Title Page

Selectivity of Delta and Kappa Opioid Ligands Depends on the Route of Central Administration in Mice

Mary M. Lunzer and Philip S. Portoghese

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455 (MML and PSP)
Running Title Page

a) Injection route determines opioid antagonist selectivity

b) Corresponding Author:
Philip S. Portoghese
U of MN - Medicinal Chemistry
308 Harvard Street S.E.
Minneapolis, MN  55455
Phone: 612-624-9174
FAX: 612-626-6891
Porto001@umn.edu

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d) List of nonstandard abbreviations: intrathecal (i.t.); intracerebroventricular (i.c.v.);
benzylidenenaltrexone, (BNTX); naltrindole, (NTI); naltriben, (NTB);
norbinaltorphimine, (norBNI); H-Tyr-D-Pen-D-Phe-D-Pen-OH (cyclic), (DPDPE); H-
Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH, (deltorphin II); 6’Guanidinonaltrindole, (6’GNTI);
3,4-dichloro-N-methyl-N-[(1R,2R)-2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide,
(U50488) and G protein-coupled receptors (GPCRs)
e) Recommended section: Neuropharmacology
Abstract

The existence of heterodimeric opioid receptors has introduced greater complexity to the \textit{in vivo} characterization of pharmacological selectivity of agonists by antagonists. Due to the possibility of cooperativity between receptors organized as heterodimers, it is conceivable that selective antagonists may antagonize an agonist bound to a neighboring, allosterically coupled receptor. Consequently, the \textit{in vivo} selectivity of an opioid antagonist may depend on the organizational state of receptors that mediate analgesia. In this regard, phenotypic delta and kappa opioid receptors have been proposed to arise from different organizational states that include oligomeric delta-kappa heterodimers and homomeric delta and kappa receptors. In view of the evidence for analgesia mediated by delta-kappa heterodimers in the spinal cord, but not the brain, we have investigated the selectivity of pharmacologically selective delta and kappa antagonists in mice by both intrathecal (i.t.) and intracerebroventricular (i.c.v.) routes of administration, in order to evaluate changes in selectivity. Using pharmacologically selective delta [benzylidennaltrexone, (BNTX), naltrindole, (NTI) and naltriben, (NTB)] and kappa [(norbinaltorphimine, (norBNI)] antagonists versus delta (DPDPE, deltorphin II) and kappa (U50488, bremazocine) agonists, the delta-1/delta-2 selectivity ratios were found to be dependent on the route of administration (i.t. vs. i.c.v.). The data from different routes of administration suggest that differences in molecular recognition between spinal delta-kappa heterodimers and supraspinal homomeric delta and kappa receptors may contribute to the divergent selectivity ratios of selective antagonists. In view of the
observed tissue-dependent selectivity, we suggest that multiple opioid antagonists be employed routinely in establishing agonist selectivity *in vivo*.
Introduction

A variety of G protein-coupled receptors (GPCRs) are known to associate as heterodimers in cultured cells (Prinster et al., 2005; Bulenger et al., 2005; Milligan, 2004; Terrilon and Bouvier, 2004). The effect of heterodimerization may be manifested in a number of ways, including changes in trafficking, function, binding, and pharmacological selectivity. Opioid receptors are among those receptors in the rhodopsin family of GPCRs that have been reported to undergo heterodimerization (Prinster et al., 2005). Studies with co-expressed delta and kappa opioid receptors in cultured cells have established they are organized as heterodimers and have led to the suggestion that the putative kappa-2 subtype may be a heterodimerized kappa receptor (Jordan and Devi, 1999). The putative subtypes of delta and kappa opioid receptors were originally proposed based on differential pharmacologic selectivity in vivo (Sofuoglu et al., 1991; Jiang et al., 1991; Horan et al., 1991).

More recently, studies using selective opioid ligands as tools to target delta-1 and kappa-2 putative subtypes in the mouse spinal cord have suggested they arise from their organization as delta-kappa heterodimers (Portoghese and Lunzer, 2003; Waldhoer et al., 2005). The physical association of these spinal receptors has been demonstrated in vivo using a specifically designed bivalent ligand, KDN-21, that is capable of bridging delta-kappa heterodimers (Bhushan et al., 2004). Significantly, KDN-21 possessed different spinal and supraspinal selectivity profiles, presumably because there appear to be no delta-kappa heterodimers in the brain that mediate antinociception. Another study with the spinally-selective opioid agonist, 6’GNTI, which selectively targets delta-kappa heterodimers, produced potent analgesia only by the spinal route of administration,
consistent with the localization of delta-kappa heterodimers in the spinal cord but not in the brain (Waldhoer et al., 2005).

Allosteric interactions have been proposed for delta and kappa receptors organized as heterodimers in the spinal cord and in cultured cells. This was attributed to the antagonism of DPDPE by the kappa opioid antagonist, norbinaltorphimine (norBNI), at its neighboring receptor in a delta-kappa heterodimer in the mouse spinal cord, and to the ability of norBNI to enhance the binding of [³H]NTI in HEK293 cells transfected with delta and kappa opioid receptors (Portoghese and Lunzer, 2003; Xie et al., 2005).

These studies have suggested that ligands known to be highly selective for a homogeneous population of opioid receptors may display different pharmacological or binding properties for heterodimeric opioid receptors. This distinction has also been observed for co-expressed delta/kappa and delta/mu heterodimeric receptors (Jordan and Devi, 1999; George et al., 2000). Consequently, it is possible that the pharmacologic selectivity of an agonist or antagonist may differ from tissue to tissue depending on the organization of receptors.

In view of the aforementioned considerations concerning the reliability of conclusions drawn from the use of selective opioid ligands, we have evaluated both intrathecal (i.t.) and intracerebroventricular (i.c.v.) selectivity of several widely employed, pharmacologically selective opioid antagonists in mice in order to compare selectivity as a function of route of administration. Here we report on the i.t. and i.c.v. antagonist ED₅₀ values of frequently employed selective antagonist ligands used as research tools in vivo. The results of this study have revealed that the pharmacologic
selectivity of an antagonist in mice depends on the route of administration and is therefore a function of the tissue (e.g., spinal cord vs. brain) targeted by the ligand.
Methods

Animals. Male ICR mice (15-20 g; Harlan, Madison, WI), were housed in groups of 5 – 10 in a temperature and humidity controlled environment with unlimited access to food and water and maintained on a 12 hour light/dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota.

Analgesic Studies. Antinociception was measured using the modified radiant heat tail-flick test (Tulunay and Takemori, 1974). Briefly, a radiant heat source was applied to the dorsal side of the tail, and the latency to flick away from the heat source was recorded. The data was made quantal by designating a positive antinociceptive response of an animal as those that increased their latency to tail flick (after drug treatment) by at least three standard deviations above the mean of the baseline latency of the whole group (Tallarida, 2000). The light source was manually turned off if the mouse did not flick its tail after the three standard deviation criteria for a positive response. At least three groups of 8-10 mice were used for each drug paradigm, and each mouse was used only once. ED_{50} values and 95% confidence intervals were calculated by using the parallel line assay (Finney, 1964).

Drugs. BNTX, NTB, NTI and norBNI were synthesized as described previously (Portoghese et al., 1992; Miyamoto et al., 1993; Portoghese et al., 1988; Portoghese et al., 1987). DPDPE, (H-Tyr-D-Pen-D-Phe-D-Pen-OH (cyclic)), U50488 and deltorphin II (H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH) were gifts from the National Institute on Drug Abuse (Mosberg et al., 1983; Szmuszkovicz and Von Voigtlander, 1982; Erspermer et al.,
Bremazocine (Simonin et al., 2001) was provided by Ping-Yee Law, Ph.D. (Department of Pharmacology, University of Minnesota).

**Experimental Protocols.** The antagonist ED$_{50}$ value was determined by challenging an ED$_{80}$ or ED$_{90}$ dose of the selected agonist with graded doses of the antagonist so that the data points in the regression either flanked or were at the antagonist ED$_{50}$ value. All solutions were dissolved in distilled water. Controls with only distilled water showed no antinociception. All drugs were administered in a 5-µl volume in conscious mice according to the method Hylden and Wilcox (1980) for i.t. and Haley and McCormick (1957) for i.c.v. injections. The drugs were administered so that the antagonist and agonist effects would peak simultaneously. The i.t. doses and peak times used for agonists were as follows: DPDPE, 12 nmol/mouse (10 minutes); deltorphin II, 6 nmol/mouse (10 minutes); U50488, 40 nmol/mouse (10 minutes); bremazocine, 0.1 nmol/mouse (20 minutes). Agonist doses and peak times for i.c.v. administration: DPDPE, 12 nmol/mouse (20 minutes); deltorphin II, 12 nmol/mouse (10 minutes); U50488, 90 nmol/mouse (10 minutes); bremazocine, 0.5 nmol/mouse (10 minutes). The peak times for the antagonists were as follows: BNTX and NTB, both i.t. and i.c.v, 10 minutes; NTI and norBNI, both 20 minutes.
Results

Routes of administration: The i.t. and i.c.v. ED$_{50}$ values of pharmacologically selective delta (DPDPE, deltorphin II) and kappa (U50488, bremazocine) agonists are displayed in Table 1. It is noteworthy that, with the exception of U50488, there were no significant potency differences between i.t. and i.c.v. administration. In this regard, U50488 was more potent by the i.t. route by a factor of ~2.

The antagonist ED$_{50}$ values expressed as delta-1/delta-2 and kappa-1/kappa-2 selectivity ratios of the antagonists were generally dependent on the route of administration, and in all but one case (i.c.v. delta-1/delta-2 ratio for BNTX), they were significantly different from unity (Table 2). It should be noted that the delta-1/delta-2 and kappa-1/kappa-2 selectivity ratios in Table 2 were obtained from values across rows, rather than from columns of data.

In contrast to the agonist data, the i.c.v./i.t. antagonist potency ratios (Table 3) frequently differed from unity when compared to the agonist potency ratios. The norBNI i.c.v./i.t. antagonist potency ratios for DPDPE, U50488, and bremazocine were in the 6-40 range. BNTX exhibited an i.c.v./i.t. antagonist ratio of 4 for DPDPE, whereas deltorphin II, U50488, and bremazocine were antagonized to the same degree via the two routes. The antagonist potency ratios for NTB were significantly different from unity for DPDPE, U50488, and bremazocine, with values of 2.1, 0.1, and 0.3, respectively; no significant difference was seen for deltorphin II. With NTI the i.c.v./i.t. antagonist potency ratios were significantly different for deltorphin II (7.6) and bremazocine (0.4); no differences in i.c.v./i.t. potency ratio was observed for the antagonism DPDPE and
U50488. The antagonism curves for the i.t. and i.c.v. routes of administration were essentially linear within the dose ranges of the antagonists employed (Fig. 1)

*Norbinaltorphimine (norBNI):* By the i.t. route, the kappa antagonist, norBNI, selectively antagonized bremazocine (kappa-2). The antagonism was ~12 times greater relative to that of U50488 (kappa-1), suggesting that norBNI functions as a selective kappa-2 antagonist in the cord. The 40-fold greater antagonism of i.t. bremazocine over the i.c.v. route by norBNI may reflect divergent phenotypic kappa receptors in the cord versus the brain. Significantly, i.t. norBNI more potently antagonized DPDPE (delta-1) over deltorphin II (delta-2) by a factor of 8 (delta-1/delta-2 = 0.13), which is consistent with earlier proposals for allosteric antagonism via coupled delta-kappa opioid receptor heterodimers in the spinal cord (Portoghese and Lunzer, 2003; Bhushan et al, 2004).

Importantly, the observation that i.t. DPDPE was more potently antagonized than U50488 by norBNI illustrates the need for caution in pharmacologic characterization based on selectivity. This is further illustrated by the data that i.t. norBNI is ~7-fold more potent than the delta antagonist, NTI, in antagonizing DPDPE. The discrimination of phenotypic receptors by i.c.v. norBNI was relatively low (selectivity ratio ~2) compared to i.t. administration.

*Benzylidenenaltrexone (BNTX):* In the cord BNTX DPDPE (delta-1) was antagonized 8-fold more potently than deltorphin-II, and exhibited a kappa-1/kappa-2 selectivity ratio of 5. These data are consistent with the well-known delta-1 antagonism of BNTX. It is not known whether BNTX antagonizes bremazocine by an allosteric mechanism through interaction with a delta-1 receptor component of a delta-kappa heterodimer or by direct interaction with the kappa-2 phenotype. In view of the absence
of significant selectivity for i.c.v.-administered BNTX for phenotypic delta receptors, heterodimers containing delta-1 and kappa-2 phenotypic receptors that mediate analgesia, are probably not abundant in the brain.

*Naltriben (NTB):* Greater antagonism of deltorphin II relative to DPDPE (delta-1/delta-2 = 4- to 6-fold) was observed by both routes of administration. NTB antagonized the kappa-opioid agonists, U50488 and bremazocine, with selectivity ratios that were in the same range as the delta agonists. Interestingly, of the four i.c.v.-administered agonists, bremazocine was most potently antagonized by NTB.

*Naltrindole (NTI):* Because NTI is a selective delta antagonist, but generally is considered not to have “subtype” selectivity, its ability to antagonize selective agonists was examined. Upon i.t. administration, NTI blocked deltorphin II 4-fold more potently than it blocked DPDPE-induced antinociception. This was in contrast to a selectivity factor of 0.4 by the i.c.v. route. While there was no significant difference between the i.c.v. and i.t. antagonism of DPDPE by NTI, i.t. deltorphin II was antagonized more potently than DPDPE by a factor of 8. The kappa-1/kappa-2 selectivity ratios for NTI by the i.t. and i.c.v. routes were in the range of 2-5.
Discussion

Heterodimerization of GPCRs has raised the possibility of greater pharmacological diversity \textit{in vivo} when compared to model systems that contain homogeneous populations of receptors (Jordan and Devi, 1999; George et al., 2000). Consequently, the \textit{in vivo} properties of agonists and antagonists may not correlate well with data derived from cell-based or in other types of \textit{in vitro} assays if the target receptors are organized differently. Because the use of selective opioid ligands employed as pharmacologic tools \textit{in vivo} have in many cases been based upon \textit{in vitro} binding selectivity or function in homogeneous populations of receptors, it is possible there may be a mismatch between \textit{in vivo} and \textit{in vitro} selectivity. Thus, it is conceivable that \textit{in vivo} tissue-specific localization of heterodimers could give rise to erroneous assignment of an opioid receptor type involved in a pharmacological response if the recognition and functional properties of a heterodimer differ from those of a homodimer. Such differences could even account for the commonly observed greater \textit{in vitro} binding selectivity of opioid ligands compared to \textit{in vivo} pharmacological selectivity.

Consistent with this concept, the results of the present study have revealed that the antinociceptive receptor selectivity ratios of selective antagonists administered i.t. often differ significantly from those obtained by the i.c.v. route. For example, the kappa opioid antagonist, norBNI, was 12-times more potent in the antagonism of i.t. bremazocine (kappa-2) relative to U50488 (kappa-1), but only minimal difference was observed on i.c.v. administration. In view of reports suggesting the presence of delta/kappa heterodimeric opioid receptors in the cord but not the brain (Portoghese and Lunzer, 2003; Bhushan et al., 2004; Waldhoer, et al., 2005), the present results may reflect
differential distribution of phenotypic kappa-1 and kappa-2 receptors. Moreover, the more potent antagonism by norBNI of DPDPE relative to U50488 is in keeping with the proposed interaction of DPDPE with spinally-localized heterodimers containing delta-1 and kappa-2 phenotypic receptors as proposed by Bhushan et al. (2004). Consistent with the antagonism of DPDPE by norBNI, binding studies in cultured cells using selective antagonists have suggested cooperativity between delta and kappa receptors organized as heterodimers (Xie et al., 2005). Thus, norBNI antagonism is mediated via competitive interaction at the kappa-1 receptor which allosterically leads to antagonism at the delta-1 receptor. Viewed from this perspective, the in vivo selectivity of norBNI would depend upon the phenotypic kappa receptor targeted, as suggested by the relatively low kappa-1/kappa-2 selectivity ratio obtained i.c.v. where such heterodimers either are not present or do not mediate antinociception.

The 8-fold greater i.t. antagonism by BNTX of DPDPE over deltorphin II is consistent with the well-known pharmacologic selectivity of this delta-1 antagonist (Portoghese et al., 1992). However, in contrast to the i.t. data, both DPDPE and deltorphin II were equally antagonized by BNTX given i.c.v. Similarly, the selective delta-kappa bivalent ligand antagonist, KDN-21, is reported to possess divergent selectivity by these different routes (Bhushan et al., 2004). These results may reflect differences between the organization of delta opioid receptors in the spinal cord versus the brain. The report that the delta-kappa heterodimer-selective agonist, 6'-GNTI, produces potent spinal analgesia, but only weak, partial agonist activity i.c.v., also is consistent with the present results (Waldhoer et al., 2005).
The delta-2 antagonist, NTB, exhibited 4-6 fold greater selectivity in favoring deltorphin II over DPDPE, which is in harmony with its generally accepted selectivity. In view of the similar i.t. and i.c.v. delta-1/delta-2 selectivity ratios for NTB antagonism of DPDPE and deltorphin II antinociception, it appears that NTB targets identical phenotypic delta opioid receptors in the cord and brain by the i.t. and i.c.v. routes of administration. The organization of this phenotypic delta-2 receptor recently has been proposed to differ from delta-1 based on studies with the bivalent ligand, KDAN-18, which contains both kappa-1 agonist and delta antagonist pharmacophores (Daniels, et al., 2005). It was proposed that KDAN-18 bridges between oligomerized kappa and delta receptor homodimers, and that kappa-1 and delta-2 ligands selectively target such receptors. These receptors are distinct from the kappa-2 and delta-1 phenotypes that have been proposed to be organized as heterodimers. In view of the report that KDAN-18 exhibits similar i.t. and i.c.v. agonist selectivity profiles, the NTB antagonism data provides additional support for the presence of both delta-2 and kappa-1 phenotypic opioid receptors in the cord and the brain. Interestingly, of the four antagonists challenged by i.c.v. NTB, bremazocine was most potently antagonized. These results emphasize the importance of using multiple selective antagonists to evaluate the selectivity of agonists in vivo.

Naltrindole (NTI) is a delta antagonist and is considered to be nonselective in regard to distinguishing between phenotypic delta-1 and delta-2 receptors. While this appeared to be the case when administered i.c.v., we have found that i.t. NTI exhibited 4-fold greater antagonism of deltorphin II relative to DPDPE. The lack of significant i.c.v. selectivity at phenotypic delta receptors and the observation that i.t. NTI antagonized
deltorphin-II ~8-fold more potently than by the i.c.v. route, suggests that NTI targeted different phenotypic delta receptors in the cord and brain. Given these data and the finding that i.c.v. NTI most potently antagonized kappa-2 receptors, again suggests that several selective opioid antagonists should be employed to establish the in vivo selectivity of an agonist.

In addition to affecting opioid receptor selectivity, the route of administration of opioid antagonists often affects potency, in part due to differences in the distribution of heterodimers in the brain and spinal cord. The finding that the i.c.v./i.t. ratios of agonists were either close to or not significantly different from unity (Table 1), while the antagonists more often exhibited i.c.v./i.t. ratios significantly greater or less than 1 (Table 3), reflects the complexity of the in vivo system. The complexity introduced by heterodimerization of opioid receptors is staggering when one considers the number of possible combinations that can exist for opioid receptor-containing heterodimers, when based upon studies of co-expressed receptors in cultured cells. In this regard, the standard selective agonists and antagonists for delta and kappa receptors employed in the present study may interact with heterodimers containing other opioid receptors and a variety of non-opioid GPCRs to produce analgesia. Unfortunately, it is not known how delta and kappa receptors that are heterodimerized with non-opioid receptors would function in vivo. Thus, without knowledge of the tissue localization and identity of heterodimers containing delta or kappa opioid receptors, the use of the standard armamentarium of opioid antagonists remains problematic for identifying specific opioid receptors in vivo. Clearly, the development of ligands that selectively target opioid receptor heterodimers would be key to their pharmacologic characterization and localization in vivo.
The most dramatic example of divergent opioid antagonist potency as a function of route of administration was seen in the antagonism of DPDPE and bremazocine by norBNI, where the i.c.v./i.t. antagonist potency ratios were 12 and 40, respectively (Table 3). The much greater antagonist potency of norBNI by the i.t. route may reflect allosteric interactions within delta-1/kappa-2 heterodimers present in the cord but not the brain (Xie et al., 2005; Bhushan et al., 2004; Waldhoer et al., 2005). However, correlation of the i.c.v./i.t. potency ratios with the receptor selectivity of other antagonists are not easily explained, given the paucity of information on their interaction with heterodimers. For example, since BNTX efficiently antagonizes DPDPE at delta-1 phenotypic receptors, it might be expected that BNTX would antagonize bremazocine through an allosteric mechanism. However, such an allosterically-mediated mechanism may not occur, since this does not correlate with the finding that the i.c.v./i.t. antagonist potency ratio for BNTX antagonism of bremazocine which was not significantly different from unity. Alternately, if DPDPE also interacts with a phenotypic delta receptor that is not present in the putative delta-1/kappa-2 heterodimer, this may also be an explantion for the lack of a significant difference between the i.t. and i.c.v. antagonism of bremazocine by BNTX. Similarly, there are multiple possible explanations for the significantly high or low i.c.v./i.t. antagonist potency ratios for NTB and NTI.

In conclusion, antagonist ED$_{50}$ selectivity ratios derived from the administration graded doses of pharmacologically selective opioid antagonists in the presence an ED$_{80-90}$ dose of selective agonists has provided a reliable estimate of the selectivity profiles at spinal and supraspinal opioid receptors. These results are in qualitative agreement with earlier studies that employed a single i.t. dose of antagonist, expressed as an ED$_{50}$ ratio in
evaluating the spinal selectivity of agonists at phenotypic delta and kappa opioid receptors. Importantly, in the present study the selectivity of an antagonist was dependent on the route of administration. The significant selectivity differences between the i.t. and i.c.v. routes of administration may be a consequence of different populations of phenotypic opioid receptors that reside in different tissues. Thus, it is suggested that the mouse spinal cord possesses delta-kappa opioid receptor heterodimers containing the delta-1 and kappa-2 phenotypes that have somewhat different recognition properties from putative homomeric receptors or other delta and kappa receptor-containing heterodimers in the brain. Given this tissue-dependent selectivity, the use of selective antagonists to characterize receptor selectivity may in some instances be problematic. Since the use of only one selective antagonist could lead to erroneous assignment of receptor selectivity, it is suggested that the antagonist ED\textsubscript{50} or ED\textsubscript{50} ratio paradigms be employed with several selective antagonists in order to obtain a more reliable assignment of selectivity. Finally, the possible existence of cooperativity among receptors organized as heterodimers introduces new challenges in the pharmacological characterization of receptors, where transactivation or allosteric antagonism complicates the assignment of selectivity. It also offers the opportunity to develop new approaches to developing analgesics that act selectively, given the potential differential tissue distribution of heterodimers and the divergent recognition properties between phenotypic opioid receptors.
Acknowledgements

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Reprint request:
Philip S. Portoghese
U of MN - Medicinal Chemistry
308 Harvard Street S.E.
Minneapolis, MN  55455
Phone: 612-624-9174
FAX: 612-626-6891
porto001@umn.edu
Legend for Figure

Figure 1. Relationships of the i.t. (A) or i.c.v. (B) opioid antagonist dose response curves of agonist-induced antinociception in mice. The regressions were obtained after administration of 3 or 4 doses of the antagonist in the presence of an ED$_{80-90}$ dose of the agonist.
Table 1.

Agonist ED$_{50}$ Values of I.T.- and I.C.V.-Administered Selective Opioid Agonists in Mice

<table>
<thead>
<tr>
<th>Route</th>
<th>DPDPE ($\delta_1$)</th>
<th>Del II ($\delta_2$)</th>
<th>U50488 ($\kappa_1$)</th>
<th>Bremazocine ($\kappa_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.t.</td>
<td>4.32 (3.50 – 5.17)</td>
<td>3.36 (2.23 – 5.20)</td>
<td>18.89 (15.49 – 22.28)</td>
<td>0.05 (0.01 - 0.17)</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>6.00 (5.01 – 7.18)</td>
<td>5.65 (3.64 – 8.49)</td>
<td>41.96 (34.06 – 49.63)</td>
<td>0.22 (0.06 – 0.68)</td>
</tr>
</tbody>
</table>
Table 2.

**Antagonist ED$_{50}$ Values of I.T.- and I.C.V.-Administered Combinations of Selective Opioid Antagonists and Agonists in Mice$^d$**

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Route</th>
<th>DPDPE  $^c$ ($\delta_1$)</th>
<th>Del II $^f$ ($\delta_2$)</th>
<th>U50488 $^g$ ($\kappa_1$)</th>
<th>Bremazocine $^h$ ($\kappa_2$)</th>
<th>$\delta_1$/ $\delta_2$ $^b$</th>
<th>$\kappa_1$/ $\kappa_2$ $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>norBNI</td>
<td>i.t.</td>
<td>515 (471 – 561)</td>
<td>3928 (3505 – 4366)</td>
<td>1030 (829 – 1251)</td>
<td>87 (62 – 128)</td>
<td>0.13 (0.10 – 0.16)</td>
<td>11.8 (7.5 – 17.3)</td>
</tr>
<tr>
<td></td>
<td>i.c.v.</td>
<td>6070 (3815 – 11420)</td>
<td>2332 (1337 – 3743)</td>
<td>6476 (5319 – 8148)</td>
<td>3535 (3380 – 3698)</td>
<td>2.6 (1.2 – 8.7)</td>
<td>2.0 (1.7 – 2.4)</td>
</tr>
<tr>
<td>BNTX</td>
<td>i.t.</td>
<td>7 (5 – 10)</td>
<td>50 (49 – 51)</td>
<td>85 (61 – 122)</td>
<td>17 (14 – 21)</td>
<td>0.13 (0.09 – 0.17)</td>
<td>5.0 (3.1 – 8.7)</td>
</tr>
<tr>
<td></td>
<td>i.c.v.</td>
<td>i.t.</td>
<td>NTB (δ2)</td>
<td>NTI (δ)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>28 (20 – 41)</td>
<td>30 (20 – 41)</td>
<td>93 (55 – 150)</td>
<td>23 (14 – 37)</td>
<td>0.9 (0.6 – 1.7)</td>
<td>3.5 (33.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90 (61 – 126)</td>
<td>23 (18 – 30)</td>
<td>187 (110 – 300)</td>
<td>45 (31 – 63)</td>
<td>4.2 (2.5 – 7.4)</td>
<td>4.1 (7.7)</td>
<td></td>
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<tr>
<td></td>
<td>192 (173 – 214)</td>
<td>32 (27 – 41)</td>
<td>25 (24.78 – 25.22)</td>
<td>14 (10 – 20)</td>
<td>5.4 (4.4 – 6.6)</td>
<td>1.8 (2.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3470 (2764 – 4610)</td>
<td>884 (845 – 925)</td>
<td>5000 (4937 – 5063)</td>
<td>2243 (1531 – 3155)</td>
<td>4.4 (3.7 – 5.4)</td>
<td>2.2 (3.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2949 (2043 – 4107)</td>
<td>6733 (3927 – 11284)</td>
<td>4454 (3168 – 6535)</td>
<td>824 (663 – 1001)</td>
<td>0.4 (0.2 – 0.8)</td>
<td>5.4 (8.5)</td>
<td></td>
</tr>
</tbody>
</table>

At least three groups of 10 male ICR-CD1 mice (Harlan Sprague Dawley) weighing between 15 - 20 g were employed in a modified tail flick assay (Tulunay and Takemori, 1974). Antinociception was considered positive if the latency to flick its tail was more than the control latency plus 3 S.D. of the mean of the reaction time.

The parallel line assay was used to calculate the ED50 values and the 95% confidence intervals.

Selectivity ratios were calculated from the antagonist ED50 values across the table.
The peak times for the antagonists were as follows: BNTX and NTB, both i.t. and i.c.v., 10 minutes; NTI and norBNI, both 20 minutes.

d PDPE (dose and peak time) i.t. - 12 nmol/mouse (10 minutes); i.c.v. - 12 nmol/mouse (20 minutes)

d deltorphin II (dose and peak time) i.t. - 6 nmol/mouse (10 minutes); i.c.v. - 12 nmol/mouse (10 minutes)

g U50488 (dose and peak time) i.t. - 40 nmol/mouse (10 minutes); i.c.v. - 90 nmol/mouse (10 minutes)

h bremazocine (dose and peak time) i.t. - 0.1 nmol/mouse (20 minutes); i.c.v. - 0.5 nmol/mouse (10 minutes)
Table 3.

**I.C.V./I.T Antagonist ED\textsubscript{50} Potency Ratios of Selective κ and δ Opioid Ligands**

<table>
<thead>
<tr>
<th>Potency Ratios (95% C.I.)</th>
<th>i.c.v./i.t.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPDPE (δ\textsubscript{1})</td>
</tr>
<tr>
<td>norBNI (κ)</td>
<td>11.8 (5.7 – 38.5)</td>
</tr>
<tr>
<td>BNTX (δ\textsubscript{1})</td>
<td>4.1 (3.0 – 5.9)</td>
</tr>
<tr>
<td>NTB (δ\textsubscript{2})</td>
<td>2.1 (1.7 – 2.8)</td>
</tr>
<tr>
<td>NTI (δ)</td>
<td>0.8* (0.4 – 1.3)</td>
</tr>
</tbody>
</table>

* I.C.V./I.T potency ratio not significantly different from unity.