Ketamine interacts with opioid receptors in the nonhuman primate amygdala and nucleus accumbens

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Background

Ketamine is an NMDAR antagonist that has rapid-acting antidepressant effects[1,2]. Despite ketamine's known action at the NMDAR, it also acts as an agonist at μ and κ opioid receptors with slightly lower binding affinity[3]. Previous studies in humans have demonstrated that activation of opioid receptors is necessary for ketamine's antidepressant effects[4], and work in rodents supports these findings[5–8]. No studies have been performed in nonhuman primates (NHPs) investigating ketamine's interactions with the opioid receptor system. NHPs are particularly well-suited for preclinical studies to investigate and develop treatments for psychiatric illnesses because NHPs have similar stress-related behaviors, social structures, and prefrontal cortical brain development to that of humans.

Methods

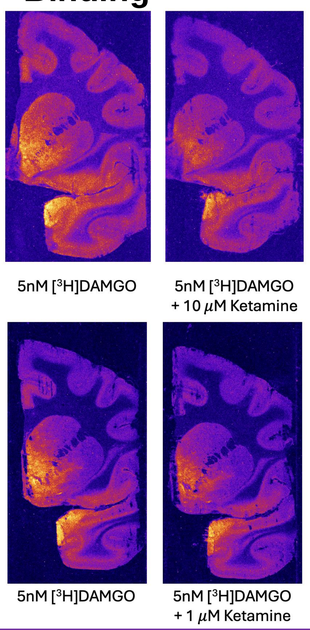
Using flash-frozen brain tissue slices from the amygdala, nucleus accumbens, and thalamus from one rhesus macaque, we conducted receptor and [^{35}S]GTPγS autoradiography to investigate ketamine's ability to bind to μ and κ opioid receptors as well as to elicit G-protein signaling. Regions of interest were determined by inspection based on the Paxinos atlas[9]. To assess ketamine binding to mu opioid receptors, tissue sections were incubated with 5 nM [^{3}H]DAMGO, a μ opioid receptor-selective ligand, in the presence of absence of 1 or 10 μM ketamine or 10 μM of the opioid antagonist naltrexone. To assess ketamine binding to κ opioid receptors, tissue sections were incubated with 15 nM [^{3}H]U-69,593, in the presence or absence of 1 or 10 μM ketamine or 10 μM naltrexone. To assess ketamine's ability to induce G-protein signaling, tissue sections were incubated with GDP and [^{35}S]GTPγS to assess basal activity. Stimulation of G-protein signaling was assessed with 10 μM ketamine or 10 μM ketamine plus 10 μM naltrexone. Some sections were incubated with 10 μM DAMGO as a positive control. Slides were placed into a Hypercassette™ covered by a BAS-SR2040 phosphor screen (FujiFilm; GE Healthcare). The slides were exposed to the phosphor screen for 3–5 days and imaged using a phosphor imager. ROIs were manually identified based on a digital atlas[10,11], and signal intensity was quantified using ImageJ. Data were analyzed using paired, one-tailed t-tests, and analyses were performed with GraphPad Prism 9 for [^{3}H]DAMGO and [^{3}H]U-69,593 data and Microsoft Excel for the [^{35}S]GTPγS data.

Results

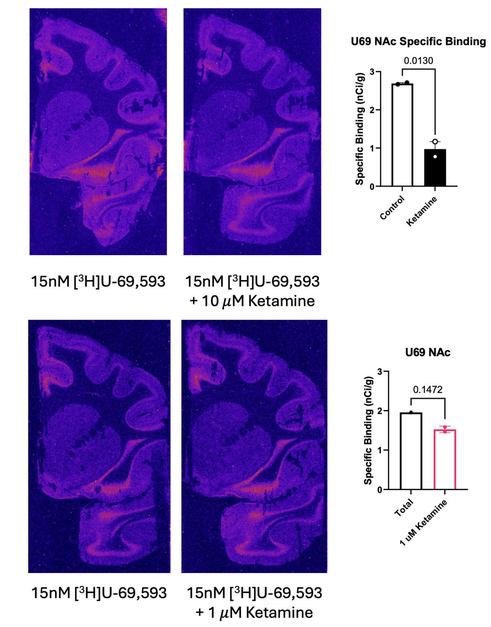
10 μM of racemic ketamine significantly reduced binding of both μ and κ opioid receptor agonists in the amygdala (p = 0.040; p = 0.046). 10 μM ketamine also significantly κ opioid receptor agonist binding in the nucleus accumbens (p = 0.013), but only tended to reduce μ opioid receptor agonist binding in the nucleus accumbens (p = 0.082). In contrast to 10 uM ketamine, 1 μM ketamine did not significantly reduce μ or κ opioid receptor agonist binding in the amygdala (p = 0.63; p = 0.34) or nucleus accumbens (p = 0.16; p = 0.15). In terms of eliciting G-protein signaling, 10 μM ketamine did not increase [^{35}S]GTPγS signal significantly more than baseline in the amygdala (p = 0.27), and it only tended to increase [^{35}S]GTPγS signal in the nucleus accumbens (p = 0.072).

Nucleus Accumbens

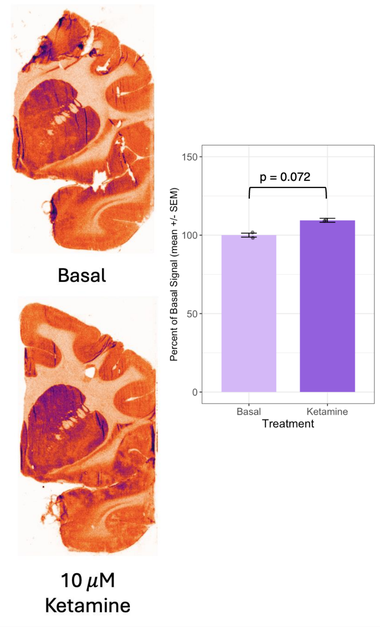
μ Opioid Receptor Binding



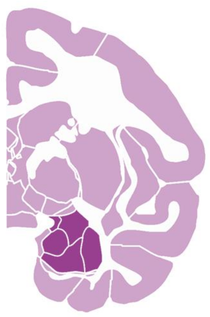
κ Opioid Receptor Binding



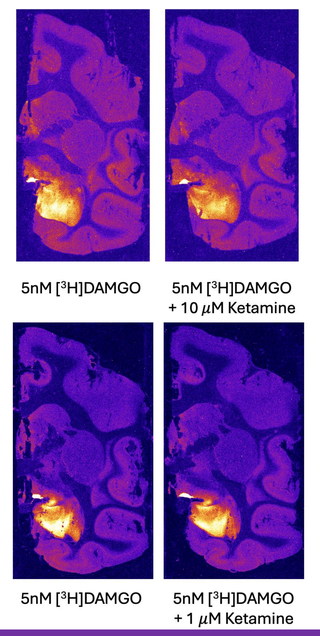
G-Protein Signaling



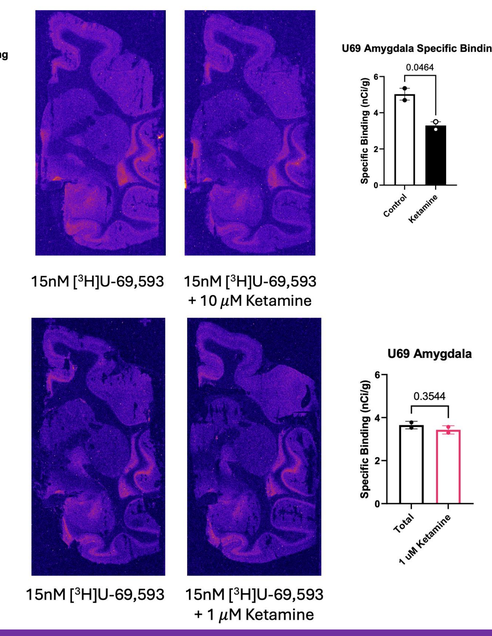
Amygdala



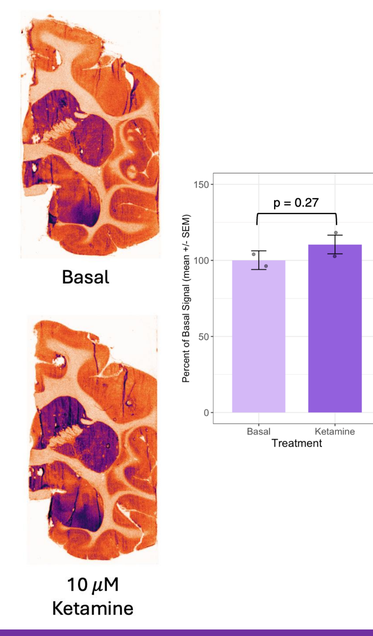
μ Opioid Receptor Binding



κ Opioid Receptor Binding



G-Protein Signaling



Conclusions

Ketamine significantly reduced both μ and κ opioid receptor agonist binding in the primate amygdala and nucleus accumbens at the higher concentration (10 μM) but not at the lower concentration (1 μM), demonstrating that ketamine competes with these high affinity opioid agonists at μ and κ opioid receptors in a concentration-dependent manner. Ketamine did not significantly increase G-protein signaling. These findings demonstrate in primates that ketamine is an opiate receptor binding compound that may be less effective in activating G-protein signaling when compared to higher affinity opioid receptor agonists. Future work will add additional animals to confirm the molecular findings and will also characterize the role of the opioid receptor system in ketamine's effects on NHP anxiety- and depressive-like behavior.

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