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Stereoselective Interaction of Ketamine with Recombinant μ, κ, and δ Opioid Receptors Expressed in Chinese Hamster Ovary Cells

Kazuyoshi Hirota, M.D., Hirobumi Okawa, M.D.,* Balraj L. Appadu M.D., F.R.C.A.,* David K. Grandy, Ph.D.,† Lakshmi A. Devi, Ph.D.‡, David G. Lambert Ph.D.§*

Background: **The authors examined the interaction of ketamine with recombinant μ, κ, and δ opioid receptors and recombinant orphan opioid receptors expressed in Chinese hamster ovary cells (CHO-μ, CHO-κ, CHO-δ, and CHO_{ORL1}, respectively).**

Methods: **CHO-μ, CHO-κ, and CHO-δ membranes were incubated with the opioid receptor radioligand [^{3}H]diprenorphine at room temperature. Ketamine (racemic, *R(−)* and *S(+)*) was included at concentrations covering the clinical range. CHO_{ORL1} membranes were incubated with [^{125}I]Tyr^{14}nociceptin and racemic ketamine at room temperature. The effects of racemic ketamine and selective opioid receptor agonists (μ: [D-Ala^2, MePhe^4, Gly(ol)^5] enkephalin (DAMGO); κ: spiradoline or δ: [D-pen^2, D-pen^5] enkephalin (DPDPE)) on forskolin-stimulated cyclic adenosine monophosphate formation also were examined. Data are mean ± SEM.**

Results: **Racemic ketamine increased the radioligand equilibrium dissociation constant for [^{3}H]diprenorphine from 85 ± 5 to 273 ± 11, 91 ± 6 to 154 ± 16, and 372 ± 15 to 855 ± 42 pM in CHO-μ, CHO-κ, and CHO-δ, respectively. The concentration of radioligand bound at saturation was unaffected. In CHO-μ and CHO-κ cells, racemic ketamine did not slow the rate of naloxone-induced [^{3}H]diprenorphine dissociation. Ketamine and its isomers also displaced [^{3}H]diprenorphine binding to μ, κ, and δ receptors in a dose-dependent manner, with pK_i values for racemic ketamine of 4.38 ± 0.02, 4.55 ± 0.04, and 3.57 ± 0.02, respectively. *S(+)*-ketamine was two to three times more potent than *R(−)*-ketamine at μ and κ receptors. Racemic ketamine displaced [^{125}I]Tyr^{14}nociceptin with an estimated affinity constant of 0.5 mM. Racemic ketamine inhibited the formation of cyclic adenosine monophosphate (naloxone insensitive) in a dose-dependent manner (concentration producing 50% inhibition ~ 2 mM) in all cell lines, including untransfected CHO cells. Ketamine (100 μM) reversed DAMGO (μ) and spiradoline (κ) inhibition of formation of cyclic adenosine monophosphate.**

Conclusions: **Ketamine interacts stereoselectively with recombinant μ and κ opioid receptors. (Key words: Adenylyl cyclase; opioid receptor antagonist; radioligand binding; radioreceptor assay.)**

OPIOID receptors are classified as μ, δ, and κ based on the pharmacology of a range of selective agonists and antagonists. All have now been cloned and sequenced, and their activation is capable of producing analgesia.[1–3] The International Union of Pharmacology, however, has reclassified the receptors as OP1 (δ), OP2 (κ), and OP3 (μ), because these receptors bind *OP*ioids.[4] Shortly after the cloning of the classic opioid receptors, a further "orphan" receptor was identified by several groups in areas of the brain involved in perception of pain. The "orphan" receptor did not bind classic opioids. An endogenous 17 amino acid ligand, nociceptin or orphanin FQ, was soon identified that produced analgesic, hyperalgesic, and antiopioid actions depending on the site of administration.[5–7] In this article, the "orphan" receptor is referred to as opioid receptor–like 1 (ORL1) and the endogenous peptide nociceptin.

Ketamine interacts with opioid receptors.[8–12] *In vitro* radioligand binding and bioassay studies have shown that ketamine interacts stereoselectively with μ and κ opioid receptors with *S(+)*-ketamine, being two to three times more potent than *R(−)*-ketamine.[9,12] In agreement with these findings, clinical observations also indicate that *S(+)*-ketamine is two- to threefold more potent as an analgesic than *R(−)*-ketamine,[8] although this is likely

* Clinical Lecturer, University Department of Anaesthesia, Leicester Royal Infirmary, Leicester, United Kingdom.

† Associate Professor, Department of Physiology, Pharmacology, Oregon Health Sciences University, Portland, Oregon.

‡ Associate Professor, Department of Pharmacology, New York University Medical Center, New York, New York.

§ Nonclinical Lecturer, University Department of Anaesthesia, Leicester Royal Infirmary, Leicester, United Kingdom.

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Address reprint requests to Dr. Lambert: University Department of Anaesthesia, Leicester Royal Infirmary, Leicester, LE1 5WW, United Kingdom. Address electronic mail to: dg13@le.ac.uk.

to result, in part, from *N*-methyl-D-aspartate (NMDA) receptor antagonism. For example, Smith *et al.*[13] reported that the analgesic action of ketamine could not be antagonized by naloxone microinjected into the periaqueductal gray region of the rat brain, an area rich in μ but not κ opioid receptors. In addition, microinjection of ketamine into the periaqueductal gray region did not produce analgesia but antagonized morphine analgesia. These findings suggested that ketamine analgesia is unlikely to be mediated through μ opioid receptors in the central nervous system.

We have examined in detail the interaction of ketamine and its optical isomers with opioid receptors using Chinese hamster ovary (CHO) cells expressing a homogenous population of recombinant μ opioid, κ opioid, δ opioid, and ORL1[14–18] receptors (designated CHO-μ, CHO-κ, CHO-δ, and CHO_{ORL1}, respectively). Opioid and ORL1 receptors are negatively coupled to adenylyl cyclase and hence inhibit the formation of cyclic adenosine monophosphate (cAMP). We used the inhibition of formation of cAMP as an index of functional opioid receptor activation, and using this system, we have examined the functional consequences of interaction of ketamine with μ, κ, and δ opioid receptors.

Materials and Methods

Materials

Racemic ketamine was purchased from Sigma (Poole, Dorset, UK). *S(+)*- Lot Q and *R(−)*- Lot U ketamine (> 90% pure) were donated from Parke Davis (Ann Arbor, MI). [^{3}H]Diprenorphine (specific activity, 41 Ci/mmol) and [^{125}I]Tyr^{14}nociceptin (specific activity, 2,000 Ci/mmol) were purchased from Amersham International (Bucks, UK). All tissue culture media and supplements were purchased from Life Technologies (Paisley, UK). All other drugs were purchased from Sigma or Calbiochem (Notts, UK). All other reagents were of the highest purity available.

Membrane Preparation and Cell Culture

Chinese hamster ovary μ, κ, and δ cells, and in some studies untransfected wild-type CHO cells, were maintained in Hams F12 medium supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml fungizone, and 10% fetal calf serum. Chinese hamster ovary cells expressing the recombinant human ORL1 receptor (CHO_{ORL1}) were maintained in Dulbecco's modified Eagle's medium:F12 (50:50) containing 5% fetal calf serum, 2 mM glutamine, 200 μg/ml hygromycin B, and 200 μg/ml G418. Cultures were maintained at 37°C in 5% CO_2/humidified air, fed every 2–3 days, and passaged every 7 days. Experiments were performed on days 5–7 after subculture. All cells were harvested for use by the addition of 0.9% saline containing HEPES (10 mM)/EDTA (0.02%). Cells were homogenized at 4°C using a tissue Tearor (setting 5,5×30-s bursts) in 50 mM Tris HCl buffer (pH 7.4). The homogenate was centrifuged at 18,000*g* for 10 min, and the pellet was resuspended in Tris HCl buffer. This homogenization and centrifugation procedure was repeated twice more. Membranes of each cell line (CHO-μ, passage number 13–16; CHO-κ, passage number 5–8; CHO-δ, passage number 7–10; CHO_{ORL1}, passage number 5–10) were prepared and used fresh daily.

Theoretical Considerations in Radioligand Binding

Three main types of radioligand binding studies are commonly used to characterize drug–receptor interaction. These are saturation, displacement, and kinetic.[19] Figure 1A shows a typical saturation experiment in which receptor-specific binding is calculated as the difference between total and nonspecific binding (*i.e.,* nonreceptor binding). As the concentration of radiolabel increases, the amount of specific binding increases until saturation occurs. The concentration of radioligand bound at saturation (B_{max}) is a measure of receptor density. The concentration at which half B_{max} is obtained is the radioligand equilibrium dissociation constant (K_d). Specific binding data can be linearized using a Scatchard[20] transformation (fig. 1B) of the data, in which the x-intercept defines B_{max} and -1/slope defines K_d. The binding characteristics of an unlabeled compound can be estimated by measuring the displacement of a fixed concentration of a receptor-specific radioligand by increasing concentrations of the displacer (fig. 1C). The concentration producing 50% inhibition (IC_{50}) is related to the affinity. The position of the displacement curve is determined by the concentration of radioligand used (*i.e.,* more radioligand requires more displacer to produce the same degree of displacement). Values of IC_{50} are corrected for this competing mass of radioligand using the Cheng and Prusoff[21] equation to yield the affinity constant (K_i). An additional estimate of drug K_d can be made kinetically in which the rate of association and dissociation are estimated. If a drug interacts with a receptor at a site other than the radioligand binding site, then the binding of the radioligand may be influenced in an allosteric fashion. This may slow the rate of radioligand dissociation from the receptor. This type of experiment is illustrated in figures 2B and 2D.

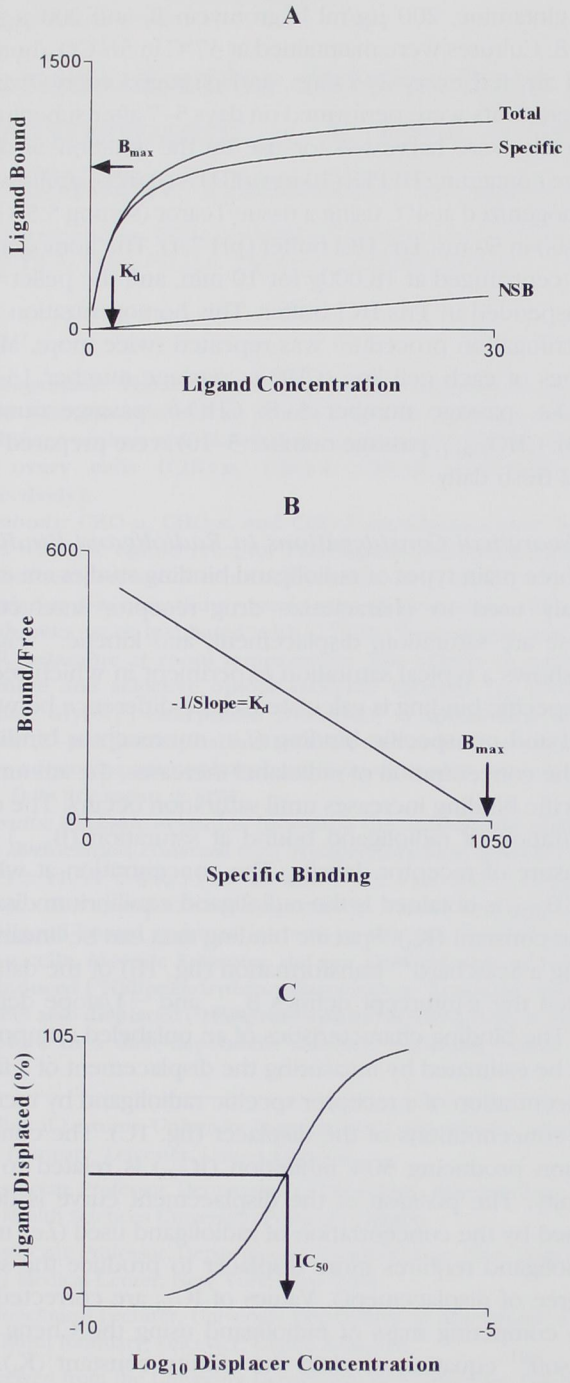

Fig. 1. Interpretation of radioligand saturation and displacement experiments. A typical saturation experiment (*A*) and Scatchard transformation (*B*) of the specific binding data, respectively. (*C*) A typical displacement curve used to calculate the affinity of a displacing drug (*e.g.*, ketamine). For further explanation, see text.

[^{3}H]Diprenorphine Binding

The binding of [^{3}H]diprenorphine was performed in 1-ml volumes of Tris HCl buffer containing ~200 μg of membranes at 20°C for 90 min. Nonspecific binding was defined in the presence of 10^{-5} M naloxone. Typically, this was < 10% in all cells around the radioligand K_d. After incubation, each sample was filtered (and washed) under vacuum through Whatman GF/B filters using a Brandel cell harvester. Filter retained radioactivity was extracted for ≥8 h in 4 ml scintillation fluid. In displacement studies, the interaction of racemic (3×10^{-7} – 10^{-2} M), *S(+)* and *R(−)* (3×10^{-6} – 10^{-3} M) ketamine with μ, κ, or δ opioid receptors was determined by displacement of 0.5 nM [^{3}H]diprenorphine (n = 6). Cyprodime (3×10^{-10} – 10^{-5} M), norbinaltorphimine (10^{-12} – 10^{-5} M), and naltrindole (10^{-11} – 10^{-5} M) were included as μ, κ, and δ selective reference compounds, respectively. Saturation analysis to determine K_d and B_{max} of CHO-μ, -κ, or -δ (n = 5) in the presence and absence of racemic ketamine (100, 50, or 350 μM, respectively) was performed using increasing concentrations of [^{3}H]diprenorphine (0.03–3.00 nM). The ketamine concentrations used are about 1.5-fold the K_i value obtained for each cell line in [^{3}H]diprenorphine displacement experiments.

Effects of Ketamine on Naloxone-induced [^{3}H]Diprenorphine Dissociation

To determine whether any interaction of ketamine with opioid receptors was allosteric, membranes were labeled with 0.5 nM [^{3}H]diprenorphine to equilibrium (90 min); then radioligand dissociation was initiated by addition of 10 μM naloxone. The time course for dissociation was followed by filtration at various times up to 90 min as described earlier. Naloxone-induced dissociation was determined in the presence and absence of ketamine (200 μM for CHO-μ and 100 μM for CHO-κ). These concentrations are higher than those used in saturation studies to maximize the chances of detecting any differences between control and ketamine-treated preparations).

[^{125}I]Tyr^{14}nociceptin Binding Assay

The effects of racemic ketamine on the binding of [^{125}I]Tyr^{14}nociceptin[22] to CHO_{ORL1} cells were determined in 1-ml volumes of Tris-HCl 50 mM, $MgSO_4$ 5 mM, and bovine serum albumin 0.5% buffer containing 30 μM of peptidase inhibitors; captopril, amastatin, bestatin, and phosphoramidon (to prevent nociceptin breakdown) at *p*H 7.4. Unlabeled nociceptin was included as

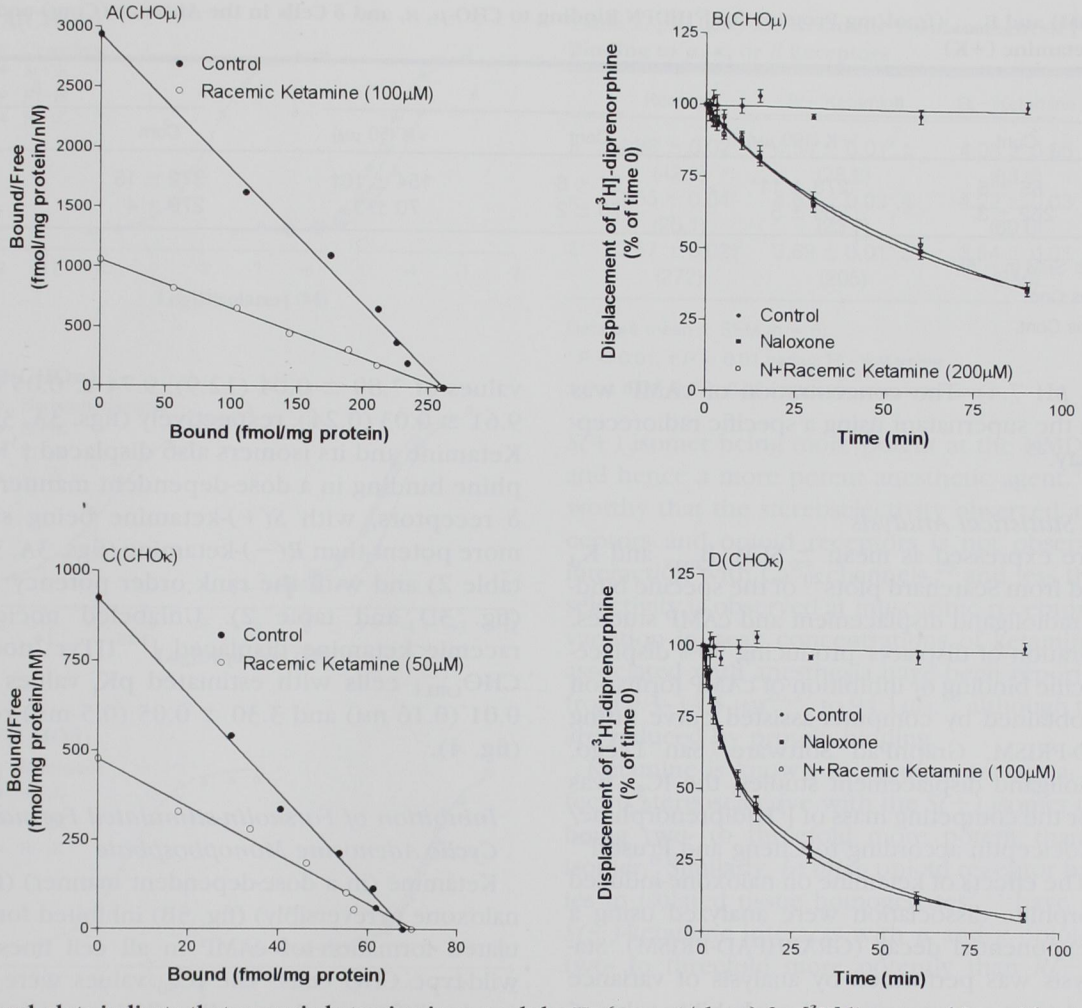

Fig. 2. Scatchard plots indicate that racemic ketamine increased the K_d (pM, −1/slope) for [^{3}H]diprenorphine without affecting the B_{max} (fmoles/mg protein, x-intercept) in Chinese hamster ovary (CHO) cells expressing the recombinant μ (*A*, 100 μM ketamine) and κ (*C*, 50 μM ketamine) opioid receptors. Data are from a typical paired experiment from n = 5. Dissociation time courses are shown for CHO cells expressing the recombinant μ (*B*, 200 μM ketamine) and κ (*D*, 100 μM ketamine) opioid receptors. Cells were labeled to equilibrium with 0.5 nM [^{3}H]diprenorphine; then dissociation was initiated (at t = 0) with 10 μM naloxone (N) in the absence and presence of racemic ketamine. In control cells, dissociation was not initiated. Data are mean ± SEM (n = 6).

a high-affinity reference compound. Membranes were incubated for 30 min at room temperature. In all studies, ~1 pM [^{125}I]Tyr^{14}nociceptin was used in the absence and presence of increasing concentrations of racemic ketamine.

Measurement of Formation of Cyclic Adenosine Monophosphate

Whole cells (CHO-μ, -κ, -δ, and wild-type cells) were suspended in 0.3 ml Krebs/HEPES buffer, *p*H 7.4, and incubated in the presence of isobutylmethylxanthine (1 mM) with or without (for the basal) forskolin (1 μM) at 37°C for 15 min. To obtain ketamine dose–response curves for inhibition of formation of cAMP, the cells were incubated additionally with or without racemic ketamine ($3 \times 10^{-6} - 10^{-2}$ M). Naloxone (10 μM) was included in some experiments. To study the nature of any interaction of ketamine with μ, κ, and δ receptors, racemic ketamine was coincubated with [D-Ala^2, MePhe^4, Gly(ol)^5] enkephalin (DAMGO; 100 nM μ-selective agonist), spiradoline (2 nM κ-selective agonist and [D-pen^2, D-pen^5] enkephalin [DPDPE; 5 nM δ-selective agonist]) in various combinations. The concentration of each selective opioid receptor agonist was approximately the IC_{50} value for inhibition of forskolin-stimulated formation of cAMP obtained in preliminary experiments. All reactions were terminated by the addition of 20 μl HCl (10 M), 20 μl NaOH (10 M), and 180 μl Tris

Table 1. K_d (pM) and B_{max} (fmol/mg Protein) for [^{3}H]DPN Binding to CHO-μ, κ, and δ Cells in the Absence (Cont) and Presence of Racemic Ketamine (+K)

	μ		κ		δ	
	Cont	+K (100 μM)	Cont	+K (50 μM)	Cont	+K (350 μM)
K_d	85 ± 5	273 ± 11*	91 ± 6	154 ± 16†	372 ± 15	855 ± 42*
B_{max}	262 ± 3	269 ± 5	70 ± 2	70 ± 3	279 ± 4	275 ± 4

Data are mean ± SEM (n = 5).

* $P < 0.01$ *versus* Cont.

† $P < 0.05$ *versus* Cont.

buffer (1 M, *p*H 7.4). The concentration of cAMP was measured in the supernatant using a specific radioreceptor mass assay.[23]

Data and Statistical Analysis

All data are expressed as mean ± SEM. B_{max} and K_d were derived from Scatchard plots[20] of the specific binding data. In radioligand displacement and cAMP studies, the concentration of displacer producing 50% displacement of specific binding or inhibition of cAMP formation (IC_{50}) was obtained by computer-assisted curve fitting (GRAPHPAD-PRISM, GraphPad Software, San Diego, CA). In radioligand displacement studies, the IC_{50} was corrected for the competing mass of [^{3}H]diprenorphine/[^{125}I]Tyr^{14}Nociceptin according to Cheng and Prusoff[21] to yield K_i. The effects of ketamine on naloxone-induced [^{3}H]diprenorphine dissociation were analyzed using a one-phase exponential decay (GRAPHPAD-PRISM). Statistical analysis was performed by analysis of variance and unpaired *t* test as appropriate. A probability value < 0.05 was considered statistically significant.

Results

[^{3}H]Diprenorphine and [^{125}I]Tyr^{14}nociceptin Binding

The specific binding of [^{3}H]diprenorphine to membranes from each cell line was dose-dependent and saturable. Scatchard analysis revealed that ketamine significantly increased the K_d in CHO-μ, -κ, and -δ cells, with B_{max} remaining unchanged (figs. 2A and 2C and table 1). Two hundred micromoles per liter of ketamine (and 100 μM, data not shown) in CHO-μ and 100 μM (and 50 μM, data not shown) in CHO-κ did not affect the dissociation of [^{3}H]diprenorphine (figs. 2B and 2D). Cyprodime, norbinaltorphimine, and naltrindole displaced [^{3}H]diprenorphine binding with high affinity in CHO-μ, -κ, or -δ cells with pK_i (mean in nM) values of 7.89 ± 0.04 (12.9), 9.74 ± 0.05 (0.18), and 9.61 ± 0.03 (0.24), respectively (figs. 3A, 3B, and 3C). Ketamine and its isomers also displaced [^{3}H]diprenorphine binding in a dose-dependent manner at μ, κ, or δ receptors, with *S(+)*-ketamine being significantly more potent than *R(−)*-ketamine (figs. 3A, 3B, 3C, and table 2) and with the rank order potency $\kappa > \mu > \delta$ (fig. 3D and table 2). Unlabeled nociceptin and racemic ketamine displaced [^{125}I]Tyr^{14}nociceptin in CHO_{ORL1} cells with estimated pK_i values of 9.80 ± 0.01 (0.16 nM) and 3.30 ± 0.05 (0.5 mM) respectively (fig. 4).

Inhibition of Forskolin-stimulated Formation of Cyclic Adenosine Monophosphate

Ketamine (in a dose-dependent manner) (fig. 5A) and naloxone (irreversibly) (fig. 5B) inhibited forskolin-stimulated formation of cAMP in all cell lines, including wild-type CHO cells. The IC_{50} values were not significantly different among all cell lines tested (table 3). Ketamine (1 mM) in conjunction with DAMGO (100 nM), spiradoline (2 nM), or DPDPE (5 nM) in CHO-μ, -κ, and -δ was neither additive nor synergistic (fig. 6). Ketamine (100 μM, a concentration that produced > 75% diprenorphine displacement in CHO-μ and CHO-κ but failed to inhibit formation of cAMP directly) reversed the inhibition of formation of cAMP by DAMGO and spiradoline (fig. 7). These studies were not performed in CHO-δ cells because ketamine concentrations that produced significant diprenorphine displacement (> 300 μM) also produced direct inhibition of cAMP.

Discussion

The dissociative anesthetic agent ketamine is a noncompetitive blocker of glutamate NMDA receptors. Ketamine is administered clinically as a racemate but is composed of two isomers, *S(+)* and *R(−)*, with the

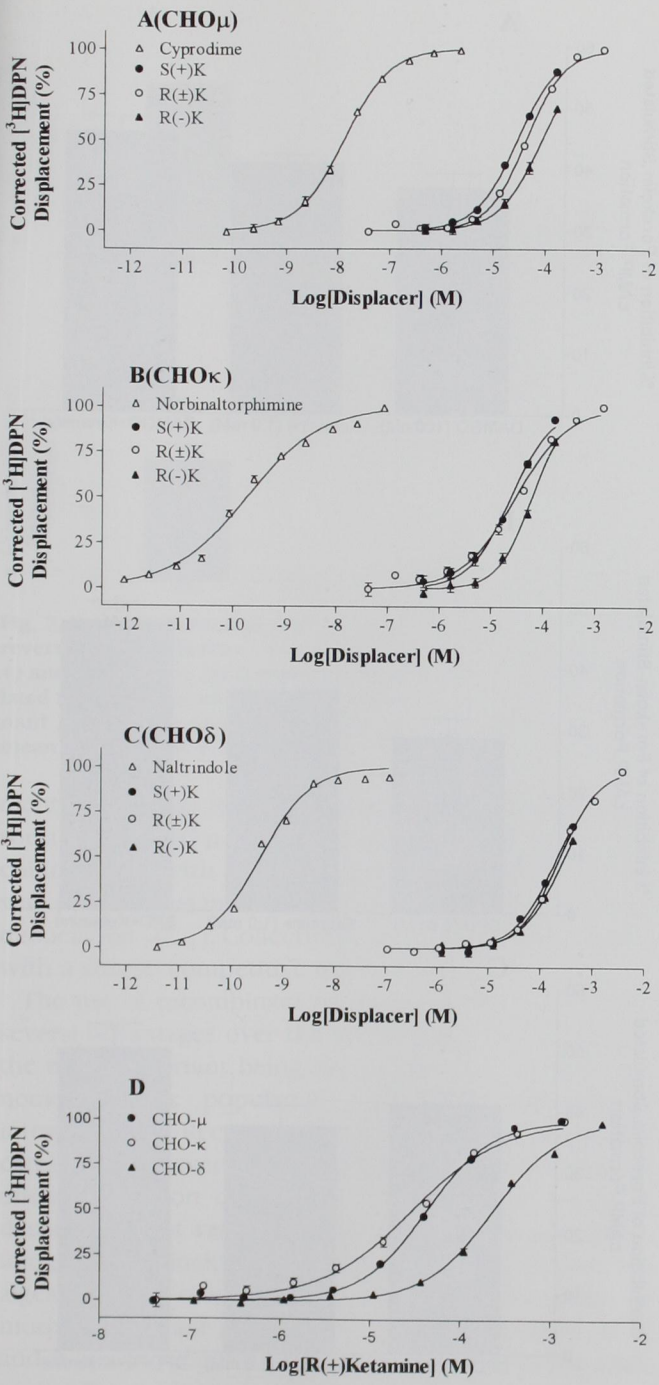

Fig. 3. Displacement of [^{3}H]diprenorphine binding to recombinant μ (*A*), κ (*B*), and δ (*C*) opioid receptors expressed in CHO cells by racemic (*R(±)*), *S(+)*, and *R(−)* ketamine (K) and selective opioid receptor antagonists (μ, cyprodime; κ, norbinaltorphimine; δ, naltrindole). (*D*) The displacement curves for racemic ketamine alone. Full displacement curves are corrected for the competing mass of [^{3}H]diprenorphine and are mean ± SEM (n = 6).

Table 2. pK_i (μM) for Ketamine Displacement of [^{3}H]DPN Binding to μ, κ, or δ Receptors

	Racemic	S(+)Ketamine	R(−)Ketamine	R(−)/S(+)
μ	4.38 ± 0.02* (42.1)	4.54 ± 0.01*,‡ (28.6)	4.08 ± 0.05 (83.8)	2.9
κ	4.55 ± 0.04* (28.1)	4.63 ± 0.03*,§ (23.7)	4.22 ± 0.03 (60.0)	2.5
δ	3.57 ± 0.02† (272)	3.69 ± 0.01*,‡ (205)	3.54 ± 0.01 (286)	1.4

Data are mean ± SEM (n = 6).

* $P < 0.01$, † $P < 0.01$ *versus* R(−)ketamine.

‡ $P < 0.01$, § $P < 0.05$ *versus* racemic ketamine.

S(+) isomer being more potent at the NMDA receptor, and hence a more potent anesthetic agent.[24] It is noteworthy that the stereoselectivity observed at NMDA receptors and opioid receptors is not observed for the interaction with Ca^{2+} channels,[25] and less than twofold selectivity is observed at muscarinic receptors.[12] A wide variation in peak concentrations of ketamine in serum associated with anesthesia have been reported, ranging from 9.3–14.5 μM[26,27] to 94.1 μM,[28] although these values are reduced by protein binding.

Ketamine is known to produce analgesia, and the effect is stereoselective with the *S(+)* isomer of ketamine being two- to threefold more potent than the *R(−)* isomer.[8] Similarly, *in vitro* opioid receptor binding studies in isolated tissue homogenates[9,12] have shown that *S(+)*-ketamine interacts with μ and κ opioid receptors two- to threefold more potently than *R(−)*-ketamine.

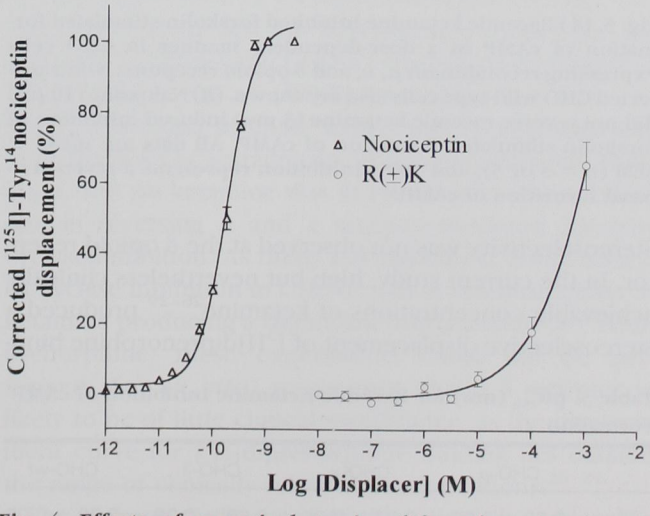

Fig. 4. Effects of racemic ketamine (K) and nociceptin on [^{125}I]Tyr^{14}nociceptin binding to recombinant ORL1 receptors. Fit for ketamine data is based on a theoretical maximum inhibition of 100%, and curves are corrected for the competing mass of [^{125}I]Tyr^{14}nociceptin. Data are mean ± SEM (n = 5).

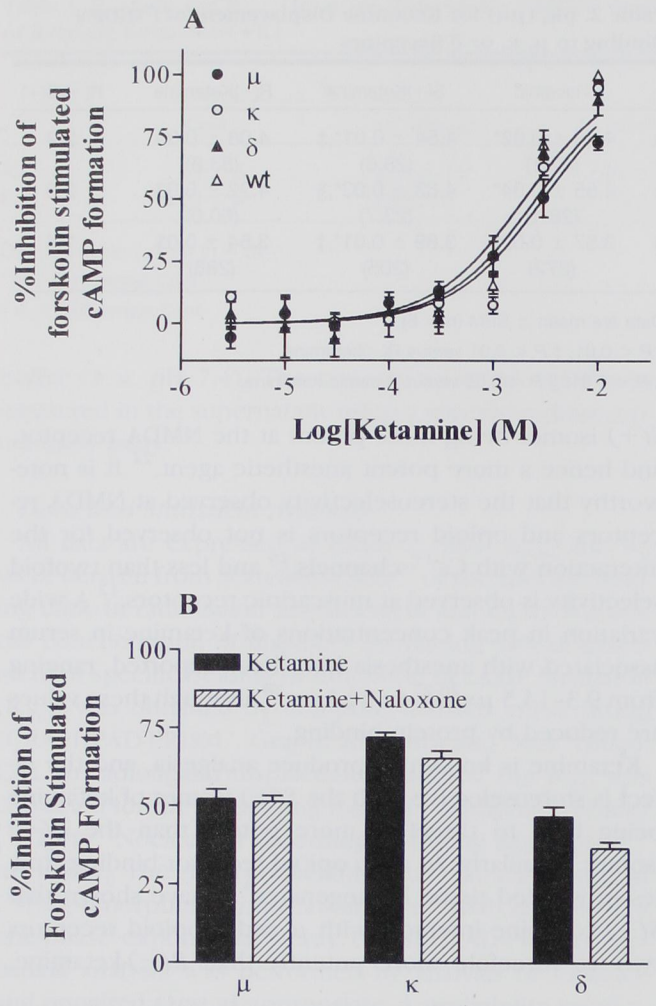

Fig. 5. (*A*) Racemic ketamine inhibited forskolin-stimulated formation of cAMP in a dose-dependent manner in CHO cells expressing recombinant μ, κ, and δ opioid receptors. Nontransfected CHO wild-type cells also are shown. (*B*) Naloxone (10 μM) did not reverse racemic ketamine (3 mM) induced inhibition of forskolin-stimulated formation of cAMP. All data are mean ± SEM (n = 4 or 5), and 100% inhibition represents a reversal to basal formation of cAMP.

Stereoselectivity was not observed at the δ opioid receptor. In the current study, high but nevertheless clinically achievable concentrations of ketamine[26-29] produced a stereoselective displacement of [^{3}H]diprenorphine binding to recombinant μ and κ but not δ opioid receptors. Supraclinical concentrations of ketamine produced a small but statistically significant stereoselective displace-

Table 3. pIC_{50} (mM) for Racemic Ketamine Inhibition of cAMP Formation

	CHO-μ	CHO-κ	CHO-δ	CHO-wt
pIC_{50}	2.68 ± 0.05 (2.07)	2.57 ± 0.05 (2.65)	2.69 ± 0.09 (2.02)	2.70 ± 0.05 (2.01)

IC_{50} values are based on a theoretical maximum inhibition of 100%. Data are mean ± SEM (n = 4).

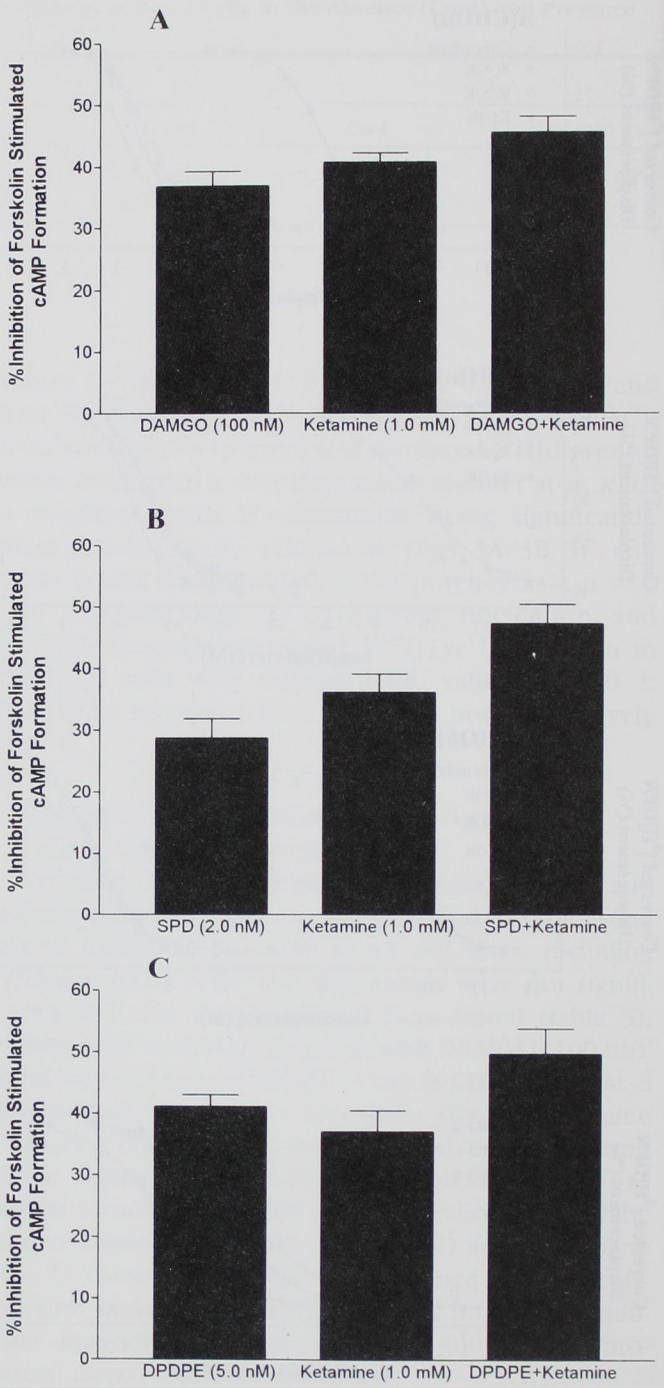

Fig. 6. Coincubation with racemic ketamine and opioid receptor subtype selective agonists (*A*, μ = [D-Ala^2, MePhe^4, Gly(ol)^5] enkephalin [DAMGO]; *B*, κ = spiradoline; *C*, δ = [D-pen^2, D-pen^5] enkephalin [DPDPE]) produced neither an additive nor synergistic interaction. Data are mean ± SEM (n = 5.)

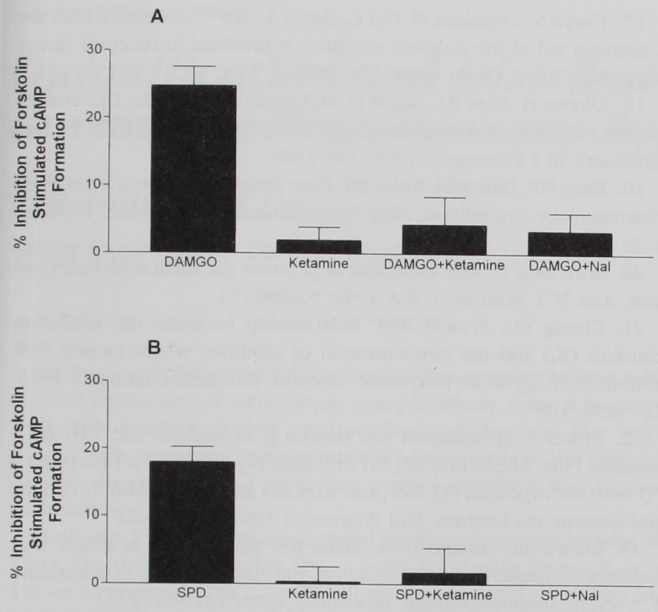

Fig. 7. Racemic ketamine (100 μM) and naloxone (Nal; 10 μM) reversed [D-Ala^2, MePhe^4, Gly(ol)^5] enkephalin (DAMGO 100 nM; *A*) and spiradoline (SPD 2 nM, *B*) inhibition of forskolin-stimulated formation of cAMP in CHO cells expressing the recombinant μ (*A*) and κ (*B*) opioid receptors, respectively. Data are mean ± SEM (n = 4–11).

ment at δ opioid receptors. The rate of radioligand dissociation in CHO-μ and CHO-κ cells was not affected by coincubation with ketamine. If an allosteric interaction was present, ketamine would have slowed the rate of dissociation (K_{-1}). Collectively, these data are consistent with a simple competitive interaction.

The use of recombinant receptors in this study offers several advantages over the use of tissue homogenates, the most important being the expression of a homogenous receptor population considerably simplifying interpretation. Receptor density should be controlled carefully, however, as second messenger coupling is often expression dependent.[4] In this study, receptor density was not vastly different from to that seen physiologically.[30] Finck *et al.*[31] suggested that ketamine analgesia may be mediated by μ or δ opioid receptors, as morphine-tolerant animals, showing upregulation of μ and δ opioid receptors,[32] are cross-tolerant to the analgesic effect of ketamine.[31] Smith *et al.*,[10] however, suggested that ketamine may be a μ antagonist and a κ agonist, as microinjection of ketamine into the rat periaqueductal gray region containing μ (but not κ) opioid receptors antagonized morphine analgesia as effectively as naloxone. The effect of ketamine was not antagonized by naloxone. In addition, it was suggested that ketamine analgesia might result from local anesthetic action as microinjection of lignocaine into the periaqueductal gray region also antagonized morphine analgesia. Consistently, we recently reported that lignocaine displaced [^{3}H]diprenorphine binding to opioid receptors.[33]

Opioid receptors are negatively coupled to adenylyl cyclase *via* a pertussis toxin-sensitive G-protein and inhibit formation of cAMP.[1–4,34] This inhibition of formation of cAMP would be expected to reduce neuronal excitability by inhibiting the hyperpolarization-activated (I_h) current.[35] In addition to inhibiting the formation of cAMP, opioid receptors close voltage-sensitive Ca^{2+} channels and enhance an outward K^+ conductance leading to hyperpolarization.[3,34] Opioids also exert excitatory effects in many systems, and we have reviewed this subject recently.[36] To examine the nature of the interaction of ketamine with opioid receptors, we measured concentrations of cAMP in CHO cells exposed to ketamine and a range of subtype selective agonists in various combinations. In the current study, we found that ketamine inhibited forskolin-stimulated formation of cAMP in a dose-dependent manner not only in CHO-μ, -κ, and -δ, but also in untransfected wild-type CHO cells with similar IC_{50} values (~2 mM). This IC_{50} value lay significantly to the right of diprenorphine displacement curves and was beyond the clinical range[26–29] and, therefore, is likely to be a nonspecific effect. Ketamine in conjunction with selective opioid receptor agonists produced neither an additive nor synergistic interaction. As the interaction was less than additive, we suspected that perhaps ketamine might be antagonizing the opioid-mediated inhibition of forskolin-stimulated formation of cAMP. To further test this hypothesis, we used 100 μM ketamine in CHO-μ and CHO-κ cells (which produced > 75% displacement of [^{3}H]diprenorphine but failed to inhibit formation of cAMP alone) and coincubated this with DAMGO or spiradoline. In this experimental paradigm, 100 μM ketamine was as effective as 10 μM naloxone in reversing μ and κ receptor-mediated adenylyl cyclase inhibition. As there was significant direct adenylyl cyclase inhibition in CHO-δ cells at concentrations of ketamine producing a significant displacement of [^{3}H]diprenorphine, these experiments could not be performed. Any *in vitro* antagonism at the δ receptor is likely to be of little clinical significance, as the displacement curve for [^{3}H]diprenorphine binding lies outside the range of clinically relevant concentrations.[26–29]

The current study suggests that clinically achievable concentrations of ketamine interact with μ and κ but not δ opioid receptors in a competitive fashion. In addition, ketamine failed to interact with the ORL1 receptor. This

produces functional antagonism of opioid receptor–mediated cellular signalling. The clinical significance of these data remains to be explored fully but suggests that ketamine may be a μ and κ antagonist, which implicates a nonopioid mechanism of analgesic action for this intriguing compound.

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