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Antagonism of δ_2-Opioid Receptors by Naltrindole-5′-isothiocyanate Attenuates Heroin Self-Administration but Not Antinociception in Rats[1]

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ABSTRACT

δ-Opioid receptors have been implicated in reinforcement processes and antagonists are available that produce long-lasting and selective antagonism of δ-opioid receptors in vivo. This experiment assessed the contribution of δ-opioid receptors to the antinociceptive and reinforcing properties of heroin. The effects of the irreversible δ-antagonist naltrindole-5′-isothiocyanate (5′-NTII) were evaluated on heroin self-administration and hot-plate antinociception in rats. 5′-NTII (10 nmol i.c.v.) shifted the dose-response curve for heroin self-administration downward, increasing the A_{50} values on the ascending and descending limbs by approximately 0.5 log units and decreasing the maximum by 33%. 5′-NTII (40 nmol i.c.v.) shifted both limbs of the heroin self-administration dose-effect curve 1.2 log units to the right and decreased the maximum by 90%. Heroin self-administration gradually returned to baseline levels over 7 or 17 days after administration of 10 or 40 nmol 5′-NTII, respectively. 5′-NTII (40 nmol i.c.v.) decreased the self-administration of 0.17 mg/infusion cocaine by 40% while having no effect on responding maintained by 0.33 or 0.67 mg/infusion. 5′-NTII attenuated the antinociceptive effects of deltorphin (δ_2) in a dose-dependent manner while having no effect on antinociception elicited after i.c.v. administration of [D-Pen^2,D-Pen^5]-enkephalin (δ_1) or [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin (μ). In addition, the antinociceptive effects of heroin were not significantly affected by 5′-NTII (40 nmol i.c.v.). Therefore, 5′-NTII can attenuate the reinforcing effects of heroin at doses that do not affect its antinociceptive effects. Long-acting δ_2-opioid antagonists may be beneficial in the treatment of heroin dependence or as adjuncts to reduce the abuse liability of opioid analgesics.

Investigation of the pharmacology of δ-opioid receptors has been significantly enhanced by the availability of selective, nonpeptidic agonists and antagonists. In the late 1980s, naltrindole was identified as the first nonpeptidic compound that displayed preferential antagonism of δ-opioid receptors compared with μ- or κ-subtypes (Portoghese et al., 1988). This compound produces selective antagonism of δ-opioid agonists in smooth muscle preparations (Portoghese et al., 1988) and in assays of antinociception (Portoghese et al., 1988). Several years later, the synthesis of the 5′-isothiocyanate analog of naltrindole (5′-NTII) was reported, and this compound was found to produce long-lasting and insurmountable antagonism of δ-agonists in vitro (Portoghese et al., 1990) and in vivo (Portoghese et al., 1990; Jiang et al., 1991; Vanderah et al., 1992) without attenuating the antinociceptive effects of μ-agonists at doses up to 17.5 nmol i.c.v. in mice (Abdelhamid et al., 1991; Jiang et al., 1991). Furthermore, 5′-NTII appears to be more selective for δ_2-opioid receptors in that it antagonizes the antinociceptive effects of deltorphin II and [D-Ser^2,Leu^5]-enkephalin-Thr but not the effects of [D-Pen^2,D-Pen^5]-enkephalin (DPDPE) (Jiang et al., 1991). Although 5′-NTII was synthesized to be a receptor-alkylating antagonist, it appears to act by decreasing the affinity of the receptor for the agonist rather than by decreasing δ-opioid receptor density as evidenced by [^{3}H][D-Ser^2,Leu^5]-enkephalin-Thr binding (Chakrabarti et al., 1993). 5′-NTII has proven to be a valuable tool for studying the pharmacology of δ-opioid receptors in vivo.

A number of studies have implicated a role for δ-opioid receptors in the reinforcing effects of abused substances. Place-preference has been conditioned in mice with i.c.v. administration of δ-opioid agonists (Shippenberg et al., 1987)

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ABBREVIATIONS: 5′-NTII, naltrindole-5′-isothiocyanate; DPDPE, [D-Pen^2,D-Pen^5]-enkephalin; HP, hot plate; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; FR, fixed ratio; DMSO, dimethyl sulfoxide; β-FNA, β-funaltrexamine.

and place-preference conditioning to β-endorphin appears to be partially mediated through δ-opioid receptors (Bals-Kubik et al., 1990). Naltrindole attenuated the reinforcing actions of heroin in rats at relatively large doses, possibly indicating a role of δ-opioid receptors in the reinforcing actions of heroin (Negus et al., 1993). Indirect evidence regarding the possible involvement of δ-opioid receptors in drug reinforcement comes from the observation that there are relatively dense populations of δ-opioid receptors in limbic structures in the central nervous system (Tempel and Zukin, 1987; Mansour et al., 1988) and that acute administration of δ-opioid agonists produces neurochemical effects in the nucleus accumbens similar to those of abused substances (DiChiara and Imperato, 1988; Spanagel et al., 1990).

These studies were performed to determine whether 5′-NTII alters the reinforcing effects of heroin and cocaine by the use of a rat self-administration paradigm and whether opioid antinociception is similarly affected. 5′-NTII is well suited for these studies because of its long duration of action. Furthermore, this long duration of action permits the use of a self-administration procedure that determines dose-effect curves in a single session for each animal (Martin et al., 1996), making assessment of effects on the full dose-effect curve more feasible. The receptor selectivity of 5′-NTII was assessed by the hot-plate (HP) assay of antinociception and selective μ- ([D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; DAMGO), δ_1- (DPDPE), or δ_2- (deltorphin) opioid agonists. These studies provide an assessment of the involvement of δ-receptors in the reinforcing effects of heroin and of the potential for long-acting δ-opioid antagonists to serve as medications for treatment of heroin dependence.

Materials and Methods

Animals. Male Fischer 344 rats (n = 111; 250–350 g; Harlan Laboratories, Indianapolis, IN) were kept on a reverse light/dark cycle (dark 5:00 AM–5:00 PM) and given ad libitum access to water except during self-administration sessions. Animals used for self-administration studies were kept at 85% of their free-feeding body weight to reduce weight fluctuations and maintain optimum health (Ator, 1991). All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health and in accordance with the Institutional Animal Care and Use Committee of the Wake Forest University School of Medicine.

Surgical Procedures. Animals were anesthetized with pentobarbital (50 mg/kg i.p.) and atropine methyl nitrate (10 mg/kg i.p.) and chronic indwelling catheters were implanted into the right exterior jugular vein extending to the right auricle with previously described procedures (Martin et al., 1995). The catheter exited between the scapulae and continued through a spring leash, terminating at a fluid swivel (Weeks, 1962). The leash was attached to the back of the animal with an implanted polypropylene plate encased in Teflon mesh. Stainless steel guide cannulas (Plastics One, Roanoke, VA) were implanted at the time of catheter implantation into the lateral ventricles (1.3 mm rostral from bregma, 1.5 mm lateral from midline, and 3.5 mm ventral from the skull surface) and were secured to the skull with dental acrylic and stainless steel, self-tapping screws (J. I. Morris and Co., Southbridge, MA). Animals were administered 75,000 U i.m. of penicillin G procaine (Butler Co., Columbus, OH) and all exterior surgical wounds were dressed with antibiotic powder (Polysporin; Wellcome-Glaxo, Research Triangle Park, NC).

Apparatus. All experimental sessions were conducted in sound-attenuated chambers and were controlled by an IBM-compatible computer through an interface (Med Associates, St. Albans, VT). The operant chamber (21 × 21 × 28 cm) contained a response lever 6.8 cm above the floor and 1.1 cm from the rear wall and a light located 4.0 cm above the response lever. Each chamber contained a house light, tone generator, and ventilator fan. The fluid swivel and catheter were connected through a 20-gauge Luer hub and a 22-gauge male connector to a 20-ml syringe on an infusion pump located outside of the sound-attenuated enclosure.

Heroin Self-Administration. Lever presses were engendered and maintained in animals with heroin infusions with a fixed-ratio (FR) schedule and a procedure that assesses the dose-response relationship within each experimental session (Martin et al., 1996). This procedure has been previously shown to generate stable dose-response curves over several weeks without the development of tolerance or physical dependence (Martin et al., 1996). After 5 to 7 days of recovery from surgery, animals were trained to self-administer varying doses of heroin under an FR1 schedule of reinforcement. The dose of heroin was varied by altering the duration that the infusion pump was activated such that operation of the pump for 1.7, 2.8, 5.6, or 9.3 s delivered infusions of 5.4, 9, 18, or 30 μg of heroin. The concentration of heroin delivered was 90 μg/ml. For self-administration sessions, the animals were placed in operant chambers and, after a 10-min acclimation period, an infusion of the dose of heroin available for the 1st h was delivered and the lever light was illuminated to indicate drug availability. On completion of each subsequent ratio requirement, the lever light was darkened and an infusion of heroin was delivered. A 30-s time-out period followed that was signaled by the operation of the house light and tone. Each successive session hour was initiated by delivery of an infusion of the dose of heroin available for that hour with the four hourly segments separated by 20-min time-out periods during which all lights were extinguished and lever presses had no programmed consequences. The order of dose presentation was randomized for each session and for each animal by use of a random number generator in the Med-PC programming language (Med Associates). Responding was considered stable when the number of infusions at each dose of heroin for each of five successive days did not vary by more than 10% of the mean. The ratio requirement was increased from 1 to 10 across experimental sessions when stable responding was established. Saline was substituted for all doses of heroin within a session after 5 days of stable responding at FR10. The ascending limb of the dose-response curve was generated by substituting 13.5 μg/ml (resulting in doses of 0.81, 1.35, 2.7, or 4.5 μg/infusion) and 27 μg/ml (resulting in doses of 1.62, 2.7, 5.4, or 9 μg/infusion) heroin for the training concentration on Tuesdays or Thursdays, provided that the number of infusions for each dose of heroin did not vary by more than 10% from the mean on the previous day. Duplicate determinations were made for each animal for each lower heroin concentration and saline.

Cocaine Self-Administration. The methods used to engender and maintain responding with infusions of cocaine were similar to those mentioned for heroin with several exceptions. Three doses of cocaine were made available for self-administration in three hourly components separated by a 10-min time-out period. Each infusion of cocaine was immediately followed with a 20-s time-out period during which lever presses had no programmed consequences. The dose of cocaine was altered by varying the time of operation of the infusion pump such that operation of the pump for 2.8, 5.6, or 11.2 s resulted in infusions of 0.17, 0.33, or 0.67 μg of cocaine, respectively. The order of dose presentation was random and the concentration of cocaine in the syringe was 1.67 mg/ml. All other aspects of the methods were the same as for the heroin self-administration experiments (n = 6).

Administration of 5′-NTII. 5′-NTII (0, 10, or 40 nmol) was administered bilaterally in dimethyl sulfoxide (DMSO) to unrestrained, conscious animals through internal cannulas (Plastics One) attached to a Hamilton gas-tight microsyringe by a polyethylene

connector (Plastics One). The total volume of injection was 8 μl (4 μl/side) and was administered at a rate of 1 μl/min with a microsyringe infusion pump (KDS Scientific, Boston, MA). The internal cannulas were left in place for 15 min after the injection to allow for pressure equilibration.

Effects of 5′-NTII on Heroin Self-Administration. Once the ascending portion of the dose-response curve and saline extinction were determined, animals were allowed to self-administer the training concentration of heroin for a minimum of five sessions and then administered 0 ($n = 6$), 10 ($n = 17$), or 40 ($n = 16$) nmol of 5′-NTII as described above. 5′-NTII was always administered on Mondays and animals were allowed to self-administer heroin on Tuesday through Friday and on Monday through Friday in successive weeks.

One group of animals was used to determine the effects of vehicle administration on heroin self-administration. These animals ($n = 6$) were allowed to self-administer the training concentration of 90 μg/ml heroin (doses of 5.4, 9, 18, or 30 μg/infusion) beginning 24 h after DMSO administration and for 30 days after vehicle treatment.

Three groups of animals were used to obtain the full dose-response curve for heroin self-administration after i.c.v. administration of 10 nmol of 5′-NTII. One group ($n = 6$) was allowed to self-administer the training concentration of 90 μg/ml heroin (doses of 5.4, 9, 18, or 30 μg/infusion). A second group ($n = 5$) was allowed to self-administer 27 μg/ml heroin (doses of 1.62, 2.7, 5.4, or 9 μg/infusion) and was necessary to generate the remainder of the ascending portion of the dose-effect curve. The third group ($n = 6$) was necessary to generate the remainder of the descending portion of the dose-response curve and was allowed to self-administer 450 μg/ml heroin (doses of 27, 45, 90, or 150 μg/infusion). After 7 days, the concentration of heroin was reduced to 90 μg/ml for three of the animals in this group and to 27 μg/ml for the other three animals.

Three groups of animals were used to obtain the full dose-effect curve for heroin self-administration after i.c.v. administration of 40 nmol of 5′-NTII. One group ($n = 6$) was allowed to self-administer the training concentration of 90 μg/ml heroin (doses of 5.4, 9, 18, or 30 μg/infusion). An additional group ($n = 5$) was necessary to generate the remainder of the ascending portion of the dose-effect curve and was allowed to self-administer 450 μg/ml heroin (doses of 27, 45, 90, or 150 μg/infusion). After 11 days, the concentration of heroin was reduced to 27 μg/ml for the latter group of animals. An additional ($n = 5$) group was required to generate the descending portion of the dose-effect curve after 5′-NTII administration and was allowed to self-administer 2.7 mg/ml heroin (doses of 162, 270, 540, or 900 μg/infusion). After 11 days, the concentration of heroin was reduced to 27 μg/ml for the latter group of animals to generate the ascending portion of the dose-effect curve.

Effects of 5′-NTII on Opioid Antinociception. The antinociceptive effect of approximate A_{90} doses of DPDPE (100 nmol), deltorphin II (30 nmol), or DAMGO (1 nmol) was determined with the HP assay at 52°C 24 h after i.c.v. administration of vehicle ($n = 6$ for each agonist or 18 total), 10 ($n = 6$ for each agonist or 18 total), or 40 ($n = 6$ for each agonist or 18 total) nmol of 5′-NTII according to previous methods (Kovelowski et al., 1999). Both 5′-NTII and agonists were administered bilaterally through guide cannulas that were stereotaxically implanted as described above. The antinociceptive effects of an approximate A_{90} dose of heroin (200 μg/kg i.v.) also was assessed with the HP assay in animals 24 h after i.c.v. administration of 5′-NTII (40 nmol; $n = 6$) or vehicle ($n = 6$). The HP test was performed by placing the rat on a heated surface and determining the latency until a nociceptive response, demonstrated by licking of a hindpaw or attempts to jump out of the enclosure, was evident. The HP latencies were determined once before drug injection and 20 or 30 min after i.c.v injection of peptide agonists or 3 min after i.v. administration of heroin. A maximum latency of 60 s was used to prevent tissue damage and animals were removed from the HP and given a maximum score after this time.

Data Analysis. Dose-response curves for heroin self-administration were fit to the general logistic form of the dose-effect equation:

$$Y = E_{\min} + \frac{E_{\max} - E_{\min}}{1 + 10^{(\log A_{50} - \log X)h}} \quad (1)$$

with a commercially available curve-fitting package for IBM-compatible computers (Prism2; GraphPad Software, San Diego, CA) where Y is the number of infusions administered in the hourly component; $E_{\min}$ and $E_{\max}$ are the minimum and maximum effects in the dose-response curve, respectively; A_{50} is the median effective dose; X is the heroin dose; and h is the Hill slope. The ascending and descending portions of the dose-effect curve were fit independently and separate values for the A_{50}, $E_{\max}$, $E_{\min}$, and Hill slope were determined for control data and for each session for 30 days after administration of 5′-NTII.

Antinociception data were expressed as percentage maximum possible effect (%MPE):

$$\%\text{MPE} = \frac{\text{latency} - \text{control latency}}{60 - \text{control latency}} \times 100 \quad (2)$$

where control latency is the latency to response before agonist administration.

Statistical Analysis. The dose responsiveness of heroin and cocaine self-administration and the effects of saline substitution were assessed with ANOVA. The 95% CI for the $E_{\min}$, $E_{\max}$, A_{50}, and Hill slope of the heroin dose-effect curves were calculated with commercially available curve-fitting software for IBM-compatible computers (Prism2; GraphPad Software). Each of these parameters was considered to be significantly affected when there was no overlap in the 95% CI for estimates from the data of treated versus control animals. The logarithm of the A_{50} values and their associated standard error are presented for statistical comparison because it is the logarithm of these parameters that is normally distributed. The data from the HP antinociception assays were analyzed by ANOVA with 5′-NTII dose as the independent variable and %MPE for each agonist as the dependent measure. Post hoc analyses were performed with a Bonferroni/Dunn t test for multiple comparisons. The antinociception data obtained with heroin were analyzed by Student's t test.

Drugs and Chemicals. Heroin hydrochloride and cocaine hydrochloride were provided by Research Triangle Institute (Research Triangle Park, NC) through the Drug Supply Program of the National Institute on Drug Abuse of the National Institutes of Health and were dissolved in 0.9% saline (w/v), pH 7.4, with 1.7 U/ml heparin sodium. DAMGO was purchased from Peninsula Laboratories (Belmont, CA). DPDPE and deltorphin also were obtained through the Drug Supply Program of the National Institute on Drug Abuse from Multiple Peptide Systems (San Diego, CA). All peptides were dissolved in 0.9% (w/v) saline, pH 7.4. Pentobarbital (Nembutal) was purchased from Abbott Laboratories (North Chicago, IL) in a vehicle of 10:40:50 ethanol:propylene glycol:water at a concentration of 50 mg/ml. Atropine methyl nitrate was purchased from Sigma Chemical Co. (St. Louis, MO) and heparin sodium was purchased from Elkins-Sinn Inc. (Cherry Hill, NJ). 5′-NTII was purchased from Research Biochemicals International (Natick, MA). All drug doses are reported in terms of the free base.

Results

Heroin Self-Administration. Heroin maintained responding in all animals at a rate greater than that maintained by saline at doses greater than 0.81 μg/infusion and the number of infusions administered was dose-dependent [$F(8,360) = 16.685$, $P = .0001$]. The A_{50} values calculated from either the ascending or descending limbs of the control dose-effect curves were not significantly different between the groups of animals that were administered vehicle or the

different doses of 5′-NTII (α = .05). Substitution of saline for heroin resulted in a significant decrease in responding [$F(1,120) = 0.021$, $P < .001$] and the number of infusions administered was 1.3 (1.4), 1.2 (1.0), 1.1 (1.0), and 0.9 (1.0) in the four respective hourly components of the session.

Effect of i.c.v. Administration of DMSO on Heroin Self-Administration. Administration of DMSO i.c.v. had a significant effect on heroin self-administration 24 h after treatment [$F(1,46) = 18.73$, $P = .001$] and there was an interaction between DMSO treatment and heroin dose [$F(3,46) = 22.16$, $P = .024$]. The only significant effect however was a decrease in the number of infusions delivered of the 5.4-μg dose 24 h later from 14.7 (1.9) to 9.1 (2.0) while having no significant effect on the number of infusions administered of the other three doses. On the second day after i.c.v. administration of DMSO, the number of infusions administered of 5.4 μg of heroin was 14.3 (1.6) and was not significantly different from the control data for this dose of heroin. The number of infusions of heroin delivered was not significantly different from control for all heroin doses for subsequent sessions up to 30 days after i.c.v. administration of vehicle (data not shown).

Effect of i.c.v. Administration of 10 nmol of 5′-NTII on Heroin Self-Administration. The dose-effect curve for heroin was shifted downward 24 h after i.c.v. administration of 10 nmol of 5′-NTII (Fig. 1). The log A_{50} was increased from a control value [mean (S.E.)] of 0.24 (0.06) (A_{50} = 1.7; 95% CL, 0.8–2.2 μg/infusion) to 0.67 (0.08) (A_{50} = 4.7; 95% CL, 2.8–6.6 μg/infusion) on the ascending portion of the dose-effect curve. The descending limb of the dose-effect curve was shifted to a similar extent, with the log A_{50} increasing from 1.27 (0.07) (A_{50} = 18.7; 95% CL, 12.9–28.8 μg/infusion) to 1.84 (0.02) (A_{50} = 69.0; 95% CL, 56.4–80.1 μg/infusion). The maximum of the dose-effect curve also was decreased from 10.2 (0.9) to 5.3 (0.7) infusions ($P \leq .05$). The A_{50} values remained significantly increased for both the ascending and descending portions of the dose-effect curve on days 2, 3, and 4 after i.c.v. administration of 5′-NTII, returning to control levels by day 7 (Fig. 2) and subsequent days thereafter. The maximum of the dose-effect curve was decreased on days 2 (6.7 ± 1.3 infusions) and 3 (7.3 ± 0.3 infusions), but was not significantly different from control on day 4 (10.5 ± 0.5 infusions) or on subsequent days thereafter (data not shown).

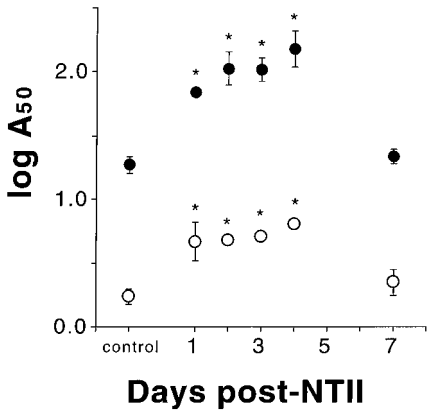

Fig. 2. Log A_{50} values for heroin self-administration versus time after 10 nmol of 5′-NTII i.c.v. The data were obtained from dose-effect curves for heroin generated with the group of animals whose data are depicted in Fig. 1. Sessions were conducted on weekdays only, and therefore data for days 5 and 6 after i.c.v. administration of 5′-NTII were not obtained. ○, ascending; ●, descending. *, significantly different from control, $P \leq .05$.

Effect of i.c.v. Administration of 40 nmol of 5′-NTII on Heroin Self-Administration. The dose-effect curve for heroin was shifted downward and to the right after i.c.v. administration of 40 nmol of 5′-NTII and affected to a greater extent than after the 10-nmol dose (Fig. 3). The log A_{50} was increased from 0.31 (0.12) (A_{50} = 2.0; 95% CL, 0.5–3.4 μg/infusion) to 1.52 (0.31) (A_{50} = 33.4; 95% CL, 15.2–62.4 μg/infusion) on the ascending limb of the dose-effect curve 24 h after i.c.v administration of 40 nmol. On the descending limb of the dose-effect curve, the log A_{50} was increased from 1.30 (0.04) (A_{50} = 19.7; 95% CL, 16.5–30.2 μg/infusion) to 2.53 (0.03) (A_{50} = 339.2; 95% CL, 290.1–443.2 μg/infusion). The maximum of the dose-effect curve was significantly decreased from 10.2 (0.8) to 2.6 (1.9) after 24 h ($P \leq .05$). The dose-effect curve for heroin self-administration gradually shifted back toward the control dose-effect curve over 17 to 21 days after i.c.v. administration of 5′-NTII (Fig. 4). The calculated log A_{50} values for the ascending and descending limbs of the dose-effect curve gradually returned to control values

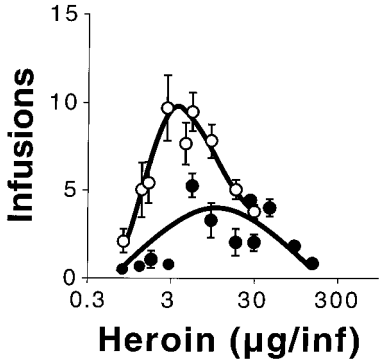

Fig. 1. Effect of 10 nmol of 5′-NTII (●) on the dose-effect curve for heroin self-administration. 5′-NTII was administered i.c.v. and animals were allowed to self-administer heroin beginning 24 h later. Control (○) data are averaged from the five self-administration sessions before 5′-NTII administration. Values are mean ± S.E. for the number of infusions administered during the hourly component for each dose of heroin. Curves are for illustrative purposes only and do not represent fitted curves. n = 17.

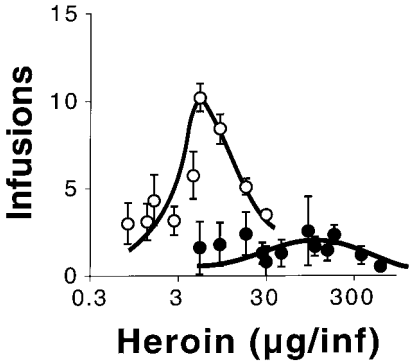

Fig. 3. Effect of 40 of nmol 5′-NTII (●) on the dose-effect curve for heroin self-administration. 5′-NTII was administered i.c.v. and animals were allowed to self-administer heroin beginning 24 h later. Control (○) data are averaged from the five self-administration sessions before 5′-NTII administration. Values are mean ± S.E. for the number of infusions administered during the hourly component for each dose of heroin. Curves are for illustrative purposes only and do not represent fitted curves. n = 16.

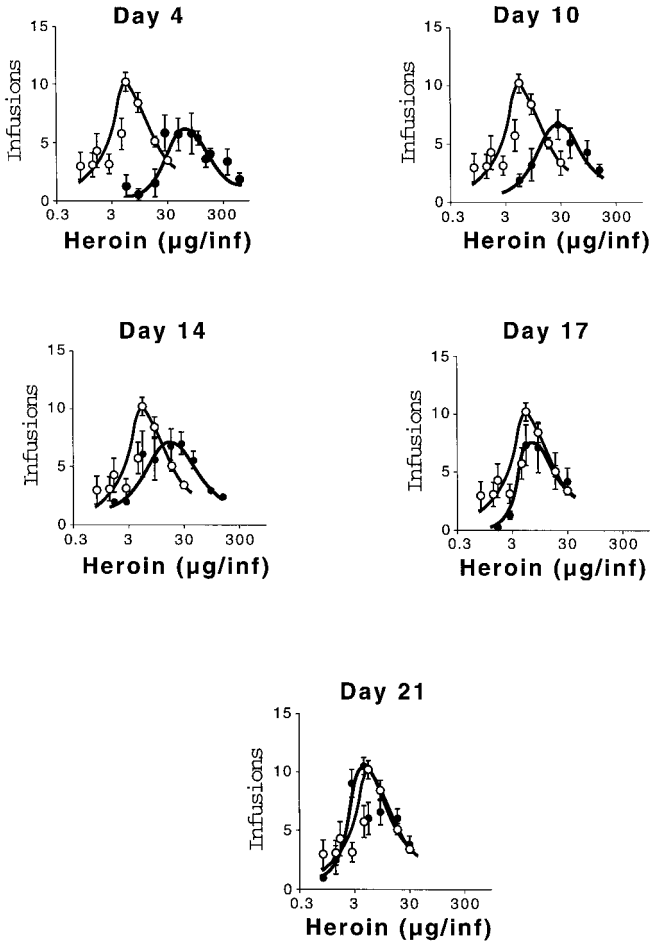

Fig. 4. Recovery of heroin self-administration after 40 nmol of 5′-NTII i.c.v. (●). Control, ○. Dose-effect curves for heroin self-administration are shown for selected days after i.c.v. administration of 5′-NTII. Data were obtained from the same groups of animals used for the data represented in Fig. 3. Curves are for illustrative purposes only and do not represent fitted curves.

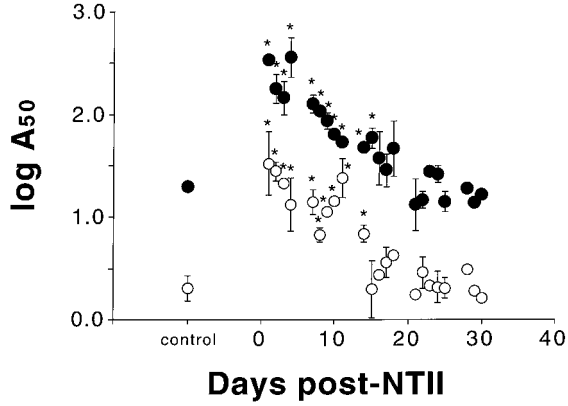

Fig. 5. Log A_{50} values for heroin self-administration versus time after 40 nmol of 5′-NTII i.c.v. The data were obtained from dose-effect curves for heroin generated with the group of animals whose data are depicted in Figs. 3 and 4. Sessions were conducted on weekdays only, and therefore data for days 5, 6, 12, 13, 19, 20, 26, and 27 after i.c.v. administration of 5′-NTII were not obtained. ○, ascending; ●, descending. *, significantly different from control, $P \leq .05$.

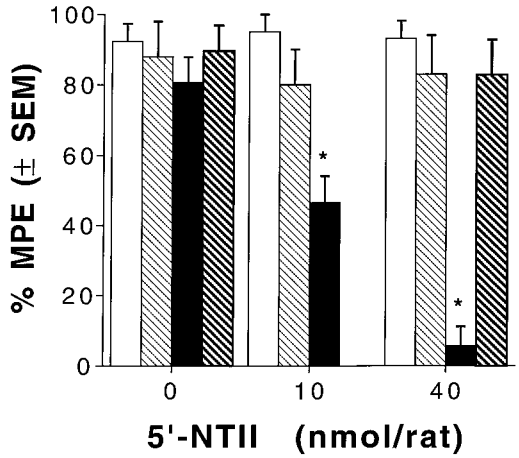

Fig. 6. Effect of 5′-NTII on antinociception induced by selective opioid agonists. 5′-NTII was administered i.c.v and antinociception was assessed 24 h later after i.c.v. administration of agonists. □, DAMGO; ▨, DPDPE; ■, deltorphin; ▩, heroin. *, significantly different from control, $P \leq .05$. n = 6/group.

over 17 days after i.c.v. treatment with 40 nmol of 5′-NTII (Fig. 5).

Effect of i.c.v. Administration of 40 nmol of 5′-NTII on Cocaine Self-Administration. Cocaine maintained responding in a dose-dependent manner [$F(2,15) = 58.36$, $P = .0001$] and substitution of saline decreased responding during all three hourly components of the session [$F(1,34) = 38.22$, $P = .0001$], resulting in administration of 1.5 (0.6), 0.9 (0.2), or 1.3 (0.2) infusions in the first, second or third hourly components, respectively. Administration of 40 nmol of 5′-NTII i.c.v. had a significant effect on cocaine self-administration [$F(1,34) = 32.73$, $P = .001$] 24 h after administration and there was a significant interaction between 5′-NTII treatment and cocaine dose [$F(2,34) = 19.16$, $P = .038$]. Post hoc analysis revealed that this dose of 5′-NTII decreased the number of infusions administered of 0.17 mg/infusion cocaine from a control value of 21.3 (3.0) to 12.3 (3.8) 24 h later ($P \leq .05$). The number of infusions administered of 0.33 or 0.67 mg/infusion cocaine was not significantly affected and was 10.3 (1.6) and 6.2 (0.9) for control versus 9.3 (2.6) and 4.5 (1.3) 24 h after 5′-NTII administration, respectively. The number of infusions of the lowest dose of cocaine was significantly decreased on the second (16.3 ± 1.4 infusions) and third (15.4 ± 1.5 infusions) day after 5′-NTII treatment, but returned to control values on the fourth day (18.6 ± 2.3 infusions) and was not significantly different thereafter (data not shown). The number of infusions of 0.33 or 0.67 mg/infusion of cocaine was not significantly affected at any time after i.c.v. administration of 40 nmol of 5′-NTII ($\alpha = .05$).

Effect of 5′-NTII on Antinociceptive Effects of Opioid Agonists. 5′-NTII decreased the antinociceptive effects of 30 nmol of deltorphin i.c.v in a dose-dependent manner 24 h after i.c.v. administration [$F(2,15) = 93.32$, $P \leq .0001$; Fig. 6]. These doses of 5′-NTII had no significant effect on the antinociception elicited by 100 nmol of DPDPE [$F(2,15) = 0.759$, $P = .486$] or 1 nmol of DAMGO [$F(2,15) = 0.124$, $P = .884$; Fig. 6]. Administration of 40 nmol of 5′-NTII had no effect on the antinociception elicited by 200 µg/kg i.v. heroin ($\alpha = .05$; Fig. 6).

Discussion

These data indicate that tonic inhibition of δ_2-opioid receptors by a long-lasting antagonist in rat brain attenuates the

reinforcing effects of heroin without influencing its antinociceptive effects. The dose-effect curve was shifted to the right and downward, suggesting a noncompetitive mechanism of action for 5′-NTII antagonism of heroin. These effects of 5′-NTII were dose responsive and do not appear to be due to nonspecific disruption of behavior because the effects of 5′-NTII on cocaine self-administration were much less than the effects on heroin. The selective antagonism of deltorphin's antinociceptive actions by 5′-NTII suggest an involvement of δ_2-opioid receptors in heroin's reinforcing effects in vivo. The inability of 5′-NTII to diminish heroin's antinociceptive actions indicates that it may be possible to develop agents that are effective analgesics with less abuse liability.

These data are consistent with previous reports that naltrindole attenuates the reinforcing effects of heroin (Negus et al., 1993). Doses of 10 or 17 mg/kg s.c. naltrindole increased the number of infusions administered of 60 μg/kg heroin by 58 or 72%, respectively. However, it is difficult to make direct quantitative comparisons between the study of Negus et al. (1993) and this study in the absence of the full dose-effect curve for heroin after naltrindole pretreatment. Complete extinction was not observed in the study of Negus et al. (1993), whereas 40 nmol of 5′-NTII i.c.v. in this study almost completely abolished the reinforcing effects of heroin. This may be due to the low relative selectivity of naltrindole for δ- versus μ-opioid receptors that limited the range of naltrindole doses that could be investigated. The partial effects of naltrindole on heroin self-administration may be due to insufficient antagonism of δ-receptors that occurs at δ-selective doses.

The antinociception data suggest that the doses of 5′-NTII that were used are selective for δ_2-opioid receptors. The antinociception data do not necessarily indicate that 5′-NTII interacts exclusively at δ_2-opioid receptors, only that the degree of antagonism at δ_1- or μ-receptors is insufficient to diminish the antinociception produced by the agonists used. It could be that 5′-NTII produces antagonism at μ-opioid receptors sufficient to diminish heroin self-administration, but not heroin- or DAMGO-mediated antinociception. Such an explanation for 5′-NTII's effects would indicate that a greater population of spare μ-opioid receptors exists for DAMGO- or heroin-mediated antinociception than for heroin self-administration. Our previous studies with the irreversible μ-opioid antagonist β-funaltrexamine (β-FNA) indicate otherwise (Martin et al., 1998). β-FNA shifted the dose-effect curve for heroin self-administration 2.5-fold to the right in a parallel manner. In other words, the effect of β-FNA could be overcome by increasing the dose of heroin, indicating the existence of spare μ-opioid receptors for heroin's reinforcing effects. A similar dose of β-FNA has been shown to completely antagonize DAMGO-mediated antinociception, and this effect cannot be overcome by increasing the dose of DAMGO (Jiang et al., 1995). These data indicate that there is a greater population of spare μ-opioid receptors for heroin reinforcement than for DAMGO-mediated antinociception. Therefore, it seems unlikely that 5′-NTII is altering the reinforcing effects of heroin through antagonism of μ-opioid receptors.

It is unlikely that heroin is producing reinforcing effects by interacting directly with δ_2-opioid receptors. Heroin is thought to exert its effects in brain through its metabolites morphine and 6-monoacetyl-morphine. Neither of these compounds has been shown to bind to δ-receptors with high affinity in vitro. Furthermore, the magnitude of the effect of the 40-nmol dose of 5′-NTII on heroin self-administration would indicate that δ-opioid receptors are the primary site of the reinforcing actions of heroin. Numerous studies indicate a significant role for μ-opioid receptors in heroin reinforcement, rendering such an explanation untenable (Koob et al., 1984; Negus et al., 1993; Martin et al., 1995). The involvement of δ-receptors in the reinforcing effects of heroin likely occurs in processes subsequent to the stimulation of μ-opioid receptors by morphine and 6-monoacetyl-morphine. Morphine has been shown to stimulate the release of met-enkephalin in the ventral pallidum (Emmett et al., 1995; Olive et al., 1995), and may therefore exert reinforcing effects through indirect δ-receptor stimulation by this mechanism. Measurement of opioid peptide levels in microdialysates taken from limbic regions during heroin self-administration is currently in progress and may lend support for this hypothesis. Others have hypothesized that repeated μ-opioid receptor stimulation results in the recruitment of δ-opioid receptors by μ-agonists as μ-opioid receptors become desensitized. This theory is largely based on data demonstrating that δ-antagonists prevent the development of tolerance to the antinociceptive properties of μ-agonists and the development of physical dependence (Miyamoto et al., 1994; Fundytus et al., 1995; Hepburn et al., 1997). The present procedure used for self-administration produces stable drug intake across several weeks or months of exposure, suggesting that tolerance does not develop to the reinforcing effects of heroin under these limited exposure conditions (Martin et al., 1996). The animals do not appear to be physically dependent as well because no overt signs of withdrawal are observed in these animals at any time. However, it is possible that acute desensitization of μ-opioid receptors occurs within the 4 h of the self-administration session and that δ-receptors are somehow sensitized or recruited into activation. Support for such a hypothesis is currently lacking and the nature of the involvement of δ_2-opioid receptors in the effects of heroin remains to be determined.

The above-mentioned explanation for the effects of 5′-NTII on heroin self-administration necessitates the involvement of δ-opioid receptors in reinforcement mechanisms. A number of studies indicate that activation of δ-opioid receptors produces a reinforcing stimulus. Met-enkephalin is self-administered by rats into the nucleus accumbens (Goeders et al., 1984) and ventral tegmental area (Devine and Wise, 1994). D-Ala^2-met-enkephalinamide, a metabolically stable but nonselective analog of met-enkephalin, is self-administered into the lateral hypothalamus by rats, and this behavior is blocked by naloxone but not naltrexone, suggesting an involvement of non-μ-opioid receptors (Olds and Williams, 1980). Place-conditioning produced by i.c.v. administration of either β-endorphin or DPDPE is attenuated by the δ-opioid antagonist ICI 174,864 (Shippenberg et al., 1987; Bals-Kubik et al., 1990). Injection of D-Ala^2-met-enkephalinamide or DL-thiorphan, an enkephalinase inhibitor, produces place-preference conditioning when injected into the ventral tegmental area in rats that is antagonized by naloxone (Glimcher et al., 1984) and administration of DPDPE into the nucleus accumbens produces conditioned reward in rats (Phillips et al., 1994). Therefore, stimulation of δ-opioid receptors by a number of

different compounds in limbic structures produces reinforcing stimuli.

The literature concerning the involvement of δ-opioid receptors in the reinforcing effects of cocaine is equivocal. Place-preference conditioning with both cocaine and amphetamine is blocked by naltriben, a δ_2-selective antagonist, and naltrindole, but not by 7-benzylidenenaltrexone (Suzuki et al., 1994). Naltrindole also blocks the facilitation of i.c. self-stimulation in the medial forebrain bundle induced by cocaine (Reid et al., 1993) and attenuates cocaine self-administration in rats (Reid et al., 1995). In contrast, others have reported a lack of effect of naltrindole on either cocaine self-administration or conditioned place-preference (de Vries et al., 1995). Naltrindole also has been reported to have only slight effects in attenuating the reinforcing and discriminative stimulus properties of cocaine in rhesus monkeys (Negus et al., 1995). These data with 5′-NTII and cocaine self-administration are most consistent with the latter findings because the effects were small and occurred only at the lowest unit dose of cocaine. The major point to be made from the data with 5′-NTII and cocaine is that the effects of 5′-NTII on heroin self-administration do not seem to be due to nonspecific disruption of behavior. These data do not support a major involvement of δ_2-opioid receptors in cocaine reinforcement, however.

Other observations that make these data intriguing are the findings that δ-opioid antagonists prevent the development of physical dependence and tolerance to the antinociceptive effects of μ-opioid agonists. Coadministration of naltrindole during chronic treatment with morphine significantly attenuates withdrawal signs and weight loss after naloxone challenge in rats (Hepburn et al., 1997). These studies also demonstrated an attenuation of the development of tolerance to the antinociceptive but not respiratory depressive effects of morphine (Fundytus et al., 1995; Hepburn et al., 1997). Other investigators have found that 5′-NTII prevents the development of acute tolerance to morphine in mice, whereas the δ_1-opioid antagonist [D-Ala^2,Leu^5,Cys^6]-enkephalin is without effect (Miyamoto et al., 1993,1994). δ-Opioid antagonists also precipitate an affective withdrawal syndrome in rats chronically treated with morphine (Funada et al., 1996). The development of a mixed μ-agonist, δ-antagonist may provide an analgesic that has lower abuse liability than current μ-opioids and with less proclivity for the development of tolerance and physical dependence.

In summary, δ_2-opioid receptors appear to be involved in the processes underlying heroin reinforcement in rats. 5′-NTII shifts the dose-effect curve for heroin in a manner consistent with noncompetitive antagonism at doses that attenuate the antinociceptive effects of δ_2-, but not δ_1- or μ-opioid agonists. The mechanism of δ_2-opioid receptors in these effects of heroin merits further investigation. The lack of antagonism of heroin's antinociceptive actions by 5′-NTII indicates that a mixed μ-agonist, δ-antagonist may provide an efficacious opioid analgesic that has limited abuse potential. These data also indicate that long-lasting δ_2-opioid antagonists may prove useful in the treatment of heroin dependence or as clinical adjuncts to reduce the abuse liability of μ-opioid analgesics.

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