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Regional hemodynamic effects of nociceptin/orphanin FQ in the anesthetized rat

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Abstract

This study examined the vasodilator action of nociceptin, an endogenous opioid receptor-like ligand (ORL1), in thiobutabarbital-anesthetized rats, via the triple-isotope microspheres technique. Nociceptin (10, 30 nmol/kg, left ventricular injection) reduced mean arterial pressure (− 27, − 29 mm Hg), total peripheral resistance (− 36, − 41% of baseline) and heart rate (− 8, − 11% of baseline), but did not significantly affect cardiac output. The vehicle (0.9% NaCl) did not alter hemodynamics. Both doses of nociceptin caused similar changes in arterial flow and conductance of all tissues. Nociceptin increased flows to the skeletal muscle, slightly reduced flows to the caecum and colon, but did not alter flows to other organs and tissues. With flow normalized by pressure to reflect intrinsic vascular tone, nociceptin was found to increase arterial conductance of all tissues, except for the intestine, spleen, caecum and colon. Its dilator influence was greater in the skeletal muscle (≈ 250% of baseline conductance) than the lungs, heart, liver, stomach, kidneys, skin, testes and brain (140–160% of baseline). Thus, nociceptin causes generalized vasodilatation; its greatest influence is on the skeletal muscle bed.

Keywords: Nociceptin; Arterial pressure, mean; Peripheral resistance, total; Blood flow; Arterial conductance

1. Introduction

Nociceptin (or orphanin FQ), the endogenous agonist for the opioid receptor-like$_1$ (ORL1) receptor, is a 17-amino acid peptide whose primary structure resembles that of dynorphin A (Meunier et al., 1995; Reinscheid et al.,1995). ORL1 is a G-protein-coupled receptor with amino acid sequence similar to those of opioid receptors (Bunzow et al., 1994; Chen et al., 1994), and is widely distributed in the central nervous system, including areas involved with blood pressure regulation (Mollereau and Mouledous, 2000). High affinity binding sites for nociceptin are also present in the rat heart (Dumont and Lemaire, 1998).

In vitro studies show that nociceptin causes relaxation of preconstricted feline renal, mesenteric, carotid and femoral arterial rings (Gumusel et al., 1997), as well as rat aortic rings (Hugghins et al., 2000), and dilatation of pressurized and preconstricted mesenteric resistance arteries (Champion et al., 1998a). I.v. injection of nociceptin reduced blood pressure and heart rate in anesthetized rats (Champion et al., 1997; Guiliani et al., 1997) and unanesthetized mice (Madeddu et al., 1999), but increased blood pressure and heart rate in conscious sheep (Arndt et al., 1999). The vasodepressor response to nociceptin was not antagonized by the opioid receptor antagonist, naloxone (Champion et al., 1998b; Czapla et al., 1998). Nociceptin also reduced the pressure of constant flow perfused rat hindquarter (Czapla et al., 1997; Champion et al., 1999). The aim of the present study was to examine the spectrum of the vasodilator action of nociceptin in the anesthetized rat.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (350–400 g) were anesthetized with thiobutabarbital (100 mg/kg i.p.). Cannulae filled with heparinized normal saline (0.9% NaCl, 25 IU/ml) were inserted into the left ventricle via the right carotid artery for

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the injection of nociceptin as well as radioactively labelled microspheres, and into the right femoral artery for blood withdrawal as required for the measurement of flow (Pang, 1983). Cannulae were also inserted into the left femoral artery for the recording of mean arterial pressure by a pressure transducer (PD23DB, Gould, Statham, CA, USA). Heart rate was derived electronically from the upstroke of the arterial pulse pressure by a Grass 7P4G tachograph.

2.2. Measurement of cardiac output and regional blood flow

A well-stirred suspension (100–200 μl) containing 20,000–40,000 microspheres (15 μm diameter) labelled with either ^{57}Co, ^{113}Sn or ^{103}Ru (Perkin-Elmer life Sciences, Boston, MA, USA) was injected and flushed over 10 s into the left ventricle. Beginning at 10 s before the injection of each set of microspheres, blood was withdrawn (Harvard infusion/withdrawal pump) from the right femoral arterial cannula into a heparinized saline-filled syringe at 0.35 ml/min for 45 s. The order of the administration of the microspheres for each group was as follows: Co–Sn–Ru ($n=2$), Sn–Ru–Co ($n=2$), Ru–Co–Sn ($n=2$). At the end of the experiments, blood samples, whole organs, as well as 30 g each of skeletal muscle (from areas of the chest, abdomen, back and forelimb) and skin were removed for the counting of radioactivity (in counts per min or cpm) using a 1185 Searle Automatic Gamma Counter (Nuclear, Chicago, IL, USA) with a 3-in. NaI crystal. Corrections were made for the spillover of radioactivity from ^{113}Sn into the ^{57}Co channel (24%), and for spillover of ^{103}Ru into the ^{57}Co and ^{113}Sn channels (30% and 7%, respectively). Cardiac output, total peripheral resistance, organ blood flow and vascular conductance were calculated as in Pang (1983).

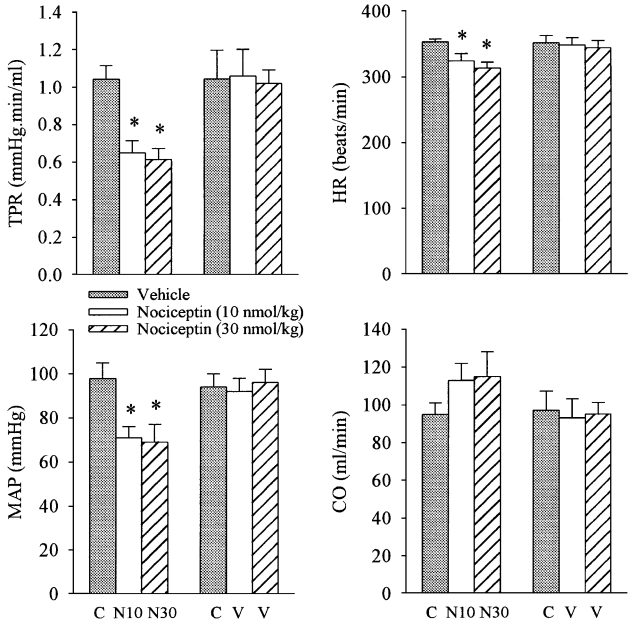

Fig. 1. Effects (means ± S.E.M.) of nociceptin (10 and 30 nmol/kg) and vehicle (0.9% NaCl) on mean arterial pressure (MAP), heart rate (HR), cardiac output (CO) and total peripheral resistance (TPR) in two groups of thiobutabarbital-anesthetized rats ($n=6$). C = pretreatment baseline, V = vehicle, N10 = nociceptin (10 nmol/kg), and N30 = nociceptin (30 nmol/kg). * Significantly different from baseline ($P<0.05$).

Table 1
Effects (mean ± S.E.M.) of vehicle (0.9% NaCl) on blood flow (ml/min) in thiobutabarbital-anesthetized rats ($n=6$ each)

Organ	Baseline	Vehicle	Vehicle
Lungs	78 ± 9	72 ± 8	79 ± 9
Heart	212 ± 9	185 ± 8	191 ± 8
Liver	20 ± 2	19 ± 2	18 ± 1
Stomach	56 ± 5	56 ± 5	61 ± 5
Intestine	103 ± 12	123 ± 14	117 ± 13
Colon + caecum	96 ± 11	96 ± 11	87 ± 10
Kidneys	479 ± 56	476 ± 56	433 ± 51
Spleen	121 ± 30	98 ± 25	103 ± 36
Muscle	56 ± 5	38 ± 4	53 ± 5
Skin	129 ± 10	112 ± 8	136 ± 10
Testes	25 ± 2	23 ± 2	24 ± 2
Brain	68 ± 6	60 ± 5	65 ± 6

Flows (per 100 g tissue), except for muscle and skin (per 1000 g tissue).

2.3. Experimental protocol

Rats were divided into two groups ($n=6$) and given 30 min to stabilize. A first set of microspheres was injected into the two groups to determine baseline flow. This was followed 10 min later by left ventricular injection of either nociceptin (10 nmol/kg) or an equal volume of saline (0.9% NaCl). A second set of microspheres was injected at the plateau phase of the depressor response to nociceptin. At 10 min after injection of the first dose of nociceptin, rats were injected with either nociceptin (30 nmol/kg) or saline, and a third set of microspheres was given at the plateau phase of the response. These two doses were chosen to allow the plateau phase of the vasodilator response to reach steady state for a long enough period to allow the determination of flow by the microspheres technique. A lower dose of nociceptin (3 nmol/kg) was not studied because the duration of the depressor response was too brief to allow reliable measurement of cardiac output and blood flow.

Fig. 2. Experimental tracing of the effect of nociceptin (30 nmol/kg) on blood pressure (BP) and mean arterial pressure (MAP) in a thiobutabarbital-anesthetized rat.

2.4. Drugs

Nociceptin (Phoenix Pharmaceuticals, CA, USA) was dissolved in distilled water, and kept in aliquots at −20° C, and an aliquot was diluted with normal saline for use on the experimental day. Thiobutabarbital (Inactin) was from Research Biochemicals International (MA, USA).

2.5. Statistical analysis

All data are shown as mean ± standard error of the mean (S.E.M.). To render homogeneous of distribution, the data of flow and conductance were log transformed prior to the analysis of variance (ANOVA, with repeated measures) followed by Tukey test, with $P<0.05$ selected as the criterion for the statistical significance.

3. Results

Left ventricular injections of saline did not significantly affect mean arterial pressure, heart rate, cardiac output and total peripheral resistance (Fig. 1) or blood flow (Table 1) to any organs or tissue. Since the vehicle did not alter mean arterial pressure or regional flows, it also did not affect arterial conductances (a ratio of flow to mean arterial pressure) of any organs or tissue (results not shown).

Both doses of nociceptin caused similar reductions of mean arterial pressure, heart rate and total peripheral resistance, but did not significantly affect cardiac output (Fig. 1). The depressor responses to nociceptin (10 or 30 nmol/kg) lasted 3–4 and 4–6 min, respectively. Fig. 2 is a representative tracing that shows the effect of nociceptin (30 nmol/kg) on blood pressure in a rat. Injection of a higher dose of nociceptin (100 nmol/kg) did not cause a greater depressor response (results not shown).

Both doses of nociceptin (10 and 30 nmol/kg) increased blood flows to the skeletal muscle, reduced flows to the caecum and colon, but did not affect flows to other organs or tissue (Fig. 3). Both doses of nociceptin also caused a similar magnitude of increase in arterial conductance in various tissues, except for the intestine, spleen, caecum and colon in which no significant changes were observed (Fig. 3). Vasodilatation was more prominent in the skeletal muscle (average ≈ 250% of baseline conductance for both doses) than the lungs, heart, liver, stomach, kidneys, skin, testes and brain (average, 140–160% of baseline for both doses).

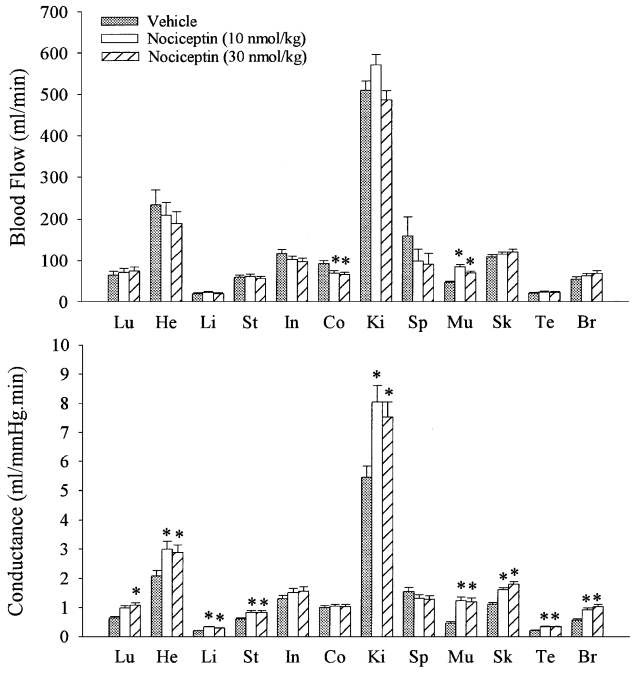

Fig. 3. Effects (means ± S.E.M.) of nociceptin (10 and 30 nmol/kg) and vehicle (0.9% NaCl) on blood flow and arterial conductance in thiobutabarbital-anesthetized rats ($n=6$). Values of flow and conductance are expressed as per 100 g of tissues from the lungs (Lu), heart (He), liver (Li), stomach (St), intestine (In), colon plus caecum (Co), kidneys (Ki), spleen (Sp), testes (Te) and brain (Br), and as per 1000 g of tissues from the skeletal muscle (Mu) and skin (Sk). * Significantly different from baseline ($P<0.05$).

4. Discussion

Nociceptin (10 and 30 nmol/kg) reduced mean arterial pressure and heart rate; these changes are in accordance to those reported previously in anesthetized rats (Champion et al., 1997; Guiliani et al., 1997). The depressor responses were of fast onset and brief duration (lasted 3 to 6 min) and associated with a significant reduction in total peripheral resistance. Cardiac output was slightly (insignificantly) increased, and was likely the result of the reduction in flow resistance. The depressor responses to nociceptin (10 and 30 nmol/kg) in pentobarbital-anesthetized rats in the Champion et al. (1997) study were, however, associated with reductions in cardiac output and total peripheral resistance. It is unclear what caused the differential effects of nociceptin on cardiac output in the two studies. Difference in the experimental conditions, for example, biological variations (source of the rats, sex, vasomotor tone) and the use of different anesthetic agents, may have affected the results of the studies.

Both doses of nociceptin caused the greatest increase in flow and arterial conductance in the skeletal muscle indicating that the skeletal muscle bed is the most sensitive to the dilator action of nociceptin. In addition to the skeletal muscle bed, nociceptin also caused significant vasodilatation of the lungs, heart, liver, stomach, kidneys, skin, testes and brain.

Since no sympathetic blockers were given, it is unclear whether dilatation responses to nociceptin were a direct action, or due to the inhibition of sympathetic vasoconstrictor tone. Nociceptin was shown to cause a depressor

response in urethane-anesthetized rats partially through the inhibition of sympathetic tone since the response was attenuated after pretreatment with guanethidine (Guiliani et al., 1997). Nociceptin is also capable of causing vasodilatation via a direct action. Nociceptin caused relaxation of isolated preconstricted vascular rings (Gumusel et al., 1997; Hugghins et al., 2000) as well as dilatation of isolated pressurized resistance mesenteric arteries, and the relaxation was unaffected by phentolamine (Champion et al., 1998a). Injections of nociceptin (1–30 nmol) caused vasodilatation of denervated and constant flow-perfused hindquarter of the rat (Czapla et al., 1997). Moreover, nociceptin caused dilatation of pial arteries of piglets partially via cAMP release, and the activation of K_{ATP} and K_{Ca} channels (Armstead, 1999). Vasodilatation following i.v. bolus injection of nociceptin in the hairless skin of pentobarbital-anesthetised, ganglionic-blocked and artificially ventilated rats has also been reported (Häbler et al., 1999).

In conclusion, i.v. injection of nociceptin causes a depressor response through generalized vasodilatation with the following rank order: skeletal muscle>lungs = heart = liver = stomach = kidneys =skin = testes = brain.

Acknowledgements

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