Nociceptin/Orphanin FQ (N/OFQ)-Evoked Bradycardia, Hypotension, and Diuresis Are Absent in N/OFQ Peptide (NOP) Receptor Knockout Mice

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ABSTRACT

Intracerebroventricular administration of the opioid-like peptide nociceptin/orphanin FQ (N/OFQ) produces bradycardia, hypotension, and diuresis in mice. We hypothesized that these responses are solely caused by selective activation of central N/OFQ peptide (NOP) receptors. To test this premise, we first examined whether i.c.v. N/OFQ produced dose-dependent diuretic and cardiovascular depressor responses in commercially available C57BL/6 mice. Next, using doses established in these studies, we examined the renal excretory and cardiovascular responses to i.c.v. N/OFQ in conscious transgenic NOP receptor knockout mice (NOP−/−). In metabolic studies, i.c.v. N/OFQ, but not saline vehicle, dose-dependently increased urine output (V) in NOP−/−; this response was significant at 3 nmol (N/OFQ, V = 0.39 ± 0.10 ml/2 h; saline, 0.08 ± 0.05 ml/2 h). The N/OFQ-evoked diuresis was absent in littermate NOP−/− (N/OFQ, V = 0.06 ± 0.06 ml/2 h; saline, 0.03 ± 0.03 ml/2 h).

There were no significant changes in urinary sodium or potassium excretion or free water clearance in either group. In telemetry studies, i.c.v. N/OFQ dose dependently lowered heart rate (HR) and mean arterial pressure (MAP). At 3 nmol N/OFQ, both HR and MAP were reduced in NOP−/− (peak ∆HR = −217 ± 31 bpm; peak ∆MAP = −47 ± 7 mm Hg) compared with saline (peak ∆HR = −14 ± 5 bpm; peak ∆MAP = 2 ± 3 mm Hg). These N/OFQ-evoked bradycardic and hypotensive responses were absent in NOP−/− (peak ∆HR = −13 ± 17 bpm; peak ∆MAP = −2 ± 4 mm Hg, respectively). Basal 24-h cardiovascular and renal excretory function were not different between NOP−/− and NOP+/+ mice. These results establish that the bradycardia, hypotension and diuresis produced by centrally administered N/OFQ are mediated by selective activation of NOP receptors.

Nociceptin/orphanin FQ (N/OFQ) is an endogenous neuropeptide that selectively binds to and activates an opioid-like receptor called the N/OFQ peptide (NOP) receptor (previously called opioid receptor-like 1) (Meunier et al., 1995; Reinscheid et al., 1995). Although the N/OFQ-NOP system belongs to the opioid receptor family, classic opioid receptor agonists do not display appreciable binding affinity to the NOP receptor and vice versa (Lachowicz et al., 1995; Molle-

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ABBREVIATIONS: N/OFQ, nociceptin/orphanin FQ; NOP, nociceptin/orphanin FQ peptide; HR, heart rate; MAP, mean arterial pressure; UFP-101, [Nphe1,Arg14,Lys15]N/OFQ-NH2; NOP−/−, NOP receptor knockout; NOP+/, NOP receptor wild-type; V, urine output; UNaV, urinary sodium excretion; UKV, urinary potassium excretion; C\textsubscript{woc}, free water clearance.
flow rate in both rodent species (Kapusta et al., 1997; Kapusta and Kenigs, 1999; Rizzi et al., 2004; Burmeister and Kapusta, 2007), which, in rats, occurs as a selective water diuresis (e.g., concurrent diuresis, antinatriuresis, and increase in free water clearance) (Kapusta and Kenigs, 1999). The pattern of the renal excretory responses to central N/OFQ is also intriguing because it is unlike the diuretic and natriuretic profile produced by central α-2/ imidazoline receptor agonists (e.g., clonidine), which also produce cardiovascular depressor and renal sympathetic inhibitory effects (Koeppke and DiBona, 1986; Patel and Zeigler, 1993; Szabo, 2002). Taken together, these findings suggest that the central N/OFQ-NOP system plays a novel role in regulating cardiovascular and fluid/electrolyte homeostasis.

We have previously shown that i.c.v. administration of UFP-101, a purported antagonist of the NOP receptor, prevents the cardiovascular depressor and diuretic responses to i.c.v. N/OFQ in conscious CD-1 mice (Burmeister and Kapusta, 2007) and Sprague-Dawley rats (Calo et al., 2005). As an alternative to pharmacological antagonists such as UFP-101, whose effects are acute, the use of transgenic NOP receptor knockout mice (N/OFQ/−/−) now offers a valuable research approach to study the role(s) of endogenous N/OFQ-NOP receptor pathways in the control of cardiovascular and renal function. However, before this genetic approach can be used to explore the role of endogenous N/OFQ in different chronic stressful or pathological conditions, it is essential to first establish that the cardiorenal responses to central N/OFQ are absent in N/OFQ/−/− mice. Furthermore, it is important to determine whether N/OFQ/−/− mice, by virtue of the genetic deletion of NOP receptors, have altered basal levels for cardiovascular function and/or daily sodium/water excretion.

Thus, the purpose of the present studies was to use NOP/−/− mice to determine whether N/OFQ-mediated bradycardia, hypotension, and diuresis are caused by selective activation of central NOP receptors. For this purpose, dose-response studies were initially performed to investigate the central N/OFQ-evoked changes in cardiovascular and renal excretory function produced by i.c.v. injection of this peptide in commercially available C57BL/6 mice (Harlan, Indianapolis, IN). C57BL/6 mice were used for these initial studies because they are similar in genetic background strain into which the transgenic NOP receptors have been deleted. Subsequent studies then used maximally effective doses of the peptide to examine the cardiovascular and renal excretory responses produced by i.c.v. N/OFQ in conscious NOP/−/− mice and NOP/+/+ littermates. Related studies also examined whether there were differences in basal 24-h cardiovascular and renal excretory function between NOP/−/− and NOP/+/+ mice. For these investigations, cardiovascular and renal excretory function was independently assessed using radiotelemetric and metabolic function methods, respectively. Here, we report that N/OFQ failed to evoke cardiodepression and diuresis after this peptide’s i.c.v. injection in NOP/−/− mice, which provides clear evidence that the responses are caused by selective activation of central NOP receptors.

Materials and Methods

Subjects

Age-matched nontransgenic C57BL/6N (Harlan) and transgenic C57BL/6J (The Jackson Laboratory, Bar Harbor, ME) mice functionally deficient in the NOP receptor gene (knockout, NOP/−/−) and their age-matched littermates (wild-type, NOP/+/+) weighing 20 to 25 g were used in these studies. NOP/−/− mice were produced as previously described (Clarke et al., 2001) and backcrossed for at least 10 generations to C57BL/J. NOP/−/− and NOP/+/+ mice used for these studies were generated from mating wild-type and homozygous NOP/−/− mice after the backcross. In previous studies, it has been shown that there is a complete absence of binding in homozygous brains from these NOP/−/− mice, thus indicating the single gene encodes for the NOP receptor and any putative subtypes (Clarke et al., 2001).

Animals were housed and tested in a climate-controlled room set to a 12-h dark/light cycle. Animals were fed a standard mouse chow diet (Lab Diet, Richmond, IN; product code 5015) and allowed tap water ad libitum. All experimental procedures were performed in accordance with Louisiana State University Health Sciences Center and National Institute of Health guidelines for the care and use of experimental animals.

Surgical Procedures

Mice were instrumented with a stainless steel guide cannula (Plastics One, Inc., Roanoke, VA) into the right lateral cerebral ventricle under anesthesia (ketamine, 200 mg/kg i.p., in combination with xylazine, 10 mg/kg i.p.). The coordinates used for cannula implantation were derived from the atlas of the mouse brain by Paxinos and Franklin (2003): 0.3 mm posterior to the bregma, 1.0 mm lateral to the midline, and 3.1 mm below the skull surface. Custom cut and fabricated guide, dummy (obturator) and internal cannula were also purchased from Plastics One, Inc. The guide cannula was fixed into position by jeweler’s screws and cranioplastic cement. Verification of cannula position in the lateral cerebroventricle was made by observing spontaneous flow of cerebrospinal fluid from the tip of the cannula after removal of the obturator (after cannula implantation and before experimentation) and after completion of the experiment by i.c.v. dye injection and subsequent post-mortem brain section verification of dye placement.

Animals were given 5 days to recover before the initiation of renal function studies. Upon completion of the renal function studies, a subset of the mice were again anesthetized (200 mg/kg i.p. ketamine in combination with 10 mg/kg i.p. xylazine) and instrumented with a TAI1P2C0 telemetry probe (Data Sciences International, St. Paul, MN), which was implanted into and secured to the left common carotid artery for chronic measurement of MAP and HR. Proper catheter placement was verified by the quality of the audio output signal on an FM radio. The body of the probe was placed in a s.c. pocket created in the right flank, and the wound was closed and sutured. Body temperature was maintained at 37°C using a heat lamp until sternal recumbency had been restored. Animals received a single injection of antibiotic (sterile potassium penicillin, 60,000 units/kg i.p.) and instrumented with a Plexiglas (walls) and stainless steel (tow of bars positioned horizontally upon which the animal sat) and were designed to allow spontaneously voided urine to be collected onto a large plastic weigh boat positioned underneath the
animal. On the day before experimentation, each mouse was removed from its home cage and "trained" by being placed individually into a metabolic cage for 20 min. The animals were then removed, and the lower abdominal region was gently massaged to void any urine already present in the bladder. Each animal was also touched in the region of the i.c.v. cannulae and then returned to the metabolic cages for 2 h. On the experimental day, the same procedure was repeated, with the exception that after the 20-min habituation period, each animal (n = 13–14/group) randomly received an i.c.v. injection of either isotonic saline vehicle (2 µl) or N/OFQ (0.03, 0.3, 1, 3, or 10 nmol). Note that the i.c.v. doses of N/OFQ used in the present cardiovascular and renal function studies are within range of those previously used (0.055, 1, 3.0, or 10 nmol i.c.v.) to test this peptide’s antinociceptive, hyperalgesic, and allodynic effects in acute thermal or mechanical tests in mice and rats (Vanderah et al., 1998).

Intracerebroventricular injections were performed by securely placing an i.c.v. injector into each animal’s indwelling i.c.v. guide cannula; the i.c.v. injector was attached by polyethylene (PE-10) tubing to a Hamilton syringe for injection of drug/vehicle. After i.c.v. drug/vehicle injection, the animals were returned to their metabolic cages. The experimental period then involved the hourly collection of urine that was spontaneously voided from mice starting after vehicle/drug administration for 2 h. Parameters analyzed were urine flow rate (V), urinary excretion of sodium (UNaV) and potassium (UKV), and free water clearance (Cf). Animals were not allowed access to food and water during testing and were returned to their home cages upon completion of each study.

Cardiovascular Function Studies. In a second set of experiments, a subset of the same animals that had completed the renal excretory function studies (described above) were randomly selected and assigned to be surgically instrumented with radiotelemetry probes to determine whether diuretic doses of N/OFQ also altered cardiovascular function. Upon recovery 7 days later (i.e., restoration of the diurnal cycle), the conscious, telemetered mice were tested in their home cages. Telemetry probes were turned on with the least possible disturbance to the animals for the measurement of baseline HR and MAP (sampling frequency, 500 Hz; sampling interval, 2 s). After baseline sampling, an i.c.v. injector was securely attached to each animal’s indwelling cannula; the i.c.v. injector was attached via PE-10 tubing to a Hamilton syringe for injection of drug/vehicle. Animals were then returned to their home cages. The PE tubing was extended outside of the cages to keep disturbance to the animals to a minimum and to prevent it from being gnawed and damaged. After stabilization and then additional baseline sampling (i.e., two 10-min predrug control periods), N/OFQ (0.03, 0.3, 1, or 3 nmol) or isotonic saline vehicle (2 µl) was randomly delivered i.c.v. via the Hamilton syringe injector assembly. MAP and HR were then measured continuously during a 120-min experimental period (12 consecutive 10-min periods). Parameters analyzed were the MAP and HR responses to i.c.v. N/OFQ and isotonic saline control.

Renal and Cardiovascular Function Studies Conducted in Transgenic C57BL/6J NOP−/− Mice and Wild-Type Littermates

Basal 24-h Renal Function Studies. After recovery from i.c.v. cannula implantation (5 days), NOP−/− and NOP+/− C57BL/6J mice were individually placed in metabolic cages for 5 days for assessment of basal 24-h renal excretory function in each group. For these studies, consecutive 24-h water intake, urine/sodium/potassium excretion, and free water clearance were measured. After the completion of these studies, these same mice were then used in renal function experiments that involved the i.c.v. injection of N/OFQ or vehicle (protocol below).

Intracerebroventricular N/OFQ Renal Excretory Function Studies. The experimental approach used for these experiments was the same as those described above for the i.c.v. N/OFQ dose-response studies (renal excretory) performed in nontransgenic mice, with the exception that transgenic NOP−/− and littermate NOP+/− C57BL/6J mice served as the experimental subjects. For these investigations, the i.c.v. doses of N/OFQ tested in NOP+/− and NOP−/− mice were those previously determined to have significant and maximal effects on renal excretory and cardiovascular function in the dose-response studies performed in commercially available (i.e., nontransgenic) C57BL/6 mice (Harlan) (protocols described above).

Basal 24-h Cardiovascular Function Studies. After completion of renal function studies (basal measurements and renal responses to i.c.v. N/OFQ/vehicle), a subgroup of the same NOP−/− and NOP+/− C57BL/6J mice were instrumented with telemetry probes and allowed to recover (7 days). After recovery of circadian rhythm, basal 24-h MAP and HR were measured over a 24-h period in NOP−/− and NOP+/− C57BL/6J mice while housed in their home cages.

Intracerebroventricular N/OFQ Cardiovascular Function Studies. After the completion of the basal 24-h cardiovascular function studies, these same telemetered mice were then used in cardiovascular function experiments that involved the i.c.v. injection of N/OFQ or vehicle (the protocol was the same as described above for nontransgenic mice).

Analytical Techniques and Data Analysis

Urine volume was determined gravimetrically. Urine sodium and potassium concentrations were measured by flame photometry (model 943; Instrumentation Laboratory, Lexington, MA). Urine osmolality was analyzed using a vapor pressure osmeter (Wescor, Inc., Logan, UT; model 5500). Telemetry data were compiled and analyzed using Dataquest A.R.T. Software (version 2.3; Data Sciences International) and Microsoft Excel (Microsoft, Redmond, WA). The output from the radiotelemetry probes was recorded (500 Hz) using receiver plates placed beneath the home cages. Data were sent to a consolidation matrix before being stored on a personal computer. Results were plotted and graphed using GraphPad Prism software (version 4.00; GraphPad Software Inc., San Diego, CA).

Statistics

Results are expressed as the mean ± S.E.M. The magnitude of the changes in cardiovascular and renal excretory parameters at different time points after i.c.v. injection of drugs were compared with respective group control values by one-way repeated measures analysis of variance with subsequent Dunnett’s test. Differences occurring between treatment groups (e.g., multiple doses of drug) were assessed by two-way repeated measures analysis of variance with treatment being one fixed effect and time the other, with the interaction included. The time (minutes) was the repeated factor. Post hoc analysis was performed using Bonferroni’s test. Where appropriate, a Student’s t test was also used to compare means between two groups. Data were verified for normality of distribution and equality of variances. In each case, statistical significance was defined as p < 0.05.

Drugs

N/OFQ (FGGFTGARKSARKLANQ) was synthesized and graciously provided by Drs. Remo Guerrini, Severo Salvadori, and Girolamo Calò (University of Ferrara, Ferrara, Italy). Stock solutions of the peptide were prepared fresh and stored frozen. N/OFQ/vehicle was administered i.c.v. in a volume of 2 µl.

Results

Nontransgenic C57BL/6N Nhsd Mouse (Harlan) Studies. The renal excretory responses produced by the i.c.v. administration of N/OFQ in conscious C57BL/6 mice (Harlan) are presented in Fig. 1, A (cumulative urine output data) and B (time course renal excretory data). As shown in Fig. 1A, i.c.v. N/OFQ (tested at 0.03, 0.3, 1, 3, and 10 nmol) elicited a
significant and dose-dependent increase in V over a 2-h period. At the 1 nmol i.c.v. dose, urine output was significantly and maximally elevated (V = 0.33 ± 0.04 ml/2 h) in comparison with the change in urine output produced by i.c.v. isotonic saline vehicle (V = 0.13 ± 0.04 ml/2 h). The 3 nmol i.c.v. dose produced a diuresis comparable with that of the 1 nmol dose. However, there was no urine excreted from animals after 10 nmol N/OFQ, most likely due to the marked hypotension caused by this high dose, which also caused sedation. Analysis of the time course data revealed that at 1 nmol, the i.c.v. N/OFQ-evoked diuresis occurred primarily during the 2nd h of the 2-h time course (Fig. 1B); there were no significant changes in UNaV or UKV excretion or CH2O (Fig. 1B).

Figure 2 illustrates the changes in HR and MAP produced by i.c.v. injection of N/OFQ (0.3, 1, or 3 nmol) or isotonic saline vehicle in conscious telemetered C57BL/6 mice (Harlan). As shown (Fig. 2), the i.c.v. administration of isotonic saline did not alter HR or MAP at any point throughout the study. However, after i.c.v. injection of N/OFQ, HR began to decrease within 30 s to 1 min, although the bradycardia was
only statistically significant at the 3 nmol dose. Concurrent with the bradycardia, i.c.v. N/OFQ also reduced MAP at the 1 and 3 nmol doses. At 3 nmol, the highest dose tested, N/OFQ decreased HR from 587±1 (C) to 428±37 bpm (20 min), and MAP from 106±3 (C) to 78±8 (20 min) mm Hg (Fig. 2). The peak reductions in each parameter occurred 20 min after central drug injection, and each response returned to baseline between 30 and 40 min postinjection, respectively.

**Transgenic C57BL/6J NOP<sup>−/−</sup> and NOP<sup>+/+</sup> Littermate Mouse Studies.** Studies were performed to compare basal 24-h levels for renal excretory and cardiovascular function in transgenic NOP<sup>−/−</sup> and NOP<sup>+/+</sup> mice (Clarke et al., 2001). As depicted in Table 1, there were no significant differences in basal 24-h HR and MAP (telemetry studies) or 24-h urine output, sodium/potassium excretion, and free water clearance (metabolic studies) between NOP<sup>+/+</sup> and NOP<sup>−/−</sup> mice.

In subsequent studies in which mice were tested during acute experimental protocols, the baseline control levels for urine output (Fig. 3, depicted as saline 2-h) and MAP (depicted as C in Fig. 4, A and B) observed in NOP<sup>−/−</sup> mice were not different from respective levels for each parameter observed in NOP<sup>+/+</sup> littermate controls. Furthermore, in these studies, both the renal excretory (Fig. 3) and cardiovascular (Fig. 4, A and B) responses elicited by i.c.v. N/OFQ were absent in NOP<sup>−/−</sup> mice compared with responses produced by this ligand in NOP<sup>+/+</sup> control littermates. More specifically, in NOP<sup>+/+</sup> animals, i.c.v. injection of 3 nmol N/OFQ produced a significant diuresis (V = 0.39 ± 0.10 ml/2 h) compared with that elicited by isotonic saline vehicle administration (V = 0.03 ± 0.03 ml/2 h; in this case, only one mouse urinated over the 2-h study). In contrast, in NOP<sup>−/−</sup> animals, urine output was not different between groups of animals treated with this dose of N/OFQ (V = 0.06 ± 0.06 ml/2 h; only one mouse urinated) or isotonic saline vehicle (V = 0.08 ± 0.05 ml/2 h). In other studies in which these same animals were instrumented with telemetry probes, N/OFQ evoked a significant bradycardia (Fig. 4A) in C57BL/6 NOP<sup>−/−</sup> mice (1 nmol, peak ΔHR = −135 ± 53 bpm, 20-min nadir; 3 nmol, −217 ± 51 bpm, 30-min nadir) compared with isotonic saline vehicle-treated animals (peak ΔHR = −14 ± 5 bpm, 20-min nadir). At each of these doses (1 and 3 nmol), as the central N/OFQ-evoked bradycardia completely recovered, this was followed by a significant rebound increase in HR (Fig. 4A).

In contrast, the bradycardia to central N/OFQ was abolished in NOP<sup>−/−</sup> animals (Fig. 4B; 1 nmol, peak ΔHR = 34 ± 10 bpm; 3 nmol, −13 ± 17 bpm) and did not differ from that produced by i.c.v. isotonic saline vehicle (peak ΔHR, −3 ± 7 bpm). In these studies, i.c.v. N/OFQ (Fig. 4A) also produced a significant hypotensive response in NOP<sup>+/+</sup> animals (1 nmol, peak ΔMAP = −10 ± 5 mm Hg, 20-min nadir; 3 nmol, −22 ± 4 mm Hg, 20-min nadir) compared with that produced by isotonic saline vehicle (peak ΔMAP = 2 ± 3 mm Hg, 20-min nadir). The N/OFQ-evoked hypotension was completely absent in NOP<sup>−/−</sup> animals (Fig. 4B; 1 nmol, peak ΔMAP = 0 ± 1 mm Hg; 3 nmol, −2 ± 4 mm Hg) and was similar to the MAP response produced by isotonic saline vehicle (Fig. 4B; peak ΔMAP = 2 ± 1 mm Hg).

**Discussion**

The results of these studies confirm our previous observations made in ICR-CD1 mice (Burmeister and Kapusta, 2007) and show that i.c.v. N/OFQ also elicits marked cardiovascular depressor and diuretic responses in conscious C57BL/6 mice. In addition, it is clear that central N/OFQ mediates these cardiovascular and renal excretory responses via selective activation of a NOP receptor pathway because the char-

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**Table 1**

Basal 24-h renal excretory and cardiovascular function in NOP<sup>+/+</sup> and NOP<sup>−/−</sup> mice

<table>
<thead>
<tr>
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<th>Renal Excretory Function (n = 9/Group)</th>
<th>Cardiovascular Function (n = 5/Group)</th>
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<tbody>
<tr>
<td></td>
<td>V (ml)</td>
<td>UnAν (μEq)</td>
</tr>
<tr>
<td>NOP&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>2.1 ± 0.02</td>
<td>480 ± 46</td>
</tr>
<tr>
<td>NOP&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>2.1 ± 0.03</td>
<td>513 ± 85</td>
</tr>
</tbody>
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C<sub>H2O</sub>, free-water clearance.
acteristic bradycardic, hypotensive, and diuretic responses to i.c.v. N/OFQ were absent in transgenic NOP / H11002 / H11002 mice. This observation is of interest because it indicates that the cardiovascular and renal responses to central N/OFQ are elicited through a NOP receptor or splice variant (Pan et al., 1998) in the brain that is derived from a single NOP gene.

As a means to understand the physiological role(s) of the endogenous N/OFQ-NOP receptor system, transgenic NOP / H11002 / H11002 mice have been phenotyped in numerous biological and behavioral paradigms, including pain sensitivity (Bertorelli et al., 2002), locomotor activity (Nishi et al., 1997), morphine dependence and tolerance (Mamiya et al., 2001; Ueda, 2004), and antinociceptive response to morphine and / H9260 -opioid peptides (Noda et al., 1999). Stress and anxiety (Gavioli et al., 2007), hyperalgesia and allodynia (Okuda-Ashitaka et al., 2006), auditory function (Nishi et al., 1997), learning and memory (Manabe et al., 1998), and depression (Gavioli et al., 2003, 2004) have all also been examined in NOP / H11002 / H11002 mice. These investigators reported that endogenous N/OFQ mediates a tonic influence on certain biological processes via NOP receptor activation because basal levels for these respective systems are significantly altered in NOP / H11002 / H11002 mice.

To date, studies have not previously explored whether

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**Fig. 3.** Changes in urine output produced by the i.c.v. injection of N/OFQ in conscious transgenic C57BL/6 NOP / H11001 / H11001 and NOP / H11002 / H11002 mice. The values are mean ± S.E.M. and illustrate the changes in cumulative V produced by the i.c.v. injection of 1 nmol N/OFQ (n = 8/group), 3 nmol N/OFQ (n = 8/group), or isotonic saline vehicle (2 μl, n = 8/group) in NOP / H11001 / H11001 and NOP / H11002 / H11002 mice. Data shown in this figure represent mean values for cumulative urine collected throughout the entire 120-min experimental protocol beginning immediately after vehicle/drug administration. *, p < 0.05 compared with isotonic saline vehicle-treated mice.

**Fig. 4.** A, time course of the cardiovascular responses produced by the i.c.v. injection of N/OFQ in conscious, telemetered C57BL/6J NOP / H11001 / H11001 control littermate mice. The values are mean ± S.E.M. and illustrate the systemic cardiovascular responses produced by the i.c.v. injection of 1 nmol N/OFQ (A, n = 5), 3 nmol N/OFQ (B, n = 5), or isotonic saline vehicle (2 μl, n = 5) in C57BL/6J NOP / H11001 / H11001 mice. HR and MAP were measured during control (C, 10-min) and immediately after i.c.v. drug/vehicle injection for 120 min, denoted as time periods 10 to 120 min (consecutive 10-min periods). *, p < 0.05 compared with respective predrug group control (time C). B, time course of the cardiovascular responses produced by the i.c.v. injection of N/OFQ in conscious, telemetered C57BL/6J NOP / H11001 / H11001 mice. The values are mean ± S.E.M. and illustrate the systemic cardiovascular responses produced by the i.c.v. injection of 1 nmol N/OFQ (A, n = 5), 3 nmol N/OFQ (B, n = 5), or isotonic saline vehicle (2 μl, n = 5) in C57BL/6J NOP / H11001 / H11001 mice. HR and MAP were measured during control (C, 10-min) and immediately after i.c.v. drug/vehicle injection for 120 min, denoted as time periods 10 to 120 min (consecutive 10-min periods). *, p < 0.05 compared with respective predrug group control (time C).
genetic deletion of the NOP receptor influences basal cardiovascular and/or renal excretory function. Despite the pronounced effects produced by exogenous N/OFQ (Madeddu et al., 1999; Burmeister and Kapusta, 2007), the results of the present study demonstrate that under resting conditions, NOP+/− and NOP−/− mice have similar cardiovascular and renal excretory phenotypes, with no major differences in 24-h basal level for any cardiorenal parameter measured. These findings indicate that the endogenous N/OFQ-NOP system does not seem to tonically influence the mechanisms that regulate daily basal levels for MAP, HR, or the renal excretion of water/sodium. This is in agreement with our recent findings that the i.c.v. administration of the selective NOP receptor antagonist UFP-101 did not elicit a change in any of these parameters, which would be expected to be opposite to those typically produced by i.c.v. N/OFQ (Burmeister and Kapusta, 2007). However, our present findings are in contrast to those in which we demonstrated that when microinjected directly into the paraventricular nucleus of the hypothalamus of conscious Sprague-Dawley rats, the NOP antagonist UFP-101 increased heart rate and renal sympathetic nerve activity and decreased urine flow rate without altering electrolyte excretion (Krowicki and Kapusta, 2006). Thus, in these previous studies, UFP-101 was shown to block a tonically active inhibitory influence of endogenous N/OFQ on central sympathetic outflow and vasopressin pathways that arise from the paraventricular nucleus of the hypothalamus to affect heart rate and urine output (Krowicki and Kapusta, 2006). There are probably several reasons underlying the discrepancy of a tonic role for N/OFQ-NOP in the previous and present studies. These probably involve differences between rat and mouse strains, locus of brain site of drug action, and genetic versus pharmacological approaches used to block the native N/OFQ-NOP system. In regards to this latter point, it should be clarified that the transgenic NOP−/− mice used in the present investigations express a whole-body gene deletion for the NOP receptor. This is in contrast to performing similar phenotype studies in which there has been a selective removal of the NOP receptor from within the brain (or specific nucleus) of an adult animal (e.g., cre-loxP technology).

Despite there being no differences in cardiovascular and renal excretory function between NOP+/− and NOP−/− mice under basal conditions, it is possible that the N/OFQ-NOP system may be activated and affect these systems during stress or pathology. This is suggested because other endorphin systems have typically been shown to remain quiescent without influence on a biological system until they are activated by a particular stress or pathology (Carr and Verrier, 1991; Kapusta and Obih, 1995). Although transgenic NOP−/− mice now provide an alternative approach in which to explore these possibilities, it was the purpose of these investigations to first determine whether or not the cardiovascular and renal excretory responses to central N/OFQ are unequivocally absent in transgenic NOP−/− mice compared with responses observed in conscious littermate NOP+/+ mice and nontransgenic C57BL/6 mice, a genetic background strain similar to the C57BL/6 mice onto which NOP−/− mice were backcrossed.

Here, we report that i.c.v. N/OFQ produced dose-dependent bradycardia, hypotension, and diuresis in nontransgenic (Harlan) and NOP+/− (The Jackson Laboratory) mice. Although i.c.v. N/OFQ evoked a similar directional change in cardiovascular function (i.e., decrease) and urine output (increase) in both groups, the magnitudes of the bradycardia and hypotension produced by this peptide were strain-dependent. More specifically, i.c.v. N/OFQ (3 nmol) produced a peak (20 min) decrease in HR of −159 ± 14 bpm in nontransgenic C57BL/6NHsd mice (Harlan). This same i.c.v. dose of N/OFQ lowered HR to a peak (30 min) of −218 ± 12 bpm in C57BL/6J NOP+/− mice. Related to this issue, we have reported previously (Burmeister and Kapusta, 2007) that in CD1 mice (Harlan), i.c.v. N/OFQ (3 nmol) also produced a pronounced bradycardia (−261 ± 19 bpm), but the magnitude was even greater than that observed in the NOP+/− mice tested in the present study. Similar to HR responses, i.c.v. N/OFQ produced differences in the peak magnitude hypotensive response observed across groups of mouse strains. In nontransgenic C57BL/6NHsd mice (Harlan), i.c.v. N/OFQ (3 nmol) significantly reduced MAP by −28 ± 3 mm Hg 20 min postdrug injection. In comparison, i.c.v. N/OFQ (3 nmol) lowered MAP by −47 ± 3 mm Hg in C57BL/6J NOP+/− mice (The Jackson Laboratory), which was similar to the decrease (−46 ± 5 mm Hg) in MAP produced 20 min after i.c.v. N/OFQ (3 nmol) as reported in ICR-CD1 mice (Harlan) (Burmeister and Kapusta, 2007). Of interest, in the present study, the durations of both the hypotension and bradycardia to i.c.v. N/OFQ were greatest in NOP+/− mice.

It is possible that underlying genetic differences between the strains of mice tested contribute to the variations in the magnitude and duration of the systemic cardiovascular depressor responses to central N/OFQ. Numerous studies have reported differences in the specific pattern (e.g., magnitude and time course) of changes in a given physiological parameter among different strains of the same species or substrains generated from the same background (e.g., C57BL/6) (Stiedl et al., 1999; Faulx et al., 2005). Although entirely speculative, it is possible that the mouse strains we studied have differences in basal sympathetic neural tone, which governs resting HR and MAP. Significant differences in neurotransmission and cellular stress-related gene expression patterns to environmental stimuli have been reported between C57BL/6J and C57BL/6NHsd mice (Grottick et al., 2005). In this case, it would be predicted that mice with a greater sympathetic influence (e.g., potentially C57BL/6J NOP+/− mice) would display a more profound and prolonged reduction in MAP and HR. This is suggested because central N/OFQ is recognized to produce hypotension and bradycardia primarily via inhibition of central sympathetic outflow (Kapusta and Kenigs, 1999). Under these conditions (i.e., hypotension caused by sustained central sympathoinhibition), it may also be predicted that after central N/OFQ injection, alternative hormonal mechanisms such as angiotensin II and epinephrine may be triggered in an attempt to restore MAP and HR to normal; in this case, the overshoot of the rebound HR and MAP responses observed in C57BL/6J NOP+/− mice in the present study could be explained by slower process for hormonal pathways to tightly and rapidly regulate these systemic cardiovascular hemodynamic parameters to normal (predrug) levels.

Despite substrain phenotypic variations, a major finding of the present investigations is that, in direct opposition to the enhanced systemic cardiovascular responses observed in NOP+/− mice, the transgenic NOP−/− mice completely failed...
to elicit any change in HR, MAP, or urine output to i.c.v. N/OFQ (1 or 3 nmol). Thus, data from these studies unequivocally demonstrate that central N/OFQ activates a single NOP receptor pathway to mediate this peptide’s cardiovascular depressor and diuretic responses.

In summary, the endogenous N/OFQ-NOP system does not seem to be involved in the tonic regulation of cardiovascular and renal excretory function in conscious mice because there were no differences in basal 24-h HR, MAP, and water/sodium excretion between N/OFQ–/– mice and their wild-type littermates. Furthermore, it is clear that the cardiovascular (bradycardia and hypotension) and renal excretory (diuresis) responses to i.c.v. N/OFQ are mediated by selective activation of central NOP receptors as demonstrated by the failure of i.c.v. N/OFQ to change cardiovascular or renal excretory function in transgenic NOP–/– mice. Together, these findings provide an important foundation for the use of N/OFQ–/– mice in future studies aimed toward elucidating a potential physiological role of the endogenous N/OFQ-NOP system in the central control of cardiovascular and renal function during acute or chronic states in which cardiovascular and/or renal function may be altered (stress) or compromised (pathology).

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References


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