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Pharmacologic Profile of NPC 168 (Naltrexone Phenyl Oxime), A Novel Compound With Activity at Opioid Receptors

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DeHAVEN-HUDKINS, D. L., P. A. BROSTROM, J. T. ALLEN, L. J. LESKO, J. W. FERKANY, P. V. KAPLITA, B. J. MAVUNKEL, W. J. RZESZOTARSKI AND L. R. STERANKA. *Pharmacologic profile of NPC 168 (naltrexone phenyl oxime), a novel compound with activity at opioid receptors.* PHARMACOL BIOCHEM BEHAV **37**(3) 497–504, 1990.—NPC 168 (naltrexone phenyl oxime) was synthesized as a novel opioid antagonist and evaluated in several in vitro and in vivo assays. NPC 168 inhibited binding to the μ, δ and κ subtypes of the opioid receptor with nanomolar potencies. The potency of NPC 168 to antagonize morphine-induced analgesia was slightly less than that of naltrexone and nalmefene following either intraperitoneal (ED_{50} = 0.07 mg/kg) or oral (ED_{50} = 0.82 mg/kg) administration. The duration of action of NPC 168 was approximately 8 hr following subcutaneous administration, compared to 4 hr for nalmefene, to antagonize oxymorphonazine-induced analgesia. The long duration of action of NPC 168 was substantiated by pharmacokinetic data that demonstrated rapid uptake and slow clearance of NPC 168 from brain. NPC 168 (5, 10 and 20 mg/kg) also inhibited cumulative 6-hr food intake in rats that were deprived of food for 24 hr, but chronic administration of this compound to rats over a three-week period resulted in a marginal reduction in cumulative body weight gain. NPC 168 at doses of up to 10 mg/kg did not produce a conditioned taste aversion. However, NPC 168 was slightly more toxic than either naltrexone or nalmefene when administered parenterally, and as toxic as nalmefene when administered by the oral route. These data demonstrate that NPC 168 is a novel opioid antagonist with a longer duration of action than either naltrexone or nalmefene.

Opioid antagonist Naltrexone Nalmefene Receptor binding Feeding Analgesia
Conditioned taste aversion Toxicity

THE role of opioid peptides as mediators of feeding behavior has been well documented (15, 27, 28, 31, 43). Morphine, numerous opioid-like peptides and opioid agonists stimulate feeding under a variety of conditions (15, 28, 43). The regulation of food intake by opioid systems appears to be under the control of the μ, δ and κ subtypes of the opioid receptor, as agonists which are selective for each of these subtypes will also stimulate eating (10).

It has long been known that opioid antagonists inhibit food intake when tested in a variety of experimental models of feeding behavior (18, 19, 27, 28, 31, 43). Numerous animal studies have demonstrated that the opioid antagonists naloxone, naltrexone and nalmefene inhibit food intake in experimental models of feeding behavior (18, 19, 27, 28, 43) and that opiate antagonists such as naloxone inhibit feeding by selectively decreasing the intake of fat (23). Thus, the use of opioid antagonists for weight reduction has been proposed, based on the hypothesis that these agents can effectively inhibit appetite and will not exhibit the side effect liabilities associated with the use of amphetamines or serotonergic compounds (7,33).

Our synthetic efforts were directed toward developing a novel opiate antagonist for the treatment of obesity with the following characteristics: 1) nanomolar potency and nonselectivity for the opioid receptor subtypes, 2) oral activity, 3) in vivo potency, duration of action and a toxicity profile superior to naltrexone and nalmefene, and 4) appetite suppression at doses which are not aversive. In an attempt to improve upon the potency and duration of action of known opioid antagonists that exhibit anorectic properties, the phenyl oxime derivative of naltrexone, NPC 168 (Fig. 1), was synthesized and tested in several in vitro and in vivo assays to determine its pharmacological actions.

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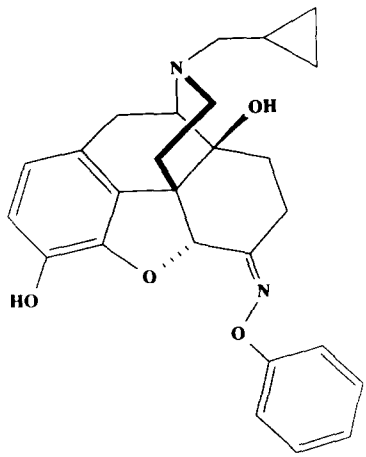

FIG. 1. Structure of NPC 168 (naltrexone phenyl oxime).

METHOD

General Method

Male Sprague-Dawley rats (175–350 g), CF-1 mice (20–25 g) or Hartley guinea pigs (300–500 g) were used in all experiments and were maintained on a 12-hr light-dark cycle at constant temperature and humidity. Animals were singly housed following cannulae implantation for intracerebroventricular (ICV) studies, and for feeding and conditioned taste aversion studies, but were group housed for all other experiments. Unless otherwise specified, animals had free access to chow and tap water.

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 (whole brain minus cerebellum and brainstem) 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 (setting 5–6, 20 sec). Following this, the suspension was centrifuged at 48,000 × g at 4°C for approximately 10 min, and the pellet was resuspended and centrifuged 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 of 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 10 μM naloxone to blank tubes. All tubes were incubated at room temperature for 90 min before termination of the reaction.

Guinea pig cerebellum was chosen as a source of tissue for κ opioid binding because of the low levels of κ receptors in rat brain (13,32). The binding to κ opioid receptors was performed with [^{3}H]U-69593 as the ligand, using a minor modification of the method of Lahti *et al.* (14). After guinea pigs were sacrificed by decapitation, cerebella were removed and homogenized in 100 volumes of cold Hepes-KOH buffer (50 mM, pH 7.4 at room temperature) using a Polytron (setting 7, 30 sec). The homogenate was centrifuged at 48,000 × g for 10 min, then incubated at 37°C for 1 hr 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 [^{3}H]U-69593 at a final concentration of approximately 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. Incubations were performed at 30°C for 90 min.

All incubations were conducted in triplicate and were terminated by rapid filtration over Whatman GF/B glass fiber filters using a Brandel cell harvester. For the [^{3}H]U-69593 assay, filters were presoaked in 0.05% polyethyleneimine for at least 2 hr. Following addition of scintillation cocktail, samples were allowed to equilibrate for 3 hr. The amount of bound radioactivity was determined by liquid scintillation spectrometry, and the potencies of the various drugs to inhibit binding were determined.

Implantation of Intracerebroventricular (ICV) Cannulae

Rats were anesthetized with 55 mg/kg pentobarbital, and cannulae made from polyethylene tubing were implanted intracerebroventricularly (ICV). The coordinates for implantation were 2 mm lateral to the sagittal suture and 1 mm posterior to bregma, at a depth of 3.6 mm from the top of the skull. Following surgery, the rats were individually housed with free access to food and water and allowed to recover for at least five days. Placements were randomly checked by infusion of dye and inspection of the brain after removal.

Antagonism of Morphine-Induced Analgesia

Tail-flick latencies were determined in rats as described by D'Amour and Smith (4) and quantitated using a Columbus Instruments Analgesia Testing Device (Columbus, OH), with the lamp intensity set to produce a flick latency of 4.0–5.0 sec in naive rats. A flick latency of 15 sec was considered a maximal response, and the trial was terminated. In experiments where drugs were administered ICV, animals were injected subcutaneously (SC) with 4 mg/kg of morphine immediately following the determination of their baseline tail-flick latencies, followed 20 min later by the ICV administration of 5 μl of the antagonist in a vehicle of saline. Tail-flick latencies were determined at 20 min following administration of the antagonist. In those experiments where the potencies of the antagonists were determined after peripheral administration, baseline flick latencies were measured and animals were then injected with the antagonist intraperitoneally (IP) or orally (PO), immediately followed by SC injection of 4 mg/kg morphine. Animals were tested at 40 min following morphine administration. Rats were food deprived overnight prior to oral drug administration. NPC 168 was dissolved in 0.3% acetic acid and administered in a volume of 4 ml/kg, and naltrexone and nalmefene were administered in a volume of 1 ml/kg of 0.9% saline.

The data for tail flick latencies were expressed as a percentage of the maximal possible effect (% MPE), calculated as:

$$100 \times \frac{(\text{postdrug latency} - \text{baseline latency})}{(15 - \text{baseline latency})}$$

Time Course of the Antagonism of Oxymorphonazine (OMZ)-Induced Analgesia

The long-acting opioid agonist OMZ (42) was used in time course studies to determine the duration of antagonism of analge-

sia for NPC 168 and nalmefene. Tail-flick latencies were determined as described above. The antagonists were administered to rats SC, immediately followed by SC treatment with 12 mg/kg of OMZ, and tail-flick latencies were recorded at 0.5, 1, 1.5, 2, 4, 6 and 8 hr after drug administration. NPC 168 was dissolved in 4 ml/kg of 0.3% acetic acid, and nalmefene was injected in a volume of 1 ml/kg of 0.9% saline. In order to control for the differences in potencies between the two antagonists, we administered various doses of each antagonist that would produce equivalent degrees of antagonism at 30 min following OMZ administration.

Pharmacokinetic Studies

A high pressure liquid chromatography (HPLC) method to measure concentrations of NPC 168 was developed, and the basic procedure was as follows. The mobile phase was a mixture of 650 ml of 10 mM phosphate buffer (pH 4.0), 350 ml of acetonitrile and 1 ml of 0.1 M sodium EDTA that was filtered through a 0.2 μm filter and degassed before use. Stock solutions of NPC 168 and the internal standard naltrexone-t-butyloxime were prepared in methanol and stored at −20°C. The samples or standards were prepared by addition of 0.1 ml of working internal standard solution and 0.5 ml of phosphate-buffered saline (0.01 M, pH 7.5, with 5 mM sodium azide) to 0.25 ml of sample or standard, and vortexed for 20 sec. Following this, samples or standards were extracted on a CN-Bondelut column, eluted with 1 ml of acetone and evaporated to dryness under nitrogen at 40°C. The dried residue was reconstituted in 0.2 ml of mobile phase, and 10–20 μl were injected into the HPLC. The flow rate was 1.2 ml/min, with retention times of 7.4 and 9.5 min for the internal standard and NPC 168, respectively. The column was at room temperature, the detector was set at +0.85 V versus an Ag/AgCl reference electrode, and the sensitivity was 20 nA full scale. The standard curve was constructed by linear regression of the peak height ratio of NPC 168/naltrexone-t-butyloxime (y axis) versus the concentration of NPC 168 (x axis) in plasma standards. The concentration of NPC 168 in unknown samples was calculated using the linear regression analysis equation generated from the standard curve.

A single dose of NPC 168 (15 mg/kg) was administered intravenously (IV) to 18 Sprague-Dawley rats. NPC 168 was formulated as an aqueous solution containing 9 mg/ml of NPC 168 and 10% Tween 80 to facilitate solubilization. Before dosing and at 1, 2, 4, 8, 24 and 32 hr after dosing, two rats were sacrificed and simultaneous blood and brain specimens were obtained. Blood samples were collected in heparinized tubes. Plasma was separated by centrifugation within 10–15 min after collection and frozen at −20°C until analysis. Brain tissue was rinsed, blotted dry, weighed and frozen for subsequent analysis of NPC 168.

Plasma samples were prepared for analysis as described above. Brain samples were prepared by homogenization in phosphate buffered saline (5 ml/g of tissue) using a Potter/Elvehjem tissue homogenizer. The internal standard solution (0.1 ml, 200 ng/ml in saline) and 1 ml of acetonitrile were added to 1 ml of brain homogenate and the mixture was vortexed for 15 sec; after centrifugation the supernatant was removed and concentrated to about half its volume by evaporation under nitrogen. Four ml of saline was added to the concentrated solution and this mixture was analyzed according to the plasma assay previously described. Standards were prepared to contain 5–1000 ng/ml of NPC 168 in blank brain homogenates and quantitation of NPC 168 in study specimens was based on peak height ratio and the standard curve.

Suppression of Feeding in 24-Hr Food-Deprived Rats

Rats had free access to powdered food and water for 7 days until food intake had stabilized, and animals were assigned to treatment groups matched on the basis of their 24-hr food consumption on the last baseline day. Rats were food deprived for 24 hr immediately before injection of the test compound. NPC 168 was administered SC in a vehicle of DMSO:water (1:1) at a volume of 3 ml/kg, and control animals were injected with 3 ml/kg of the vehicle. Food intakes (including spillage) and water intakes were recorded at 1300 and 1900 hr.

Chronic Administration of Drug in Schedule-Adapted Rats

Rats were allowed to eat on a daily 3-hr feeding schedule. Baseline data were collected for a period of 8 days, and animals were assigned to treatment groups matched on the basis of their food intake for the total 3-hr period. Animals were injected SC at 1300 hr with 5 mg/kg/day of either NPC 168 (in 2 ml/kg of 0.3% acetic acid) or naltrexone (in 1 ml/kg of 0.9% saline). The dose of both drugs was increased to 7.5 mg/kg/day from days 8–14, and to 10 mg/kg/day from days 15–21. Control rats were injected daily with the appropriate vehicles. Intakes were recorded at 1600 hr, and cumulative food intake and body weight gain were calculated for the entire period of drug administration.

Conditioned Taste Aversion

The procedure of Wagner *et al.* (40) was used to assess conditioned taste aversion. Rats were adapted to water deprivation by restricting their access to water to a single 30-min presentation occurring at the same time every day. Baseline water intakes were determined for 7 days until intakes had stabilized, and animals were assigned to treatment groups based on their intakes on the final baseline day. On the following day (day 8), all rats received a bottle of condensed milk (diluted 2:1 water:milk) in place of the water, and intakes were recorded for 30 min. Fifteen min after the milk was removed, all rats were administered an IP injection of vehicles, lithium chloride (LiCl; 12.7 mg/kg in a volume of 1 ml/kg of 0.9% saline) or various doses of NPC 168 (4 ml/kg of 0.3% acetic acid). This procedure was repeated on day 9. On day 10, all rats were given a two-bottle choice of water or the milk:water solution, and intakes were recorded for 30 min.

Acute Toxicity

Compounds were evaluated for acute toxicity in CF-1 mice and an approximate LD_{50} value was determined (17, 34, 44). Initially drugs were administered PO (3 mice per group) as a single dose of 500 mg/kg. The animals were observed for overt signs of toxicity (morbidity and mortality) over the following 14 days. When the animals did not show signs of morbidity and mortality at the end of day 1, a second group was administered 1000 mg/kg PO. When no effects were observed with the higher dose, the experiment was ended and the approximate LD_{50} was reported as >1000 mg/kg. When mice exhibited considerable morbidity and mortality at the end of day 1, additional groups of 3 mice each received doses of 250, 125, 62.5 and 31.25 mg/kg PO or until a nonlethal dose was determined. At the completion of the oral phase of experiments, the compounds were administered IP to separate groups of three mice, starting with 250 mg/kg, and the dosing schedule was repeated.

Data Analysis

Inhibition constants (K_i values) for the binding of test compounds to receptors were calculated using the EBDA program (20). ED_{50} values for each antagonist were calculated using the %

TABLE 1

POTENCIES OF VARIOUS OPIOID ANTAGONISTS TO INHIBIT THE SPECIFIC BINDING OF [^{3}H]DAGO, [^{3}H]DADLE AND [^{3}H]U-69593 TO μ, δ AND κ OPIOID RECEPTORS

	K_i (nM)		
	μ	δ	κ
Compound	[^{3}H]DAGO	[^{3}H]DADLE	[^{3}H]U-69593
NPC 168	0.33 ± 0.04	0.68 ± 0.07	1.59 ± 0.31
Naltrexone	0.60 ± 0.07	1.40 ± 0.71	0.51 ± 0.12
Nalmefene	0.64 ± 0.16	0.87 ± 0.11	0.24 ± 0.15

Rat forebrain was used as the source of membranes for the [^{3}H]DAGO and [^{3}H]DADLE assays, and guinea pig cerebellum was used as the source of membranes for the [^{3}H]U-69593 assay.

Each value represents the mean ± SEM of at least three individual experiments performed on separate occasions.

TABLE 2

ED_{50} VALUES FOR THE ANTAGONISM OF MORPHINE-INDUCED ANALGESIA IN RATS BY ADMINISTRATION OF VARIOUS OPIOID ANTAGONISTS

	ED_{50} Values		
Route of Administration	NPC 168	Naltrexone	Nalmefene
ICV (nmol)	2.2	2.7	ND
IP (mg/kg)	0.07	0.04	0.01
PO (mg/kg)	0.82	0.27	0.12

For ICV administration, rats were treated with each antagonist at 20 min following the SC injection of 4 mg/kg morphine. Tail-flick latencies were determined immediately prior to and 20 min after the administration of antagonist. For peripheral administration, rats were treated with each antagonist immediately followed by 4 mg/kg morphine, and tested at 40 min postinjection. N = 4–12 per dose group. ND = not determined.

MPE values from the log dose-response curves by least squares regression analysis. In experiments where the antagonism of analgesia was measured as a function of time, the tail-flick latency data were used to calculate the area under the time-effect curve by the computer programs of Tallarida and Murray (37). In the acute toxicity experiments, LD_{50} estimates for each drug (17, 29, 34) were calculated by means of the SAS Probit program (SAS Institute, Cary, NC). Analysis of variance was used for the analysis of all other data by means of the BMDP Statistical Software Package (UCLA, Los Angeles, CA), and post hoc comparisons were performed using the Tukey test (6).

RESULTS

The binding of NPC 168 to opioid receptors demonstrated a two- to five-fold selectivity for the μ and δ sites (Table 1). NPC 168 was twice as potent as naltrexone or nalmefene to inhibit [^{3}H]DAGO binding, and was also slightly more potent than the reference compounds to inhibit [^{3}H]DADLE binding to sites in rat forebrain. However, NPC 168 was less potent than naltrexone or nalmefene to displace binding to κ opioid receptors of guinea pig cerebellum.

Following ICV administration, NPC 168 was approximately equipotent to naltrexone to antagonize the analgesia produced by 4 mg/kg morphine (Table 2). However, NPC 168 was approximately two- to three-fold less potent than naltrexone, and seven-fold less potent than nalmefene, to antagonize morphine-induced analgesia when given by either the IP or PO routes of administration.

Figure 2 presents the tail-flick latency data for the time-dependent antagonism of OMZ-induced analgesia produced by administration of NPC 168 or nalmefene. Analysis of the areas under the curves for each dose group revealed significant differences for both NPC 168, $F(5,22) = 17.23$, $p<0.0001$, and nalmefene, $F(5,20) = 9.32$, $p<0.0001$. The mean levels of antagonism produced by NPC 168 for the eight-hr testing period were significantly different from vehicle at doses of 1.0, 2.5, 5.0 and 10.0 mg/kg ($p<0.01$, Tukey); this was also true for nalmefene at doses of 1.0 ($p<0.05$, Tukey) and 5.0 ($p<0.01$, Tukey).

An examination of the latency data suggested that at doses of 1 or 5 mg/kg of nalmefene, the antagonism of OMZ-induced analgesia began to subside by 3–4 hr. This was not the case with NPC 168, where a constant level of analgesia was observed for the 8 hr of testing, regardless of the dose.

The long duration of action of NPC 168 in the antagonism of OMZ-induced analgesia was confirmed by pharmacokinetic studies, which demonstrated that substantial concentrations of NPC 168 were present in brain from 24–32 hr after a single IV injection (Fig. 3A). The brain:plasma ratio data demonstrate the high capacity of brain tissue to retain NPC 168 during the 24–32 hr after its administration, and the clearance of NPC 168 from plasma appeared to be faster than the clearance from brain (Fig. 3B).

Figure 4 presents the data on the effects of NPC 168 on 6-hr food intakes in food-deprived rats. NPC 168 suppressed 6-hr food intake in rats that were deprived of food for 24 hr, $F(5,24) = 6.77$, $p = 0.0005$, and the amount of food eaten was decreased by approximately 40%, regardless of the dose administered. Post hoc analysis of the means revealed that the mean 6-hr intakes for all doses of NPC 168 were significantly different from the saline mean ($p<0.05$, Tukey), and that the 5, 10 and 20 mg/kg group means were significantly different from the vehicle mean ($p<0.05$, Tukey). Cumulative water intake at this time point was not affected by any dose of NPC 168, $F(5,24) = 0.85$, $p = 0.5259$ (data not shown).

Following chronic administration of either NPC 168 or naltrexone to rats adapted to a daily 3-hr feeding schedule, cumulative body weight gain, $F(3,23) = 2.95$, $p = 0.054$ (Fig. 5) and cumulative food intake, $F(3,23) = 3.00$, $p = 0.051$ (data not shown) were marginally decreased following treatment with either compound, and post hoc analysis did not reveal any significant differences between the individual group means.

The data from the conditioned taste aversion experiment are shown in Fig. 6. Analysis of variance revealed significant effects of treatment on milk intake, $F(5,24) = 10.86$, $p<0.0001$, and this effect was entirely due to the aversion produced by lithium chloride ($p<0.01$, Tukey). NPC 168 did not produce a conditioned taste aversion at the lower doses which caused suppression of appetite, suggesting that the anorectic properties of this compound were not due to general malaise.

The estimated LD_{50} values for naltrexone, nalmefene and NPC 168 after oral or intraperitoneal administration to mice are shown in Table 3. Oral doses of naltrexone produced tremors and seizures, but were not lethal at doses up to 1000 mg/kg PO. Intraperitoneal administration of naltrexone caused lethality at doses of 500 mg/kg, but produced ptosis, tremors and seizures at lower doses. Nalmefene elicited decreases in activity, tremors and seizures after oral administration. The compound was lethal at doses of 500 mg/kg PO or greater and the LD_{50} was estimated between 500–1000 mg/kg PO. Nalmefene was more toxic when injected IP, causing death at doses of 250 mg/kg or greater, and

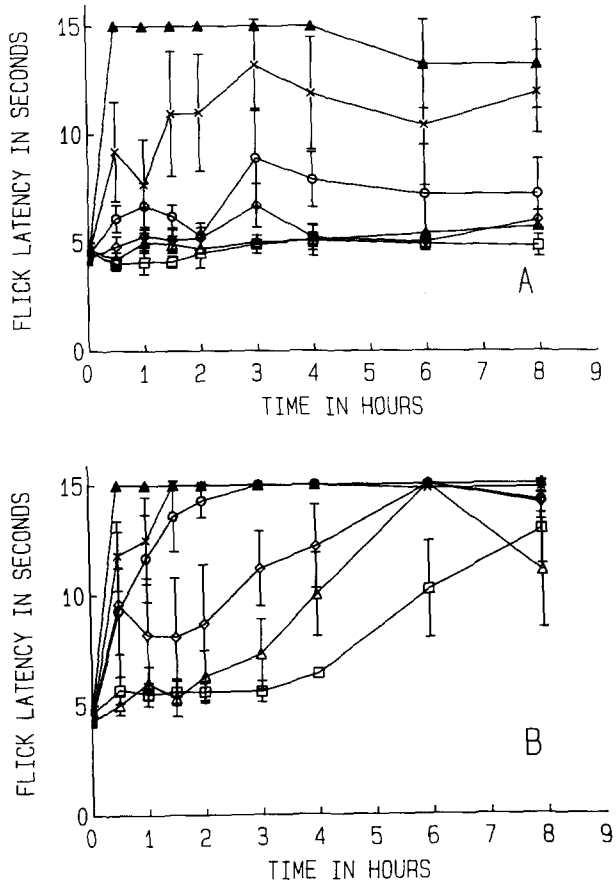

FIG. 2. Time course of the effects of NPC 168 or nalmefene administered SC on OMZ-induced analgesia in rats. Antagonists were administered to rats SC, immediately followed by SC treatment with 12 mg/kg of OMZ, and tail-flick latencies were recorded at 0.5, 1, 1.5, 2, 4, 6 and 8 hr after drug administration. Data are expressed as the mean ± SEM flick latencies in sec for each dose group at the individual time points. (A) NPC 168. ▲ = Vehicle. × = 0.5 mg/kg. ○ = 1.0 mg/kg; ◇ = 2.5 mg/kg; △ = 5.0 mg/kg; □ = 10.0 mg/kg. (B) Nalmefene. ▲ = Vehicle. × = 0.05 mg/kg. ○ = 0.1 mg/kg; ◇ = 0.5 mg/kg; △ = 1.0 mg/kg; □ = 5.0 mg/kg. N = 4–5 per group.

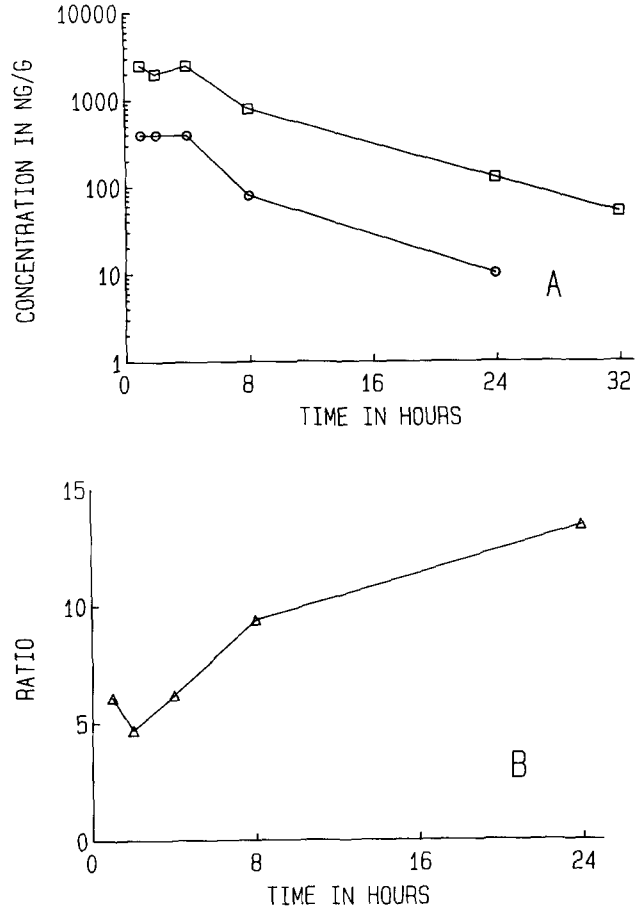

FIG. 3. Time course of the concentrations of NPC 168 in brain homogenates and plasma. NPC 168 (15 mg/kg) was administered intravenously (IV) to 18 Sprague-Dawley rats. Before dosing and at 1, 2, 4, 8, 24 and 32 hr after dosing, two rats were sacrificed and simultaneous blood and brain specimens were obtained. Data represent the mean values for two rats at each time point. (A) Total concentrations of NPC 168 expressed as ng/g, where □ = concentrations in brain and ○ = concentrations in plasma. (B) Brain:plasma ratios of NPC 168.

producing ptosis, decreases in activity, tremors and seizures. The LD_{50} for nalmefene was estimated as between 125–250 mg/kg IP. Animals that received oral doses of NPC 168 exhibited ptosis, decreases in activity, tremors and seizures. NPC 168 was lethal at doses of 500 mg/kg and greater; the LD_{50} was estimated as between 500–1000 mg/kg PO. Like naltrexone and nalmefene, NPC 168 was also more toxic after IP administration, also producing ptosis, decreased activity, tremors and seizures, but causing death at doses of 125 mg/kg IP. The LD_{50} was estimated as between 62.5–125 mg/kg IP.

DISCUSSION

Naltrexone was originally synthesized as an attempt to improve upon the pharmacological activity of the opiate antagonist naloxone. Although naloxone is potent, its duration of action is extremely short and its oral bioavailability is poor (2). Substitution of a cyclopropyl moiety for the ethylene group of naloxone resulted in the compound naltrexone, with a substantial improvement in the oral activity and duration of action when compared to the parent compound, as reflected in a half-life of 4–10 hr and rapid and complete absorption from the gastrointestinal tract (2). Studies in animals (28,31) demonstrated that administration of naltrexone, like naloxone, inhibited food intake, although clinical trials of naltrexone for the treatment of obesity yielded conflicting results (1, 8, 21, 26).

Nalmefene, with a methylene substitution at the 6 position of naltrexone, was equipotent to naltrexone to displace binding to μ receptors, and roughly two-fold more potent than naltrexone to displace binding to δ and κ opioid receptors (25). Further, the potency of nalmefene to inhibit the antagonism produced by 6-azidomorphine on electrically stimulated contractions of the guinea pig ileum-longitudinal muscle was also two-fold greater than naltrexone (39). Nalmefene proved to be superior to naltrexone in vivo because of its potency to antagonize morphine-induced analgesia after oral administration (11) and its long half-life after IV injection in humans (5). Nalmefene effectively decreased meal size in obese rats at a dose of 1 mg/kg, and decreased food and water intake and weight gain during a three-week period in obese rats (18).

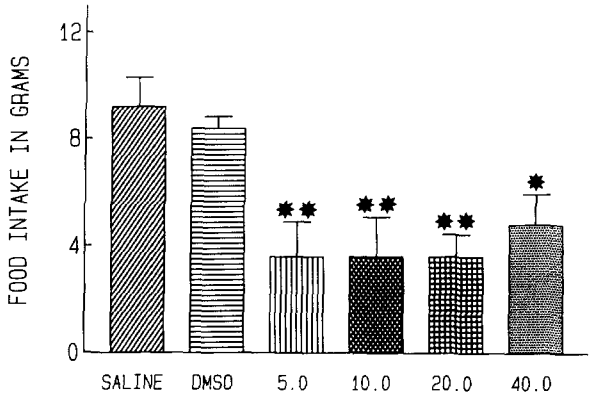

FIG. 4. Effects of NPC 168 on food intake in rats deprived of food for 24 hr. NPC 168 was injected SC immediately before reintroduction of food, and doses are expressed in mg/kg. Data are expressed as cumulative 6-hr food intakes in g and represent the mean ± SEM of 5 rats per group. **Significantly different from saline, $p<0.01$, and DMSO, $p<0.05$, Tukey. *Significantly different from saline, $p<0.05$, Tukey.

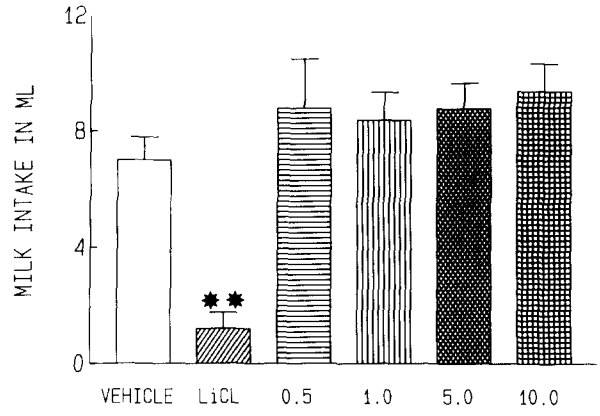

FIG. 6. Effects of NPC 168 or lithium chloride on the formation of a conditioned taste aversion in rats. Various doses of NPC 168 or LiCl (12.7 mg/kg) were injected IP on two consecutive days immediately following a 15-min access period to a milk:water solution. On the third day, all rats were given a two-bottle choice of water or the milk:water solution, and intakes were recorded for 30 min. Doses of NPC 168 are expressed in mg/kg, and data represent the mean ± SEM of 5 animals per group. **$p<0.01$, Tukey.

NPC 168, the o-phenyl oxime of naltrexone, was synthesized by the reaction of naltrexone with o-phenylhydroxylamine as described (24). This compound possessed pharmacological activity that was generally consistent with the actions of an opioid antagonist and that was similar to both naltrexone and nalmefene. The compound demonstrated nanomolar potency to displace binding to the μ and δ opioid receptor subtypes and was more potent than naltrexone or nalmefene. In contrast, NPC 168 was less potent than the reference drugs to displace binding to the κ receptor. NPC 168 was equipotent to naltrexone after ICV administration, but less potent than both reference compounds to antagonize morphine-induced analgesia in the tail-flick procedure when administered IP or PO. Further, NPC 168 generally was more toxic than either naltrexone or nalmefene. The primary improvement in the pharmacological activity of NPC 168 compared to naltrexone and nalmefene was that the duration of action of NPC 168 to antagonize the analgesia produced by OMZ was longer than that of nalmefene, and pharmacokinetic studies confirmed that concentrations of NPC 168 were present in plasma and brain at detectable levels for up to 24 hr after in vivo administration. Also, like other opioid antagonists, NPC 168 was an effective appetite suppressant in food-deprived rats at doses that did not produce a conditioned taste aversion.

The marginal effects of either NPC 168 or naltrexone to cause a decrease in cumulative body weight following chronic administration were somewhat surprising, but may be due to the fact that lean rats were used in these studies. The opioid antagonists naloxone, naltrexone and nalmefene inhibit weight gain in genetically obese rats or in rats that have been rendered obese by feeding of a cafeteria diet, but the inhibition of weight gain in lean animals generally is not as pronounced, and at times has been difficult to demonstrate at all (19, 27, 28, 31). It has been suggested that with chronic treatment, opioid antagonists may be less effective in animals of normal size and physiology, since opioid antagonists may act to decrease body weight in the obese animals by a combination of lowering food intake and increasing energy expenditure (27). Alternatively, it is possible that the doses used or the length of time of drug administration in our study were insufficient to produce significant changes in body weight.

The prolonged duration of action of NPC 168 to antagonize analgesia and feeding behavior is reminiscent of that of other long-acting or irreversible opioid antagonists. The irreversible opioid antagonists β-chlornaltrexamine [β-CNA; (30)] and the

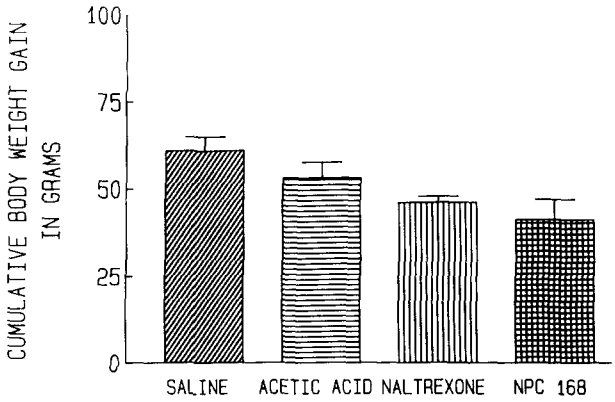

FIG. 5. Effects of chronic administration of NPC 168 or naltrexone on cumulative body weight gain in rats adapted to a 3-hr feeding schedule. Animals were injected SC with 5 mg/kg/day of either NPC 168 or naltrexone. The dose of both drugs was increased to 7.5 mg/kg/day from days 8–14, and to 10 mg/kg/day from days 15–21. Control rats were injected daily with the appropriate vehicles. Data are expressed as cumulative body weight gain in grams and represent the mean ± SEM of 6–7 animals per group.

TABLE 3

ACUTE TOXICITY OF OPIOID ANTAGONISTS IN MALE CF-1 MICE

Route of Administration	Estimated LD_{50} (mg/kg) NPC 168	Naltrexone	Nalmefene
IP	62.5–125	250–500	125–250
PO	500–1000	>1000	500–1000

See text for experimental details. N = 3 per dose group.

μ-selective compound β-funaltrexamine [β-FNA; (41)] effectively antagonized morphine-induced analgesia in mice (41) and rats (12) for 48 to 72 hr. When administered ICV, β-CNA (10 μg) suppressed food intake in rats deprived of food for 24 hr, and studies with ICV administration of selective agonists demonstrated that the effect of β-CNA lasted for approximately three days and was not selective for μ, δ or κ opiate receptors (9). Ukai and Holtzman (38) also reported long-lasting antagonism of food intake with ICV administration of 5 μg β-FNA to free-feeding rats, which took 24 hr to become apparent. Naloxonazine given ICV (36) or IV (16) at 24 hr before morphine challenge effectively antagonized analgesia, with a prolonged duration of action that was attributed to an irreversible binding to μ_1 sites and not to a slow rate of elimination (16). This compound inhibited feeding in free-feeding or food-deprived rats following administration of 10 or 20 mg/kg IV (36), and daily administration of 10 mg/kg IV of naloxonazine over a 14-day period suppressed food intake by 24% and body weight gain by 53% in rats of comparable size to the ones used in these studies (22). We have observed that naloxonazine at doses of 2 to 20 mg/kg SC suppressed food intake at shorter time intervals in food-deprived rats, and that only the 20 mg/kg dose produced a CTA (3).

Direct comparisons among all of the irreversible antagonists and NPC 168 cannot be made due to the variety of experimental designs, testing paradigms and routes of administration that were used. The antagonism of OMZ-induced analgesia produced by SC administration of NPC 168 lasted up to 8 hr, and preliminary pharmacokinetic studies by the IV route using direct measurement of NPC 168 concentrations indicated that its rate of elimination from brain and plasma was quite slow (Fig. 3). In contrast, the half-life for elimination of [^{3}H]naloxonazine (10 mg/kg IV) from plasma was approximately 3 hr, with undetectable levels in brain at 60 min (16). The use of radiolabelled naloxonazine as an indicator of plasma concentrations of naloxonazine did not permit direct measurement of parent compound, and these investigators did not measure brain levels at longer time intervals, so it is unknown if naloxonazine accumulates into brain in a manner similar to NPC 168. Although we did not systematically study the effects of NPC 168 at longer time intervals, based on the pharmacokinetic data one would anticipate the duration of action of this compound to be longer than 8 hr. We do not know to what extent metabolism or irreversible binding might contribute to the pharmacological actions of NPC 168, so that the precise mechanism for the duration of action of NPC 168 is unknown at this time.

In summary, the current results demonstrate that the phenyl oxime derivative of naltrexone, NPC 168, antagonized both morphine- and OMZ-induced analgesia, and the duration of action of NPC 168 to antagonize OMZ-induced analgesia was longer than either naltrexone or nalmefene when doses were adjusted for antagonist potency. Lower doses of NPC 168 that suppressed feeding did not result in the production of a conditioned taste aversion. This compound also demonstrated an acute toxicity profile that was slightly worse than the comparison drugs naltrexone or nalmefene. Thus, NPC 168 represents a novel compound with antagonist activity at opioid receptors.

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