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Amnestic Concentrations of Etomidate Modulate $GABA_{A,slow}$ Synaptic Inhibition in Hippocampus

Shuiping Dai, M.D., Misha Perouansky, M.D.,† Robert A. Pearce, M.D., Ph.D.‡*

Background: **γ-Aminobutyric acid type A ($GABA_A$) receptor-mediated inhibition in the central nervous system exists in two forms: phasic (inhibitory postsynaptic currents, IPSCs) and tonic (nonsynaptic). Phasic inhibition is further subdivided into fast ($GABA_{A,fast}$) and slow ($GABA_{A,slow}$) IPSCs. By virtue of its dendritic location and kinetics, $GABA_{A,slow}$ has been proposed to control synaptic plasticity and memory. Etomidate is a nonbarbiturate, intravenous anesthetic that selectively modulates $GABA_A$ receptors and produces amnesia at low doses *in vivo*. This study tested whether correspondingly low concentrations of etomidate *in vitro* alter $GABA_{A,fast}$ and $GABA_{A,slow}$ phasic inhibition.**

Methods: **Electrophysiological recordings were obtained from hippocampal slices prepared from postnatal day 3–8 mice and maintained in organotypic culture for 10–14 days. Etomidate was applied at concentrations corresponding to one-half to four times the half maximal effective concentration that impairs hippocampus-dependent learning and memory – *i.e.*, 0.125–1.0 μM.**

Results: **Etomidate 0.25 μM (the half maximal effective concentration) doubled the time constant of decay of $GABA_{A,slow}$ IPSCs, but it had no detectable effect on $GABA_{A,fast}$ IPSCs. Higher concentrations of etomidate had stronger effects on both types of phasic inhibition: 0.5 and 1 μM etomidate prolonged the time constant of decay by 310% and 410% for $GABA_{A,slow}$ and by 25% and 78% for $GABA_{A,fast}$. Concentrations of etomidate up to 1 μM had no significant effects on the amplitudes of either $GABA_{A,fast}$ or $GABA_{A,slow}$ IPSCs.**

Conclusions: **At concentrations that impair hippocampus-dependent memory, etomidate modulates $GABA_{A,slow}$ more strongly than $GABA_{A,fast}$ IPSCs. Effects of etomidate on $GABA_{A,slow}$ IPSCs may contribute to etomidate-induced amnesia.**

MODULATION of γ-aminobutyric acid type A ($GABA_A$) receptor-mediated inhibition is thought to play a key role in the mechanism of action of general anesthetics.[1] $GABA_A$ receptors exist in a variety of subunit combinations that confer distinct physiologic and pharmacological properties to the individual receptors.[2] Moreover, it has been recently recognized that $GABA_A$ receptor-mediated inhibition presents in three distinct forms: two transient (phasic) types mediated by synaptic receptors, and one continuous (tonic) type mediated by receptors that are largely extrasynaptic.[3] Therefore, even anesthetics with a relatively narrow and well-defined receptor-level activity profile (such as etomidate and propofol)[1] may differentially engage different types of $GABA_A$-ergic inhibition to achieve the desirable components of the anesthetic state (*e.g.*, sedation, amnesia, hypnosis, and immobility).

Significant progress has been made recently in understanding the chain of events linking molecular to behavioral actions of etomidate. Evidence obtained from knockout animals has suggested a role for α5 subunit-containing $GABA_A$ receptors in the control of learning and memory.[4,5] The α5 subunit is expressed most strongly in the hippocampus, a structure that plays a central role in the formation of declarative memories. Located at extrasynaptic sites on pyramidal neurons, receptors containing α5 subunits have been shown to mediate tonic inhibition.[6] The finding that tonic inhibition is enhanced at low anesthetic concentrations, together with the demonstration that mice lacking the α5 subunit are resistant to the amnestic action of etomidate, thus pointed to tonic inhibition in the hippocampus as the principal mechanism for this behavioral effect.[7]

However, recent findings indicate that receptors containing α5 subunits are also located at dendritic synapses[8] and that they underlie a slow form of synaptic (phasic) inhibition in hippocampal CA1 pyramidal neurons.[9,10] By virtue of its dendritic location and slow kinetics, which match those of *N*-methyl-D-aspartate receptor–mediated excitation, this inhibitory current (which has been termed $GABA_{A,slow}$) is well suited to control synaptic plasticity and memory formation.[11,12] It represents an additional or alternative effector mechanism by which etomidate and other drugs that enhance $GABA_A$ receptor function may produce amnesia.

To test whether $GABA_{A,slow}$ shows the requisite sensitivity to etomidate to be considered a viable candidate for contributing to etomidate-induced amnesia, we examined the effects of a range of concentrations of etomidate bracketing the half maximal effective concentration that impairs hippocampus-dependent learning and memory (EC50-amnesia) on $GABA_{A,fast}$ and $GABA_{A,slow}$ inhibitory postsynaptic currents (IPSCs). We found that in contrast to $GABA_{A,fast}$ IPSCs, which were unaffected or only weakly modulated by etomidate at amnestic concentrations, $GABA_{A,slow}$ IPSCs were strongly enhanced. Therefore, along with tonic GABAergic inhibition, $GABA_{A,slow}$ represents a potentially important effector mechanism for suppression of hippocampal learning and memory.

* Assistant Scientist, † Professor, ‡ Professor and Chair, Department of Anesthesiology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin.

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Address correspondence to Dr. Pearce: Department of Anesthesiology, University of Wisconsin, B6/319 Clinical Science Center, 600 Highland Avenue, Madison, Wisconsin 53792. rapearce@wisc.edu.

Materials and Methods

Organotypic Hippocampal Slice Cultures (OTChip)

All experiments conformed to the guidelines laid out in the Guide for the Care and Use of Laboratory Animals, and were conducted with the approval of the University of Wisconsin-Madison (Madison, Wisconsin) Animal Care and Use Committee.

OTC*hip* were prepared from 3- to 8-day-old B6/129 hybrid mice as described by Stoppini *et al.*[13] In brief, mice were anesthetized with isoflurane and decapitated. The brain was quickly removed and immersed in ice-cold dissecting medium containing 50% Gey's balanced salt solution, 10 mM $MgCl_2$, and 60 mM glucose. The brain was then transferred to a dissecting chamber, blocked, and mounted on the stage of a vibrating microtome (Vibratome series 1000; Ted Pella, Inc., Redding, CA). Brain slices were cut 350 μm thick and placed on Milli-Cell-CM membrane inserts (Millipore, Billerica, MA) in a 6-well culture plate. Each well contained one membrane insert and 1 ml of culture medium. The culture medium was composed of 25% Hank's salt solution, 25% horse serum with 30 mM D-glucose, 50% minimum essential medium with Earl's salts, L-glutamine, and sodium bicarbonate. After 1 day in culture, cytostatics (10 μM Uridine, 10 μM ARA-C, and 10 μM 5-Fluoro-2′deoxyuridine; Sigma-Aldrich, St. Louis, MO) were added to the culture medium. The slice cultures were maintained in an incubator at 36°C in 5% carbon dioxide. The culture medium was exchanged (90% of volume) twice per week, with cytostatics added to the medium once weekly. Slices were used between 10 and 14 days in culture, at which time hippocampal structures remained easily identifiable (fig. 1A).

Electrophysiology

A brain slice recording chamber was mounted on the stage of an upright microscope (Olympus BX50WI; Olympus America Inc., Center Valley, PA) with a long working distance water immersion objective and differential interference contrast optics, which was connected to a video camera (Hamamatsu C2400; Hamamatsu, Tokyo, Japan). A single OTC*hip* was removed from the six-well culture plate by cutting the membrane from the well with a scalpel blade, and it was then transferred to the recording chamber by using a Pasteur glass pipette. For experiments, slices were continuously superfused with artificial cerebrospinal fluid at 34 ± 1°C bubbled with carbogen (95% O_2 + 5% CO_2).

Whole cell pipettes were fabricated from borosilicate glass (KG33; Garner Glass, Claremont, CA). Pipettes were filled with a solution containing (in mM): CsCl 135 (for evoked IPSCs recording, CsCl was partially replaced by 40 mM K-gluconate), Na-HEPES 10, EGTA 10, MgATP 3, GTP 0.5, lidocaine *N*-ethyl bromide (QX-314) 5, pH = 7.25. Whole-cell patch clamp recordings were obtained from neurons within the pyramidal cell layer of the CA1 area that had pyramidal cell-like morphology. Recordings generally remained stable for up to 1.5 h at a holding potential of −50 to −60 mV. Signals were amplified using a MultiClamp 700A amplifier (Axon Instruments, Foster City, CA) and ClampEx software (Axon Instruments), filtered at 5 kHz and sampled at 10 kHz by using a Digidata 1322A (Axon Instruments). Open tip resistance ranged from 1.5–4 MΩ. Whole-cell access resistance was less than 15 MΩ before compensation by 50–80%.

In the absence of any excitatory or inhibitory antagonists, recordings revealed frequent large-amplitude spontaneous inward currents (fig. 1B). In chloride-loaded

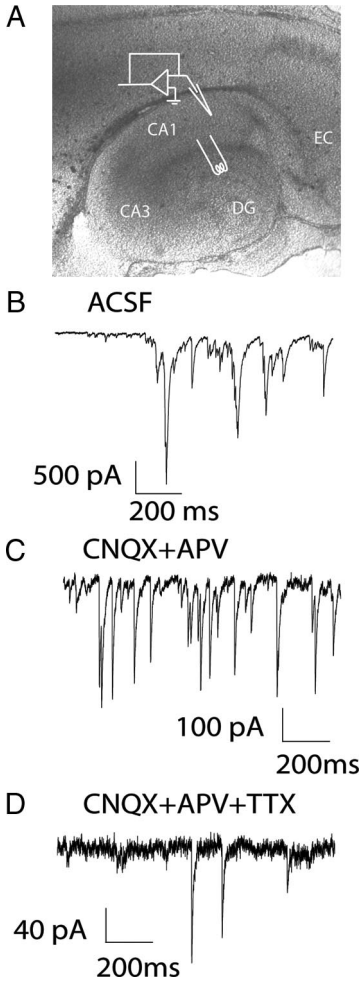

Fig. 1. Synaptic currents in organotypic hippocampal slices *in vitro*. (*A*) Photograph of a slice maintained *in vitro* for 14 days. Note that major structural features are maintained, enabling identification of specific subfields and laminae. Typical positions of the recording and stimulating electrodes are illustrated schematically. CA1 and CA3 = Cornu Ammonis areas 1 and 3; DG = dentate gyrus; EC = entorhinal cortex. (*B–D*) Recordings from a pyramidal-shaped neuron in *stratum pyramidale*: (*B*) under control conditions, with no drugs added to the artificial cerebrospinal fluid (ACSF); (*C*) in the presence of the ionotropic glutamate antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and (2R)-amino-5-phosphonovaleric acid (APV), which reveals abundant large-amplitude spontaneous inhibitory postsynaptic currents; (*D*) after the addition of tetrodotoxin (TTX), which leaves only small-amplitude miniature (action potential-independent) inhibitory postsynaptic currents.

neurons, these events include both GABAergic IPSCs and glutamatergic excitatory postsynaptic currents. Even in the presence of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 40 μM (2R)-amino-5-phosphonovaleric acid (APV), which together block all ionotropic glutamatergic synaptic input, there remained sufficiently frequent and large spontaneous fast IPSCs that individual events were difficult to separate for kinetic analysis (fig. 1C). Therefore, to characterize the effects of etomidate on $GABA_{A,fast}$, we examined miniature IPSCs (mIPSCs) by adding tetrodotoxin to the superfusate (fig. 1D). To characterize the effects of etomidate on $GABA_{A,slow}$ IPSCs, we placed a glass electrode filled with artificial cerebrospinal fluid in the stratum lacunosum-moleculare (SL-M, fig. 1A) and stimulated using current pulses of 40–60 μA, 0.1 ms in duration, at a frequency of 0.05 Hz.

Drugs

All drugs were purchased from Sigma-Aldrich. Etomidate was dissolved in dimethyl sulfoxide, frozen, and freshly dissolved in artificial cerebrospinal fluid before adding it to the bath perfusion by using a syringe pump with the flow rate adjusted to yield the desired concentration.

Data Analysis

Data were analyzed offline on a personal computer by using Mini Analysis (Synaptosoft, Decatur, GA) and ClampFit (Molecular Devices, Sunnyvale, CA). The threshold for event detection was set at three times the root mean square noise level. Events were analyzed by automated event detection that acquires amplitude, 10–90% rise times, and the time to 63% decay.

The decay phase of averaged mIPSCs was fit with a double exponential function using Microcal Origin (version 6.0, 7.0; OriginLab Corporation, Northampton, MA). The weighted time constant (τ_{wt}) was calculated as $[A_1\tau_1 + (1 - A_1)\tau_2]$, where A_1 is the amplitude of the fast component of the biexponential equation and where τ_1 and τ_2 are the time constants of the fast and slow components, respectively. The decay phase of averaged $GABA_{A,slow}$ IPSCs was fit with a single exponential function.

Concentration-response data were fit by using microcal origin to the logistic function:

$$i = i\text{max} + (i\text{min} - i\text{max})/(1 + ([\text{etomidate}]/\text{EC50})^n)$$

where i is the current amplitude normalized to the control response, $i$max is the maximal current amplitude, $i$min is the minimum current amplitude, EC50 is the concentration of etomidate eliciting half maximal current amplitude, and n is the slope factor. During the fitting procedure, all values were allowed to float freely, with the exception of $i$min, which was fixed to a value of "1."

Statistics

Statistical analysis was performed by using Microcal Origin (version 7; OriginLab Corporation) or GraphPad Prism (version 7; GraphPad Software, San Diego, CA). Data are presented as mean ± SD. Statistical comparisons were made by using a one-tailed Student t test to examine effects of etomidate on decay, and a two-tailed t test to examine effects of etomidate on amplitude. Effects were considered significant at $P < 0.05$. Fits to the logistic equation were compared by examining 95% confidence intervals; nonoverlapping confidence intervals indicated significant differences.

Results

Etomidate Slows the Decay of Evoked $GABA_{A,slow}$ IPSCs

We examined the effects of etomidate on $GABA_{A,slow}$ IPSCs at concentrations ranging from 0.125–1 μM (*i.e.*, 0.5–4 times EC50 amnesia) by using electrically evoked, action potential–dependent responses. At its EC50–amnesia concentration, etomidate markedly prolonged the decay time constant (τ_{decay}) of $GABA_{A,slow}$ IPSCs: 0.25 μM slowed τ_{decay} approximately two-fold (fig. 2A). The effect was rapid in onset, reversible (fig. 2B), and concentration-dependent (fig. 2C). Figure 3 summarizes the effects of etomidate on the amplitude and τ_{decay} of $GABA_{A,slow}$ obtained from 6 cells. Over the range of concentrations tested, etomidate slowed IPSC decay in a concentration-dependent manner (fig. 3, left axis; $P < 0.05$ for all concentrations); 0.5 and 1 μM etomidate prolonged τ_{decay} by 310 and 410%. By contrast, etomidate did not alter the amplitude of evoked $GABA_{A,slow}$ IPSCs at any concentration (fig. 3, right axis; $P > 0.05$).

Miniature $GABA_{A,fast}$ IPSCs: Relative Resistance

We examined the effects of etomidate on $GABA_{A,fast}$ mIPSCs at concentrations ranging from 0.25–1 μM (*i.e.*, 1–4 times EC50 amnesia). Examples of action potential–independent mIPSCs under control conditions and in the presence of 0.5 and 1 μM etomidate are shown in figure 4A. Individual mIPSCs were characterized by their amplitude and interevent interval and plotted as cumulative distributions (fig. 4, B and C). In addition, several hundred events under each condition were averaged to evaluate their kinetics (fig. 4D). Just as we found for $GABA_{A,slow}$ IPSCs, the amplitude of $GABA_{A,fast}$ mIPSCs was not changed by etomidate (fig. 4B). The aggregate data confirmed that mIPSC amplitude was unchanged by 1 μM etomidate, the highest concentration tested (33.6 ± 14.1 pA and 32.2 ± 14.3 pA, for control and drug, respectively, n = 4, $P > 0.05$). Similarly, there was no significant effect of etomidate on mIPSC frequency (interevent interval 47.3 ± 10.3 ms and 58.9 ± 10.1 ms for control and 1 μM etomidate, respectively, $P > 0.05$).

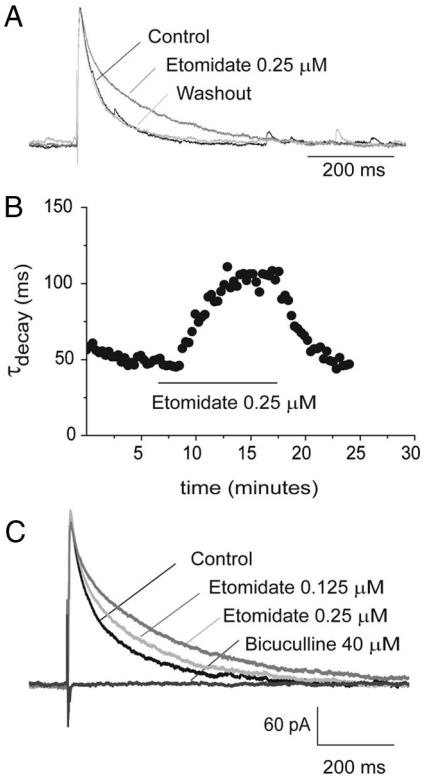

Fig. 2. Effect of etomidate on slow evoked γ-aminobutyric acid type A ($GABA_{A,slow}$) inhibitory postsynaptic currents (IPSCs). (*A*) $GABA_{A,slow}$ IPSCs evoked by electrical stimulation of stratum lacunosum-moleculare, under control conditions, in the presence of etomidate 0.25 μM, and after drug washout (currents are normalized to peak amplitude). (*B*) Time course of onset and washout of etomidate effects on decay time constant (τ_{decay}). (*C*) Pharmacological sensitivity of $GABA_{A,slow}$. Responses in the absence of bicuculline are averaged and normalized to peak current. Bicuculline completely blocked the response.

However, 0.5 and 1 μM etomidate did appear to slow $GABA_{A,fast}$ mIPSC decay modestly (fig. 4D).

To characterize the effect of etomidate on decay kinetics of $GABA_{A,fast}$ in detail, we fit the averaged currents to biexponential functions (table 1). This analysis showed that the effect of etomidate was restricted to a slowing of the slow component (τ_2) of the biexponential decay,

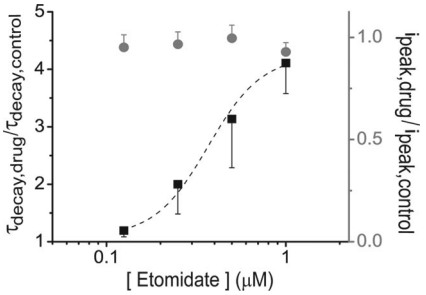

Fig. 3. Summary of effects of etomidate on slow evoked γ-aminobutyric acid type A ($GABA_{A,slow}$) inhibitory postsynaptic currents (IPSCs). Etomidate 0.125–1.0 μM prolonged IPSC decay in a dose-dependent manner, but had no effect on IPSC amplitude. *Error bars* represent SD; n = 4 at each concentration. The *dasbed line* shows the best fit to a logistic function, with EC50 = 0.37 ± 0.17 μM.

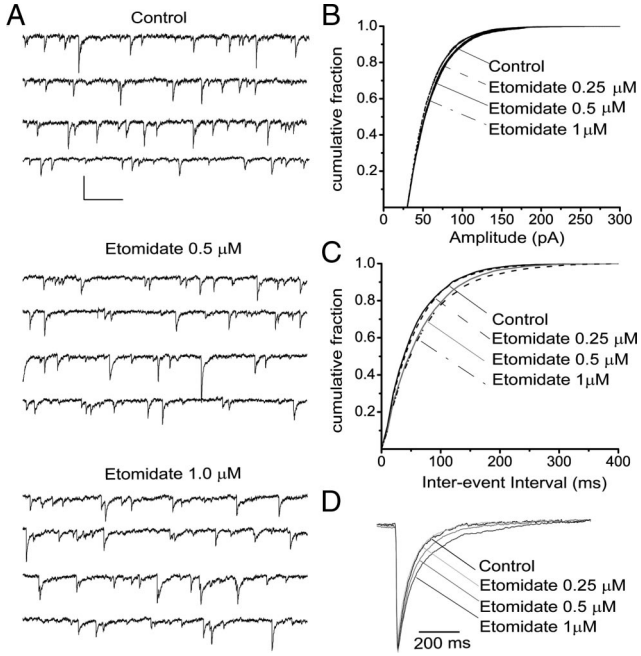

Fig. 4. Effect of etomidate on fast γ-aminobutyric acid type A ($GABA_{A,fast}$) inhibitory postsynaptic currents (IPSCs). (*A*) Representative recordings of miniature IPSCs under control conditions (*top traces*) and in the presence of etomidate 0.5 μM (*middle traces*) and 1.0 μM (*bottom traces*). (*B–D*) Analysis of etomidate effects on amplitude (*B*), interevent interval (*C*), and kinetics (*D*) of $GABA_{A,fast}$ miniature IPSCs (currents normalized to peak current) for the cell illustrated in *panel A*.

with no changes in either the relative amplitude (A_1) or the decay rate (τ_1) of the fast component. The value of τ_2 was nearly doubled (from 12.3 to 21.1 ms) at 1 μM, but there was no appreciable effect of etomidate on τ_{wt} at EC50 for amnesia (0.25 μM; table 1, $P > 0.05$).

Differential Effects of Etomidate on $GABA_{A,fast}$ versus $GABA_{A,slow}$

Our experiments showed that etomidate did exert pronounced effects on the kinetics of $GABA_A$ receptor-mediated synaptic currents but not on peak amplitude (or frequency, for $GABA_{A,fast}$ mIPSCs). However, the degree to which the decay time constant (and hence the charge transfer) was altered differed substantially between the two forms of phasic inhibition. The difference is illustrated in figure 5, which compares τ_{decay} of $GABA_{A,slow}$ with the weighted time constant of decay of $GABA_{A,fast}$ (τ_{wt}).

To compare the overall effects of etomidate on the two different types of phasic inhibition, we compared the parameters of the fits of the logistic equation to these two data sets. Both *i*max ($GABA_{A,slow}$ 4.3 ± 0.9 *vs.* $GABA_{A,fast}$ 1.9 ± 0.1) and EC50 values ($GABA_{A,slow}$ 0.37 ± 0.17 μM *vs.* $GABA_{A,fast}$ 0.61 ± 0.07 μM) favored greater modulation of $GABA_{A,slow}$ compared to $GABA_{A,fast}$, with nonoverlapping 95% confidence intervals supporting a statistical difference in these parameters. The steepness of the two curves did not differ ($GABA_{A,slow}$ 2.5 ± 1.0 *vs.*

Table 1. Effect of Etomidate on Kinetics of $GABA_{A,fast}$ Miniature Inhibitory Postsynaptic Currents

	Amplitude, pA	τ_1, ms	A_1, %	τ_2, ms	τ_{wt}, ms
Control	−33.6 ± 14.1	4.8 ± 1.7	56% ± 11%	12.3 ± 6.7	6.2 ± 1.7
Etomidate 0.25 μm	−32.9 ± 13.6	4.8 ± 1.8	53% ± 20%	12.1 ± 9.7	6.9 ± 2.5
Etomidate 0.5 μm	−32.9 ± 13.0	6.0 ± 2.8	53% ± 12%	19.2 ± 3.1	9.6 ± 4.6
Etomidate 1.0 μm	−32.2 ± 14.3	5.4 ± 2.5	56% ± 11%	21.1 ± 12.1*	11.2 ± 4.9*

Data are presented as mean ± SD, n = 3–5 cells at each concentration. The decay phase was fit by the sum of two exponentials; the weighted time constant (τ_{wt}) was calculated as ($A_1\tau_1 + [1 - A_1]\tau_2$), where A_1 is the amplitude of the fast component of the biexponential equation, and τ_1 and τ_2 are the time constants of the fast and slow components, respectively. * $P < 0.05$ *vs.* control.

$GABA_{A,fast}$ 3.8 ± 1.3) These results suggest that, to the extent that etomidate exerts its amnesic action *via* synaptic $GABA_A$-ergic inhibition in the hippocampus, it involves primarily $GABA_{A,slow}$.

Discussion

Our principal finding is that etomidate, at concentrations associated with amnesia *in vivo*, enhances $GABA_{A,slow}$ inhibition to a substantially greater extent than $GABA_{A,fast}$. Therefore, we conclude that $GABA_{A,slow}$ does have the requisite sensitivity to serve as an effector mechanism for etomidate-induced impairment of hippocampal-dependent learning and memory.

The Organotypic Culture (OTC)

Since its introduction two decades ago,[14] different types of OTCs have been studied from a variety of brain areas. They have become accepted as useful preparations, complementary to acute brain slices and profitably employed in the study of synaptic transmission, plasticity, and long-term drug exposure.[15] In the context of anesthetic mechanisms, hippocampal OTCs have been used primarily to study the effects of anesthetics on spontaneous network activity.[16–18]

The OTC*hip* offered some advantages as a preparation for this project. The hippocampus plays a central role for the formation of explicit memories and offers important targets for amnesia-inducing drugs acting *via* inhibitory and excitatory receptors. Inhibition in the OTC*hip* resembles that in age-matched acute hippocampal slices.[19] However, due to the thinness of the preparation (just several cell layers, or 100-μm-thick, by 1–2 weeks in culture), even lipophilic drugs such as etomidate equilibrate quickly enough to allow experiments to be performed at known tissue concentrations within the time limitations imposed by whole-cell recording techniques. Indeed, we found that etomidate's effect on the most sensitive parameter, the time constant of decay of $GABA_{A,slow}$ IPSCs, stabilized within 10 min of drug application (fig. 2). This is substantially faster than the 1–2 h required in experiments with thicker acute brain slices.[20]

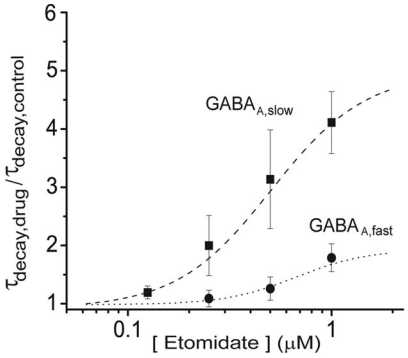

Fig. 5. Comparison of etomidate effects on decay kinetics of fast and slow γ-aminobutyric acid type A ($GABA_{A,fast}$ and $GABA_{A,slow}$, respectively) inhibitory postsynaptic currents. The time constant of decay in the presence of etomidate ($\tau_{decay,drug}$) divided by the time constant of decay under control conditions ($\tau_{decay,control}$) is plotted as a function of the concentration of etomidate. *Dashed lines* are best fits of the data to the logistic equation.

One important difference between the OTC and the more commonly used acute brain slice preparation is that the rate of ongoing spontaneous inhibitory currents is so high in the OTC that essentially all spontaneous $GABA_{A,fast}$ ISPCs overlap temporally (fig. 1). This high frequency of spontaneous synaptic currents complicated kinetic analysis of spontaneous $GABA_{A,fast}$ IPSCs and completely obscured spontaneous $GABA_{A,slow}$ IPSCs. Thus, it precluded a direct comparison of these types of IPSCs under identical conditions. However, we were able to characterize the effect of etomidate on $GABA_{A,fast}$ currents by measuring miniature IPSCs because the addition of tetrodotoxin resulted in a lower frequency that permitted kinetic analysis. Since miniature $GABA_{A,slow}$ IPSCs occur too infrequently to use for this purpose,[21] instead we measured evoked $GABA_{A,slow}$ IPSCs. Although these conditions of activation did differ for the two forms of phasic inhibition, raising the possibility that the observed difference in their modulation reflected the methods used to activate them rather than their intrinsic synaptic responsiveness, we feel that this explanation is unlikely. Our previous studies have shown that the kinetic characteristics of each of the two types of IPSCs are independent of whether they are expressed as miniature, spontaneous, or evoked responses. Rather, their differences reflect intrinsic properties of the two types of synapses, which arise from different classes of presynaptic neurons, utilize different postsynaptic receptors, and produce different neurotransmitter profiles. Which of these factors accounts for

the differences in modulation of $GABA_{A,fast}$ *versus* $GABA_{A,slow}$ by etomidate remains unknown.

The Physiology and Pharmacology of Slow Phasic Inhibition

Synaptic currents of the $GABA_{A,slow}$ type were discovered in hippocampal neurons, where they were found to underlie the "early IPSP" and to control the excitability of pyramidal cells.[11] Subsequently, similar synaptic currents were found in other brain areas as well.[22–24] To date, their properties are best characterized in the hippocampus.[25–27]

The slow time course of decay of $GABA_{A,slow}$ (30–70 ms as opposed to 3–8 ms for $GABA_{A,fast}$ at 36°C) is its most striking characteristic. A number of explanations for the slow decay, as well as for its functional implications, have been proposed. The time course could be due to the intrinsic kinetic properties imparted by the subunit combination of the underlying receptors. This includes α_5 subunits, probably in combination with β_3 and γ_2 subunits.[9,10] Their high affinity, which is associated with a slow unbinding rate, would be expected to impart slow deactivation, even after a brief stimulus. However, additional subunits, primarily α_1 and α_2, also have been shown to contribute to $GABA_{A,slow}$ IPSCs,[9] so other factors evidently also contribute to their slow decay. One such factor is likely to be the prolonged presence of transmitter at these synapses. This could be produced by either of two mechanisms. First, there may be spillover of transmitter onto high-affinity receptors located at perisynaptic or extrasynaptic sites. This would be analogous to the situation in granule cells of the dentate gyrus, where it has been shown that the 'slow' time course of IPSCs in the molecular layer arises from spillover onto perisynaptically located δ-subunit containing receptors.[28] In the hippocampal CA1 area, α_5-containing receptors have been found perisynaptically,[9] lending support to this possibility. Alternatively, the release of GABA into a large-volume synaptic cleft, possibly surrounded by a glial wrap that limits diffusion out of the cleft, may lead to the prolonged presence of a low concentration of transmitter. This mechanism has been proposed for the $GABA_{A,slow}$ IPSC produced by neurogliaform cells on neocortical pyramidal cells.[23] Multiple types of interneurons can produce slow IPSCs, including neurogliaform,[23,27] oriens lacunosum-moleculare,[29] and ivy cells[30]; therefore, it is possible that different types of slow synapses employ each of these two proposed mechanisms.

In addition to its slow kinetics and dendritic location, another important feature of $GABA_{A,slow}$ that supports its role in controlling synaptic plasticity is its susceptibility to modulation by presynaptic $GABA_B$ receptors. Unlike $GABA_{A,fast}$ IPSCs, $GABA_{A,slow}$ IPSCs are strongly suppressed by repeated stimuli at frequencies that are effective in producing long-term potentiation.[25] This presynaptic effect, which is mediated by $GABA_B$ autoreceptors, leads to unopposed excitation. The resulting dendritic depolarization relieves a voltage-dependent block of *N*-methyl-D-aspartate receptors by magnesium, permitting calcium entry and activating an ensuing cascade of calcium-dependent processes that ultimately leads to a persistent increase in synaptic strength.[31] In the presence of the $GABA_B$ antagonist CGP35348, $GABA_{A,slow}$ inhibition is maintained, and this prevents the depolarization required for induction of long-term potentiation.[32] The ability of $GABA_B$ antagonists to impair long-term potentiation demonstrates the effectiveness of $GABA_{A,slow}$ in controlling dendritic depolarization and synaptic plasticity. The enhancement of $GABA_{A,slow}$ by amnestic concentrations of etomidate and other drugs may similarly prevent memory formation by enhancing the hyperpolarizing influence of dendritic inhibitory inputs, thereby maintaining a hyperpolarized dendritic membrane potential and preventing sufficient depolarization to initiate synaptic plasticity. However, even if these drugs do modulate the same receptors or synapses and even if these actions do contribute to their amnestic properties, different classes of drugs might achieve amnesia by way of quantitatively different balances of effects on IPSCs *versus* other targets – or even *via* qualitatively different types of effects on IPSCs. For example, whereas we found that the major effect of etomidate was to slow $GABA_{A,slow}$ IPSC decay, benzodiazepines were shown recently to increase $GABA_{A,slow}$ amplitude rather than decay,[9] and halothane-modulated $GABA_{A,fast}$ and $GABA_{A,slow}$ currents similarly, altering decay but not amplitude.[33]

$GABA_A$ Receptor α_5 Subunits, Tonic and Phasic Inhibition, and Amnesia

The existence of a tonic $GABA_A$ receptor-mediated conductance was initially documented in cerebellar granule cells.[34,35] In those neurons, tonic current arises from receptors located within a glomerulus that are exposed to relatively high and sustained levels of GABA. Similar currents have been identified in a number of other neuron types, including thalamic,[36] cortical,[37] dentate gyrus,[38,39] and hippocampal[40] neurons. Though these cells lack similar specialized structures, they do contain receptors that are sufficiently sensitive to GABA that ambient levels of neurotransmitter in the extracellular space can activate them.

In hippocampal CA1 pyramidal neurons, tonic current is mediated by $GABA_A$ receptors that contain α_5 subunits.[6,9] This current is enhanced by a variety of anesthetics, including amnestic concentrations of isoflurane and etomidate.[7,41] Behavioral experiments, paired with genetic[4,42,43] and pharmacologic[44] manipulations, strongly support a memory-modulating role of α_5-containing $GABA_A$ receptors in hippocampal learning paradigms. Furthermore, mice lacking α_5 subunits are resistant to the effects of etomidate on memory in behavioral experiments.[7] Taken together, this evidence has pointed to tonic current carried by α_5 subunit-containing $GABA_A$

receptors as playing a causal role in anesthetic-induced amnesia. However, since the α_5 subunit-containing receptors that contribute to $GABA_{A,slow}$ IPSCs in CA1 pyramidal cells may very well be the same high-affinity, slowly decaying receptors that produce tonic current in these cells,[6] slow phasic inhibition provides an additional, or alternative, explanation for these findings.

Our results do not provide experimental support for a prominent role of fast phasic inhibition in etomidate-induced amnesia. In this respect, they are in agreement with previous work that used a genetic approach to conclude that $GABA_{A,fast}$ IPSCs do not play an important role for this anesthetic endpoint.[7] However, the finding that the decay of fast IPSCs is slowed by etomidate at higher concentrations (fig. 4D and fig. 5) is compatible with a role for modulation of $GABA_{A,fast}$ IPSCs in other anesthetic end points, such as impaired perception or unconsciousness. Indeed, the decay of fast IPSCs is an important determinant of oscillation frequency in the γ range (40–90 Hz),[45] a rhythm that has been tied to cognitive processes.[46] Thus, anesthetic modulation of distinct forms of $GABA_A$ receptor-mediated inhibition may contribute to specific components of the anesthetic state.

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References

1. Rudolph U, Antkowiak B: Molecular and neuronal substrates for general anaesthetics. Nat Rev Neurosci 2004; 5:709–20
2. Sieghart W, Sperk G: Subunit composition, distribution and function of GABA(A) receptor subtypes. Curr Top Med Chem 2002; 2:795–816
3. Mody I, Pearce RA: Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci 2004; 27:569–75
4. Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW: Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. J Neurosci 2002; 22:5572–80
5. Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Bluthmann H, Mohler H, Rudolph U: Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. Proc Natl Acad Sci U S A 2002; 99:8980–5
6. Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA: Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 2004; 101:3662–7
7. Cheng VY, Martin LJ, Elliott EM, Kim JH, Mount HT, Taverna FA, Roder JC, Macdonald JF, Bhambri A, Collinson N, Wafford KA, Orser BA: Alpha5GABAA receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. J Neurosci 2006; 26:3713–20
8. Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL: Synaptic and nonsynaptic localization of GABAA receptors containing the alpha5 subunit in the rat brain. J Comp Neurol 2006; 499:458–70
9. Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE: Specific subtypes of GABAA receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. J Neurophysiol 2006; 96: 846–57
10. Zarnowska ED, Keist R, Rudolph U, Pearce RA: GABAA receptor alpha5 subunits contribute to GABAA,slow synaptic inhibition in mouse hippocampus. J Neurophysiol 2009; 101:1179–91
11. Pearce RA: Physiological evidence for two distinct GABAA responses in rat hippocampus. Neuron 1993; 10:189–200
12. Banks MI, Pearce RA: Kinetic differences between synaptic and extrasynaptic GABA(A) receptors in CA1 pyramidal cells. J Neurosci 2000; 20: 937–48
13. Stoppini L, Buchs PA, Muller D: A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 1991; 37:173–82
14. Gahwiler BH: Organotypic cultures of neural tissue. Trends Neurosci 1988; 11:484–9
15. Gahwiler BH, Capogna M, Debanne D, McKinney RA, Thompson SM: Organotypic slice cultures: A technique has come of age. Trends Neurosci 1997; 20:471–7
16. Hentschke H, Antkowiak B: NMDA receptor-mediated changes of spontaneous activity patterns in thalamocortical slice cultures. Brain Res 1999; 830: 123–37
17. Hentschke H, Schwarz C, Antkowiak B: Neocortex is the major target of sedative concentrations of volatile anaesthetics: Strong depression of firing rates and increase of GABAA receptor-mediated inhibition. Eur J Neurosci 2005; 21:93–102
18. Drexler B, Roether CL, Jurd R, Rudolph U, Antkowiak B: Opposing actions of etomidate on cortical theta oscillations are mediated by different gamma-aminobutyric acid type A receptor subtypes. ANESTHESIOLOGY 2005; 102:346–52
19. De Simoni A, Griesinger CB, Edwards FA: Development of rat CA1 neurones in acute *versus* organotypic slices: Role of experience in synaptic morphology and activity. J Physiol 2003; 550:135–47
20. Benkwitz C, Liao M, Laster MJ, Sonner JM, Eger EI 2nd, Pearce RA: Determination of the EC50 amnesic concentration of etomidate and its diffusion profile in brain tissue: Implications for *in vitro* studies. ANESTHESIOLOGY 2007; 106:114–23
21. Banks MI, Hardie JB, Pearce RA: Development of GABA(A) receptor-mediated inhibitory postsynaptic currents in hippocampus. J Neurophysiol 2002; 88:3097–107
22. Kapur A, Pearce RA, Lytton WW, Haberly LB: GABAA-mediated IPSCs in piriform cortex have fast and slow components with different properties and locations on pyramidal cells. J Neurophysiol 1997; 78:2531–45
23. Szabadics J, Tamas G, Soltesz I: Different transmitter transients underlie presynaptic cell type specificity of GABAA,slow and GABAA,fast. Proc Natl Acad Sci U S A 2007; 104:14831–6
24. Sceniak MP, Maciver MB: Slow GABA(A) mediated synaptic transmission in rat visual cortex. BMC Neurosci 2008; 9:8
25. Pearce RA, Grunder SD, Faucher LD: Different mechanisms for use-dependent depression of two GABAA-mediated IPSCs in rat hippocampus. J Physiol 1995; 484(Pt 2):425–35
26. Banks MI, Li TB, Pearce RA: The synaptic basis of GABAA,slow. J Neurosci 1998; 18:1305–17
27. Price CJ, Cauli B, Kovacs ER, Kulik A, Lambolez B, Shigemoto R, Capogna M: Neurogliaform neurons form a novel inhibitory network in the hippocampal CA1 area. J Neurosci 2005; 25:6775–86
28. Wei W, Zhang N, Peng Z, Houser CR, Mody I: Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. J Neurosci 2003; 23:10650–61
29. Maccaferri G, Roberts JD, Szucs P, Cottingham CA, Somogyi P: Cell surface domain specific postsynaptic currents evoked by identified GABAergic neurones in rat hippocampus *in vitro*. J Physiol 2000; 524 Pt 1:91–116
30. Fuentealba P, Begum R, Capogna M, Jinno S, Marton LF, Csicsvari J, Thomson A, Somogyi P, Klausberger T: Ivy cells: A population of nitric-oxide-producing, slow-spiking GABAergic neurons and their involvement in hippocampal network activity. Neuron 2008; 57:917–29
31. Malenka RC, Nicoll RA: Long-term potentiation–a decade of progress? Science 1999; 285:1870–4
32. Mott DD, Lewis DV: Facilitation of the induction of long-term potentiation by GABAB receptors. Science 1991; 252:1718–20
33. Lukatch HS, MacIver MB: Voltage-clamp analysis of halothane effects on GABA(A fast) and GABA(A slow) inhibitory currents. Brain Res 1997; 765: 108–12
34. Kaneda M, Farrant M, Cull-Candy SG: Whole-cell and single-channel currents activated by GABA and glycine in granule cells of the rat cerebellum. J Physiol 1995; 485:419–35
35. Farrant M, Nusser Z: Variations on an inhibitory theme: Phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 2005; 6:215–29
36. Porcello DM, Huntsman MM, Mihalek RM, Homanics GE, Huguenard JR: Intact synaptic GABAergic inhibition and altered neurosteroid modulation of thalamic relay neurons in mice lacking delta subunit. J Neurophysiol 2003; 89:1378–86
37. Yamada J, Furukawa T, Ueno S, Yamamoto S, Fukuda A: Molecular basis for the GABAA receptor-mediated tonic inhibition in rat somatosensory cortex. Cereb Cortex 2007; 17:1782–7
38. Nusser Z, Mody I: Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J Neurophysiol 2002; 87:2624–8
39. Herd MB, Haythornthwaite AR, Rosahl TW, Wafford KA, Homanics GE, Lambert JJ, Belelli D: The expression of GABAA beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. J Physiol 2008; 586:989–1004
40. Bai D, Zhu G, Pennefather P, Jackson MF, MacDonald JF, Orser BA: Distinct functional and pharmacological properties of tonic and quantal inhibitory

postsynaptic currents mediated by gamma-aminobutyric acid(A) receptors in hippocampal neurons. Mol Pharmacol 2001; 59:814–24

41. Caraiscos VB, Newell JG, You-Ten KE, Elliott EM, Rosahl TW, Wafford KA, MacDonald JF, Orser BA: Selective enhancement of tonic GABAergic inhibition in murine hippocampal neurons by low concentrations of the volatile anesthetic isoflurane. J Neurosci 2004; 24:8454–8

42. Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW: Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. J Neurosci 2002; 22:5572–80

43. Yee BK, Hauser J, Dolgov VV, Keist R, Mohler H, Rudolph U, Feldon J: GABA receptors containing the alpha5 subunit mediate the trace effect in aversive and appetitive conditioning and extinction of conditioned fear. Eur J Neurosci 2004; 20:1928–36

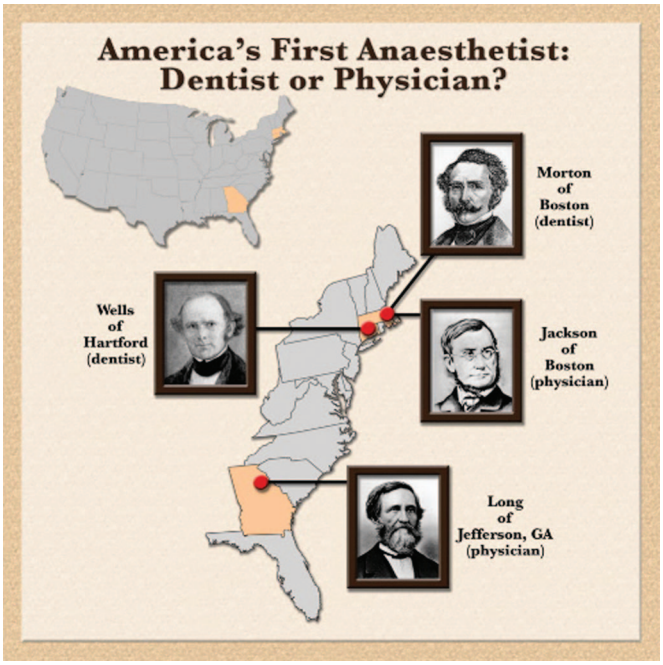
44. Chambers MS, Atack JR, Broughton HB, Collinson N, Cook S, Dawson GR, Hobbs SC, Marshall G, Maubach KA, Pillai GV, Reeve AJ, MacLeod AM,: Identification of a novel, selective GABA(A) alpha5 receptor inverse agonist which enhances cognition. J Med Chem 2003; 46:2227–40

45. Whittington MA, Jefferys JG, Traub RD: Effects of intravenous anaesthetic agents on fast inhibitory oscillations in the rat hippocampus *in vitro*. Br J Pharmacol 1996; 118:1977–86

46. Singer W: Synchronization of cortical activity and its putative role in information processing and learning. Annu Rev Physiol 1993; 55:349–74

ANESTHESIOLOGY REFLECTIONS

Long, Wells, Morton, or Jackson?



Who was the first "anaesthetist"? Was it pharmacist-physician Crawford W. Long (1815–1878) of Georgia? Or was it dentist Horace Wells (1815–1848) of Connecticut? Or was it perhaps dentist William T. G. Morton (1819–1868) or even geologist-chemist-physician Charles T. Jackson (1805–1880), both of Massachusetts? These are the Americans most frequently cited by the physicians and historians who pursue Wood Library-Museum Fellowships. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA's Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.