

Nalmefene Induced Elevation in Serum Prolactin in Normal Human Volunteers: Partial Kappa Opioid Agonist Activity?

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In humans, mu- and kappa-opioid receptor agonists lower tuberoinfundibular dopamine, which tonically inhibits prolactin release. Serum prolactin is, therefore, a useful biomarker for tuberoinfundibular dopamine. The current study evaluated the unexpected finding that the relative mu- and kappa-opioid receptor selective antagonist nalmefene increases serum prolactin, indicating possible kappa-opioid receptor agonist activity. In all, 33 healthy human volunteers (14 female) with no history of psychiatric or substance use disorders received placebo, nalmefene 3 mg, and nalmefene 10 mg in a double-blind manner. Drugs were administered between 0900 and 1000 on separate days via 2-min intravenous infusion. Serial blood specimens were analyzed for serum levels of prolactin. Additional *in vitro* studies of nalmefene binding to cloned human kappa-opioid receptors transfected into Chinese hamster ovary cells were performed. Compared to placebo, both doses of nalmefene caused significant elevations in serum prolactin ($p < 0.002$ for nalmefene 3 mg and $p < 0.0005$ for nalmefene 10 mg). There was no difference in prolactin response between the 3 and 10 mg doses. Binding assays confirmed nalmefene's affinity at kappa-opioid receptors and antagonism of mu-opioid receptors. [³⁵S]GTPγS binding studies demonstrated that nalmefene is a full antagonist at mu-opioid receptors and has partial agonist properties at kappa-opioid receptors. Elevations in serum prolactin following nalmefene are consistent with this partial agonist effect at kappa-opioid receptors. As kappa-opioid receptor activation can lower dopamine in brain regions important to the persistence of alcohol and cocaine dependence, the partial kappa agonist effect of nalmefene may enhance its therapeutic efficacy in selected addictive diseases.

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INTRODUCTION

Three opioid antagonists, naloxone, naltrexone, and nalmefene, are currently approved for human use. Naloxone and nalmefene have been approved for parenteral administration and are used in the reversal of opioid (eg heroin) overdose. Naltrexone is available for oral administration and is approved for the treatment of opioid and alcohol dependence. Naltrexone's effectiveness is poor for the former and moderate for the latter (San *et al*, 1991; Volpicelli *et al*, 1992; O'Malley *et al*, 1992, 2002). Nalmefene also has a moderate treatment effect for alcoholism but is not yet available for this indication (Mason *et al*, 1994; Anton *et al*, 2004).

Naloxone, naltrexone, and nalmefene are moderately selective ligands for the three opioid receptor subtypes

(mu, kappa, and delta). While in the rat each antagonist has highest affinity for mu-opioid receptors, in non-human primates and humans they have similar affinity at mu- and kappa-opioid receptors followed by much lower affinity at delta-opioid receptors (Michel *et al*, 1985; Raynor *et al*, 1994; Emmerson *et al*, 1994; Toll *et al*, 1998). Respective reported K_i (nM) values for naloxone, naltrexone, and nalmefene at cloned human receptors expressed on Chinese hamster ovary (CHO) cells are: 1.4 at mu, 2.5 at kappa, and 67.5 at delta; 0.2 at mu, 0.4 at kappa, and 10.8 at delta; and 0.3 at mu, 0.3 at kappa, and 7.3 at delta (Toll *et al*, 1998). These values are not dissimilar to those measured by Emmerson *et al* (1994), using monkey brain cortex homogenates.

To date, data from receptor binding studies have demonstrated that naloxone, naltrexone, and nalmefene are primarily mu-opioid receptor antagonists; however, there have been a few reports suggesting that nalmefene may also have agonist, or partial agonist properties at kappa-opioid receptors. For example, nalmefene has been shown to stimulate [³⁵S]GTPγS binding to a level of approximately 20% above baseline (compared to a level of approximately 75% for cyclazocine, a predominantly kappa agonist with mu partial agonism) in a C6 glioma cell

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membrane preparation expressing human kappa-opioid receptors, although another study showed no effect on [35 S]GTP γ S binding in CHO cells compared to U69,593 (a full kappa agonist) stimulation (Toll *et al*, 1998; Remmers *et al*, 1999). *In vivo* work also suggests that nalmefene has kappa activity. These reports include ethylketocyclazocine-like discrimination (a sign of kappa agonist effect) in rhesus monkeys following nalmefene infusion (Woods *et al*, 1986) and increased food intake (an effect associated with kappa agonism) in mice following chronic oral nalmefene administration (Chen *et al*, 2004).

In humans, prolactin is secreted from the anterior pituitary and is primarily regulated through tonic inhibition by tuberoinfundibular dopaminergic (TIDA) neurons (Freeman *et al*, 2000). Dopamine from TIDA neurons acts at dopamine D2 receptors present on pituitary lactotrophs to inhibit prolactin release (Freeman *et al*, 2000). The endogenous opioid system, more specifically mu- and kappa-opioid receptors localized on dopaminergic neurons within the mesolimbic-mesocortical and TIDA systems, modulates dopamine release. Therefore, serum prolactin may also serve as a biomarker of opioid effects on TIDA (NIH Definitions Working Group, 2000). Human studies have shown that the mu-opioid receptor agonist methadone and the kappa-opioid receptor agonist dynorphin A(1–13) each stimulate prolactin release; and non-human primate and human studies have shown that the increase in serum prolactin following acute administration of mu- and kappa-opioid agonists is blocked by administration of an opioid antagonist (Kreek, 1978; Wehrenberg *et al*, 1981; Pfeiffer *et al*, 1986; VanVugt *et al*, 1989a; Ur *et al*, 1997; Kreek *et al*, 1999). While mu- and kappa-opioid antagonists have generally been shown to have no effect on serum prolactin, there are conflicting data in females indicating that prolactin response to these antagonists may be affected by menstrual cycle phase (Ellingboe *et al*, 1980; Mendelson *et al*, 1986; VanVugt *et al*, 1989b; Butelman *et al*, 1999; Mello *et al*, 2000).

Based on our observation in humans that nalmefene may cause elevations in serum prolactin (unpublished data), the current study was conducted to explore the possible kappa-opioid agonist effects of nalmefene by conducting a dose-response study in healthy humans. As nalmefene shows binding affinity to mu- and kappa-opioid receptors similar to naloxone, and we have not observed alterations in serum prolactin following naloxone 30 mg intravenously (unpublished data), an increase in serum prolactin following nalmefene may indicate that, unlike naloxone, nalmefene has agonist or partial agonist properties at kappa-opioid receptors. To further assess the possibility that nalmefene has kappa-agonist properties, binding studies and measures of [35 S]GTP γ S activity were performed in CHO cells stably expressing cloned human kappa-opioid receptors.

METHODS

Subjects

The study was approved by the Institutional Review Board of the Rockefeller University Hospital General Clinical Research Center (NIH-GCRC) and performed in accordance with the Helsinki Declaration of 1975. Written informed

consent was obtained prior to participation; confidentiality was strictly maintained. Nalmefene HCl 1 mg/ml (Revix) was purchased from Baxter Health Care (Deerfield, IL) and its administration was conducted under Investigational New Drug status (held by Mary Jeanne Kreek, MD, The Rockefeller University) approved by the United States Food and Drug Administration. The study cohort consisted of 33 subjects (14 female) with a mean age of 34.6 years (range 19–59 years; SD 10.3 years). The mean age of the male subjects was 34.4 years (range 19–59 years; SD 10.5 years) and the mean age of the female subjects was 34.9 years (range 19–52 years; SD 10.4 years). Menstrual cycle phase data were not available.

Healthy normal volunteers were recruited from the local community by word of mouth and by advertisement. Subjects were initially screened for the study by phone contact with a registered nurse. Evaluation for medical and psychiatric inclusion and exclusion criteria were made by an internist or psychiatrist using clinical interview, physical exam, and review of laboratory and corroborative data. Evaluations included general medical, psychiatric, and substance abuse histories, physical examination, ECG, and laboratory testing including complete blood cell count, metabolic panel, thyroid function tests, and urinalysis. Subjects were counseled about, and gave informed consent for, HIV testing. Urine was collected on each of the outpatient visits and on a 24-h basis during inpatient studies. Aliquots of urine were tested daily, both during the screening process and during the inpatient stay, for the presence of opioids, cocaine, cannabinoids, or benzodiazepines. Subjects included in the study were free of significant medical problems, were HIV antibody negative, were not pregnant (serum beta-hCG confirmation upon admission), did not meet DSM IV criteria for any axis I diagnosis such as schizophrenia, major depression, bipolar disorder, panic disorder, PTSD, or generalized anxiety disorder, and substance and/or alcohol abuse or dependence. Eight subjects who were current smokers all smoked <1 pack per day and none met DSM IV criteria for nicotine dependence. Female subjects were not on hormone replacement therapy or using hormonal contraceptives. Subjects were not taking prescription medications, and were not regularly using over-the-counter medications or herbal preparations.

Inpatient Studies

Subjects were admitted to the in-patient unit at least one evening prior to the testing days. In most cases, testing was completed on 3 consecutive days, and in some cases, testing was carried out during separate, closely scheduled admissions to accommodate time constraints of subjects. Subjects fasted at least 9 h prior to the beginning of testing and were allowed to eat only after the first 2 h of testing had elapsed. Test substances were administered through, and blood was withdrawn from, an indwelling 20-G intravenous catheter (BD Angiocath Autoguard, Becton, Dickinson and Company, New Jersey), inserted at least an hour prior to the beginning of testing. In some cases, a functioning catheter from the previous day was used. Total blood volume sampled, including that taken during screening and testing, did not exceed 550 ml. In a random order double-blind

manner, nalmefene 10 mg, nalmefene 3 mg, or saline placebo was administered intravenously over 2 min in a volume of 10 ml. Each subject served as his or her own control receiving a placebo, and each of the two doses of nalmefene, on separate days.

At several intervals (just prior to each blood draw, described below) on each test day, all subjects completed two 100 mm visual analog scales (VAS) related to mood (anchors terrible and terrific) and drug effect (anchors sick and euphoria).

Prolactin Assay

Serum prolactin levels were determined in blood samples drawn at sequential time points. Time points started 10 min prior to test substance administration, at time zero (immediately prior to test substance administration), and then at 10, 20, 30, 40, 50, 60, 75, 90, 120, and 240 min following nalmefene or placebo administration. Blood was drawn into plain vacutainers, and immediately placed on ice. Samples were stored on ice for up to 40 min until centrifuged at 4°C at 1500 g for 5 min. Serum was then removed, aliquoted, and stored at -40°C until assayed. Serum prolactin levels were determined by IRMA procedures, with slight modifications (ICN, Pharmaceuticals Inc., Orangeburg, NY). Prolactin analytical range was 2.5–100 ng/ml and intra- and interassay coefficients of variation were 8.6 and 14.3%, respectively.

Estradiol Assay

Estradiol was measured at a single time point (generally in the morning) as part of admission laboratory screening. Blood was drawn into plain vacutainers and sent to the clinical laboratories of Memorial Sloan-Kettering Cancer Center for evaluation within 2 h of sampling. Serum estradiol levels were determined by magnetic separation assay (Estradiol (ES2), Bayer Immuno 1 System, Bayer Corporation, Tarrytown, NY). Estradiol analytical range was 5–3600 pg/ml and intra- and interassay coefficients of variation were 5.3 and 7.7%, respectively.

Statistics

Statistical analyses of serum prolactin response was performed using area under the curve (AUC) from 0 to 90 min after each dose of nalmefene or placebo administration, to focus on the main effects of nalmefene and to minimize the influence of other factors such as enterohepatic recycling, which may have occurred later in the studies when subjects were allowed to have their first meal. To examine the effects of nalmefene 10 mg and nalmefene 3 mg, compared with placebo, two-way analysis of variance (ANOVA), Condition by Gender, followed by Newman-Keuls *post hoc* tests was also used to evaluate the differences between conditions and any possible effect of gender.

Binding Studies

CHO cells stably transfected with the human delta- and kappa-opioid receptors were obtained from Dr Larry Toll (SRI International, Palo Alto, CA, USA); human mu-opioid

receptors were obtained from Dr George Uhl (NIDA Intramural Program, Bethesda, MD, USA). The cells were grown in 100 mm dishes in Dulbecco's modified Eagle's media supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin (10 000 U/ml) at 37°C in a 5% CO₂ atmosphere. The affinity and selectivity of the compounds for the multiple opioid receptors was determined by incubating the membranes with radiolabeled ligands and 12 different concentrations of the compounds at 25°C in a final volume of 1 ml of 50 mM Tris-HCl, pH 7.5. Incubation times of 60 min were used for the mu-opioid receptor selective peptide [³H]DAMGO and the kappa-opioid receptor selective ligand [³H]U69,593. A 3-h incubation was used with the delta-opioid receptor selective antagonist [³H]naltrindole. To determine the IC₅₀ values for the inhibition of binding by the compounds, the final concentrations of [³H]DAMGO, [³H]naltrindole, and [³H]U69,593 were 0.25, 0.2, and 1 nM, respectively. Non-specific binding was measured by inclusion of 10 μM naloxone. Binding was terminated by filtering the samples through Schleicher & Schuell No. 32 glass fiber filters (Keene, NH, USA) using a Brandel 48-well cell harvester. Filters were soaked for at least 60 min in 0.25% polyethylenimine for [³H]naltrindole and [³H]U69,593 binding experiments. After filtration, filters were washed three times with 3 ml of cold 50 mM Tris-HCl, pH 7.5, and were counted in 2 ml of Ecoscint A scintillation fluid. The K_i values of unlabeled compounds were calculated from the equation $K_i = IC_{50}/(1 + S)$, where $S = (\text{concentration of radioligand})/(K_d \text{ of radioligand})$ (Cheng and Prusoff, 1973).

[³⁵S]GTPγS Binding Studies

Membranes from CHO cells stably expressing either human kappa- or mu-opioid receptors were used in the experiments. Cells were scraped from tissue culture plates, and then centrifuged at 1000 g for 10 min at 4°C. The cells were resuspended in phosphate-buffered saline, pH 7.4, containing 0.04% EDTA. After centrifugation at 1000 g for 10 min at 4°C, the cell pellet was resuspended in membrane buffer, which consisted of 50 mM Tris-HCl, 3 mM MgCl₂, and 1 mM EGTA, pH 7.4. The membranes were homogenized with a Dounce homogenizer, followed by centrifugation at 40 000 g for 20 min at 4°C. The membrane pellet was resuspended in membrane buffer and the centrifugation step was repeated. The membranes were then resuspended in assay buffer, which consisted of 50 mM Tris-HCl, 3 mM MgCl₂, 100 mM NaCl, and 0.2 mM EGTA, pH 7.4. The protein concentration was determined by the Bradford assay (Bradford, 1976) using bovine serum albumin as the standard. The membranes were frozen at -80°C until use.

CHO cell membranes expressing either the human kappa-opioid receptor (15 μg of protein per tube) or mu-opioid receptor (7.5 μg of protein per tube) were incubated with 12 different concentrations of the agonist (ie DAMGO or U50,488) in assay buffer for 60 min at 30°C in a final volume of 0.5 ml. The reaction mixture contained 3 μM GDP and 80 pmol [³⁵S]GTPγS. Basal activity was determined in the presence of 3 μM GDP and in the absence of an agonist, and nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. Then, the membranes were filtered onto glass fiber filters by vacuum filtration, followed by

three washes with 3 ml ice-cold 50 mM Tris-HCl, pH 7.5. Samples were counted in 2 ml Ecoscint A scintillation fluid. Data represent the percent of agonist stimulation [35 S]GTP γ S binding over the basal activity, defined as: ((specific binding/basal binding) \times 100) – 100. All experiments were repeated at least three times, and were performed in triplicate. To determine antagonist activity of a compound at the mu-opioid receptor, CHO membranes expressing mu-opioid receptors were incubated with the compound in the presence of 200 nM of the mu-opioid receptor agonist DAMGO. To determine antagonist activity of a compound at the kappa-opioid receptor, CHO membranes expressing kappa-opioid receptors were incubated with the compound in the presence of 100 nM of the kappa-opioid receptor agonist U50,488.

RESULTS

Subjective Effects of Nalmefene

VAS measures of mood and drug effect did not reveal any significant findings related to nalmefene. However, upon asking subjects several hours after the completion of testing 'how did the study go?', two subjects provided information about feeling 'dream-like' following nalmefene.

Prolactin Dose Response to Nalmefene

Dose-response curves showing all mean (\pm SEM) prolactin measurements from 0 min before to 240 min after each nalmefene dose (0, 3, and 10 mg) are shown in Figure 1a. AUCs of serum prolactin from 0 to 90 min after administration of nalmefene are shown in Figure 1b. ANOVA of nalmefene dose (0, 3, and 10 mg) by gender showed a significant main effect of dose, $F(2,62) = 9.78$, $p < 0.0002$, but no effect of gender, $F(1,31) < 1$. Newman-Keuls *post hoc* tests showed that prolactin following each dose of nalmefene was greater than placebo (nalmefene 3 mg $p < 0.002$; nalmefene 10 mg $p < 0.0005$), but there was no difference between nalmefene 3 and 10 mg.

Although there was no effect of gender on prolactin response, a regression analysis of estradiol effects on prolactin response was performed in a subset of five female subjects for whom serum estradiol levels were available. There was the expected correlation between basal estradiol and prolactin following placebo ($r = 0.947$, $p < 0.02$). However, the change in prolactin AUC following nalmefene 10 mg from baseline was not correlated with basal estradiol (see Figure 2).

Radiolabeled Receptor Binding Assay

The affinity and selectivity of nalmefene for mu- delta-, and kappa-opioid receptors was determined by the use of tritiated receptor binding assays. Membranes from CHO cells that stably expressed either the human mu- delta-, or kappa-opioid receptors were incubated with 12 different concentrations of nalmefene. Table 1 shows that nalmefene had high affinity for both mu- and kappa-opioid receptors. Nalmefene had a three-fold greater affinity for kappa- than mu-opioid receptors, and a 200-fold greater affinity for kappa- than delta-opioid receptors.

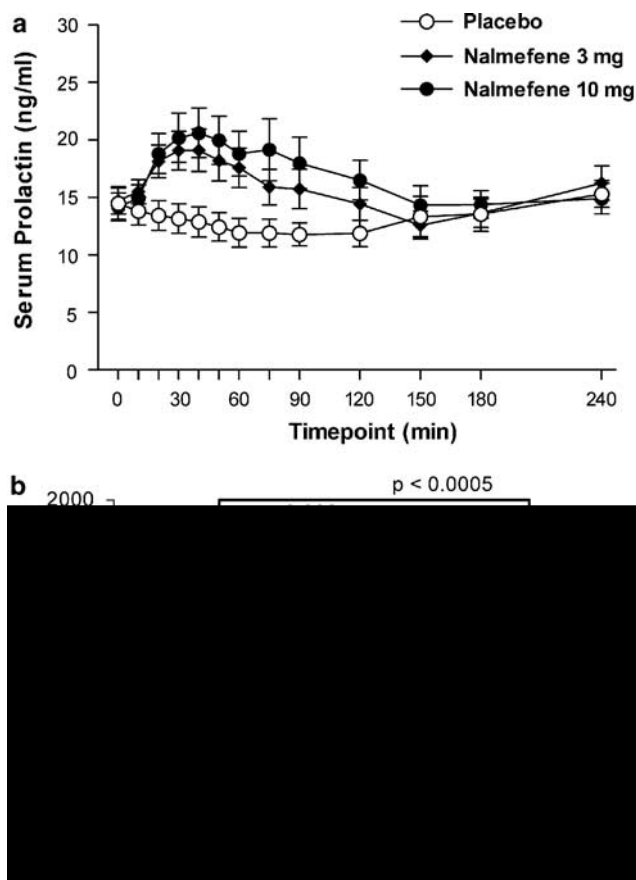


Figure 1 (a) Mean (\pm SEM) level of serum prolactin at each time point from 0 to 240 min after each dose of nalmefene infusion ($n = 33$). (b) Dose response of serum prolactin measured as AUC from 0 to 90 min after each infusion. Values are shown as mean \pm SEM. There were significant increases in serum prolactin after both 3 mg ($p < 0.002$) and 10 mg ($p < 0.0005$) doses of nalmefene, but there was no difference between the 3 and 10 mg doses.

[35 S]GTP γ S Binding to Determine the Efficacy of Nalmefene at Mu-Opioid Receptors

In order to determine if nalmefene was an agonist, antagonist, or mixed agonist/antagonist at mu-opioid receptors, CHO cell membranes that expressed mu-opioid receptors were incubated with 12 different concentrations of nalmefene in the presence of [35 S]GTP γ S. As shown in Figure 3a, nalmefene did not stimulate [35 S]GTP γ S binding mediated by the mu-opioid receptor. Figure 3b shows that nalmefene inhibited [35 S]GTP γ S binding that was induced by the mu-opioid receptor selective peptide DAMGO. Nalmefene had an I_{\max} value of $91 \pm 3.7\%$ and an IC_{50} value of 13 ± 2.3 nM. Thus, nalmefene was an antagonist at the mu-opioid receptor.

[35 S]GTP γ S Binding to Determine the Efficacy of Nalmefene at Kappa-Opioid Receptors

In order to determine if nalmefene was an agonist, antagonist, or mixed agonist/antagonist at the kappa-opioid receptor, CHO cell membranes that expressed kappa-opioid receptors were incubated with 12 concentrations of nalmefene in the presence of [35 S]GTP γ S. Figure 3c shows

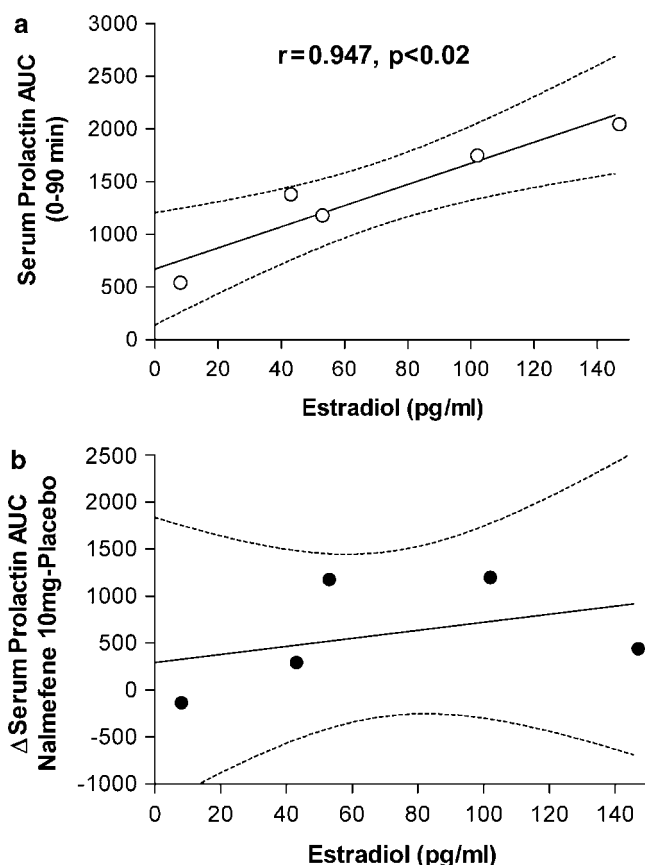


Figure 2 (a) Regression analysis of the effects of basal levels of estradiol in a subset of five women for whom such data was available showed a correlation between basal estradiol and prolactin following placebo of $r = 0.947$, $p < 0.02$. (b) There was no correlation between basal estradiol and change in prolactin AUC from placebo following nalmefene 10 mg.

Table 1 K_i Values \pm SEM for the Inhibition Mu-, Delta-, and Kappa-Opioid Receptor Binding to CHO Membranes by Nalmefene

Compound	[³ H]DAMGO (mu)	[³ H]Naltrindole (delta)	[³ H]U69,593 (kappa)
		K_i (nM \pm SE)	
Nalmefene	0.24 \pm 0.006	16 \pm 1.7	0.083 \pm 0.0008

that nalmefene stimulated [³⁵S]GTP γ S binding, mediated by the kappa-opioid receptor. Nalmefene produced a maximal stimulation, with an E_{\max} value of $29 \pm 1.8\%$ and had an EC_{50} value of 4.2 ± 1.6 nM. In addition to producing some agonist effects at the kappa-opioid receptor, nalmefene inhibited [³⁵S]GTP γ S binding induced by 100 nM U50,488, as shown in Figure 3d, nalmefene produced maximal inhibition, having an I_{\max} value of $57 \pm 7.3\%$ and an IC_{50} value of 18 ± 1.0 nM. These data demonstrate that nalmefene had both agonist and antagonist properties at the kappa-opioid receptor.

In summary, nalmefene has very high affinity for mu- and kappa-opioid receptors. It has a three-fold greater affinity for kappa- than mu-opioid receptors, and a 200-fold greater affinity for kappa- than delta-opioid receptors. In the

[³⁵S]GTP γ S binding assay, nalmefene had both agonist and antagonist properties that were mediated by the kappa-opioid receptor. Nalmefene was an antagonist at mu-opioid receptors.

DISCUSSION

The results of this study demonstrate that nalmefene is a partial agonist at the human kappa-opioid receptor. This conclusion is supported by increased levels of serum prolactin following nalmefene infusion in healthy human volunteers and by cell binding assays using cloned human kappa-opioid receptors.

Nalmefene is a modestly selective opioid antagonist with affinity for mu-, delta-, and kappa-opioid receptors. In non-human primates and humans, nalmefene's affinity is relatively equivalent at mu- and kappa-opioid receptors followed by delta-opioid receptors. It may, therefore, be difficult to assess in humans what pharmacological effects are specific to nalmefene's kappa activity. While nalmefene may have limited delta-opioid receptor agonist properties (Toll *et al*, 1998), its affinity at the delta-opioid receptor is very low ($K_i \sim 16$ nM, or 200-fold less than its affinity at the kappa-opioid receptor) and delta-opioid receptor agonists have not been shown to alter prolactin, the biomarker used in this study (Butelman *et al*, 2002). It is, therefore, unlikely that delta-opioid receptor effects of nalmefene contributed to our findings and will not be further considered.

Dopamine and the Mu- and Kappa-Opioid Systems

The mu- and kappa-opioid receptor systems modulate diverse processes including response to pain and analgesia, the stress-responsive hypothalamic-pituitary-adrenal axis (HPA), the hypothalamic-pituitary-gonadal axis, gastrointestinal motility, immune function, and mood. The mu- and kappa-opioid receptor systems also modulate dopaminergic neurons. In rodents, systemically administered mu-opioid receptor agonists stimulate dopamine release in the nucleus accumbens through their inhibition of GABAergic interneurons in the ventral tegmental area (Johnson and North, 1992; Bergevin *et al*, 2002). GABAergic interneurons in the VTA inhibit dopamine firing in mesolimbic-mesocortical dopaminergic neurons, thus mu-opioid receptor agonists stimulate dopamine release through disinhibition (Di Chiara and Imperato, 1988; Spanagel *et al*, 1991). Systemically or locally administered synthetic and locally administered natural peptidic kappa-opioid receptor agonists (ie dynorphin) inhibit dopamine release in the mesolimbic-mesocortical dopaminergic systems (Di Chiara and Imperato, 1988; Spanagel *et al*, 1990; Claye *et al*, 1997; Zhang *et al*, 2004a, b). While mu- and kappa-opioid receptor antagonists can each reverse these effects, neither administered alone have been shown to alter dopamine release in either rodent or non-human primate models (Deyo *et al*, 1979; Durham *et al*, 1996; Butelman *et al*, 2002). One possible exception is a positron emission tomography (PET) study in rats, which found increased binding potential in the striatum of the displaceable dopamine D1 ligand [¹¹C]SCH23,390 following acute but not chronic nalmefene administration (Unterwald *et al*, 1997). Interestingly, there was no change in binding

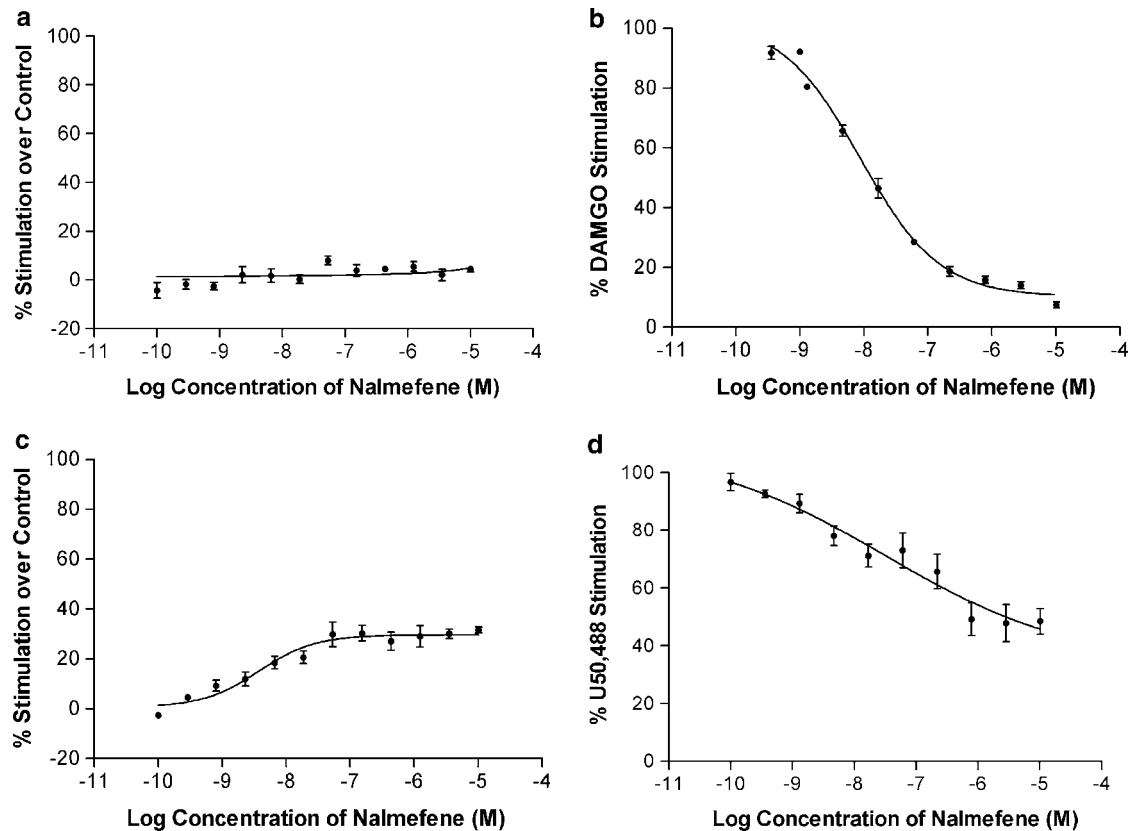


Figure 3 (a) Effect of nalmefene on $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding mediated by the mu-opioid receptor. Basal activity was determined in the presence of $3\text{ }\mu\text{M}$ GDP and in the absence of an agonist, and nonspecific binding was determined in the presence of $10\text{ }\mu\text{M}$ unlabeled $\text{GTP}\gamma\text{S}$. (b) Nalmefene inhibition of $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding stimulated by DAMGO. DAMGO stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding by $102 \pm 1.2\%$ above basal values. (c) Nalmefene stimulation of $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding mediated by the kappa-opioid receptor. Basal activity was determined in the presence of $3\text{ }\mu\text{M}$ GDP and in the absence of an agonist, and nonspecific binding was determined in the presence of $10\text{ }\mu\text{M}$ unlabeled $\text{GTP}\gamma\text{S}$. (d) Nalmefene inhibition of $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding stimulated by U50,488. U50,488 stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding by $68 \pm 6.3\%$ above basal values.

potential of the nondisplaceable dopamine D2 ligand $[^{11}\text{C}]\text{N}$ -methylspiperone following either acute or chronic nalmefene.

TIDA neurons, however, are suppressed by both mu- and kappa-opioid receptor agonists. In humans, peripheral administration of the mu-opioid receptor agonist beta-endorphin or the kappa-opioid receptor agonist dynorphin A(1–13) will stimulate peripheral levels of serum prolactin (Reid *et al*, 1981; Kreek *et al*, 1999). As neither of these peptides has extensive penetrance across the blood–brain barrier, their effects are likely limited to TIDA as opposed to mesolimbic–mesocortical dopaminergic neurons. In humans, dopamine D2 receptors on anterior pituitary lactotrophs act through tonic inhibition from TIDA neurons as the principal modulator of peripheral prolactin release (Durham *et al*, 1996; Freeman *et al*, 2000). As kappa-opioid receptors present on presynaptic dopamine terminals and mu-opioid receptors present on GABAergic interneurons inhibit TIDA, serum prolactin also serves as a biomarker for kappa- and mu-opioid receptor agonist activity.

Prolactin and Mu- and Kappa-Opioidergic Drugs

There are human data that show both short- and long-acting mu-opioid receptor agonists stimulate peripheral levels of prolactin (Kreek, 1978; Ellingboe *et al*, 1980).

Despite development of tolerance to many of the effects of chronically administered methadone, we have shown that serum prolactin levels increase for 2–4 h following each daily dose in methadone maintained subjects (Kreek, 1978; Bart *et al*, 2003). In humans, the natural sequence kappa-opioid receptor ligand dynorphin A(1–13) and also synthetic kappa agonists increase serum prolactin (Ur *et al*, 1997; Kreek *et al*, 1999; Bart *et al*, 2003), although the effects following chronic dosing are unknown.

Other than one human study reporting an increase in serum prolactin following intramuscular administration of naloxone 210 mg (Kumor *et al*, 1988), extensive studies in non-human primates and humans have not found an effect of this mu-opioid receptor antagonist on serum prolactin (Volavka *et al*, 1980; Morley *et al*, 1980; Naber *et al*, 1981; Cohen *et al*, 1983, 1985; van Bergeijk *et al*, 1986; VanVugt *et al*, 1989b). Data for naltrexone and nalmefene, the two antagonists with greater affinity at mu- and kappa-opioid receptors than naloxone, are, however, mixed. One non-human primate study found that nalmefene suppressed basal prolactin, whereas its quaternary ammonium form, which does not penetrate the blood–brain barrier, had no effect on prolactin (Simpkins *et al*, 1991). Another study found that nalmefene had no effect on serum prolactin in male rhesus monkeys (Mello *et al*, 2000), while another group found that nalmefene stimulated prolactin release in

normal female rhesus monkeys but not in ovariectomized monkeys (VanVugt *et al*, 1989b). Naltrexone 50 mg stimulated prolactin release in normal male volunteers, early follicular phase female volunteers, and, following daily dosing for 1 week, in luteal phase female volunteers (Volavka *et al*, 1979; Mendelson *et al*, 1986; Gindoff *et al*, 1988), although, when studied in males, naltrexone 100 mg had no effect on prolactin (Volavka *et al*, 1979). In an all male study of oral nalmefene 10 mg, mean prolactin levels were not different compared to placebo- or naloxone-treated volunteers (Graves *et al*, 1993). As the current study did not control for menstrual cycle phase and gave nalmefene intravenously, it is difficult to make direct comparisons with the aforementioned studies.

Rodent and non-human primate studies have not shown an effect of kappa-opioid receptor antagonists alone on serum prolactin. We previously found that both naloxone and nalmefene attenuate dynorphin A(1–13)-stimulated increase in serum prolactin in normal human volunteers (Kreek *et al*, 1999). Interestingly, nalmefene had a greater effect in attenuating dynorphin A(1–13)-induced prolactin release than did an equivalent dose of naloxone. Owing to their unavailability for human use, the effect of kappa-opioid receptor selective antagonists on serum prolactin in humans remains unknown.

Mu- and Kappa-Opioid System and Addiction

The neurobiological effects of drugs of abuse are mediated in part either directly or indirectly by the mu-opioid receptor system. Both rodent and human studies have shown that chronic cocaine and alcohol use lead to altered mu-opioid receptor expression measured as change in levels of mRNA, by quantitative autoradiography, or by PET (Unterwald *et al*, 1992, 1993, 1994; Zubietta *et al*, 1996; Yuferov *et al*, 1999; Bencherif *et al*, 2004). In mu-opioid receptor knockout mice, opiate self-administration is blocked and both cocaine and alcohol self-administration are attenuated (Roberts *et al*, 2000; Hall *et al*, 2001). In addition, several animal models have shown that mu-opioid receptor antagonists will eliminate or curtail alcohol and psychostimulant self-administration and conditioned place preference (Altshuler *et al*, 1980; Myers *et al*, 1986; Trujillo *et al*, 1991; Middaugh and Bandy, 2000).

Chronic cocaine administration has also been shown to increase kappa-opioid receptor expression in rats, a finding also seen in human cocaine overdose deaths (Unterwald *et al*, 1994; Staley *et al*, 1997). Natural and synthetic kappa-opioid receptor agonists directly infused into the striatum or administered systemically to rodents will both attenuate cocaine-induced dopamine surges in the nucleus accumbens and block cocaine-induced conditioned place preference (Maisonnette *et al*, 1994; Zhang *et al*, 2004a,b). Centrally penetrating kappa-opioid receptor agonists can be aversive in rodents, so studies showing a reduction in alcohol intake but an increased alcohol deprivation effect and a decrease in morphine and cocaine self-administration may be difficult to interpret (Glick *et al*, 1995; Höltér *et al*, 2000; Lindholm *et al*, 2001). Studies of kappa-opioid receptor antagonists have not found an effect of these agents on alcohol, morphine, or cocaine administration (Glick *et al*, 1995; Höltér *et al*, 2000).

Acute administration of cyclazocine or butorphanol, compounds with mixed activity at mu- and kappa-opioid receptors, in human cocaine users did not alter the effects of investigator-administered intranasal cocaine or reduce intravenous cocaine self-administration (Walsh *et al*, 2001; Preston *et al*, 2004). Enadoline, a selective kappa-opioid receptor agonist, also did not attenuate the effects or self-administration of intravenous cocaine (Walsh *et al*, 2001). Chronic treatment with the mixed kappa- and mu-opioid receptor agonist MCL-101, however, did reduce cocaine self-administration in a non-human primate model (Bowen *et al*, 2003).

A preliminary study on the effects on euphoria of naltrexone in normal volunteers given amphetamine indicates that opioid antagonism may be clinically efficacious in the treatment of amphetamine addiction (Jayaram-Lindström *et al*, 2004). Other studies also indicate that naltrexone may be useful in the treatment of cocaine addiction (Kosten *et al*, 1992; Schmitz *et al*, 2001). There are inadequate studies to delineate whether naltrexone's affinity at kappa-opioid receptors contributes to this and, if so, whether a partial-agonist at kappa-opioid receptors would add further benefit.

Naltrexone is effective in treating some individuals with moderate to severe alcoholism (Volpicelli *et al*, 1992). While studies of the treatment of alcoholism using nalmefene have been mixed (Mason *et al*, 1994; Anton *et al*, 2004), initial studies of nalmefene in the treatment of compulsive gambling have been promising (SW Kim, personal communication). Naltrexone and nalmefene do have comparable affinity at mu- and kappa-opioid receptors. The current study shows, in human and cellular binding studies, that nalmefene acts as a partial agonist at kappa-opioid receptors. It is unclear whether this effect may impart an advantage for the use of nalmefene in the treatment of specific addictive diseases. Using a similar experimental design, it will be important to determine whether the treatment effects of naltrexone may also be related to a partial-agonist effect at kappa-opioid receptors.

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