Ketamine Increases the Function of γ-Aminobutyric Acid Type A Receptors in Hippocampal and Cortical Neurons

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ABSTRACT

Background: The "dissociative " general anesthetic ketamine is a well-known *N*-methyl-D-aspartate receptor antagonist. However, whether ketamine, at clinically relevant concentrations, increases the activity of inhibitory γ-aminobutyric acid (GABA) receptor type A ($GABA_A$) receptors in different brain regions remains controversial. Here, the authors studied the effects of ketamine on synaptic and extrasynaptic $GABA_A$ receptors in hippocampal neurons. Ketamine modulation of extrasynaptic $GABA_A$ receptors in cortical neurons was also examined.

Methods: Whole cell currents were recorded from cultured murine neurons. Current evoked by exogenous GABA, miniature inhibitory postsynaptic currents, and currents directly activated by ketamine were studied.

Results: Ketamine did not alter the amplitude, frequency, or kinetics of postsynaptic currents but increased a tonic inhibitory current generated by extrasynaptic $GABA_A$ receptors in hippocampal neurons. For example, ketamine (100 μM) increased the tonic current by 33.6 ± 6.5% (mean ± SEM; 95% CI, 18.2 to 48.9; n = 8, $P < 0.001$). Ketamine shifted the GABA concentration–response curve to the left, but only when $GABA_A$ receptors were activated by low concentrations of GABA (n = 6). The selective increase in tonic current was attributed to ketamine increasing the apparent potency of GABA at high-affinity extrasynaptic $GABA_A$ receptors. Ketamine also increased a tonic current in cortical neurons (n = 11). Ketamine directly gated the opening of $GABA_A$ receptors, but only at high concentrations that are unlikely to occur during clinical use.

Conclusions: Clinically relevant concentrations of ketamine increased the activity of high-affinity extrasynaptic $GABA_A$ receptors in the hippocampus and cortex, an effect that likely contributes to ketamine's neurodepressive properties.
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KETAMINE is a phencyclidine derivative that is widely used as a short-acting "dissociative" general anesthetic.[1] Relative to most other general anesthetics, ketamine causes limited cardiovascular and respiratory depression. Thus, it is frequently administered for the induction of anesthesia in hemodynamically unstable patients, for brief surgical procedures, for the treatment of mass casualties in disaster relief efforts, and for combat-related injuries.[1–3]

The primary target for ketamine is generally thought to be excitatory glutamatergic *N*-methyl-D-aspartate (NMDA) receptors, as ketamine acts as a noncompetitive and uncompetitive antagonist of these receptors.[1,4] However, ketamine also modifies the function of other neurotransmitter receptors and ion channels including acetylcholine receptors, hyperpolarization-activated cyclic nucleotide-gated channel 1, voltage-gated ion channels, and dopamine and opioid receptors, and these actions likely contribute to the drug's neurodepressive properties.[5–10]

Most other commonly used general anesthetics increase the activity of inhibitory γ-aminobutyric acid (GABA) receptor type A ($GABA_A$) receptors, which are broadly classified into two major groups, postsynaptic and extrasynaptic.[11–13]

What We Already Know about This Topic

- The anesthetic properties of ketamine are usually attributed to inhibition of *N*-methyl-D-aspartate-type glutamate receptors; however, evidence also exists for involvement of other ion channels and receptors
- While potentiation of inhibitory γ-aminobutyric acid (GABA) receptor type A ($GABA_A$) receptors is a property of nearly all clinically used general anesthetics, their role in ketamine anesthesia is controversial

What This Article Tells Us That Is New

- Ketamine increased tonic inhibitory currents in isolated mouse hippocampal and cortical neurons with minimal effects on synaptic $GABA_A$ receptors
- Ketamine has $GABA_A$ receptor subtype-specific effects that result in selective potentiation of extrasynaptic $GABA_A$ receptor–mediated tonic inhibition

Postsynaptic $GABA_A$ receptors are activated by high concentrations of GABA that are released from presynaptic terminals. These receptors generate transient inhibitory postsynaptic currents that regulate rapid communication between neurons. In contrast, extrasynaptic $GABA_A$ receptors are activated by low, ambient concentrations of GABA and generate a tonic inhibitory current that regulates neuronal excitability, network

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synchrony, and cognitive behavior.[11,12] Extrasynaptic $GABA_A$ receptors are broadly grouped as those that contain the δ subunit (*e.g.*, α6βδ, α4βδ, α4δ, α6δ, and α1βδ) and those that lack the δ subunit (*e.g.*, α5βγ, αβ, α3βγ2, and αβε).[14,15] Some overlap occurs as some $GABA_A$ receptor subtypes are expressed at both synaptic and extrasynaptic locations.[12,16] Whether ketamine, at clinically relevant concentrations, increases inhibitory neurotransmission mediated by different subtypes of $GABA_A$ receptors remains controversial.

Studies that examined the effects of ketamine on inhibitory neurotransmission have produced inconsistent results, depending on the experimental model. Ketamine does not enhance $GABA_A$ receptor–mediated dorsal root potentials recorded in the spinal cord *in vitro* or the inhibitory effects of GABA on the excitatory firing of spinal neurons *in vivo*.[17] In addition, ketamine does not modify the function of recombinant α1β2γ2s $GABA_A$ receptors, likely the most ubiquitous subtype of postsynaptic $GABA_A$ receptor.[18] In contrast to these findings, other *in vitro* studies show that ketamine may enhance $GABA_A$ receptor–mediated inhibition.[19–23] Behavioral studies in mice found that bicuculline, a competitive and allosteric $GABA_A$ receptor antagonist, attenuates ketamine-induced loss of the righting reflex.[24,25] In addition, brain imaging studies of humans suggest that *S*-ketamine increases the function of $GABA_A$ receptors in the prefrontal cortex.[26]

The direct effects of ketamine on native $GABA_A$ receptors were studied with whole cell recordings from cerebellar granule neurons.[27] Clinically relevant concentrations of ketamine (100 μM) increased $GABA_A$ receptor current in cerebellar granule neurons by about 40%. Ketamine failed to increase the current in cerebellar granule neurons from transgenic functionally α6 and δ subunit null-mutant mice, prompting the suggestion that ketamine potentiation of native $GABA_A$ receptors is restricted to subtypes containing α6 and δ subunits. Furthermore, it was suggested that ketamine effects on $GABA_A$ receptors are limited to the cerebellum.[27]

Given the multiple behavioral effects of ketamine including memory loss, sedation, and hypnosis, we reasoned that ketamine modulates $GABA_A$ receptor activity in different brain regions other than the cerebellum and that the discordant results of previous studies could be attributed to the differential effects of ketamine on $GABA_A$ receptor subtypes. The aim of this study was to determine whether ketamine modifies the function of extrasynaptic and synaptic $GABA_A$ receptors in hippocampal neurons. Ketamine effects on extrasynaptic $GABA_A$ receptors in cortical neurons were also studied. The results show that ketamine increases the function of extrasynaptic $GABA_A$ receptors but has no effect on synaptic currents. We also studied the ketamine sensitivity of $GABA_A$ receptors in neurons from δ subunit null-mutant ($Gabrd^{-/-}$) mice and the effects of furosemide, an α6 subunit–containing $GABA_A$ receptor antagonist, on the ketamine-sensitive tonic current. The results showed that neither δ nor α6 subunit–containing $GABA_A$ receptors are required for ketamine's action. Collectively, these findings suggest that extrasynaptic $GABA_A$ receptors, which are expressed in multiple brain regions, are targets for ketamine's neurodepressive properties.

Materials and Methods

Experimental Animals

All experimental procedures were approved by the Animal Care Committee of the University of Toronto (Toronto, Ontario, Canada). Mice of either sex were used to produce cultures of hippocampal and cortical neurons. Swiss white mice were obtained from Charles River (USA). $Gabrd^{-/-}$ mice and wild-type (WT) mice (C57BL/6 × SvJ129) were generously provided by Dr. Gregg E. Homanics from the University of Pittsburgh (Pittsburgh, PA, USA). The generation, genotyping, and characterization of $Gabrd^{-/-}$ mice have been previously described.[28] Mice were housed in the animal care facility of the University of Toronto.

Cell Culture and Whole Cell Voltage Clamp Recordings

Primary cultures of hippocampal and cortical neurons were prepared from the hippocampi or cortex that were dissected from each fetus (embryonic day 18) as previously described.[29,30] Cell cultures prepared under these conditions primarily contain neurons. The density of neurons per 35-mm culture dish was approximately 1×10^6 cells. Cells were maintained in culture for 14 to 20 days before use.

Conventional whole cell voltage clamp recordings were performed at room temperature to reduce the frequency of miniature inhibitory postsynaptic currents (mIPSCs) and slow down the decay kinetics of mIPSCs for improved analysis.[31,32] Extracellular recording solution contained (in mM) 140 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 5.4 KCl, 25 HEPES, and 28 glucose (pH 7.4, 320 to 330 mOsm). Intracellular solution contained (in mM) 140 CsCl, 10 HEPES, 11 EGTA, 4 MgATP, 2 $MgCl_2$, 1 $CaCl_2$, 2 TEA, and 4 MgATP (pH 7.3 with CsOH, 285 to 295 mOsm). The cell access resistance was 13.7 ± 0.7 MΩ (95% CI, 12.2 to 15.1; n = 30), and cells were not studied further if the access resistance was greater than 20 MΩ. Series resistance was compensated at 70%, and the holding potential was set at −60 mV. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) and (2R)-amino-5-phosphonovaleric (APV) acid (20 μM) were added to the extracellular solution to block ionotropic glutamate receptors. Tetrodotoxin (0.2 μM) was also added to the extracellular solution to block voltage-dependent sodium channels. No randomization methods were applied. For most studies, currents were recorded before and during the application of ketamine or the $GABA_A$ receptor antagonist bicuculline. The experimenters were not blinded to the drug application conditions.

Drugs and Chemicals

Tetrodotoxin was purchased from Alomone Labs (Israel). CNQX, APV, and bicuculline were obtained from Abcam (United Kingdom). GABA, picrotoxin, and furosemide were from Sigma–Aldrich (Canada), while ketamine was from

Wyeth (Canada). Dizocilpine (MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo [*a*,*d*] cyclohepten-5,10-imine maleate) was obtained from Tocris Bioscience (United Kingdom).

Data Analysis

Currents were analyzed with pClamp 10 software (Molecular Devices, USA). There were no missing data from the results presented in this manuscript. mIPSCs were analyzed with MiniAnalysis 6.0.3 (USA). Manual inspection of each file was also performed, to allow the rejection of false events caused by noise and inclusion of events that were not detected automatically. At least 100 individual mIPSC events were detected under each experimental condition. Graphs, cumulative distribution plots of the amplitudes, and frequency of mIPSCs were generated with GraphPad Prism 5 (GraphPad Software Inc., USA). The effects of ketamine were normalized to control response: % of control = $I_{GABA + ketamine}/I_{GABA} \times 100$ for GABA-evoked current, and % of control = $I_{ketamine + bicuculline}/I_{bicuculline} \times 100$ for tonic GABA current.

Statistical Analyses

Data are represented as mean ± SEM together with the 95% CI of the mean. A Student's *t* test (paired or unpaired) was used to compare groups, where appropriate. For comparing three or more groups, ANOVA followed by Newman–Keuls *post hoc* test or two-way ANOVA followed by Bonferroni *post hoc* test was used. Cumulative distributions of the amplitude and frequency of mIPSCs were compared using the Kolmogorov–Smirnov test. Pearson correlation coefficient was used to measure the strength of concentration-dependent effects. A two-tailed hypothesis test was used, and statistical significance was set at $P < 0.05$. No statistical power calculation was conducted before the study. The sample size was based on our previous experience with this experimental design.

Results

Ketamine Increases $GABA_A$ Receptors, Only When These Receptors Are Activated by Low Concentrations of GABA

We first studied ketamine modulation of postsynaptic $GABA_A$ receptors in hippocampal neurons by recording spontaneous mIPSCs before and during an application of ketamine. Ketamine had no effect on the amplitude, frequency, or time course of mIPSCs, even at a concentration as high as 300 μM (fig. 1, A to C; table 1). Consistent with these results, the amplitude of the current evoked by a saturating concentration of exogenous GABA (1 mM) was not modified by coapplication of ketamine (1 mM; GABA: 8.0 ± 2.1 nA; 95% CI, 2.9 to 13.0 *vs.* GABA plus ketamine: 7.7 ± 1.9 nA; 95% CI, 3.0 to 12.3; n = 7; $P = 0.1$, Student's paired *t* test).

We next examined whether ketamine modulated the current evoked by lower concentrations of exogenous GABA. The minimum concentration of GABA that consistently produced a detectable inward current in hippocampal neurons was 0.3 μM. Therefore, the initial studies were performed using this concentration of GABA. Coapplication of ketamine (30 μM) increased the inward current by 35.3 ± 11.4% (95% CI, 9.1 to 61.5; n = 9; $P < 0.001$; fig. 1D). The potentiating effect of ketamine was rapid, reversible, and concentration-dependent (Pearson correlation coefficient = 0.91; $P = 0.045$). Interestingly, when the concentration of GABA was increased from 0.3 to 0.5 μM, the increase in current by ketamine (300 μM) was reduced ($P < 0.01$, one-way ANOVA followed by Newman–Keuls *post hoc* test). Specifically, ketamine increased the current to 170.0 ± 10.0% of control (95% CI, 146.3 to 193.7; n = 8) with 0.3 μM GABA, but increased the current to only 144.4 ± 1.9% of control (95% CI, 139.9 to 148.9; n = 8) with 0.5 μM GABA (fig. 1E). When the concentration of GABA was further increased to 10 μM, ketamine no longer increased the current (101.5 ± 1.5% of control; 95% CI, 98.1 to 104.9; n = 9). Thus, ketamine preferentially potentiated the currents evoked by low concentrations of GABA.

Ketamine Increases Tonic GABA Current

The sensitivity to low concentrations of GABA suggests that ketamine increases the activity of the high-affinity extrasynaptic $GABA_A$ receptors that generate tonic inhibitory currents.[11,12] To mimic the tonic current generated *in vivo*, neurons were continuously perfused with a low "ambient" concentration of GABA (0.3 μM), as this concentration is similar to the low physiologic levels of GABA that occur *in vivo*.[11,33] To probe the effects of ketamine, different concentrations were coperfused with GABA (0.3 μM; fig. 2A). The amplitude of the tonic current was assessed by applying the $GABA_A$ receptor antagonist bicuculline (20 μM) and measuring the change in holding current (fig. 2A).[24] Ketamine (10 to 1,000 μM) increased the amplitude of tonic current in a dose-dependent manner at all concentrations studied (Pearson correlation coefficient = 0.98; $P = 0.0004$; fig. 2, B and C). When the extracellular concentration of GABA was increased from 0.3 to 0.5 μM, the ketamine-induced increase in tonic current was reduced (fig. 2D). This observation is similar to the previous effects of ketamine on GABA-evoked currents, as shown in fig. 1E.

Ketamine Increases the Apparent Potency of GABA at $GABA_A$ Receptors

The previous results showed that ketamine preferentially increased the activity of high-affinity extrasynaptic $GABA_A$ receptors that were sensitive to low concentrations of GABA, whereas ketamine failed to modulate lower-affinity synaptic $GABA_A$ receptors that were activated by millimolar concentrations of GABA. Together, these findings suggest that ketamine might increase the apparent potency of low concentrations of GABA at $GABA_A$ receptors. To test this postulate, GABA (0.1 μM to 1 mM) concentration–response plots were constructed for current recorded in the absence

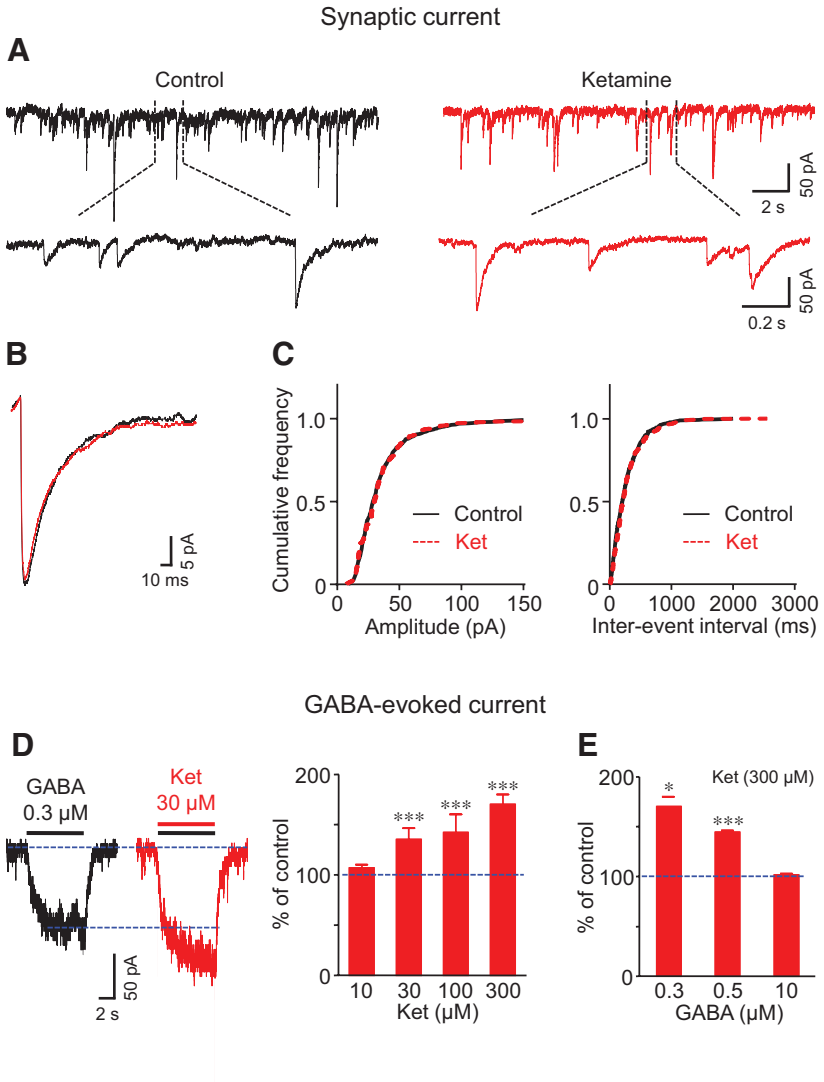

Fig. 1. Ketamine (Ket) had no effect on synaptic γ-aminobutyric acid (GABA) currents but increased the currents evoked by low concentrations of GABA in hippocampal neurons. (*A*) Representative recordings of GABA receptor type A ($GABA_A$) receptor–mediated miniature inhibitory postsynaptic currents (mIPSCs) from the same neuron, before and during application of ketamine. (*B*) Traces were averaged from 557 (control) and 522 (ketamine) individual mIPSCs. (*C*) Cumulative distributions of the amplitude (*left*) and frequency (*right*) of mIPSCs showing that both the amplitude and frequency were not affected by ketamine. $P = 0.07$ for the amplitude, $P = 0.12$ for the frequency, Kolmogorov–Smirnov test. (*D*) Representative traces and summarized data showing that current evoked by GABA (0.3 μM) was increased by ketamine. Two-way ANOVA, effect of ketamine treatment: $F(1,54) = 121.1$, $P < 0.0001$; effect of ketamine concentration: $F(3,54) = 3.6$, $P = 0.03$; effect of interaction: $F(3,54) = 27.4$, $P < 0.0001$; n = 6 for 10 μM ketamine, n = 9 for 30 μM ketamine, n = 8 for 100 and 300 μM ketamine, ***$P < 0.001$ compared with control, Bonferroni *post hoc* test. (*E*) Summarized data showing the effects of ketamine (300 μM) on currents evoked by different concentrations of GABA. Two-way ANOVA, effect of ketamine treatment: $F(1,44) = 27.3$, $P < 0.0001$; effect of GABA concentration: $F(2,44) = 137.3$, $P < 0.0001$; effect of interaction: $F(2,44) = 3.3$, $P = 0.06$; n = 8 for 0.3 and 0.5 μM GABA, n = 9 for 10 μM GABA, *$P < 0.05$, ***$P < 0.001$ compared with control, Bonferroni *post hoc* test.

or presence of ketamine (1 mM; fig. 3, A and B). We predicted that ketamine would modify the GABA concentration–response plot, but only for currents evoked by lower concentrations of GABA. Consistent with this hypothesis, ketamine increased the currents evoked by GABA at 0.1 to 3 μM and shifted the concentration–response plot to the left, but only for the lower components of the curve (fig. 3B). The effective concentrations of GABA that induce 10 to 60% of the maximum response (EC_{10}–EC_{60} values) were significantly reduced by ketamine, whereas the EC_{70}–EC_{90} values were unchanged (table 2). Therefore, the apparent potency of low concentrations of GABA at the $GABA_A$ receptors was increased by ketamine.

Desensitization of $GABA_A$ receptors can also modify the magnitude of tonic current, and a variety of general anesthetics, including propofol and etomidate, have been shown

Table 1. Ketamine (300 μM) Did Not Modify γ-Aminobutyric Acid Miniature Inhibitory Postsynaptic Currents

	Amplitude, pA	Frequency, Hz	Rise time, ms	Decay time, ms	Area, pA.ms
Control	34.7 ± 3.4 (25.9–43.5)	2.4 ± 0.7 (0.7–4.2)	6.2 ± 0.3 (5.6–6.9)	20.2 ± 2.8 (13.1–27.3)	748.2 ± 107.5 (471.8–1025)
Ketamine	34.7 ± 3.3 (26.3–43.1)	2.3 ± 0.5 (0.9–3.6)	6.5 ± 0.2 (5.9–7.1)	20.2 ± 3.9 (10.1–30.3)	798.8 ± 174.9 (349.2–1248)

Data are presented as mean ± SEM, and the 95% CI of the mean is presented in parentheses. For all the parameters, $P > 0.05$, Student's paired *t* test, n = 6.

to modulate receptor desensitization.[11,34–36] Therefore, we investigated whether ketamine altered the apparent extent of $GABA_A$ receptor desensitization. We recorded whole cell currents evoked by longer applications of GABA (16 s) before and during application of ketamine (1 mM; fig. 3C). The ratio of current at 16 s relative to the initial peak current was measured. As shown in fig. 3D, ketamine had no effect on the apparent extent of $GABA_A$ receptor desensitization for currents evoked by GABA (0.3 to 10 μM).

Ketamine at High Concentrations Directly Activates $GABA_A$ Receptors

Intravenous and inhalational general anesthetics, including propofol, etomidate, pentobarbital, alfaxalone, isoflurane, and sevoflurane, directly activate or gate $GABA_A$ receptors at higher concentrations.[37–41] Therefore, we examined whether ketamine also acts as a $GABA_A$ receptor agonist. Application of high concentrations of ketamine evoked inward currents in all of the hippocampal neurons tested (fig. 4A). Ketamine-evoked current was first detected at a concentration of 300 μM (12.6 ± 4.9 pA; 95% CI, 1.1 to 26.3; n = 5). The amplitude of the current increased with higher concentrations of ketamine, and the maximal response was observed at 30 mM. The ketamine concentration–response plot revealed an EC_{50} of 6.4 ± 0.6 mM (95% CI, 5.1 to 7.6) and a Hill coefficient of 2.2 ± 0.4 (95% CI, 1.5 to 2.9).

To confirm that the ketamine-evoked current was mediated by $GABA_A$ receptors, the effects of bicuculline and a noncompetitive $GABA_A$ receptor antagonist, picrotoxin, were studied (fig. 4, B and C). Both antagonists blocked the current evoked by 3 mM ketamine in a concentration-dependent manner. The half-maximal inhibitory concentrations (IC_{50}) were 1.3 ± 0.1 μM (95% CI, 1.0 to 1.6) for bicuculline and 15.2 ± 2.0 μM (95% CI, 11.1 to 19.4) for picrotoxin, respectively.

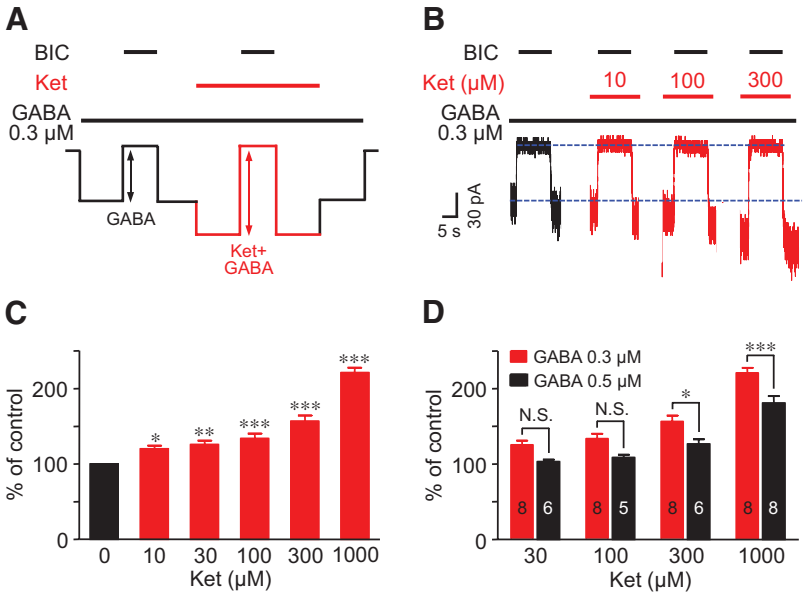

Fig. 2. Ketamine (Ket) increased the amplitude of the tonic γ-aminobutyric acid (GABA) current. (*A*) Schematic diagram showing the experimental design. To mimic the tonic current generated *in vivo*, neurons were continuously perfused with a low concentration of GABA (0.3 μM), which generated a persistent inward current. The amplitude of the tonic current was quantified by applying the GABA receptor type A ($GABA_A$) receptor antagonist bicuculline (BIC, 20 μM) and measuring the change in holding current. Perfusion of ketamine, in the presence of GABA (0.3 μM), further increased the inward current as reflected by larger reduction in holding current when bicuculline was applied. (*B*) Representative traces demonstrating the dose-dependent enhancing effects of ketamine on tonic current. (*C*) Summarized data showing the dose-dependent effects of ketamine on the amplitude of the tonic current (n = 8 for each concentration). One-way repeated measures ANOVA, $F(5,35) = 70.0$, $P < 0.0001$; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* 0 μM ketamine, Newman–Keuls *post hoc* test. (*D*) Quantified data showing that ketamine-induced increase in tonic current was decreased when GABA concentration was increased from 0.3 to 0.5 μM. Two-way ANOVA, effect of GABA concentration: $F(1,49) = 35.1$, $P < 0.0001$; effect of ketamine concentration: $F(3,49) = 68.0$, $P < 0.0001$; effect of interaction: $F(3,49) = 0.7$, $P = 0.6$; the numbers in the columns show the n number; no significance (N.S.), $^*P < 0.05$, $^{***}P < 0.001$, Bonferroni *post hoc* test.

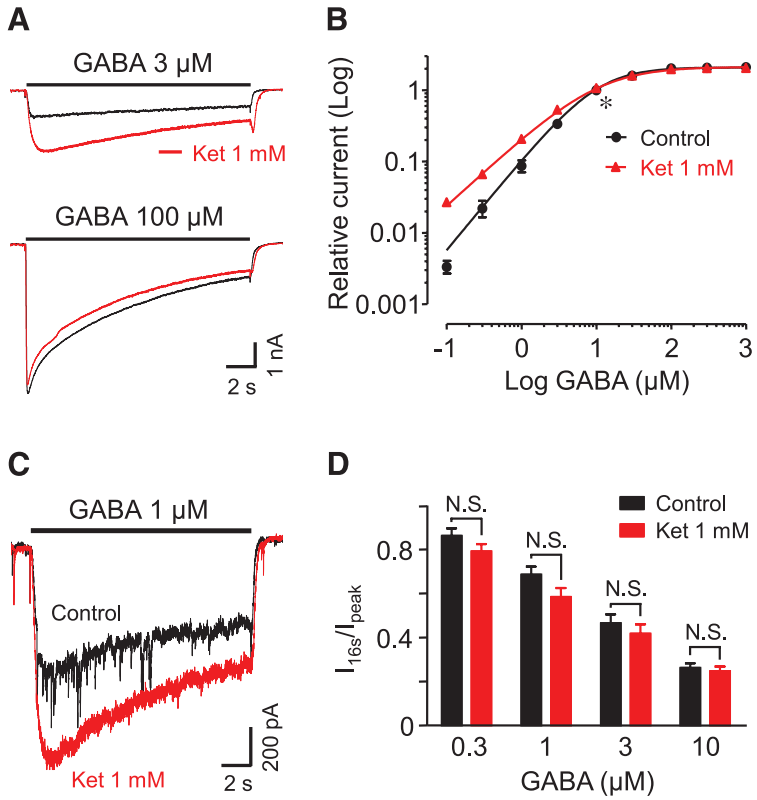

Fig. 3. Ketamine (Ket) increased the apparent potency of low concentrations of γ-aminobutyric acid (GABA) without modifying the desensitization properties. (*A*) Representative traces showing the effects of ketamine (1 mM) on the currents evoked by 3 μM and 100 μM of GABA. (*B*) Concentration–response plots in the absence and presence of ketamine (n = 6). Responses were normalized to current evoked by GABA (10 μM) alone as indicated by *. (*C*) Representative traces showing currents evoked by GABA (1 μM) before and during application of ketamine (1 mM). (*D*) Summarized data showing no effects of ketamine on desensitization of currents evoked by increasing concentrations of GABA. Two-way ANOVA, effect of ketamine: $F(1,40) = 8.8$, $P = 0.008$; effect of GABA concentration: $F(3,40) = 59.2$, $P < 0.0001$; effect of interaction: $F(3,40) = 0.7$, $P = 0.6$; n = 6 for each column; no significance (N.S.), Bonferroni *post hoc* test.

Potentiating and Direct Gating by Ketamine Do Not Require $GABA_A$ Receptors that Contain δ or α6 Subunits

Hevers *et al.*[27] proposed that ketamine modulates $GABA_A$ receptors, but only if the neurons express δ and α6 subunits. We used both genetic and pharmacologic approaches to determine whether the effects of ketamine were restricted to these $GABA_A$ receptor subtypes. First, the effects of ketamine were studied using neurons that were harvested from *Gabrd*$^{-/-}$ mice and WT mice. Ketamine produced a similar increase in the current evoked by GABA (0.3 μM) and the tonic GABA current in both genotypes (fig. 5A). Also, the current directly activated by ketamine (3 mM) was similar in magnitude between *Gabrd*$^{-/-}$ and WT neurons (fig. 5A). Next, the effects of furosemide (300 μM), an α6-containing $GABA_A$ receptor antagonist,[42,43] were studied on the GABA-evoked current, the tonic GABA current, and the current directly evoked by ketamine (fig. 5B). Furosemide did not significantly reduce the potentiating effects of ketamine on GABA-evoked current or tonic current, although a slight reduction was observed. In addition, the direct ketamine-evoked current was unaffected by furosemide. Thus, $GABA_A$ receptors containing δ or α6 subunits are not required for the potentiating or direct gating effects of ketamine on $GABA_A$ receptors in hippocampal neurons.

Table 2. The Effective Concentrations of γ-Aminobutyric Acid that Induce 10 to 90% of the Maximum Response (EC10–90 values, μM) Were Calculated from the Concentration–Response Plots

	GABA	Ketamine
EC10	2.1 ± 0.1 (1.7–2.4)	1.1 ± 0.1 (0.8–1.4)*
EC20	3.8 ± 0.2 (3.4–4.2)	2.4 ± 0.2 (1.8–3.0)†
EC30	5.7 ± 0.3 (4.9–6.5)	4.0 ± 0.4 (2.8–5.1)†
EC40	8.1 ± 0.6 (6.5–9.6)	6.1 ± 0.8 (4.1–8.1)†
EC50	11.0 ± 1.0 (8.3–13.7)	9.1 ± 1.3 (5.7–12.4)*
EC60	15.2 ± 1.8 (10.6–19.8)	13.6 ± 2.2 (7.9–19.3)‡
EC70	21.5 ± 3.1 (13.6–9.5)	21.4 ± 4.0 (10.9–31.3)
EC80	33.7 ± 5.8 (18.2– 8.2)	36.5 ± 8.0 (15.9–57.1)
EC90	64.2 ± 14.5 (26.9–101.5)	84.1 ± 22.9 (25.2–43.1)

Data are presented as mean ± SEM, and the 95% CI of the mean is presented in parentheses. Student's paired *t* test, n = 6.

*$P < 0.01$. †$P < 0.001$. ‡$P < 0.05$.

GABA = γ-aminobutyric acid.

Ketamine Increases $GABA_A$ Receptors in Cortical Neurons

We investigated whether the effects of ketamine could be extended to neurons from other brain regions. Cortical

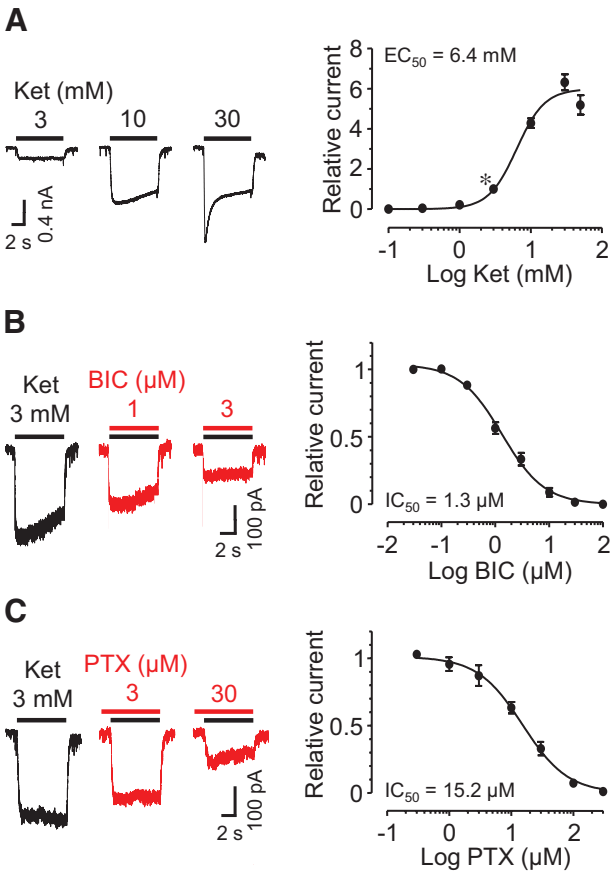

Fig. 4. Ketamine (Ket), at high concentrations, directly gated γ-aminobutyric acid receptor type A receptors. (*A*) *Left*: Representative responses to application of increasing concentrations of ketamine. *Right*: Concentration–response plot for ketamine-evoked currents. Responses were normalized to current evoked by 3 mM ketamine (*). n = 5 for 0.1 and 0.3 mM, n = 6 for 1 mM, n = 13 for 3 and 10 mM, n = 12 for 30 mM, and n = 7 for 50 mM. (*B*, *C*) Representative traces and the concentration–response plot showing concentration-dependent inhibition of ketamine-evoked currents by bicuculline (BIC; *B*) and picrotoxin (PTX; *C*). (*B*) n = 5 for 0.03, 0.1, and 100 μM; n = 6 for 1 and 30 μM; n = 7 for 0.3, 3, and 10 μM. (*C*) n = 5 for 0.3, 3, 30, and 300 μM; n = 6 for 1, 10, and 100 μM.

neurons are of particular interest, as the cortex is thought to contribute to the clinically important behavioral properties of ketamine such as sedation.[1] Similar to the effects observed in hippocampal neurons, ketamine (30 and 300 μM) increased the current evoked by GABA (0.3 μM), as well as the tonic current (fig. 6, A and B). Ketamine (3 mM) alone evoked a current that was inhibited by bicuculline (fig. 6C). Thus, the effects of ketamine on GABA$_A$ receptors are similar in hippocampal and cortical neurons.

MK-801 Fails to Increase Tonic GABA Current in Hippocampal Neurons

Others have proposed that ketamine inhibition of NMDA receptors is necessary but not sufficient to produce general anesthesia and that modulation of other receptors is required to produce profound neurodepression.[1,27] In support of this notion, the high-affinity NMDA receptor antagonist MK-801 is ineffective for clinical use as a general anesthetic, possibly because it fails to increase GABA$_A$ receptors.[27,44,45] We next tested whether MK-801 modified the activity of extrasynaptic GABA$_A$ receptors in hippocampal neurons. Even high concentrations of MK-801 (50 μM) failed to increase the amplitude of the tonic current (control: 48.0 ± 2.7 pA; 95% CI, 40.6 to 55.4 *vs.* MK-801: 45.7 ± 2.3 pA; 95% CI, 39.2 to 52.1; n = 5; $P = 0.2$, Student's paired *t* test). These results support the argument that ketamine potentiation of extrasynaptic GABA$_A$ receptors is necessary to produce general anesthesia.

Discussion

The results show that ketamine preferentially increases a tonic inhibitory current generated by extrasynaptic GABA$_A$ receptors but has no effect on synaptic GABA$_A$ receptors. The increase in tonic current but not synaptic currents is attributed to the selective increase in potency of GABA at high-affinity extrasynaptic GABA$_A$ receptors. The ketamine-induced increase in tonic GABA current in hippocampal neurons does not require GABA$_A$ receptors that contain either the δ or α6 subunit. Furthermore, potentiation by ketamine is not restricted to GABA$_A$ receptors expressed in cerebellar granule cells.

Several of the findings reported here indicate that ketamine selectively increases GABA$_A$ receptors that are activated by low concentrations of GABA. First, ketamine fails to modify the mIPSCs that are generated by near-saturating, millimolar concentrations of GABA at postsynaptic GABA$_A$ receptors. Second, when high concentrations of exogenous GABA are applied to neurons (to maximize the binding of GABA at GABA$_A$ receptors), ketamine fails to increase the amplitude of the current. Similar findings have been reported for cerebellar granule cells.[27] Third, ketamine shifts the GABA concentration–response curve to the left, but only for low concentrations of GABA. Finally, ketamine does not modify the apparent extent of desensitization, which suggests that it does not modify the intrinsic gating properties of the receptors. Collectively, these results suggest that ketamine increases the activity of high-affinity extrasynaptic GABA$_A$ receptors by increasing the potency for GABA. Interestingly, hydrogen peroxide, like ketamine, selectively increases current evoked by low but not high concentrations of GABA.[30] As such, both hydrogen peroxide and ketamine may target similar receptor subtypes or low agonist binding states of GABA$_A$ receptors.

We attribute the differential ketamine sensitivity of tonic and synaptic currents to differences in the subtypes of underlying GABA$_A$ receptors. The whole cell macroscopic currents reflect the activation of ensembles of GABA$_A$ receptors that differ in their sensitivity to both GABA and ketamine. Extrasynaptic GABA$_A$ receptors typically have a greater affinity for GABA and are

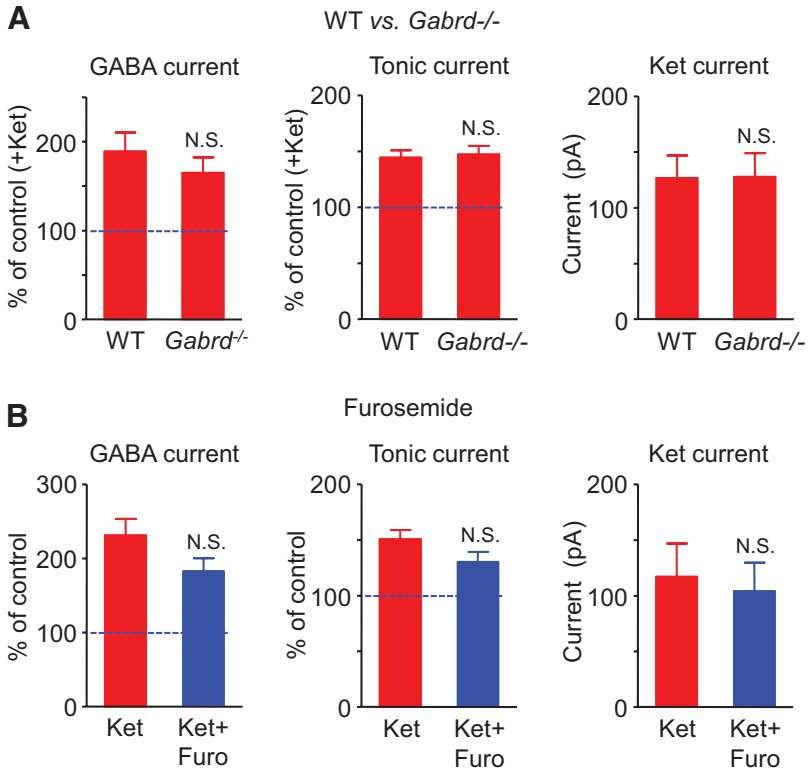

Fig. 5. γ-Aminobutyric acid (GABA) receptor type A receptors containing either δ or α6 subunits were not required for ketamine (Ket) effects. (*A*) Quantified data summarizing the potentiating effects of ketamine (300 μM) on current evoked by GABA (0.3 μM, wild type [WT]: n = 7, *Gabrd*$^{-/-}$: n = 9), on tonic current with GABA (0.3 μM; n = 7) in the bath, as well as on the current evoked by ketamine (3 mM; n = 8) in neurons from *Gabrd*$^{-/-}$ and WT mice. N.S.: no significance, Student's unpaired *t* test. (*B*) Furosemide (300 μM) did not alter the potentiating effects of ketamine on current evoked by GABA (0.3 μM; n = 9), on tonic current (GABA, 0.3 μM in the bath; n = 8), and on ketamine-evoked current (n = 8). No significance (N.S.), Student's paired *t* test.

more sensitive to ketamine compared with postsynaptic $GABA_A$ receptors. Such subtype-dependent differences also likely account for ketamine effects on the GABA concentration–response plot. Specifically, ketamine increases the currents evoked by low concentrations of GABA and shifts the lower component of the concentration–response plot to the left. Ketamine thereby increases the apparent potency of GABA at the $GABA_A$ receptors, but only for low concentrations of GABA. It should be noted, however, that it is impossible to disentangle the contribution of extrasynaptic *versus* synaptic $GABA_A$ receptors to GABA-evoked macroscopic currents using the methods employed in this study.

Ketamine effects are not restricted to native $GABA_A$ receptors containing δ and α6 subunits, nor are they restricted to cerebellar granule neurons.[27] In hippocampal and cortical neurons, the tonic current is generated by multiple different subtypes including those that contain the δ subunit (*e.g.*, α6βδ, α4βδ, α4δ, α6δ, and α1βδ) and those that lack the δ subunit (*e.g.*, α5βγ, αβ, α3βγ2, and αβε).[14,15,30] The selective effect of ketamine on tonic current but not synaptic currents in hippocampal neurons is likely attributed to the intrinsic pharmacologic properties of the underlying $GABA_A$ receptor subtypes. Consistent with this notion, others have shown that the efficacy of ketamine potentiation of $GABA_A$ receptor function depends on the subunit composition of the underlying receptors. Incorporating the γ subunit in recombinant $GABA_A$ receptors, a subunit that is present in most synaptic receptors, renders receptors less sensitive to ketamine.[27] Ketamine potentiation of recombinant α1β2γ2 $GABA_A$ receptors (a ubiquitous synaptic subtype) is markedly less than the potentiation of current generated by α6β2δ $GABA_A$ receptors (a typical extrasynaptic receptor subtype), even when these receptor subtypes are activated by equivalent concentrations of GABA (EC_3).[27]

Our results, together with those reported in a previous study, show that ketamine potentiation of native $GABA_A$ receptors expressed in neurons is considerably less than the potentiation of recombinant $GABA_A$ receptors.[27] Interestingly, ketamine (100 μM) increases tonic current recorded from native $GABA_A$ receptors in hippocampal neuron by about 34%. Extrasynaptic $GABA_A$ receptors composed of α1β2 and α4β2δ are expressed in hippocampal neurons, yet ketamine increases recombinant α1β2 and α4β2δ receptors by about 81% and 66%, respectively.[27] Ketamine (100 μM) increases GABA (EC_3)–evoked current from recombinant α6β2δ and α6β3δ receptors by 260% and 416%, respectively.[27] In contrast, the ketamine-induced increase in

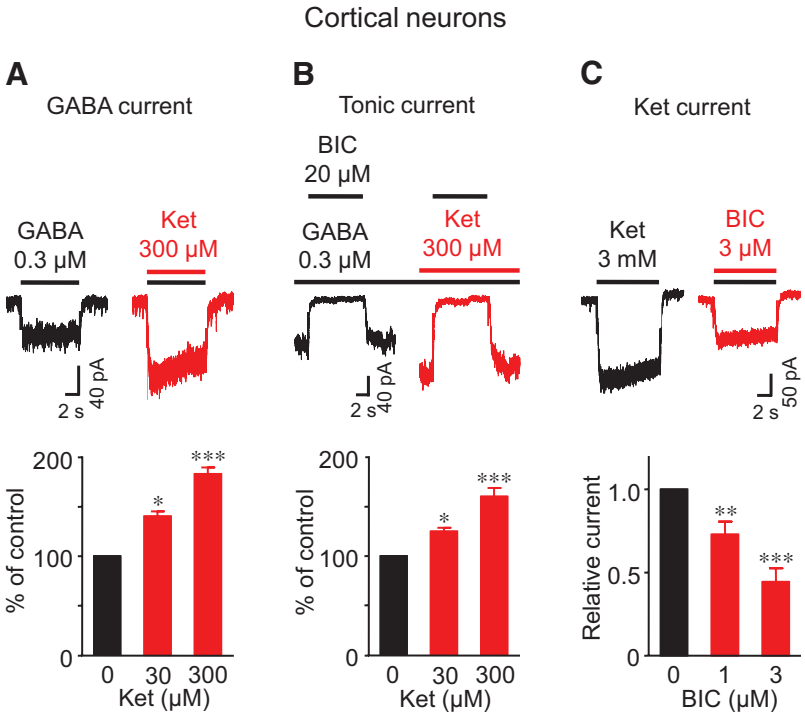

Fig. 6. Ketamine (Ket) had similar effects in cortical neurons. (*A*) Representative traces and summarized data showing that ketamine similarly increased current evoked by γ-aminobutyric acid (GABA; 0.3 μM) in cortical neurons. n = 9, one-way repeated measures ANOVA, $F(2,16) = 20.8$, $P < 0.0001$; $^*P < 0.05$, $^{***}P < 0.001$ *versus* 0 μM ketamine, Newman–Keuls *post hoc* test. (*B*) Ketamine increased tonic current (GABA, 0.3 μM in the bath). n = 11, one-way repeated measures ANOVA, $F(2,20) = 17.0$, $P < 0.0001$; $^*P < 0.05$, $^{***}P < 0.001$ *versus* 0 μM ketamine, Newman–Keuls *post hoc* test. (*C*) Ketamine-evoked current was inhibited by bicuculline (BIC). n = 5, one-way repeated measures ANOVA, $F(2,8) = 36.7$, $P < 0.0001$; $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* 0 μM bicuculline, Newman–Keuls *post hoc* test.

current from native $GABA_A$ receptors in cerebellar neurons is about 40%, even though α6β2δ and α6β3δ receptors are highly expressed in this cell type.[27] The reasons for a discrepancy between the ketamine effects on current recorded from native and recombinant $GABA_A$ receptors are uncertain but most likely result from differences in the subunit composition of the underlying receptors.[11,12] Posttranslational modifications such as receptor phosphorylation or interactions with cytosolic anchoring proteins could also reduce ketamine effects on native $GABA_A$ receptors.[46,47]

It is notable that high ketamine concentrations directly activate native $GABA_A$ receptors in the absence of exogenous GABA. This direct gating property of ketamine is similar to that of most other intravenous and inhaled general anesthetics, including propofol, etomidate, pentobarbital, alfaxalone, isoflurane, and sevoflurane.[37–41] Given that direct gating of $GABA_A$ receptors by ketamine occurs only at high concentrations (greater than or equal to 300 μM), it is unlikely to contribute to the clinical effects of ketamine.

Concentrations of ketamine in the brain during anesthesia are generally about 10 to 100 μM but can reach as high as 300 μM in both rodents and humans.[48–50] Our current results show that ketamine, at concentrations that occur during clinical use (10 to 300 μM), increases $GABA_A$ receptors. More specifically, ketamine (100 μM) increases the tonic current in hippocampal neurons by about 34%. Similarly, ketamine (100 μM) increases the GABA current in dissociated cerebellar granule neurons by about 40%,[27] which suggests that the effect of ketamine for high-affinity $GABA_A$ receptors may be similar across different brain regions. Interestingly, the IC_{50} for inhibition of NMDA receptor–mediated neuronal events by ketamine is about 10 to 20 μM.[7,51] Thus, at concentrations that occur during clinical use, ketamine both enhances extrasynaptic $GABA_A$ receptor activity and inhibits NMDA receptors.

Extrasynaptic $GABA_A$ receptors in the hippocampus and cortex are plausible targets to mediate the neurodepressive properties of ketamine. The tonic current generated by these receptors is a powerful regulator of neuronal excitability, synaptic plasticity, and cognition.[12] Indeed, the contribution of tonic inhibition to the overall inhibitory conductance has been estimated as at least threefold greater than that of phasic inhibition.[52] Various drugs, including anesthetics that preferentially increase the tonic current, typically cause profound neurodepression.[11,12] Thus, increase in tonic GABA current by ketamine likely contributes to the acute neurodepressive properties of this drug, such as memory loss and sedation.

The potentiation of extrasynaptic $GABA_A$ receptors by ketamine may also contribute to the long-term adverse

consequences of chronic illicit use, such as persistent cognitive deficits.[53] Persistent illicit use of ketamine, which has been increasing rapidly in many countries (including the United States, Australia, and China), leads to cognitive deficits, dependence, and drug tolerance.[53–57] Interestingly, long-term treatment of mice with a subanesthetic dose of ketamine for 1 or 3 months significantly up-regulated messenger RNA and protein levels of extrasynaptic α5 subunit–containing $GABA_A$ ($\alpha5GABA_A$) receptors in the prefrontal cortex.[58] Other general anesthetics that directly increase extrasynaptic $GABA_A$ receptors, such as etomidate and isoflurane, trigger a sustained increase in $\alpha5GABA_A$ receptor–mediated tonic inhibitory current that lasts for days after the anesthetics are eliminated.[59] The increased tonic current is due to an increase in the cell-surface expression of $\alpha5GABA_A$ receptors, which interferes with memory processes.[59–61] Long-term treatment of mice with ketamine causes memory deficits in a hippocampus-dependent spatial navigation task.[58] In future studies, it will be important to investigate whether a persistent increase in extrasynaptic $GABA_A$ receptor function contributes to the cognitive deficits that result from long-term use.

In summary, the current study shows that ketamine increases a tonic inhibitory conductance generated by high-affinity extrasynaptic $GABA_A$ receptors in hippocampal and cortical neurons. The ketamine-induced increase in tonic current likely contributes to the drug's desired anesthetic properties. Further studies are warranted to determine the role of extrasynaptic $GABA_A$ receptors in the adverse consequences of long-term ketamine abuse.

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Competing Interests

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Address correspondence to Dr. Orser: Department of Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada. beverley.orser@utoronto.ca. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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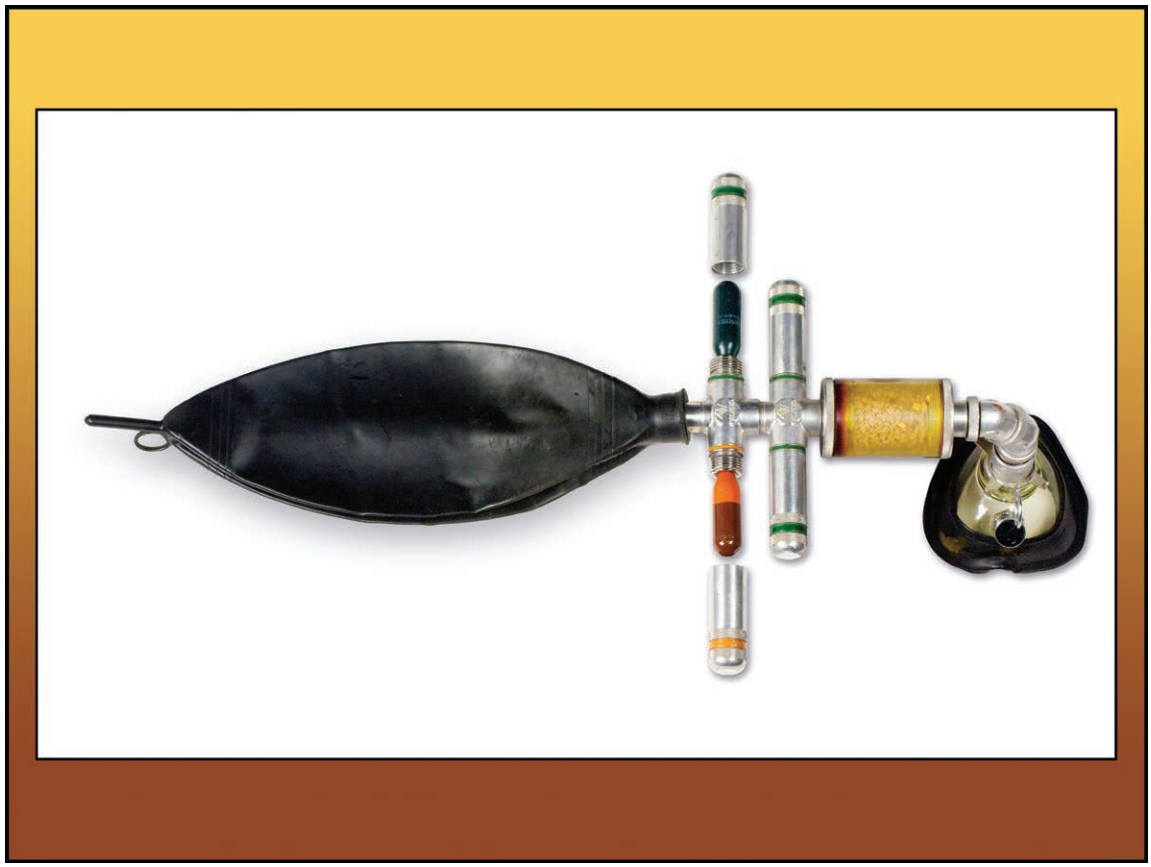
Ziherl, Kish, and Hingson's "Portable Anesthesia Machine, Oxygen Inhalator and Resuscitator"



In December 1955, Frank A. Ziherl (1912 to 2002), Arthur S. Kish (1920 to 2002), and Western Reserve University anesthesiologist Robert A. Hingson, M.D. (1913 to 1996) filed for a U.S. patent on their invention of a "Portable Anesthesia Machine, Oxygen Inhalator and Resuscitator." Granted in July 1960, U.S. Patent No. 2,944,547 was assigned to Z and W Machine Products, Inc., of Wickliffe, Ohio. The invention comprised "a central axial body assembly, a soda-lime canister assembly, an elbow fitting assembly, a slide valve assembly, a face mask assembly, and a rebreather bag." The tiny colorful compressed gas cylinders permitted brief anesthetics or short resuscitations "in the field." Each full green cartridge contained 1.65 l of oxygen; each orange-and-brown cartridge, 2.2 l of oxygen mixed with 1.18 l of helium. Besides this clever device, Dr. Hingson also pioneered many advances, including jet injection, mass immunization, and both caudal and epidural anesthesia. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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