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Retrodialysis of N/OFQ into the nucleus accumbens shell blocks cocaine-induced increases in extracellular dopamine and locomotor activity

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ABSTRACT

Nociceptin (N/OFQ) has been implicated in a variety of neurological disorders, most notably in reward processes and drug abuse. N/OFQ suppresses extracellular dopamine in the nucleus accumbens (NAc) after intracerebroventricular injection. This study sought to examine the effects of retrodialyzed N/OFQ on the cocaine-induced increase in extracellular dopamine levels in the NAc, as well as locomotor activity, in freely moving rats. 1.0 μM, 10 μM, and 1 mM N/OFQ, in the NAc shell, significantly suppressed the cocaine-induced dopamine increase in the NAc, while N/OFQ alone had no significant effect on dopamine levels. Co-delivery of the selective NOP receptor antagonist SB612111 ([(−)-cis-1-Methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5 H-benzocyclohepten-5-ol] reversed the N/OFQ suppression of cocaine-induced dopamine in the NAc, suggesting that this is an NOP receptor-mediated effect. Using a novel system to assess locomotion, we measured various motor activities of the animals with simultaneous microdialysis from the home cage. Cocaine produced an expected increase in total activity, including horizontal movement and rearing behavior. Retrodialysis of N/OFQ with cocaine administration affected all motor activities, initially showing no effect on behavior, but over time inhibiting cocaine-induced motor behaviors. These results suggest that N/OFQ can act directly in the NAc shell to block cocaine-induced increases in extracellular dopamine levels. Extracellular dopamine and locomotor activity can be dissociated within the NAc and may reflect motor output differences in shell versus core regions of the NAc. These studies confirm the widespread involvement of NOP receptors in drug addiction and further validate the utility of an NOP receptor agonist as a medication for treatment of drug addiction.

1. Introduction

The endogenous neuropeptide, Nociceptin/Orphanin FQ (N/OFQ) (Meunier et al. 1995; Reinscheid et al. 1995), and its cognate receptor, NOP, are found throughout the brain and are involved in a large number of neurological processes including anxiety, memory, feeding, pain, and reward, among others. There is a moderate to high density of NOP receptors in areas implicated in drug abuse and reward, including the nucleus accumbens (NAc), ventral tegmental area (VTA), medial prefrontal cortex, lateral hypothalamus, amygdala, and the bed nucleus of stria terminalis (Neal et al., 1999a, 1999b). N/OFQ injected intracerebroventricularly (i.c.v.) attenuates the rewarding properties of several common drugs of abuse. Specifically, N/OFQ blocks conditioned place preference (CPP) induced by morphine, cocaine, amphetamines, and alcohol (Ciccocioppo et al., 2000; Kotlinska et al., 2003b; Murphy et al., 1999; Sakoori and Murphy, 2004; Zhao et al., 2003), alcohol self-administration, and stress-induced reinstatement of alcohol self-administration (Ciccocioppo et al., 2004; Martin-Fardon et al., 2000). N/OFQ also blocks the stimulant effects associated with drugs of abuse. For example, i.c.v. injections of N/OFQ attenuate the acute motor stimulatory effects of cocaine (Lutfy et al., 2001; Narayanan et al., 2004).

Microdialysis studies, measuring extracellular dopamine following N/OFQ administration, have complemented the behavioral findings discussed above. N/OFQ decreases basal dopamine levels and attenuates morphine and cocaine-induced increases of dopamine levels in the NAc (Di Giannuario et al., 1999; Lutfy et al., 2001; Murphy et al.,

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1996). Furthermore, an injection of N/OFQ (1 mM) directly into the VTA, is sufficient to attenuate cocaine-induced increases in NAc dopamine and locomotor activity (Murphy and Maidment, 1999). However, it should be noted that striatal administration of high concentrations of N/OFQ can have opioid-like activity, inducing a naloxone reversible increase in dopamine levels in the nucleus accumbens (NAc) (Konya et al., 1998).

To address whether the NOP receptor system can directly influence dopamine release in the NAc, the effect of retrodialyzed N/OFQ in the NAc shell on the cocaine-induced increases in extracellular dopamine levels, in this region, was examined. In parallel we also examined whether intra-NAc N/OFQ could alter cocaine-induced activity, using the novel SmartCage™ apparatus, under identical experimental conditions. Results indicated that 1 μM–1000 μM N/OFQ dialyzed within the NAc shell blocked the cocaine-induced increase in extracellular dopamine in this brain region. Additionally, intra-NAc N/OFQ administration also induced a delayed decrease in cocaine-induced hyperactivity.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g; Harlan Laboratories, Hayward, CA) were housed under constant ambient conditions on a 12 h light/dark cycle (lights on at 06:00 h) with food and water ad libitum. All animals were handled and habituated to the microdialysis testing procedures for 1 week prior to surgery and 1 week post-surgery. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences Press, Washington, DC, 1996) and were approved by the Institutional Animal Care and Use Committee at SRI International. Every effort was made to minimize animal discomfort throughout the experimental protocols.

2.2. Drugs

N/OFQ was obtained from the NIDA drug supply program. The NOP receptor antagonist SB612111 ([(−)-cis-1-Methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol] was synthesized at SRI International using previously reported methodology (Barlocco et al., 2006). Drugs that were delivered by reverse dialysis were dissolved in artificial cerebrospinal fluid (aCSF, 124 mM NaCl, 2.2 mM KCl, 1.3 mM KH_2PO4, 1.3 mM $MgSO_4$, 20 mM $NaHCO_3$, and 2.0 mM $CaCl_2$; pH 6.0–6.2). Cocaine (20 mg/kg, Sigma, St. Louis, MO) was prepared in 0.9% saline and administered intraperitoneally (i.p.) to the animals. All drug solutions were made on the day of each experiment and serially diluted to their final concentrations. The 1 μM, 10 μM, and 1000 μM concentrations of N/OFQ and the 100 μM of SB612111 (NOP antagonist) were delivered by reverse dialysis.

2.3. Surgery

The animals were anesthetized with isoflurane (2–3%) and their core temperature maintained at 37 °C using a thermoregulated heating pad in conjunction with a rectal probe. The rats were implanted with microdialysis guide cannulae (CMA/12; CMA Microdialysis, Chelmsford, MA), which were stereotaxically implanted 2 mm above the NAc (shell) [AP,+1.7; L, +0.8; DV,−6.0 as calculated relative to bregma and skull surface, according to Paxinos and Watson (Paxinos and Watson, 1997). The cannulae were affixed to the head with two small screws and dental cement. All animals were allowed at least 1 week of recovery and several hours (4–6 h) of daily habituation to the microdialysis environment prior to the initiation of experiments.

2.4. Assessment of dopamine levels using microdialysis and high performance liquid chromatography (HPLC) with electrochemical detection (EC)

Concentric microdialysis probes with 2 mm exposed membrane (0.5 mm diameter, 20 kDa cutoff; CMA 12, CMA Microdialysis) were inserted into the microdialysis cannula 18 h prior to sample collection, to allow for dopamine stabilization, and perfused with aCSF at a rate of 1 μl/min. Overnight perfusate was discarded. On the morning of each microdialysis session, samples were collected at 20 min intervals into vials containing 5 μL of 1 mM oxalic acid (to prevent degradation of monoamine transmitters) and kept at 4 °C for the duration of the experiment. The samples were subsequently frozen at −70 °C and later analyzed in several experiment batches using HPLC/EC (ESA-Dionex, Inc. Sunnyvale, CA).

Microdialysis samples were then injected into an HPLC system via an ESA model 542 autosampler and separated on an ESA MD-150/RP-C18 analytical column (150 × 3.2 mm, 3 μm), perfused with ESA mobile phase MD-TM type II (#70-5049 P, Chelmsford, MA) at a flow rate of 0.6 mL/min. Dopamine was detected by oxidation using an ESA CouloChem III detector equipped with an ESA 5020 guard cell (+300 mV) and an ESA 5014b dual electrode analytical cell (E1, −100 mV; E2, +200 mV). Chromatographic data were acquired and analyzed using EZChrome Elite software. The HPLC system was calibrated at the start of each set of experimental batch of samples, using external dopamine standards.

2.5. Assessment of motor behavior using SmartCage™

In a separate series of experiments, motor behavior in freely moving animals was assessed using the SmartCage™ technology (AfaSci, Inc., Redwood City, CA) (Khroyan et al., 2012; Xie et al., 2011). SmartCage™ uses a USB-cable linked, noninvasive rodent behavior monitoring system in conjunction with the animal's home cage. Microdialysis balance arms and dual channel swivels (CMA Microdialysis) were adapted to a home cage, with the SmartCage™ apparatus positioned around the home cage, so that the animals could remain in their home environment while motor activity could be measured. Motor traces were generated to represent 20 min of activity/interval over time in the animal's home cage from left to right. Motor behavior was quantified as percent active time, locomotion (cm), and the incidence of rearing. Calculation of locomotion was based on breakage of the lower infra-red beams of the SmartCage™, and rearing was defined as the breakage of the upper infra-red beams. Percent active time encompassed the time that the animal was moving across the cage (i.e. breaking consecutive horizontal beams), rearing, and also more refined movements including sniffing and grooming behavior.

2.6. Experimental design

In the first series of microdialysis experiments animals received cocaine alone (20 mg/kg, i.p.; $N=8$), N/OFQ alone (1000 μM, by reverse dialysis; $N=7$), or cocaine (20 mg/kg, i.p.) with N/OFQ (reverse dialysis; 1.0 μM, 10 μM, or 1000 μM; $N=$ 6–11/group). The dose of cocaine was chosen to be similar to other microdialysis studies (Chefer et al., 2003). Initially the 1000 μM dose of N/OFQ was chosen since it was the concentration used previously in the literature (Murphy and Maidment,

1999), however, given that it produced a large effect we examined lower doses as well. For all treatment groups, five baseline samples of dialysate at 20 min intervals were collected, after which animals received their assigned drug treatment by the i.p. route (cocaine) and/or by reverse dialysis (N/OFQ). Following drug treatment, dialysate samples were then collected for 200 min.

To verify that the attenuation of cocaine-induced increases in dopamine levels were through the activation of NOP receptors, a separate group of animals were given the NOP antagonist SB612111 (100 μM) co-delivered through the probe with N/OFQ (10 μM) with a simultaneous injection of cocaine (20 mg/kg; i.p.; $N=6$). Dialysate samples were collected in a similar manner as described above.

In the experiments assessing motor behavior, animals ($N=6$/group) received cocaine alone (20 mg/kg, i.p.), N/OFQ alone (10 μM by reverse dialysis), or N/OFQ plus cocaine. One group served as controls and received a vehicle injection prior to testing. Animals receiving cocaine were given an i.p. injection and were immediately placed back in their own home cage (L 47.6 × W 25.9 × H 20.9 cm) surrounded by the SmartCage™ apparatus. Animals that received N/OFQ by reverse dialysis, simultaneously received cocaine systemically and were subsequently placed back in the cage. Animals that only received N/OFQ were not removed from the cage and were given N/OFQ via the dialysis probe. Behavior was recorded for 260 min using the SmartCage™ system described in 2.5.

2.7. Histology

After the final experiment, animals were deeply anesthetized, decapitated and brains removed and sunk in 10% formalin. The brains were post-fixed and serial, coronal sections (30 μm) were cut on a cryostat and stained with Hematoxylin and Eosin (H&E) and microdialysis sites were localized by comparison with the tissue sections, in the atlas of Paxinos and Watson (Paxinos and Watson, 1997).

2.8. Data Analyses

Pre- and post-experimental probe recoveries obtained from dialysates of a known concentration of dopamine were analyzed by *t*-test to ensure that changes in dopamine were not an artifact of probe changes during the experiment. The data presented here were obtained from experiments that showed no statistically significant change in probe recovery ensuring that measured changes in dopamine resulted from drug delivery and not due to changes in the permeability of the dialysis membrane.

Neurochemical and behavioral data were analyzed using repeated measures analysis of variance (ANOVA), with drug treatment (i.e., baseline, vehicle, cocaine, N/OFQ, SB 612111) as between group variables and post-drug treatment time intervals as the repeated measure. For neurochemical data examining drug group differences across drug conditions collapsed across time (i.e. Fig. 2B) a one way ANOVA with drug condition as the between group variable was used. In these experiments, the mean of the five baseline samples before any drug treatments was considered to represent basal release (100%) of neurotransmitter level, and the absolute basal releases were calculated based on the real concentrations. Significant effects from the overall ANOVA were further analyzed by one way ANOVAs and/or Fisher's least significant difference (LSD) post-hocs for multiple comparisons. The results are presented as means ± S.E.M. and the level of overall statistical significance was set at $p \leq 0.05$.

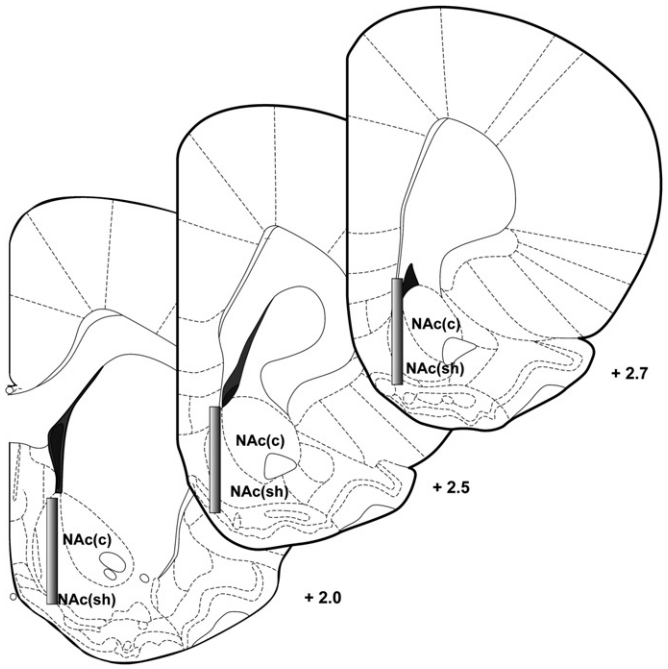

Fig. 1. Reconstructed coronal sections showing probe placement in the NAc. The semi-permeable membrane (2 mm length tip) was largely centered in the NAc shell and dialysates obtained from this region were used to quantify dopamine. Representative probe tracts are shown as gray cylinders and depending on the anterior to posterior level, occasionally showed small portions of tract that extended into the cortex or islands of Calleja ventrally, or dorsally into the caudate putamen. Coronal sections were adapted from the atlas of Paxinos and Watson (1997). The numbers refer to the approximate anterior–posterior level, relative to Bregma. Abbreviations: NAc(c), nucleus accumbens, core; NAc(sh), nucleus accumbens, shell.

3. Results

3.1. N/OFQ suppresses the cocaine-induced increase in dopamine levels in the NAc

Fig. 1 summarizes the approximate placement for all microdialysis probe sites, in animals whose data were included in the final analyses. Histological analysis showed that the majority of the probes sites were localized to the shell region of the NAc (Fig. 1, representative gray cylinders) with coordinates that ranged from A 2.0 to A 2.8, L 0.6 to L1.0. and V 7.5 to V 8.0, based on the atlas of Paxinos and Watson (1997).

Fig. 2A summarizes sequential dopamine measures obtained during baseline dialysis and changes in extracellular dopamine following an injection of 20 mg/kg cocaine, with and without retrodialysis delivery of three different concentrations of N/OFQ, to the NAc. Two-way repeated measures ANOVA indicated that N/OFQ significantly altered cocaine-mediated extracellular dopamine levels (Fig. 2A), as indicated by significant drug x time interaction ($F_{49,449}=1.773$; $P=0.0031$). Post-hoc revealed that control dopamine levels were similar between experiments and over time (comparing gray symbols from min 20 to 100). Cocaine produced an increase in extracellular dopamine that was apparent within 20 min post-injection. Significant increases in dopamine continued for approximately 80 min following cocaine injection and dopamine levels, while they began decreasing after the first hour post injection, remained elevated relative to baseline until the last 100 min of the experiment (min 140–200, $P<0.05$).

Retrodialyzed N/OFQ was able to attenuate the cocaine-induced increase in dopamine levels. For initial experiments, we used 1000 μM N/OFQ to be consistent with previous published

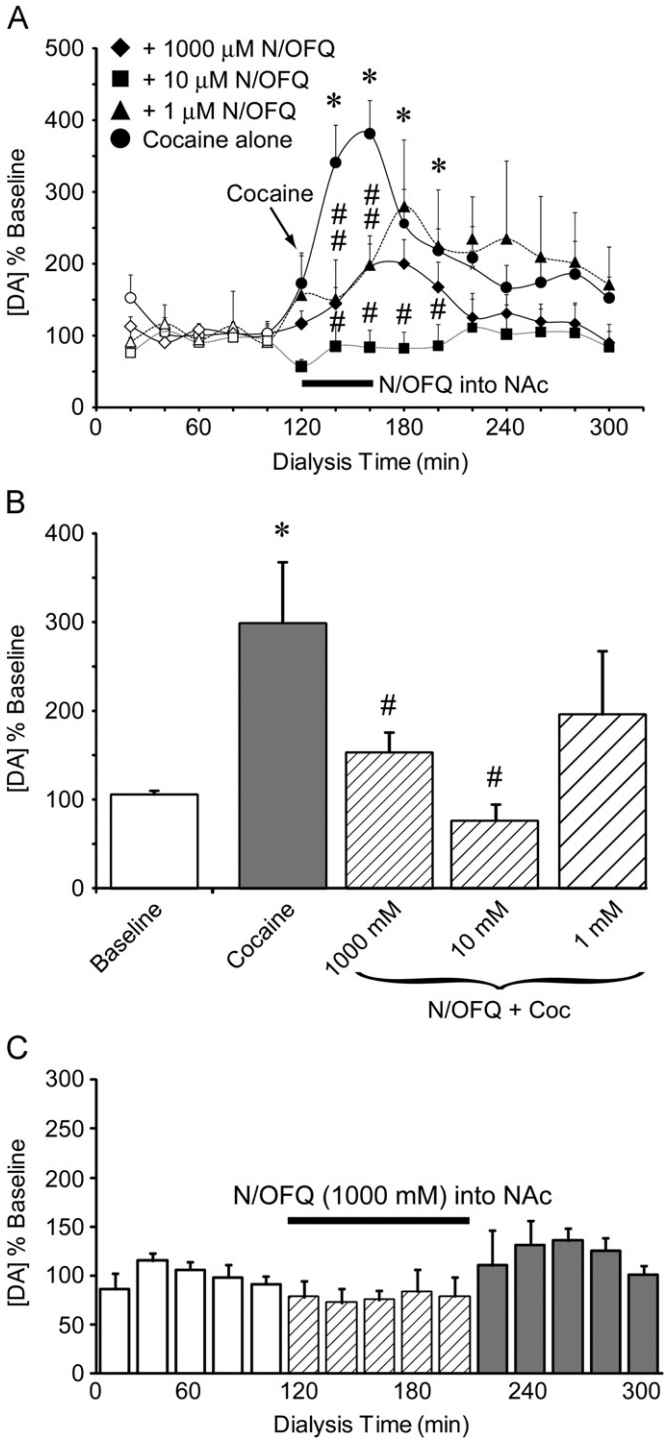

Fig. 2. N/OFQ suppresses the cocaine-induced dopamine increase in NAc shell. (A) The effects of 20 mg/kg cocaine (i.p.) alone (●) or with retrodialysis delivery of N/OFQ (1 μM (▲); 10 μM (■); 1000 μM N/OFQ (◆)), on extracellular levels of dopamine (DA) in the NAc shell over time. (B) The effects of various N/OFQ doses on cocaine-stimulated increases in dopamine release relative to cocaine alone and to baseline, averaged during the 60 min period of N/OFQ retrodialysis. (C) The effect of 1000 μM N/OFQ retrodialyzed into the NAc shell on basal dopamine levels, in the absence of cocaine over time. Data are means ± S.E.M. ∗, significant difference from baseline; #, significant difference from cocaine alone ($P < 0.05$).

results (Murphy and Maidment, 1999). During the 60 min retrodialysis of N/OFQ, the 1000 μM dose suppressed dopamine levels, relative to cocaine injection alone (Fig. 2A; min 140 and min 160; $P < 0.05$). Following the removal of 1000 μM N/OFQ, dopamine levels were no different than cocaine alone, and by 220 min dialysis time, the dopamine concentration returned to baseline levels. Because of the dramatic decrease in cocaine-induced extracellular dopamine by retrodialyzed N/OFQ (1000 μM), we determined the effects of two lower doses of N/OFQ (10 μM, and 1.0 μM) on cocaine-induced extracellular dopamine levels. Both doses of N/OFQ attenuated the cocaine-induced increase in extracellular dopamine levels. As evident in Fig. 2A, 10 μM N/OFQ completely blocked the cocaine-induced increase in extracellular dopamine (140 to 200 min dialysis time; $P < 0.05$). Post-hoc tests showed that 1.0 μM N/OFQ also suppressed cocaine-induced extracellular dopamine in the NAc shell, but only during the retrodialysis, similar to 1000 μM N/OFQ ($P < 0.05$). Fig. 2B summarizes the main-effect of N/OFQ suppression on cocaine-stimulated dopamine release ($P < 0.05$) in the NAc ($F_{8, 63} = 2.09$; $P < 0.05$) compared to cocaine alone collapsed across the first 60 min period following N/OFQ dialysis. To examine whether N/OFQ alone could alter dopamine levels in the NAc shell, a separate group of animals received retrodialysis delivery of the high dose of N/OFQ (1000 μM) alone in this region. As shown in Fig. 2C, under these conditions, there were no significant changes in dopamine levels in comparison to baseline levels across time (n.s.).

3.2. NOP antagonist SB612111 reverses the effects of N/OFQ suppression on cocaine-induced dopamine

Additional experiments were conducted to determine whether administering an NOP antagonist would have the ability to reverse N/OFQ's suppression of cocaine-induced dopamine output (Fig. 3). There was a significant drug x time interaction effect for NOP antagonist SB612111, on the inhibition by N/OFQ of cocaine-induced extracellular dopamine. ($F_{28,276} = 1.54$; $P < 0.0001$). Post hoc tests revealed that 100 μM of SB612111, co-delivered with 10 μM N/OFQ by reverse dialysis, in the presence of cocaine, caused a significant increase in extracellular dopamine compared to cocaine plus 10 μM N/OFQ (min 120 to 220; $P < 0.05$; Fig. 3). In fact, in the presence of SB612111, extracellular dopamine was significantly greater than with cocaine alone at two time points (min 140 and min 200; $P < 0.05$).

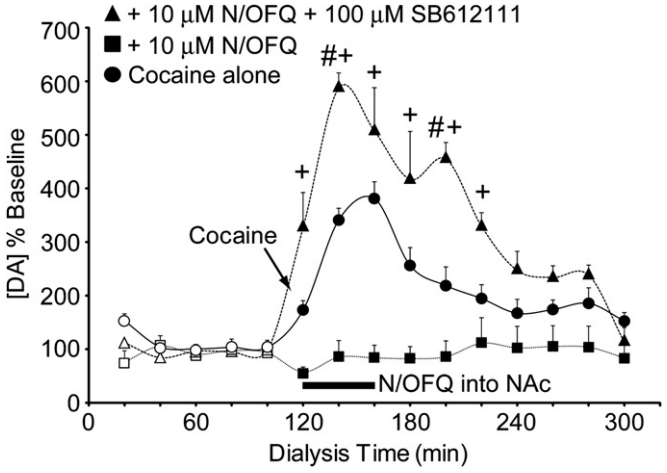

Fig. 3. NOP receptor antagonist, SB612111, reverses N/OFQ suppression of cocaine-induced dopamine in the NAc shell. The effects of cocaine alone (●), cocaine with retrodialyzed N/OFQ (■), and cocaine with retrodialyzed N/OFQ and SB 612111 (▲) on % dopamine (DA) levels in the NAc shell. Data are means ± S.E.M. #, significant difference from cocaine alone; +, significant difference from cocaine with retrodialyzed N/OFQ ($P < 0.05$).

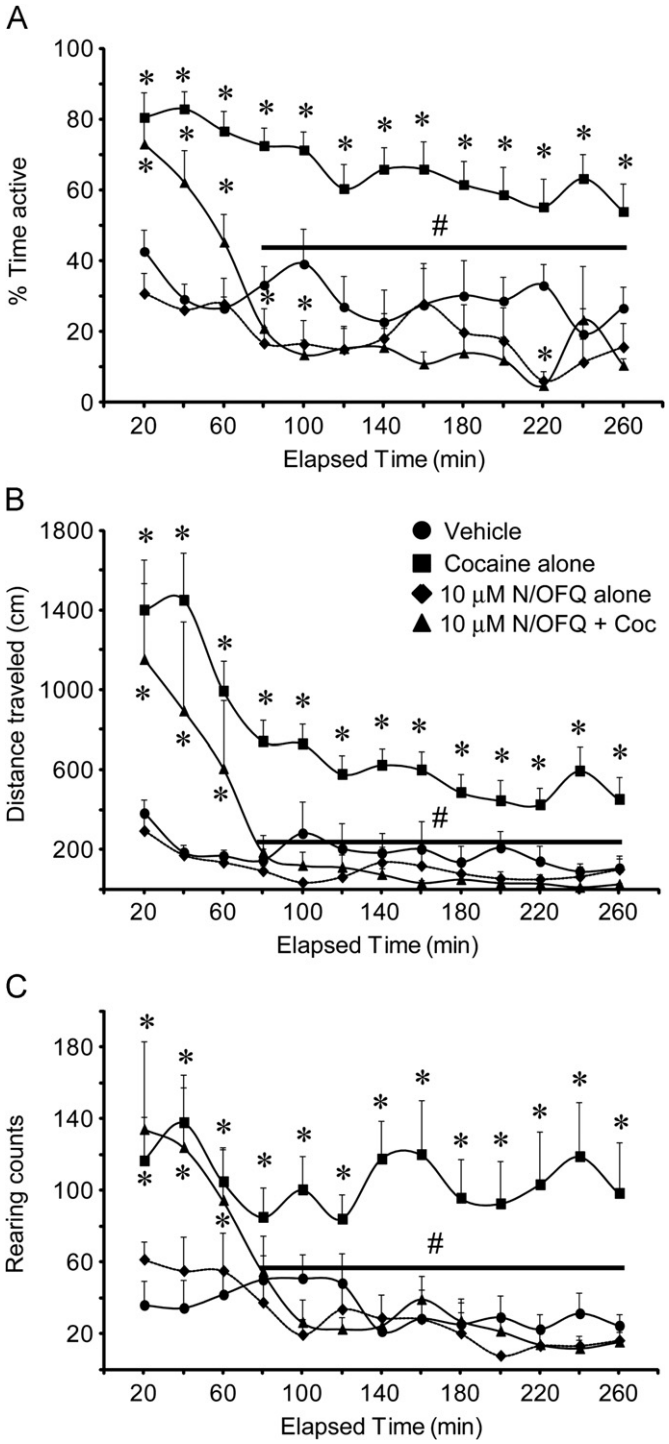

Fig. 4. Retrodialyzed N/OFQ attenuates cocaine-induced behaviors. The effects of 20 mg/kg cocaine alone (■), 10 μM N/OFQ alone (♦), or cocaine with N/OFQ (▲) on percent active time (A), mean distance traveled (B), and rearing counts (C). These groups were compared to the vehicle baseline (●). Data are means ± S.E.M. ∗, significant difference from vehicle; #, significant difference from cocaine alone ($P<0.05$).

3.3. N/OFQ dialysis attenuates the motor stimulatory effect of cocaine.

We also assessed motor activity in the rat home cages, while attached to the dialysis probes, to measure activity under the exact conditions as those with the microdialysis experiments. Looking at the percent time active, the overall ANOVA indicated that there was a significant drug × time interaction ($F_{36,228}=3.444$; $P<0.0001$) on motor activity. Elevated levels of activity were immediately apparent after i.p. injection of cocaine, relative to vehicle controls, and continued for the duration of the experiment ($P<0.05$; Fig. 4A). Retrodialysis of 10 μM N/OFQ (black triangles) decreased cocaine-induced hyperactivity but this was not evident until 80 min post-cocaine injection ($P<0.05$). It is interesting to note that retrodialyzed N/OFQ alone did not produce a robust decrease in overall activity levels in comparison to vehicle alone, across the entire testing period, although there was a significant decrease at the 80, 100, and 220-min time points ($P<0.05$).

When we examined the effects of N/OFQ on distance traveled (horizontal movement), not surprisingly similar results as above were observed (Fig. 4B). The overall ANOVA indicated a significant drug x time interaction ($F_{36,228}=3.369$; $P<0.0001$) on the overall distance traveled (cm). Cocaine produced an increase in distance traveled, relative to vehicle controls, evident in the first 20 min, and lasting for the duration of the experiment ($P<0.05$). Reverse dialysis of 10 μM N/OFQ produced a time dependent effect on cocaine-induced locomotor activity that was significantly different from cocaine alone from 80 min onward (Fig. 4B, $P<0.05$). Reverse dialysis of 10 μM N/OFQ, in the absence of cocaine, had no effect on the distance the animals traveled (cm), and was comparable to vehicle controls for the entire experiment.

Drug-induced stereotypic rearing behavior was also examined using the SmartCage™ system (Fig. 4C). The overall ANOVA indicated that there was a significant drug × time interaction ($F_{36,228}=3.451$; $P<0.0001$) on stereotypic rearing. There was an increase in the incidence of rearing behavior immediately after i.p. injection of cocaine (Fig. 4C) relative to vehicle controls and continued for the duration of the experiment ($P<0.05$). In animals that received dialysis of 10 μM N/OFQ plus an i.p. injection of cocaine, rearing activity was similar to cocaine alone for the 60 min during dialysis delivery (Fig. 5C, black triangles, $P<0.05$) but subsequently tapered off to control levels for the rest of the experiment. Retrodialysis of 10 μM N/OFQ had no significant effect on rearing behavior, relative to saline controls.

4. Discussion

The effects of N/OFQ, and small molecule NOP receptor agonists, on rewarding/reinforcing and stimulant behavior induced by drugs of abuse, have been extensively examined in rodents. Maidment and colleagues demonstrated that i.c.v.-administered N/OFQ decreases basal dopamine levels in the NAc (Murphy et al., 1996). Based upon these results, the effect of N/OFQ on various drugs of abuse was tested and it has been shown to block drug-induced CPP (Ciccocioppo et al., 2000, 2002; Kotlinska et al., 2003a; Murphy et al., 1999; Sakoori and Murphy, 2004; Zhao et al., 2003), as well as alcohol self-administration and stress-induced reinstatement of extinguished self-administration (Ciccocioppo et al., 2004; Martin-Fardon et al., 2000). Small molecule NOP agonists can have similar reward-attenuating effects. Ro 64-6198 can block both the acquisition and reinstatement of morphine CPP in mice (Shoblock et al., 2005), and SR16835 can attenuate morphine CPP, an effect that is blocked by the selective NOP receptor antagonist SB612111 (Toll et al., 2009). Some evidence shows that the μ-receptor partial agonist, buprenorphine, has the ability to attenuate alcohol consumption in rats, due to activation of NOP receptors at relatively high doses (Ciccocioppo et al., 2007). Taken together, these studies confirm the involvement of NOP receptors in drug addiction.

The sites at which N/OFQ acts to attenuate dopamine output in the mesolimbic pathways have not been thoroughly examined. I.c.v. administered N/OFQ attenuates morphine and cocaine-induced increases in extracellular dopamine in the NAc (Lutfy

et al., 2001). However, when delivered i.c.v., there are numerous sites that can be affected by N/OFQ administration. For example, N/OFQ acts in the lateral hypothalamus to block release of the neuropeptide hypocretin (Xie et al., 2008). Hypocretin has been demonstrated to be required for morphine CPP and reinstatement of cocaine self-administration (Boutrel et al., 2005; Harris et al., 2005). Localized intra-VTA injections of N/OFQ decreases dopamine accumulation in the NAc (Murphy and Maidment, 1999). So it is possible that N/OFQ can act in the lateral hypothalamus (to block hypocretin release), the VTA (to block dopamine output directly) and potentially additional sites to attenuate drug reward.

NOP receptors are found in the NAc itself (Neal et al., 1999a). Our experiments were conducted to test whether stimulation of NOP receptors in the NAc could attenuate cocaine-induced increases in extracellular dopamine, in the same brain region. More specifically, we concentrated on the NAc shell, rather than the core, because previous research seems to indicate that the dopaminergic input into the NAc shell is important for drug reward (McBride et al., 1999; Sellings and Clarke, 2003). As shown in Fig. 2, N/OFQ reverse dialyzed into the NAc shell blocks cocaine-induced accumulation of dopamine captured in the same dialysis probe. N/OFQ produced an inverted U-shaped dose-response curve. 10 μM N/OFQ was most potent at inhibition of cocaine-induced dopamine accumulation in the NAc shell, whereas 1000 μM N/OFQ, the concentration used previously in the literature (Murphy and Maidment, 1999), was only partially effective, inhibiting only as much as a 1 μM concentration. Because N/OFQ is over 100-fold selective for NOP over mu-opioid receptors (Spagnolo et al., 2008), this U-shaped dose response could be due to opioid agonist activity induced by the very high, 1000 μM, concentration of N/OFQ (Konya et al., 1998). It is also important to note that it is likely that only a small fraction of the total concentration of N/OFQ ($<20\%$) was delivered to the NAc through the active membrane (Hocht et al., 2007).

Importantly since the NOP receptor antagonist SB612111, which is over 100-fold selective for NOP over the opioid receptors (Zaratin et al., 2004), can reverse the effects of N/OFQ on cocaine-induced dopamine release, this is clearly a NOP receptor-mediated effect. It is interesting to note that the combination of SB612111 plus N/OFQ, given with cocaine, produced an increase in dopamine release that surpassed cocaine alone. It is possible that this effect is due to blocking other neurotransmitter systems that would also inhibit dopamine release, or might suggest an endogenous tone for N/OFQ in the NAc shell. In the absence of cocaine, N/OFQ does not decrease basal extracellular dopamine. These findings are not consistent with those reported by Maidment and colleagues, who demonstrated a sizable decrease in basal dopamine in the NAc after i.c.v. administration of N/OFQ (Lutfy et al., 2001; Murphy et al., 1996). However, there were experimental differences. In the present experiments, N/OFQ was microinjected directly into the NAc, rather than using i.c.v. administration, and the rats used were freely moving, rather than under anesthesia during dopamine recovery.

The relative influence of dopamine levels in various subregions of the NAc, on locomotor activity, is not conclusive given the many conflicting reports subsequent to microinjection of drugs and lesioning experiments (Choi et al., 2000; Ikemoto, 2002; Swanson et al., 1997). Using the SmartCage™, we were able to measure motor activities of the rats, while attached to the dialysis probes, in a home cage, where the microdialysis took place, allowing us to determine directly whether changes in extracellular dopamine in the NAc shell correspond to changes in locomotor activity. The results demonstrated that systemic cocaine induced a predictable large increase in locomotor behavior, including overall activity, locomotor activity, and rearing behavior. Retrodialysis of 10 μM N/OFQ alone into the NAc shell had no effect on locomotor or rearing behavior. However, when N/OFQ was administered at the same time as cocaine, intra-NAc N/OFQ produced a gradual decrease in cocaine-induced hyperactivity. The onset of the effect of N/OFQ on cocaine-induced hyperactivity was not evident until 60 min post-cocaine administration. These results clearly demonstrate that the effect of N/OFQ on extracellular dopamine levels and acute cocaine-induced hyperactivity can be dissociated. Administration of N/OFQ in the NAc shell, can immediately and completely block the cocaine-induced increase in dopamine levels in the NAc shell, whereas the N/OFQ-induced decrease in cocaine-mediated hyperactivity is delayed until 60–80 min after cocaine administration. The delayed onset of N/OFQ on cocaine-induced locomotor activity might reflect the peptide traveling over into the NAc core and/or other brain regions, such as anterior medial caudate putamen (Baker et al., 1998, 1996), and thereby reducing locomotor activity by reducing dopamine levels, in those more distal brain regions. Alternately, cocaine might induce an increase in behavioral activity by some mechanism other than by inducing an increase in NAc dopamine levels. It is interesting to note that the half-life of both cocaine and N/OFQ is relatively short, in the range of 20 min (Pan et al., 1998; Yu et al., 1996), yet both the stimulatory actions of cocaine and the inhibitory actions of N/OFQ can last for up to 4–5 h.

In conclusion, our data suggest that presynaptic NOP receptors on dopamine terminals in the NAc shell modulate extracellular dopamine in this region. This is consistent with the hypothesis that NOP receptor activation can attenuate reward induced by so many abused drugs because N/OFQ and small molecule agonists can attenuate dopamine levels in several brain regions. These data also show that, although NOP receptors can play a role in the regulation of cocaine-induced motor stimulating effects, the delayed onset of action suggests that this is probably not due to stimulation of NOP receptors in the NAc shell. Collectively, the present findings further elaborate the effects of the N/OFQ–NOP system on cocaine-mediated extracellular dopamine and support the pursuit of an NOP receptor agonist, as a medication for treatment of addiction disorders.

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