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Microinjection of the δ -Opioid Receptor Selective Antagonist Naltrindole 5'-Isothiocyanate Site Specifically Affects Cocaine Self-Administration in Rats Responding under a Progressive Ratio Schedule of Reinforcement

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Abstract

Whether the δ -opioid receptor (DOR) system can modulate behavioral effects of cocaine remains equivocal. We examined whether site- and subtype-selective blockade of DORs within the rat mesocorticolimbic system affects cocaine self-administration. The DOR antagonist naltrindole 5'-isothiocyanate (5'-NTII; 5 nmol) was microinjected into the nucleus accumbens (NAcc), ventral tegmental area (VTA), or amygdala (AMYG) in rats self-administering 1.5 mg/kg cocaine under a progressive ratio (PR) schedule. Intra-NAcc 5'-NTII significantly decreased cocaine self-administration, while 5'-NTII administration into the VTA significantly increased cocaine-maintained responding. 5'-NTII administration into the AMYG produced no effect. These data support a site-specific role of DORs in cocaine's behavioral effects.

Whereas the initial sites of action governing cocaine reinforcement are thought to be dopamine (DA) transporters within the mesocorticolimbic system (see Koob et al. 1998 for review), a number of other neurotransmitter systems co-localized within the mesocorticolimbic system can modulate the reinforcing effects of cocaine, including the endogenous opioid system. For example, non-selective opioid receptor blockade with naloxone or naltrexone can decrease cocaine self-administration in both non-human primates (Mello et al 1990) and rodents (Carroll et al 1986; De Vry et al 1989; Corrigall and Coen 1991; Ramsey and Van Ree 1991; Ramsey et al 1999). The relative contributions of μ , δ , and κ opioid receptor subtype-specific antagonism by these compounds on decreased cocaine self-administration remain equivocal. For example, our laboratory reported that site specific microinjections of the μ -opioid receptor (MOR) selective antagonist beta-funaltrexamine (β -FNA) attenuated responding for cocaine under a progressive ratio (PR) schedule of reinforcement in rats (Ward et al 2003). However, MOR blockade has been shown by others to be ineffective in altering cocaine's reinforcing effects (Martin et al 1998; Corrigall et al 1999). Similarly, Kuzmin et al (1998) reported that κ -opioid antagonism decreased cocaine self-administration in rats, while others have reported no effect of κ -opioid antagonism on cocaine self-administration in rats (Glick et al 1995) and rhesus monkeys (Negus et al 1997).

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Evidence also suggests that δ -opioid receptor (DOR) selective compounds and cocaine may interact with common neural substrates (Mansour et al 1987; Fowler et al 1989; Madras et al 1989; Kaufman et al 1991; Svingos et al 1999). For over a decade, researchers have been investigating whether selective DOR antagonism can attenuate the reinforcing effects of cocaine in laboratory animals, but results again are ambiguous. For example, Reid et al. (1995) demonstrated that i.p. administration of the DOR selective antagonist naltrindole decreased responding for cocaine in rats regardless of the schedule of reinforcement. Conversely, de Vries et al (1995) reported that only a high dose of naltrindole which also decreased locomotor activity (10 mg/kg i.p.) attenuated cocaine self-administration. In rhesus monkeys, i.v. naltrindole administration produced decreases in cocaine self-administration; however these effects were inconsistent across animals and sessions and were not dose-related (Negus et al 1995). The 5'-isothiocyanate analog of naltrindole, 5'-NTII, significantly decreased cocaine self-administration in rats when administered i.c.v, but this attenuation was also modest in comparison to the effect of i.c.v. 5'-NTII on heroin self-administration in the same study (Martin et al. 2000).

The present studies were performed to determine whether site-specific administration of 5'-NTII to brain regions within the mesocorticolimbic system alters motivation to self-administer cocaine under a progressive ratio (PR) schedule of reinforcement in rats. 5'-NTII is well suited for these self-administration studies because of its long duration of action; 5'-NTII has been shown to produce selective, insurmountable antagonism of DOR agonists *in vitro* and *in vivo* (Portoghese et al 1990). Interestingly, although 5'-NTII was synthesized to be a receptor-alkylating antagonist, it appears to act primarily by decreasing the affinity of the receptor for the agonist rather than by decreasing DOR density (Chakrabarti et al 1993). DORs have been located in the nucleus accumbens (NAcc), ventral tegmental area (VTA) and amygdala (AMYG) (Cahill et al 2001); therefore, we investigated the effect of bilateral microinjection of 5 nmol 5'-NTII into the NAcc, VTA, or AMYG, on cocaine self-administration maintained under a PR schedule of reinforcement.

Male Fischer 344 rats (n = 44; 275-300 g; Charles River, Raleigh, North Carolina) were used for the following experiments. Following arrival at the facility all rats were acclimated for a 2 week period and maintained on a reverse light/dark cycle (dark 3:00 AM-3:00 PM). The care and treatment of all animals was in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003; <http://nap.edu>) and conformed to the standards of the Wake Forest University Animal Care and Use Committee and the National Institutes of Health. Food and water were available *ad libitum* throughout all phases of the experiment. Following acclimation, rats were implanted with intravenous cannulae into the right jugular vein under anesthesia as described previously (Ward et al 2003). Rats were individually housed and trained in 25-cm $\times$ 25-cm $\times$ 25-cm operant testing chambers containing a retractable lever and stimulus light mounted directly above the lever. A motor driven syringe pump was located in front of the chamber. The cannula was connected through a stainless steel protective spring to a counterbalanced swivel apparatus that allowed free movement within the operant chamber. Following recovery from jugular cannulation, animals were given access to a single response lever that controlled the delivery of 1.5 mg/kg/infusion cocaine (cocaine hydrochloride, RTI, Research Triangle Park, NC) over 3-5 seconds based on body weight under a fixed ratio (FR) 1 schedule. Concurrent with the start of each drug infusion, a stimulus light located above the lever was activated to signal a 20-s post-infusion time-out period, during which the lever was retracted and no response could be made. After establishing a stable daily pattern of cocaine intake (3 consecutive days of >30 injections/6 h and regular post-infusion pauses) on an FR1 schedule, rats were switched to a PR schedule of reinforcement. Under this schedule animals were required to make a progressively greater number of responses to obtain each subsequent infusion throughout the test session. Drug infusions were contingent upon completion of ratio requirements

incremented through the following progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc. The final ratio completed before a 1-h time period elapsed with no responses was defined as the break point. After achieving three consecutive days of stable break points (break points stayed within a range of three increments with no upward or downward trends), animals were anesthetized and received bilateral microinjections of 5'-NTII (Research Biochemicals International, Natick, MA) or vehicle into the NAcc (n = 8/group), VTA (n = 8/group), or AMYG (n = 6/group).

5'-NTII (5 nmol) or vehicle was administered bilaterally in dimethyl sulfoxide (DMSO) to anesthetized rats (combination 75 mg/kg ketamine and 8 mg/kg xylazine) placed in a stereotaxic apparatus with the nose piece set at 2.5 mm below the horizontal. A micro-injector was attached to the arm of the stereotax via polyethylene tubing to a Hamilton gas-tight micro-syringe which was fitted into a syringe pump (Razel Scientific Instruments Inc., Stamford, CT). The micro-injector was lowered stereotactically into the NAcc (+ 7.5 mm anterior to lambda, $\pm$ 1.5 mm lateral to the midline, and - 6.5 mm ventral to skull surface), VTA (+ 3.2 mm anterior to lambda, $\pm$ 1.0 mm lateral to the midline, and - 8.2 mm ventral to the skull surface) or AMYG (-2.8 posterior to bregma, $\pm$ 5.0 lateral to the midline, and -8.2 ventral to the skull surface). The total volume of injection was 1 μ L (0.5 μ L into each hemisphere) administered at a rate of 1 μ L/min using the syringe pump, with flow of the proper injection volume confirmed by observing the movement of an air bubble for a pre-determined distance along the polyethylene tubing connecting the micro-syringe to the micro-injector. The micro-injector was left in place for 5 min to allow for pressure equilibration and then slowly withdrawn. The exterior incision was then sutured and dressed with antibiotic (Neosporin ointment; Pfizer, Inc. Consumer Healthcare, Morris Plains, NJ).

Animals were given one day of recovery following microinjection before being given access again to 1.5 mg/kg/infusion cocaine under the PR schedule. Break points were reassessed for 7 consecutive days. In order to conform to the requirement of homogeneity of variance for these statistical analyses, break points were log transformed; this conversion is equivalent to using the number of infusions self-administered during the session. Figure 1 illustrates that microinjection of 5'-NTII into the NAcc significantly decreased responding for cocaine under the PR schedule as compared to vehicle. Figure 1 illustrates the effect of 5'-NTII administration into the NAcc. Two-way analysis of variance (ANOVA; Prism4, GraphPad Software) revealed a significant main effect of treatment ($F_{(1,112)} = 19.51$, $p < 0.01$), with no significant effect of time ($F_{(6,112)} = 0.55$, n.s.) and no interaction ($F_{(6,112)} = 1.67$, n.s.). Bonferroni posttest analysis revealed no statistical differences in means at specific time points. Figure 2 illustrates the effect of 5'-NTII administration into the VTA. Two-way ANOVA revealed a significant main effect of treatment ($F_{(1,112)} = 18.52$, $p < 0.01$), with no significant effect of time ($F_{(6,112)} = 0.20$, n.s.) and no interaction ($F_{(6,112)} < 1$, n.s.). The high level of variability in cocaine-maintained responding following intra-VTA administration of 5'-NTII resulted from subjects' increased break points occurring at different time points across the retest period (see Table 1). Bonferroni posttest analysis revealed no statistical differences in means at specific time points. Figure 3 illustrates the effect of 5'-NTII administration into the AMYG. Two-way ANOVA revealed no significant main effect of treatment ($F_{(1,70)} = 0.08$, n.s.) or time ($F_{(6,70)} = 0.23$, n.s.) and no interaction ($F_{(6,70)} = 0.58$, n.s.).

These results are the first to suggest site-specific effects of tonic inhibition of DORs in the rat brain by a long-lasting δ selective antagonist on the reinforcing effects of cocaine. Irreversible blockade of DORs within the NAcc decreased responding for cocaine below baseline levels as measured by the PR schedule of reinforcement. In contrast, administration of 5'-NTII into the VTA significantly increased cocaine-maintained responding under the PR schedule as compared to vehicle. Microinjection of 5'-NTII into the AMYG was without effect.

The NAcc data are consistent with previous reports that DOR antagonism can significantly attenuate responding for cocaine (Reid et al 1995), although this effect had been characterized as modest (Negus et al 1995; Martin et al 2000) and inconsistent (Negus et al 1995). The present findings are most similar to Martin et al (2000), who reported that i.c.v. administration of 40 nmol 5'-NTII decreased responding for cocaine maintained under an FR5 schedule. The time course of this effect paralleled the time course of intra-NAcc 5'-NTII, with responding for cocaine returning to baseline levels by the fourth retest day in both studies. While the present result does not provide information as to the neurobiology underlying the effect of intra-NAcc 5'-NTII on cocaine reinforcement, it suggests that endogenous activation of DORs within the NAcc during cocaine self-administration contributes to reinforcement mechanisms. Indeed, DOR activation and cocaine administration produce comparable effects, including hyperlocomotion (Kalivas et al 1983), positive reinforcement (Shippenberg et al 1987) and increases in extracellular DA and its metabolites in the NAcc (Spanagel et al 1990; Longoni et al 1991; Manzanares et al 1993). Furthermore, immunohistochemical data reveal that DORs and DA transporters are co-localized within the NAcc in a way which indicates that DOR agonists can both directly and indirectly modulate extracellular dopamine levels (Svingos et al 1999). This suggests that increases in extracellular DA during cocaine self-administration may be further augmented by activation of DORs within the NAcc, and that intra-NAcc 5'-NTII may attenuate cocaine self-administration via inhibition of this facilitation.

While the present results suggest that blockade of DORs within the NAcc decrease cocaine's reinforcing effects, they also demonstrate that intra-VTA 5'-NTII increased responding for cocaine, suggesting that inactivation of DORs within the VTA enhanced the reinforcing effects of cocaine. This is somewhat surprising given that activation of DORs within the VTA produce cocaine-like effects similar to those reported when DOR agonists are administered into the NAcc. For example, intra-VTA administration of the DOR agonist DPDPE increases both DA and DOPAC levels in the NAcc (Devine et al 1993) and stimulates locomotor activity (Klitenick and Wirtshafter 1995). Interestingly, however, Ukai et al (1994) reported that while i.c.v. injection of the μ -selective agonist DAMGO inhibited cocaine-induced locomotor behavior, the δ -selective agonist DPLPE enhanced cocaine-induced locomotor activity. Furthermore, the locomotor-activating effects of cocaine are also enhanced in transgenic mice lacking the DOR (Chefer et al 2004). While the localization of DORs within the NAcc has been well characterized (Svingos et al 1999), less is known regarding the microstructural distribution of DORs within the VTA, as well as the chemical and anatomical profile of neurons containing these DORs (e.g. GABAergic or DAergic projection neurons). Preliminary evidence does suggest that these receptor subtypes within the VTA have distinct microstructural localization, in that both MORs and DORs have been located on neurons projecting to the medial prefrontal cortex (mPFC), and that the DOR labeling pattern appears to be complementary to that of the MOR (Svingos et al 2001; Svingos personal communication). One early interpretation of the present data is that this enhanced response to cocaine following blockade of DORs is mediated by a disinhibition of projections from the VTA to the mPFC, a brain region implicated in the development of drug sensitization (see Steketee 2003 for review).

Microinjection of 5'-NTII into the AMYG did not affect motivation to self-administer cocaine under a PR schedule. The AMYG has been shown by others to play a weak role in cocaine self-administration maintained under a PR schedule (McGregor and Roberts 1993, 1994; but see Loh and Roberts 1990). Instead, a wealth of evidence demonstrates the importance of the AMYG specifically in cocaine-seeking behavior (Whitelaw et al 1996; Meil and See 1997; Katak et al 2002; Yun and Fields 2003; see See 2005 for review).

In summary, the present results support the hypothesis that the DOR system can modulate the reinforcing effects of cocaine. The δ -opioid selective antagonist 5'-NTII decreased cocaine-

maintained responding when microinjected in the NAcc but increased cocaine self-administration when administered into the VTA. Administration of 5'-NTII into the amygdala was without effect. These results support the hypothesis that the DOR system can site-specifically modulate the reinforcing effects of cocaine; however, the mechanism by which 5'-NTII differentially modulates cocaine's reinforcing effects within the NAcc versus VTA merits further investigation.

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Figure 1.

Effect of intra-NAcc 5'-NTII on responding for 1.5 mg/kg/infusion cocaine maintained under a PR schedule. Points represent the mean ($\pm$ SEM) break point reached following microinjection of DMSO (■, $n=8$) or 5 nmol 5'-NTII (□, $n=8$) into the NAcc. Asterisk indicates significant difference from Vehicle ($P < 0.05$).

Figure 2.

Effect of intra-VTA 5'-NTII on responding for 1.5 mg/kg/infusion cocaine maintained under a PR schedule. Points represent the mean ($\pm$ SEM) break point reached following microinjection of DMSO (■, n=8) or 5 nmol 5'-NTII (□, n=8) into the VTA. Asterisk indicates significant difference from Vehicle ($P < 0.05$).

Figure 3. Effect of intra-AMYG 5'-NTII on responding for 1.5 mg/kg/infusion cocaine maintained under a PR schedule. Points represent the mean ($\pm$ SEM) break point reached following microinjection of DMSO ($\blacksquare$, $n=6$) or 5 nmol 5'-NTII ($\square$, $n=6$) into the AMYG.

Table 1

Increases in break points following intra-VTA 5'-NTII microinjection

Bolded cells indicate day(s) on which highest break point was achieved.

Subject	Baseline	Retest day(s)						
		1	2	3	4	5	6	7
S370	95	20	95	178	603	603	492	95
S372	268	402	492	219	77	118	219	145
S375	62	145	118	77	77	62	77	40
S377	40	50	40	62	118	40	40	50
S384	178	145	286	219	178	178	1646	1347
S388	492	1646	1347	603	328	402	268	219
S418	402	402	402	1102	1102	1102	1102	1102
S419	118	118	145	145	178	95	328	603