Inflammation Increases Neuronal Sensitivity to General Anesthetics

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ABSTRACT

Background: Critically ill patients with severe inflammation often exhibit heightened sensitivity to general anesthetics; however, the underlying mechanisms remain poorly understood. Inflammation increases the number of γ-aminobutyric acid type A (GABA\(_A\)) receptors expressed on the surface of neurons, which supports the hypothesis that inflammation increases up-regulation of GABA\(_A\) receptor activity by anesthetics, thereby enhancing the behavioral sensitivity to these drugs.

Methods: To mimic inflammation in vitro, cultured hippocampal and cortical neurons were pretreated with interleukin (IL)-1β. Whole cell patch clamp methods were used to record currents evoked by γ-aminobutyric acid (GABA) (0.5 μM) in the absence and presence of etomidate or isoflurane. To mimic inflammation in vivo, mice were treated with lipopolysaccharide, and several anesthetic-related behavioral endpoints were examined.

Results: IL-1β increased the amplitude of current evoked by GABA in combination with clinically relevant concentrations of either etomidate (3 μM) or isoflurane (250 μM) (n = 5 to 17, P < 0.05). Concentration–response plots for etomidate and isoflurane showed that IL-1β increased the maximal current 3.3-fold (n = 5 to 9) and 1.5-fold (n = 8 to 11), respectively (P < 0.05 for both), whereas the half-maximal effective concentrations were unchanged. Lipopolysaccharide enhanced the hypnotic properties of both etomidate and isoflurane. The immobilizing properties of etomidate, but not isoflurane, were also increased by lipopolysaccharide. Both lipopolysaccharide and etomidate impaired contextual fear memory.

Conclusions: These results provide proof-of-concept evidence that inflammation increases the sensitivity of neurons to general anesthetics. This increase in anesthetic up-regulation of GABA\(_A\) receptor activity in vitro correlates with enhanced sensitivity for GABA\(_A\) receptor–dependent behavioral endpoints in vivo. (Anesthesiology 2016; 124:417-27)

Anesthetic dose requirements vary widely among different patients and, for the same patient, under different clinical conditions. Anesthetic hypersensitivity, a phenomenon that is frequently observed in clinical practice, occurs when a standard dose of anesthetic that is appropriate for most patients causes excessive neurodepression in others. Inflammation likely plays an important role in anesthetic hypersensitivity. In high inflammatory states, such as sepsis, the neurodepressive properties of anesthetics are increased, as evidenced by electroencephalographical recordings from both humans and laboratory animals. The mechanisms by which inflammation causes anesthetic hypersensitivity are poorly understood but have been attributed to pharmacokinetic factors, including an increase in the concentration of anesthetics in the brain. In this study, we investigated whether inflammation modifies a key pharmacodynamic factor, specifically, the response of the neurons themselves to a given concentration of anesthetic.

Most of the commonly used anesthetics are positive allosteric modulators of γ-aminobutyric acid type A (GABA\(_A\)) receptors. GABA\(_A\) receptors are classified into two main groups: synaptic and extrasynaptic. Synaptic GABA\(_A\) receptors generate transient inhibitory postsynaptic currents, whereas extrasynaptic receptors generate a persistent, low-amplitude tonic inhibitory current. Inflammation...
preferentially increases the number of extrasynaptic GABA_\textsubscript{A} receptors expressed on the surface of neurons, without increasing the number of synaptic receptors.\textsuperscript{11} For example, the proinflammatory cytokine interleukin (IL-1β) increases the cell-surface expression of extrasynaptic GABA_\textsubscript{A} receptors in hippocampal neurons. This increase in the number of GABA_\textsubscript{A} receptors enhances a tonic inhibitory current that impairs synaptic plasticity and reduces performance for hippocampus-dependent memory tasks. In contrast, IL-1β reduces the amplitude of postsynaptic GABA_\textsubscript{A} receptor currents.\textsuperscript{11} Using confocal microscopy and immunolabeling, other investigators showed that IL-1β increases the cell-surface expression of GABA_\textsubscript{A} receptors and that this increase in receptor number correlates with an increase in GABA_\textsubscript{A} receptor-mediated currents.\textsuperscript{12} Thus, inflammation primarily enhances extrasynaptic rather than synaptic GABA_\textsubscript{A} receptor function by increasing the cell-surface expression of receptors.

An increase in the number of GABA_\textsubscript{A} receptors is predicted to enhance the behavioral neurodepressive properties of general anesthetics. Extrasynaptic GABA_\textsubscript{A} receptors are highly sensitive to positive allosteric modulation by commonly used general anesthetics.\textsuperscript{13,14} Up-regulation of extrasynaptic GABA_\textsubscript{A} receptor activity powerfully depresses neuronal and network excitability.\textsuperscript{15} In addition, extrasynaptic GABA_\textsubscript{A} receptors are expressed in brain regions that play critical roles in anesthetic-induced behavioral endpoints, including the hippocampus,\textsuperscript{16} cortex,\textsuperscript{17} thalamus,\textsuperscript{18} and spinal cord.\textsuperscript{19} Accordingly, we hypothesize that inflammation increases anesthetic neurodepression by up-regulating GABA_\textsubscript{A} receptor activity.

To test this hypothesis, we first used an in vitro neuronal culture model. The effects of IL-1β on γ-aminobutyric acid (GABA)-evoked currents, recorded in the absence or presence of the prototypic IV anesthetic (etomidate) or volatile anesthetic (isoflurane), were studied. Next, in vivo behavioral assays were used to determine whether treatment of mice with a low dose of the endotoxin lipopolysaccharide, which elicits a strong inflammatory response, potentiated the hypnotic, immobilizing, and memory-blocking properties of the anesthetics. Finally, the results from these in vitro and in vivo studies were compared to elucidate the role of GABA_\textsubscript{A} receptors in anesthetic hypersensitivity.

**Materials and Methods**

**Cell Cultures**

Primary cultures of hippocampal and cortical neurons were prepared from CD1 mice (Charles River, Canada), as previously described.\textsuperscript{20} In brief, fetal pups (embryonic day 18) were removed from maternal mice that had been sacrificed by cervical dislocation. The hippocampus or cortex of each fetus was collected and placed on an ice-cooled culture dish. Neurons were then dissociated by mechanical trituration with a Pasteur pipette (tip diameter: 150 to 200 μm) and plated on 35-mm culture dishes at a density of approximately 1 × 10^6 cells per milliliter. The culture dishes were coated with collagen or poly-d-lysine (Sigma-Aldrich, USA). Two hours later, the medium was changed to a neurobasal medium that was supplemented with 2% B27 and 1% GlutaMAX (Life Technologies, Canada). The medium was changed every 3 days, and the cells were maintained in culture for 12 to 16 days before use. For all reported results, data were acquired from at least three brain dissections.

**Whole Cell Voltage Clamp Recordings in Cultured Neurons**

Whole cell currents were recorded at room temperature under voltage clamp (−60 mV) conditions with an Axopatch 1D amplifier (Molecular Devices, USA) controlled with pClamp 8.0 software (Molecular Devices) via a Digidata 1322 interface (Molecular Devices). Patch pipettes were pulled from thin-walled borosilicate glass capillary tubes with open-tip resistance of 3 to 5 MΩ. Patch electrodes were filled with an intracellular solution containing 140 mM cesium chloride, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 11 mM ethylene glycol tetraacetic acid, 2 mM magnesium chloride, 1 mM calcium chloride, 4 mM magnesium adenosine triphosphate, and 2 mM triethanolamine. The pH was adjusted to 7.3 with cesium hydroxide, and osmolarity was adjusted to 290 to 300 mOsm. The extracellular solution contained 140 mM sodium chloride, 1.3 mM calcium chloride, 2 mM potassium chloride, 1 mM magnesium chloride, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 28 mM glucose. The pH was adjusted to 7.4 with sodium hydroxide, and osmolarity was adjusted to 320 to 330 mOsm. The extracellular solution was applied to the neurons by a computer-controlled, multibarrel perfusion system (SF-77B; Warner Instruments, USA). Membrane capacitance was measured according to the membrane test protocol in the pClamp 8.0 software. Tetrodotoxin (300 nM) was added to the extracellular solution to block voltage-sensitive sodium channels, and 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) and 2-amino-4-phosphonovaleric acid (40 μM) were added to inhibit the ionotropic glutamate receptors.

To mimic inflammation, neurons were pretreated with IL-1β (60 ng/ml; R&D Systems, USA) or saline vehicle for 3 h before GABA-evoked currents were recorded. This concentration of IL-1β was selected because others have reported that it mimics levels that occur in the brain during sepsis.\textsuperscript{12} To activate low-amplitude tonic currents, GABA (0.5 μM) was applied to the neurons. This concentration is similar to the ambient extracellular concentration of GABA that occurs in the central nervous system in vivo.\textsuperscript{21-23} The studies focused on the tonic GABA current because previous data showed that under similar conditions, IL-1β preferentially enhanced extrasynaptic GABA_\textsubscript{A} receptor expression.\textsuperscript{11} Two different anesthetics were used in these experiments, including etomidate (Bedford Laboratory, USA).
and isoflurane (Abbott Laboratories, USA). Etomidate was chosen as the prototypic injectable anesthetic because it is relatively selective for GABA<sub>A</sub> receptors and causes minimal hemodynamic instability when administered in vivo. Isoflurane was chosen as the prototypic inhaled anesthetic because it is widely used in clinical practice and its effects have been extensively characterized both in vitro and in vivo. Both anesthetics were prepared fresh daily by diluting the anesthetics with an extracellular solution that contained GABA (0.5 μM) to yield the final concentrations. The isoflurane solution was prepared and applied as described previously. In brief, 100 ml isoflurane was added to a 500-ml gas-tight glass bottle containing 200 ml of the extracellular solution. The highest concentration of isoflurane in the undiluted, saturated aqueous solution was 2.5 mM. An aqueous-phase concentration of isoflurane equivalent to 1 minimum alveolar concentration (MAC) was chosen for the initial electrophysiological studies, on the basis of previously recommended doses for in vitro use. We previously measured the concentration of isoflurane in the aqueous solution that was collected at the tip of the perfusion electrode using gas chromatography and showed that it was similar to the predicted isoflurane concentration.

Experimental Animals

All experiments were approved by the Animal Care Committee of the University of Toronto (Toronto, Ontario, Canada) and conformed to guidelines set by the Canadian Council on Animal Care. Three-month-old male mice (C57Bl/6 × Sv129Ev) were used for the behavioral studies. The colony was located in a pathogen-free transgenic facility in an air-conditioned room maintained at a temperature of 25° ± 1°C on a 12 h light–dark cycle (lights on at 7:00 AM; lights off at 7:00 PM). Mice were housed in groups of up to four mice per cage, with free access to food and water. To reduce variability in performance, and to prevent acute stress reactions during conditioning, all mice were handled for 10 min each day for a minimum of 1 week before the start of the behavioral experiments. The experimenters who analyzed the behavior were blinded to the treatment groups.

To induce systemic inflammation, a low dose of lipopolysaccharide (125 μg/kg; Sigma-Aldrich) was administered by intraperitoneal injection (ip) 3 h before testing. We selected this experimental model as it mimics several features of acute systemic illness in humans, such as increased plasma levels of IL-1β and impaired memory performance. This dose is not expected to cause significant hemodynamic consequences such as septic shock. The 3-h interval between injecting lipopolysaccharide and behavioral assays reflects the timing of peak levels of proinflammatory cytokines, including IL-1β.

Anesthesia Treatment In Vivo

Mice were treated with either etomidate (ip) or physiological saline (ip), which was used as the vehicle control. Isoflurane was administered by placing each mouse in an airtight Plexiglas chamber (27 cm wide × 10 cm deep × 10 cm high) that had been preflushed with the anesthetic gas mixture or the vehicle gas, delivered at 1 l/min. Oxygen (30% for 20 min) mixed with air was used as the vehicle treatment for isoflurane. The concentrations of isoflurane, oxygen, and expired carbon dioxide in the chamber were continuously analyzed with a commercial gas analyzer (Datex Ohmeda, Canada). To prevent hypothermia, the temperature of the chamber was maintained at 35°C with a heating pad and was monitored by a digital thermometer.

Loss of the Righting Reflex Assay

To investigate the effect of inflammation on the hypnotic properties of anesthetics, the latency to loss of the righting reflex (LORR) was measured. This behavioral endpoint was selected as a surrogate measure for the latency to loss of consciousness in humans. Mice were injected with lipopolysaccharide and 3 h later were treated with etomidate or isoflurane. Testing for the LORR started immediately after administration of the anesthetic. A mouse was considered to have lost its righting reflex and entered a hypnotic state when its ability to stand on three of four paws twice within a 30-s period was impaired. The latency to LORR was defined as the interval between administration of the anesthetic and the LORR. Behavior was scored using direct observation by an investigator who was blinded to the treatment groups.

Tail Withdrawal Reflex Assay

To investigate the effect of inflammation on the immobilizing properties of the anesthetics, the loss of the tail-pinch withdrawal reflex (LTWR) was used. Mice were injected with lipopolysaccharide and 3 h later were treated with etomidate or isoflurane. LTWR is a binary score (movement vs. immobilization) in response to noxious stimulation, such as tail pinch. In brief, movement to tail clamp was tested by placing an alligator clip on the proximal one third of the tail. To ensure that no tissue damage occurred, the clamp was modified by covering the tips of the blades with tape, and the safety of the modified clamp was assessed by a veterinarian from the animal facility. In order for the clamp to be suitable for all the subjects, mice of similar age group (about 3 months old) were used.

Etomidate-induced immobility was quantified by dividing the number of mice that were immobilized 5 min after the administration of etomidate (30 mg/kg) by the total number of mice that received the drug. Isoflurane-induced immobility was used to determine the MAC of isoflurane, as previously described. Isoflurane was administered at an initial subanesthetizing concentration of 0.9%. After a 30-min equilibration period, movement to tail clamp was tested. At this subanesthetizing concentration, all mice responded with movement to tail clamp. The concentration of isoflurane was then increased by 0.1% for another equilibration period, and the response was tested again. If no movement was
observed, the clamp was left in place for a 1-min period. The concentration at which the mice lost their tail-clamp reflex was noted. The MAC value for each mouse was calculated by averaging the concentrations of isoflurane that permitted movement.

**Fear Conditioning Assay**

To investigate the effect of inflammation on the amnestic properties of anesthetics in mice, a fear conditioning (FC) assay was used. FC allowed the examination of hippocampus-dependent contextual fear memory and hippocampus-independent auditory-cued fear memory. Specifically, the conditioning chamber (20 × 20 × 35 cm) had a shock grid floor consisting of stainless steel bars (5 cm apart, ø = 4 mm; Technical & Scientific Equipment Systems Inc., USA). During the training phase, each mouse was allowed to explore the chamber for 180 s. Next, a 20 s, 2.8 kHz tone was presented. The tone was produced by a frequency generator and amplified to 70 decibels. The last 2 s of the auditory tone was paired with an electric foot shock (0.5 mA). This tone-shock pairing was presented three times (designated S1, S2, and S3), where consecutive presentations were separated by a 60-s interval. On day 2 (24 h after training), contextual FC memory was assessed by returning the mouse to the FC context for 8 min and measuring the percentage of time that it spent freezing (absence of movement except respiration). On day 3 (48 h after training), the conditioning chamber was modified to measure the freezing response to the auditory tone (auditory-cued FC memory). The modified context had a significantly different shape, scent, and visual appearance than the original chamber. Mice were monitored for 180 s for freezing to the modified context, to rule out contextual influences. After the initial monitoring period, the auditory tone was presented for 5 min, and the percentage of time that each mouse spent freezing was determined. FreezeView software 2.26 (Actimetrics Inc., USA) was used to determine the freezing score. Mice that successfully acquired the association demonstrate freezing behavior when reexposed to either context or tone. A higher freezing score suggests a stronger memory. For this assay, only etomidate was studied as it could be easily administered by injection. Studies of isoflurane require the FC test to be performed in an airtight chamber that maintains a stable concentration of a volatile anesthetic. Because this equipment was not readily available, isoflurane was not studied.

**Statistical Analysis**

Data are expressed as mean ± SEM. Sample size was estimated on the basis of previous experience and published data from our laboratory. The cell-culture dishes and animals were randomly assigned to the various experimental conditions, and there were no lost or missing data. Statistical analysis was performed by using GraphPad Prism software 5.0 (USA) and SPSS 20.0 software (IBM Corporation, USA). An unpaired, two-tailed Student’s t test was used for all the electrophysiological experiments and for the study of isoflurane on LTWR. The lower and upper 95% CIs are reported for these data sets. For the LORR assay, a two-way ANOVA with two between-subject factors, followed by a Bonferroni post hoc test was used. The two factors were inflammation (lipopolysaccharide or vehicle) and drug dose (etomidate 14, 20, or 26 mg/kg and isoflurane 1, 1.3, or 1.5%). To study the LTWR to etomidate, a Fisher test was used. For the FC experiments, freezing behavior on the training day was analyzed by using a 2 × 3 multivariate ANOVA with the between-subjects factors of inflammation (vehicle or lipopolysaccharide) and etomidate (0, 6, and 12 mg/kg) as well as the within-subjects factor of time interval (baseline, S1, S2, and S3). For the training data, a post hoc analysis was performed using a paired, two-tailed Student’s t test. For the baseline data, a post hoc analysis was performed by using the Fisher least significant difference (LSD) test. As suggested by a reviewer, we also considered the more conservative Tukey post hoc test to analyze the baseline data. The two statistical methods yielded similar results. Since the LSD test was selected during the initial design of the study, the results from the LSD test are presented. Freezing behavior in contextual and cued FC experiments was analyzed by using a 2 × 3 two-way ANOVA with the between-subjects factors of inflammation (vehicle or lipopolysaccharide) and etomidate (0, 6, and 12 mg/kg). Significance was set at the P value of less than 0.05 level.

**Results**

**IL-1β Increases GABA A Receptor Current in the Presence of General Anesthetics**

First, to determine whether inflammation modified anesthetic up-regulation of GABA A receptor activity, hippocampal and cortical neurons were pretreated with either IL-1β (60 ng/ml) or vehicle solution. Whole cell currents were activated by applying a low concentration of GABA (0.5 μM), in the absence or presence of either etomidate or isoflurane.

In the absence of anesthetics, GABA (0.5 μM) activated a small inward current in both hippocampal and cortical neurons (fig. 1). In hippocampal neurons, IL-1β increased the peak current 2.1-fold (control: 0.97 ± 0.13 pA/pF, 95% CI, 0.71 to 1.23 vs. IL-1β: 1.99 ± 0.25 pA/pF, 95% CI, 1.48 to 2.5; n = 24 to 28; P = 0.001; Student’s t test; fig. 1A), as previously reported.11 Similarly, in cortical neurons, IL-1β increased the peak current 1.8-fold (control: 1.11 ± 0.1 pA/pF, 95% CI, 0.83 to 1.38 vs. IL-1β: 2.02 ± 0.39 pA/pF, 95% CI, 1.03 to 3.01; n = 6, P = 0.04, Student’s t test; fig. 1B).

In the presence of a clinically relevant concentration of etomidate (3 μM), the amplitude of the GABA A receptor current was increased in both vehicle and IL-1β-treated hippocampal neurons (fig. 2A); however, the amplitude was markedly greater in IL-1β-treated neurons (control: 5.44 ± 1.19 pA/pF, 95% CI, 2.13 to 8.74 vs. IL-1β: 10.88 ± 1.45 pA/pF, 95% CI, 7.55 to 14.22; n = 5 to 9,
Fig. 1. Interleukin (IL-1β) increases γ-aminobutyric acid (GABA) type A receptor current in cultured hippocampal and cortical neurons. Representative traces and summarized data show an approximately twofold increase in currents evoked by GABA (0.5 μM) after pretreatment with IL-1β (60 ng/ml, 3 h) in both hippocampal neurons (A) and cortical neurons (B). *P < 0.05, **P < 0.01 versus control (Con), unpaired two-tailed Student's t test.

$P = 0.02$, Student's $t$ test; fig. 2B). Interestingly, the relative change in the peak current produced by etomidate (3 μM) was similar in vehicle and IL-1β-treated neurons. Etomidate caused a 5.6-fold increase in control neurons (GABA alone: $0.97 ± 0.13$ pA/pF; $n = 24$ vs. GABA + etomidate: $5.44 ± 1.19$ pA/pF; $n = 5$, $P < 0.0001$, Student's $t$ test) and a 5.4-fold increase in IL-1β-treated neurons (GABA alone: $1.99 ± 0.25$ pA/pF; $n = 28$ vs. GABA + etomidate: $10.88 ± 1.45$ pA/pF; $n = 9$, $P < 0.0001$, Student's $t$ test; fig. 2B).

The larger current evoked by etomidate plus GABA in IL-1β-treated neurons could result from either an increase in the number of GABA$_A$ receptors or an increase in the pharmacological sensitivity of the receptors. Based on previous studies, the increased anesthetic-sensitive current was likely due to an increased expression of GABA$_A$ receptors. If this postulate was true, then etomidate concentration–response plots should reveal an enhanced anesthetic efficacy, as indicated by an increase in the maximal current ($I_{\text{max}}$), whereas potency, indicated by the half-maximal effective concentration ($EC_{50}$), should be unchanged. Indeed, IL-1β increased $I_{\text{max}}$ 3.3-fold (control: $1.0 ± 0.2$ nA, 95% CI, 0.45 to 1.58 vs. IL-1β: $3.3 ± 0.3$ nA, 95% CI, 2.7 to 3.91; $n = 5$ to 9, $P < 0.0001$; fig. 2C), whereas potency was unchanged ($EC_{50}$ in control neurons: $11 ± 7$ μM, 95% CI, −8 to 29 vs. $EC_{50}$ IL-1β: $13 ± 3$ μM, 95% CI, 6 to 20; $n = 5$ to 9, $P = 0.7$).

The magnitude of the IL-1β-dependent increase in the GABA and etomidate-evoked current was substantial. A comparison of the fitted curves (fig. 2C) showed that the current evoked by GABA and etomidate (1 μM) in IL-1β-treated neurons was equivalent to the current evoked by a much higher concentration of etomidate (4.3 μM) in control neurons. With a higher concentration of etomidate (3 μM), the evoked current in IL-1β-treated neurons was comparable to that produced by a saturating concentration of etomidate (100 μM) and GABA in control neurons. Thus, after treatment with IL-1β, low clinically relevant concentrations of etomidate produced a current that was equivalent to the current generated by much higher concentrations of the anesthetic in vehicle-treated neurons.

Next, we investigated whether IL-1β also modified the response of hippocampal neurons to isoflurane. A clinically relevant concentration of isoflurane (250 μM, approximately 1 MAC) was coapplied with GABA (0.5 μM) to IL-1β- or vehicle-treated hippocampal neurons. IL-1β increased the peak current evoked by isoflurane and GABA (control: $3.84 ± 0.73$ pA/pF; 95% CI, 2.26 to 5.43 vs. IL-1β: $7.15 ± 0.81$ pA/pF; 95% CI, 5.38 to 8.93; $n = 13$ to 14, $P = 0.005$, Student's $t$ test; fig. 3, A and B). The relative increase in amplitude produced by isoflurane was similar...
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We then investigated whether an increase in anesthetic sensitivity by IL-1β occurred in neurons from other brain regions. Cortical neurons are particularly interesting as the cortex is thought to contribute to the hypnotic properties of anesthetics. The same protocol that was described for hippocampal neurons was used for cortical neurons. IL-1β increased the current evoked by GABA (0.5 μM) plus etomidate (3 μM) (control: 3.1 ± 0.49 pA/pF, 95% CI, 1.74 to 4.46 vs. IL-1β: 5.6 ± 0.78 pA/pF, 95% CI, 3.58 to 7.62; n = 5 to 6, P = 0.03, Student’s t test, fig. 4A). Similarly, the current evoked by GABA (0.5 μM) plus isoflurane (250 μM) was greater in IL-1β-treated neurons (control: 2.94 ± 0.53 pA/pF, 95% CI, 1.45 to 4.42 vs. IL-1β: 5.7 ± 0.75 pA/pF, 95% CI, 3.77 to 7.64; n = 5 to 6, P = 0.01, Student’s t test, fig. 4B). A similar relative increase in the peak current produced by GABA and both etomidate (control: 2.8-fold vs. IL-1β: 2.7-fold) and isoflurane (control: 2.6-fold vs. IL-1β: 2.8-fold) was observed.

Collectively, the results showed that IL-1β markedly increased the current evoked by GABA plus etomidate or isoflurane in both hippocampal and cortical neurons. Thus, IL-1β increases the sensitivity of the neurons, from at least two brain regions, to anesthetics.

![Image](image_url)

**Fig. 3.** Interleukin (IL)-1β markedly increases γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor responses evoked by coapplication of γ-aminobutyric acid (GABA) (0.5 μM) and isoflurane in hippocampal neurons. (A) Representative traces of GABA<sub>A</sub> receptor currents in the absence and presence of isoflurane (Iso, 250 μM) in neurons treated with vehicle (control) or IL-1β (60 ng/ml, 3 h). (B) Summarized data show that isoflurane increases GABA<sub>A</sub> receptor currents in both vehicle- and IL-1β-treated neurons. The amplitude of the peak current is markedly greater in IL-1β-treated neurons, but the relative increase in the peak current by isoflurane is similar in both treatment conditions. **P < 0.01, unpaired two-tailed Student’s t test.** (C) The isoflurane concentration-response plots demonstrate that the maximal current, but not the half maximal effective concentration, is increased by IL-1β. The missing isoflurane concentrations on the abscissa are 500 and 750 μM.

![Image](image_url)

**Fig. 4.** Interleukin (IL)-1β markedly increases γ-aminobutyric acid (GABA) type A receptor responses evoked by coapplication of GABA (0.5 μM) and anesthetics in cortical neurons. Representative traces and summarized data show that pretreatment with IL-1β (60 ng/ml, 3 h) increases GABA type A receptor responses mediated by coapplication of GABA with either etomidate (Etom, A) or isoflurane (Iso, B). The amplitude of the peak current is markedly greater in IL-1β-treated neurons, but the relative increase in the peak current by the anesthetic is similar in both treatment conditions. *P < 0.05, unpaired two-tailed Student’s t test.
Inflammation Potentiates Anesthetic Depression of Behavioral Responses

We next investigated whether the inflammation-induced up-regulation of GABA<sub>A</sub> receptor activity in vitro correlated with an increased sensitivity for anesthetic-related behavioral endpoints in vivo. To induce inflammation, mice were treated with a low dose of the bacterial endotoxin lipopolysaccharide (125 μg/kg, ip). Three hours later, the effects of etomidate or isoflurane on several different behavioral endpoints were assessed (figs. 5 and 6). To determine whether lipopolysaccharide potentiated the hypnotic, immobilizing, and amnestic properties of anesthetics, the LORR, LTWR, and FC assays were used, respectively.

We first investigated whether lipopolysaccharide increased the hypnotic properties of etomidate (14, 20, and 26 mg/kg) and isoflurane (1.0, 1.3, and 1.5%). Etomidate or isoflurane were administered to lipopolysaccharide- or vehicle-treated mice and the latency to LORR was measured (fig. 5B). None of the mice treated with lipopolysaccharide alone lost their righting reflex (data not shown). Etomidate reduced the latency to LORR. Lipopolysaccharide further reduced the latency time, and there was a statistically significant interaction between lipopolysaccharide and etomidate ($F_{(2,42)} = 5.08, n = 8, P = 0.01$, two-way ANOVA). Isoflurane alone also reduced the latency to LORR and the latency was further reduced by lipopolysaccharide. There was a significant interaction between lipopolysaccharide and isoflurane to reduce the latency to LORR ($F_{(2,42)} = 3.72, n = 8, P = 0.03$, two-way ANOVA). Interestingly, lipopolysaccharide did not significantly reduce the latency to LORR produced by the highest dose of etomidate (26 mg/kg) or isoflurane (1.5%). This lack of effect could be attributed to a “basement effect” where a further reduction in latency by lipopolysaccharide was not detected. Collectively, the results show that lipopolysaccharide enhanced the hypnotic properties of both etomidate and isoflurane.

Next, the effects of lipopolysaccharide on the immobilizing properties of etomidate and isoflurane were studied. Lipopolysaccharide alone did not cause immobility (data not shown). In mice treated with etomidate alone (30 mg/kg, ip), immobility was observed in 2 of 15 control mice. The same dose of etomidate caused immobility in 12 of 15 lipopolysaccharide-treated mice. Thus, lipopolysaccharide increased the relative number of mice that were immobilized sixfold, from 13 to 80% ($P = 0.0007$, Fisher test; fig. 5C, left panel). To study the effects of lipopolysaccharide on immobility caused by isoflurane, a standard upward and downward dosing method was used, as described previously. Isoflurane caused a concentration-dependent loss of movement in both lipopolysaccharide- and vehicle-treated mice. Unexpectedly, the isoflurane concentration–response plots showed that the EC<sub>50</sub> values were similar in both groups (control: 1.4 ± 0.05%, 95% CI, 1.27 to 1.51 vs. lipopolysaccharide: 1.4 ± 0.06%, 95% CI, 1.24 to 1.54; n = 8, $P = 1.0$; fig. 5C, right panel). Thus, lipopolysaccharide potentiated the immobilizing properties of etomidate, but it failed to potentiate the immobilizing properties of isoflurane.

The effects of lipopolysaccharide on anesthetic-induced memory loss were studied by using two FC memory paradigms. Mice were trained to associate an electric foot shock (unconditioned stimulus) with both an environmental context and a tone (conditioned stimuli; fig. 6A). During the training phase, mice were habituated to the chamber for 3 min and were subsequently exposed to a sequence of three tone-shock pairings (S1, S2, and S3). The baseline period during the training phase was defined as the 3 min before exposure to the first shock.

During the training phase, freezing behavior increased for both vehicle- and lipopolysaccharide-treated groups with...
Inflammation Increases Sensitivity to Anesthetics

**Fig. 6.** The effects of lipopolysaccharide (LPS) on etomidate-induced learning and memory impairment. (A) Timeline showing the experimental design. (B) Summarized freezing scores during training. Consecutive exposure to three shocks is indicated as S1, S2, and S3. There was a significant interaction between etomidate, LPS, and presentation of the shocks on freezing behavior ($F_{(2,34)} = 2.61, P < 0.05$). All mice acquired fear memory as shown by increased freezing scores from baseline to S3 (2 × 34 multivariate two-way ANOVA with a paired two-tailed Student’s *t* test post hoc analysis, $*P < 0.05, **P < 0.01, ***P < 0.001$). (C) Summarized data replotted from B showing that the baseline freezing score is enhanced by increasing concentrations of etomidate. A *post hoc* analysis was performed using the Fisher least significant difference test. Coadministration of a low dose of LPS (125 μg/kg) only increases the baseline freezing score of etomidate (6 mg/kg, $P < 0.05$). (D) Summarized data showing freezing scores for contextual fear memory. LPS and etomidate reduced freezing scores, but there is only a marginal interaction between LPS and etomidate. (E) Summarized data showing freezing scores for cued fear memory. LPS, but not etomidate, reduces the freezing scores, and there is no interaction between LPS and etomidate.

Each tone-shock pairing (fig. 6B). There was a significant interaction between etomidate, lipopolysaccharide, and the presentation of shocks on freezing behavior ($F_{(6,195)} = 2.61, P < 0.05$). A *post hoc* analysis showed that freezing increased significantly (all $P < 0.05$) from baseline to S3, suggesting that the mice acquired fear memory. In addition, both etomidate and lipopolysaccharide increased baseline freezing (fig. 6C). The baseline freezing scores for both vehicle- and lipopolysaccharide-treated mice at etomidate (12 mg/kg) are significantly increased when compared with the scores at lower concentrations of etomidate (0 and 6 mg/kg, $P < 0.05$, LSD test). For etomidate (6 mg/kg), the lipopolysaccharide-treated mice showed higher baseline freezing scores compared with vehicle-treated mice ($P < 0.05$, LSD test). These results suggest that etomidate caused sedation during training, as reflected by the higher baseline freezing scores, and lipopolysaccharide potentiated this effect.

Contextual fear memory was tested by reexposing the mice to the training context 24 h later. Etomidate caused a concentration-dependent reduction in freezing scores, suggesting impaired contextual fear memory ($F_{(1,65)} = 8.63, n = 10 to 17, P = 0.0005$, two-way ANOVA; fig. 6D). Lipopolysaccharide alone also reduced the freezing scores ($F_{(1,65)} = 20.56, n = 10 or 11, P < 0.0001$, two-way ANOVA; fig. 6D). The interaction between lipopolysaccharide and etomidate was marginal, but not significant ($F_{(2,65)} = 2.25, n = 10 to 17, P = 0.11$). This lack of a significant interaction could be due to a “basement effect” where lipopolysaccharide could not further reduce the freezing scores.

To test cued fear memory, mice were reexposed to the auditory tone 48 h after training. Control mice showed high freezing scores indicating strong cued fear memory (fig. 6E). Lipopolysaccharide reduced the freezing scores ($F_{(1,65)} = 12.35, n = 10 to 11, P = 0.0008$). Interestingly, etomidate had no effect on freezing ($F_{(2,65)} = 1.68, n = 10 to 17, P = 0.19$), and there was no interaction between lipopolysaccharide and etomidate ($F_{(2,65)} = 0.38, n = 10 to 17, P = 0.68$).

Collectively, these results show that lipopolysaccharide alone impaired both contextual and cued fear memory. Etomidate caused a concentration-dependent impairment of contextual fear memory, but not cued fear memory. There was a trend toward an interaction between lipopolysaccharide and etomidate for impairment of contextual fear memory, but the interaction was not significant.

**Discussion**

The results of this study showed that IL-1β markedly increased the current evoked by GABA and clinically relevant concentrations of either etomidate or isoflurane. Thus, IL-1β increased the anesthetic sensitivity of hippocampal and cortical neurons to both an injectable and an inhaled anesthetic *in vitro*. The results of our behavioral studies showed that lipopolysaccharide, which stimulates an innate immune response, increased anesthetic sensitivity *in vivo*. The hypnotic properties of etomidate and isoflurane were increased in lipopolysaccharide-treated mice. In contrast, lipopolysaccharide enhanced the immobilizing properties of etomidate, but not isoflurane.

The simplest mechanism to account for the enhanced current in IL-1β-treated neurons is an increase in the cell-surface expression of anesthetic-sensitive GABA$_A$ receptors. Consistent with this mechanism, concentration–response plots showed that IL-1β increased the efficacy but not the
potency of etomidate and isoflurane. For several reasons, extrasynaptic GABA<sub>A</sub> receptors, rather than synaptic GABA<sub>A</sub> receptors, are likely major contributors to the increase in anesthetic-sensitive current in IL-1β-treated neurons. First, previous studies have shown that the low concentration of GABA used in the current study (0.5 μM) preferentially activates high-affinity extrasynaptic GABA<sub>A</sub> receptors rather than lower-affinity postsynaptic GABA<sub>A</sub> receptors. Second, biotinylation assays have shown that IL-1β increases cell-surface expression of extrasynaptic GABA<sub>A</sub> receptors and decreases the expression of synaptic GABA<sub>A</sub> receptors. Third, electrophysiological studies have shown that IL-1β increases the amplitude of a tonic current generated by extrasynaptic GABA<sub>A</sub> receptors but reduces the amplitude of postsynaptic currents activated by saturating concentrations of GABA. Thus, IL-1β likely increases the number of anesthetic-sensitive extrasynaptic GABA<sub>A</sub> receptors without markedly altering their intrinsic pharmacological properties.

Behavioral studies support the notion that inflammation increases anesthetic sensitivity, at least in part, by increasing GABA<sub>A</sub> receptor activity. IV anesthetics, including etomidate, are more selective for GABA<sub>A</sub> receptors than are inhaled anesthetics. Etomidate primarily modifies GABA<sub>A</sub> receptors that contain either a β2 subunit or a β3 subunit, as point mutations of specific amino acids in these subunits decrease the effects of etomidate on GABA-evoked current in vitro and reduce etomidate-induced LORR and LTWR in vivo. Other studies have shown that the GABA<sub>A</sub> receptor antagonist bicuculline reverses the effects of propofol, another IV anesthetic, on hippocampal neuronal excitability. In contrast, volatile anesthetics, such as isoflurane, are more promiscuous and modify a broad range of neurotransmitter receptors and other membrane proteins. Whereas the hypnotic properties of isoflurane are mediated primarily by GABA<sub>A</sub> receptors, the immobilizing properties of this anesthetic are not likely mediated by these receptors. Indeed, intrathecal treatment with the GABA<sub>A</sub> receptor antagonists picrotoxin failed to antagonize the immobilizing properties of isoflurane. Other target receptors that likely mediate isoflurane immobility include glycine receptors, N-methyl-D-aspartate receptors, and two-pore domain potassium channels.

An increased GABA<sub>A</sub> receptor activity is known to impair learning and memory in laboratory animals. The FC data in the current study reveal an interaction between etomidate, lipopolysaccharide, and the presentation of shocks on freezing behavior during the training phase of the study (fig. 6B). However, all mice, including those treated with the highest dose of etomidate (12 mg/kg) eventually learned to associate the conditioned stimulus (context) with the unconditioned stimulus (foot shock), as evidenced by the final high freezing scores. Baseline freezing, measured before presentation of the foot shocks, increased with increasing doses of etomidate (6 and 12 mg/kg). This result suggests that movement was reduced and freezing scores were increased due to the sedative properties of etomidate. Lipopolysaccharide alone did not increase baseline freezing, as shown in figure 6C; however, lipopolysaccharide increased the freezing scores for mice treated with etomidate (6 mg/kg) and therefore exacerbated the sedative properties of etomidate. This effect of lipopolysaccharide was not observed at a higher dose of etomidate (12 mg/kg), possibly because of a “ceiling effect” whereby lipopolysaccharide failed to potentiate the sedative effects of etomidate.

Lipopolysaccharide alone impaired memory performance for contextual fear, as shown on day 2 of the FC study (fig. 6D). Mice treated with lipopolysaccharide plus etomidate (6 mg/kg) exhibited lower freezing scores than mice treated with etomidate (6 mg/kg) alone; however, the interaction between lipopolysaccharide and etomidate was not statistically significant. Importantly, it is difficult to demonstrate longitudinal deficits in cognitive performance caused by drug–drug interactions when the two drugs independently impair cognition. The lack of a statistically significant interaction between lipopolysaccharide and etomidate could be due to etomidate confounding the acquisition of memory.

The studies of cued fear memory showed that lipopolysaccharide alone, but not etomidate, impaired memory for an auditory conditioned stimulus (fig. 6E). These results are consistent with previous studies showing that recall for auditory-cued fear memory is relatively resistant to the memory-blocking properties of general anesthetics.

Collectively, the behavioral results obtained in this study show that lipopolysaccharide increased anesthetic sensitivity to GABA<sub>A</sub> receptor–dependent behavioral endpoints, including etomidate-induced LORR and LTWR and isoflurane-induced LORR. However, a GABA<sub>A</sub> receptor–independent behavioral endpoint (isoflurane-induced LTWR) was not affected by lipopolysaccharide. Therefore, inflammation-enhanced GABA<sub>A</sub> receptor activity might have contributed to the observed lipopolysaccharide-induced behavioral hypersensitivity.

Hemodynamic parameters were not measured in the current study; however, it is unlikely that lipopolysaccharide-induced hypotension or septic shock accounted for the increased anesthetic sensitivity. Lipopolysaccharide is known to cause septic shock and hemodynamic instability, but only at high doses (1 to 25 mg/kg). In the current study, a low dose of lipopolysaccharide (125 μg/kg) was administered to stimulate an innate immune response but not to induce septic shock. In previous studies, doses of lipopolysaccharide that were 8-fold to 40-fold higher than the dose used in this study caused minimal changes in blood pressure and heart rate at 2 to 3 h after lipopolysaccharide injection in mice. In another study, echocardiographic analysis showed that left ventricular contractility and heart rate were unchanged in mice treated with isoflurane anesthesia and low-dose lipopolysaccharide (0.1 to 1 mg/kg). Thus, hemodynamic consequences are unlikely to account for the increased drug sensitivity observed in the current study.

Although these findings from animal studies cannot be directly extrapolated to patients, they suggest that clinical trials are required to identify the appropriate anesthetic doses for patients in high inflammatory states. Such clinical studies...
should probe multiple anesthetic behavioral endpoints, particularly for volatile anesthetics. The current results suggest that MAC values may not accurately predict the doses required to cause hypnosis or memory loss. Indeed, it may be appropriate to significantly reduce the amount of anesthetics administered to patients in high inflammatory states. Lowering the anesthetic dose may also decrease the incidence of anesthetic-related complications, such as postoperative delirium and postoperative cognitive dysfunction. Thus, studies on the impact of inflammation on anesthetic requirements in patients are of considerable importance.

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Competing Interests
The authors declare no competing interests.

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