ELSEVIER

European Journal of Pharmacology 345 (1998) 47–53

ejp

# Chronic naltrexone differentially affects supraspinal δ-opioid receptor-mediated antinociception

Benjamin Kest, Cynthia E. Lee, Shirzad Jenab, Bernard Kao, Charles E. Inturrisi *

*Department of Pharmacology, LC-524, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, USA*

Received 4 December 1997; accepted 9 December 1997

**Abstract**

The effects of chronic treatment with naltrexone, an opioid receptor antagonist, on $\delta_1$- and $\delta_2$-opioid receptor agonist-induced antinociception and ligand binding were investigated in mice. Antinociception by intracerebroventricular (i.c.v.) [D-Pen$^{2,5}$]enkephalin (DPDPE) and [D-Ala$^2$]deltorphin II, agonists selective for $\delta_1$- and $\delta_2$-opioid receptors, respectively, was blocked following subcutaneous (s.c.) implantation of a naltrexone pellet (7.5 mg) for 7 days. Removal of the naltrexone pellet was followed 24 h later by a decrease of 7.5-fold in the $ED_{50}$ value of [D-Ala$^2$]deltorphin II, but not that of DPDPE. In a whole brain homogenate the binding of [$^3$H][D-Ala$^2$]deltorphin II was increased twice as much as that of [$^3$H]DPDPE. Chronic naltrexone treatment also produced an 8.6-fold decrease in the $ED_{50}$ value of i.c.v. administered morphine. The increase in morphine potency was reversed to a control (placebo-treated mice) value by the selective $\delta_2$-opioid receptor antagonist, naltriben (25 pmol, i.c.v.). Thus, chronic naltrexone selectively increases $\delta_2$-opioid receptor-mediated antinociception, supporting the existence of $\delta$ opioid receptor subtypes with distinct adaptive characteristics. The data also indicate that $\delta_2$-opioid receptors are critically involved in the expression of morphine supersensitivity. © 1998 Elsevier Science B.V.

*Keywords:* δ-Opioid receptor; Morphine; Naltrexone; Naltriben; Supersensitivity; Up-regulation

## 1. Introduction

The ability of the δ-opioid receptor to mediate supraspinal antinociceptive processes in mice is now well established. This effort has been facilitated by the intracerebroventricular (i.c.v.) administration of opioid ligands with high affinity and great selectivity for the δ-receptor including deltorphin peptides and the conformationally restricted cyclic peptide [D-Pen$^{2,5}$]enkephalin (DPDPE) (Mosberg et al., 1983; Erspamer et al., 1989; Suh and Tseng, 1990; Mattia et al., 1991; Sanchez-Blazquez and Garzon, 1993). In support of the specificity of these opioids for the δ-receptor, their antinociceptive effects are antagonized by the δ-opioid receptor-selective ligand *N,N*-diallyl,Tyr-($Aib_2$)-Phe-Leu-OH (ICI174,864), but not by the μ-opioid receptor-selective antagonist β-funaltrexamine (Jiang et al., 1990; Suh and Tseng, 1990). Although both ligands act through the same transducer protein and produce identical cellular effects (Sanchez-Blazquez and Garzon, 1993; Buzas et al., 1994), the ability of several different δ-opioid receptor-selective antagonists to block the antinociceptive effects of the deltorphins and DPDPE is ligand-specific, suggesting that δ-opioid receptors can be dissociated into $\delta_1$-(DPDPE-sensitive) and $\delta_2$-(deltorphin-sensitive) opioid receptor subtypes (Jiang et al., 1991; Sofuoglu et al., 1991). Further support for different sites of action following DPDPE and [D-Ala$^2$]deltorphin II administration comes from the report that, whereas both agonists produce antinociception in CD-1 mice, only DPDPE produces analgesia in the CXBK mouse (Raffa et al., 1992). $\delta_1$- and $\delta_2$-opioid receptor subtypes may also undergo adaptive alterations independently of each other. For example, no cross-tolerance between the deltorphins and DPDPE is observed when either compound is tested following the chronic i.c.v. administration of the other (Mattia et al., 1991). Mice rendered diabetic following chronic streptozotocin treatment display a selective enhancement of DPDPE ($\delta_1$) but not [D-Ala$^2$]deltorphin II ($\delta_2$), antinociception (Kamei et al., 1994).

Chronic treatment of mice and rats with naltrexone, an opioid receptor antagonist, produces functional supersensitivity (i.e. increased antinociceptive potency) of δ-opioid receptor agonists (Zukin et al., 1982; Tempel et al., 1985;

* Corresponding author. Tel.: +1-212-7466235; fax: +1-212-7468835; e-mail: ceintur@mcd.cornell.edu

Morris et al., 1988; Yoburn et al., 1988, 1990). These changes are accompanied by increased δ-opioid receptor binding (i.e. receptor up-regulation). In these studies however, δ-opioid receptor-mediated antinociception and ligand binding were assessed using [D-Ala$^2$, D-Leu$^5$]enkephalin, which does not discriminate well between δ- and μ-opioid receptors or between δ-opioid receptor subtypes (Corbett et al., 1984). Duttaroy et al. (1992) have reported increased whole brain [$^3$H]DPDPE binding in naltrexone treated mice. However, the effects of chronic naltrexone on supraspinal DPDPE antinociception, or on supraspinal $\delta_2$-opioid receptor antinociception and binding are unknown. Furthermore, chronic administration of 7-benzylidine-7-dehydronaltrexone, a selective $\delta_1$-opioid receptor antagonist enhances the antinociceptive potency of DPDPE but not that of [D-Ala$^2$]deltorphin II, while chronic administration of naltriben, a selective $\delta_2$-opioid receptor antagonist enhances the antinociceptive potency of [D-Ala$^2$]deltorphin II but not that of DPDPE (Bhargava et al., 1996). Thus, since δ-opioid receptor subtypes can display different pharmacological and functional profiles and can undergo functional changes independently, they may also display differential responses to chronic naltrexone treatment. Therefore, the present study examined the effects of 7 days of naltrexone treatment on the antinociceptive potency of DPDPE and [D-Ala$^2$]deltorphin II on day 8 in mice. Additionally, to assess δ-opioid receptor up-regulation following chronic naltrexone treatment, [$^3$H]DPDPE and [$^3$H][D-Ala$^2$]deltorphin II binding was measured in whole brain homogenate. Finally, the $\delta_2$-opioid receptor has been demonstrated to modulate morphine analgesia (Porreca et al., 1992) and to contribute to the adaptive changes that produce morphine tolerance and dependence following chronic administration (Abdelhamid et al., 1991; Kest et al., 1996). Therefore, the role of $\delta_2$-opioid receptors in the adaptive changes contributing to the expression of morphine supersensitivity following chronic naltrexone treatment was also investigated using the selective $\delta_2$-opioid receptor antagonist naltriben (Sofuoglu et al., 1991).

## 2. Materials and methods

### 2.1. Subjects

Male adult CD-1 mice (25–35 g) were used throughout. Animals were housed five to a cage and maintained in a temperature controlled environment provided with unrestricted food and water. $ED_{50}$ values for each drug condition were determined on separate groups of 7–10 mice each and were replicated once.

### 2.2. Tail-flick assay

A standardized tail-flick apparatus (EMDIE, Richmond, VA) with a radiant heat source connected to an automatic timer was used to assess nociceptive thresholds. Withdrawal latency was measured from the onset of the heat stimulus applied to the distal 2 cm of the tail and was terminated upon the flick of the tail. Baseline latencies were comprised of the mean of two such determinations. The intensity was adjusted to yield baseline latencies of between 2.5–3.5 s. A maximum latency of 10 s was employed to prevent tissue damage.

### 2.3. Drug administration

Standard naltrexone (30 mg of naltrexone, 105 mg of cholesterol and 15 mg of tristearin) and placebo pellets (135 mg of cholesterol and 15 mg of tristearin) were obtained from the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse (Rockville, MD). A pellet was cut into 4 pieces, each containing approximately 7.5 mg of naltrexone. Each piece was wrapped in nylon mesh and implanted subcutaneously (s.c.) under halothane anesthesia at the nape of the neck for 7 days and then removed. DPDPE, obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD), [D-Ala$^2$]deltorphin II (Peninsula Labs, Belmont, CA), naltriben (Research Biochemicals, Natick, MA), and morphine (Mallinckrodt, St. Louis, MO) were dissolved in physiological saline and injected into the lateral cerebral ventricle (i.c.v.) according to the method of Haley and McCormick (1957). Under halothane anesthesia, an incision was made in the scalp and bregma located. Drug or vehicle were then injected directly through the skull at a point 2 mm caudal and 2 mm lateral to bregma at a depth of 3 mm using a Hamilton (Hamilton Co., Reno, NV) microliter syringe with a 27-gauge needle. All i.c.v. injections were made with a volume of 5 μl. Following each injection, the incision was closed with a stainless steel wound clip.

### 2.4. Dose–response studies

Changes in the relative potency of [D-Ala$^2$]deltorphin II, DPDPE, and morphine are expressed as changes in their respective $ED_{50}$ values, determined by constructing cumulative dose–response curves derived by administering increasing log doses (approximately 0.25 increments) of drug until each animal became a responder. A responder was operationally defined as an animal whose tail-flick latency on two consecutive determinations was equal to or greater than double that animal's mean baseline score assessed just prior to the testing session. [D-Ala$^2$]deltorphin II and DPDPE $ED_{50}$ values were assessed on day 7 in the presence of naltrexone and 24 h after pellet removal (day 8), when naltrexone has been eliminated and naltrexone-induced supersensitivity to morphine is maximal (Yoburn and Inturrisi, 1988). Assessment of morphine $ED_{50}$ values began 10 min after saline or naltriben (25 pmol) administration. Naltriben is a selective $\delta_2$-receptor antagonist and,

Table 1
The intracerebroventricular (i.c.v.) antinociceptive potency of DPDPE and [D-Ala$^2$]deltorphin II during and after chronic naltrexone treatment. A naltrexone (7.5 mg) or a placebo pellet was implanted subcutaneously for 7 days. DPDPE and [D-Ala$^2$]deltorphin II were administered i.c.v. on day 7 and 24 h after pellet removal (day 8) and $ED_{50}$ values were determined using a cumulative dose–response method

| δ Agonist | Treatment | Day | $ED_{50}$ ( μg/mouse) | 95% CI | Relative potency |
|---|---|---|---|---|---|
| DPDPE | naltrexone | 7 | > 100.0 | ND | ND |
| | placebo | 8 | 29.2 | (23.0–35.7) | 1.0 |
| | naltrexone | 8 | 27.5 | (21.5–33.5) | 1.1 |
| [D-Ala$^2$]deltorphin II | naltrexone | 7 | > 100.0 | ND | ND |
| | placebo | 8 | 10.8 | (8.5–13.5) | 1.0 |
| | naltrexone | 8 | 1.4[a] | (1.2–1.7) | 7.7 |

CI = confidence intervals.
[a] Significantly different ( $p < 0.05$) from the corresponding day 8 placebo value.
ND: could not be determined (see text).

at the dose presently employed, does not alter tail-flick latencies or morphine analgesia (Takemori et al., 1992). All agonists were tested 10 min following each dose of drug, respectively, corresponding to their peak antinociceptive effect as previously reported (Jiang et al., 1990; Mattia et al., 1991).

### 2.5. [$^3$H]DPDPE and [$^3$H][D-Ala$^2$]deltorphin II binding assay

Brains (minus cerebellum) were removed on day 8 and prepared as previously described (Cheng et al., 1992). Briefly, the tissue was rapidly homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in ice-cold buffer (50 mM Tris, 1 mM EGTA, 0.1 M NaCl, pH 7.7) containing 1 μM PMSF to inhibit protease activity. The homogenate was then incubated at 25°C for 15 min and centrifuged at 4°C for 30 min. Pellets were resuspended in buffer (50 mM $K_2PO_4$, 5 mM $MgSO_4$, pH 7.2) to a final tissue concentration of 1 mg protein/ml as determined by the Lowry assay (Lowry et al., 1951). [$^3$H]DPDPE and [$^3$H][D-Ala$^2$]deltorphin II (New England Nuclear, Boston, MA) binding assays were performed in 1 ml aliquots of mouse brain homogenate. Assays were filtered through Whatman B glass fiber filters (Biomedical Research and Development Laboratories, Gaithersburg, MD) and washed twice with 4 ml of ice-cold buffer (5mM Tris, pH 7.4) on a cell harvester (Brandel, Gaithersburg, MD). Nonspecific binding was determined with 1 μM levallorphan (Research Biochemicals International, Natick, MA). All measurements were performed in triplicate and each assay was replicated 3–5 times.

### 2.6. Data analysis

Baseline tail-flick latencies for all groups were subject to analysis of variance. The quantal dose–response data was analyzed using the BLISS-21 computer program. This program maximized the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose–response data and provides $ED_{50}$ values, 95% confidence intervals (CI) and estimates of relative potency (Umans and Inturrisi, 1981). The mean and mean difference of the counts/minute (c.p.m.) for each radiolabeled ligand in placebo and naltrexone treated brain homogenates were compared using the Student's *t*-test.

## 3. Results

The solubility of DPDPE and [D-Ala$^2$]deltorphin II limited the dosage that could be administered i.c.v. There-

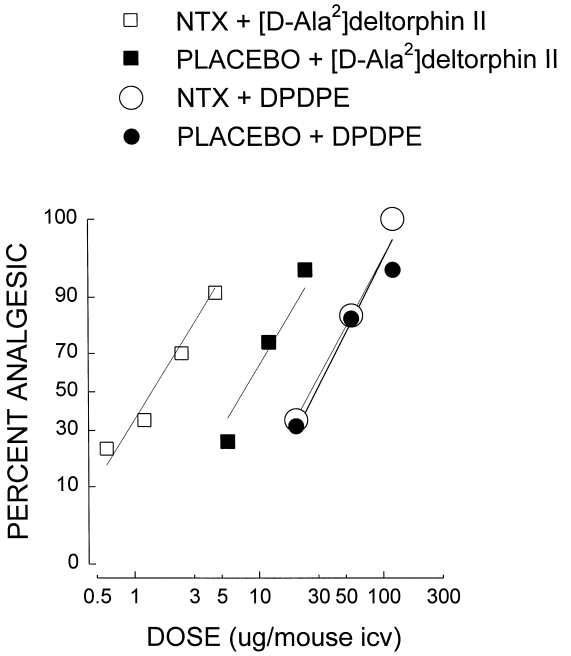


Fig. 1. Naltrexone (NTX) treatment produces supersensitivity to the antinociceptive effects of [D-Ala$^2$]deltorphin II (DELT II) but not to [D-Pen$^{2,5}$]enkephalin (DPDPE). Supersensitivity was assessed by a leftward shift in the cumulative dose–response curve in mice following the subcutaneous implantation of a 7.5 mg NTX pellet or a placebo pellet for 7 days. The testing was conducted 24 h after removal of the pellet. The ordinate indicates the percentage of animals that achieved an analgesic (antinociceptive) response using the tail-flick test. The estimated $ED_{50}$ values are given in Table 1.

fore, the $ED_{50}$ values for DPDPE and [D-Ala$^2$]deltorphin II in the presence of naltrexone on day 7 can only be estimated. However, the blockade by naltrexone appeared complete (Table 1). In contrast, the $ED_{50}$ values assessed on day 8, 24 h after the removal of naltrexone pellets, indicate that the potency of i.c.v. DPDPE was not significantly altered in naltrexone treated mice relative to placebo treated controls (Table 1, Fig. 1). The $ED_{50}$ value for i.c.v. [D-Ala$^2$]deltorphin II, however was significantly decreased from 10.7 to 1.4 $\mu$g/mouse, indicating a 7.5-fold increase in potency resulting from naltrexone treatment (Table 1). Fig. 1 illustrates this leftward shift in the [D-Ala$^2$]deltorphin II dose–response curve. Both [$^3$H]DPDPE and [$^3$H][D-Ala$^2$]deltorphin II binding were significantly increased in the whole brain homogenate from naltrexone treated mice relative to placebo treated controls (Fig. 2). However, the increase in [$^3$H][D-Ala$^2$]deltorphin II binding was twice that observed for [$^3$H]DPDPE ($P < 0.05$). An increase in the potency of morphine, indicated by an 8.6-fold decrease in the morphine $ED_{50}$ value (Table 2), was also observed in naltrexone treated mice relative to placebo treated controls. However, no increase in the morphine $ED_{50}$ was observed in naltrexone pelleted mice who received naltriben before the morphine challenge dose. Naltriben alone did not affect baseline tail-flick latencies (data not shown) or morphine potency in placebo treated mice.

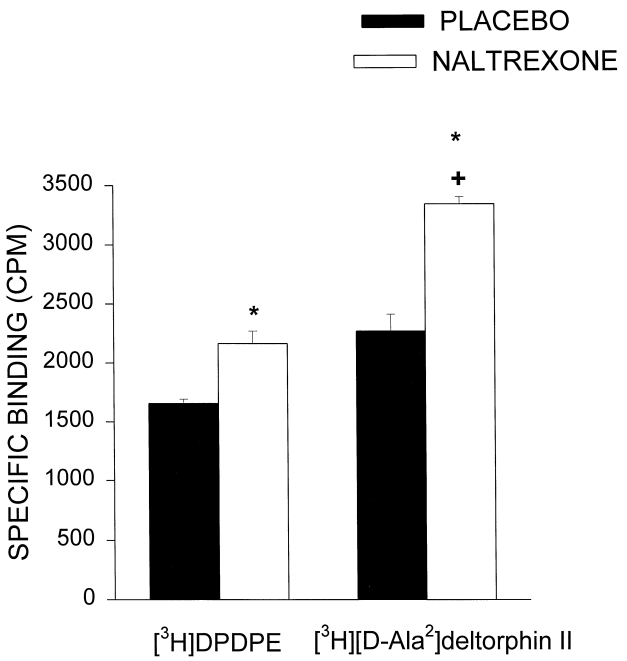


Fig. 2. Ligand binding after chronic in vivo treatment with naltrexone or a placebo pellet. Aliquots of mouse whole brain (minus cerebellum) homogenate were incubated with [$^3$H]DPDPE or [$^3$H][D-Ala$^2$]deltorphin II. Results are specific binding and are the means + SEM of three to five determinations. The mice were implanted subcutaneously for 7 days with a 7.5 mg naltrexone or a placebo pellet. The brain homogenates were prepared 24 h after removal of the pellet. * Significantly different ($P < 0.05$) from the corresponding placebo treated group. + A significantly different ($P < 0.05$) increase compared to [$^3$H]DPDPE binding in the naltexone treated group.

Table 2
The effect of naltriben on the intracerebroventricular (i.c.v.) antinociceptive potency of morphine in placebo and chronic naltrexone treated mice. A naltrexone (7.5 mg) or a placebo pellet was implanted subcutaneously for 7 days and removed. On day 8, mice received a single i.c.v. injection of naltriben (25 pmol). 10 min later, i.c.v. morphine $ED_{50}$ values were determined using a cumulative dose–response method

| Treatment | $ED_{50}$ ($\mu$g/mouse) | 95% CI | Relative potency |
|---|---|---|---|
| Placebo-pelleted | | | |
| Saline + morphine | 2.13 | (1.28–3.06) | 1.00 |
| Naltriben + morphine | 2.07 | (1.10–3.34) | 1.02 |
| Naltrexone-pelleted | | | |
| Saline + morphine | 0.18[a] | (0.09–0.30) | 11.80 |
| Naltriben + morphine | 1.80 | (1.12–2.71) | 1.18 |

CI = confidence intervals.
[a] Significantly different ($p < 0.05$) from placebo-pelleted/saline + morphine value.

## 4. Discussion

In the present experiments, chronic naltrexone treatment for 7 days produced functional supersensitivity to [D-Ala$^2$]deltorphin II induced antinociception on day 8 (Table 1). Although morphine analgesia was also up-regulated in the following experiment, the antinociceptive effect of [D-Ala$^2$]deltorphin II is not antagonized by the $\mu$-opioid receptor-selective antagonist $\beta$-funaltrexamine (Jiang et al., 1990) nor cross-tolerant with the selective $\mu$-opioid receptor agonist [D-Ala$^2$,NMPhe$^4$, Gly-ol]enkephalin (Mattia et al., 1991), suggesting that $\mu$-opioid receptors do not mediate the presently observed [D-Ala$^2$]deltorphin II supersensitivity. In contrast, no change in the antinociceptive potency of DPDPE was observed despite evidence that the naltrexone treatment produced antagonism at $\delta$-opioid receptors and that the antinociceptive potency of DPDPE can be increased following the selective antagonism of $\delta_1$-opioid receptors by 7-benzylidene-7-dehydronaltrexone (Bhargava et al., 1996). The present results also demonstrate that $\delta$-opioid receptor subtypes are regulated by mechanisms which differentially adapt to chronic naltrexone treatment. This, however, is not the first instance where $\delta$-opioid receptor subtypes display different adaptive responses in response to chronic treatment. In mice rendered diabetic following chronic streptozotocin administration, there is a selective enhancement of DPDPE ($\delta_1$), but not [D-Ala$^2$]deltorphin II ($\delta_2$), antinociception (Kamei et al., 1994). Thus, a change in the activity mediated at $\delta$-opioid receptor subtypes is not limited to either subtype and may occur independently of each other.

The absence of increased supraspinal DPDPE antinociceptive potency in the present study contrasts with the

report of Yoburn et al. (1989) who found DPDPE supersensitivity following intrathecal administration in naltrexone treated mice. We believe that the absence of increased DPDPE antinociceptive potency reported here may be due to the different route of administration used. Whereas an intrathecal route of administration was used in that study, DPDPE in the present study was injected i.c.v. Thus, the differential effects of DPDPE in the two studies may reflect the circumscribed delivery of DPDPE to, and the consequent assessment of, spinal and supraspinal $\delta_1$-opioid receptor populations, respectively. The site of opioid drug administration in naltrexone treated mice is particularly relevant since up-regulation of opioid receptors may not occur uniformly across brain regions. Indeed, all three opioid receptor subtypes have been observed to undergo region-specific up-regulation in response to chronic naltrexone treatment (Morris et al., 1988; Belcheva et al., 1994). This differential up-regulation between spinal and supraspinal $\delta_1$-opioid receptor populations would be expected to produce uneven changes in spinal and supraspinal DPDPE antinociceptive potency following chronic naltrexone. Interestingly, it has been reported that spinal DPDPE antinociception is antagonized by naltrindole-5′-isothiocyanate (Mattia et al., 1992) and an antisense oligodeoxynucleotide to the cloned δ-opioid receptor DOR-1 (Standifer et al., 1994), both characterized as selectively antagonizing the $\delta_2$-opioid receptor subtype (Jiang et al., 1991; Lai et al., 1994). On the basis of these findings, it has been suggested that DPDPE does not discriminate very well between δ-opioid receptor subtypes in the spinal cord. It is therefore possible that the increased antinociceptive potency of DPDPE in the spinal cord following chronic naltrexone treatment as reported by Yoburn et al. (1989) was actually mediated by the $\delta_2$-opioid receptor subtype.

The present data are the first to report that the level of $\delta_2$-opioid receptor subtype binding, as assayed by [$^3$H]-[D-Ala$^2$]deltorphin II, is increased on day 8 (Fig. 2). Previous work in this laboratory has shown, however, that levels of the DOR-1 mRNA, a putative $\delta_2$-opioid receptor subtype transcript, is not altered in mice displaying [D-Ala$^2$]deltorphin II supersensitivity following the identical naltrexone treatment paradigm used here (Jenab et al., 1995). Thus, it appears that up-regulation of the $\delta_2$-opioid receptor in naltrexone treated mice occur via mechanisms other than alterations in transcript abundance. The functional supersensitivity demonstrated to [D-Ala$^2$]deltorphin II was associated with a proportionally greater increase in [$^3$H][D-Ala$^2$]deltorphin II binding, compared to [$^3$H]DPDPE binding (Fig. 2). It may be that the increase in DPDPE binding was not sufficient to produce a functional change as assessed by an increase in DPDPE potency (Fig. 2). However, Duttaroy et al. (1992) observed a dissociation between receptor up-regulation and functional supersensitivity in that increased $\mu$-opioid receptor analgesia does not accompany increased $\mu$-receptor binding in mice receiving D-amphetamine during chronic naltrexone treatment. Additionally, Shah et al. (1994) have demonstrated that while intrathecal naltrindole administration in mice selectively affects $\delta_2$-opioid receptor selective [D-Ser$^2$, Leu$^5$, Thr$^6$]enkephalin (DSLET) potency, the specific binding of both [$^3$H]DPDPE and [$^3$H]DSLET are both reduced. Furthermore, in NG108-15 cells, a mouse neuroblastoma–rat glioma hybrid cell line expressing only the $\delta_2$-opioid receptor subtype, DPDPE binding was reduced by 40% following treatment with an antisense oligodeoxynucleotide targeting DOR-1 mRNA, demonstrating its lack of absolute specificity for $\delta_1$-opioid receptors (Standifer et al., 1994). Therefore, although deltorphin II and DPDPE have distinct selectivity profiles for the δ-opioid receptor subtypes, it is important to remember that these ligands do not exhibit absolute specificity for these subtypes. Due to this degree of DPDPE nonspecificity, it is possible that the increase in both [$^3$H][D-Ala$^2$]deltorphin II and [$^3$H]DPDPE binding measure the same up-regulated $\delta_2$-opioid receptor population.

Our data support the finding that neither baseline tail-flick latencies nor morphine analgesia in placebo treated mice are affected by the $\delta_2$-opioid receptor-selective antagonist naltriben at the dose used in the present study (Takemori et al., 1992). This is consistent with a predominantly $\mu$-opioid receptor site of action in morphine analgesia. What needs to be understood is why naltriben was effective in blocking morphine analgesia in naltrexone treated mice. It must be noted that morphine is not selective for the $\mu$-opioid receptor and can produce analgesia at δ-opioid binding sites as well (Takemori and Portoghese, 1987). Given the up-regulation of $\delta_2$-opioid receptors in the present study, it is thus possible that morphine interacted with this population of receptors. Therefore, morphine analgesia would be blocked by naltriben in naltrexone, but not placebo, treated mice as presently reported. However, since $\mu$-opioid receptors are also up-regulated following chronic naltrexone (Duttaroy et al., 1992), the fact that naltriben completely reversed morphine supersensitivity but did not block morphine analgesia, implies that $\delta_2$-opioid receptors are either increased to a greater extent or have a more critical role in morphine supersensitivity relative to $\mu$-receptors following chronic naltrexone. Alternatively, it is possible that morphine acts at up-regulated $\mu$-opioid receptors, resulting in morphine supersensitivity. In this case, the reversal of morphine supersensitivity by naltriben may reflect the ability of the $\delta_2$-opioid receptor subtype to modulate morphine analgesia (Porreca et al., 1992) as well as the adaptive responses following chronic drug treatment that contribute to morphine tolerance and dependence (Abdelhamid et al., 1991; Miyamoto et al., 1993; Kest et al., 1996). Regardless of the mechanism of action, the reversal of morphine supersensitivity, but not morphine analgesia, by naltriben indicates a role for the $\delta_2$-opioid receptor in the expression of morphine supersensitivity following chronic naltrexone and is consistent with

our recent observation that an antisense oligodeoxynucleotide targeting the coding sequence of the cloned δ-opioid receptor (DOR-1) blocks the development of morphine supersensitivity (Kest et al., 1997).

In summary, the following experiments demonstrate that, although both δ-opioid receptor subtypes are up-regulated, functional supersensitivity of only the $\delta_2$-opioid receptor subtype is observed following chronic naltrexone treatment. Thus, the data support distinct pharmacological profiles and adaptive responses for δ-opioid receptor subtypes. Furthermore, the ability of the $\delta_2$-opioid receptor-selective antagonist naltriben to block morphine supersensitivity in naltrexone treated mice suggests a modulatory role for the $\delta_2$-opioid receptor in morphine supersensitivity. Further work is needed however to clarify both the mechanism and the significance of receptor up-regulation, as well as the processess contributing to opioid functional supersensitivity, following chronic naltrexone treatment.

## Acknowledgements

Supported in part by NIDA Grant DA01457 (C.E.I.) and NIDA Training Grant DA07274 (SJ,CEL). C.E.I. is a recipient of a Research Scientist Award from NIDA (DA00198). B.K. is an Aaron Diamond Foundation Fellow and this work was supported in part by a grant from The Aaron Diamond Foundation.

## References

Abdelhamid, E.E., Sultana, M., Portoghese, P.S., Takemori, A.E., 1991. Selective blockage of δ opioid receptors prevents the development of morphine tolerance and dependence in mice. J. Pharmacol. Exp. Ther. 258, 299–303.

Bhargava, H.N., Zhao, G.-M., House, R.V., Thomas, P.T., 1996. Effects of chronic administration of 7-benzylidene-7-dehydronaltrexone and naltriben on the antinociceptive actions of $\delta_1$ and $\delta_2$-opioid receptor agonists. Eur. J. Pharmacol. 311, 127–132.

Belcheva, M.M., Barg, J., Mchale, R., Coscia, C.J., 1994. Naltrexone induces down- and up-regulation of δ-opioid receptors in rat brain regions. Brain Res. Bull. 35, 69–72.

Buzas, B., Izenwasser, S., Portoghese, P.S., Cox, B.M., 1994. Evidence for δ opioid receptor subtypes regulating adenylyl cyclase activity in the rat brain. Life Sci. 54, 101–106.

Cheng, J., Roques, B.P., Gacel, G.A., Huang, E., Pasternak, G.W., 1992. $K_3$ opiate receptor binding in the mouse and rat. Eur. J. Pharmacol. 226, 15–20.

Corbett, A.D., Gillan, M.G.C., Kosterlitz, H.W., McKnight, A.T., Paterson, S.J., Robson, L.E., 1984. Selectivities of opioid peptide analogues as agonists and antagonists at the δ-receptor. Br. J. Pharmacol. 83, 271–279.

Duttaroy, A., Billings, B., Candido, J., Yoburn, B.C., 1992. Chronic D-amphetamine inhibits opioid receptor antagonist-induced supersensitivity. Eur. J. Pharmacol. 221, 211–215.

Erspamer, V., Melchiorri, P., Falconieri-Erspamer, G., Negri, L., Corsi, R., Severini, C., Barra, D., Simmaco, M., Kreil, G., 1989. Deltorphins: A family of naturally occurring peptides with high affinity and selectivity for δ-opioid binding sites. Proc. Natl. Acad. Sci. USA 86, 5188–5192.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12–15.

Jenab, S., Kest, B., Inturrisi, C.E., 1995. Assessment of δ opioid antinociception and receptor mRNA levels in mouse after chronic naltrexone treatment. Br. Res. 691, 69–75.

Jiang, Q., Mosberg, H.I., Porreca, F., 1990. Antinociceptive effects of [D-Ala$^2$]Deltorphin II, a highly selective δ-agonist in vivo. Life Sci. 47, PL43–PL47.

Jiang, Q., Takemori, A.E., Sultana, M., Portoghese, P.S., Bowen, W.D., Mosberg, H.I., Porreca, F., 1991. Differential antagonism of opioid δ antinociception by [D-Ala$^2$,Leu$^5$,Cys$^6$]Enkephalin and naltrindole 5′-isothiocyanate: Evidence for δ receptor subtypes. J. Pharmacol. Exp. Ther. 257, 1069–1075.

Kamei, J., Iwamoto, Y., Misawa, M., Nagase, H., Kasuya, Y., 1994. Streptozotocin-induced diabetes selectively enhances antinociception mediated by $\delta_1$- but not $\delta_2$-opioid receptors. Life Sci. 55, 121–126.

Kest, B., Lee, C.E., McLemore, G.M., Inturrisi, C.E., 1996. An antisense oligodeoxynucleotide for the δ opioid receptor (DOR-1) inhibits morphine tolerance and acute dependence. Brain Res. Bull. 39, 185–188.

Kest, B., Lee, C.E., Mogil, J.S., Inturrisi, C.E., 1997. Blockade of morphine supersensitivity by an antisense oligodeoxynucleotide targeting a δ opioid receptor (DOR-1). Life Sci. 60, PL155–PL159.

Lai, J., Bilsky, E.J., Rothman, R.B., Porreca, F., 1994. Treatment with antisense oligodeoxynucleotide to the opioid δ receptor selectively inhibits $\delta_2$-agonist antinociception. Neuroreport 5, 1049–1052.

Lowry, O.H., Rosebrough, A.L., Farr, J., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mattia, A., Vanderah, T., Mosberg, H.I., Porreca, F., 1991. Lack of antinociceptive cross-tolerance between [D-Pen$^2$, D-Pen$^5$]enkephalin and [D-Ala$^2$]deltorphin II in mice: Evidence for δ receptor subtypes. J. Pharmacol. Exp. Ther. 258, 583–587.

Mattia, A., Farmer, S.C., Takemori, A.E., Sultana, M., Portoghese, P.S., Mosberg, H.I., Bowen, W.D., Porreca, F., 1992. Spinal opioid δ antinociception in the mouse: Mediation by a 5′-NTII-sensitive δ receptor subtype. J. Pharmacol. Exp. Ther. 260, 518–525.

Miyamoto, Y., Portoghese, P.S., Takemori, A.E., 1993. Involvement of $\delta_2$ opioid receptors in the development of morphine dependence in mice. J. Pharmacol. Exp. Ther. 264, 1141–1145.

Morris, B.J., Millan, M.J., Herz, A., 1988. Antagonist-induced opioid receptor up-regulation. II. Regionally specific modulation of μ, δ, and κ binding sites in rat brain revealed by quantitative autoradiography. J. Pharmacol. Exp. Ther. 247, 729–736.

Mosberg, H.I., Hurst, R., Hruby, V.J., Gee, K., Yamamura, H.I., Galligan, J.J., Burks, T.F., 1983. Bis-penicillamine enkephalins possess highly improved specificity toward δ-opioid receptors. Proc. Natl. Acad. Sci. USA 80, 5871–5874.

Porreca, F., Takemori, A.E., Sultana, M., Portoghese, P.S., Bowen, W.D., Mosberg, H.I., 1992. Modulation of μ-mediated antinociception in the mouse involves opioid $\delta_2$-receptors. J. Pharmacol. Exp. Ther. 263, 147–152.

Raffa, R.B., Martinez, R.P., Porreca, F., 1992. Lack of antinociceptive efficacy of intracerebroventricular [D-Ala$^2$,Glu$^4$]deltorphin, but not [D-Pen$^2$,D-Pen$^5$]enkephalin, in the μ-opioid receptor deficient CXBK mouse. Eur. J. Pharmacol. 216, 453–456.

Sanchez-Blazquez, P., Garzon, J., 1993. δ-opioid supraspinal antinociception in mice is mediated by $G_{i3}$ transducer proteins. Life Sci. 53, PL129–PL134.

Shah, S., Davis, T., Yoburn, B.C., 1994. The effect of naltrindole on spinal and supraspinal δ opioid receptors and analgesia. Life Sci. 55, 1451–1458.

Sofuoglu, M., Portoghese, P.S., Takemori, A.E., 1991. Differential antagonism of δ opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: Evidence for δ opioid receptor subtypes. J. Pharmacol. Exp. Ther. 257, 676–680.

Standifer, K.M., Chien, C.-C., Wahlestedt, C., Brown, G.P., Pasternak, G.W., 1994. Selective loss of δ opioid analgesia and binding by antisense oligodeoxynucleotides to a δ-opioid receptor. Neuron 12, 805–810.

Suh, H.H., Tseng, L.F., 1990. Different types of opiate receptors mediating analgesia induced by morphine, DAMGO, DPDPE, DADLE, and β-endorphin in mice. Naunyn-Schmiedebergs Arch. Pharmacol. 342, 67–71.

Takemori, A.E., Portoghese, P.S., 1987. Evidence for the interaction of morphine with κ and δ opioid receptors to induce analgesia in β-funaltrexamine-treated mice. J. Pharmacol. Exp. Ther. 243, 91–104.

Takemori, A.E., Sultana, M., Nagase, H., Portoghese, P.S., 1992. Agonist and antagonist activities of ligands derived from naltrexone and oxymorphone. Life Sci. 50, 1491–1495.

Tempel, A., Gardner, E.L., Zukin, R.S., 1985. Neurochemical and functional correlates of naltrexone-induced opiate receptor up-regulation. J. Pharmacol. Exp. Ther. 232, 439–444.

Umans, J.G., Inturrisi, C.E., 1981. Pharmacodynamics of subcutaneously administered diacetylmorphine, 6-acetylmorphine, and morphine in mice. J. Pharmacol. Exp. Ther. 218, 409–415.

Yoburn, B.C., Inturrisi, C.E., 1988. Modification of the response to opioid and nonopioid drugs by chronic opioid antagonist treatment. Life Sci. 42, 1689–1696.

Yoburn, B.C., Luke, M.C., Pasternak, G.W., Inturrisi, C.E., 1988. Upregulation of opioid receptor subtypes correlates with potency changes of morphine and DADLE. Life Sci. 43, 1319–1324.

Yoburn, B.C., Paul, D., Azimuddin, S., Lutfy, K., Sierra, V., 1989. Chronic opioid antagonist treatment increases μ and δ receptor mediated spinal opioid analgesia. Brain Res. 485, 176–178.

Yoburn, B.C., Sierra, V., Lutfy, K., 1990. Simultaneous development of opioid tolerance and opioid antagonist-induced receptor upregulation. Brain Res. 529, 143–148.

Zukin, R.S., Sugarman, J.R., Fitz-Syage, M.L., Gardner, E.L., Zukin, S.R., Gintzler, A.R., 1982. Naltrexone-induced opiate receptor supersensitivity. Brain Res. 245, 285–292.