage increase of basal electrical field stimulation-induced twitch force. The mean peak force (in g) of four electrical field stimulation-induced contractile responses were measured in the presence of each concentration of agonist and compared to the mean peak force of the four electrical field stimulation-induced contractile responses immediately preceding the administration of each drug concentration. The mean estimates of apparent dissociation constants (K_B) for one concentration of antagonist were calculated from these estimates using the equation K_B = antagonist concentration/CR − 1 (Furchgott, 1972). A student's paired *t*-test was used to evaluate the mean differences in control electrical field stimulation-induced contractions between the control and treatment groups. Differences in concentration–response curves were statistically evaluated using a two-way repeated measure analysis of variance (ANOVA). Values of $P < 0.05$ were considered significant.

2.3.3. Drugs

The following drugs were used: neurokinin A, senktide and substance P (Auspep); thiorphan, atropine, carbachol and noradrenaline (Sigma); endothelin-1 (American Pep-

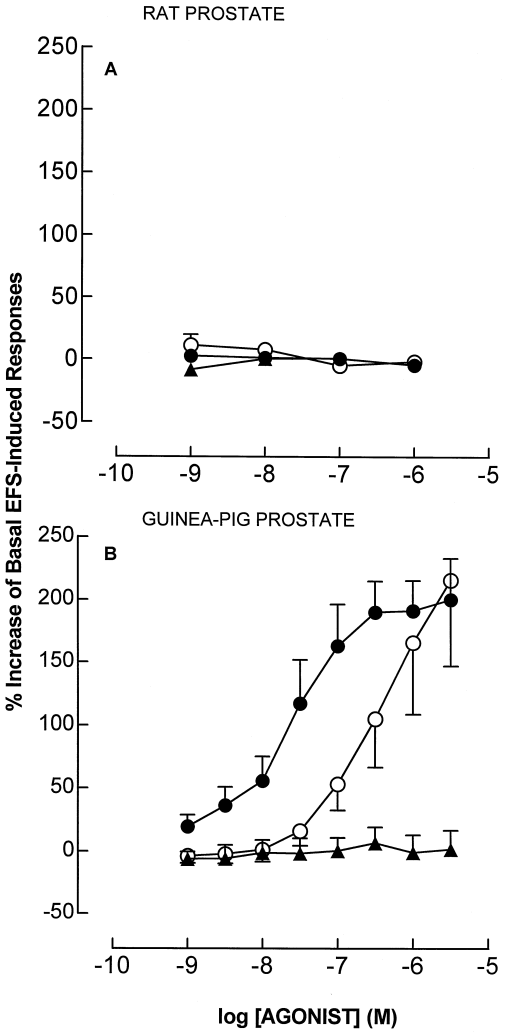

Fig. 2. Mean log concentration–response curves for tachykinin agonists in the rat (A) and guinea-pig (B) prostate. Substance P (●), neurokinin A (○) and senktide (▲). Each point represents the mean ± S.E.M. of 4–9 experiments. Results are expressed as the percentage increase of basal electrical field stimulation-induced responses.

tide). SCH 39370 (*N*-[*N*-[1-(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenyl-alanyl-(*S*)-isoserine) was a gift to J.N.P. from Dr. E.J. Sybertz, Schering-Plough Research Institute. SR 140333 [(*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane, chloride] and SR 48968 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl] benzamide were gifts to J.N.P. from Dr. Xavier Emonds-Alt, Sanofi Recherche.

Noradrenaline was dissolved and diluted to the required concentrations in a catecholamine diluent (mM: NaCl 154.0, NaH_2PO_4 1.2, ascorbic acid 0.2). Thiorphan, atropine and carbachol were dissolved in distilled water. Stock solutions of SCH 39370 (1 mM) were dissolved in 1% Na_2CO_3. All peptides were dissolved in acidified water (0.01 M HCl). Stock aliquots were stored in plastic vials, frozen at −20°C and were not thawed more than twice. Once thawed, neurokinin A and substance P were diluted in the modified Krebs Henseleit solution. Senktide was diluted in distilled water. SR 140333 and SR 48968 (both 1 mM) were dissolved in absolute ethanol and diluted in distilled water.

3. Results

3.1. Immunohistochemistry

Thin and varicose substance P and neurokinin A immunoreactive nerve fibres were sparsely distributed throughout the fibromuscular stroma and sub-epithelial connective tissue of the prostate in the guinea-pig (Fig. 1). Sparse immunoreactivity was also seen in association with glandular elements. Substance P and neurokinin A immunoreactivity showed a similar distribution profile in the rat prostate but was much more sparsely distributed. This distribution was seen in the prostates taken from all five rats and all five guinea-pigs.

Thin and varicose tyrosine hydroxylase immunoreactive nerve fibres were densely distributed throughout the fibromuscular stroma of the prostate in both rat and guinea-pig. No immunoreactivity was seen in association with subepithelial connective tissue or glandular elements in prostate sections from either species. Stromal tyrosine hydroxylase immunoreactivity showed no colocalization or consistent relationship to the distribution of immunoreactivity to either tachykinin (Fig. 1).

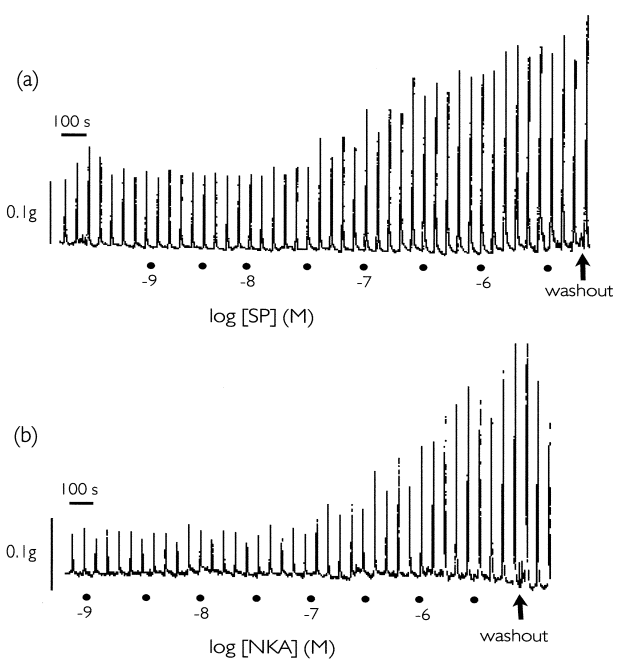

Fig. 3. Representative traces showing the effects of cumulative addition of substance P and neurokinin A on the electrical field stimulation-induced contractions in the guinea-pig prostate (a,b).

3.2. Isolated organ bath studies

3.2.1. Agonist responses

In the rat prostate, the sequential addition of substance P, neurokinin A or senktide (1 nM–1 μM) had no effect on either basal prostatic smooth muscle tone or electrical field stimulation-induced responses (Fig. 2). Conversely, substance P and neurokinin A (1 nM–3 μM) caused concentration-dependent enhancement of field stimulation-induced contractile responses in the guinea-pig prostate (Figs. 2 and 3). Senktide was inactive up to 3 μM. The rank order of agonist potency of substance P > neurokinin A ≫ senktide was observed (Fig. 3) ($n = 5$–9). All tachykinin agonists were without effect on basal guinea-pig prostatic smooth muscle tone.

In the guinea-pig prostate, thiorphan (10 μM) did not potentiate field stimulation-induced responses to substance P ($n = 6$; $P = 0.60$) or neurokinin A ($n = 5$; $P = 0.60$), suggesting that neutral endopeptidase 24.11 and/or angiotensin converting enzyme are not involved in the degradation of tachykinin peptides in this tissue. SCH 39370, a potent inhibitor of neutral endopeptidase 24.11 (Sybertz et al., 1989), did not potentiate responses to substance P at 10 μM ($n = 5$; $P = 0.16$). In contrast, the effect of neurokinin A to cause potentiation of field stimulation-induced contractions were markedly enhanced in the presence of this inhibitor ($n = 5$; $P < 0.01$). SCH 39370 was used with neurokinin A in further antagonist studies, while thiorphan was used with substance P to ensure against any possible peptide degradation.

3.2.2. Effects of SR 140333 and SR 48968 on agonist responses in the guinea-pig prostate

In the presence of thiorphan (10 μM), the tachykinin NK_1 receptor selective antagonist SR 140333 (10 nM) consistently caused a parallel rightward shift to the log concentration–response curve of substance P (Fig. 4). The mean concentration ratio was 12.9 (95% confidence limits 11.3–14.5, $df = 78$) and the apparent pK_B value was estimated at 9.10 ± 0.30 ($n = 5$). In the presence of SCH 39370 (10 μM), SR 140333 (10 nM) also caused a rightward shift to the log concentration–response curve of neurokinin A (Fig. 4), further confirming the presence of a tachykinin NK_1 receptor population. The mean concentration ratio was 9.2 (95% confidence limits 7.2–11.8, $df = 62$) with an apparent pK_B value of 8.90 ± 0.25 ($n = 4$). SR 140333 had no significant effect on prostatic basal tone. SR 140333 at the concentration tested selectively depressed the effects of tachykinins as it did not affect the potentiation of electrically evoked contractions by carbachol ($n = 4$, $P = 0.89$) or endothelin-1 ($n = 4$, $P = 0.93$).

SR 48968 did not inhibit control baseline electrical field-stimulation-induced responses. Similarly, the selective tachykinin NK_2 receptor antagonist, SR 48968 (10 nM) did not antagonise the potentiating effects of substance P or neurokinin A (Fig. 4). Responses to high concentrations of neurokinin A were paradoxically increased in the presence of the tachykinin NK_2 receptor antagonist, SR 48968 (Fig. 4).

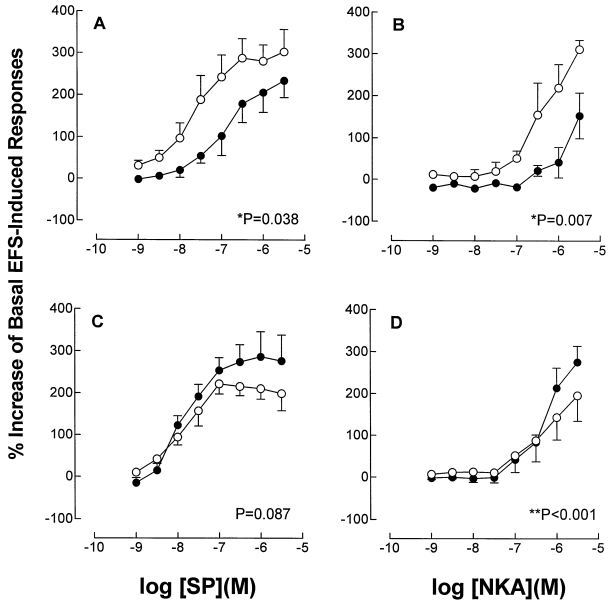

Fig. 4. In the guinea-pig prostate, mean log concentration–response curves to substance P were constructed in the absence control (○) and presence (●) of (A) SR 140333 (10 nM; $n = 5$) or (C) SR 48968 (10 nM; $n = 5$). Likewise, mean log concentration–response curves to neurokinin A were constructed in the absence and presence of (B) SR 140333 (10 nM; $n = 4$) or (D) SR 48968 (10 nM; $n = 5$). Each point represents the mean ± S.E.M. Asterisks indicate a significant difference ($^*P < 0.05$; $^{**}P < 0.005$; one-way repeated measures ANOVA).

3.2.3. Postjunctional effects of substance P on guinea-pig prostate

Discrete concentration–response curves to noradrenaline (0.1 μM–1 mM) on unstimulated isolated guinea-pig prostate preparations were unaffected in the presence of substance P (100 nM) ($n = 4$, $P = 0.88$).

3.2.4. Effects of atropine on tachykinin responses of the guinea-pig prostate

Preincubation of guinea-pig prostates with atropine (0.1 μM) did not affect mean log concentration–response curves to exogenous substance P ($n = 5$; $P = 0.11$) and neurokinin A ($n = 4$; $P = 0.14$).

4. Discussion

The aims of this study were to investigate both the presence and effects of tachykinin peptides within the rat and guinea-pig prostate glands. It was confirmed that substance P and neurokinin A immunoreactive nerves were present within the stromal tissue of both species. These nerve fibres were more abundant in prostates taken from guinea-pigs compared to those taken from rats. Functional

studies further revealed that tachykinins facilitate neurotransmission to prostatic smooth muscle of the guinea-pig, but not that of the rat. This effect is most probably mediated by a tachykinin NK_1 receptor subtype.

Our immunohistochemical studies demonstrate for the first time the presence of neurokinin A containing nerves closely associated with prostatic stromal tissue in the rat and guinea-pig. Our demonstration of the presence of sparse substance P containing nerves closely associated with prostatic stromal tissue in the rat and guinea-pig is consistent with observations in the fetal and adult human prostate, where sparse substance P immunoreactive nerve fibres were exclusively associated with the smooth muscle (Gu et al., 1983; Jen and Dixon, 1995; Tainio, 1995). Substance P immunoreactivity has been previously demonstrated in the prostate of the guinea-pig (Lamano Carvalho et al., 1986; Dhami and Mitchell, 1994). In contrast to our study, it has been previously reported that the rat prostate does not possess any substance P immunoreactive nerve fibres (Alm et al., 1978; Lamano Carvalho et al., 1986). This anomaly may merely be a reflection of the improvement of tachykinin antibodies and basic immunohistochemical techniques. In addition our study demonstrates that in both species the tachykinins were present in a separate population of nerve fibres from those containing tyrosine hydroxylase.

Neither the smooth muscle tone nor the electrically-evoked contractions of rat prostatic preparations were affected by the application of tachykinin agonists. This observation is consistent with that of Watts and Cohen (1991) who reported that substance P had no contractile effect in the prostate gland of the rat. The presence of tachykinins within nerve fibres, however, suggest that these neuropeptides may be implicated in primary sensory neurotransmission.

The relative potencies of the tachykinin agonists enhancing electrical field stimulation-induced contractions of the guinea-pig prostate, indicate the presence of a population of tachykinin NK_1 receptors. The rank order of agonist potency showed substance P to be more potent than neurokinin A while senktide, the tachykinin NK_3 receptor agonist was inactive. Although neurokinin A is tachykinin NK_2 receptor-preferring, at high doses it can activate tachykinin NK_1 receptors.

In this study, the tachykinin NK_1 selective antagonist, SR 140333 (Croci et al., 1995) significantly caused a rightward shift to the log concentration–response curves of both substance P and neurokinin A. This was not a nonspecific depressant effect of the drug since SR 140333 had no effect on the potentiation of excitatory neurotransmission by either carbachol or endothelin-1. This suggests the possible presence of a tachykinin NK_1 receptor subtype, especially since the pK_B estimate is comparable with those previously reported. Basal electrical field stimulation-induced contractile responses were not, however, affected by SR 140333 alone suggesting that endogenous tachykinins are not normally involved in excitatory neurotransmission to the prostatic smooth muscle of adult guinea-pigs.

The fact that the selective tachykinin NK_2-receptor antagonist SR 48968 (Maggi et al., 1993) did not antagonize responses to substance P or neurokinin A, indicates the absence of tachykinin NK_2 receptors in this tissue. Thus substance P and neurokinin A may modulate neurotransmission to the smooth muscle of the guinea-pig prostate through a tachykinin NK_1 receptor subtype. Previous studies have suggested that the tachykinin NK_1 receptor subtype may also include a further ''septide sensitive'' atypical tachykinin NK_1 receptor subtype for which substance P has low affinity (Glowinski, 1995) but is susceptible to blockade by selective tachykinin NK_1 receptor antagonists (Zeng and Burcher, 1994) and potently activated by neurokinin A (Glowinski, 1995). As electrically-evoked contractile responses to neurokinin A were antagonized by SR 140333 in this study, it would be interesting to investigate the possible existence of a ''septide'' receptor in the guinea-pig prostate.

The mechanism by which tachykinins enhance the contractile response of the guinea-pig prostate to electrical field stimulation is not known. The tachykinins do not seem to act at a postjunctional site since substance P and neurokinin A do not increase baseline smooth muscle tone nor does substance P potentiate the contractile effect of exogenously added noradrenaline. The experiments involving atropine, carbachol and endothelin-1 also suggest that the tachykinins act independently of cholinergic and endothelin related mechanisms. Stimulation of the tachykinin NK_2 receptors present in the guinea-pig prostate may somehow be increasing the concentration of noradrenaline at the neuromuscular junction, either by increasing its release or by blocking its removal from the biophase.

In conclusion, the present finding has demonstrated substance P- and neurokinin A-immunoreactive nerves in the rat and guinea-pig prostate gland. Furthermore, isolated organ bath studies have revealed a species difference in the functional effects of sensory neuropeptides. In the rat prostate, tachykinin agonists were without effect. In the guinea-pig prostate, tachykinin-induced neuromodulation of electrically-evoked contractile responses occurred through activation of a tachykinin NK_1 receptor subtype. Further studies are required to determine the site/s at which these peptides act to modulate neurotransmission to the prostate. The involvement of tachykinin peptides within the prostate gland with the urinary symptoms associated with prostate disease including benign prostatic hyperplasia remains to be established.

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