Involvement of sympathetic efferents but not capsaicin-sensitive afferents in nociceptin-mediated dual control of rat synovial blood flow

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McDougall, Jason J. Involvement of sympathetic efferents but not capsaicin-sensitive afferents in nociceptin-mediated dual control of rat synovial blood flow. Am J Physiol Regul Integr Comp Physiol 284: R1477–R1485, 2003. First published February 6, 2003; 10.1152/ajpregu.00733.2002.—This study set out to examine the vasomotor effects of the opioid-like peptide nociceptin on knee joint capsular blood flow in urethane-anaesthetized rats. Topical application of nociceptin (10⁻¹⁵–10⁻¹⁰ mol) caused a progressive fall in joint perfusion that was significantly inhibited by the specific nociceptin receptor antagonist [Phe¹-(CH₂-NH)-Gly²] Nociceptin (1–13)-NH₂ as well as by the nonspecific opioid antagonist naloxone. To test whether this constrictor response was sympathetically mediated, we administered nociceptin in animals treated with guanethidine to produce sympathetic blockade or in the presence of the α-adrenoceptor antagonist phentolamine. Both guanethidine treatment and phentolamine coadministration attenuated the constrictor response to nociceptin. Inhibition of nociceptin-mediated vasoconstriction revealed a supplementary hyperemic response that persisted in animals whose knee joints were treated with 1% capsaicin to destroy the articular unmyelinated nerve supply. These results show that, in the rat knee, peripheral administration of nociceptin primarily causes a sympathetically mediated vasoconstriction. In addition, high-dose nociceptin produces a vasodilatory response that is likely due to the direct action of nociceptin on vascular smooth muscle and not by a neurogenic mechanism.

neuropeptides; neurogenic inflammation; orphanin FQ; sympathetic nervous system

The opioid system has long been associated with chronic pain management, but there is increasing evidence to suggest that classic opioid agonists have the potential to modulate inflammatory changes in peripheral tissues. Morphine, for example, has been shown to reduce edema formation in the rat paw and trachea (20, 22, 33) as well as being able to reduce knee joint plasma extravasation in response to capsaicin treatment (17). Indeed, a growing body of evidence seems to suggest that opioid agonists can successfully attenuate the severity of experimentally induced arthritis when administered either locally to the affected joint or systemically (6, 24, 41). The mechanism by which opioids exert their antiarthritic effects is still unclear, but this supplementary attribute could have major therapeutic implications.

Three classes of opioid receptor (termed μ, δ, and κ) were originally described on the basis of extensive pharmacological and localization criteria. Morphine, for example, acts via the μ-opioid receptor, whereas the endogenous opioid peptide dynorphin A shows a high affinity for the κ-opioid receptor type. In the early 1990s, a number of investigators successfully cloned the receptor genes that encode for the μ-opioid receptor (39), δ-opioid receptor (12), and the κ-opioid receptor (25). Arising from these receptor cloning studies emerged a novel receptor that exhibited ~65% homology to the δ-opioid receptor but did not bind any of the known endogenous opioids (10, 31, 40). With no known agonist, the receptor was termed the opioid-like orphan receptor (ORL-1). The endogenous ligand for the ORL-1 receptor was later found to be a heptadecapeptide called nociceptin (also known as orphanin FQ), whose amino acid sequence is similar to that of dynorphin (30, 34). Immunolocalization studies have identified nociceptin in the central nervous system (32, 35, 38), where it acts as a modulator of pain, long-term potentiation, and locomotor activity (11, 16). More recently, nociceptin has also been identified in a number of peripheral tissues including rat ankle joints (1). Information regarding the physiological function of nociceptin in the periphery is scarce, but a few studies have found that nociceptin can influence cardiovascular regulation as well as modulate nociception. In vivo studies in rats revealed that systemic nociceptin administration produces transient hypotension and bradycardia (5, 9, 15). These cardiovascular effects seem to be autonically controlled, since sympathetic blockade by guanethidine treatment and bilateral cervical vagotomy altered the level of bradycardia and hypotension in nociceptin-treated animals (15). Conflicting results were reported by Arndt et al. (4), who found in alert sheep that nociceptin caused an increase in systemic blood pressure and heart rate. Pretreatment of the animals with the nonspecific α-adrenoceptor antagonist phenoxybenzamine abrogated these effects, suggesting once again that the cardiovascular changes produced by nociceptin were sympathetically dependent.

In normal rat knee joints, nociceptin has been found to cause a hypersensitization of articular afferents,
demonstrating the existence of functional ORL-1 receptors in joint tissues (27, 29). The observation that nociceptin-containing nerves occur in close proximity to articular blood vessels (1) implies that, in addition to its nociceptive properties, nociceptin may also be involved in joint vasomotor control mechanisms and neurogenic inflammation. Although nociceptin does not itself affect synovial plasma extravasation, it is able to inhibit articular vascular leakiness in response to 5-hydroxytryptamine (18). The present study set out to investigate whether peripherally administered nociceptin could alter joint capsular blood flow and to see whether sympathetic nerve fibers are involved in the vasomotor changes. To fully characterize the ORL-1 receptor in the rat knee joint, nociceptin dose-response curves were repeated in the presence of the recently described ORL-1-specific receptor antagonist [Phe\(^1\)-(CH\(_2\)-NH)-Gly\(^2\)] Nociceptin(1-13)-NH\(_2\) (19), as well as the nonspecific opioid antagonist naloxone. The pseudopeptide antagonist [Phe\(^1\)-(CH\(_2\)-NH)-Gly\(^2\)] Nociceptin(1-13)-NH\(_2\) has been shown to successfully inhibit the hypotensive and bradycardia actions of nociceptin (5) and does not have any affinity for other opioid receptors (5, 19), confirming the validity of this agent as a suitable antagonist for the experiments outlined here.

**MATERIALS AND METHODS**

Experiments were performed on 65 male Wistar rats (215–382 g) deeply anesthetized by intraperitoneal injection of urethane (1.5 g/kg), and depth of anesthesia was confirmed by an absence of the pedal withdrawal reflex. The skin covering the joint was shaved, and the rat was placed supine by an absence of the pedal withdrawal reflex using barbital sodium, 240 mg intracardiac, and a scan of the dead animal knee joint was obtained. This “biological zero” measurement, which corresponds to tissue noise (e.g., Brownian motion flux), was subtracted from all images before data calculations.

**Studies to test sympathetic involvement.** It has been suggested that the cardiovascular effects of nociceptin in some species are achieved via the activation of the sympathetic nervous system (4). To investigate whether the nociceptin-induced blood flow changes described here were sympathetically mediated, two sets of experiments were carried out. The first involved treating a group of rats with guanethidine (50 mg·kg\(^{-1}\)·day\(^{-1}\)·ip for 3 consecutive days), which results in a submaximal blockade of sympathetic neurotransmission rendering them functionally inert at the time of blood flow assessment. This treatment regimen should not be confused with chronic guanethidine treatment, which results in general sympatholysis. A dose-response curve using nociceptin was generated in the guanethidine-treated animals. In a separate group of rats, postjunctional \(\alpha\)-adrenoceptors were pharmacologically blocked by administration of the nonspecific \(\alpha\)-adrenoceptor antagonist phentolamine (10\(^{-6}\) mol topical) for 6 min before and concurrently with each dose of nociceptin. To test the effectiveness of guanethidine blockade and phentolamine antagonism on sympathetic activity, we performed additional experiments in a subset of treated animals in which the knee joint sympathetic nerve supply was electrically stimulated. Here, the saphenous nerve was isolated in the inguinal region of the hindlimb and then centrally transected. The cut nerve end was placed over silver bipolar electrodes and electrically stimulated by a
Harvard Stimulator (model 6012; Harvard Apparatus, Saint Laurent, Quebec, Canada) with the stimulating parameters set at delay of 1 ms, pulse width of 1 ms, voltage of 15 V, and frequency of 30 Hz. This stimulation regimen has previously been shown to elicit a potent sympathetically mediated vasoconstriction of rat knee joint blood vessels (28).

Capsaicin treatment. Destruction of unmyelinated knee joint afferent nerves was produced by intraarticular injection of 1% capsaicin (vehicle consisting of 5% ethanol, 5% cremophor, and physiological saline). Rats were deeply anaesthetized by intraperitoneal injection of diazepam (2.5 mg/kg) and intramuscular injection of Hypnorm (0.2 ml/kg), and the right knee joint was shaved and swabbed with alcohol. The capsaicin solution (0.2 ml) was injected into the joint cavity with 0.1 ml being introduced into the posterior and 0.1 ml into the anterior compartments of the joint. Animals were allowed to recover for 1 wk, which has been shown to be the optimal time point for almost complete destruction of rat knee joint unmyelinated nerve fibers (13). As such, I am confident that unmyelinated and thinly myelinated nerve fibers were destroyed in this study.

Laser Doppler image analysis and statistics. Each Doppler image of the knee joint was analyzed with Moor Image Processor software (Moor Instruments). A region of interest analysis area was selected that encompassed the knee joint and the mean flux for the knee was calculated and reported in arbitrary perfusion units (PU). Blood flow changes in response to drug administration were expressed as a percent change in PU between the test scan and the control scan taken immediately before drug administration. The relative potency of nociceptin alone and in the presence of the antagonists was determined by ED50 comparisons. The ED50s were calculated from linear regression analyses of the dose-response curves with GraphPad Prism software (GraphPad Software, San Diego, CA). All data were tested for normality by the Kolmogorov-Smirnov test with GraphPad Prism software. All data were found to be not significantly different from a Gaussian distribution ($P > 0.10$) and were therefore analyzed by parametric statistical tests (Student’s $t$-test, one- and two-factor ANOVA). Dunnett’s and Bonferroni multiple comparison posttests were performed to compare different time points or individual doses in certain data sets. Data are presented as means ± SE and were considered significantly different if $P < 0.05$.

Drugs. Nociceptin (orphanin FQ), naloxone hydrochloride, [Phe$^1$-CH$_2$-NH$^2$] Nociceptin[1–13]-NH$_2$, phentolamine hydrochloride, guanethidine monosulphate, capsaicin, cremophor, and urethane were all obtained from Sigma-Aldrich Canada (Ontario, Canada). Hypnorm was supplied by Janssen Pharmaceutica (Beerse, Belgium) and diazepam by Sabe (Boucherville, Canada). Test drugs were dissolved in 0.9% saline to give the necessary concentrations, aliquotted into 250-μl volumes and stored at −20°C until required.

Fig. 1. Laser Doppler perfusion images of rat knee joints showing the time course in response to nociceptin administration. A: photograph of an exposed rat knee joint after skin and fascia removal. The joint capsule roughly corresponds to the central lighter region of the knee. B: perfusion images of the joint capsule before (control) and immediately, 2, 4, and 6 min after topical application of 10$^{-12}$ mol nociceptin. The greatest reduction in joint blood flow occurred at the 2-min time point with perfusion gradually recovering over the proceeding 4 min. The color-coding system used in the images is shown at left with bands ranging from dark blue (0 perfusion units) to white (255 perfusion units).
RESULTS

Effects of nociceptin on knee joint blood flow. Application of nociceptin onto the surface of rat knee joints was found to cause a progressive reduction in articular blood flow (Fig. 1). However, this vasoconstrictor response to nociceptin was significantly different from vehicle control only with the 10^{-12}- to 10^{-8}-mol doses (Fig. 2). For the 10^{-12} to 10^{-10}-mol doses, the maximal effect occurred at 2 min after nociceptin administration, whereas the maximum response associated with the 10^{-9} and 10^{-8} mol doses occurred slightly later at 4 min. A one-factor ANOVA revealed that the vasoconstricter effect of nociceptin was dose dependent (P < 0.05; n = 12–19) with an ED_{50} of 3.5 \times 10^{-10} mol. Saline vehicle had no significant effect on knee joint perfusion (P = 0.17).

When nociceptin was applied in the presence of the selective nociceptin receptor antagonist [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2, the vasoconstrictor effect of nociceptin was significantly attenuated (P < 0.05 two-factor ANOVA; n = 14–19). The ED_{50} in the presence of the ORL-1 receptor antagonist was 3.4 \times 10^{-10} mol, confirming a rightward shift in the nociceptin dose-response curve. Interestingly, the nonspecific opioid antagonist naloxone was also found to significantly (P < 0.05 two-factor ANOVA; n = 13–19) inhibit the vasoactive effects of nociceptin (Fig. 2), resulting in an ED_{50} of 1.1 \times 10^{-10} mol. It should be noted that [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 and naloxone alone had no significant effect on joint blood flow (P = 0.15 and P = 0.21 for [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 and naloxone, respectively). The inability of [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 to alter blood flow is probably due to the animal being deeply anesthetized, and as such the basal release of nociceptin from peripheral nerve endings would be inhibited. Mean arterial blood pressure remained stable during all of the nociceptin experiments, confirming a local effect of the drugs on the joint vasculature (Table 1).

Latent vasodilator effect of nociceptin. A surprising observation with the antagonist experiments was that the highest dose of nociceptin caused a transient but profound hyperemic response in the presence of [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 or naloxone, whereas a similar effect was also detected in guanethidine-injected animals (Fig. 3). Coincubation of [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 and 10^{-8} mol nociceptin produced an initial 67.7 \pm 9.66% increase in capsular blood flow (P < 0.05 repeated-measures one-factor ANOVA), whereas perfusion rose by 75.6 \pm 13.13% in the presence of naloxone (P < 0.05). In guanethidine-treated rats, 10^{-8} mol nociceptin produced an initial rise in perfusion by 24.8 \pm 5.54% (P < 0.05). These dilator effects were apparent immediately after drug administration and returned back to control levels after 4 min. Rats pretreated with 1% capsaicin to destroy unmyelinated knee joint afferents also showed a 65.3 \pm 15.33% increase in joint blood flow immediately after application of the [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 and nociceptin cocktail (Fig. 3D). A one-factor ANOVA confirmed that this hyperemic response was statistically significant (P < 0.05; n = 10).

Effects of guanethidine or phentolamine treatment. In control knees, electrical stimulation of the saphe nous nerve produced a 47.2 \pm 4.59% fall in perfusion (Fig. 4). This vasoconstrictor response was significantly inhibited by either guanethidine treatment or phentolamine coadministration (P < 0.05 one-factor ANOVA; n = 5–8), clearly showing that these treatments block sympathetic activity in the joint.

As shown in Fig. 5, guanethidine treatment caused a significant inhibition of nociceptin-induced vasocon striction in the rat knee (P < 0.05 two-factor ANOVA; n = 15–16). Similarly, antagonism of smooth muscle \(a\)-adrenoceptors by coadministration of the \(a\)-adrenergic antagonist phentolamine also attenuated the constrictor capacity of nociceptin (P < 0.05). Mean arterial pressure was unaffected by drug application in these

Fig. 2. Effect of nociceptin on knee joint perfusion compared with vehicle (saline) control or in the presence of 10^{-9} mol of the opioid-like orphan receptor (ORL)-1 receptor antagonist [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 or 10^{-8} mol of the nonspecific opioid antagonist naloxone. The time point chosen for each dose corresponds to the maximal response to nociceptin (i.e., 2 min for the 10^{-15}–10^{-10} mol doses and 4 min for the 10^{-9} and 10^{-8} mol doses). The vascular response to nociceptin was only significantly different from saline control across the dose range 10^{-12}–10^{-8} mol (P < 0.05; n = 12–19). For the antagonist experiments, both [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2 and naloxone significantly attenuated the vasoconstrictor effect of nociceptin (P < 0.05 two-factor ANOVA; n = 13–19). Nociceptin(1–13); [Phe^1-(CH_2-NH)-Gly^2]-Nociceptin(1–13)-NH_2. Means are shown with their respective SE.
experiments, confirming that blood flow changes were due to local vascular responses and not a consequence of blood pressure fluctuations (Table 1).

**DISCUSSION**

The identification of the opioid-like peptide nociceptin has yielded a plethora of information regarding the role of this neuropeptide in the control of pain. Less known is the ability of nociceptin to modulate other physiological processes such as vasoregulation. The present study found that peripherally administered nociceptin produced a reduction in rat knee joint perfusion that could be inhibited by the specific ORL-1 receptor antagonist \( \text{[Phe}^1-(\text{CH}_2-\text{NH})\text{Gly}^2] \text{Nociceptin}(1-13)-\text{NH}_2 \) as well as the nonspecific opioid antagonist naloxone. It should be noted that neither antagonist alone nor the vehicle in which all drugs were dissolved (0.9% saline) had any effect on synovial blood flow. The vasoconstrictor effect of nociceptin is somewhat unexpected, since nociceptin has mainly been

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**Fig. 3.** Transient hyperemic response to \( 10^{-8} \text{ mol nociceptin} \) as revealed during \( \text{[Phe}^1-(\text{CH}_2-\text{NH})\text{Gly}^2] \text{Nociceptin}(1-13)-\text{NH}_2 \) treatment. In guanethidine-treated animals (C), \( 10^{-8} \text{ mol nociceptin} \) alone also caused a significant vasodilatation in the joint capsule. One week after intraarticular capsaicin injection (D), rat knee joints still showed an increase in blood flow in response to coadministration of \( 10^{-8} \text{ mol nociceptin} \) and \( \text{[Phe}^1-(\text{CH}_2-\text{NH})\text{Gly}^2] \text{Nociceptin}(1-13)-\text{NH}_2 \). NC, Nociceptin; NC (1–13), \[\text{[Phe}^1-(\text{CH}_2-\text{NH})\text{Gly}^2] \text{Nociceptin}(1–13)-\text{NH}_2 \); PU, perfusion units. *\( P < 0.05 \); Dunnett’s multiple comparison posttest. Means ± SE are shown; \( n = 10–16 \).
shown to cause systemic hypotension and peripheral vascular relaxation in a number of species including rats (5, 9, 15). In these studies, however, nociceptin was administered systemically and as such may be exerting its inhibitory effects on the cardiovascular system via a central mechanism or by direct activation of the heart. Conversely in the experiments described here, nociceptin was localized to the periphery, as shown by the lack of any effect of the neuropeptide on mean arterial blood pressure (see Table 1). Hence, the vasoconstrictor response of nociceptin in the rat knee is purely a microcirculatory effect that is independent of cardiac stimulation or baroreceptor reflexes.

The inhibitory effect of [Phe\(^1\)-(CH\(_2\)-NH)-Gly\(^2\)] Nociceptin(1–13)-NH\(_2\) on nociceptin-induced vasoconstriction confirms the presence of ORL-1 receptors in the joint capsule. This finding is supported by electrophysiological studies that have successfully demonstrated functional ORL-1 receptors on joint afferents, as revealed by nociceptin-mediated changes in articular mechanosensitivity (27, 29). The attenuation of the vasoconstrictor response by naloxone suggests that nociceptin may to some extent also be acting through \(\mu\), \(\delta\), or \(\kappa\)-opioid receptors to alter joint perfusion. The naloxone result is quite surprising, since the physiological effects of nociceptin are generally believed to be naloxone insensitive, although some reports do cite naloxone inhibition. Rossi et al. (36), for example, described an analgesic action of nociceptin that was reversed by naloxone, whereas Arndt and colleagues (4) have eloquently shown that naloxone attenuates a pressor and tachycardia response to nociceptin. Interestingly, in both of these studies as well as the present investigation, nociceptin was producing atypical effects i.e., analgesia instead of hyperalgesia, hypertension rather than hypotension, tachycardia as opposed to bradycardia, and vasoconstriction instead of vasodilation. Thus nociceptin appears to be able to exert distinct and conflicting actions in vivo of which one set of responses appears to be naloxone sensitive. Some of the peripheral effects of nociceptin have been attributed to the ability of the peptide to modulate neurotransmitter release at a prejunctional level from sensory and sympathetic nerves (for review, see Ref. 14).

The present investigation, therefore, tested whether the vasoconstrictor effects of nociceptin are sympathetically mediated. As shown in the nerve stimulation experiments, supramaximal sympathetic blockade was achieved by systemic guanethidine pretreatment, whereas \(10^{-6}\) mol phenolamine was sufficient in producing \(\alpha\)-adrenoceptor antagonism. A slight vasodilation was observed during nerve stimulation in

![Fig. 4. Vasoconstrictor response of capsular blood vessels to electrical stimulation of joint sympathetic nerves (nerve stim.) and its inhibition by the \(\alpha\)-adrenoceptor antagonist phenolamine (10\(^{-6}\) mol) and by guanethidine treatment. \(P < 0.05\) 1-factor ANOVA; \(n = 5–8\). Data are means ± SE.](#)

![Fig. 5. Effect of supramaximal sympathetic blockade with guanethidine pretreatment or \(\alpha\)-adrenergic antagonism by 10\(^{-6}\) mol phenolamine coadministration on nociceptin-induced vasoconstriction. Across the dose range tested, guanethidine and phenolamine significantly attenuated the constrictor effect of nociceptin (for guanethidine treated, \(P < 0.05\) 2-factor ANOVA, \(n = 15–19\); for phenolamine treated \(P < 0.05\), \(n = 9–19\)). Data shown as means ± SE.](#)
guanethidine-treated rats (see Fig. 4), and this effect was probably due to a small population of joint afferents being stimulated. Some of these sensory nerves contain inflammatory peptides such as substance P, which will be released into the joint, resulting in the reported mild vasodilatation. Both guanethidine treatment and phentolamine coadministration significantly inhibited nociceptin-induced vasoconstriction in the knee. This finding supports the idea that nociceptin is acting prejunctionally on sympathetic nerve endings in the joint to cause the secondary release of norepinephrine, which in turn is responsible for the synovial vasoconstriction. In light of the \([\text{Phe}1-(\text{CH}_2\text{-NH})\text{-Gly}^2]\) Nociceptin(1–13)-NH\(_2\) and naloxone results, the data seem to indicate that nociceptin is acting through ORL-1 and classic opioid receptors present on sympathetic nerve endings.

A curious observation in the antagonist experiments is that administration of the top dose of nociceptin in the presence of naloxone or \([\text{Phe}1-(\text{CH}_2\text{-NH})\text{-Gly}^2]\) Nociceptin(1–13)-NH\(_2\) caused a transient but significant hyperemic response. This increase in joint blood flow could also be seen in guanethidine-treated rats, suggesting that this secondary vasodilator effect of nociceptin probably occurred concurrently with the more potent vasoconstrictor response but was only revealed when the constrictor action was blocked. Whether this alternate outcome was due to nociceptin acting on a novel receptor subtype (e.g., ORL-2) requires further investigation, but it would explain why the ORL-1-specific antagonist \([\text{Phe}1-(\text{CH}_2\text{-NH})\text{-Gly}^2]\) Nociceptin(1–13)-NH\(_2\) was unable to inhibit the vasodilatation as well. This dual action of nociceptin is probably a real physiological event, since similar outcomes have been described in other studies. For example, Grond et al. (18) found that low- and high-dose nociceptin produced disparate effects on 5-hydroxytryptamine-induced protein extravasation in the rat knee. Additional evidence comes from electrophysiology studies in which nociceptin caused conflicting effects on knee joint mechanosensitivity depending on the dose of the peptide and the inflammatory status of the tissue (27, 29).

One possible explanation for the increased perfusion of the joint could be nociceptin causing secondary release of proinflammatory peptides into the knee. Hyperalgesia caused by peripherally applied nociceptin appears to be dependent on substance P release from unmyelinated afferent nerve endings (21, 27), and this same mechanism could be responsible for the dilator activity of nociceptin in joints. This possibility was tested by treating a group of rats with intraarticular injection of 1% capsaicin, which has been shown to destroy unmyelinated nerve endings in the rat knee at 1 wk after treatment (13). In these capsaicin-treated rats, coadministration of \([\text{Phe}1-(\text{CH}_2\text{-NH})\text{-Gly}^2]\) Nociceptin(1–13)-NH\(_2\) and 10⁻⁸ mol nociceptin still produced a potent vasodilatation, indicating that nociceptin is exerting its dilator effects directly on the vascular smooth muscle and not via secondary release of neurogenically derived inflammatory mediators such as substance P into the joint. It could be argued that nociceptin is causing the release of endothelially derived factors such as nitric oxide; however, an abundance of evidence contests the notion that nociceptin does not interact with the vascular endothelium to alter blood vessel tone (3, 8).

The model system employed in this study, although not purely physiological, does extend the opportunity to test drug responses and thereby deduce the physiological activity of the endogenous receptors. The physiological significance of the presented findings is complicated by the fact that endogenous nociceptin levels in the rat knee are not known. Circulating nociceptin in humans is thought to be as low as 10 pg/ml (7), which when applied to the present investigation would suggest that nociceptin plays only a minor role in the basal modulation of joint blood flow. However, the concentration of the peptide in the tissues is likely to be considerably higher than circulating levels, indicating that nociceptin may still contribute to the physiological control of joint perfusion. Of greater importance is the role of nociceptin during inflammation, where levels of the peptide are known to increase (2). Here, accumulation of nociceptin in the joint would attenuate synovial hyperemia and potentially reduce joint inflammation. As peptide levels continue to rise, however, nociceptin would eventually become proinflammatory as its dilator characteristics are suddenly invoked. More information re-

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Table 1. Mean arterial blood pressure (mmHg) in response to topical nociceptin application either by itself or in the presence of \([\text{Phe}1-(\text{CH}_2\text{-NH})\text{-Gly}^2]\) Nociceptin (1–13)-NH\(_2\), naloxone, phentolamine, or after guanethidine treatment

<table>
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<th>Dose of Nociceptin, mol</th>
<th>Control</th>
<th>10⁻¹⁵</th>
<th>10⁻¹⁴</th>
<th>10⁻¹³</th>
<th>10⁻¹²</th>
<th>10⁻¹¹</th>
<th>10⁻¹⁰</th>
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<td>48 ± 3</td>
<td>52 ± 3</td>
<td>58 ± 5</td>
<td>54 ± 5</td>
<td>51 ± 5</td>
<td>50 ± 5</td>
<td>49 ± 7</td>
<td>0.8506</td>
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<tr>
<td>With 10⁻⁸ mol naloxone</td>
<td>58 ± 2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>59 ± 6</td>
<td>56 ± 6</td>
<td>56 ± 6</td>
<td>53 ± 4</td>
<td>50 ± 4</td>
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<td>N/A</td>
<td>N/A</td>
<td>67 ± 5</td>
<td>67 ± 4</td>
<td>66 ± 4</td>
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<tr>
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Values are means ± SE. N/A, not available.
garding nociceptin levels in normal and inflamed joints is therefore required to provide a clearer appreciation of the involvement of this neuropeptide in joint homeostasis and pathology.

As alluded to earlier, nociceptin can produce parallel but opposing physiological responses (18, 27, 29, 37). A dual action of nociceptin has been described here in the rat knee where high-dose nociceptin causes a transient capsular hyperemia that is not neurogenically mediated. This dilator response is succeeded by a more prolonged vasoconstrictor response that is sympathetically mediated. Further investigation into the complex function of nociceptin and its diverse effects in vivo may yield meaningful information regarding the role of this peptide in the control of joint pain and inflammation.

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REFERENCES


