

## [Tyr<sup>1</sup>]-Nociceptin and Nociceptin Have Similar Naloxone-Insensitive Erectile Activity in the Cat

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**ABSTRACT:** The heptadecapeptide nociceptin, also known as Orphanin FQ, is a newly discovered endogenous ligand for the opioid-like G-protein-coupled receptor ORL<sub>1</sub>. The present study was undertaken to investigate responses to intracavernosal injections of the nociceptin analog [Tyr<sup>1</sup>]-nociceptin and to investigate the effects of naloxone on erectile responses in anesthetized cats to [Tyr<sup>1</sup>]-nociceptin and to nociceptin. Intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin and of nociceptin in doses of 0.3–30 nmol elicited dose-related increases in cavernosal pressure, which, at the highest dose studied, were comparable to increases induced by the triple-drug standard (papaverine, phentolamine, and prostaglandin E<sub>1</sub>), a preparation used in the treatment of erectile dysfunction. Responses to

[Tyr<sup>1</sup>]-nociceptin were rapid in onset and had a time course similar to responses to nociceptin. Metenkephalin increased cavernosal pressure, whereas injections of nociceptin-(2-17), dynorphin A, and  $\beta$ -endorphin did not alter cavernosal pressure. Erectile responses to nociceptin and to [Tyr<sup>1</sup>]-nociceptin were not altered after administration of the opioid receptor antagonist naloxone at a time when erectile responses to metenkephalin were attenuated. These data show that [Tyr<sup>1</sup>]-nociceptin and nociceptin have similar naloxone-insensitive erectile activity in the cat.

**Key words:** Penile erection, Orphanin FQ, intracavernosal injection, hypotensive activity.

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It has been suggested that there exists an interaction between the opioid peptides and sexual activity (Dail et al, 1985; Berendsen and Gower, 1986; Fabbri et al, 1989). Chronic administration of opiates has been associated with loss of libido and sexual potency in men and experimental animals (Fabbri et al, 1989). Fabbri et al (1989) reported that administration of the opioid receptor antagonist naltrexone increased erectile activity in humans not being treated with opioids, suggesting that endogenous opioid peptides play a role in inhibiting erectile function. Previous studies, however, have focused on the central effect of opioid peptides on sexual activity (Berendsen and Gower, 1986; Fabbri et al, 1989). While opioid receptors are present in the testis, little, if anything, is known about the presence of opioid receptors or responses to the opioids in the corpora cavernosa of the penis (Wittert et al, 1996).

ORL<sub>1</sub> is a novel G-protein-coupled receptor that shares sequence homology to the  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid receptors (Bunzow et al, 1994; Chen et al, 1994; Fukuda et al,

1994; Mollereau et al, 1994; Nishi et al, 1994; Wang et al, 1994a; Wick et al, 1994; Halford et al, 1995). Although this novel receptor possessed the ability to inhibit adenylyl cyclase, it did not exhibit high-affinity binding to the known opioid peptides (Bunzow et al, 1994; Chen et al, 1994; Fukuda et al, 1994; Mollereau et al, 1994, 1996; Nishi et al, 1994; Wang et al, 1994a; Wick et al, 1994, 1995; Halford et al, 1995; Lachowicz et al, 1995; Nothacker et al, 1996; Pan et al, 1996). ORL<sub>1</sub> receptor transcripts are widely distributed in the central nervous system, as well as peripherally in organs, such as the spleen, kidney, vas deferens, and intestine (Bunzow et al, 1994; Chen et al, 1994; Fukuda et al, 1994; Nishi et al, 1994; Wang et al, 1994a; Lachowicz et al, 1995; Wick et al, 1995; Mollereau et al, 1996; Nothacker et al, 1996; Pan et al, 1996). However, evidence for the existence of ORL<sub>1</sub> transcripts in the corpora cavernosa has not been obtained (Wick et al, 1995; Mollereau et al, 1996; Nothacker et al, 1996; Pan et al, 1996).

Nociceptin (H<sub>2</sub>N-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-COOH), also known as Orphanin FQ, is an endogenous ligand for the "orphan" opioid receptor (ORL<sub>1</sub>). Nociceptin is a 17-amino acid peptide that shares structural homology with the dynorphin family of peptides (Meunier et al, 1995; Reinscheid et al, 1995). Nociceptin differs from other opioid peptides in that it does not possess the *N*-terminal tyrosine residue essential for activity at the  $\mu$ -,  $\delta$ -, and

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$\kappa$ -opioid receptors (Meunier et al, 1995; Reinscheid et al, 1995). The isolation of nociceptin was based on the ability of the peptide to inhibit adenylyl cyclase in ORL<sub>1</sub>-transfected cells; and, although this mechanism is similar to that induced by the traditional opioid peptides, it has been reported that responses to nociceptin are not altered by naloxone (Meunier et al, 1995; Reinscheid et al, 1995, 1996; Giudiani and Maggi, 1996; Nicol et al, 1996; Vaughan and Christie, 1996; Patel et al, 1997). Nociceptin has been shown to have novel hypotensive activity, as well as potent diuretic and antinatriuretic activity, in the rat (Champion and Kadowitz, 1997; Kapusta et al, 1997).

It has been postulated that the opioid receptors have a common binding site that interacts with the *N*-terminal pentapeptide moiety (Tyr-Gly-Gly-Phe-Met/Leu) and that the C-terminal amino acids determine the degree of relative selectivity for the ligand's respective receptor subtype (Meunier et al, 1995; Reinscheid et al, 1995). It has been suggested, therefore, that the Phe in the first position of the nociceptin sequence conveys the selectivity of this novel ligand for the ORL<sub>1</sub> receptor (Meunier et al, 1995; Reinscheid et al, 1995).

It has recently been reported that intracavernosal injection of nociceptin induces penile erection in the cat (Champion et al, 1997). However, little, if anything, is known about the effects of naloxone on the nociceptin-induced erectile response, and the effects of [Tyr<sup>1</sup>]-nociceptin, nociceptin-(2-17),  $\beta$ -endorphin, metenkephalin, or dynorphin A on penile erection have not been determined. The present study was therefore undertaken to investigate erectile function response to intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin in a cat model, which has been used in our laboratory to study changes in cavernosal pressure and penile length and to investigate the role of a naloxone-sensitive mechanism in mediating responses to nociceptin and [Tyr<sup>1</sup>]-nociceptin.

## Materials and Methods

Adult male cats weighing 2.8 to 4.8 kg were sedated with ketamine hydrochloride (10–15 mg/kg IM) and anesthetized with sodium pentobarbital (30 mg/kg IV). Supplemental doses of pentobarbital were administered, as needed, to maintain a uniform level of anesthesia. A vertical, circumcision-like incision was made to expose the two ventral corpora cavernosa and the dorsal corpus spongiosum. A 30-gauge needle was placed into the right corpus to permit administration of drugs into the penis. A 25-gauge needle was placed midway into the left corpus for the measurement of intracavernosal pressure. Systemic arterial and intracavernosal pressures were measured with Statham P23 transducers and recorded on a Grass Model 7 polygraph. Mean pressures were derived from the pulsatile signals by electronic averaging. Penile length in millimeters was measured with a ruler. These procedures have been previously described (Wang et al, 1993, 1994b; Champion et al, 1997).

Agonist injections were made when cavernosal pressure was at baseline value, and the pressure response was monitored until cavernosal pressure returned to preinjection level. The next injection of an agonist was made after a period of 15–25 minutes from the end of the preceding response to ensure a stable baseline.

In the first series of experiments, the effects of intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin on cavernosal pressure and penile length were investigated, and responses to injections of [Tyr<sup>1</sup>]-nociceptin (10 and 30 nmol) were again obtained after a 25–30-minute interval, to determine if responses were reproducible with respect to time. In each animal, [Tyr<sup>1</sup>]-nociceptin in doses of 0.3–30 nmol in a volume of 200  $\mu$ l was injected intracavernosally, and after the dose-response curve for [Tyr<sup>1</sup>]-nociceptin was obtained, the control injection of papaverine, PGE<sub>1</sub>, and phentolamine was administered for comparative purposes.

In a separate series of experiments, the increases in cavernosal pressure in response to [Tyr<sup>1</sup>]-nociceptin were compared to increases in response to intracavernosal injection of nociceptin, nociceptin-(2-17), metenkephalin, dynorphin A, and  $\beta$ -endorphin. Doses were expressed on a nanomole basis to take molecular weight into account. The duration of erectile responses and changes in systemic arterial pressure in response to [Tyr<sup>1</sup>]-nociceptin, nociceptin, and the triple-drug standard were compared.

In the last series of experiments, the influence of traditional opioid receptors in mediating erectile responses to [Tyr<sup>1</sup>]-nociceptin, nociceptin, and metenkephalin was investigated. Increases in cavernosal pressure in response to intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin, nociceptin, and metenkephalin were compared before and after injection of naloxone in a dose of 2 mg/kg IV. Naloxone injections did not alter systemic arterial pressure or baseline cavernosal pressure.

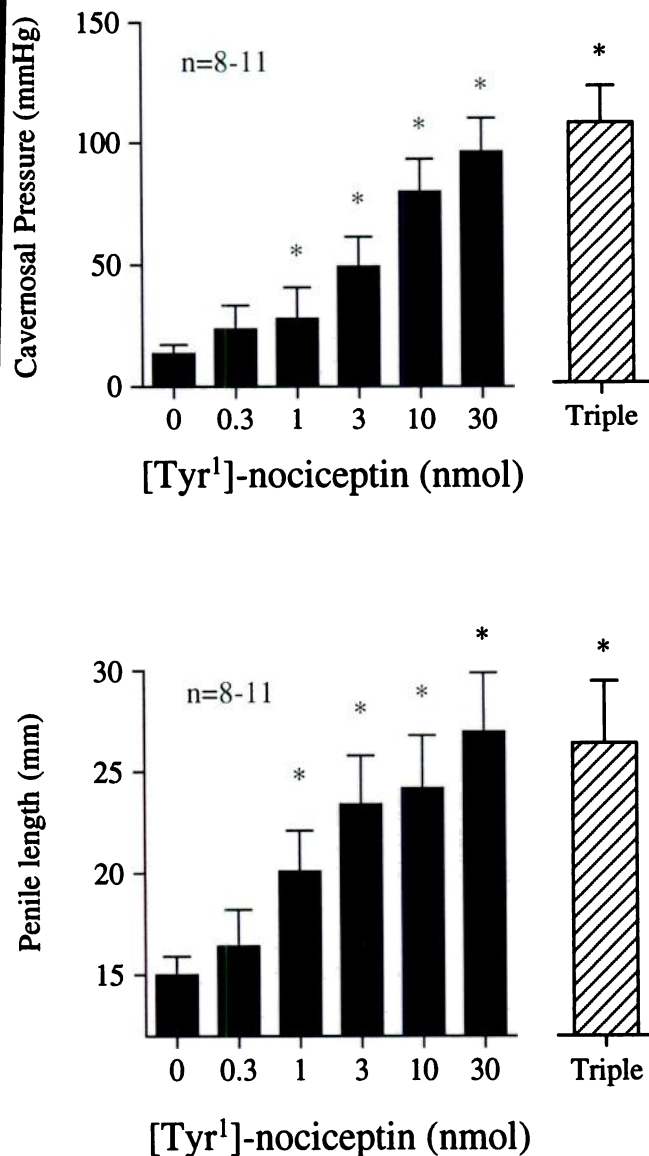
Nociceptin (Orphanin FQ), [Tyr<sup>1</sup>]-nociceptin, nociceptin-(2-17), metenkephalin, dynorphin A, and  $\beta$ -endorphin (Phoenix Pharmaceuticals, Mountain View, California) were dissolved in 0.9% NaCl, and the solutions were divided into aliquots and stored in 1-ml plastic tubes. The aliquots were stored frozen and were thawed on the day of an experiment. During the experiment, the agonist solutions were kept on crushed ice. The agonists were administered intracavernosally in small volumes (200  $\mu$ l), and, in control experiments, injection of the saline vehicle for nociceptin and [Tyr<sup>1</sup>]-nociceptin had no effect on cavernosal or systemic arterial pressure. The control triple-drug standard composed of papaverine (1.65 mg), PGE<sub>1</sub> (0.5  $\mu$ g), and phentolamine (25  $\mu$ g) (Sigma Chemical Co., St. Louis, Missouri) was prepared and injected as previously described (Wang et al, 1993, 1994b). The triple-drug standard was injected in a single dose and was used as a reference for comparison for erectile responses.

The data are expressed as mean  $\pm$  SEM and were analyzed by Student's *t*-test for single group comparison and by one-way analysis of variance with Tukey's test for multiple-group comparisons. *P* < 0.05 was the criterion for statistical significance (Snedecor & Cochran, 1987).

## Results

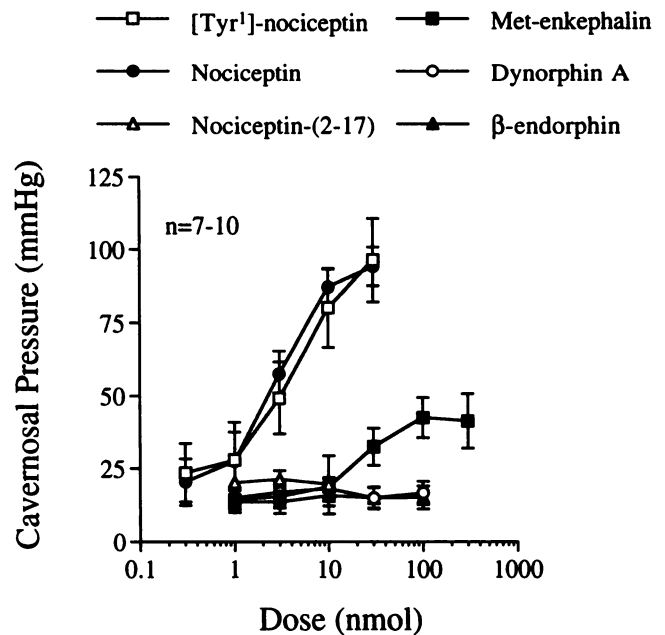
### Responses to [Tyr<sup>1</sup>]-Nociceptin and Opioid Peptides

The effects of intracavernosal injection of [Tyr<sup>1</sup>]-nociceptin on intracavernosal pressure and penile length in the



**FIG. 1.** Bar graphs showing dose-dependent increases in cavernosal pressure and penile length in response to intracavernosal injection of [Tyr<sup>1</sup>]-nociceptin. "Triple" denotes response to injection of a control triple-drug standard (papaverine, phentolamine, and PGE<sub>1</sub>), which is used as a standard for comparison with [Tyr<sup>1</sup>]-nociceptin. The ratios for the change in cavernosal pressure/systemic arterial pressure for the peak response to nociceptin (at 10 and 30 nmol) are  $0.86 \pm 0.10$  and  $0.95 \pm 0.12$ , and the ratios for [Tyr<sup>1</sup>]-nociceptin (at 10 and 30 nmol) are  $0.86 \pm 0.09$  and  $0.96 \pm 0.11$ . *n* indicates number of experiments; asterisk indicates that the response is significantly different from baseline value,  $P < 0.05$ .

cat are illustrated in Figure 1. Intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin (0.3–30 nmol) caused dose-dependent increases in cavernosal pressure and penile length (Fig. 1). The 30-nmol dose of [Tyr<sup>1</sup>]-nociceptin caused approximately an eightfold increase in cavernosal pressure and an 80% increase in penile length (Fig. 1) when compared to baseline values for cavernosal pressure ( $14 \pm 4$  mm/Hg) and penile length ( $15 \pm 1$  mm). Injections



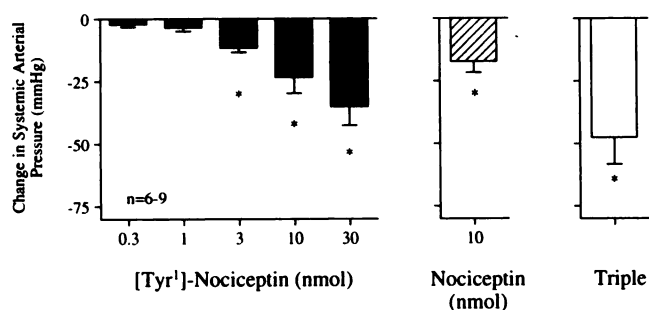
**FIG. 2.** Dose-response curves comparing increases in intracavernosal pressure in response to [Tyr<sup>1</sup>]-nociceptin, nociceptin, nociceptin-(2-17), metenkephalin, dynorphin A, and β-endorphin. Doses of the agonists are expressed on a nanomole basis to take molecular weight into account. *n* indicates number of experiments.

of [Tyr<sup>1</sup>]-nociceptin in a dose of 30 nmol and of the triple-drug reference standard caused similar increases in cavernosal pressure and penile length (Fig. 1). Increases in cavernosal pressure and penile length in response to injections of [Tyr<sup>1</sup>]-nociceptin in doses of 10 and 30 nmol were similar in four experiments when responses were compared during the control period and after a period of 25–30 minutes (data not shown).

Increases in cavernosal pressure in response to [Tyr<sup>1</sup>]-nociceptin were also compared with responses to nociceptin, nociceptin-(2-17), metenkephalin, dynorphin A, and β-endorphin (Fig. 2). When compared on a nanomole basis, the dose-response curves for [Tyr<sup>1</sup>]-nociceptin and nociceptin were similar, indicating that the two peptides had similar activity (Fig. 2). Intracavernosal injections of metenkephalin (1–300 nmol) resulted in dose-dependent increases in cavernosal pressure. The maximal increase in cavernosal pressure in response to metenkephalin was a twofold increase from baseline value (Fig. 2). Nociceptin-(2-17), dynorphin A, and β-endorphin did not alter cavernosal pressure when injected in doses of 1–100 nmol (Fig. 2).

#### Effect on Systemic Arterial Pressure

Intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin (0.3–1 nmol) did not produce a significant decrease in systemic arterial pressure, whereas injections of [Tyr<sup>1</sup>]-nociceptin (3–30 nmol) and the triple-drug standard caused signifi-



**FIG. 3.** Bar graphs comparing the decrease in systemic arterial pressure in response to intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin and nociceptin. "Triple" denotes response to control triple-drug standard (1.65 mg papaverine, 25  $\mu$ g phentolamine, and 0.5  $\mu$ g PGE<sub>1</sub>). *n* indicates number of experiments; asterisk indicates that the response is significantly different from baseline value,  $P < 0.05$ .

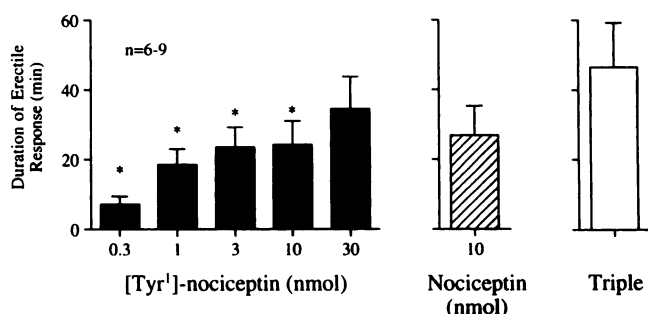
cant decreases in systemic arterial pressure (Fig. 3). Systemic arterial pressure returned to baseline value over a 15–30-minute period after agonist injection (data not shown). The magnitude of the decreases in systemic arterial pressure induced by intracavernosal injection of [Tyr<sup>1</sup>]-nociceptin and nociceptin in a dose of 10 nmol was similar (Fig. 3).

#### Duration of Erectile Response

The duration of the erectile response was significantly shorter for the 0.3–10 nmol doses of [Tyr<sup>1</sup>]-nociceptin than was the duration of the response to the triple-drug reference standard (Fig. 4). However, erectile responses to [Tyr<sup>1</sup>]-nociceptin in doses up to 3 nmol were significantly smaller than were responses to the triple-drug reference standard (Fig. 4). The duration of erectile responses to intracavernosal injection of the 10-nmol doses of [Tyr<sup>1</sup>]-nociceptin and nociceptin was similar (Fig. 4).

#### Effect of Naloxone

The effects of the opioid receptor antagonist naloxone on increases in cavernosal pressure and penile length in response to [Tyr<sup>1</sup>]-nociceptin, nociceptin, and metenkephalin were investigated, and these data are summarized in Figure 5. Naloxone did not alter systemic arterial pressure when administered in a dose of 2 mg/kg IV (data not shown). Increases in cavernosal pressure in response to [Tyr<sup>1</sup>]-nociceptin and nociceptin were not changed following administration of naloxone in a dose of 2 mg/kg IV (Fig. 5). Although increases in cavernosal pressure in response to [Tyr<sup>1</sup>]-nociceptin or nociceptin were not altered by naloxone, the increase in cavernosal pressure in response to metenkephalin (100 nmol) was significantly reduced by the opioid receptor antagonist (Fig. 5). The decreases in systemic arterial pressure in response to intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin and nociceptin were not altered after administration of naloxone (data not shown). Decreases in systemic arterial pressure in re-



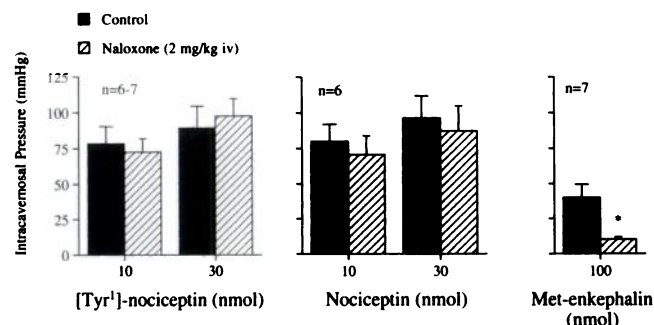
**FIG. 4.** Bar graphs comparing the duration of the erectile response to intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin and nociceptin. "Triple" denotes response to control triple-drug standard (1.65 mg papaverine, 25  $\mu$ g phentolamine, and 0.5  $\mu$ g PGE<sub>1</sub>). *n* indicates number of experiments; asterisk indicates that responses to [Tyr<sup>1</sup>]-nociceptin at 0.3, 1, and 3 nmol are significantly different from those to the triple-drug standard.

sponse to metenkephalin and  $\beta$ -endorphin were reduced significantly following administration of naloxone (data not shown).

## Discussion

Results of the present study show that erectile responses to [Tyr<sup>1</sup>]-nociceptin were similar in magnitude and duration to responses induced by nociceptin and that metenkephalin increased cavernosal pressure. Nociceptin-(2-17), dynorphin A, and  $\beta$ -endorphin did not alter cavernosal pressure. Erectile responses to [Tyr<sup>1</sup>]-nociceptin and nociceptin were not altered by the opioid receptor antagonist naloxone in a dose that significantly reduced the response to metenkephalin. These data indicate that responses to nociceptin and to [Tyr<sup>1</sup>]-nociceptin are mediated by a naloxone-insensitive mechanism, whereas responses to metenkephalin are mediated by a naloxone-sensitive mechanism.

It has been suggested that endogenous opioid peptides



**FIG. 5.** Bar graphs comparing increases in cavernosal pressure in response to intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin, nociceptin, and metenkephalin, before and after administration of the opioid receptor antagonist naloxone in a dose of 2 mg/kg IV. *n* indicates number of experiments; asterisk indicates that the response is significantly different from baseline value,  $P < 0.05$ .

are involved in mediating and modulating sexual activity and erectile responses in humans and experimental animals. Nociceptin is an endogenous ligand for the ORL<sub>1</sub> receptor, which shares considerable sequence homology with the  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid receptors (Dail et al, 1985; Berendsen and Gower, 1986; Fabbri et al, 1989). However, the ORL<sub>1</sub> receptor mediates activity that is opposite to that found with other opioid peptides (Meunier et al, 1995; Reinscheid et al, 1995). While ORL<sub>1</sub> transcripts are found in the brain and in peripheral organs, such as the spleen, kidney, vas deferens, and intestine, little is known about the existence of ORL<sub>1</sub> receptors in the corpora cavernosa of the penis (Bunzow et al, 1994; Chen et al, 1994; Fukuda et al, 1994; Mollereau et al, 1994, 1996; Nishi et al, 1994; Wang et al, 1994a; Wick et al, 1994, 1995; Halford et al, 1995; Lachowicz et al, 1995; Nothacker et al, 1996; Pan et al, 1996). The existence of ORL<sub>1</sub> transcripts in the vas deferens suggests that the ORL<sub>1</sub> receptor may be present in other genitourinary tissues; however, the presence of the receptor in these tissues and in the feline penis has not yet been determined and requires ligand-binding studies and studies with specific ORL<sub>1</sub> receptor antagonists.

It has been reported that intracavernosal injection of nociceptin induces dose-related increases in cavernosal pressure and penile length in the cat, suggesting that ORL<sub>1</sub> receptors are present and that activation of the ORL<sub>1</sub> receptor induces an erectile response in the cat (Champion et al, 1997). Results of the present study with the nociceptin analog [Tyr<sup>1</sup>]-nociceptin are consistent with the hypothesis that the ORL<sub>1</sub> receptor may be present in the corpora cavernosum of the cat; however, studies with specific ORL<sub>1</sub> receptor antagonists are required to validate this hypothesis. Intracavernosal injection of [Tyr<sup>1</sup>]-nociceptin produced dose-related increases in cavernosal pressure and penis length. The increases in cavernosal pressure and penile length induced by this novel nociceptin analog were similar to responses to nociceptin and, at a dose of 30 nmol, to responses to the triple-drug standard reference, suggesting that [Tyr<sup>1</sup>]-nociceptin possesses the ability to induce a marked erectile response. We have not obtained a full dose-response curve for nociceptin and [Tyr<sup>1</sup>]-nociceptin, and since larger doses and a definite plateau in the dose-response curve have not been obtained, no statement about maximum erectile response can be made. Moreover, since only one dose of the triple-drug reference standard was used, no statement about maximal erectile response can be made. Interestingly, dynorphin A, the opioid peptide that shares the greatest sequence homology with nociceptin, was without effect on cavernosal pressure or penile length when injected in doses up to 100 nmol. Metenkephalin had far less activity when compared with nociceptin or with [Tyr<sup>1</sup>]-nociceptin, and  $\beta$ -endorphin did not alter caver-

nosal pressure or penile length. These data may suggest that  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid receptor activation in cavernosal tissue probably do not play a major role in inducing penile erection in the cat model.

Although nociceptin shares significant structural homology with the  $\mu$ -,  $\delta$ -, or  $\kappa$ -opioid receptor agonists, it binds with high affinity only to the ORL<sub>1</sub> receptor (Meunier et al, 1995; Reinscheid et al, 1995). It has been suggested that opioid receptors have a common binding site that interacts with the *N*-terminal moiety (Tyr-Gly-Gly-Phe-Met/Leu) and that the C-terminal amino acids determine the degree of relative selectivity for the ligand's respective receptor subtype (Meunier et al, 1995; Reinscheid et al, 1995). The most striking difference between the amino acid sequence of nociceptin and the traditional opioid peptide sequence is the substitution for the tyrosine residue with a phenylalanine in the first position (Meunier et al, 1995; Reinscheid et al, 1995). It has been suggested, therefore, that the Phe in the first position of the nociceptin sequence conveys the selectivity of this novel ligand for the ORL<sub>1</sub> receptor (Meunier et al, 1995; Reinscheid et al, 1995). The results of the present study show that [Tyr<sup>1</sup>]-nociceptin possesses erectile activity similar to nociceptin and suggest that the increase in cavernosal pressure in response to nociceptin is not altered when the *N*-terminal Phe is replaced with a Tyr residue. These data may suggest that the Phe residue in the first position of the nociceptin sequence is not critical for binding of nociceptin to its receptor. Moreover, nociceptin-(2-17) had no erectile activity and did not alter systemic arterial pressure when administered intracavernosally, suggesting that peptide chain length is important in determining penile erectile and vasodepressor activity in the cat. The observation that nociceptin and [Tyr<sup>1</sup>]-nociceptin have similar erectile activity in the cat is consistent with the observation that both peptides have comparable affinity at the ORL<sub>1</sub> receptor in human 293 cells and guinea pig brain preparations (Shimohigashi et al, 1996).

It is interesting to note that [Tyr<sup>1</sup>]-nociceptin and nociceptin produced dose-related decreases in systemic arterial pressure when injected into the corpora cavernosa. The mechanism of this hypotensive response is uncertain but may suggest that nociceptin has vasodilator activity when the peptide is absorbed into the systemic circulation. Although metenkephalin and  $\beta$ -endorphin did not induce erectile responses in the cat, systemic arterial pressure was decreased significantly, suggesting that these peptides may have activity in other vascular beds or may have a centrally mediated vasodepressor action. The decreases in systemic arterial pressure in response to injections of nociceptin and of [Tyr<sup>1</sup>]-nociceptin in a dose of 10 nmol were similar in magnitude and were significantly less than the decrease in systemic arterial pressure observed in response to the triple-drug standard. However,

decreases in systemic arterial pressure in response to the 30-nmol dose of [Tyr<sup>1</sup>]-nociceptin, nociceptin, and the triple-drug reference standard were similar.

Results of the present study show that increases in cavernosal pressure and penile length in response to [Tyr<sup>1</sup>]-nociceptin are not altered by naloxone at a time when vasodepressor responses to metenkephalin are reduced. Moreover, the decrease in systemic arterial pressure in response to nociceptin and to [Tyr<sup>1</sup>]-nociceptin is not altered by naloxone, suggesting that the hypotensive activity in response to intracavernosal injection of the peptides is mediated by a naloxone-insensitive mechanism, as well. However, the decrease in systemic arterial pressure in response to intracavernosal injection of metenkephalin was reduced significantly following administration of naloxone. These data suggest that increases in cavernosal pressure and penile length, as well as decreases in systemic arterial pressure, in response to intracavernosal injection of [Tyr<sup>1</sup>]-nociceptin are mediated by a naloxone-insensitive mechanism. Although the mechanism by which ORL<sub>1</sub> receptor activation induces erection is unknown, the control and regulation of erection must involve interactions at multiple levels to ensure viability (Adams et al, 1997). In regard to the role of nitric oxide in the erectile response, the release of this mediator from neurons innervating the corpora undoubtedly represents a major vasodilator pathway with actions that could be reinforced or enhanced by the release of a nociceptin-like molecule, since nociceptin has recently been shown to have nitric oxide-dependent vasodilator activity in the hindlimb vascular bed of the rat (Champion and Kadowitz, 1998).

The mechanism mediating responses to nociceptin is not well understood and appears to vary with the experimental model used. While nociceptin was isolated because of its ability to inhibit adenylyl cyclase activity (Meunier et al, 1995; Reinscheid et al, 1995), other mechanisms have been reported to mediate responses to this ORL<sub>1</sub> ligand. It has recently been reported that nociceptin increases K<sup>+</sup> channel activity in dorsal raphe nucleus neurons and rat locus coeruleus neurons (Connor et al, 1996a; Vaughan and Christie, 1996). However, responses to nociceptin in the feline penis and in the peripheral vascular bed in the cat and rat are not mediated by activation of K<sup>+</sup><sub>ATP</sub> channels, since responses are not inhibited by K<sup>+</sup><sub>ATP</sub> channel antagonists (Champion et al, 1997). Although responses to nociceptin are not mediated by activation of K<sup>+</sup><sub>ATP</sub> channels, a role for other K<sup>+</sup> channels, such as the calcium-activated K<sup>+</sup> channel, cannot be ruled out at this time. In addition, nociceptin has been shown to inhibit calcium channel activation in human neuroblastoma cells (Connor et al, 1996b). The mechanism mediating erectile and hypotensive responses to nociceptin and [Tyr<sup>1</sup>]-no-

ciceptin is uncertain and will be addressed in future studies.

In summary, the results of the present study demonstrate that intracavernosal injection of [Tyr<sup>1</sup>]-nociceptin induces dose-related increases in cavernosal pressure and penile length in the cat. Erectile responses to [Tyr<sup>1</sup>]-nociceptin were similar to responses induced by nociceptin, suggesting that the first-position substitution of Phe with Tyr residue does not alter the erectile activity of the peptide. The erectile response to [Tyr<sup>1</sup>]-nociceptin was rapid in onset, suggesting that this novel peptide exerts its activity at the level of the corpora cavernosum; however, the role of a centrally mediated mechanism cannot be ruled out. Nociceptin-(2-17), dynorphin A, and β-endorphin did not alter cavernosal pressure, and metenkephalin induced only partial erection. Intracavernosal injections of [Tyr<sup>1</sup>]-nociceptin and of nociceptin produced dose-related decreases in systemic arterial pressure. The mechanism of this hypotensive response is uncertain but may involve a direct effect in the peripheral vascular bed or a centrally mediated vasodepressor action. The results of the present study suggest that erectile and vasodepressor responses to intracavernosal injection of [Tyr<sup>1</sup>]-nociceptin and of nociceptin are mediated by a naloxone-insensitive mechanism. The results of the present study provide support for the hypothesis that ORL<sub>1</sub> receptors are present in the corpora cavernosum of the penis. However, validation of this hypothesis requires additional studies with a specific ORL<sub>1</sub> antagonist.

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