Activation of the micturition reflex by NK₂ receptor stimulation in the anaesthetized guinea-pig

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- 1 The mechanisms underlying stimulation of bladder contractions and bronchoconstriction by the selective NK₂ receptor agonist, [β -Ala⁸]NKA(4–10), were examined in the anaesthetized guinea-pig.
- 2 Atropine, α,β -methylene-ATP and ganglion blocking agents were used to examine the contribution of reflex arc activation and/or potentiation of efferent mechanisms to the NK₂ receptor-mediated responses seen in these two tissues.
- 3 [β -Ala⁸]NKA(4–10)-induced bronchoconstriction was immediate, dose-dependent and was unaffected by pretreatment with ganglion blockers (hexamethonium or chlorisondamine), blockade of muscarinic receptors by atropine, or desensitization of P₂ purinoceptors by α,β -methylene-ATP.
- 4 At doses of 5 μ g kg⁻¹ and above, [β -Ala⁸]NKA(4–10) induced bladder contractions that appeared to be of an 'all-or-nothing' nature. These contractions occurred after a delay of 10 to 30 s and were often biphasic, comprised of an initial rapid component followed by a slower tonic component.
- 5 Pretreatment of the animals with either atropine or the desensitizing purinoceptor agonist α,β -methylene-ATP, resulted in partial inhibition of bladder contractile responses to [β -Ala⁸]NKA(4–10). The combination of atropine and α,β -methylene-ATP pretreatment resulted in additive inhibition leading to complete blockade of the response.
- 6 The bladder responses to [β -Ala⁸]NKA(4–10) (5 μ g kg⁻¹) were inhibited by pretreatment with the ganglion blockers, hexamethonium and chlorisondamine, indicating a preganglionic mechanism of action.
- 7 These findings demonstrate the indirect nature of the bladder contractions induced by activation of NK₂ receptors in the anaesthetized guinea-pig. Contractions occur secondary to the release of endogenous cholinergic and NANC transmitters by activation of neuronal NK₂ receptors located at a preganglionic site, possibly on capsaicin-sensitive sensory afferent nerves, where NK₂ sites have been demonstrated autoradiographically. In contrast, [β -Ala⁸]NKA(4–10)-induced bronchoconstriction in the anaesthetized guinea-pig is a direct smooth muscle contractile response that is unaffected by ganglion blockade or blockade of muscarinic receptors.

Keywords: Tachykinins; NK₂ receptors; [β -Ala⁸]NKA(4–10); guinea-pig urinary bladder; guinea-pig airways; micturition reflex; sensory nerves

Introduction

Mammalian airways and bladder are richly innervated by capsaicin-sensitive primary afferent neurones (Holzer *et al.*, 1982; Polak & Bloom, 1982; Lundberg *et al.*, 1984; Su *et al.*, 1986) that are characterized by high levels of tachykinin immunoreactivity (Holzer *et al.*, 1982; Lundberg & Saria, 1986). The endogenous tachykinins, substance P (SP), neurokinin A (NKA) and NKB, are potent contractile agents in bladder (Falconieri-Erspamer *et al.*, 1973; Maggi *et al.*, 1988; Giuliani *et al.*, 1993) and airways (Deviellier *et al.*, 1988; Frossard *et al.*, 1989; Frossard & Advenier, 1991) from various species. These effects are mediated by activation of tachykinin NK₁ (SP-preferring) and NK₂ (NKA-preferring) receptors (Frossard & Advenier, 1991; Maggi *et al.*, 1991d; Hall *et al.*, 1992; Longmore & Hill, 1992; Giuliani *et al.*, 1993).

The main functional roles of NK₁ and NK₂ receptors in airways and bladder vary, depending upon the species under examination. NK₂ receptors, predominantly, mediate direct contractile effects of tachykinins in guinea-pig and human trachea and bronchi preparations (Dion *et al.*, 1990; Frossard & Advenier, 1991; Maggi *et al.*, 1991c). However, NK₁ receptors also have a direct smooth muscle contractile role in guinea-pig, but not human, airways (Dion *et al.*, 1990; Frossard & Advenier, 1991; Ireland *et al.*, 1991; Maggi *et al.*, 1991c). NK₁ receptors, predominantly, mediate tachykinin-

induced airway mucosecretion, vasodilatation, microvascular leakage and inflammatory cell activation (Rogers *et al.*, 1988; Joos & Pauwels, 1990; Frossard & Advenier, 1991). This has led to the suggestion that NK₁ receptors play a major role in neurogenic inflammation and that selective NK₁ and/or NK₂ antagonists, respectively, may be useful for the treatment of airway hyperresponsiveness and bronchoconstriction associated with asthma (Barnes, 1986; Manzini, 1993).

Tachykinin-induced contractions of rat, guinea-pig and hamster bladder preparations are mediated by both NK₁ and NK₂ receptors (Falconieri-Erspamer *et al.*, 1973; Maggi *et al.*, 1991a; Hall *et al.*, 1992), while human bladder contracts in response to activation of NK₂ but not NK₁ receptors (Dion *et al.*, 1990; Giuliani *et al.*, 1993). NK₁ receptors mediate SP-induced microvascular leakage and contribute to inflammatory responses in the bladder (Abelli *et al.*, 1989). Several *in vivo* studies have demonstrated contractile effects of NK₂ agonists on the bladder and airways in anaesthetized rats (Joos *et al.*, 1986; Maggi *et al.*, 1991a; Palea *et al.*, 1993; Lecci *et al.*, 1993) and guinea-pigs (Maggi *et al.*, 1991b; Buckner *et al.*, 1993; Belvisi *et al.*, 1994). The presence of NK₂ receptors on both smooth muscle and sensory neuronal sites in the bladder (Burcher & Buck, 1986; Carstairs *et al.*, 1993) indicates that the effects of NK₂ agonists in the bladder may be either direct, via smooth muscle contraction, and/or indirect, via activation of the afferent limb of the micturition reflex. In the present study we have compared the *in vivo* bronchoconstriction and bladder contractile responses induced by the selective NK₂ receptor

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agonist, [β -Ala⁸]NKA(4–10) (Rovero *et al.*, 1989), in the anaesthetized guinea-pig. In particular, we have used ganglion blocking and antimuscarinic agents, as well as α,β -methylene-ATP ([α,β -Me]ATP) (Kasakov & Burnstock, 1983), to examine the contribution of reflex arc activation and/or potentiation of efferent mechanisms to the NK₂ responses seen in these two tissues. Our results indicate that in the guinea-pig bladder, unlike the airways, [β -Ala⁸]NKA(4–10)-induced contractions are indirect and result from NK₂-dependent activation of the micturition reflex. Some of this work has been presented in preliminary form to the British Pharmacological Society (Metcalf *et al.*, 1994).

Methods

Animals

Female Dunkin Hartley guinea-pigs (350–450 g) were obtained from Charles River, UK, and were anaesthetized with urethane (1.2 g kg⁻¹, i.p.) and placed on a heating pad to maintain body temperature at 37°C. The carotid artery was

cannulated (Portex Luer IV cannula) for blood pressure recording (Druck pressure transducer) and the left jugular vein cannulated for i.v. drug administration. After tying off the external urethra, a catheter was inserted via the dome into the bladder and connected via a three-way tap to a Millar microtip catheter (model MPC500) and transducer control unit (TCB500) for intraluminal pressure recording. The guinea-pigs were artificially ventilated (60 strokes min⁻¹; 10 ml kg⁻¹) with a Harvard small animal respirator (model 683) and the tracheal insufflation pressure recorded. Data were recorded and analysed with a Macintosh MacLab/8 data recording system controlled by a Macintosh IICI computer with Chart software. Animals were allowed to stabilize for 15 to 20 min after surgery before drug additions.

Drugs used in the study

Hexamethonium and [α,β -Me]ATP were obtained from Sigma (UK) and atropine was from Research Biochemicals Inc (UK). [β -Ala⁸]NKA(4–10) was purchased from Bachem, U.K., Ltd. ($\pm$)-SR48968 (N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl] benzamidine) (Emonds-Alt *et al.*, 1992) and ($\pm$)-SR140333 (1-{2-[3-(3,4-

Figure 1 Traces illustrating the effects of [β -Ala⁸]NKA(4–10) on bladder pressure and tracheal insufflation pressure in the anaesthetized guinea-pig. Intravenous administration of the indicated doses of [β -Ala⁸]NKA(4–10) resulted in immediate and dose-related increases in tracheal insufflation pressure while delayed all-or-nothing bladder contractions were induced by doses of 5 μ g kg⁻¹ and above. All doses of [β -Ala⁸]NKA(4–10) induced transient and variable increases (2 to 30 mmHg) in blood pressure that returned to baseline levels within 5 min. The traces shown are representative of 5 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]-ethyl)-4-phenyl-1-azoniabicyclo[2.2.2]octane) (Emonds-Alt *et al.*, 1993) were synthesized by Mike Stace and Chris Burns, Discovery Chemistry Department, Pfizer Central Research, Sandwich. All other drugs and chemicals were obtained from Aldrich (U.K.) or BDH (U.K.) and were of the highest grade available. All drugs were dissolved in de-ionised water apart from [β -Ala⁸]NKA(4–10) and ($\pm$)-SR140333 which were dissolved at 1 mg ml⁻¹ in 100% and 50% dimethylsulphoxide (DMSO), respectively, and further dilutions were made in de-ionised water. All drugs were administered intravenously in a volume of 1 ml kg⁻¹.

Results

Airway and bladder responses to [β -Ala⁸]NKA(4–10)

Administration of the NK₂ receptor agonist, [β -Ala⁸]NKA(4–10) (5 μ g kg⁻¹, i.v.) caused a contraction of the bladder and an increase in tracheal insufflation pressure. The bladder response to [β -Ala⁸]NKA(4–10) occurred after a variable lag period of 10 to 30 s and displayed at least two distinct phases: an initial fast component and a slower tonic component. In some cases, the tonic phase of the contraction was accompanied by a series of transient rhythmic contractions. Dose-response studies

showed the bladder contraction to be of an 'all or nothing' nature, with the threshold dose being between 1 and 5 μ g kg⁻¹ (Figure 1). In contrast, the tracheal response to [β -Ala⁸]NKA(4–10) was immediate and dose-dependent (Figure 1) and displayed an ED₅₀ of 0.63 μ g kg⁻¹ (geometric mean, range = 0.34–1.29, n = 5). The maximal response was achieved with between 5 and 10 μ g kg⁻¹.

Effects of NK₁ and NK₂ antagonists on responses to [β -Ala⁸]NKA(4–10)

In order to confirm the NK₂ receptor involvement in the responses of the anaesthetized guinea-pig to [β -Ala⁸]NKA(4–10), we examined the effects of pretreatment with selective NK₁ and NK₂ receptor antagonists. The NK₂ receptor-selective (Emonds-Alt *et al.*, 1992) antagonist, ($\pm$)-SR48968 (1–300 μ g kg⁻¹), abolished the bladder contraction in response to a threshold dose of [β -Ala⁸]NKA(4–10) (5 μ g kg⁻¹, i.v.) at doses of 30 μ g kg⁻¹ and above (Figure 2). ($\pm$)-SR48968 inhibited the [β -Ala⁸]NKA(4–10) (5 μ g kg⁻¹, i.v.)-induced increase in tracheal insufflation pressure in a dose-dependent manner (Figure 2), with an ED₅₀ of 75.0 μ g kg⁻¹ (geometric mean, range = 35.5–1.29, n = 6). The tracheal response to a submaximal dose of [β -Ala⁸]NKA(4–10) (1 μ g kg⁻¹, i.v.) was inhibited with an ED₅₀ of 25.4 μ g kg⁻¹ (geometric mean, range = 18.0–38.5, n = 4). In contrast, the

Figure 2 Traces illustrating the inhibition of [β -Ala⁸]NKA(4–10)-induced bladder and tracheal responses by ($\pm$)-SR48968 was administered at the indicated doses 5 min prior to injection of [β -Ala⁸]NKA(4–10) (5 μ g kg⁻¹). Agonist-induced increases in tracheal insufflation pressure were inhibited in a dose-dependent manner while doses of 1 μ g kg⁻¹ and above of [β -Ala⁸]NKA(4–10) were required to abolish the tracheal response. Traces shown are representative of 6 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

NK₁-selective (Emonds-Alt *et al.*, 1993) antagonist, ($\pm$)-SR-140333, at doses (10–1000 $\mu\text{g kg}^{-1}$) more than twenty fold higher than those necessary to inhibit SP-induced bronchoconstriction (Edmonds-Alt *et al.*, 1993), had no effect on $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ (5 $\mu\text{g kg}^{-1}$, i.v.)-induced bladder on tracheal responses (not shown).

Effects of ganglion blockade and inhibition of cholinceptors and purinoceptors

Hexamethonium (0.1 to 30 mg kg^{-1} , i.v.) treatment 5 min prior to $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ had no effect on the increase in tracheal pressure. However, there was a dose-dependent inhibition of the bladder response (Figure 3), giving a maximal degree of inhibition of $80 \pm 6\%$ (mean $\pm$ s.e.mean, $n=7$). Similar results were seen with chlorisondamine (1–1000 $\mu\text{g kg}^{-1}$, i.v.) treatment which produced dose-dependent inhibition of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ (5 $\mu\text{g kg}^{-1}$, i.v.) induced bladder ($\text{ED}_{50} = 56.9 \text{ g kg}^{-1}$, geometric mean, range = 36.2–128, $n=4$) but not tracheal responses (Figure 4).

Injection of atropine (1 to 1000 $\mu\text{g kg}^{-1}$, i.v.) 5 min prior to $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ resulted in a dose-dependent partial inhibition of the bladder contraction. A maximum reduction of $63.2 \pm 6.7\%$ (mean $\pm$ s.e.mean, $n=5$) was achieved at the highest dose (Figure 5). The desensitising purinoceptor agonist, $[\alpha, \beta\text{-Me}]\text{ATP}$ (1–300 $\mu\text{g kg}^{-1}$) induced a direct stimulation of bladder smooth muscle contraction and inhibited the response to subsequent administration of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$. The direct bladder contractions in response to $[\alpha, \beta\text{-Me}]\text{ATP}$ were dose-related (Figure 6), unlike the all-or-nothing responses to $[\beta\text{-Ala}^8]\text{NKA}(4-10)$. Pretreatment with $[\alpha, \beta\text{-Me}]\text{ATP}$ (1–300 $\mu\text{g kg}^{-1}$) 3 min prior to the NK₂ agonist, resulted in dose-dependent inhibition of the bladder contraction (Figure 7) with a maximum inhibition of $34.8 \pm 6.7\%$ (mean $\pm$ s.e.mean, $n=5$). Atropine pretreatment had no significant effect on the direct bladder smooth muscle contractile

effect of $[\alpha, \beta\text{-Me}]\text{ATP}$. However, the combination of atropine (300 $\mu\text{g kg}^{-1}$) and $[\alpha, \beta\text{-Me}]\text{ATP}$ (300 $\mu\text{g kg}^{-1}$) completely abolished the NK₂ agonist-induced bladder contraction (Figure 8). Neither atropine nor $[\alpha, \beta\text{-Me}]\text{ATP}$ modified the $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ (5 $\mu\text{g kg}^{-1}$) induced increase in tracheal insufflation pressure (Figure 8).

Discussion

The results of this study indicate that NK₂ receptor-dependent bronchoconstriction *in vivo* is mediated by a direct contractile effect on airway smooth muscle that is independent of cholinergic neurotransmission. These findings are in agreement with *in vitro* studies that have identified only direct smooth muscle contractile effects of NK₂ receptors in guinea-pig trachea (Devillier *et al.*, 1988; Frossard & Advenier, 1991). Tachykinin-induced enhancement of cholinergic neurotransmission has been demonstrated in guinea-pig airways. However, this effect was shown to be mediated by activation of NK₁ but not NK₂ receptors (Hall *et al.*, 1989; Watson *et al.*, 1993; Belvisi *et al.*, 1994).

In contrast to the direct bronchoconstrictor effect of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ in the anaesthetized guinea-pig, the results of this study indicate that the *in vivo* bladder responses to NK₂ receptor activation are indirect. The NK₂ agonist-induced response was shown to be of an 'all-or-nothing' nature and occurred after a distinct lag period following injection of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$, indicative of an indirect mechanism of action, possibly via activation of a reflex arc. This possibility was further explored by use of blocking drugs to interfere with the post-junctional action of the main neurotransmitters implicated in micturition contractions. Activation of micturition contractions in the guinea-pig and other mammalian species is mediated by both cholinergic and purinergic neurotransmitters (Burnstock *et al.*, 1972; Fujii, 1988; Brading & Williams, 1990).

Figure 3 Traces illustrating the inhibition by hexamethonium of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ -induced bladder but not tracheal responses. Animals were pretreated for 5 min with the indicated doses of hexamethonium prior to dosing with $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ (5 $\mu\text{g kg}^{-1}$). Agonist-induced bladder responses were inhibited by hexamethonium but tracheal responses were not significantly changed. Mean arterial blood pressure, before addition of hexamethonium, was $58.4 \pm 2.4 \text{ mmHg}$ (mean $\pm$ s.e.mean, $n=4$). Hexamethonium produced a dose-dependent depression in blood pressure that reached $38 \pm 5.3\%$ of control at the highest dose tested. Traces shown are representative of 4 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

Figure 4 Traces illustrating the inhibition by chlorisondamine of [β -Ala⁸]NKA(4–10)-induced bladder but not tracheal responses. Animals were pretreated for 5 min with the indicated doses of chlorisondamine prior to injection of [β -Ala⁸]NKA(4–10) ($5 \mu\text{g kg}^{-1}$). Agonist-induced bladder responses were inhibited by chlorisondamine but tracheal responses were not significantly changed. Mean arterial blood pressure, before addition of chlorisondamine, was $70.2 \pm 5.2 \text{ mmHg}$ (mean $\pm$ s.e. mean, $n=4$). Chlorisondamine produced a dose-dependent depression in blood pressure that reached $53.3 \pm 5.5\%$ of control at the highest dose tested. Traces shown are representative of 4 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

Figure 5 Traces illustrating the inhibition by atropine of [β -Ala⁸]NKA(4–10)-induced bladder but not tracheal responses. Animals were pretreated for 5 min with the indicated doses of atropine prior to injection of [β -Ala⁸]NKA(4–10) ($5 \mu\text{g kg}^{-1}$). Agonist-induced bladder responses were partially inhibited by atropine but tracheal responses were not significantly changed. Traces shown are representative of 5 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

Inhibition of these cholinergic and purinergic components by atropine and $[\alpha, \beta\text{-Me}]\text{ATP}$, respectively, was shown to produce additive inhibition of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ induced bladder contractions, indicating a prejunctional site of action of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ *in vivo*. This effect could be mediated either by direct enhancement of efferent transmitter release or via activation of a reflex arc.

NK₂ receptor-dependent activation of the micturition reflex arc was confirmed by use of the ganglion blockers, hexam-

ethonium and chlorisondamine, which both caused almost complete blockade of the $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ response. Taken together, these results indicate that i.v. administration of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ induced reflex bladder contractions by an action on preganglionic efferent or afferent NK₂ receptors. Autoradiographic studies with $[^{125}\text{I}]\text{-NKA}$ in rat and guinea-pig tissues have localized bladder NK₂ receptor sites to both smooth muscle and epithelia (Burcher & Buck, 1986; Carstairs *et al.*, 1993). Epithelial binding of $[^{125}\text{I}]\text{-NKA}$ was absent in

Figure 6 Traces illustrating the effects of $[\alpha, \beta\text{-Me}]\text{ATP}$ on bladder pressure in the anaesthetized guinea-pig. Intravenous administration of the indicated doses of $[\alpha, \beta\text{-Me}]\text{ATP}$ resulted in immediate dose-related transient increases in bladder pressure but had no effect on tracheal insufflation pressure. In one example (d), the animal had been pretreated with atropine ($300 \mu\text{g kg}^{-1}$, i.v.) 15 min prior to the administration of $[\alpha, \beta\text{-Me}]\text{ATP}$. The traces shown are representative of 5 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

Figure 7 Traces illustrating the inhibition by $[\alpha, \beta\text{-Me}]\text{ATP}$ of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ -induced bladder but not tracheal responses. Animals were pretreated for 3 min with the indicated doses of $[\alpha, \beta\text{-Me}]\text{ATP}$ prior to injection of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ ($5 \mu\text{g kg}^{-1}$). $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ -induced bladder responses were partially inhibited by $[\alpha, \beta\text{-Me}]\text{ATP}$ pretreatment but tracheal responses were not significantly changed. Traces shown are representative of 5 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

Figure 8 Traces illustrating the additive inhibitory effect of atropine and $[\alpha,\beta\text{-Me}]\text{ATP}$ on $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ -induced bladder but not tracheal responses. Animals were pretreated for 5 min with atropine ($300\text{ }\mu\text{g kg}^{-1}$) and for 3 min with $[\alpha,\beta\text{-Me}]\text{ATP}$ ($300\text{ }\mu\text{g kg}^{-1}$) prior to dosing with $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ ($5\text{ }\mu\text{g kg}^{-1}$). $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ -induced bladder responses were abolished by pretreatment with the combination of atropine and $[\alpha,\beta\text{-Me}]\text{ATP}$ but tracheal responses were not significantly changed. Traces shown are representative of 5 experiments in different animals. Bladder pressure, continuous line trace; tracheal pressure, shaded trace.

bladder from rats that had been treated with capsaicin as neonates (Carstairs *et al.*, 1993), indicating a possible location on capsaicin-sensitive afferent nerve terminals. Activation of these neuronal NK₂ receptors *in vivo* may underlie the stimulation of the micturition reflex by $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ seen in this study.

In a previous study, Maggi and co-workers demonstrated facilitation of reflex micturition by intravesical administration of $[\beta\text{-Ala}^8]\text{NKA}(4-10)$, indicating a peripheral site of action (Maggi *et al.*, 1991b). These results were interpreted as reflecting a direct smooth muscle contraction by $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ leading to increased afferent discharge by bladder mechanoreceptors. However, a direct stimulant action on capsaicin-sensitive bladder afferent nerves could not be ruled out from this work or in related studies in guinea-pig and rat (Maggi *et al.*, 1987; 1991d; Lecci *et al.*, 1994). Indeed, results with $[\alpha,\beta\text{-Me}]\text{ATP}$, in the present study, indicate that it is possible to induce large amplitude bladder contractions without activating the micturition reflex. Therefore, it is likely that NK₂ receptor-dependent activation of the micturition reflex is not merely a result of bladder smooth muscle contraction but involves an additional site of action. *In vivo* electrophysiological studies will be required to determine whether NK₂ receptor agonists can directly stimulate bladder afferent nerve firing, in the absence of detrusor contraction.

The existence of NKA in capsaicin-sensitive bladder afferent nerves, as well as the presence of receptors for this peptide

on the same nerve terminals, raises the possibility of a role for endogenous NKA and NK₂ receptors in the control of reflex micturition. The stimulatory effect of exogenous NK₂ receptor agonists on bladder reflexes suggests that these receptors may play a positive feedback role in the regulation of micturition frequency. In a study in rats, using the selective NK₂ antagonist, SR48968, Lecci and co-workers presented evidence against a role for peripheral NK₂ receptors in the normal control of micturition (Lecci *et al.*, 1993). However, there is evidence from rat studies for a role of NK₂ receptors in bladder hypersensitivity induced by irritants such as xylene (Maggi *et al.*, 1991a; Pietra *et al.*, 1992). Whether the contribution of NK₂ receptors to the control of micturition in guinea-pigs is also restricted to conditions of irritation or inflammation remains to be determined. However, we have found that there are marked species differences in *in vivo* bladder sensitivity to neurokinins, with guinea-pigs more sensitive than rats to $[\beta\text{-Ala}^8]\text{NKA}(4-10)$ -induced stimulation of the micturition reflex (unpublished observations). Autoradiographic studies in human bladder indicate the presence of submucosal (presumably neuronal) NK₂ receptors, in addition to those receptor sites located on detrusor smooth muscle (Nimmo *et al.*, 1993). As with rats and guinea-pigs, these receptors may contribute to sensitization of the micturition reflex in conditions of ongoing irritation and may contribute to the pathophysiology of diseases such as cystitis or urinary urge incontinence.

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