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Opioid Antagonist Profile of SC Nor-Binaltorphimine in the Formalin Paw Assay

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WETTSTEIN, J. G. AND A. GROUHEL. *Opioid antagonist profile of SC nor-binaltorphimine in the formalin paw assay.* PHARMACOL BIOCHEM BEHAV **53**(2) 411–416, 1996.—The antinociceptive effects of μ and κ agonists were examined after the systemic administration of the opioid antagonists nor-binaltorphimine (nor-BNI) and naloxone in the late response or tonic nociceptive phase of the mouse formalin assay. Initially, SC morphine (ED_{50}, 0.97 mg/kg), racemic U-50488H (ED_{50}, 0.79 mg/kg), (−)U-50488 (ED_{50}, 0.41 mg/kg), and another agonist PD 117,302 (ED_{50}, 0.28 mg/kg) were found to produce graded increases in the level of antinociception as measured by this procedure; naloxone, administered immediately before morphine and U-50488H, antagonized their antinociceptive actions. The effects of morphine and U-50488H then were evaluated 10 min to 96 h after the administration of nor-BNI. Subcutaneous nor-BNI at 30.0 mg/kg, but not at 3.0 or 10.0 mg/kg, attenuated the antinociceptive effects of morphine and U-50488H when the interval separating nor-BNI and the agonists was kept constant at 1 h. Time-course analysis of the effects of combinations of nor-BNI with morphine led to irregular findings: 10.0 mg/kg of nor-BNI lessened the effects of morphine (2.0 mg/kg) if the dosing interval was 10 min, whereas 30.0 mg/kg of nor-BNI attenuated the effects of morphine (2.0 mg/kg) if the dosing interval was 1 or 4 h; 10.0 mg/kg of nor-BNI also diminished the antinociceptive effects of U-50488H (1.7 mg/kg) only if the interval spacing the two drugs was 24 h. In comparison, a threefold higher dose of nor-BNI (30.0 mg/kg) reduced the effects of U-50488H (1.7 mg/kg) if the interval was 1 h or more. In these latter experiments, the antagonist effects of SC nor-BNI (30.0 mg/kg) were evident up to 96 h posttreatment. These results show that the μ opioid antagonist activity of nor-BNI is variable and that the κ opioid antagonist selectivity of nor-BNI is a function of dose and treatment interval and is long-lasting even after systemic administration.

Nor-binaltorphimine Morphine U-50488H Naloxone Formalin Pain Antinociception Mice μ Agonists κ Agonists

NOR-BNI (11,13) is a bivalent ligand having unusual properties as an opioid antagonist that are best illustrated by two features. Nor-BNI has been reported to have very selective, centrally mediated κ opioid antagonist effects in mice if the time separating the injections of nor-BNI and a κ agonist are at least 24 h (4). Furthermore, the κ opioid antagonist activity and selectivity of nor-BNI can last up to 21 days in rats (9) and 28 days in mice (2,6). Antagonist selectivity at κ opioid receptors, however, is not apparent under all conditions with nor-BNI. If it is given shortly before morphine, nor-BNI can attenuate the antinociceptive effects of the μ agonist (4). Also, repeated ICV administration of nor-BNI (30 μg/b.i.d./10 days) results in both μ and δ opioid receptor blockade (17). It has been suggested that the unique mechanisms of action of nor-BNI may be due a number of factors, including resistance to metabolism, induction of conformational receptor change, and slow distribution and passage across cell membranes (6,19).

The purpose of the present study was to examine the opioid antagonist profile of systemically administered (SC) nor-BNI in the late nociceptive phase of the mouse formalin assay. Injection of a 5% solution of formalin into the paw of a mouse or rat leads to two nociceptive periods, one immediate, likely due to the direct action of formalin on sensory recep-

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tors, and the other delayed, due to local inflammation (3,7, 23). This deferred localized inflammation and pain can have a duration of up to 30 min in mice and is a particularly good model for studying the effects on drugs under a constant tonic pain state (3,7,10,23). Also, it has been recommended that use of the formalin procedure is beneficial in understanding κ opioid pharmacology in the brain (12), in part because non-thermal pain may be primarily a κ phenomena (1,5,12) and that the procedure can detect a robust antinociceptive effect with κ opioids (12,23). In addition to examining the antinociceptive effects of morphine and U- 50488H in the presence of nor-BNI using varied treatment intervals, the effects of two other κ agonists (PD 117,302 and (−)U-50488) and the antagonist naloxone also were evaluated in the formalin procedure for comparison.

METHOD

Animals

Male Swiss mice (Iffa-Credo, L'Arbresle, France), obtained 1 week before experiments, were housed 12 per cage with water and food (AO3 pellets, Usine Alimentation Rationnelle) freely available. The animal housing room was maintained at a temperature of 21 ± 1°C, with a regular light cycle (lights on 0700 h and off at 1900 h). Individual mice were used only once and weighed approximately 25 g at the time of experiments.

Analgesic Assay

A modified version of the formalin procedure as described previously was used (7). On the morning of the experiment, mice were brought into and allowed to acclimate for a minimum of 1 h in a laboratory maintained at 24.7 ± 0.2°C. Twenty microliters of a 5% solution of formalin prepared in sterile 0.9% saline was injected into the plantar arch of the posterior left paw with a 26.5 gauge needle attached to a 25 μl Hamilton syringe. Afterwards, mice were placed in cages with paper towel as bedding. Fifteen minutes after the injection of formalin, each mouse was placed in a transparent Plexiglas cylinder (height, 25 cm; diameter, 20 cm) onto a paper towel and 5 min were allowed to pass before measurements were begun. A mirror was located behind the cylinder to facilitate observation. The degree of pain intensity was measured as the time spent licking the posterior left paw for a 5-min period, 20 to 25 min after the injection of formalin. This period of time corresponds to the late response or tonic nociceptive phase of the procedure (7,23). Experiments were conducted blind, Monday through Friday from 0830 h to 1630 h.

Drugs and Injection Procedures

Nor-BNI 2HCl was synthesized in the Department of Medicinal Chemistry at the Institut de Recherche Jouveinal. Morphine HCl was obtained from Francopia (Paris), racemic U-50488H methanesulfonate (referred to as U-50488H throughout the text) from Upjohn (Kalamazoo, MI), (−)U-50488 HCl from Cookson Chemicals (Southampton, England), PD 117,302 HCl from Parke-Davis (Ann Arbor, MI), and naloxone HCl from Sigma (St. Louis, MO). All drugs were dissolved in sterile distilled water and administered SC in volumes of 0.2 ml/20 g body weight; doses are expressed as the base. Control (vehicle) injections were similar volumes of distilled water. For all experiments, morphine, U-50488H, (−)U-50488 and PD 117,302 were administered 10 min before formalin was injected, i.e., 30 min before the analgesic assay; naloxone was given immediately before morphine and U-50488H.

In one study, mice were given nor-BNI (3.0, 10.0, and 30.0 mg/kg) 60 min before the administration of a range of doses of morphine or U-50488H. In a second study designed to evaluate the onset and duration of action of the antagonist effects of nor-BNI, mice were given nor-BNI (10.0 and 30.0 mg/kg) 10 or 30 min or 1, 2, 4, 24, 48, or 96 h prior to a fixed dose of morphine (2.0 mg/kg) or U-50488H (1.7 mg/kg).

Statistics

Mean and standard error values for the time spent licking during the 5 min period were calculated for each group of mice. Comparisons between control and treatment groups were made using a one-way analysis of variance followed by Dunnett's test (20). Results are expressed as the percent inhibition of licking time as compared to the control group values. ED_{50} values (the dose of drug reducing licking time by 50% as compared to control) were determined by linear regression analysis (20).

RESULTS

Effects of 5% Formalin in Vehicle-Treated Mice

Intraplantar injection of 5% formalin resulted in a consistent paw-licking response in all mice. A typical vehicle-treated group of 10 mice exhibited licking behavior for 80 ± 15 s (mean ± SEM) in a 5-min span, 20 to 25 min after 5% formalin was given.

Effects of Opioid Agonists in the Absence or Presence of Naloxone

Morphine, U-50488H, (−)U-50488, and PD 117,302 produced dose-related increases in the level of antinociception as measured in the formalin assay (Fig. 1). Morphine (ED_{50}, 0.97

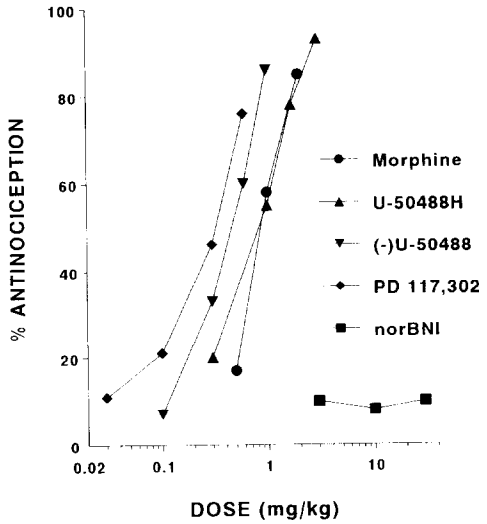

FIG. 1. Effects of morphine, U-50488H, (−)U-50488, PD 117,302, and nor-BNI in the late nociceptive phase of the mouse formalin procedure. The agonists were given 30 min and nor-BNI 90 min prior to the analgesic assay. Abscissa: dose, log scale. Ordinate: antinociception expressed as the percent inhibition of paw licking compared to control mice. Symbols are means obtained from groups of 10 mice.

mg/kg) and U-50488H (ED_{50}, 0.79 mg/kg) were almost equipotent in the assay. The dose-response function for (−)U-50488 (ED_{50}, 0.41 mg/kg) was shifted somewhat to the left of that of racemic U-50488H. PD 117,302 (ED_{50}, 0.28 mg/kg) was the most efficacious of the agonists examined in the formalin paw assay.

Naloxone produced a dose-related, surmountable blockade of the antinociceptive effects of morphine and U-50488H (Fig. 2). Although naloxone was an effective antagonist against both opioids, at least 30-fold higher doses of naloxone were required to attenuate the effects of U-50488H as opposed to morphine. For example, coadministration of 0.03 mg/kg naloxone with morphine resulted in an approximately threefold rightward shift in the morphine dose-response function. To achieve a similar shift in the U-50488H function, 3.0 mg/kg of naloxone was utilized.

Effects of Nor-BNI Alone

In no instance did nor-BNI alter the nociceptive effects produced by the injection of 5% formalin in mice. In one experiment, nor-BNI, given 90 min before the analgesic assay over a dose range of 3.0–30 mg/kg, did not alter the nociceptive threshold in mice (Fig. 1, squares). In other experiments, for example, when nor-BNI (10.0 mg/kg) was administered 10 min, 70 min, or 24 h prior to formalin, the resulting licking behavior in the 5 min observation phase occurred for 76 ± 11, 73 ± 10 or 79 ± 11 s, respectively (control values for vehicle-treated mice, 80 ± 15 s).

Dose-Response Effects of Morphine and U-50488 After Nor-BNI: Fixed 60 Min Treatment Interval

When given 60 min prior to the administration of morphine, nor-BNI altered morphine antinociception (Fig. 3, left panel). The effects of morphine were relatively unaffected by pretreatment with the lower dose of nor-BNI (10.0 mg/kg), whereas the morphine dose-response function was shifted almost one log unit to the right in a surmountable manner following treatment with the higher dose of nor-BNI (30.0 mg/kg).

When nor-BNI was administered 60 min prior to U-50488H, only the highest dose of nor-BNI altered U-50488H antinociception (Fig. 3, right panel). Neither pretreatment with 3.0 nor 10.0 mg/kg of nor-BNI antagonized the antinociceptive effects of U-50488H at the 1 h time point. However, the U-50488H dose-response function was shifted approximately a one-half log unit to the right in a surmountable manner after the administration of 30.0 mg/kg of nor-BNI.

Single Dose Effects of Morphine and U-50488H after Nor-BNI: Varied Treatment Intervals

The effects of near-maximal antinociceptive doses of morphine (2.0 mg/kg) and U- 50488H (1.7 mg/kg) were examined 10 or 30 min or 1, 2, 4, 24, 48, or 96 h after 10.0 and 30.0 mg/kg of nor-BNI. In these experiments, 10.0 mg/kg of nor-BNI lessened the effects of morphine if the dosing interval was 10 min; longer treatment intervals (e.g., 1 h or more) did not significantly result in any attenuation of the morphine effect (Fig. 4, unfilled circles). A higher dose of nor-BNI (30.0 mg/kg) lessened the effects of morphine if the dosing interval was 1 or 4 h but not 10 or 30 min or more than 4 h (Fig. 4, unfilled squares). Soon after the injection of 30.0 mg/kg of nor-BNI, there was a trend for morphine's effects to be attenuated, this reaching significance at 1 h. In addition, the magnitude of the blockade at the 2 h mark, although not significant, was equivalent to that measured at 4 h.

In a different manner, nor-BNI also diminished the antino-

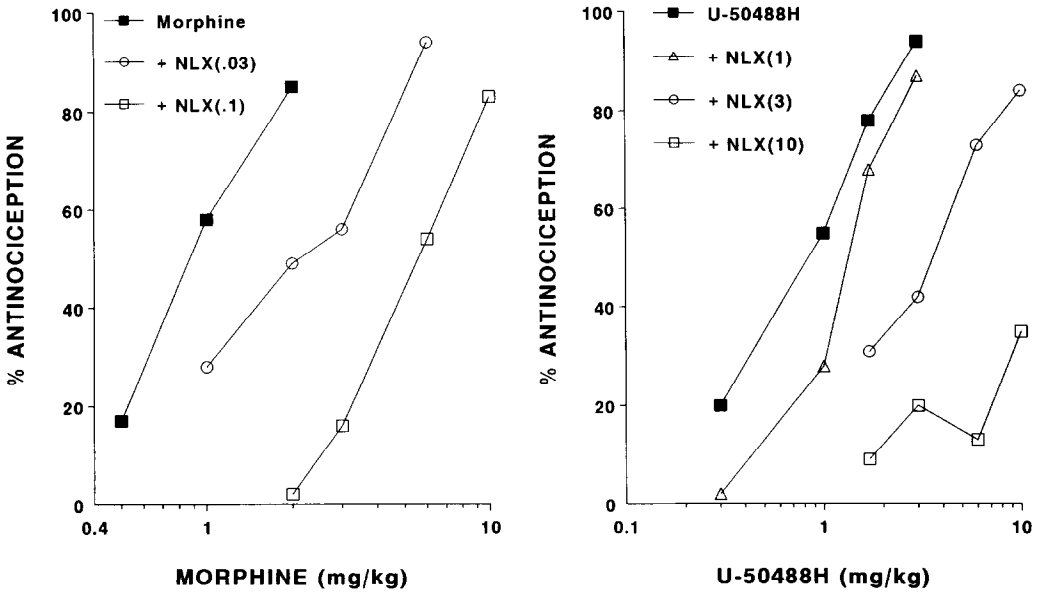

FIG. 2. Effects of morphine and U-50488H after naloxone (NLX) in the late nociceptive phase of the mouse formalin procedure. Naloxone was given immediately before the agonists. Abscissas: dose, log scale. Ordinates: antinociception expressed as the percent inhibition of paw licking compared to control mice. Symbols are means obtained from groups of 10 mice. Doses of naloxone administered with morphine and U-50488H are indicated in parentheses as mg/kg.

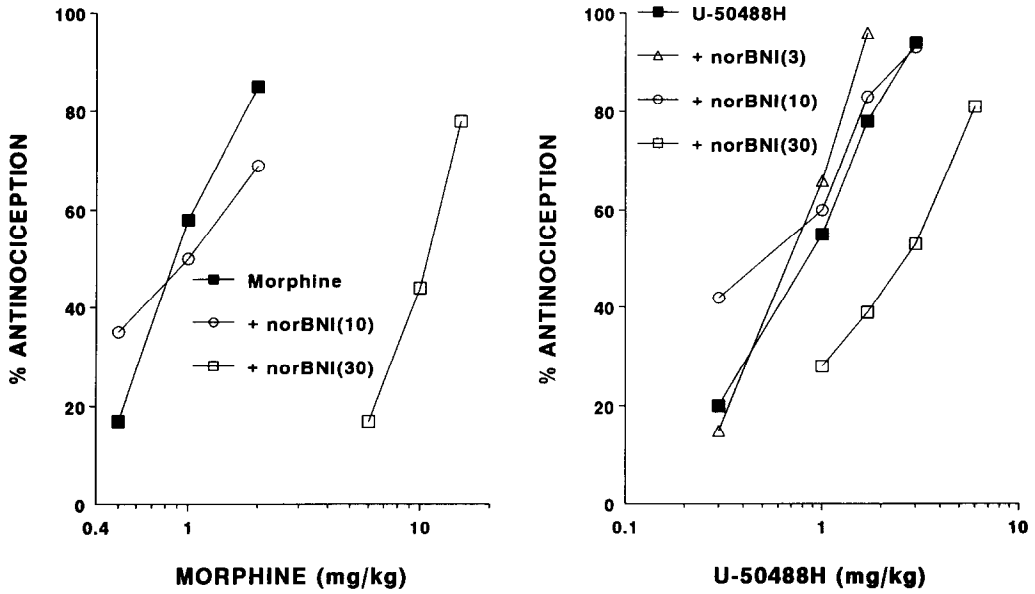

FIG. 3. Effects of morphine and U-50488H after nor-BNI in the late nociceptive phase of the mouse formalin procedure. Nor-BNI was given at a constant time, 60 min prior to the agonists. Abscissas: dose, log scale. Ordinates: antinociception expressed as the percent inhibition of paw licking compared to control mice. Symbols are means obtained from groups of 10–20 mice except in the experiment U-50488H + nor-BNI(3), six mice per group. Doses of nor-BNI administered prior to morphine or U-50488H are indicated in parentheses as mg/kg.

ciceptive effects of a fixed dose of U-50488H (1.7 mg/kg). Pretreatment with the low dose of nor-BNI (10.0 mg/kg) blocked U-50488H antinociception only if the interval spacing the two drugs was 24 h (Fig. 4, filled circles). In comparison, 30.0 mg/kg of nor-BNI reduced the effects of U-50488H if the interval was 1 h or more. In these latter experiments, the antagonist effects of SC nor-BNI (30.0 mg/kg) were evident up to 96 h posttreatment and were near maximal in magnitude at the two latter time points (Fig. 4, filled squares).

DISCUSSION

U-50488H, (−)U-50488, and PD 117,302, along with morphine, produced graded, dose-related increases in the level of antinociception as measured in the late nociceptive phase of the mouse formalin procedure. Each of these drugs had an analgesic ED_{50} of less than 1.0 mg/kg. The antinociceptive effects of morphine and U-50488H were antagonized in a surmountable manner by naloxone. Although there was not a substantial difference between the analgesic ED_{50}s for the three κ agonists, relative potencies in the mouse formalin assay correlate well with those from radioligand binding studies using guinea pig brain [cf., (8,14)]. Moreover, (−)U-50488 was reported to be two to four times more potent as an analgesic than U-50488H in rhesus monkeys (14), a difference found with these same compounds in mice using the formalin paw test. Together, the results confirm that the formalin procedure is sensitive to the analgesic effects of κ opioid agonists.

The present study demonstrates that systemically administered nor-BNI, which itself lacks antinociceptive action, has κ and some μ opioid antagonist effects in mice using the formalin analgesic assay. Mu antagonist effects were observed albeit only at selected time points and in an irregular manner: 10.0

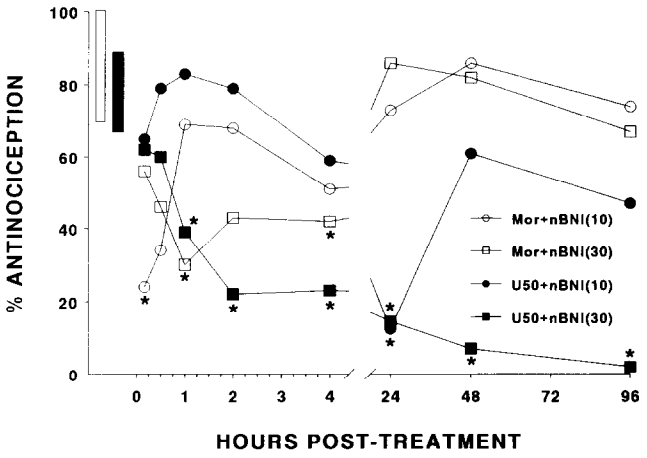

FIG. 4. Effects of morphine (Mor) or U-50488H (U50) with nor-BNI (nBNI) after varied treatment intervals in the late nociceptive phase of the mouse formalin procedure. The agonists were given 10 min to 96 h after nor-BNI. Abscissa: time in hours separating nor-BNI and agonist treatments (the abscissa has been broken to better represent the data). Ordinate: antinociception expressed as the percent inhibition of paw licking compared to control mice. Symbols are means obtained from groups of 10 to 20 mice. The doses of morphine and U-50488H were kept constant at 2.0 and 1.7 mg/kg, respectively. Doses of nor-BNI administered prior to morphine and U-50488H are indicated in parentheses as mg/kg. An asterisk indicates that opioid-induced antinociception was significantly ($p \leq 0.05$) antagonized (i.e., combination drug treatment did not reduce licking time when compared to control, vehicle-treated mice). Vertical unfilled and filled bars show the range (mean ± SEM) of effects of single injections of morphine (2.0 mg/kg) and U-50488H (1.7 mg/kg), respectively.

mg/kg of nor-BNI lessened the effects of morphine if the dosing interval between the two was short (i.e., 10 min) whereas a threefold higher dose of nor-BNI attenuated the effects of morphine if the dosing interval was 60 min (in a dose-related manner) or 4 h. A recent report has shown that SC nor-BNI antagonized the antinociceptive effects of morphine in the acetic acid writhing and tail pinch assays in mice if the treatment period between the drugs was either 30 or 60 min (4). The antinociceptive effects of the μ agonist [*d*-Ala(2),*N*-methyl-Phe(4),Gly(5)-ol]enkephalin (DAMGO) in the tail pressure test also were blocked by nor-BNI administered ICV daily for 10 days (17). In the present study, a single SC injection of nor-BNI did not reduce morphine's antinociceptive effect if given 24–96 h prior to the μ agonist, results consistent (at the 24 h mark) with other accounts (4,9). Little is known about the physical distribution of nor-BNI or its effects on μ receptor conformation, and these factors could contribute to the antagonist profile of this drug. Nonetheless, the current results support the idea that increasing the dose of nor-BNI will expand the time during which nor-BNI has μ opioid antagonist effects.

Nor-BNI blocked U-50488H antinociception in the late nociceptive phase of the mouse formalin procedure. First, with a treatment interval of 1 h, the antagonist effects of 30 mg/kg of nor-BNI were surmountable. Thus, the κ opioid antagonist effects of nor-BNI are not limited to the utilization of long treatment intervals. Elsewhere, it was observed that both 5.0 and 10.0 mg/kg of nor-BNI diminished the antinociceptive effects of U-50488H in an acetic acid writhing procedure with only a 1 h separation between drugs (4). It also has been reported that a low dose of nor-BNI (5.0 mg/kg) can attenuate the effects of a high dose of U-50488H (10.0 mg/kg) in the mouse tail pinch and 55°C hot-plate assays if 2 h separate the two injections (4,18). Second, blockade by nor-BNI was particularly evident when the interval between κ antagonist and agonist injections was 24 h or more. For sustained blocked in the formalin procedure, however, 10 mg/kg of nor-BNI was not a sufficient dose and a threefold higher dose was required. In general, these results are comparable to those found with similar combinations of drugs using ICV or parenteral routes of administration in the tail flick (21) and acetic acid writhing (4,13,19) assays in mice with antagonist-agonist separation periods of up to 28 days (2,6). The marked κ antagonist effects of nor-BNI observed in both the present and related studies that were evident 2 or more days after treatment are consistent with the idea that nor-BNI induces an alteration of opioid receptor conformation. In this regard, κ receptor radioligand binding studies using whole mouse brain demonstrated a 30-fold decrease in [^{3}H]U- 69593 affinity 3 days after nor-BNI injection (6). Moreover, a significant decrease in [^{3}H]U-69593 affinity was observed up to 56 days posttreatment without any alteration of the number of binding sites (6).

It is likely that the opioid antagonist effects of nor-BNI are, in part, dependent on the nature, location, and intensity of the noxious stimulus employed during experiments; in turn, the κ antinociceptive effects of agonists also are contingent on these methodological issues (10,15,16,22). Furthermore, the selectivity of nor-BNI action can be controlled by allowing a substantial time span between its injection and the administration of opioid agonists. It also is apparent that doses of nor-BNI capable of reducing U-50488H antinociception, also can attenuate morphine antinociception if the factors of treatment interval and antagonist dose are taken into consideration.

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