Involvement Of Sympathetic Efferents But Not Capsaicin-Sensitive Afferents In Nociceptin-Mediated Dual Control Of Rat Synovial Blood Flow

Jason J. McDougall

Department of Physiology & Biophysics, University of Calgary, Calgary, Alberta. T2N 4N1, Canada

Running Title: Dual action of nociceptin on blood flow

Corresponding author: Dr. Jason J. McDougall

Department of Physiology & Biophysics,
3330, Hospital Drive NW,
University of Calgary,
Calgary, AB.
T2N 4N1, Canada
Tel: (403) 220 4507 Fax: (403) 270 0617 E-mail: mcdougaj@ucalgary.ca

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ABSTRACT

This study set out to examine the vasomotor effects of the opioid-like peptide nociceptin on knee joint capsular blood flow using urethane anaesthetised rats. Topical application of nociceptin (10^{-15} – 10^{-8} mol) caused a progressive fall in joint perfusion which was significantly inhibited by the specific nociceptin receptor antagonist [Phe^{1}-(CH_{2}-NH)-Gly^{2}] Nociceptin(1-13)-NH_{2} as well as the non-specific opioid antagonist naloxone. To test whether this constrictor response was sympathetically mediated, nociceptin was administered in animals treated with either guanethidine to produce sympathetic blockade, or in the presence of the α-adrenoceptor antagonist phentolamine. Both guanethidine treatment and phentolamine co-administration attenuated the constrictor response to nociceptin. Inhibition of nociceptin-mediated vasoconstriction revealed a supplementary hyperaemic response which 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 vasodilatatory response which is likely due to the direct action of nociceptin on vascular smooth muscle and not by a neurogenic mechanism.
INTRODUCTION

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 oedema 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 anti-arthritic 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 based on extensive pharmacological and localisation criteria. Morphine, for example, acts via the µ-opioid receptor while 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 which 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 which exhibited about 65% homology to the δ-opioid receptor but which 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). Immunolocalisation 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 autonomically 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. Pre-treatment of the animals with the non-specific 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 hypersensitisation 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 fibres are involved in the vasomotor changes. To fully characterise 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 [Phe¹-(CH₂-NH)-Gly²] Nociceptin(1-13)-NH₂ (19) as well as the non-specific opioid antagonist
naloxone. The pseudopeptide antagonist \([\text{Phe}^1-(\text{CH}_2-\text{NH})-\text{Gly}^2] \text{Nociceptin}(1-13)-\text{NH}_2\) 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 & METHODS**

Experiments were performed on 65 male Wistar rats (215-382g) deeply anaesthetised by intraperitoneal injection of urethane (1.5g/kg) and depth of anaesthesia was confirmed by an absence of the pedal withdrawal reflex. The skin covering the joint was shaved and the rat was placed supine on a thermostatically controlled heating pad (Fine Science Tools Inc., North Vancouver, Canada). Core body temperature was maintained in the 36 – 37°C range as measured by a rectally inserted thermosensitive probe.

**Surgical procedures**

All surgical and experimental procedures had received prior approval from the University of Calgary Animal Care Committee which is in accordance with the Canadian Council for Animal Care guidelines.

A longitudinal incision was made in the neck of the rat and the exposed trachea cannulated to allow unrestricted breathing. Two animals experienced respiratory irregularities and were therefore artificially ventilated with 100% \(\text{O}_2\). The left carotid artery was then isolated and cannulated with a heparinised saline-filled cannula (1.0mm outer diameter polyethylene tubing; Portex, Kent, UK). The carotid cannula was connected to a pressure transducer (Stoelting Co., Illinois, USA) which allowed mean arterial pressure to be recorded on a blood pressure...
monitor (World Precision Instruments, Florida, USA). An ellipse of skin overlying the knee joint was excised and the fascia covering the joint was removed to expose the joint capsule (synovium and associated fibrous tissues – see Figure 1A). To prevent desiccation of articular structures, warmed (37°C) physiological saline (0.9% NaCl) was regularly superfused over the surface of the knee which in itself has no measurable effect on joint blood flow (26). While observing the knee under a dissecting microscope, black cloth was carefully placed around the circumference of the joint to cloak non-articular structures thereby delimiting the field of view to the joint.

**Blood flow assessment**

Relative changes in knee joint perfusion were ascertained by a laser Doppler imager (Moor Instruments Ltd., Axminster, UK) using previously described protocols which have been validated for rat knee joint perfusion studies (23). The technique involves a low power (2mW) red laser beam (wavelength = 633nm) scanning over the surface of a tissue of interest in a raster pattern. At each point in the scan a perfusion measurement is dynamically acquired based on the concentration and velocity of circulating erythrocytes as they flow through the microcirculation of a discrete volume of tissue. This information is processed in real-time to give a 2-dimensional colour-coded image of joint perfusion. The scanner head was positioned 20cm above the knee and angled in such a way so as to obviate tissue reflectance artefacts which would introduce errors to the perfusion values. Image resolution was set at 93 X 88 pixels with a scan speed of 4ms/pixel. Knee joint scans were taken before (control) and then at 0, 2, 4 and 6min following topical application of nociceptin. This mode of drug administration has been consistently found to be advantageous in restricting the vasoactive effects of the drug to the joint without the confounding effects of blood pressure changes. Nociceptin was administered as a 0.1ml bolus in
the dose range $10^{-15} - 10^{-8}$ mol with the order of dose being randomised between animals to minimise any potential tachyphylaxis. Since nociceptin was only effective over the dose range $10^{-12} - 10^{-8}$ mol, then the following experiments were restricted to this dose range. In separate groups of animals, nociceptin dose response curves were repeated in the presence of either the specific nociceptin antagonist [Phe$^1$-(CH$_2$-NH)-Gly$^2$] Nociceptin(1-13)-NH$_2$ $(10^{-9}$ mol), or the non-specific opioid antagonist naloxone $(10^{-9}$ mol). Antagonists were also administered topically to the joint as a 0.1ml bolus both before the first dose of nociceptin as well as concurrently with each dose of the agonist. In a further group of rats, control experiments were performed in which the effect of vehicle (0.9% saline) on knee joint blood flow was assessed. At the completion of an experiment, the rat was killed by an anaesthetic overdose (pentobarbital sodium, 240mg intracardiac) and a scan of the dead animal knee joint was obtained. This “biological zero” measurement, which corresponds to tissue noise (eg. Brownian motion flux), was subtracted from all images prior to 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 (50mg/kg/day i.p. for 3 consecutive days) which results in supramaximal 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 administering the non-specific $\alpha$-adrenoceptor antagonist phentolamine ($10^{-6}$ mol topical) for 6min prior to and concurrently with each dose of nociceptin. To test the effectiveness of guanethidine blockade and phentolamine antagonism on sympathetic activity, additional experiments were performed 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 inguineal 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, Canada) with the stimulating parameters set at delay 1ms, pulse width 1ms, voltage 15V and frequency 30Hz. 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 anaesthetised by intraperitoneal injection of diazepam (2.5mg/kg) and intramuscular injection of Hypnorm (0.2ml/kg) and the right knee joint was shaved and swabbed with alcohol. 0.2ml of the capsaicin solution was injected into the joint cavity with 0.1ml being introduced into the posterior and 0.1ml into the anterior compartments of the joint. Animals were allowed to recover for 1 week which has been shown to be the optimal time point for almost complete destruction of rat knee joint unmyelinated nerve fibres (13). As such, I am confident that unmyelinated and thinly myelinated nerve fibres were destroyed in this study.
Laser Doppler image analysis and statistics

Each Doppler image of the knee joint was analysed using Moor Image Processor software (Moor instruments Ltd., Axminster, UK). A region of interest analysis area was selected which 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 prior to drug administration. The relative potency of nociceptin alone and in the presence of the antagonists was determined by ED_{50} comparisons. The ED_{50}s were calculated from linear regression analyses of the dose-response curves using GraphPad Prism software (GraphPad Software, San Diego, USA). All data were tested for normality using 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 analysed using parametric statistical tests (Student t-test, One and two factor ANOVA). Dunnett’s and Bonferroni Multiple Comparison post-tests were performed to compare different time points or individual doses in certain data sets. Data were presented as means ± S.E.M. and were considered significantly different if P<0.05.

Drugs

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

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 (Figure 1). However, this vasoconstrictor response to nociceptin was only significantly different from vehicle control with the $10^{-12} - 10^{-8}$ mol doses (Figure 2). For the $10^{-12} - 10^{-10}$ mol doses the maximal effect occurred at 2min after nociceptin administration while the maximum response associated with the $10^{-9}$ and $10^{-8}$ mol doses occurred slightly later at 4min. A one factor ANOVA revealed that the vasoconstrictor effect of nociceptin was dose-dependent (P<0.05; n = 12 - 19) with an ED$_{50}$ of $3.5 \times 10^{-13}$ 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 non-specific opioid antagonist naloxone was also found to significantly (P<0.05 two factor ANOVA; n = 13 – 19) inhibit the vasoactive effects of nociceptin (Figure 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 [Phe1-(CH2-NH)-Gly2] Nociceptin(1-13)-NH2 to alter
blood flow is probably due to the animal being deeply anaesthetised 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 hyperaemic response in the presence of [Phe\(^1\)-(CH\(_2\)-NH)-Gly\(^2\)] Nociceptin(1-13)-NH\(_2\) or naloxone, while a similar effect was also detected in guanethidine injected animals (Figure 3). Co-administration of [Phe\(^1\)-(CH\(_2\)-NH)-Gly\(^2\)] Nociceptin(1-13)-NH\(_2\) and \(10^{-8}\) mol nociceptin produced an initial 67.7 ± 9.66% increase in capsular blood flow (P<0.05 repeated measures one factor ANOVA) while perfusion rose by 75.6 ± 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 ± 5.54% (P<0.05). These dilator effects were apparent immediately following drug administration and returned back to control levels after 4 min. Rats pre-treated with 1% capsaicin to destroy unmyelinated knee joint afferents also showed a 65.3 ± 15.33% increase in joint blood flow immediately following application of the [Phe\(^1\)-(CH\(_2\)-NH)-Gly\(^2\)] Nociceptin(1-13)-NH\(_2\) and nociceptin cocktail (Figure 3D). A one factor ANOVA confirmed that this hyperaemic response was statistically significant (P<0.05; \(n = 10\)).

**Effects of guanethidine or phentolamine treatment**

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

As shown in Figure 5, guanethidine treatment caused a significant inhibition of nociceptin-induced vasoconstriction in the rat knee (P<0.05 two factor ANOVA; \(n=15-16\)). Similarly, antagonism of smooth muscle \(\alpha\)-adrenoceptors by co-administration of the \(\alpha\)-adrenergic antagonist phentolamine also attenuated the constrictor capacity of nociceptin (P<0.05). Mean arterial pressure was unaffected by drug application in these 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 which could be inhibited by the specific ORL-1 receptor antagonist \([\text{Phe}^1-(\text{CH}_2-\text{NH})-\text{Gly}^2]\) Nociceptin(1-13)-NH\(_2\) as well as the non-specific 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 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 localised to the periphery as evidenced 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 which is independent of cardiac stimulation or baroreceptor reflexes.

The inhibitory effect of [Phe\textsuperscript{1}-(CH\textsubscript{2}-NH)-Gly\textsuperscript{2}] Nociceptin(1-13)-NH\textsubscript{2} on nociceptin-induced vasoconstriction confirms the presence of ORL-1 receptors in the joint capsule. This finding is supported by electrophysiological studies which have successfully demonstrated functional ORL-1 receptors on knee 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 which was reversed by naloxone while 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 \textit{ie.} analgesia instead of hyperalgesia, hypertension rather than hypotension, tachycardia as opposed to bradycardia, and vasoconstriction instead of vasodilatation. Thus, nociceptin appears to be able to exert distinct and conflicting actions \textit{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 14). The present investigation, therefore, tested whether the vasoconstrictor effects of nociceptin were sympathetically mediated. As evidenced in the
nerve stimulation experiments, supramaximal sympathetic blockade was achieved by systemic
guanethidine pre-treatment while $10^{-6}$ mol phentolamine was sufficient in producing $\alpha$-
adrenoceptor antagonism. A slight vasodilatation was observed during nerve stimulation in
guanethidine treated rats (see Figure 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 co-administration 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 noradrenaline which in turn is responsible for the synovial vasoconstriction.

In light of the [Phe$^1$-(CH$_2$-NH)-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 was that administration of the top
dose of nociceptin in the presence of naloxone or [Phe$^1$-(CH$_2$-NH)-Gly$^2$] Nociceptin(1-13)-NH$_2$
caused a transient but significant hyperaemic 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 (eg. ORL-2) requires further investigation,
but it would explain why the ORL-1 specific antagonist [Phe$^1$-(CH$_2$-NH)-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. found that low and high dose nociceptin produced disparate effects on 5-hydroxytryptamine-induced protein extravasation in the rat knee (18). Additional evidence comes from electrophysiology studies in which nociceptin caused conflicting effects on knee joint mechanosensitivity depending upon 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 due to nociceptin causing secondary release of pro-inflammatory peptides into the knee. Hyperalgesia caused by peripherally applied nociceptin appears to be dependent upon 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 one week following treatment (13). In these capsaicin treated rats, co-administration of [Phe\(^1\)-(CH\(_2\)-NH)-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 that nociceptin does not interact with the vascular endothelium to alter blood vessel tone (3, 8).

The model system employed in this study, while 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 hyperaemia and potentially reduce joint inflammation. As peptide levels continue to rise, however, nociceptin would eventually become pro-inflammatory as its dilator characteristics are suddenly invoked. More information regarding 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 hyperaemia which is not neurogenically mediated. This dilator response is succeeded by a more prolonged vasoconstrictor response which 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.
ACKNOWLEDGEMENTS

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REFERENCES


**Figure 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 following 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 following topical application of $10^{-12}$ mol nociceptin. The greatest reduction in joint blood flow occurred at the 2min time point with perfusion gradually recovering over the proceeding 4 min. The colour coding system used in the images is shown at left with bands ranging from dark blue (0 perfusion units) to white (255 perfusion units).

**Figure 2:** Effect of nociceptin on knee joint perfusion compared to vehicle (saline) control, or in the presence of $10^{-9}$ mol of the ORL-1 receptor antagonist [Phe$^1$-(CH$_2$-NH)-Gly$^2$] Nociceptin(1-13)-NH$_2$, or $10^{-9}$ mol of the non-specific opioid antagonist naloxone. The time point chosen for each dose corresponds to the maximal response to nociceptin (*ie.* 2min for the $10^{-15}$ – $10^{-10}$ mol doses and 4min 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 S.E.M.
Figure 3: Transient hyperaemic response to 10^{-8} mol nociceptin as revealed during [Phe^1-(CH_2-NH)-Gly^2] Nociceptin(1-13)-NH_2 (A), or naloxone (B) treatment. In guanethidine treated animals (C), 10^{-8} 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 co-administration of 10^{-8} mol nociceptin and [Phe^1-(CH_2-NH)-Gly^2] Nociceptin(1-13)-NH_2. NC: Nociceptin; NC (1-13): [Phe^1-(CH_2-NH)-Gly^2] Nociceptin(1-13)-NH_2. *P<0.05; Dunnett’s Multiple Comparison post-test. Means ± S.E.M. are shown; n = 10 – 16.

Figure 4: Vasoconstrictor response of capsular blood vessels to electrical stimulation of joint sympathetic nerves and its inhibition by the \(\alpha\)-adrenoceptor antagonist phentolamine (10^{-6} mol) and by guanethidine treatment. P<0.05 one-factor ANOVA; n = 5 – 8. Data are means ± S.E.M.

Figure 5: Effect of supramaximal sympathetic blockade with guanethidine pre-treatment or \(\alpha\)-adrenergic antagonism by 10^{-6} mol phentolamine co-administration on nociceptin-induced vasoconstriction. Across the dose range tested, guanethidine and phentolamine significantly attenuated the constrictor effect of nociceptin (for guanethidine treated P<0.05 two factor ANOVA; n = 15 – 19. For phentolamine treated P<0.05; n = 9 – 19). Data shown as means ± S.E.M.
Table 1: Mean arterial blood pressure (mmHg) in response to topical nociceptin application either by itself or in the presence of [Phe\(^1\)-(CH\(_2\)-NH)-Gly\(^2\)] Nociceptin(1-13)-NH\(_2\), naloxone, phentolamine, or following guanethidine treatment. Means ± S.E.M are shown.

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<th>Dose of Nociceptin (mol)</th>
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<th>(10^{-14})</th>
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</tr>
<tr>
<td>With (10^{-9}) 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>
<td>(P = 0.6024)</td>
</tr>
<tr>
<td>With (10^{-9}) mol [Phe(^1)-(CH(_2)-NH)-Gly(^2)] Nociceptin(1-13)-NH(_2)</td>
<td>70±2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>67±5</td>
<td>67±4</td>
<td>66±4</td>
<td>69±6</td>
<td>66±7</td>
<td>(P = 0.7945)</td>
</tr>
<tr>
<td>With (10^{-6}) mol phentolamine</td>
<td>60±2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>65±5</td>
<td>58±4</td>
<td>56±3</td>
<td>57±3</td>
<td>56±3</td>
<td>(P = 0.6537)</td>
</tr>
<tr>
<td>Guanethidine treated</td>
<td>74±2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>73±5</td>
<td>77±6</td>
<td>72±6</td>
<td>71±5</td>
<td>75±6</td>
<td>(P = 0.9815)</td>
</tr>
</tbody>
</table>
Figure 1:
Figure 2
Figure 3

A

B

C

D

Guanethidine Treated

Capsaicin Treated
Figure 4

Nerve Stim. (Control)  Nerve Stim. (Phentolamine Treated)  Nerve Stim. (Guanethidine Treated)

% Change in Perfusion

-60  -50  -40  -30  -20  -10  0  10

Figure 4
Figure 5

- ■ Nociceptin
- ○ Guanethidine treated
- □ Phentolamine treated

% change in perfusion

Dose Nociceptin (mol)