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Opioid Agonist Properties of Two Oxime Derivatives of Naltrexone, NPC 831 and NPC 836

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DEHAVEN-HUDKINS, D. L., K. M. KOMER, J. A. PETERSON, B. J. MAVUNKEL AND W. J. RZESZOTARSKI. *Opioid agonist properties of two oxime derivatives of naltrexone, NPC 831 and NPC 836.* PHARMACOL BIOCHEM BEHAV **44**(1) 45–50, 1993.—Two novel oxime derivatives of naltrexone, 6-[2-phenylethyl]-oximino naltrexone (NPC 831) and 6-[3-phenylpropyl]-oximino naltrexone (NPC 836) were potent agonists at opioid receptors. Both compounds inhibited binding to all three opioid receptor subtypes with nanomolar affinities. In vivo, NPC 831 and NPC 836 were equipotent to morphine and more potent than the κ-selective agonist U-50,488H to produce analgesia. ED_{50} values of 4.02 mg/kg for NPC 831 and 2.24 mg/kg for NPC 836 were generated for inhibition of the tail-flick response in the rat, and ED_{50} values of 0.05 mg/kg for NPC 831 and 0.02 mg/kg for NPC 836 were calculated for inhibition of the writhing response in the mouse. Bombesin-induced scratching was used to evaluate NPC 831 and NPC 836 for κ-agonist properties, and the A_{50}, defined as the percent antagonism of the bombesin-induced response, was 1.86 mg/kg for NPC 831 and 0.08 mg/kg for NPC 836, compared to an A_{50} of 1.54 mg/kg for U-50,488H. These data suggest that NPC 831 and NPC 836 possess potent μ- and κ-agonist properties in vivo, with NPC 836 being approximately twice as potent as NPC 831 to produce analgesia and 20 times as potent as NPC 831 to inhibit the scratching response produced by bombesin.

Analgesia Bombesin scratch test Naltrexone Opioid agonist Opioid antagonist Receptor binding

THERE are three subtypes of the opioid receptor, designated μ, δ, and κ (8,18,32), and the physiological functions subserved by these subtypes have been the focus of much research (18,24). The μ-, δ-, and κ-subtypes act via supraspinal and/or spinal sites to modulate analgesia measured by thermal, chemical, and pressure stimuli (15,21,23,25,30). Euphoria is mediated by the μ-, and dysphoria by the κ-, opioid receptor (22). The respiratory depression produced by opioid agonists is mediated by the μ_2-subtype (17), and the δ-receptor is hypothesized to play a role in endotoxic and hemorrhagic shock (1). Agonists at κ-receptors exhibit anticonvulsant (26,27), diuretic (13,28), and neuroprotective (2) properties. Lastly, feeding behavior is stimulated by agonists acting at all three opioid receptor subtypes (9).

As part of our drug discovery program searching for opioid antagonists with appetite suppressant properties, several oxime derivatives of naltrexone were synthesized as novel opioid antagonists (6,19) and originally tested for antagonist properties in a variety of in vitro and in vivo assays. Interestingly, two of these compounds, 6-[2-phenylethyl]-oximino naltrexone (NPC 831) and 6-[3-phenylpropyl]-oximino naltrexone (NPC 836) (Fig. 1) demonstrated potent agonist activity in the tail-flick and acetic acid-induced writhing assays. In this article, we describe the binding profile of these compounds at opioid receptor subtypes, their analgesic effects in measures of chemical and thermal pain, and their ability to inhibit bombesin-induced scratching, a measure of κ-agonist activity in vivo (7).

METHOD

Animals

Male Sprague-Dawley rats (180–350 g), mice (CF-1 strain, 20–25 g), or guinea pigs (300–500 g) from Charles River were used in the assays described below. Unless otherwise stated, animals were housed in group cages and had free access to chow and tap water. Animals were maintained on a 12 L : 12 D cycle at constant temperature and humidity.

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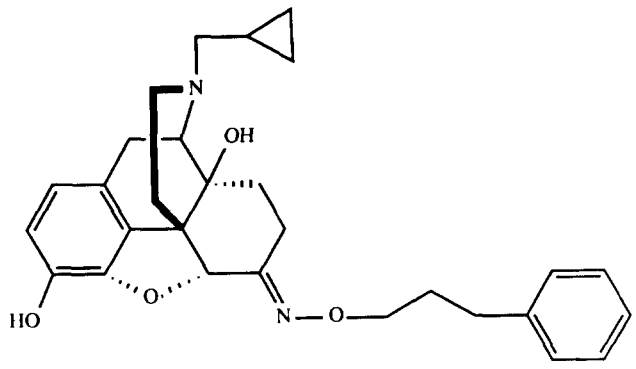
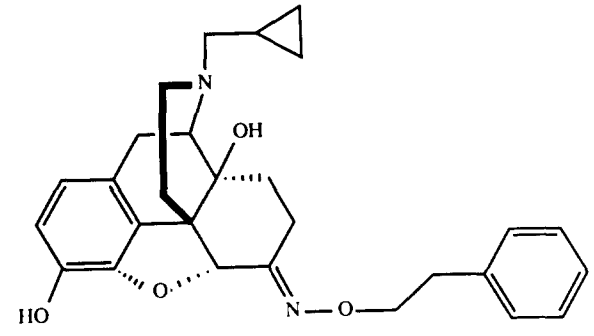


NPC 836



NPC 831

FIG. 1. Structures of 6-[2-phenylethyl]-oximino naltrexone (NPC 831) and 6-[3-phenylpropyl]-oximino naltrexone (NPC 836).

Receptor Binding Assays

The binding of [^{3}H]*D*-Ala^2-MePhe^4-Gly-ol^5-enkephalin (DAGO) and [^{3}H]*D*-Ala^2-*D*-Leu^5-enkephalin (DADLE) to μ- and δ-receptors was performed as follows. Rats were sacrificed by decapitation and forebrain was dissected and homogenized in Tris-HCl buffer (50 mM; pH 7.4 at room temperature) at a concentration of 40 mg/ml using a Polytron (Brinkmann Instruments, Westbury, NY) (setting 5–6, 20 s). Following this, the suspension was centrifuged at 40,000 × *g* at 4°C for approximately 10 min. The above centrifugation procedure was repeated once. The tissue suspension was then incubated at 37°C for 10 min and washed and centrifuged twice as described above. The pellet was resuspended in Tris-HCl buffer and stored on ice until use. Each assay tube contained 100 μl of either [^{3}H]DAGO or [^{3}H]DADLE at a final concentration of 1 nM, 100 μl of various concentrations of the compounds of interest, 1 ml tissue to a final concentration of 20 mg/ml, and sufficient buffer to a final assay volume of 2 ml. Nonspecific binding was defined by addition of 2 × 10^{-4} M naloxone to blank tubes. All tubes were incubated at room temperature for 90 min before termination of the reaction.

The binding to κ-opioid receptors was performed with [^{3}H]ethylketocyclazocine (EKC) and [^{3}H]U-69593 as ligands. After guinea pigs were sacrificed by decapitation, cerebella were removed and homogenized in 100 vol cold Tris-HCl buffer (50 mM, pH 7.4 at room temperature) using a Polytron (setting 7, 30 s). The homogenate was centrifuged at 49,000 × *g* for 10 min and then incubated at 37°C for 1 h with occasional stirring. Following this, centrifugation was repeated once as described above and the pellet was resuspended at a concentration of 35 mg/ml with the addition of bacitracin (0.25 mg/ml) to the homogenate. Each assay tube contained 100 μl of either [^{3}H]EKC at a final concentration of 0.23 nM or [^{3}H]U-69,593 at a final concentration of 1 nM, 100 μl of various concentrations of the compounds of interest, 1 ml of the homogenate to a final tissue concentration of 17.5 mg/ml, and sufficient buffer to a final assay volume of 2 ml. Nonspecific binding was determined using 10 μM U-50,488H. For [^{3}H]EKC binding, final concentrations of 100 nM DAGO and 500 nM DADLE were added to all tubes to block binding of the [^{3}H]EKC to μ- and δ-receptor sites. All tubes were incubated for 90 min and the reaction was terminated.

All incubations were conducted in triplicate and terminated by rapid filtration over Whatman GF/B glass fiber filters (Whatman, Clifton, NJ) using a Brandel cell harvester. Following addition of scintillation cocktail, samples were allowed to equilibrate for at least 3 h. The amount of bound radioactivity was determined by liquid scintillation spectrometry, and the potencies of the various drugs to inhibit binding were determined. Inhibition constants (K_i values) were calculated using the EBDA program (20).

Analgesic Properties in the Tail-Flick Assay

Tail-flick latencies were determined as described by D'Amour and Smith (5) and quantitated using a Columbus Instrument Analgesia Testing Device (Columbus, OH), with the lamp intensity set to produce a flick latency of 4.5–5.5 s in naive rats. Rats were lightly restrained by enclosure in a cloth bag, and the lamp intensity was focused on an area approximately one third of the distance from the tip of the tail. A flick latency of 15 s was considered a maximal response, and the trial was terminated. Tail-flick latencies were determined immediately prior to as well as 40 min after administration of the agonist, with the order of testing counterbalanced across treatment groups. All drugs were dissolved in distilled water or saline and administered IP in volumes of 1–2 ml/kg. The compounds were also tested for antagonism of morphine-induced analgesia as described by DeHaven-Hudkins et al. (6).

Individual postdrug latencies were determined and expressed as a percentage of the maximal possible effect (% MPE) using the following formula:

$$100 \times \frac{\text{(post drug latency} - \text{baseline latency)}}{\text{(15} - \text{baseline latency)}} .$$

ED_{50} values for each antagonist were calculated using the % MPE values from the log dose-response curves by least-squares regression analysis.

Analgesic Properties in the Acetic Acid-Induced Writhing Assay

Mice were divided into treatment groups and administered various doses of test compounds SC in a volume of 10 ml/kg

of either distilled water or saline 30 min prior to testing. Five minutes prior to testing, a 0.6% acetic acid solution was administered IP in a volume of 10 ml/kg. Mice were then placed into observation chambers and the number of writhing responses was recorded for 10 min. A writhing response consisted of full hind-limb extension and retraction. The mean number of writhing responses was calculated for vehicle-treated control mice, and the percent inhibition (% I) of writhing was calculated for each mouse that was treated with drug using the following formula:

$$\%\ I = 100 \times \frac{(\text{mean control writhing responses} - \text{individual test responses})}{\text{mean control writhing responses}}$$

ED_{50} values were calculated from the mean % I values for each group from the log dose–response curves by least-squares regression analysis.

Acetic Acid-Induced Writhing Assay With 24-H β-Funaltrexamine Pretreatment

The evaluation of acetic acid-induced writhing was determined as above except separate groups of animals were pretreated with either distilled water or the irreversible μ-antagonist β-funaltrexamine (β-FNA, 80 mg/kg, SC) 24 h prior to testing (29,31). ED_{50} values were calculated from the mean % I values for each group from the log dose–response curves by least-squares regression analysis.

Bombesin Scratch Test

Rats were implanted ICV with cannulae made from polyethylene tubing. The coordinates for implantation were 2 mm lateral to the sagittal suture and 1 mm posterior to bregma. The cannulae tips were 3.5–4.0 mm long and cut at a 45° angle. Following surgery, rats were individually housed with free access to food and water and allowed to recover for at least 5 days. The testing procedure used was essentially that of Gmerek and Cowan (7). Rats were injected SC with various doses of the test compounds 30 min prior to testing, and all drugs were administered in a volume of 1 ml/kg distilled water. At the time of testing, each rat was injected with the standard submaximal dose of bombesin (0.1 μg in 3 μl ICV over 20 s). One group received a saline injection as a control. A 1-ml injection of saline followed each injection of bombesin to flush drug from the cannula. Four animals were observed at one time and injections were counterbalanced across each period of observation. Each rat was observed for 5 of every 20 s for a total of 30 min. A positive score was given for grooming behavior exhibited within that 5 s, such as scratching, licking, and washing, with a maximum possible score of 90 points. The number of positive scores was converted into percent antagonism (% A) of grooming as follows:

$$\%\ A = 1 - \frac{(\text{score in drug-pretreated rats} - \text{saline controls})}{(\text{score in vehicle-pretreated rats} - \text{saline controls})} \times 100$$

A_{50} values for each drug were calculated from the mean % A values for each group from the log dose–response curves by least-squares regression analysis.

RESULTS

The receptor binding data for the two NPC compounds and the reference drugs morphine, naltrexone, and U-50,488H are presented in Table 1. In general, NPC 831 and NPC 836 were equipotent or slightly more potent than naltrexone to displace binding to opioid receptor subtypes. However, both NPC 831 and NPC 836 were more potent than morphine or U-50,488H to displace binding to the μ-, δ-, or κ-subtypes. Unlike U-50,488H and morphine, both NPC compounds were not remarkably selective for any receptor subtype.

In contrast to other compounds of this series (6,19), both NPC 831 and NPC 836 exhibited analgesic properties in the tail-flick and acetic acid-induced writhing assays (Table 2). NPC 836 was equipotent to morphine and NPC 831 was approximately twofold less potent than morphine in the tail-flick assay. In the writhing assay, NPC 836 was 25-fold and NPC 831 10-fold more potent than morphine. Further, NPC 836 was 100-fold and NPC 831 40-fold more potent than the selective κ-agonist U-50,488H (28). Neither compound antagonized morphine-induced analgesia at doses up to 5 mg/kg for NPC 831 and 10 mg/kg for NPC 836.

The agonist activities of both compounds in the acetic acid-induced writhing assay were further examined in experiments where mice were pretreated with the irreversible μ-antagonist β-FNA (30). As shown in Fig. 2, pretreatment with β-FNA

TABLE 1

POTENCIES OF VARIOUS COMPOUNDS TO INHIBIT SPECIFIC BINDING OF OPIOID SUBTYPE-SELECTIVE LIGANDS TO BRAIN MEMBRANES

	K_i (nM)			
Compound	μ [³H]DAGO	δ [³H]DADLE	κ [³H]EKC	κ [³H]U-69593
Morphine	4.26 ± 1.54	9.18 ± 1.45	125 ± 8.50	ND
Naltrexone*	0.60 ± 0.07	1.40 ± 0.71	0.51 ± 0.12	ND
U-50, 488H	1,560 ± 104	1,380 ± 262	1.61 ± 0.14	0.60 ± 0.03
NPC 831	0.88 ± 0.09	0.60 ± 0.04	0.28 ± 0.10	0.04 ± 0.02
NPC 836	0.32 ± 0.15	0.43 ± 0.03	0.12 ± 0.07	0.04 ± 0.01

Each value represents the mean ± SEM of at least three separate experiments performed in triplicate. ND, not determined.

*Data taken from DeHaven-Hudkins et al. (6).

TABLE 2

POTENCIES OF VARIOUS OPIOID AGONISTS TO PRODUCE ANALGESIA

Compound	ED_{50} (mg/kg)	
	Acetic Acid-Induced Writhing in the Mouse	Tail-Flick Response in the Rat
Morphine	0.50 (0.12–0.54)	2.60 (1.76–3.21)
U-50,488H	2.14 (1.19–2.96)	>30
NPC 831	0.05 (0.01–0.18)	4.02 (1.05–5.46)
NPC 836	0.02 (0.01–0.08)	2.24 (0.95–7.74)

Acetic acid-induced writhing: Drugs were injected SC followed 30 min later by IP injection of 0.6% acetic acid. The number of writhing responses that occurred during the 5–10 min following injection of acetic acid were recorded. n = 6–8 per group. Tail-flick response: Drugs were injected IP 40 min before testing in the tail-flick apparatus. n = 4–6 per group. Confidence limits are shown in parentheses.

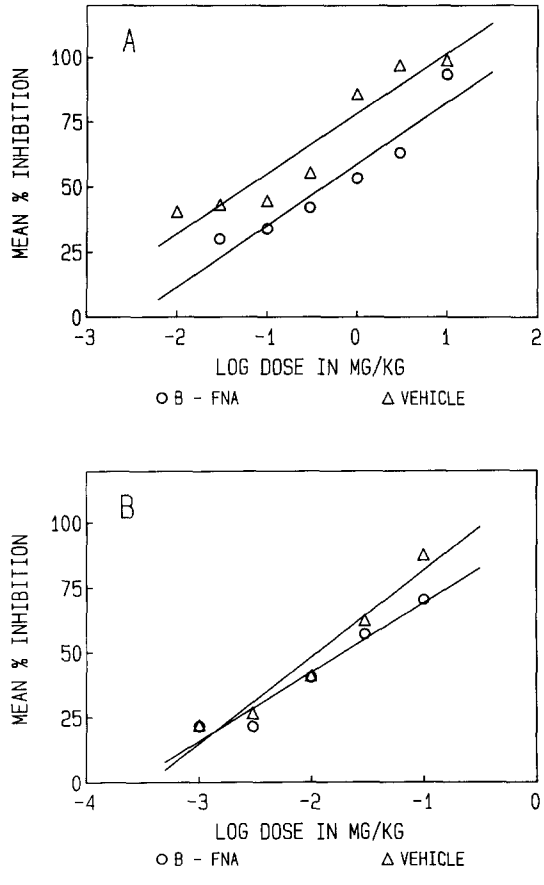


FIG. 2. Acetic acid-induced writhing assay with 24-h β-funaltrexamine (β-FNA) pretreatment. Mice were pretreated with either distilled water or β-FNA (80 mg/kg, SC) 24 h prior to testing. (A) 6-[2-Phenylethyl]-oximino naltrexone (NPC 831). ED_{50} without β-FNA pretreatment, 0.07 mg/kg (0.03–0.14); with β-FNA pretreatment, 0.44 mg/kg (0.22–0.86). (B). 6-[3-Phenylpropyl]-oximino naltrexone (NPC 836). ED_{50} without β-FNA pretreatment, 0.01 mg/kg (0.007–0.02); with β-FNA pretreatment, 0.02 mg/kg (0.01–0.03).

caused a rightward shift in the dose-response curve for NPC 831, with a sixfold decrease in the ED_{50} value from 0.07 mg/kg to 0.44 mg/kg. In contrast, no shift was observed for NPC 836 following β-FNA pretreatment (ED_{50} values of 0.01 and 0.02 mg/kg, respectively). The dose-response curve for NPC 836 in the writhing assay was shifted to the right when mice were pretreated with 1 mg/kg naloxone (ED_{50} = 0.9 mg/kg).

The bombesin scratch test has been proposed as a measure of κ-agonist activity (7) and was utilized here to further characterize the involvement of κ receptors in the actions of these drugs. Figure 3 presents the data for NPC 831 and NPC 836, where both compounds demonstrated κ-agonist activity in this assay. The A_{50} values were 1.86 mg/kg for NPC 831 and 0.08 mg/kg for NPC 836, compared to an A_{50} of 1.54 mg/kg for U-50,488H.

DISCUSSION

Although both NPC 831 and NPC 836 potently inhibited binding to all three opioid receptor subtypes in vitro, these compounds failed to demonstrate marked increases in potency or selectivity when compared to the opioid antagonist naltrexone. However, in comparison to the opioid agonists morphine and U-50,488H, NPC 831 and NPC 836 were at least 5-fold more potent than morphine, and over 1,000-fold more potent than the κ-agonist U-50,488H at μ-sites. The binding affinities of NPC 831 and NPC 836 at the μ-subtype were similar to the potent analgesic fentanyl [K_i = 1.6 nM (16)]. Both compounds were also more potent than morphine and U-50,488 at δ-sites (approximately 15- and 2,300-fold, respectively). At κ-sites labeled by [^{3}H]U-69,593 or [^{3}H]ethylketocyclazocine, both compounds were slightly more potent than U-50,488H, and this difference in potency was greater when [^{3}H]U-69,593 was used as a κ-ligand. Slight selectivity for the κ-subtype was also observed when [^{3}H]U-69,593 was used as the ligand, with NPC 831 exhibiting a 15-fold and NPC 836 an 8-fold selectivity for the κ-site. Although the binding affinity of NPC 831 and NPC 836 at κ-sites is slightly greater than that of newer

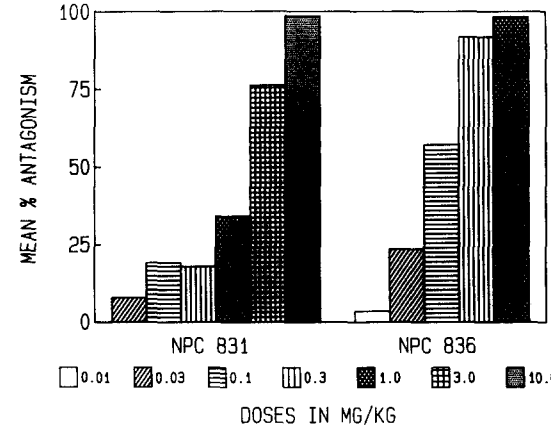


FIG. 3. Bombesin scratch test. Rats were injected SC with various doses of the test compounds at 30 min prior to ICV administration of bombesin (0.1 μg). Data are presented as percent antagonism (% A) values. A_{50} values were: (6-[2-Phenylethyl]-oximino naltrexone (NPC 831), 1.86 mg/kg; 6-[3-Phenylpropyl]-oximino naltrexone (NPC 836), 0.08 mg/kg.

κ-agonists such as CI-977, PD-117302, and spiradoline (12), the selectivity of NPC 831 and NPC 836 for the κ-opioid subtype is at least 100-fold less than that of other κ-agonists.

The profiles of NPC 831 and NPC 836 in measures of thermal and chemical pain were consistent with both μ- and κ-agonism. Like morphine, NPC 831 and NPC 836 potently inhibited the tail-flick response in the rat, while the κ-agonist U-50,488H was inactive. In comparison to other opioid agonists, fentanyl [0.017 mg/kg (21)] was more potent and morphine (Table 2) and buprenorphine [1.6 mg/kg (4)] approximately equipotent to the two NPC compounds. The κ-agonists are less sensitive to this procedure (11,15,21,28).

Both NPC compounds were also potent inhibitors of the writhing response in the mouse and were more potent than either morphine or U-50,488H. The agonism produced by NPC 831 in the writhing assay was partially attributable to its μ-activity, whereas NPC 836 appeared to be a pure κ-agonist in this assay. Pretreatment with β-FNA shifted the dose-response curve for NPC 831 to the right, whereas a decrease in potency was not observed for NPC 836 following β-FNA pretreatment. However, pretreatment with naloxone caused a pronounced shift in the ED_{50} for NPC 836, confirming an opioid mechanism of action for the analgesia produced by this compound. Following SC administration to mice, the potencies of the NPC compounds were comparable to the μ-agonist fentanyl [0.026 mg/kg (31)] and the mixed agonist-antagonists buprenorphine [0.014-0.043 mg/kg (4,31)], ethylketocyclazocine [0.034 mg/kg (31)], and cyclazocine [0.05 mg/kg (31)], and were more potent than the κ-agonist U-50,488H (Table 2) in the acetic acid-induced writhing assay. Although NPC 831 and 836 were more potent than κ-agonists such as PD 117302 [0.8 mg/kg (14)] and spiradoline [0.3 mg/kg (12)], both compounds were at least 10-fold less potent than CI-977 [0.005 mg/kg (12)] and at least 800-fold less than GR 103545 [0.00025 mg/kg (10)] in the writhing assay when acetylcholine was used as the stimulus.

Bombesin-induced scratching was originally proposed as a behavioral measure of benzomorphan-selective analgesics, as only those compounds that were benzomorphan analgesics were able to antagonize the scratching response to ICV administration of bombesin (7). In their original report, Gmerek and Cowan (7) demonstrated that other nonselective opioid agonists and antagonists, compounds with μ- and/or δ-selectivity, and SKF 10,047, a benzomorphan compound with σ-selectivity, were ineffective in antagonizing the scratching produced by bombesin. Subsequently, these investigators expanded their studies and determined that the κ-selective, nonbenzomorphan agonists tifluadom and U-50,488 also inhibited the response to bombesin (3). Together, their data suggested that the bombesin scratch test was a new measure of κ-agonist activity in vivo. Both NPC compounds were potent to antagonize bombesin-induced scratching, suggesting that these drugs possess κ-agonist activity in vivo. The A_{50} value for NPC 831 was equivalent to that of U-50,488H, while NPC 836 was approximately 20 times more potent than U-50,488H in this assay. NPC 836 was also approximately four-fold more potent than the benzomorphans ethylketocyclazocine or bremazocine in this assay (3).

In summary, the 2-phenylethyl and 3-phenylpropyl oxime derivatives of naltrexone inhibited binding to the μ-, δ-, and κ-opioid receptor subtypes in vitro and were potent opioid agonists in vivo when tested in the tail-flick and acetic acid-induced writhing assays. Like other κ-opioid agonists, NPC 831 and NPC 836 inhibited bombesin-induced scratching behavior. The agonist properties of these compounds are attributable to their actions in vivo at μ- and/or κ-opioid receptors. Given the unique profile of the compounds in vivo, NPC 831 and NPC 836 may prove to be useful tools with which to study opioid pharmacology.

ACKNOWLEDGEMENTS

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REFERENCES

1. Bernton, E. W.; Long, J. B.; Holaday, J. W. Opioids and neuropeptides: Mechanisms in circulatory shock. Fed. Proc. 44:290–299; 1985.
2. Birch, P. J.; Rogers, H.; Hayes, A. G.; Hayward, N. J.; Tyers, M. B.; Scopes, D. I. C.; Naylor, A.; Judd, D. B. Neuroprotective actions of GR89696, a highly potent and selective κ-opioid receptor agonist. Br. J. Pharmacol. 103:1819–1823; 1991.
3. Cowan, A.; Gmerek, D. E. In-vivo studies on kappa opioid receptors. Trends Pharmacol. Sci. 7:69–72; 1986.
4. Cowan, A.; Lewis, J. W.; MacFarlane, I. R. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. Br. J. Pharmacol. 60:537–545; 1977.
5. D'Amour, F. E.; Smith, D. L. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74–79; 1941.
6. DeHaven-Hudkins, D. L.; Brostrom, P. A.; Allen, J. T.; Lesko, L. J.; Ferkany, J. W.; Kaplita, P. V.; Mavunkel, B. J.; Rzeszotarski, W. J.; Steranka, L. R. Pharmacologic profile of NPC 168 (naltrexone phenyl oxime), a novel compound with activity at opioid receptors. Pharmacol. Biochem. Behav. 37:497–504; 1990.
7. Gmerek, D. E.; Cowan, A. In vivo evidence for benzomorphan-selective receptors in rats. J. Pharmacol. Exp. Ther. 230:110–115; 1984.
8. Goldstein, A.; James, I. F. Multiple opioid receptors: Criteria for identification and classification. Trends Pharmacol. Sci. 5: 503–505; 1984.
9. Gosnell, B. A.; Levine, A. S.; Morley, J. E. The stimulation of food intake by selective agonists of mu, kappa and delta opioid receptors. Life Sci. 38:1081–1088; 1986.
10. Hayes, A. G.; Birch, P. J.; Hayward, N. J.; Sheehan, M. J.; Rogers, H.; Tyers, M. B.; Judd, D. B.; Scopes, D. I. C.; Naylor, A. A series of novel, highly potent and selective agonists for the κ-opioid receptor. Br. J. Pharmacol. 101:944–948; 1990.
11. Hayes, A. G.; Sheehan, M. J.; Tyers, M. B. Differential sensitivity of models of antinociception in the rat, mouse and guinea-pig to μ- and κ-opioid receptor agonists. Br. J. Pharmacol. 91:823–832; 1987.
12. Hunter, J. C.; Leighton, G. E.; Meecham, K. G.; Boyle, S. J.; Horwell, D. C.; Rees, D. C.; Hughes, J. CI-977, a novel and selective agonist for the κ-opioid receptor. Br. J. Pharmacol. 101: 183–189; 1990.
13. Leander, J. D. A kappa opioid effect: Increased urination in the rat. J. Pharmacol. Exp. Ther. 224:89–94; 1983.
14. Leighton, G. E.; Johnson, M. A.; Meecham, K. G.; Hill, R. G.; Hughes, J. Pharmacological profile of PD 117302, a selective κ-opioid agonist. Br. J. Pharmacol. 92:915–922; 1987.
15. Leighton, G. E.; Rodriguez, R. E.; Hill, R. G.; Hughes, J. κ-Opioid agonists produce antinociception after i.v. and i.c.v. but not intrathecal administration in the rat. Br. J. Pharmacol. 93: 553–560; 1988.
16. Leysen, J. E.; Gommeren, W.; Niemegeers, C. J. E. [^{3}H]Sufentanil, a superior ligand for μ-opiate receptors: Binding properties and regional distribution in rat brain and spinal cord. Eur. J. Pharmacol. 87:209–225; 1983.

17. Ling, G. S. F.; Spiegel, K.; Lockhart, S. H.; Pasternak, G. W. Separation of opioid analgesia from respiratory depression: Evidence for different receptor mechanisms. J. Pharmacol. Exp. Ther. 232:149–155; 1985.
18. Martin, W. R. Pharmacology of opioids. Pharmacol. Rev. 35: 283–323; 1984.
19. Mavunkel, B. J.; Rzeszotarski, W. J. Oximes of oxymorphone, naltrexone and naloxone as potent, selective opioid receptor agonists and antagonists. U.S. Patent 4,760,069, July 6, 1988.
20. McPherson, G. A. A practical computer-based approach to the analysis of radioligand binding experiments. Comp. Prog. Biomed. 17:107–114; 1983.
21. Millan, M. J. Kappa-opioid receptor-mediated antinociception in the rat. I. Comparative actions of mu- and kappa-opioids against noxious thermal, pressure and electrical stimuli. J. Pharmacol. Exp. Ther. 251:334–341; 1989.
22. Millan, M. J. κ-Opioid receptors and analgesia. Trends Pharmacol. Sci. 11:70–76; 1990.
23. Millan, M. J.; Czlonkowski, A.; Lipkowski, A.; Herz, A. Kappa-opioid receptor-mediated antinociception in the rat. II. Supraspinal in addition to spinal sites of action. J. Pharmacol. Exp. Ther. 251:342–350; 1989.
24. Pasternak, G. W., ed. The opiate receptors. Clifton, NJ: The Humana Press; 1988.
25. Porreca, F.; Mosberg, H. I.; Omnaas, J. R.; Burks, T. F.; Cowan, A. Supraspinal and spinal potency of selective opioid agonists in the mouse writhing test. J. Pharmacol. Exp. Ther. 240:890–894; 1987.
26. Tortella, F. C.; Robles, L.; Holaday, J. W. U50,488, a highly selective kappa opioid: Anticonvulsant profile in rats. J. Pharmacol. Exp. Ther. 237:49–53; 1986.
27. VonVoigtlander, P. F.; Hall, E. D.; Camacho Ochoa, M.; Lewis, R. A.; Triezenberg, H. J. U-54494A: A unique anticonvulsant related to kappa opioid agonists. J. Pharmacol. Exp. Ther. 243: 542–547; 1987.
28. VonVoigtlander, P. F.; Lahti, R. A.; Ludens, J. A. U-50,488: A selective and structurally novel non-mu (kappa) opioid agonist. J. Pharmacol. Exp. Ther. 224:7–12; 1983.
29. Ward, S. J.; Portoghese, P. S.; Takemori, A. E. Pharmacological characterization in vivo of the novel opiate, β-funaltrexamine. J. Pharmacol. Exp. Ther. 220:494–498; 1982.
30. Ward, S. J.; Takemori, A. E. Relative involvement of mu, kappa and delta receptor mechanisms in opiate-mediated antinociception in mice. J. Pharmacol. Exp. Ther. 224:525–530; 1983.
31. Zimmerman, D. M.; Leander, J. D.; Reel, J. K.; Hynes, M. D. Use of β-funaltrexamine to determine mu opioid receptor involvement in the analgesic activity of various opioid ligands. J. Pharmacol. Exp. Ther. 241:374–378; 1987.
32. Zukin, R. S.; Zukin, S. R. The case for multiple opiate receptors. Trends Pharmacol. Sci. 5:160–164; 1984.