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$GABA_A$ receptor antagonism increases NMDA receptor inhibition by isoflurane at a minimum alveolar concentration

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Abstract

Objective—At the minimum alveolar concentration (MAC), isoflurane potentiates $GABA_A$ receptor currents and inhibits NMDA receptor currents, and these actions may be important for producing anesthesia. However, isoflurane modulates $GABA_A$ receptors more potently than NMDA receptors. The objective of this study was to test whether isoflurane would function as a more potent NMDA receptor antagonist if its efficacy at $GABA_A$ receptors was decreased.

Study design—Prospective experimental study.

Animals—Fourteen 10-week-old male Sprague–Dawley rats weighing 269 ± 12 g.

Methods—Indwelling lumbar subarachnoid catheters were surgically placed in isoflurane-anesthetized rats. Two days later, the rats were anesthetized with isoflurane, and artificial CSF containing either 0 or 1 mg kg^{-1} picrotoxin, a $GABA_A$ receptor antagonist, was infused intrathecally at 1 μL $minute^{-1}$. The baseline isoflurane MAC was then determined using a standard tail clamp technique. MK801 (dizocilpine), an NMDA receptor antagonist, was then administered intravenously at 0.5 mg kg^{-1}. Isoflurane MAC was re-measured.

Results—Picrotoxin increased isoflurane MAC by 16% compared to controls. MK801 significantly decreased isoflurane MAC by 0.72% of an atmosphere in controls *versus* 0.47% of an atmosphere in rats receiving intrathecal picrotoxin.

Conclusions and clinical relevance—A smaller MK801 MAC-sparing effect in the picrotoxin group is consistent with greater NMDA antagonism by isoflurane in these animals, since it suggests that fewer NMDA receptors are available upon which MK801 could act to decrease isoflurane MAC. Decreasing isoflurane $GABA_A$ potentiation increases isoflurane NMDA antagonism at MAC. Hence, the magnitude of an anesthetic effect on a given channel or receptor at MAC may depend upon effects at other receptors.

Keywords

intrathecal; isoflurane; minimum alveolar concentration; rat

Introduction

Although the mechanism of action of inhaled anesthetics remains a mystery (Sonner et al. 2003a; Solt & Forman 2007), the effects of anesthetics on many cellular channels and receptors in the CNS are known. In general, class A γ-aminobutyric acid ($GABA_A$)

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receptors are positively modulated by conventional inhaled anesthetics whereas *N*-methyl-D-aspartate (NMDA) receptors are negatively modulated by these same agents. Increasing inhibitory $GABA_A$ chloride currents and decreasing excitatory NMDA cation currents both cause neuronal hyperpolarization that reduces cell excitability and likely contributes to the immobilizing (Stabernack et al. 2003) and amnestic (Sonner et al. 2005) effects of anesthetics.

The magnitude of $GABA_A$ and NMDA receptor modulation varies by agent. At minimum alveolar concentration (MAC), some anesthetics such as cyclopropane, nitrous oxide, and xenon potently inhibit NMDA currents, but have little effect on $GABA_A$ currents (Franks et al. 1998; de Sousa et al. 2000; Yamakura & Harris 2000; Raines et al. 2001; Hara et al. 2002; Eger et al. 2006; Solt et al. 2006; Kelly et al. 2007). Conversely, contemporary haloether volatile agents, such as isoflurane, greatly potentiate $GABA_A$ currents at MAC, but have modest effects on NMDA receptors at similar concentrations (Lin et al. 1992; Harrison et al. 1993; de Sousa et al. 2000; Yamakura & Harris 2000; Eger et al. 2006; Ogata et al. 2006; Solt et al. 2006; Kelly et al. 2007). Are these reciprocal activities at $GABA_A$ *versus* NMDA receptors at an immobilizing anesthetic concentration a fixed property of the agent, or does activity at one anesthetic sensitive site change as a function of the sum of actions at other anesthetic sensitive targets? In other words, if a drug like isoflurane is rendered a less effective GABA agonist, does isoflurane then function more as an NMDA antagonist at MAC?

Picrotoxin is a noncompetitive $GABA_A$ receptor antagonist (Akaike et al. 1985) that does not alter excitatory glutamate neurotransmission in the brain (Davidson & Southwick 1971) or spinal cord (Barker et al. 1975). Depending on the dose and route administered, picrotoxin increases isoflurane MAC by up to 60% in rats (Zhang et al. 2001a,b). *In vitro*, isoflurane does cause dose-dependent NMDA inhibition (Hollmann et al. 2001; Ogata et al. 2006). Consequently, if picrotoxin increases the isoflurane concentration necessary to produce immobility, then NMDA antagonism should play a greater role in mediating immobility, assuming NMDA receptors are important mediators of MAC (Stabernack et al. 2003; Dutton et al. 2006). How could augmented NMDA effects on MAC be measured? If an inhaled agent causes immobility primarily through cellular targets other than NMDA receptors, then administration of an NMDA antagonist, such as MK801 (dizocilpine), should potently decrease MAC because NMDA receptors are available for drug binding and because antagonism of NMDA receptors decreases MAC (Kuroda et al. 1993; Stabernack et al. 2003). In other words, if NMDA receptor inhibition contributes a larger effect to anesthetic immobility, then administration of an NMDA antagonist should have a smaller effect on MAC, since many of the NMDA receptors will be already bound to – and at least partially inhibited by – the inhaled agent (Eger et al. 2006).

If correct, these predictions would support a more general paradigm about how inhaled anesthetics work at the cellular level. Potent volatile agents typically negatively modulate excitatory cell membrane receptors and positively modulate inhibitory cell membrane receptors (Sonner et al. 2003a), thereby reducing neuronal excitability. In a whole animal, these anesthetic effects will manifest themselves in a manner commensurate with the location and function of the affected neurons. For example, net inhibition of thalamocortical neuron activity by anesthetics produces amnesia and unconsciousness, whereas net inhibition of spinal cord neuron function by anesthetics produces immobility (Antognini & Carstens 1998). It is postulated here that the anesthetic-sensitive cell receptors responsible for a given anesthetic pharmacologic endpoint – such as immobility, amnesia, or unconsciousness – are dependent upon five factors: the types of anesthetic-sensitive receptors present at the neuroanatomical site of action, the relative density of a given anesthetic-sensitive receptor at these sites, the binding affinity between the anesthetic and its

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cell target, the efficacy by which anesthetic-mediated modulation of a given ion channel decreases neuronal excitability, and the role of an affected neuron within a neural circuit (i.e., for movement, memory, or consciousness). If inhaled anesthetics act by a summation of ion channel effects that hyperpolarize cells, such as by inhibition of NMDA receptors and by potentiation of $GABA_A$ receptors, then a decrease in the contribution of one cell target effect must be compensated by a reciprocal increase in the contribution of other cell targets at relevant sites of action. Indeed, *in vitro* electrophysiologic measurements in frog oocytes indicate that agents producing large $GABA_A$ potentiation at concentrations equivalent to MAC cause comparatively small inhibition of NMDA receptor currents, and *vice-versa* (Solt et al. 2006; Kelly et al. 2007). A likely corollary is that if the apparent $GABA_A$ affinity of an inhaled anesthetic is reduced by co-administration of a $GABA_A$ antagonist, then MAC will increase and the relative contribution of NMDA receptor inhibition – as well as those of other relevant anesthetic-sensitive targets – in reducing neuronal excitability at the new MAC will also be increased.

In this study, spinal administration of picrotoxin was used to increase isoflurane MAC in rats, using immobility in response to a noxious stimulus as an endpoint (Eger et al. 1965). The increased isoflurane concentration needed to produce immobility in the picrotoxin rats should inhibit NMDA receptors more than in control rats that have a normal isoflurane MAC. Therefore, it is hypothesized that administration of intravenous MK801 to both groups will decrease isoflurane requirement more in the control rats than in the picrotoxin rats, suggesting that a decreased apparent potency of isoflurane increases the relative NMDA effects of isoflurane at MAC.

Materials and methods

Fourteen male 10-week-old Sprague–Dawley rats (Harlan, CA, USA) weighing 269 ± 12 g (mean ± SD) were studied. Rats were individually housed in a vivarium with a 12-hour light/dark cycle and with food and water available *ad libitum.* The Institutional Animal Use and Care Committee approved this protocol.

Intrathecal catheter placement

In each rat, anesthesia was induced with isoflurane (Attane; Minrad, NY, USA) in oxygen delivered in an acrylic glass chamber and then maintained with isoflurane in oxygen delivered by mask through a coaxial Mapleson E circuit. The rat was positioned in a stereotaxic frame (Model 1900; David Kopf Instruments, CA, USA) using non-rupture tip ear bars with the head positioned in cervical ventro-flexion. Eyes were lubricated with an ophthalmic ointment, and body temperature was monitored using a rectal probe. The dorsal aspect of the head and neck were clipped, scrubbed with isopropyl alcohol and povidone iodine, and covered with a sterile iodophor impregnated adhesive and a sterile polylined drape.

Using aseptic technique, the skin and underlying median fibrous raphe were incised along the median plane from the parietal bone rostrally, along the sagittal crest, and terminating over the caudal edge of the spinous process of the axis vertebra. Blunt dissection was used to divide the midline fascial plane of the trapezius, cleidocervicalis, splenius, and semispinalis muscles which were then elevated and retracted to expose the atlantooccipital space. Using a 20-gauge intravenous needle tip, a 0.5-mm incision was made in the dorsal atlantooccipital membrane through which a 32-gauge intrathecal catheter (Part 0046; RecathCo, PA, USA) was advanced approximately 10–12 cm to the mid-lumbar vertebral region. The catheter collar insertion point was affixed to the surrounding deep tissues using sterile cyanoacrylate glue (Tissuemend II; Veterinary Products Laboratories, AZ, USA), and the incision was sutured closed in two layers, leaving a few cm of intrathecal catheter exposed. This end of

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the intrathecal catheter was plugged with a sterile 0.5 cm long 0.035 cm diameter stainless steel wire (Small Parts, FL, USA). The incision site was infiltrated with 0.15 mL of 1% lidocaine and 0.5 mg of flunixin meglumine was administered intramuscularly for analgesia.

Rats were recovered with facemask oxygen, and blankets and heating blankets were used as needed to correct hypothermia. Rats were returned to individual cages once they were moving vigorously. All rats were eating and drinking within 1 hour after completion of surgery, and no motor deficits were evident in any animal. Prior to further study, rats were allowed 48 hours recovery time, during which no appreciable tissue inflammation, lethargy or anorexia were present in any animal.

Preparation of solutions

Rat aCSF (Zhang et al. 2001b) solutions were made daily using aseptic techniques and ACS grade chemicals (Fisher Scientific, PA, USA). A monovalent stock solution was prepared by dissolving 3.6963 g NaCl, 1.1551 g $NaHCO_3$, 0.0895 g KCl, and 0.0340 g KH_2PO_4 in sterile distilled water to yield 500 mL. A divalent stock solution was prepared by dissolving 0.8086 g $CaCl_2 \cdot 2H_2O$ and 0.8437 g $MgCl_2 \cdot 6H_2O$ in sterile distilled water to yield 10 mL. Rat aCSF was prepared by adding 0.0266 g glucose to 25 mL of monovalent stock solution plus 50 μL of divalent stock solution. Carbon dioxide (99.999%; Matheson Trigas, CA, USA) was added to adjust pH. The solution was then transferred to a stoppered sterile vial, and the following gas pressures and solute concentrations were measured at 37 °C using an automated analyzer (ABL700; Radiometer America, OH, USA): pH = 7.62, PCO_2 = 33 mmHg, PO_2 = 156 mmHg, K^+ = 4.1 mEq L^{-1}, Na^+ = 147 mEq L^{-1}, Ca^{2+} = 0.85 mEq L^{-1}, Cl^- = 125 mEq L^{-1}, glucose = 68 mg dL^{-1}.

Isoflurane MAC measurement

Rats were placed in four 8 ×30 cm acrylic glass cylinders that were connected in parallel with each tube receiving isoflurane in oxygen through a one-holed rubber stopper from an agent-specific and temperature-compensated vaporizer. Fresh gas flow rate was ≥1 L $minute^{-1}$ $cylinder^{-1}$. A stopcock sealed sampling port permitted gas sampling near the tube inflow. The other end of each acrylic tube was sealed with a 2-holed stopper; rat tails were drawn through one hole and a passive gas scavenging system was attached to the second hole. A thermistor probe (400 series; YSI, OH, USA) that was calibrated using a certified mercury thermometer (SRM934-FC; ERTCO, IA, USA) also traversed a sealed portion of each 2-holed stopper to measure rectal temperature, which was maintained at 37 ± 1 °C using heating pads as needed. A 24-gauge catheter was placed percutaneously in the lateral tail vein as distally as possible and sealed with an intermittent infusion plug.

Once rats were anesthetized, the steel plug was removed from the end of the intrathecal catheter and was replaced with a 27-gauge blunt needle that was connected by a primed extension set to a 500 μL gastight glass syringe (series 1700; Hamilton, NV, USA) containing either aCSF (control) or 1 mg mL^{-1} picrotoxin (Sigma, MO, USA) in aCSF. Control or picrotoxin aCSF infusions were administered throughout the entire study at 1 μL $minute^{-}{}_{1}$ using a multisyringe infusion pump (PHD 2000; Harvard Apparatus, MA, USA). Solutions were administered intrathecally for at least 30 minutes at a constant inspired sub-MAC isoflurane dose prior to the first isoflurane MAC test.

After equilibration, an alligator clip was placed on the distal tail and rotated back and forth for 1 minute. The presence or absence of movement in response to tail clamp for each rat was recorded, and gas was removed via the sampling port via a glass syringe for analysis. The isoflurane concentration was then increased by 10–15%, and rats were allowed 20 minutes for equilibration between the new inspired and expired partial pressures. Again,

responses to clamping the tail slightly cranial to the previous test were recorded and gas samples were sampled and analyzed. This process was repeated until all animals did not move in response to a tail clamp. The arithmetic mean of isoflurane concentrations that bracketed move/no move responses defined the first individual MAC (Eger et al. 1965) baseline value (MAC1). Isoflurane concentration was then decreased by 10–15% for 20 minutes followed by re-testing of tail clamp responses and re-measuring of cylinder isoflurane concentrations. This process was repeated until all animals did move in response to tail clamp. The arithmetic mean of isoflurane concentrations that bracketed no move/move responses defined the second individual baseline MAC value (MAC2).

After baseline measurements, 0.15 mg of +MK801 (Sigma), prepared as a 1 mg mL^{-1} solution in 0.9% NaCl, was administered in a lateral tail vein of each rat, and the inspired isoflurane was reduced to a sub-MAC concentration. This dose (0.5 mg kg^{-1}) and route of MK801 was selected because it produces a near-maximum isoflurane MAC-sparing effect (Kuroda et al. 1993; Stabernack et al. 2003) that persists for at least 3 hours (Kuroda et al. 1993) in rats. Following 30 minutes of equilibration between inspired and expired concentrations, a cylinder gas sample was collected for analysis and the tail clamp response was recorded. Isoflurane concentration was then increased 10–15% for 20 minutes, followed by assessment of tail clamp responses and analysis of cylinder gas samples. This process was repeated, as previously described, until all animals did not move in response to a tail clamp. The arithmetic mean of isoflurane concentrations that bracketed move/no move responses defined the first individual post-MK801 MAC value. The isoflurane concentration was then decreased 10–15% for 20 minutes, and tail clamp responses were re-assessed and cylinder gas samples were re-measured. This process was repeated until all animals did not move in response to tail clamp. The arithmetic mean of isoflurane concentrations that bracketed no move/move responses defined the second individual post-MK801 MAC value. At the end of the study, rats were euthanized by exsanguination and bilateral thoracotomy under deep isoflurane anesthesia.

Isoflurane concentration measurement

Isoflurane concentrations were measured using a gas chromatograph (Clarus 500; Perkin Elmer, MA, USA). Gas samples were injected via a 0.25 mL sample loop directly onto a 3 m SF-96 packed column with Chromasorb WHP support (Perkin Elmer) having a hydrogen flow of 35 mL $minute^{-1}$, zero air flow of 350 mL $minute^{-1}$, and a surrounding oven temperature of 150 °C. After a 1.3-minute retention time, isoflurane was quantitated using a flame ionization detector. Commercial data acquisition software (TotalChrom; Perkin Elmer) was used to calculate the resulting area under the curve (AUC) in μV seconds. The AUC was calibrated against secondary isoflurane standard gas mixtures that encompassed the range of experimental isoflurane measurements; calibration curves were always linear. Concentrations of secondary isoflurane gas standards were, in turn, previously determined using multiple primary gas isoflurane standard mixtures that were prepared utilizing ideal gas laws. The AUC of unknown gas mixtures was divided by the slope of the calibration curve (V s $\%^{-1}$) to calculate the % isoflurane in the sample.

Statistical analysis

Data were summarized as mean ± SD, and normal distributions were verified by Kolmogorov–Smirnov tests calculated using commercial software (v.11; SPSS, IL, USA). Differences between the first and second isoflurane MAC values for baseline and post-MK801 determinations were assessed using paired two-tailed Student's *t*-tests. Differences between the control and picrotoxin MAC determinations were assessed using unpaired two-tailed Student's *t*-tests. The ΔMAC was calculated as the difference between the mean baseline isoflurane MAC and the mean post-MK801 isoflurane MAC for each rat. The

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difference between the ΔMAC for the control rats *versus* the ΔMAC for the picrotoxin rats was analyzed using an unpaired two-tailed Student's *t*-test. Differences were deemed significant if $p < 0.05$.

Results

A summary of isoflurane MAC measurements for rats in the control and picrotoxin groups is shown in Table 1. There was no difference between the first and second MAC for either group at either the baseline measurement or the post-MK801 measurement. This suggests that the effects of picrotoxin intrathecal infusions on MAC remained similar following infusions from 30 minutes, the equilibration time before first tail clamp, to 297 ± 41 minutes, the average total picrotoxin infusion time. Similarly, the effect of the single intravenous MK801 injection on MAC did not appear to wane over the course of 107 ± 31 minutes, the average time from MK801 injection until the completion of the experiment.

Intrathecal picrotoxin increased isoflurane MAC from 1.54 ± 0.03% to 1.78 ± 0.21%, a statistically significant ($p = 0.009$) difference of 16%. No rats seized during picrotoxin infusions, although spontaneous muscle twitching was occasionally observed at lower isoflurane doses. MK801 administration significantly reduced isoflurane MAC to 0.82 ±0.09% ($p = 0.00001$) and 1.31 ± 0.23% ($p = 0.0003$) in control and picrotoxin rats, respectively. After MK801, isoflurane MAC was 60% higher in picrotoxin rats than control rats, a difference that was also statistically significant ($p = 0.0006$). No seizure activity or muscle twitching was evident after MK801.

MK801 significantly ($p = 0.0008$) reduced isoflurane MAC more in control rats than in rats receiving picrotoxin (Table 1). Hence, increasing isoflurane MAC by picrotoxin antagonism of $GABA_A$ receptors reduced the magnitude of MAC reduction caused by MK801 antagonism of NMDA receptors.

Discussion

Intrathecal picrotoxin significantly increased isoflurane MAC, but MAC reductions due to MK801 were lower in picrotoxin rats compared to aCSF control rats. Isoflurane at MAC normally produces large $GABA_A$ current potentiation and much smaller NMDA inhibition (de Sousa et al. 2000; Yamakura & Harris 2000). $GABA_A$ receptor antagonism increases MAC, and the resulting increased isoflurane dose increases NMDA receptor inhibition. Consequently, the relatively small NMDA receptor effects of isoflurane normally observed at MAC are due to much larger cell hyperpolarizing effects of isoflurane at other anesthetic-sensitive sites at this same concentration. If isoflurane is rendered less effective as a $GABA_A$ receptor agonist, then it functions more as an NMDA antagonist at the greater MAC. The magnitude of an anesthetic's effect at any receptor at MAC is thus potentially dependent upon the magnitude to which that anesthetic also modulates the activity of other cell targets that reduce neuronal excitability.

Although *in vitro* data (Ogata et al. 2006) support the notion of augmented NMDA receptor antagonism at higher inspired isoflurane concentrations *in vivo*, the same does not necessarily hold true for comparisons of homologous compounds. Although polyfluorination of benzene rings decreases inhibition of NMDA receptors expressed in frog oocytes and increases MK801 MAC-sparing in rats, no such correlation exists for conventional anesthetic agents (Eger et al. 2006). Perhaps this is because *in vitro* models do not accurately model the magnitude of *in vivo* responses equally well for all agents. Such a phenomenon might be explained by the diversity in NMDA receptor subtypes, the presence or absence of co-expressed anesthetic-sensitive channels or receptors, dissimilar resting membrane

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potentials, the variation in extracellular or intracellular fluid compositions, the variation in cell membrane compositions, and the differences between second messenger systems between *in vitro* model cells and *in vivo* CNS sites responsible for MAC. Nonetheless, even if responses measured in model cells are not quantitatively identical to responses in whole animal models, an *in vitro* dose-effect for a single drug likely still reflects a relative dose-effect *in vivo*. It is possible that increased inhibition of NMDA receptors expressed in frog oocytes may not reveal the precise magnitude of increased inhibition of NMDA receptors in a rat spinal cord, but rather it may simply correlate with a relative increase in spinal cord NMDA receptor inhibition of unknown magnitude.

In this study, $GABA_A$ receptor antagonism was used to decrease the apparent potency of isoflurane in order to measure *in vivo* NMDA antagonism at a new increased blood isoflurane EC_{50}. This is not to imply that $GABA_A$ receptors contribute significantly to the mechanism by which isoflurane causes immobility. To the contrary, several studies suggest that while $GABA_A$ receptor agonists (Schwieger et al. 1994) and antagonists (Zhang et al. 2001a) can modify MAC, they in fact may not mediate immobility (Zhang et al. 2001b, 2004; Sonner et al. 2003b). Again, the purpose of $GABA_A$ antagonism in this study was simply to increase the blood isoflurane EC_{50} by a technique that spared NMDA receptor function and in a manner that neither induced spinal neuronal damage nor interfered with the detection of gross movement in response to a noxious stimulus. Assuming these same criteria are satisfied, it is postulated that results reported here would be little changed if a different specific receptor agonist or antagonist other then picrotoxin were used instead to increase isoflurane MAC. Moreover, it is postulated that, at the increased isoflurane MAC caused by picrotoxin administration, the contributions of other relevant cell targets to anesthetic immobility will also be increased, so long as these other targets – like NMDA receptors – are not directly modulated by picrotoxin and do not exhibit a maximal effect at 1.0×MAC of isoflurane.

The intrathecal administration route for picrotoxin served three purposes. First, it permitted isoflurane MAC changes to achieve a maximum ceiling (Zhang et al. 2001b), at which the anesthetic effect of GABA antagonism would be unchanged over the duration of several MAC determinations. This is because the spinal cord represents the anatomical site of action for inhaled anesthetic-mediated immobility (Antognini & Schwartz 1993). Second, achieving a maximum increase in isoflurane MAC likely helped maximize the effect size for MK-801 NMDA receptor antagonism between picrotoxin treated animals and controls. Lastly, if the intravenous route were used, larger picrotoxin doses would be required to achieve a MAC effect similar to intrathecal infusions. But due to picrotoxin toxicity (Pericic et al. 1986; Drummer & Woolley 1991; Ishimaru et al. 1995), these larger intravenous doses would confound interpretation of MAC-sparing results. The present study was designed to measure the *in vivo* effect of diminished apparent isoflurane potency – using $GABA_A$ receptor antagonism – on NMDA receptor antagonism at MAC. However, this study does not address whether increased NMDA receptor antagonism at increased isoflurane MAC is occurring at the level of the spinal cord, brain, or both.

Whereas a previous study reported that intrathecal picrotoxin infusions ≥1 μg $minute^{-1}$ in rats increased isoflurane MAC by 30–40% (Zhang et al. 2001b), similar infusions in the present study produced only about half of this effect. The reason for this difference is unclear, although the previous study also reported that, without picrotoxin, isoflurane MAC was 1.28%, considerably lower than measurements in this study that are also within the range of published values for rats (Mazze et al. 1985; Kuroda et al. 1993; Eger et al. 2006; Brosnan et al. 2007a,b). A lower baseline MAC measurement could possibly exaggerate the effect of intrathecal picrotoxin, and vice versa. Also, though descriptions of aCSF preparation methods were identical, measurements at 37 °C in this study differed by 0.2 pH

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units – and more modestly with respect to electrolytes – from the aCSF composition calculated by Zhang and co-workers during their studies of intrathecal picrotoxin (Zhang et al. 2001b). Changes in CSF pH caused by carbon dioxide decrease MAC (Eisele et al. 1967; Brosnan et al. 2007a), and perhaps these differences between pH and PCO_2 in aCSF solutions might explain some of the discrepancy between picrotoxin responses. Fortunately, since each rat served as its own control before and after MK801 administration and, since aCSF composition was constant throughout each experiment, it seems unlikely that this systematic difference, if important, should affect ΔMAC.

In this study, intravenous MK801 decreased isoflurane MAC by 47% in control rats. Previous studies (Kuroda et al. 1993; Eger et al. 2006) have shown similar results with both MK801 infusions of ≥8 μg kg^{-1} $minute^{-1}$ and bolus doses of 0.5 mg kg^{-1}, with the latter decreasing isoflurane MAC by half for at least 3 hours. This is consistent with the relatively constant MAC-sparing effect observed for the first and second post-MK801 isoflurane measurements for both control and picrotoxin rats.

Both picrotoxin and MK801 have the potential to cause CNS injury. Thus, it is essential that pharmacologic effects be distinguished from toxic effects. The total picrotoxin dose used in this study is at least more than 10–60 times lower than the median lethal dose for a bolus intraperitoneal injection in rats (Pericic et al. 1986; Drummer & Woolley 1991). Moreover, even after administration of intrathecal picrotoxin at 1.2 μg $minute^{-1}$, a dose higher than the one used in the present study, isoflurane MAC returns to baseline within 2 days (Zhang et al. 2001b). A rapid and reversible effect on isoflurane requirement is more consistent with a pharmacologic response than a toxic one. MK801 can cause neuronal vacuolization, but histologic changes are prevented by co-administration of an inhaled anesthetic (Ishimaru et al. 1995). Rather than increasing toxicity, MK801 actually decreases seizure severity and lethality induced by picrotoxin (Veliskova & Velisek 1992). Finally, if the combination of MK801 plus picrotoxin was toxic, then ΔMAC with the two agents should be more than ΔMAC for control rats receiving only picrotoxin since toxicity should reduce anesthetic requirement (Brosnan et al. 2007a,b). In fact, just the opposite result was obtained.

In this study, MAC was determined by an individual who was not blinded to the intrathecal infusion drug (aCSF + picrotoxin *versus* aCSF alone). Although MAC is often considered an objective measure of anesthetic potency, the potential for subjective bias remains (Liao et al. 2006), particularly when the treatment effect is relatively small. Certainly, the large MAC decrease with MK801 would seem to render a saline control unnecessary, since the presence or absence of the drug effect would be immediately obvious. A large increase in isoflurane MAC was also anticipated from intrathecal picrotoxin, but the actual change was more modest. Even though picrotoxin-induced muscle twitching may make blinding of spinal infusions difficult, it nonetheless warrants consideration in the development of future experimental designs.

Inhaled agents likely cause anesthesia by actions on multiple cell channels and receptors that generally decrease CNS excitability. If an anesthetic state represents the sum of these target effects, then the magnitude of a single receptor effect at MAC should equal that fraction of CNS depression required for immobility that is not produced by the sum of all other anesthetic-sensitive cellular targets at MAC. Hence, were it not for its potent positive modulation of GABA receptors, isoflurane would be a more effective negative modulator of NMDA receptors at MAC. This study demonstrates that anesthetic efficacy at one cell target is, in part, likely a function of efficacy and potency at all other targets. Moreover, anesthetic efficacy at anesthetic-sensitive targets is mutable. It can be manipulated pharmacologically, as in this study, by drugs or states that alter MAC.

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Table 1

Summary of isoflurane MAC measurements (%, mean ± SD) in rats receiving intrathecal aCSF (control) *versus* rats receiving intrathecal picrotoxin in aCSF. There was no difference between the first and second MAC measurements for any treatment in either group. Post-MK801 MAC was significantly decreased from baseline in both groups. Isoflurane MAC was significantly higher in the picrotoxin animals than the control animals. The ΔMAC was significantly lower in the picrotoxin animals than the control animals

Measurement	Control (n = 5)	Picrotoxin (n = 9)
Baseline		
MAC 1	1.56 ± 0.08	1.80 ± 0.21
MAC 2	1.53 ± 0.02	1.76 ± 0.23
Post-MK801		
MAC 1	0.78 ± 0.11	1.32 ± 0.26
MAC 2	0.86 ± 0.08	1.30 ± 0.21
ΔMAC	0.72 ± 0.07	0.47 ± 0.14