Cardiovascular Effects of Nociceptin in Unanesthetized Mice

Paolo Madeddu, Maria Bonaria Salis, Anna Franca Milia, Costanza Emanuelli, Remo Guerrini, Domenico Regoli, Girolamo Calò

Abstract—We evaluated the systemic hemodynamic effects induced by nociceptin (NC) and NC-related peptides, including the NC receptor antagonist [Phe1 ψ(CH2–NH)Gly2]NC(1–13)NH2 ([F/G]NC(1–13)NH2) in unanesthetized normotensive Swiss Morini mice. Bolus intravenous injection of NC decreased mean blood pressure and heart rate. The hypotensive response to 10 nmol/kg NC lasted <10 minutes, whereas a more prolonged hypotension was evoked by 100 nmol/kg (from 114±3 to 97±2 mm Hg at 10 minutes, P<0.01). The latter dose reduced heart rate from 542±43 to 479±31 beats/min (P<0.05) and increased aortic blood flow by 41±5% (P<0.05). Hypotension and bradycardia were also evoked by NC(1–17)NH2 and NC(1–13)NH2 fragments, whereas NC(1–13)OH and NC(1–9)NH2 were ineffective. Thiorphan, an inhibitor of neutral endopeptidase 24.11, enhanced the hypotension induced by NC(1–13)NH2 and revealed the ability of NC(1–13)OH to decrease mean blood pressure. [F/G]NC(1–13)NH2, a recently synthesized antagonist of the NC receptor, did not alter basal mean blood pressure or heart rate, but it prevented the hypotension, bradycardia, and increase in aortic blood flow evoked by NC. In contrast, [F/G]NC(1–13)NH2 did not alter the hypotension induced by bradykinin or endomorphin-1 (a μ-receptor agonist), and the bradycardia induced by leu-enkephalin (a δ-receptor agonist) or U504885 (a synthetic κ-receptor agonist). In conclusion, NC and some of its fragments cause hypotension and bradycardia and increase aortic blood flow in mice, with the NC(1–13) sequence being critical for these biological effects. Our results also demonstrate that the compound [F/G]NC(1–13)NH2 is a potent and selective antagonist of the NC receptor in vivo. (Hypertension. 1999;33:914-919.)

Key Words: hypotension ▪ blood pressure ▪ heart rate ▪ nociceptin ▪ [Phe1 ψ(CH2–NH)Gly2]NC(1–13)NH2 ▪ mouse

In 1994, a new opioid receptor, referred to as opioid receptor–like 1 (ORL1), was identified; 1 year later, the heptadecapeptide nociceptin (NC) (also known as orphanin FQ) was demonstrated to be the endogenous ligand for this receptor (hereafter called NC receptor).1 Peripheral biological activities of NC are similar to those exerted by opioids.1 In particular, both NC and endomorphins induce hypotension and bradycardia and reduce peripheral resistance.2–6 However, the opioid receptor antagonist naloxone does not alter the effect of NC, thus suggesting that the latter peptide activates receptors different from those of opioids.2

The cardiovascular effects of NC have been characterized mainly in rats.1–6 Similar to opioids, NC causes hypotension without reflex tachycardia; instead, bradycardia occurs after acute intravenous injection.6 The role, if any, of endogenous NC and related peptides in the regulation of cardiovascular function remains unknown, mainly because of the lack of potent and selective receptor antagonists. Recently, this limitation has been overcome, at least partially, by the discovery of [Phe1 ψ(CH2–NH)Gly2]NC(1–13)NH2 ([F/G]NC(1–13)NH2). This compound has been shown to act as a selective antagonist in peripheral NC-sensitive preparations such as the guinea pig ileum and mouse vas deferens.7 We exploited the availability of new technologies for measuring hemodynamic parameters in the mouse8,9 to design a series of experiments aimed to characterize the cardiovascular action of exogenous NC and NC-related peptides. In addition, we tested the antagonistic property of [F/G]NC(1–13)NH2 in vivo to investigate the role of endogenous NC in the regulation of cardiovascular function.

Methods

Animals

Male Swiss Morini mice (28 to 35 g body wt), purchased from Morini (Reggio Emilia, Italy), were housed at constant room temperature (24±1°C) and humidity (60±3%) with a 12-hour light/dark cycle. All experimental protocols complied with standards for the care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the local Animal Care and Use Committee.
**Results**

**Experiment 1: Effect of NC and NC-Related Peptides on MBP and HR**

No difference was detected between groups given vehicle or NC (1, 10, or 100 nmol/kg) in basal MBP (112±4, 112±3, 110±5, and 114±3 mm Hg, respectively; \( P=NS \)) or HR values (551±46, 556±24, 585±51, and 542±43 beats/min, respectively; \( P=NS \)). Intravenous bolus injection of NC decreased the MBP of Swiss Morini mice (Figure 1, left). The hypotensive response to 10 nmol/kg lasted <10 minutes, whereas a more prolonged hypotension was evoked by 100 nmol/kg (from 114±3 to 97±2 mm Hg at 10 minutes, \( P<0.01 \)). No incremental MBP effect was observed with higher doses (data not shown). As also shown in Figure 1 (right), 100 nmol/kg NC reduced HR from 542±43 to 479±31 beats/min (\( P<0.05 \)). A similar effect was observed with 10 nmol/kg NC. No change in MBP and HR was observed after administration of vehicle.

As shown in Figure 2, 100 nmol/kg NC(1–17)NH₂ or NC(1–13)NH₂ caused hypotension and bradycardia similar to that caused by NC. By contrast, the same dose of NC(1–13)OH, NC(1–9)NH₂, or [F/G]NC(1–13)NH₂ did not alter MBP or HR. A tendency of HR to increase following occlusion of the aorta. Then the probe wires were tunneled out of the back of the neck, and the animals were allowed to recover for 5 hours. Intra-arterial MBP was measured with a Statham transducer connected to the carotid catheter, and heart rate (HR) was measured with a counter triggered by the pressure waveform.

In a set of experiments, aortic blood flow (ABF) was measured with a pulsed Doppler flowmeter (Pulse-Doppler, University of Iowa). With mice anesthetized, a Doppler probe (0.8 mm internal diameter, Crystal Biotech) was implanted and secured with 6-0 ophthalmic silk around the abdominal aorta just below the origin of the left renal artery. The electronic zero, established by turning off the ultrasound signal, was equal to zero flow obtained by a short-lasting occlusion of the aorta. Then the probe wires were tunneled out of the back of the neck, and the animals were allowed to regain consciousness. Experiments were performed 5 hours after implantation of the probe. During basal and experimental periods, hemodynamic parameters were continuously recorded in unanesthetized, free-moving mice using a Quartet polygraph (Ugo Basile Biological Apparatus). A 50-μL syringe (Hamilton) was used for drug injection. Injection volume was 30 μL, followed by 10 μL saline to flush the jugular catheter.

**Experiment 2: Effect of Thiophan on Hemodynamic Changes Induced by NC or NC Fragments**

Mice received thiophan at 2.5 mg/kg body wt (n=5 each group) or vehicle intravenously (n=4 each group) and then were injected with NC(1–13)NH₂ or NC(1–13)OH at 100 nmol/kg. MBP and HR were recorded continuously for an additional 10 minutes. In preliminary experiments, we found that the dose of thiophan used in the present study enhanced the MBP-lowering effect of exogenously administered bradykinin (38±3 mm Hg at 25±3 mm Hg in controls, \( P<0.01 \)), whereas it does not alter the hypotension evoked by verapamil, a compound not metabolized by neutral endopeptidase (NEP) 24.11 (27±2 vs 25±2 mm Hg in controls, \( P=NS \)).

**Experiment 3: Effect of [F/G]NC(1–13)NH₂ on NC-Evoked Changes in MBP, HR, and ABF**

After stabilization, [F/G]NC(1–13)NH₂ (at 1, 10, or 100 nmol/kg body wt) or vehicle was injected in unanesthetized mice. Five minutes later, NC (1, 10, or 100 nmol/kg body wt) was administered intravenously. Each animal received a single dose of agonist and antagonist (n=5 each group). MBP and HR were recorded continuously for 10 minutes after NC injection. In a subgroup of animals, ABF was also measured.

**Experiment 4: Selectivity of [F/G]NC(1–13)NH₂**

To test the selectivity of the antagonist, we pretreated mice with [F/G]NC(1–13)NH₂ (100 nmol/kg body wt) or vehicle. Five minutes later, NC (10 nmol/kg body wt), bradykinin (20 nmol/kg body wt), endomorphin-1 (100 nmol/kg body wt), leu-enkephalin (100 nmol/kg body wt), or U50488S (100 nmol/kg body wt) was given (n=5 each group). Hemodynamic measurements were obtained for an additional 10 minutes.

**Statistical Analysis**

All data are expressed as mean±SEM. Multivariate repeated-measures ANOVA was performed to test for interaction between time and grouping factor. Univariate ANOVA then was used among groups and over time. Differences within and between groups were determined using paired or unpaired Student’s t test, respectively. A value of \( P<0.05 \) was considered significant.
Experiment 3: Effect of [F/G]NC(1–13)NH₂ on NC-Evoked Changes in MBP, HR, and ABF

As shown in Figure 4, the dose-response curves to NC were shifted to the right by [F/G]NC(1–13)NH₂. Complete blockade of NC-induced hemodynamic effects was observed with a 10:1 antagonist/agonist stochiometric ratio.

As shown in Figure 5, NC-induced hypotension was associated with an increase in ABF, with this response also being prevented by [F/G]NC(1–13)NH₂.

Experiment 4: Selectivity of [F/G]NC(1–13)NH₂

Although preventing the hypotension and bradycardia evoked by NC, [F/G]NC(1–13)NH₂ did not alter the MBP effect induced by bradykinin (−22±2 versus −20±1 mm Hg in controls, P=NS) or by the μ-receptor agonist endomorphin-1 (−10±1 versus −11±2 mm Hg in controls, P=NS). No
A significant MBP change occurred after leu-enkephalin or U504885 in mice given the NC antagonist or its vehicle (data not shown). Leu-enkephalin and U504885 induced bradycardia (−35±6 and −43±7 beats/min, respectively), with these changes being unaltered by [F/G]NC(1–13)NH₂ (−38±5 and −44±5 beats/min, respectively).

**Discussion**

The cardiovascular effects of NC, a novel opioid peptide that has received a great deal of attention from the neuroscience community, have now been investigated. The present study describes the hemodynamic action of NC, NC fragments, and
selectivity of [F/G]NC(1–13)NH2 is indicated by the failure of the ORL1 antagonist to interfere with the hypotensive action of a structurally unrelated peptide, such as bradykinin, and more importantly, with the hypotension induced by endomorphin-1, a μ-receptor agonist. In addition, [F/G]NC(1–13)NH2 did not alter the bradycardia evoked by leu-enkephalin or by the synthetic κ-receptor agonist U504885. These results are consistent with those showing the selectivity of the antagonist in vitro.

The observation that [F/G]NC(1–13)NH2 does not affect basal systemic hemodynamics does not favor a major role of endogenous NC in the regulation of cardiovascular function under normal conditions. NC acts as a modulator of pain perception at the level of the central nervous system and peripheral neuronal endings. Therefore, the vasodilator property of NC could become evident under pathological conditions characterized by nociception in association with hypotension. It is hoped that this possibility will be addressed by the use of the antagonist [F/G]NC(1–13)NH2.

The present study deals with the acute pharmacological effects of NC on cardiovascular function. Recent evidence indicates that NC exerts diuretic and antinatriuretic responses in rats.18 It would be interesting to evaluate whether NC antagonism is able to alter sodium homeostasis, thus producing long-term effects on blood pressure.

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References


Characterization of the cardiovascular effects of NC fragments revealed that NC(1–13)NH2 retains a potent agonistic activity in mice, whereas NC(1–9)NH2 is ineffective. These results are consistent with in vitro studies showing that NC(1–13)NH2 is the smallest peptide maintaining the same efficacy and potency as the natural peptide.11,12 Interestingly, the fragment NC(1–13)OH does not exert any cardiovascular effect. This could be due to a high susceptibility to enzymatic degradation and/or to a low affinity for the receptor. The former possibility is favored by the observation that the protease inhibitor thiorphan significantly enhances the hypotensive and bradycardic effect induced by NC(1–13)OH. Once again, these data are consistent with those obtained in the mouse vas deferens: in fact, pretreating this tissue with thiorphan caused a 10-fold increase in NC(1–13)OH potency, whereas the potency of NC(1–13)NH2 was not significantly modified.13 Altogether, these findings suggest that NEP 24.11 plays an important role in the peripheral degradation of NC and NC-related peptides. NEP 24.11 appears to be less important in the brain, where aminopeptidase N and endopeptidase 24.15 have been indicated as the main enzymes responsible for NC degradation.14,15 We found that the fragment NC(1–9)NH2, which does not alter MBP, tends to induce tachycardia, an effect opposite to that evoked by NC. Further studies are necessary to determine whether this action is due to interaction of NC(1–9)NH2 with unrelated receptors or to other unforeseen properties of the fragment.

Hemodynamic effects similar to those exerted by NC have been reported with the μ-opioid receptor ligands endomorphin-1 and -2. Despite the structural similarity of NC and its receptor with the opioid peptides and receptors, the cardiovascular effects mediated by NC are not modified by naloxone.4 Thus, the two systems (NC and opioids) appear to exert a similar depressant action on the cardiovascular system by activating distinct receptors. Only recently, the lack of selective antagonists has been overcome by the discovery that [F/G]NC(1–13)NH2, an analogue designed to be resistant to degradation by aminopeptidases, is instead a fairly potent NC receptor antagonist in various preparations in vitro.7 Actually, the putative ORL1 antagonist has been found to exert residual agonistic activity in Chinese hamster ovary cells transfected with the human ORL1,16 with this property being directly proportional to receptor expression.17 However, we found that in vivo [F/G]NC(1–13)NH2 is devoid of any residual agonistic activity at doses from 1 to 100 nmol/kg. In addition, the same range of doses was able to antagonize NC-induced cardiovascular effects.

As far as we know, this is the first report indicating that NC exerts hypotensive and bradycardic effects in mice. The range of doses showing pharmacological activity and the duration of the hemodynamic effects were similar to those already reported in rats.2,6 At variance with most vasodilators, NC-induced hypotension is associated with a decrease in HR. In rats, this bradycardia is significantly reduced by bilateral vagotomy and abolished by the combination of this maneuver with guanethidine. These findings suggest that the bradycardic effect of NC is mediated by a concomitant inhibition and activation, respectively, of the sympathetic and parasympathetic outflows to the heart.6


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