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Short communication

Tachykinin-mediated effect of nociceptin in the rat urinary bladder in vivo

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Abstract

The application of nociceptin (5–50 nmol/rat) onto the serosa in the urinary bladder of urethane-anaesthetized rats, with the intravesical volume kept below threshold for activation of the micturition reflex, induced a low amplitude tonic contraction (local, i.e., resistant to ganglionectomy) with high amplitude phasic contractions (reflex, i.e., abolished by ganglionectomy) superimposed. The pharmacology of the local contraction was studied in animals with acute bilateral ablation in the pelvic ganglia: the combined administration of tachykinin NK_1 (*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl-acetyl)-piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2.]octane chloride (SR 140333) and NK_2 c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β–5β)} (MEN 11420) receptor antagonists (given at doses of 1 + 0.1 μmol/kg, intravenous (i.v.), respectively) abolished the local bladder contraction induced by topical nociceptin (50 nmol/rat). These results indicate that the topical application of nociceptin onto the bladder evokes a tachykinin-mediated contraction.

Keywords: Micturition reflex; Tachykinin NK_1 receptor; Tachykinin NK_2 receptor; Orphanin FQ; Tachykinin

1. Introduction

Nociceptin is a 17 aminoacid neuropeptide that acts as the endogenous ligand of opioid-like receptors (ORL1) (Henderson and McKnight, 1997). Previous studies have shown that nociceptin can inhibit transmitter release from sympathetic, parasympathetic and sensory nerves, thereby influencing several autonomic functions such as blood pressure, heart rate, and micturition (Giuliani et al., 1998). Nociceptin can inhibit the micturition reflex by acting at a peripheral and supraspinal site (Giuliani et al., 1998; Lecci et al., 2000). In particular, we observed that the intravenous (i.v.) administration of nociceptin triggers a prolonged inhibition of the volume-evoked micturition reflex in urethane-anesthetized rats (Giuliani et al., 1998). This peripheral effect was ascribed to inhibition of afferent discharge from bladder sensory nerves and may also involve, to a minor extent, an inhibitory prejunctional modulation of pelvic efferent neurotransmission to the detrusor muscle (Giuliani et al., 1998). Capsaicin-sensitive afferent nerves play important roles in setting the gain for activation of micturition reflex (Lecci et al., 1998 for review). Subsequent studies showed that i.v. nociceptin can inhibit the reflex bladder contractions stimulated by topical application of capsaicin onto the rat urinary bladder (chemoceptive micturition reflex) (Giuliani et al., 1999). Moreover, systemic capsaicin pretreatment antagonizes the inhibitory effect induced by the systemic administration of nociceptin on the volume-evoked micturition reflex (Lecci et al., 2000).

Although an inhibitory effect of nociceptin on the release of sensory neuropeptides from peripheral endings of capsaicin-sensitive afferent nerves has been repeatedly described (e.g., Giuliani and Maggi, 1996; Helyes et al., 1997), there have also been reports suggesting that nociceptin may actually excite capsaicin-sensitive afferent nerves and induce the release of sensory neuropeptides such as tachykinins (Inoue et al., 1998).

In this study, we aim to assess whether nociceptin exerts a tachykinin-mediated stimulant effect on bladder motility if tested under experimental conditions suitable to detect such an excitatory effect, i.e., following its topical application onto the bladder when the volume of fluid in the viscus is kept below threshold for activation of the micturition reflex (cf. Maggi et al., 1984).

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2. Material and methods

The experimental procedures used for this study have been carried out according to the Italian laws for the care and the use of laboratory animals. Male albino Wistar rats (Charles River, Calco, Italy) weighing 340–400 g were anaesthetized with urethane (1.2 g/kg, s.c.). The body temperature was kept constant at 36.5°C and the animals were tracheotomized. The right jugular vein was cannulated for i.v. administration of drugs. Both ureters were ligated to maintain a constant bladder volume throughout the experiment. The urinary bladder was prepared for intraluminal pressure recording by the transurethral route as described previously (Giuliani et al., 1998). For the topical application onto the bladder serosa, the bladder was exposed, kept separated from the adjoining viscera by a piece of Parafilm®, and covered with a saline-moistened cotton gauze. The effects induced by the topical application of nociceptin (5 and 50 nmol/50 μl of saline/rat) onto the bladder were studied in two different models: (i) when the bladder was filled with a subthreshold volume (0.1–0.4 ml) for triggering the micturition reflex; (ii) when the bladder was filled with a volume (0.8–1.5 ml) sufficient to maintain ongoing distension-induced micturition reflex contractions. In the first model, the incidence of activation and the number of high-amplitude (> 15 mm Hg) reflex contractions in response to the topical application of saline or nociceptin were evaluated. In the second model, the early excitatory effect of nociceptin was evaluated as the increase in amplitude of distension-induced micturition reflex contractions with respect to the basal, whereas the late inhibitory effect (10–12 min delay) was calculated as duration of suppression of distension-induced micturition reflex contractions. In this model, the influence of guanethidine pretreatment (68 μmol/kg s.c. administered as two equally divided doses, 18 and 1 h before) on nociceptin (5 and 50 nmol/rat) evoked bladder motor responses was also evaluated. In another series of experiments, the topical application of nociceptin onto the bladder was carried out in animals subjected to the acute (2 h) bilateral ablation of pelvic ganglia: the bladders of these animals were filled with 0.5 ml of saline but no micturition contractions could be elicited given the surgical interruption of the reflex arc. In these experiments, the rats received (*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl-acetyl)-piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2.] octane chloride (SR 140333), (1 μmol/kg/100 μl of dimethylsulphoxide, i.v.) and c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β–5β)} (MEN 11420), (0.1 μmol/kg/100 μl of saline, i.v.) or the respective vehicles 10 min before topical application of saline (50 μl/rat) and 30 min before nociceptin (50 nmol/50 μl of saline/rat). The preparations were thereafter challenged with carbachol (50 nmol/50 μl of saline/rat) and capsaicin (2.5 nmol/50 μl of saline containing 0.7% ethanol/rat) 60 and 120 min from tachykinin antagonists administration, repectively. In this series of experiments, the amplitude of contraction and the area under the curve (AUC) in response to various agonist challenges were evaluated. Nociceptin was purchased from Neosystem (Strasbourg, France); capsaicin, guanethidine and carbachol from Sigma-Aldrich (Milano, Italy); SR 140333 was a kind gift from Dr. X. Emonds-Alt; MEN 11420 was synthesized by the Chemistry Research Department of Berlin-Chemie (Berlin, Germany). The data were analyzed by means of Fisher's exact test or Student's *t*-test for unpaired or paired values, when applicable; $P < 0.05$ was considered as significant.

3. Results

When the urinary bladder was filled with an amount of fluid below the threshold (0.1–0.4 ml) for activating the micturition reflex, the application of nociceptin (5 or 50 nmol/rat) onto the serosal surface of the viscus triggered a low-amplitude tonic contraction (local) with high-amplitude phasic contractions (reflex) superimposed (Fig. 1A) in six out of eleven (dose, 5 nmol/rat) or six out of six (dose, 50 nmol/rat) preparations. The average number of nociceptin-induced reflex contractions was 4 ± 2 and 14 ± 3 for duration of 6 ± 3 and 12 ± 2 min at the dose of 5 and

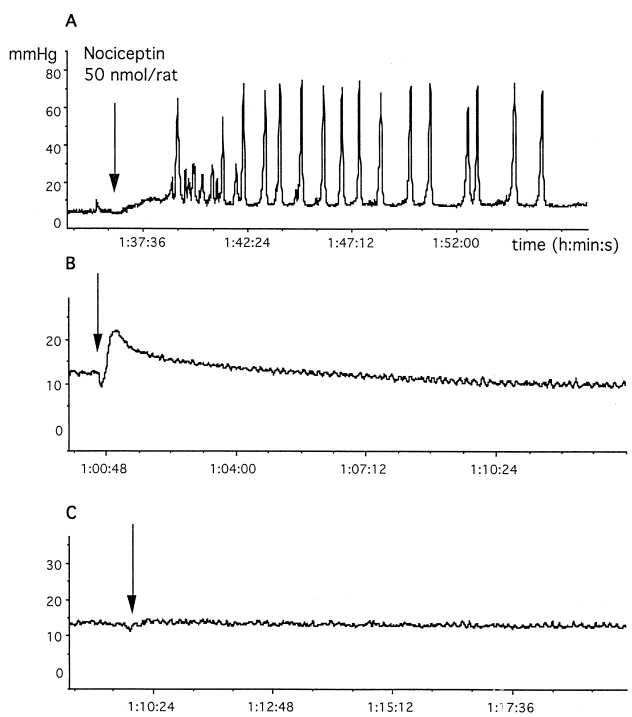

Fig. 1. Traces from three different animals showing the effect of application of nociceptin (50 nmol/rat) onto the urinary bladder serosa (arrows) on the intravesical pressure in different experimental conditions: (A) in control intact rat; (B) after acute (2 h before) bilateral ablation of pelvic ganglia; (C) after acute bilateral ablation of pelvic ganglia and pretreated (30 min before) with tachykinin NK_1 (SR 140333, 1 μmol/kg, i.v.) and NK_2 receptor antagonist (MEN 11420, 0.1 μmol/kg, i.v.).

Table 1
Effect of co-administration of SR 140333 (1 μmol/kg, i.v.) and MEN 11420 (0.1 μmol/kg, i.v.) on bladder contractions induced by nociceptin (50 nmol/rat, 50 μl), carbachol (50 nmol/rat, 50 μl) or capsaicin (2.5 nmol/rat, 50 μl) in rats subjected to acute, bilateral ablation of pelvic ganglia
MAC, maximal amplitude of contraction; AUC, area under the curve. Each value represents the mean ± S.E.M. of eight experiments.

Treatment	Saline		Nociceptin		Carbachol		Capsaicin	
	MAC (mm Hg)	AUC (mm Hg s)	MAC (mm Hg)	AUC (mm Hg s)	MAC (mm Hg)	AUC (mm Hg s)	MAC (mm Hg)	AUC (mm Hg s)
Vehicle	3.6 ± 0.7	918 ± 165	8.4 ± 0.8[a]	1649 ± 157[a]	39.5 ± 4.4[a]	15776 ± 1690[a]	15.6 ± 1.5[a]	3394 ± 472[a]
SR 140333 + MEN 11420	3.7 ± 0.6	834 ± 143	4.4 ± 1.1[b]	1063 ± 198[c]	32.4 ± 5.5[a]	12118 ± 2031[a]	6.8 ± 1.1[a,b]	1087 ± 247[b]

[a] Student's *t*-test for paired data: $P < 0.01$ vs. saline.
[b] Student's *t*-test for unpaired data: $P < 0.01$ vs. vehicle.
[c] Student's *t*-test for unpaired data: $P < 0.05$ vs. vehicle.

50 nmol/rat, respectively. The topical application of saline (50 μl, $n = 6$) did not elicit any response.

When nociceptin (5 and 50 nmol/rat) was topically applied to preparations with the bladder filled with a volume (0.8–1.5 ml) sufficient to maintain ongoing distension-induced micturition reflex contractions, an early excitatory effect was followed by an inhibition of distension-evoked motility. The excitatory effect consisted of enhancement of the amplitude of distension-induced micturition reflex contractions that was significant at both 5 (from 40 ± 3 to 58 ± 5 mm Hg, $n = 6$, $P < 0.01$) and 50 nmol/rat (from 37 ± 4 to 49 ± 4 mm Hg, $n = 6$, $P < 0.01$) of nociceptin, whereas the topical application of saline (50 μl/rat) had no effect (from 42 ± 4 to 40 ± 2 mm Hg, $n = 6$ n.s.). A slight increase in basal bladder pressure was also consistently observed with both doses of nociceptin (not shown). These excitatory effects lasted 9 ± 1 and 11 ± 2 min for the doses of 5 and 50 nmol/rat, respectively. Thereafter, there was a complete suppression of distension-induced micturition reflex contractions and bladder motility remained inhibited for 18 ± 4 and 32 ± 5 min for the two doses tested, respectively (saline: 0.4 ± 0.3 min, $P < 0.01$ vs. dose of 5 and 50 nmol/rat). Neither the inhibitory nor the excitatory effects of nociceptin (5 or 50 nmol/rat) were altered by guanethidine pretreatment (68 μmol/kg, s.c., 18 and 1 h before, $n = 5–6$, data not shown).

In rats with bilateral ablation of pelvic ganglia (1–2 h before), the topical application of nociceptin (50 nmol/rat) only elicited a low amplitude tonic-type contraction (Fig. 1B), which was abolished by the co-administration of tachykinin NK_1 (SR 140333, 1 μmol/kg, i.v.) and NK_2 (MEN 11420, 0.1 μmol/kg, i.v.) receptor antagonists (Fig. 1C and Table 1). SR 140333 and MEN 11420 also reduced the tonic-type contraction elicited by the topical application of capsaicin (2.5 nmol/rat) but not that induced by carbachol (50 nmol/rat) (Table 1).

4. Discussion

Previous studies have shown that i.v. administration of nociceptin inhibits the volume- or capsaicin-evoked micturition reflex in anesthetized rats and this effect involves the inhibition of excitability of capsaicin-sensitive bladder afferent nerves (Giuliani et al., 1999; Lecci et al., 2000). The present results indicated that, when nociceptin is applied topically onto the rat urinary bladder, its inhibitory effect of on distension-induced micturition reflex contractions is preceded by a transient excitatory effect that was evident as increased amplitude of distension-induced micturition reflex contractions. The excitatory effect of topically applied nociceptin was best appreciated when the bladder was kept quiescent, below the volume threshold for eliciting distension-induced micturition reflex contractions. Under these conditions, topically applied nociceptin

produced a local contractile effect mediated by tachykinin release from afferent nerves, and triggered the micturition reflex. Overall, the excitatory effects of topically applied nociceptin (potentiation of distension-induced micturition reflex contractions, induction of a tachykinin-mediated contraction and reflex contraction in the quiescent bladder) are super-imposable on the effects produced by topical application of capsaicin under comparable experimental conditions (cf. Maggi et al., 1984; Lecci et al., 1998). It has to be noted that an excitatory effect of nociceptin on the micturition reflex was never observed following its i.v. administration at doses up to 100 nmol/kg (Giuliani et al., 1998, 1999; Lecci et al., 2000). It remains possible that higher i.v. doses of neuropeptide are required to obtain an excitatory effect on the micturition reflex. However, an inhibitory effect of nociceptin on distension-induced micturition reflex contractions, similar to that observed after its i.v. administration, was evident after topical application of neuropeptide, when excitatory effect had subsided. Moreover, both doses tested in this study (5 and 50 nmol/rat) sequentially determined the excitatory and inhibitory effects on the micturition reflex, it was only the duration of the excitatory and inhibitory effect that was related to the dose of nociceptin applied to the viscus. These observations suggest that a differential access of nociceptin to different regions of sensory nerves (axons vs. nerve terminals) after i.v. and topical administration, respectively, may be involved in determining whether a pure inhibitory effect (i.v. administration) or a mixed excitatory and inhibitory effect (topical administration) can be observed in response to this neuropeptide.

The present findings are similar to the observations of Inoue et al. (1998) and Sakurada et al. (1999) who reported that the intraplantar or intrathecal administration of nociceptin in mice induces a pain-related behavioral response ascribable to the release of tachykinins from capsaicin-sensitive afferent nerves. In this context, it is worth mentioning that the opioid-like receptors (ORL1) can couple with a variety of transduction systems, some of which (the opening of K^+ channels) decrease neuronal excitability (Ikeda et al., 1997), whereas others (e.g., phospholipase C, inhibition of adenylate cyclase) could increase it (Cheng et al., 1997; Lou et al., 1997). Inoue et al. (1998) proposed that phospholipase C activation be involved in the sensory nerve stimulation induced by the intraplantar administration of nociceptin in mice. Also in the experiments of Inoue et al. (1998), the route of administration (intraplantar) probably allowed direct access of nociceptin to peripheral sensory nerve terminals.

The biphasic effect of topical nociceptin on excitability of sensory nerves, which regulate the micturition reflex in rats, is reminiscent of the biphasic effect on the pain threshold that is observed after intrathecal administration of nociceptin in rodents (Xu et al., 1999). The findings of Inoue et al. (1998) and the present findings suggest that access to the regions of sensory nerves from which transmitters are released could be an important factor to detect the excitatory effect of nociceptin.

In conclusion, the present findings provide further evidence that nociceptin is capable of profoundly affecting the micturition reflex through a peripheral site of action and that capsaicin-sensitive afferent nerves are a major target for the action of this neuropeptide. The present findings also indicate that if nociceptin is allowed to have access to peripheral endings of sensory nerves, its action is biphasic, a transient excitation preceding the inhibitory effect. The mechanisms underlying the two effects of nociceptin on micturition reflex await further investigation.

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