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Interaction of ketamine with μ_2 opioid receptors in SH-SY5Y human neuroblastoma cells

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Abstract
Purpose. Ketamine is known to interact with opioid receptors. However, because this agent does not produce opioid-like respiratory depression, it might not interact with μ_2 opioid receptors. Therefore, we have studied the interaction of ketamine with μ_2 opioid receptors expressed in SH-SY5Y cells.
Methods. SH-SY5Y cells (passage 70–80) were used to obtain ketamine dose-response curves for inhibition of 0.4 nM [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5] enkephalin (DAMGO) binding to μ_2 opioid receptors and of forskolin (1 μM)-stimulated cyclic AMP (cAMP) formation.
Results. Ketamine displaced [^{3}H]DAMGO binding in SH-SY5Y cells with a K_i of 12.1 μM. However, this concentrations did not inhibit forskolin-stimulated cAMP formation, although at supraclinical concentrations, significant inhibition was observed with an estimated IC_{50} of 700 μM.
Conclusion. The present study indicates that a clinically relevant concentration of ketamine interacts with μ_2 opioid receptors. However, no agonist activity was observed.

Key words: Ketamine, μ_2 Opioid receptors, SH-SY5Y human neuroblastoma cells

Introduction

Ketamine is known to interact with opioid receptors [1–6]. We and others have reported a stereoselective interaction at μ and κ opioid receptors with the (+)-enantiomer, *S*(+)-ketamine being two to three times more potent than the (−)-enantiomer, *R*(−)-ketamine [2,5,6]. These data are in good agreement with the effects of ketamine seen clinically, where *S*(+)-ketamine is two to three times more potent than *R*(−)-ketamine in analgesic studies [1]. In contrast to the reported analgesic effects of ketamine, this anesthetic agent does not produce opioid-like respiratory depression.

On pharmacological grounds, the μ opioid receptor is subdivided into μ_1 and μ_2, with some evidence for a μ_3 subtype [7]. μ_1 Receptors produce analgesia and μ_2 receptors produce both analgesia and respiratory depression [7]. We would therefore predict that ketamine would not interact with the μ_2 opioid receptor.

SH-SY5Y human neuroblastoma cells express both μ and δ opioid receptors, with the μ receptor predominating [8]. Utilizing naloxonazine and the dermorphin analogue Tyr-D-Arg^2-Phe-sarcosine^4, we have previously shown that SH-SY5Y cells appear to express a homogeneous population of μ_2 opioid receptors [8,9].

In this study we examined the interactions of ketamine with μ_2 opioid receptors expressed in SH-SY5Y cells.

Materials and methods

Cell culture and harvesting

SH-SY5Y cells (passage 70–80) were maintained in minimum essential medium supplemented with 100 iu·ml^{-1} penicillin, 100 μg·ml^{-1} streptomycin, 2.5 μg·ml^{-1} fungizone, and 10% fetal calf serum. Cultures were maintained at 37°C in 5% CO_2/humidified air at 37°C, fed every 2 to 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%).

Membrane preparation and [^{3}H]DAMGO binding

Cells were homogenized at 4°C using a Tissue Tearor (985-370, Biospec, USA) (setting 5.5 × 30-s bursts) in 50 mM Tris HCl buffer (pH 7.4). The homogenate was

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centrifuged at 18000*g* for 10 min and the pellet was resuspended in Tris HCl buffer. This procedure was repeated two more times. The membranes were prepared and used fresh daily. Opioid receptors in SH-SY5Y cell membranes were radiolabeled with [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5] enkephalin (DAMGO) to avoid labeling the δ-receptor population. The binding assay was performed in 1-ml volumes of Tris HCl buffer containing approximately 200 μg of membranes at 20°C for 90 min. Nonspecific binding was defined in the presence of 10^{-5} M naloxone. Following incubation, each sample was filtered (and washed) under vacuum through Whatman GF/B filters using a Brandel cell harvester. Radioactivity retained on the filter was extracted for at least 8 h in 4 ml of scintillation fluid. The interaction of ketamine (3×10^{-6}–10^{-3} M) with the opioid receptor was determined by displacement of 0.4 nM [^{3}H] DAMGO ($n = 6$).

Measurement of cAMP formation

Whole SH-SY5Y cells were suspended in 0.3 ml Krebs/HEPES buffer, pH 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. The cells were incubated additionally with or without ketamine (5×10^{-7}–10^{-3} M) to obtain dose-response curves ($n \geq 4$ for each point) for inhibition of cyclic AMP (cAMP) formation.

Data analysis

The concentration of ketamine producing 50% displacement of specific binding and 50% inhibition of cAMP formation (IC_{50}) was obtained by computer-assisted curve fitting: sigmoid function with variable slope for a curve over the 0–100 range (GRAPHPAD-PRISM). The IC_{50} for specific binding displacement was corrected for the competing mass of [^{3}H]DAMGO using a K_d of 1.04 nM previously obtained [10] and the Cheng and Prusoff equation to yield K_i: $K_i = IC_{50}/[1+([^3H]DAMGO\ used/K_d)]$.

Results

Ketamine displaced [^{3}H]DAMGO binding in SH-SY5Y cells with a K_i of 12.1 ± 0.8 (SEM) μM (Fig. 1). Ketamine also inhibited forskolin-stimulated cAMP formation in a concentration-dependent manner with an estimated IC_{50} of 700 μM (Fig. 2).

Discussion

In this study, we report an interaction of ketamine with μ_2 opioid receptors expressed in SH-SY5Y cells with a K_i (12.1 μM) in the clinically relevant range (<20 μM [1]). If ketamine were to produce opioid-like respiratory depression, it might be anticipated that ketamine would act as a μ_2-receptor agonist. However, ketamine did not inhibit cAMP formation (an index of opioid receptor activation) in the present study. We have previously suggested that ketamine acts as an antagonist at recombinant μ receptors expressed in Chinese hamster ovary cells [6]. Although the present study excludes agonist activity, we cannot exclude an antagonist action of ketamine at μ_2 opioid receptors.

If ketamine is an opioid antagonist, how does ketamine produce analgesia? NMDA antagonists have been reported to produce analgesia, and ketamine is a

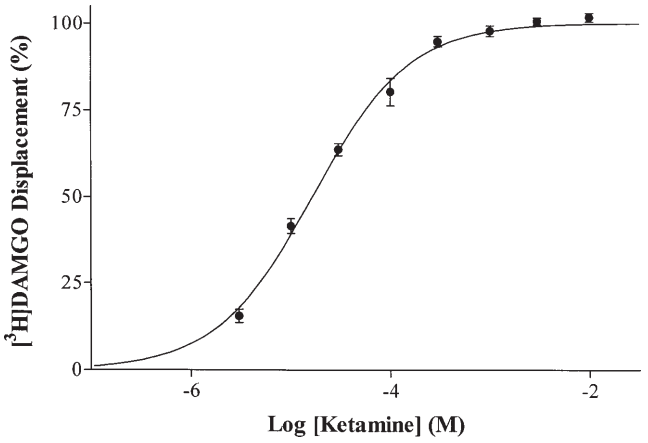

Fig. 1. Ketamine dose-dependently displaced [^{3}H]DAMGO binding to μ_2-opioid receptors in SH-SY5Y cell membranes. The dose-response curve was corrected for the competing mass of [^{3}H]DAMGO. All data are means ± SEM

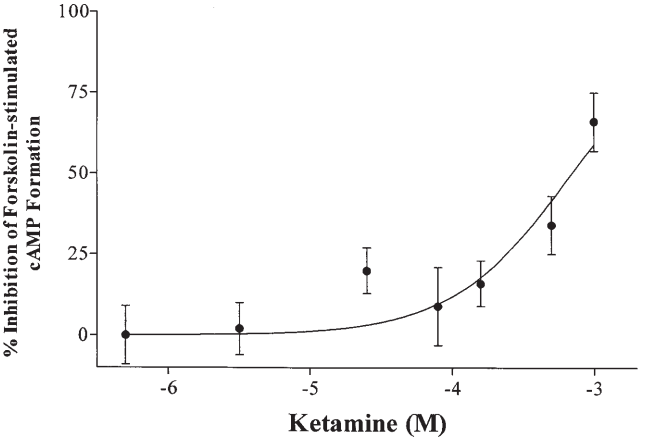

Fig. 2. Supraclinical concentration of ketamine inhibited forskolin (1 μM)-stimulated cAMP formation in a concentration-dependent manner. Each point is based on $n \geq 4$. All data are means ± SEM

noncompetitive antagonist of the NMDA receptor Ca^{2+} channel pore [11]. Moreover, clinically relevant concentrations of ketamine show stereoselectivity for this receptor [11]. Therefore, ketamine analgesia is likely to result from NMDA receptor blockade. Additionally, the antinociceptive actions of ketamine may involve descending inhibitory monoaminergic pain pathways [11].

In conclusion, the present data suggest that ketamine interacts with μ_2 opioid receptors at clinically relevant concentrations, but the functional consequences of that interaction remain to be determined.

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