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The inhibitory effect of nociceptin on the micturition reflex in anaesthetized rats

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1 We have investigated the effect of nociceptin on the micturition reflex evoked by distension or topical application of capsaicin on the urinary bladder of urethane-anaesthetized rats.

2 Nociceptin produced a dose-dependent (3–100 nmol kg^{-1} i.v.) transient suppression of the distension-evoked micturition reflex: its effect was not modified by guanethidine (68 μmol kg^{-1} s.c.) nor by bilateral cervical vagotomy, alone or in combination, and by naloxone (1.2 μmol kg^{-1} i.v.).

3 Nociceptin (100 nmol/kg i.v.) slightly (about 30%) inhibited the contractions of the rat bladder produced by pre- or postganglionic electrical stimulation of the pelvic nerve.

4 Nociceptin almost totally abolished the reflex component of the response to topical capsaicin (1 μg in 50 μl).

5 In the rat isolated bladder, submaximal contractions produced by electrical field stimulation were slightly reduced (25±4% inhibition) by 1 μM nociceptin. Nociceptin did not affect the contraction of the rat bladder induced by acetylcholine (10 μM) or ATP (1 mM).

6 These findings indicate that nociceptin exerts a naloxone-resistant suppression of the volume-evoked micturition reflex which involves inhibition of transmitter release from postganglionic bladder nerves. An inhibitory effect on bladder afferent nerves is also suggested.

Keywords: Nociceptin; micturition reflex; urinary bladder; pelvic stimulation

Introduction

The novel neuropeptide nociceptin is considered the putative endogenous ligand for the opioid-like ORL_1 receptor (Wang *et al*., 1994; Meunier *et al*., 1995; Henderson and McKnight, 1997 for review). In the peripheral nervous system, nociceptin exerts a number of biological actions which, in several instances are at least qualitatively comparable to those exerted by opioid peptides: as an example, nociceptin inhibits the depolarization-evoked transmitter release from both cholinergic, sympathetic and sensory nerve endings as demonstrated, for e.g. in the guinea-pig ileum, left atrium, trachea and renal pelvis, mouse vas deferens and rat trachea (Giuliani & Maggi, 1996; 1997; Calò *et al*., 1996; Berzetei-Gurske *et al*., 1996; Helyes *et al*., 1997; Patel *et al*., 1997).

In all these instances, the inhibitory effect of nociceptin has been shown to be resistant to naloxone or other relevant opioid receptor antagonists. This indicates that nociceptin exerts its effects through its own receptor(s), putatively the ORL_1 receptor, which is expressed at various sites in the peripheral nervous system (Wang *et al*, 1994).

Although the physiological implications of nociceptin effects in the peripheral nervous system are unclear, a few studies have documented the ability of this neuropeptide to affect autonomic functions *in vivo*. The i.v. administration of nociceptin elicits a profound and long lasting hypotension and bradycardia in anaesthetized rats, an effect which seems dependent upon a concomitant suppression of the sympathetic and parasympathetic outflows to the cardiovascular system (Giuliani *et al*., 1997). Moreover, nociceptin could have a direct vasodilator action, at least in certain vascular beds (Gumusel *et al*., 1997; Czapla *et al*., 1997).

The peptides of the opioid family are known to exert a powerful inhibition of the micturition reflex in rats (Dray *et al*., 1985; Dray & Metsch, 1984a,b; Maggi *et al*., 1989a). The suppressant effect of opioid peptides is easily documented after the administration in the central nervous system and may involve different mechanisms, both spinal and supraspinal sites of action (Maggi & Meli, 1986; Maggi, 1991 for reviews). In this study we present evidence that intravenously (i.v.) administered nociceptin exerts a profound and long lasting suppressant effect on the micturition reflex in urethane-anaesthetized rats via a naloxone-resistant mechanism and we have partly characterized the mechanisms involved.

Methods

In vivo *experiments*

Male albino rats of Wistar strain (Charles River, Calco, Italy), weighing 340–400 g, were anaesthetized with urethane (1.2 g kg^{-1} s.c.). The body temperature was kept constant at 36.5°C and the animals were tracheotomized.

The urinary bladder was prepared for recording of intraluminal pressure by the transurethral route as described previously (Maggi *et al*., 1984a,b; 1986). Briefly, through a midline incision of the abdomen, the urinary bladder was exposed, emptied of urine and cannulated with a polyethylene tubing (PE 90, Clay Adams) through a small urethral incision and secured in place by means of a silk ligature. The tubing was connected to a pressure transducer and the whole system filled with saline (0.9% NaCl). The intravesical pressure signal was delivered to a Hewlett-Packard (HP) carrier amplifier and displayed on a HP four channel polygraph 7754A. The ureters were ligated in the middle region to maintain constant the bladder volume. The right jugular vein was cannulated for i.v. administration of drugs.

In some experiments the blood pressure was recorded through a polyethylene catheter inserted into the left carotid artery, connected to a pressure transducer and a HP 8805D

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pressure amplifier. The blood pressure signal was used to trigger a cardiotachometer (HP 15050A) for heart rate recording.

The activity of nociceptin was tested in two models of micturition reflex as follows: (a) distension-induced rhythmic contractions (DIRCs); and (b) contractions induced by topical application of capsaicin. In a first series of experiments the micturition reflex was evoked by filling the urinary bladder via the transurethral catheter with an amount of saline (>0.5 ml) sufficient to trigger a regular series of distension-induced rhythmic contractions (DIRCs) which in previous studies, were characterized as being sustained by the activation of a supraspinal vesico-vesical micturition reflex (Maggi *et al*., 1986). Under isovolumetric recording conditions and urethane anaesthesia the DIRCs are a suitable model for quantitative studies on the effects of drugs on micturition reflex (Maggi *et al*., 1984a; 1987): a similar model has been used previously to study the effect of opioid peptides on micturition (Dray & Metsch, 1984a,b).

Nociceptin was administered after an observation period of at least 30 min with regular values of frequency and amplitude of DIRCs. Only one dose of nociceptin was administered to each animal and its effect was followed for 1 h. Some experiments were performed after the bilateral section of the vagi at the cervical level (bilateral vagotomy). These animals were artificially ventilated by means of a respirator for small rodents (Basile, Italy) (70 strokes min^{-1}, 0.8 ml/100 g body weight). Some experiments were performed in rats pretreated with guanethidine (68 μmol kg^{-1} s.c., administered two times, 18 and 1 h before the experiment). The effect of nociceptin was calculated as Δ intercontraction interval which is the difference between the intercontraction interval after and before nociceptin administration.

In separate experiments, we studied the effect of nociceptin on the motor response of the rat urinary bladder produced by the topical application of capsaicin (1 μg in 50 μl) onto the serosal surface of the viscus as described in previous studies (Maggi *et al*., 1984b; Lecci *et al*., 1995). In these experiments the bladder were filled with an amount of saline (<0.5 ml) which is below the threshold for activating the volume-evoked micturition reflex. As shown previously, the topical application of capsaicin produces two distinct motor responses in these conditions: a transient, tonic-type local response produced by the release of tachykinins from sensory nerves in the bladder wall and a more sustained series of phasic contractions which represents a vesico-vesical supraspinal micturition reflex initiated by chemical stimulation of afferent nerves (chemoceptive micturition reflex) (Maggi *et al*., 1984b; 1986; Lecci *et al*., 1993; 1995).

The preparations were allowed to equilibrate for 2 h. Nociceptin was administered by the i.v. route 5 min before the challenge with topical capsaicin. In some experiments the pelvic nerves were bilaterally sectioned to study the effect of nociceptin in deafferented bladder. The contractile activity induced by topical administration of capsaicin was calculated as the area under the curve (AUC) by using a Mini-mop apparatus (Kontron, Germany). Two AUC measurements were made: (a) the total AUC of the response to capsaicin; and (b) the AUC underlying the rhythmic contractions superimposed onto the tonic-type contractions.

Pre- and postganglionic electrical stimulation of the pelvic nerve

In another series of experiments the pelvic nerve, which provides the excitatory nerve supply to the urinary bladder, was electrically stimulated at a preganglionic and postganglionic site, as described previously (Maggi *et al*., 1985a). The preganglionic or the postganglionic branch of the left pelvic nerve, both lying on the lobar prostatic wall, were exposed and gently isolated (0.3–0.4 cm) for mounting on a bipolar platinum hook electrode. The contralateral nerve was sectioned at the postganglionic level to avoid activation of reflex motility. The ipsilateral nerves were sectioned proximally to the stimulating electrode toward the spinal cord. The abdomen was filled with mineral oil and the nerve/electrode site protected with cotton pellets soaked with mineral oil to avoid the spread of current. The bladder was filled with an amount of saline (<0.5 ml) insufficient to trigger the volume-evoked micturition reflex.

Square wave pulses were delivered by means of a Grass S88 stimulator, connected to the electrode through a stimulus isolation unit (SIU 5): the electrical pulses were delivered at a frequency of 5 Hz with trains of 2 s every 60 s (submaximal parameters ranging from 0.01 and 0.1 ms for pulse width at 10–15 V). In each preparation the pulse width was then adjusted (between 0.01 and 0.1 ms) to produce contractions of submaximal amplitude. In each preparation, after having recorded the effect of nociceptin on the bladder contractions produced by electrical stimulation of the pelvic nerve, i.v. hexamethonium (0.11 mmol kg^{-1}) was administered to check the correct pre- or postganglionic placement of the stimulating electrode (Figure 4, see results).

In vitro *experiments*

The activity of nociceptin was also evaluated on the electrically-induced contractile activity in isolated bladder strips. The animals were sacrificed by cervical dislocation. The whole urinary bladder was rapidly removed, half bladder strips excised and placed in a 5 ml organ bath containing oxygenated (96% O_2 and 4% CO_2, pH 7.4 at 37°C) Krebs solution, of the following composition: NaCl, 119 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.5 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM and glucose 11 mM. A resting load of 10 mN was applied and tension was recorded by means of an isometric force transducer connected to a Basile 7050 Unirecord. After 60–90 min equilibration period the preparations were stimulated with single electrical pulses delivered at a frequency of 0.1 Hz and 0.5 ms pulse width, maximal voltage until steady state responses had been obtained. The voltage and pulse width were then reduced to obtain submaximal contractile responses approaching about 80% the maximal response to single pulse electrical field stimulation (EFS). In separate experiments, the contraction of bladder strips to acetylcholine (10 μM) or ATP (1 mM) was recorded at 20 min intervals until two reproducible responses were obtained. Nociceptin (1 μM) was administered 5 min before the next challenge with the agonist.

Statistical analysis

All values in the text, tables or figures are means ± s.e.mean. Statistical analysis was performed by means of Student's *t*-test for unpaired data or by means of one-way analysis of variance (ANOVA), followed by Dunnett test for multiple comparisons, when applicable. Regression analysis was performed by the Graph Pad InStat 2.01 computer program (parametric Pearson correlation). A P level <0.05 was considered statistically significant.

Drugs

Drugs used were: nociceptin (Neosystem, Strasbourgh, France), hexamethonium bromide, naloxone, acetylcholine chloride, capsaicin, adenosine 5′-triphosphate (ATP) and guanethidine sulfate (Sigma, St. Louis, MO, U.S.A.).

Results

Effect of nociceptin on DIRCs

Isovolumetric distension (>0.5 ml) of the rat urinary bladder by the transurethral route, activated a regular series of reflex rhythmic contractions of the viscus whose amplitude was 29 ± 4 mmHg with frequency of 31 ± 6 contractions h^{-1} ($n=7$) (Figure 1). Nociceptin produced a dose-dependent (3–100 nmol kg^{-1} i.v.) transient suppression of the distention-induced reflex contractions (DIRCs) within 0.5–3 min from administration, the duration of this suppressant effect being related to the dose administered (Figure 2). At the highest dose tested (100 nmol kg^{-1}, $n=7$) the suppressant effect of nociceptin lasted for 22 ± 4 min (Figures 1 and 2). Both onset and offset of the action of nociceptin had an all-or-none character (Figure 1): even at the lower doses tested, the DIRCs were totally suppressed by the neuropeptide and, upon recovery, DIRCs showed similar amplitude as those recorded before nociceptin administration. A dose of 100 nmol kg^{-1} was selected for further experiments. This dose induces a consistent and long lasting suppression of the micturition reflex, in particular, a suppressant effect which clearly exceeds the duration of the hypotension and bradycardia induced by nociceptin (Figure 1).

We reported previously that i.v. nociceptin induces a profound hypotension and bradycardia in anaesthetized rats which involve changes in both sympathetic and parasympathetic (vagal) control of cardiovascular function (Giuliani *et al*, 1997). The resting values of systolic, diastolic blood pressure and heart rate of urethane-anaesthetized rats are showed in Table 1. In this series of experiments, i.v. nociceptin lowered systolic and diastolic blood pressure by 28 ± 6 and 40 ± 5 mmHg, respectively and reduced heart rate by 141 ± 28 beats/min ($n=6$) (Table 2). As shown in Table 2, vagotomy alone did not affect the hypotension induced by nociceptin but inhibited the bradycardic response by about 70%; on the other hand, guanethidine pretreatment alone significantly reduced the reduction in systolic and diastolic blood pressure induced by nociceptin (by about 68 and 75%, respectively) and at a lesser extent (about 37% inhibition) also significantly inhibited the nociceptin-induced bradycardia (Table 2). Combining vagotomy plus guanethidine pretreatment almost totally abolished all the cardiovascular responses to i.v. nociceptin (Table 2).

Neither guanethidine pretreatment nor vagotomy alone or in combination, did significantly affect the characteristics of DIRCs (Table 3). Moreover as shown in Figure 2, neither

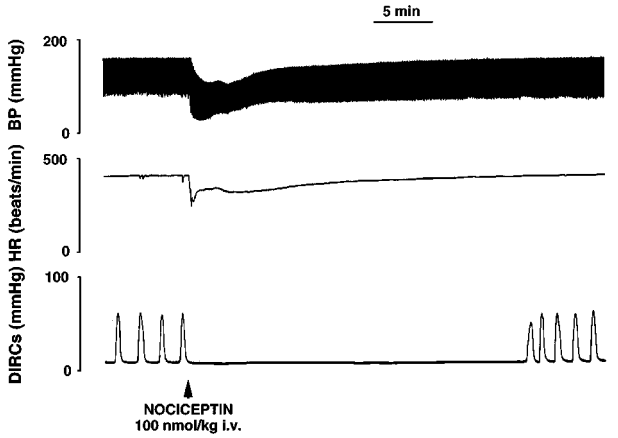

Figure 1 Typical traces from the same animal showing the effect of i.v. nociceptin (100 nmol kg^{-1}) on blood pressure (BP, panel a), heart rate (HR, panel b) and distention-induced rhythmic contractions (DIRCs) of the urinary bladder (panel c) in urethane-anaesthetized male rats.

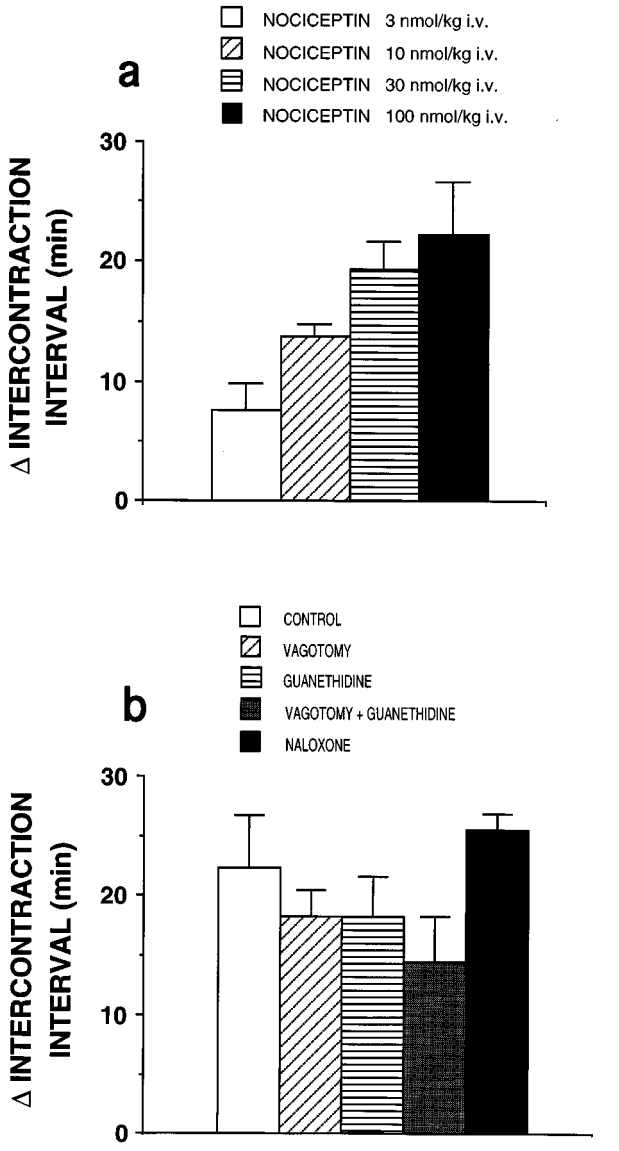

Figure 2 Dose-dependent suppressant effect of nociceptin (3–100 nmol kg^{-1} i.v.) on the distension-induced micturition reflex (a) and effect of acute vagotomy, guanethidine pretreatment (68 μmol kg^{-1} s.c. in two times 18 and 1 h before experiment), a combination of both treatments or naloxone (1.2 μmol kg^{-1} i.v.) on the suppression of micturition reflex, expressed as Δ intercontraction interval, induced by nociceptin (100 nmol kg^{-1} i.v.) in urethane-anaesthetized male rats (b). Each values is the mean ± s.e.mean of 5–7 experiments. Δ intercontraction interval is the difference between the intercontraction interval after and before nociceptin administration.

Table 1 Resting cardiovascular parameters (systolic blood pressure, SBP, diastolic blood pressure, DBP and heart rate, HR) in controls or after bilateral vagotomy, guanethidine pretreatment or the combination of both in urethane-anaesthetized rats

	No.	*SBP* (mmHg)	*DBP* (mmHg)	*HR* (beats/min)
Controls	6	143±7	73±6	410±17
Vagotomy	4	140±10	76±6	415±14
Guanethidine	4	129±14	50±5	370±16
Vagotomy + guanethidine	4	154±13	66±3	421±15

Each value is the mean±s.e.mean. The values are not significantly different from controls (Dunnett test for multiple comparisons).

Table 2 Effect of nociceptin (100 nmol/kg i.v.) on systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in controls, after bilateral vagotomy, guanethidine pretreatment or the combination of both in urethane-anaesthetized rats

	No.	*SBP* (mmHg)	*DBP* (mmHg)	*HR* (beats/min)
Controls	6	−28±6	−40±5	−141±28
Vagotomy	4	−37±8	−42±4	−43±8*
Guanethidine	4	−9±1*	−10±1*	−89±13*
Vagotomy + guanethidine	4	0±0*	−8±1*	−4±2*

Each value is the mean±s.e.mean. *$P<0.05$, significantly different from controls (Dunnett test for multiple comparisons).

Table 3 Resting parameters of the distension induced rhythmic contractions (DIRCs) of the urinary bladder in controls, following bilateral vagotomy and guanethidine or naloxone pretreatment in urethane-anaesthetized rats

	No.	*Amplitude* (mmHg)	*Frequency* (contractions/h)
Controls	7	29±4	31±6
Vagotomy	5	35±6	18±6
Guanethidine	6	39±4	42±5
Vagotomy + guanethidine	5	42±1	43±6
Naloxone	7	41±4	45±6

Each value is the mean±s.e.mean. The values are not significantly different from controls (Dunnett test for multiple comparisons).

bilateral vagotomy nor guanethidine pretreatment, alone or in combination, modified the suppressant effect of i.v. nociceptin (100 nmol kg^{-1}) on DIRCs.

After naloxone pretreatment the DIRCs had higher amplitude and frequency than controls, although the effect did not reach the level of statistical significance (Table 3). Naloxone (1.2 μmol kg^{-1} i.v., 5 min before, $n=7$) did not prevent the inhibitory effect of nociceptin (100 nmol kg^{-1} i.v.) on DIRCs (Figure 2).

Effect of nociceptin on the urinary bladder response to topical capsaicin

The topical application of capsaicin (1 μg 50 μl^{-1}) onto the serosal surface of the rat urinary bladder induced a prompt tonic-type contraction on which a series of rhythmic contractions superimposed whose amplitude was

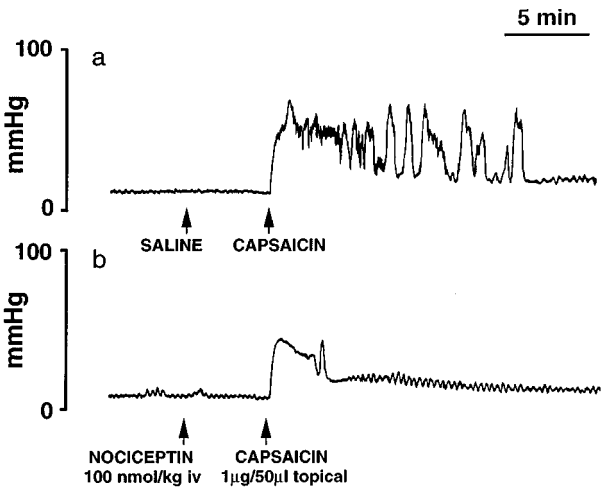

Figure 3 Typical traces from two separate experiments showing the effect of topical capsaicin (1 μg 50 μl^{-1}) applied on the serosal surface of the urinary bladder in urethane-anaesthetized male rats after vehicle (a) or nociceptin administration (b). Nociceptin (100 nmol kg^{-1} i.v., 5 min before capsaicin) almost abolished the rhythmic contractions of reflex origin leaving the initial tonic type effect unaffected.

52±5 mmHg (Figure 3, $n=8$). The total AUC of the response to capsaicin was slightly but not significantly smaller in rats pretreated with nociceptin (100 nmol kg^{-1} i.v. 5 min before) than in controls, (the AUC averaged 394±59 and 274±40 mm^2 in vehicle- and nociceptin-treated rats, respectively, $n=8$ in each group, n.s.). However, at a close inspection, it was evident (Figure 3) that nociceptin prevented the occurrence of phasic contractions produced by topical capsaicin. This effect was quantified by separately measuring the AUC of the phasic contractions superimposed onto the tonic response: the inhibitory effect of nociceptin averaged about 87% (155±39 and 21±5 mm^2 in vehicle and nociceptin-treated rats, respectively, $n=8$ in each group, $P<0.05$). These results suggest that the inhibitory effect of nociceptin is preferentially if not exclusively exerted on the sensory or reflex component of the response to capsaicin, whereas the local or 'efferent' part of the response would be less affected. To verify this hypothesis, separate experiments were performed in which topical capsaicin was applied to the rat bladder after bilateral section of the pelvic nerves: in these conditions, the topical application of capsaicin only produces a tonic-type contraction ascribable to tachykinins released from sensory nerves in the bladder wall (Maggi *et al.*, 1984b; Lecci *et al.*, 1993). The tonic type contraction produced by topical capsaicin in acutely denervated bladders was slightly but not significantly reduced by nociceptin pretreatment (the AUC was 234±48 *vs* 144±47 mm^2 in vehicle- and nociceptin-treated rats, respectively, $n=8$ in each group, n.s.).

Effect of nociceptin on the response to electrical stimulation of the pelvic nerve

The pelvic nerve was unilaterally stimulated *in vivo*, at either pre- or postganglionic level (5 Hz for 2 s, 0.01–0.1 ms pulse width at 10–15 V every 60 s). The pre- or postganglionic site of stimulation was verified at the end of the experiments by checking the effect of i.v. hexamethonium (Figure 4). The bladder contractions produced by preganglionic stimulation (8.7±1.7 mmHg, $n=5$) were markedly reduced (82±4%

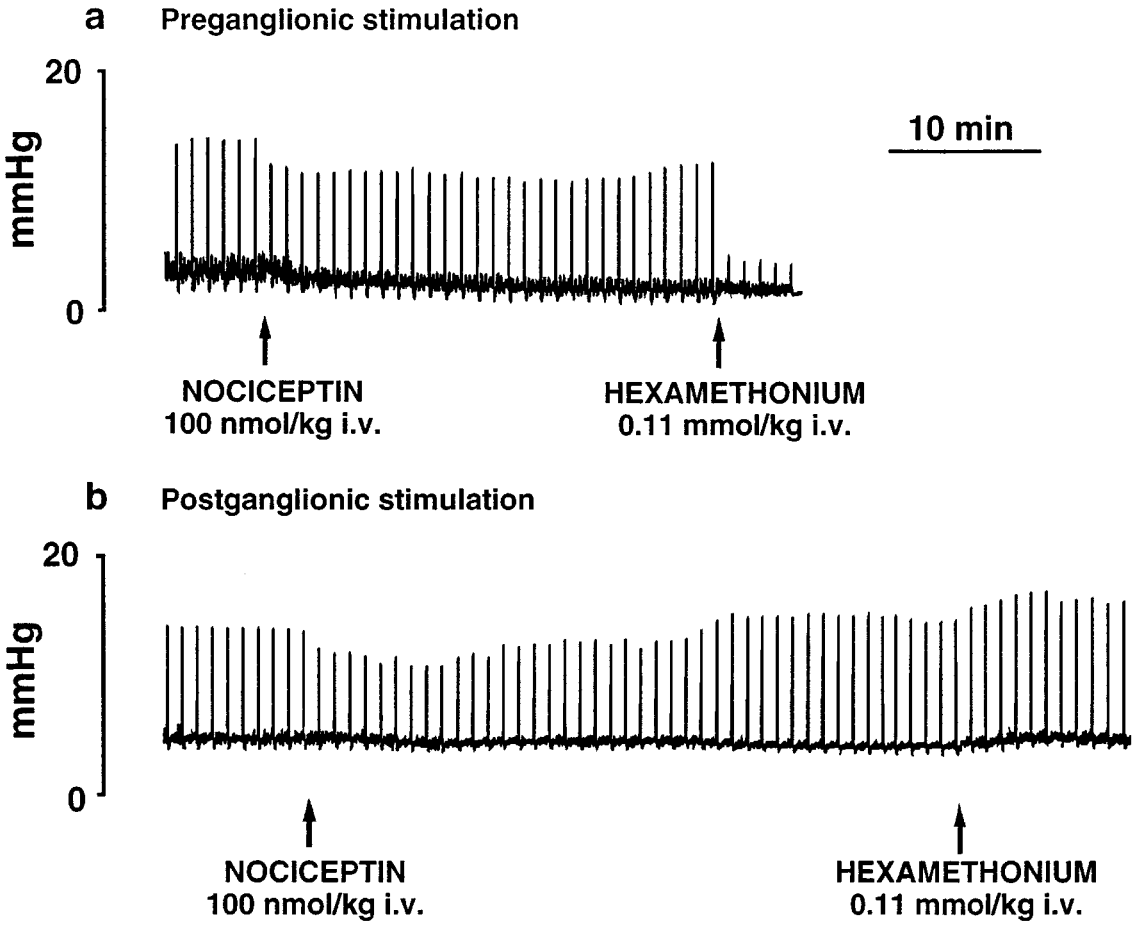

Figure 4 Typical traces from two separate experiments showing the effect of nociceptin (100 nmol kg^{-1} i.v.) on contractions of the urinary bladder produced by preganglionic (a) or postganglionic (b) stimulation (5 Hz for 2 s, 10–15 V, 0.01–0.1 ms every 60 s) of the left pelvic nerve (the contralateral pelvic nerve had been sectioned) in urethane-anaesthetized rats. Hexamethonium (0.11 mmol kg^{-1} i.v.) inhibits the contractions to preganglionic but not to postganglionic stimulation.

inhibition) by hexamethonium (0.11 mmol kg^{-1} i.v.); the bladder contractions produced by postganglionic stimulation (9.1 ± 2.2 mmHg, $n=4$) were slightly increased ($16 \pm 13\%$) by hexamethonium.

Nociceptin (100 nmol kg^{-1} i.v.) reduced significantly the bladder contractions to preganglionic nerve stimulation ($31 \pm 7\%$ inhibition, $n=5$). The peak inhibitory effect was attained within 3 min and a complete recovery of the amplitude of contractions was observed after 16 ± 2 min. Nociceptin (100 nmol kg^{-1} i.v.) inhibited significantly the bladder contractions to postganglionic nerve stimulation by $32 \pm 4\%$ ($n=4$). The peak effect was attained within 3 min and a complete recovery of the amplitude of contractions was observed after 14 ± 2 min.

In vitro *experiments*

Single pulse EFS (0.1 Hz, 0.5 ms, 60 V) produces twitch contractions of bladder strips averaging 12.6 ± 1.2 mN ($n=11$). When the amplitude of EFS-evoked twitches had reached a steady state, the parameters of stimulation were lowered in each preparation: pulse width was lowered to 0.2 ms and voltage was adjusted to 35–50 V to obtain twitch contractions of submaximal amplitude (<95% of control). At steady state the amplitude of twitches averaged $81 \pm 4\%$ (range 59–95%, $n=11$) of the maximal response to single pulse EFS.

In preliminary experiments we observed that, if applied cumulatively, nociceptin (0.01–3 μM) produces an inconsistent inhibition of twitches ($-5 \pm 5\%$ at 1 μM) with a flat concentration-response curve. Therefore we studied the effect of a single concentration of nociceptin (1 μM): this concentration inhibited EFS-induced twitches by $25 \pm 4\%$ ($n=11$) peaking at about 2 min from administration; no sign of recovery from the inhibition was observed during a 30 min observation period in the presence of the peptide. A highly significant inverse correlation ($r=-0.763$, $n=11$, $P<0.01$) was found between the intensity of the inhibitory effect exerted by 1 μM nociceptin and the amplitude of twitches, expressed as % of the maximal twitch amplitude to single pulse EFS, as determined at steady state before lowering of parameters of stimulation. In other words the intensity of twitches depression induced by nociceptin was more intense when the amplitude of twitches was lower..

The response of the rat isolated bladder to single pulse EFS consists of a fast and a slow component, ascribable to ATP and acetylcholine release, respectively (Maggi *et al.*, 1985b). In this series of experiments the slow component was well evident in 4 out of 11 strips tested: nociceptin (1 μM) inhibited the fast and slow component of the response to single pulse EFS by 25 ± 4 and $23 \pm 2\%$, respectively ($n=11$ and 4, n.s.). To assess a possible postjunctional site of action, the effect of nociceptin (1 μM, 5 min before) was evaluated on contraction of bladder strips induced by 10 μM acetylcholine and 1 mM ATP, the two

mediators released from postganglionic excitatory nerves in the rat bladder. Nociceptin had no inhibitory effect on the response to either agent: the response to acetylcholine averaged 21 ± 3 and 20 ± 3 mN in the absence and presence of nociceptin ($n = 4$, n.s.); the response to ATP averaged 18 ± 2 and 18 ± 2 mN in the absence and presence of nociceptin ($n = 4$, n.s.).

Discussion

Our findings indicate that nociceptin exerts a profound naloxone-insensitive depressant action on the micturition reflex in rats. Although i.v. nociceptin elicits a transient and intense bradycardia and hypotension (Giuliani *et al*., 1997), its suppressant effect on the micturition reflex is not secondary to an action on cardiovascular parameters: in fact, the action of nociceptin on reflex bladder contractions was not significantly affected by combining guanethidine pretreatment and bilateral vagotomy, a procedure which almost completely eliminates the cardiovascular depressant effects of this neuropeptide. In our experiments, the sympathetic nerve supply to the bladder was intact: therefore we cannot totally exclude that the inhibitory effect of nociceptin on the micturition reflex may have involved an activation of sympathetic bladder innervation by releasing some inhibitory transmitters stored in these nerves. However, the observation that guanethidine, at a dose which inhibits the depressant action of nociceptin on cardiovascular function, was without effect on the inhibitory effect exerted by nociceptin on the micturition reflex argues against this interpretation.

At least part of the inhibitory action of nociceptin may involve a depressant action on the release of excitatory transmitters from postganglionic efferent nerves. In fact nociceptin inhibited (by about 30%) the *in vivo* bladder contractions produced by electrical stimulation of the pelvic nerve at either pre- or postganglionic level. In some species there is evidence for opioidergic control of transmission at pelvic ganglia level (De Groat & Kawatani, 1989; Maggi and Meli, 1986 for review). Kummer and Fischer (1997) recently reported that both nociceptin and the ORL_1 receptor are expressed in guinea-pig sympathetic ganglia. No information of this type is available at present with regard to parasympathetic ganglia in general or about rat pelvic ganglia in particular: since the inhibitory effect of i.v. nociceptin had a similar intensity and time course toward bladder contractions induced by pre- and postganglionic electrical stimulation of the pelvic nerve, our data indicate a site of action on postganglionic nerves whereas an inhibitory effect on ganglionic transmission can apparently be excluded. A modulatory influence on the release of excitatory transmitters from postganglionic nerves is further indicated by experiments on the rat isolated bladder in which nociceptin was shown to inhibit twitches produced by single pulse EFS without affecting the contractile response to acetylcholine or ATP.

It is interesting to note that the time course of the transient suppressant action of i.v. nociceptin on DIRCs (mean suppression time 22 min at 100 nmol kg^{-1} i.v.) was comparable to the duration (about 15 min) of the transient inhibitory action on the contractile response to pelvic nerve stimulation. On the other hand, no recovery from inhibitory exerted by nociceptin was observed in organ bath experiments until washout of the peptide (data not shown). These observations suggest that degradation by peptidases (cf. Montiel *et al*., 1997) could be a limiting factor in the action of nociceptin *in vivo*.

The ability of nociceptin to negatively modulate transmitter release from postganglionic nerves in the urinary bladder of adult rats is interesting since morphine does not cause this effect (Carpenter *et al*., 1986; Maggi *et al*., 1989b), even if using submaximal parameters of stimulation and single pulse EFS (the same conditions used in this study). Indeed the micturition suppressant effect of systemically-administered morphine is blocked by centrally-administered naloxone and is therefore thought to involve a purely central effect (Dray & Metsch 1984b). Nociceptin and opioids share the ability to exert a prejunctional neuromodulatory effect on cholinergic, noradrenergic and peripheral sensory nerve terminals in various species (see Introduction for references). The rat urinary bladder therefore represents a notable exception, being sensitive to the neuromodulatory action of nociceptin but not to that of opioids.

An interesting character of the response to nociceptin is the all-or-none onset and offset of its inhibitory action on the micturition reflex (see Figure 1): this contrasts with the inhibitory effects produced by other type of drugs on micturition reflex in this model which, before producing a total suppression of DIRCs, can also induce graded inhibitory effect by affecting the frequency and amplitude of the volume-evoked contractions (Maggi *et al*., 1984a,b; 1987). In addition, although the time course of the depressant effect on efferent nerves and the suppression of micturition reflex by i.v. nociceptin are similar, the recovery from the former inhibitory effect is graded (Figure 4) while the recovery from the latter effect has an all-or-none character. This element further argues for the existence of multiple sites of action through which nociceptin inhibits the micturition reflex in rats. A possibility for producing an all-or-none inhibitory effect on the micturition reflex is that of a drug acting at one or more sites in the central nervous system, including the spinal cord, by switching off the neural pathways which sustain the vesico-vesical micturition reflex. At the present time, there is no published information as to whether peripherally-administered nociceptin is capable to gain access to the central nervous system in biological relevant amounts.

If assuming a purely peripheral site of action of i.v. administered nociceptin, our data suggest that, in addition to its effect on postganglionic efferent nerves, nociceptin suppresses micturition reflex through an action on afferent nerves as well. In isolated organs, nociceptin was shown to inhibit the release of sensory neuropeptides from the peripheral endings of primary afferent neurones in response to depolarizing electrical stimuli but was ineffective when capsaicin itself is used as a stimulant (Giuliani & Maggi, 1996; 1997; Helyes *et al*., 1997). In the present study we demonstrated that the contraction of the acutely denervated rat urinary bladder to topical capsaicin, which involves the release of tachykinins from sensory nerve endings in the bladder wall (Maggi *et al*., 1984b; Lecci *et al*., 1993; 1995), was unaffected by nociceptin at a dose which almost totally prevented the sensory stimulant action of capsaicin on the micturition reflex. It is important to remember that the two components of the response to capsaicin are concomitant but independent of each other. In fact, i.v. administration of peripherally-acting tachykinin receptor antagonists at doses which block the tonic-type contraction to capsaicin does not prevent the ability of capsaicin to stimulate the phasic reflex contractions (chemoceptive micturition reflex) (Lecci *et al*., 1993). By contrast, the intrathecal administration of a tachykinin NK_1 receptor antagonist blocks the chemoceptive micturition reflex induced by capsaicin without affecting the tonic-type contraction produced by tachykinins released in the

bladder wall. The present findings raise the possibility that sensory nerves themselves are a target for nociceptin action: without affecting the neurosecretory process in general (peptide release induced by capsaicin being unaffected by nociceptin) the neuropeptide may impair the generation of afferent impulses. It had to be mentioned that the bladder contraction induced by topical capsaicin in acutely denervated bladders was slightly inhibited (by about 30%) by nociceptin: although this effect did not reach the statistical significance it is possible that topical capsaicin had activated axon reflexes in the bladder *in vivo* which could be partially sensitive to the neuromodulatory effect of nociceptin (cf. Giuliani & Maggi, 1997).

In conclusion, the present findings demonstrate that nociceptin exerts a profound naloxone-resistant depressant action on the micturition reflex in rats: the effect, which occurs independently from nociceptin-induced depression of cardiovascular function, was evidenced in models involving both mechanical and chemical stimulation of bladder sensory nerves. The inhibitory effect of nociceptin on the micturition reflex may partly involve a depression of excitatory transmitter release from postganglionic nerves; if assuming a purely peripheral site of action of i.v. administered nociceptin, an impairment in generation of afferent impulses can be hypothesized.

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