Electroencephalographic Recovery, Hypnotic Emergence, and the Effects of Metabolite after Continuous Infusions of a Rapidly Metabolized Etomidate Analog in Rats

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ABSTRACT

Background: Methoxycarbonyl etomidate is an ultrarapidly metabolized etomidate analog. It is metabolized to methoxycarbonyl etomidate carboxylic acid (MOC-ECA), which has a hypnotic potency that is 350-fold less than that of methoxycarbonyl etomidate. The authors explored the relationships between methoxycarbonyl etomidate infusion duration, recovery time, metabolite concentrations in blood and cerebrospinal fluid (CSF), and methoxycarbonyl etomidate metabolism in brain tissue and CSF to test the hypothesis that rapid metabolism of methoxycarbonyl etomidate may lead to sufficient accumulation of MOC-ECA in the brain to produce a pharmacologic effect.

Methods: A closed-loop system with burst suppression ratio feedback was used to administer methoxycarbonyl etomidate infusions of varying durations to rats. After infusion, recovery of the electroencephalogram and righting reflexes were assessed. MOC-ECA concentrations were measured in blood and CSF during and after methoxycarbonyl etomidate infusion, and the in vitro half-life of methoxycarbonyl etomidate was determined in rat brain tissue and CSF.

Results: Upon termination of continuous methoxycarbonyl etomidate infusions, the burst suppression ratio recovered in a biexponential manner with fast and slow components having time constants that differed by more than 100-fold and amplitudes that varied inversely with infusion duration. MOC-ECA concentrations reached hypnotic concentrations in the CSF with prolonged methoxycarbonyl etomidate infusion and then decreased during a period of several hours after infusion termination. The metabolic half-life of methoxycarbonyl etomidate in brain tissue and CSF was 11 and 20 min, respectively.

Conclusion: In rats, methoxycarbonyl etomidate metabolism is sufficiently fast to produce pharmacologically active MOC-ECA concentrations in the brain with prolonged methoxycarbonyl etomidate infusion.

What We Already Know about This Topic

• Methoxycarbonyl etomidate is an etomidate analog that produces hypnosis of short duration after bolus administration to rats
• It is rapidly metabolized by esterases to methoxycarbonyl etomidate carboxylic acid, which has less than 1% of its hypnotic potency
• With prolonged methoxycarbonyl etomidate administration, the electroencephalographic burst suppression ratio remains increased long after termination of the infusion

What This Article Tells Us That Is New

• Prolonged methoxycarbonyl etomidate administration to rats produces brain methoxycarbonyl etomidate carboxylic acid concentrations that are sufficiently high to cause electroencephalographic burst suppression

Methods: A closed-loop system with burst suppression ratio feedback was used to administer methoxycarbonyl etomidate infusions of varying durations to rats. After infusion, recovery of the electroencephalogram and righting reflexes were assessed. MOC-ECA concentrations were measured in blood and CSF during and after methoxycarbonyl etomidate infusion, and the in vitro half-life of methoxycarbonyl etomidate was determined in rat brain tissue and CSF.

Results: Upon termination of continuous methoxycarbonyl etomidate infusions, the burst suppression ratio recovered in a biexponential manner with fast and slow components having time constants that differed by more than 100-fold and amplitudes that varied inversely with infusion duration. MOC-ECA concentrations reached hypnotic concentrations in the CSF with prolonged methoxycarbonyl etomidate infusion and then decreased during a period of several hours after infusion termination. The metabolic half-life of methoxycarbonyl etomidate in brain tissue and CSF was 11 and 20 min, respectively.

Conclusion: In rats, methoxycarbonyl etomidate metabolism is sufficiently fast to produce pharmacologically active MOC-ECA concentrations in the brain with prolonged methoxycarbonyl etomidate infusion.
methoxycarbonyl etomidate carboxylic acid (MOC-ECA), is a carboxylic acid whose hypnotic and γ-aminobutyric acid receptor modulatory potencies are 350-fold less than that of methoxycarbonyl etomidate. After single-bolus administration to rats, methoxycarbonyl etomidate produces hypnosis of extremely short duration, even when given at doses that far exceed its hypnotic ED₅₀, because it is very rapidly metabolized.

With many drugs (including sedative-hypnotics), the duration of action after terminating a continuous infusion increases with infusion duration. After a single bolus or brief infusion, drug is cleared from the effect site by redistribution, and the recovery time is relatively insensitive to the terminal elimination rate. However, with longer infusions that significantly fill peripheral compartments with drug, clearance from the effect site slows as drug diffuses from these compartments back to the effect site, and recovery depends more heavily on terminal elimination. A drug’s duration of action may also increase with infusion duration if its metabolite possesses pharmacologic activity and accumulates at the effect site during infusion. This mechanism is of particular relevance for soft analogs that may be given as prolonged continuous infusions, resulting in the production of relatively high quantities of metabolites that often are not completely devoid of pharmacologic activity.

In a previous study, we found that the electroencephalographic burst suppression ratio (BSR) of rats increased with methoxycarbonyl etomidate administration and then decreased rapidly once administration was stopped, consistent with the drug’s known hypnotic action and rapid rate of metabolism. With intravenous boluses, the BSR promptly returned to its baseline value after methoxycarbonyl etomidate administration. However, with prolonged infusions, the BSR remained above the baseline preinfusion value for many minutes after infusion termination, suggesting a residual pharmacologic effect. In the current study, we explored the relationship between methoxycarbonyl etomidate infusion time and postinfusion recovery time, metabolite concentrations in blood and cerebrospinal fluid (CSF), and the rate of methoxycarbonyl etomidate metabolism in brain tissue and CSF in a rat model to test the hypothesis that prolonged methoxycarbonyl etomidate infusion can lead to sufficient MOC-ECA accumulation within the central nervous system to produce persistent electroencephalographic and hypnotic effects.

Materials and Methods

Animals

All studies were conducted in accordance with rules and regulations of the Subcommittee on Research Animal Care at the Massachusetts General Hospital, Boston, Massachusetts. Adult male Sprague-Dawley rats (230–350 gm) were purchased from Charles River Laboratories (Wilmington, MA) and housed in the Massachusetts General Hospital Center for Comparative Medicine animal care facility. Drugs were administered through a femoral venous catheter, and blood draws were from a femoral arterial catheter. CSF was sampled from an intracisternal cannula. All catheters and cannulae were reimplemented by the vendor before animal delivery to our animal care facility.

Drugs and Chemicals

Methoxycarbonyl etomidate was synthesized (more than 99% purity) by Aberjona Laboratories (Beverly, MA), as described previously. MOC-ECA was synthesized as reported previously. Methoxycarbonyl etomidate and MOC-ECA were diluted in normal saline for infusion. Isoflurane was purchased from Baxter (Deerfield, IL). Bupivacaine and heparin were from APP Pharmaceuticals (Schaumburg, IL).

Methodological Overview

In the first series of experiments, rats implanted with electroencephalographic electrodes were sedated with 1% isoflurane and administered methoxycarbonyl etomidate by closed-loop continuous infusion for varying durations of time (5, 15, or 30 min). The purpose of these experiments was to (1) determine the methoxycarbonyl etomidate dosing protocol required to maintain a constant hypnotic depth (80% BSR); (2) define the rate of electroencephalographic recovery after methoxycarbonyl etomidate infusion termination; and (3) measure the blood concentrations of MOC-ECA achieved during and after methoxycarbonyl etomidate infusion. Our rationale for using BSR as an objective measure of hypnotic depth has been discussed previously. In the second series of experiments, rats were sedated with isoflurane (1%) and administered either methoxycarbonyl etomidate or MOC-ECA for 30 min (using the protocol defined in the first series of experiments), and the concentrations of MOC-ECA and methoxycarbonyl etomidate achieved in CSF were determined. In the third series of experiments, rats were administered methoxycarbonyl etomidate by continuous infusion (again using the protocol defined in the first series of experiments) without isoflurane for either 5 or 30 min, and the time required for righting reflexes to return after infusion termination was determined. In the fourth series of experiments, the in vitro metabolic half-life of methoxycarbonyl etomidate was defined in rat blood and CSF.

Electroencephalographic Electrode Placement and Recording

Electroencephalographic electrodes were placed as described previously. Briefly, rats were anesthetized with inhaled isoflurane (2–3%) in 100% oxygen and placed in a stereotactic frame fitted with a nose cone. The skin was infiltrated with bupivacaine (0.5%) containing epinephrine 1:200,000, the skull was exposed, the periosteum removed, and four 1.59-mm OD, 3.2-mm long bone anchor screws (Stoelting, Wood Dale, IL) with attached 4-mm Teflon-coated stainless steel wire (A-M Systems, Sequim, WA) were inserted through the bone and reinforced with dental acrylic at the
stereotactic coordinates described by Vijn and Sneyd.10 The wires were connected to a P511 AC preamplifier (Grass Technologies, West Warwick, RI). The electroencephalographic signal was amplified 5,000-fold, filtered (low frequency pass: 0.3 Hz, high frequency pass: 0.03 kHz), digitized at 128 Hz using a USB-6009 data acquisition board (National Instruments, Austin, TX), and the BSR measured in real time with LabView Software (version 8.5 for Macintosh OS X; National Instruments) to provide feedback for a closed-loop infusion system and to monitor BSR recovery after infusion termination.

BSR Extraction and Closed-loop Infusion of Methoxycarbonyl Etomidate

Methods described in Cotten et al., Vijn and Sneyd, and Rampil and Laster were used to continuously estimate BSR, where BSR is the percentage time the electroencephalographic signal spent in suppression during each 6-s time epoch.2,10,11 Temporal differentiation (the difference between two successive data samples in the digitized electroencephalographic signal) was used to enhance BSR sensitivity.10 Suppression was defined as an interval during which the time-differentiated electroencephalographic signal amplitude stayed within a suppression voltage window for at least 100 ms. To account for variable signal noise among rats, this suppression voltage window was defined individually in each rat as described previously.2 Rats were then equilibrated with background of inhaled isoflurane (1%) to facilitate BSR measurements and blood and CSF sampling.

A KDS Model 200 Series infusion pump (KD Scientific, Holliston, MA) was used for continuous methoxycarbonyl etomidate or MOC-ECA infusion. The pump was controlled remotely via its RS 232 serial port by a Macintosh computer using a Keyspan USB-Serial port adapter (Tripp Lite, Chicago, IL). A LabView 8.5 instrument driver using Virtual Instrument Software Architecture protocols provided computer-to-pump communication. For closed-loop methoxycarbonyl etomidate infusions, we used the algorithm described by Vijn and Sneyd.10 In this approach, the methoxycarbonyl etomidate infusion rate is increased (if the current BSR is less than 80%) or decreased (if the current BSR is more than 80%) every 6 s. The magnitude of the change in the infusion rate is dependent upon the difference between the current BSR measured in the rat and our target BSR of 80%. The algorithm was modified with a maximum infusion rate of 60 mg · kg⁻¹ · min⁻¹ to prevent inadvertent overdosage and a minimum rate of 5 mg · kg⁻¹ · min⁻¹ to assure continuous methoxycarbonyl etomidate infusion. Because it was not possible to place electroencephalographic electrodes in rats with intracisternal cannulae, we used an infusion protocol determined from previous closed-loop infusion experiments for in vivo studies to determine CSF MOC-ECA and methoxycarbonyl etomidate concentrations. This approach was also used for studies to define the duration of loss of righting reflexes after methoxycarbonyl etomidate infusion.

Analysis of BSR Recovery after Terminating Closed-loop Infusion of Methoxycarbonyl Etomidate

Because the fast and slow components of the BSR recovery occurred over vastly different time scales, each component was analyzed separately by fitting the time-dependent change in BSR to an exponential equation over the appropriate time scale. Thus, the fast component was analyzed by fitting the first 5 min of BSR data recorded immediately after methoxycarbonyl etomidate infusion termination. The slow component was analyzed by fitting the BSR data beginning 2 min after infusion termination (to exclude the fast component) and ending at least 3.5 h later.

Measurement of Blood and CSF Concentrations of MOC-ECA and Methoxycarbonyl Etomidate

Before, during, and after closed-loop methoxycarbonyl etomidate infusion, blood samples (200 μl/sample) were intermittently collected through the femoral arterial catheter and immediately mixed with acetonitrile (200 μl). To minimize dehydration, this blood was replaced with an equal volume of normal saline. The samples were centrifuged and the resultant plasma was collected and stored at −20°C until analyzed. After the samples were thawed, the MOC-ECA concentration in each was determined by high-performance liquid chromatography using a Varian Prostar system with a 4.6 × 250 mm Proto 300 C18 column (Nest Group, Southborough, MA) with the ultraviolet detector set at 240 nm. A linear gradient 20–90% acetonitrile in water with trifluoroacetic acid (0.05%; Thermo Scientific, Rockford, IL) over 30 min was used with a flow rate of 1 ml/min. MOC-ECA standards were prepared in rat blood and processed identically to experimental samples.

Before, during, and after methoxycarbonyl etomidate and MOC-ECA infusion, CSF samples (25 μl/sample) were collected through the intracisternal catheter, immediately mixed with acetonitrile (50 μl), centrifuged, and the supernatant stored at −20°C until analyzed. The concentrations of MOC-ECA and methoxycarbonyl etomidate in CSF were determined by high-performance liquid chromatography as described for blood using standards prepared in methanol.

The lower limits of quantitation, precision, and accuracy of our chromatographic analyses were determined generally as described by Hubbard et al.12 For the lower limit of quantitation, we used samples within the lower range of our calibration curve (1, 3, 5, and 10 μM MOC-ECA or methoxycarbonyl etomidate) and defined a lower limit of quantitation of 3 μM for both compounds. We also obtained lower limit of quantitation estimates of 2.1 μM for MOC-ECA and 3.7 μM for methoxycarbonyl etomidate,
respectively, based on the SD of the responses and the slopes of the calibration curves. Intraday precision and accuracy were determined at 100 μM H9262 M and 1 mM MOC-ECA and 10 μM methoxycarbonyl etomidate. At these concentrations, the relative SD were less than 5% and the accuracy of all samples was within 15% of their nominal values. Standard curves were linear across the entire concentration ranges with r² > 0.99.

Measurement of Methoxycarbonyl Etomidate In Vitro

Metabolic Half-life in Brain Tissue and CSF

Rat brain homogenate (20 mg/ml in phosphate buffered saline media pH 7.4) pooled from four rats was purchased from Bioreclamation LLC (Hicksville, NY). Methoxycarbonyl etomidate (100 μl from a 1 mM stock solution in saline) was added to 1 ml homogenate. After the desired incubation period at 37°C, a 100-μl aliquot was removed, and the metabolism was stopped by mixing with 100 μl acetonitrile. The samples were centrifuged and the supernatant collected and stored at −20°C until analyzed by high-performance liquid chromatography as described for blood.

Cerebrospinal fluid (150 μl) was drawn from the preimplanted intracisternal cannula of a rat sedated with isoflurane (1%). Methoxycarbonyl etomidate (16.5 μl from a 1 mM stock solution in saline) was added to the CSF and, after the desired incubation period at 37°C, a 25-μl aliquot was removed and mixed with 50 μl acetonitrile. The samples were centrifuged, and the supernatant collected and stored at −20°C until analyzed by high-performance liquid chromatography as described for blood.

Recovery from Methoxycarbonyl Etomidate Infusions


Recovery of Righting Reflexes after Infusion of Methoxycarbonyl Etomidate

Methoxycarbonyl etomidate was infused through a femoral venous catheter for either 5 or 30 min. Three minutes after the infusion was started, rats were turned supine. The time to recovery of righting reflexes after methoxycarbonyl etomidate infusion was defined as the time from infusion termination until spontaneous righting onto all four legs.

Statistical Analysis

All data are reported as mean ± SD. Statistical analyses were done using Prism v5.0 for the Macintosh (GraphPad Software, Inc., LaJolla, CA) or Igor Pro 6.1 (Wavemetrics, Lake Oswego, OR). For multiple comparisons, we performed either a one-way or a two-way analysis of variance followed, respectively, by a Dunnett or Bonferroni posttest. P < 0.05 was considered statistically significant. The built-in fitting functionality of the Igor Pro 6.1 was used to fit data to an exponential equation.

Results

The Relationship between the Duration of Methoxycarbonyl Etomidate Infusion and the Kinetics of BSR Recovery

After equilibration with isoflurane (1%), rats were assigned to receive a 5- (n = 4 rats), 15- (n = 4 rats), or 30-min (n = 4 rats) closed-loop methoxycarbonyl etomidate infusion with a target BSR of 80%. In all experiments, the BSR was recorded for 5 min before the methoxycarbonyl etomidate infusion was begun, during infusion, and for at least 3.5 h

Fig. 1. Mean burst suppression ratio (BSR) during each 6-s epoch recorded from rats before, during, and after 5- (A; n = 4 rats), 15- (B; n = 4 rats) or 30-min (C; n = 4 rats) closed-loop methoxycarbonyl etomidate infusions. In each large panel, the curve is an exponential fit of the data to obtain the amplitude and time constant of the slow component of the BSR recovery. The inset in each panel expands the time period beginning 2 min before infusion termination until 5 min after infusion termination to better reveal the fast component of the recovery, and the curve is an exponential fit of the data to obtain the amplitude and time constant of the fast component of the BSR recovery. Closed-loop infusions were begun after the baseline BSR was measured for 5 min. The target burst suppression ratio during methoxycarbonyl etomidate infusion was 80% for all experiments. Studies were done in a background of isoflurane (1%).
after the infusion ended. Figure 1 shows the mean BSR recorded from rats before, during, and after 5- (panel A), 15- (panel B), and 30-min (panel C) closed-loop methoxycarbonyl etomidate infusions. In these three groups of rats, baseline BSR values measured during the 5 min before methoxycarbonyl etomidate etomidate infusion averaged 80% ± 2%, 11% ± 2%, and 13% ± 3%, respectively. The BSR reached the target value of 80% approximately 3 min after beginning methoxycarbonyl etomidate etomidate infusion and remained near that value for the remainder of the closed-loop infusion period. After the infusion was complete, the BSR of rats in all three groups decreased from the 80% target toward the preinfusion baseline value; however, the kinetics of this recovery varied with infusion duration.

Upon terminating 5-min infusions (fig. 1A), the BSR recovered in a biexponential manner. There was a relatively large fast component that was complete within 1 min of infusion termination and a smaller slow component that was barely perceptible above the signal noise and persisted for more than an hour. A fit of the fast component to an exponential equation yielded an amplitude of 45% ± 4% and a time constant of 18 ± 3 s (fig. 1A inset), whereas a fit of the slow component yielded an amplitude of 15.5% ± 0.3% and a time constant of 40 ± 2 min.

Upon termination of the 15-min infusions (fig. 1B), the BSR decayed in a manner similar to that observed upon termination of the 5-min infusions. However, the amplitude of the fast component was smaller and that of the slow component was larger. A fit of the fast component to an exponential equation yielded an amplitude of 31% ± 4% and a time constant of 12 ± 3 s (fig. 1B inset), whereas a fit of the slow component yielded an amplitude of 57.5% ± 0.3% and a time constant 71 ± 1 min.

Upon termination of 30-min infusions (fig. 1C), there was no detectable fast component (fig. 1C inset), and the BSR decayed over several hours. A fit of this slow decay to an exponential equation yielded an amplitude of 68.0 ± 0.4% and a time constant of 90 ± 1.7 min.

Figure 2A plots the average methoxycarbonyl etomidate etomidate dose delivered each minute by the closed-loop infusion system and shows that the methoxycarbonyl etomidate etomidate infusion rate required to maintain an 80% BSR decreased with infusion time. A fit of this infusion data to an exponential equation yielded a maximum of 58 ± 2 mg/kg, a plateau of 6 ± 2 mg/kg, and a time constant of 7.3 ± 0.8 min (A). Cumulative methoxycarbonyl etomidate etomidate doses as a function of infusion time delivered during closed-loop infusions of 5-, 15-, or 30-min duration. All data represent the mean ± SD from four rats (B).

**MOC-ECA Concentrations in Blood with 30-min Intravenous Infusions of Methoxycarbonyl Etomidate**

To assess the extent to which MOC-ECA accumulated in the blood during the closed-loop methoxycarbonyl etomidate etomidate infusions, we also drew arterial blood samples from each rat before starting the infusion, when the BSR first reached 80%, at the end of the infusion, and intermittently after the infusion was complete. Figure 3 reveals that the blood concentration of MOC-ECA in these samples increased progressively during methoxycarbonyl etomidate etomidate infusion. At the time that the BSR first reached 80%, the MOC-ECA concentration in the blood was not significantly different among the three groups of rats and averaged 0.39 ± 0.06 mM. In all three groups, the MOC-ECA concentration peaked at the end of the closed-loop methoxycarbonyl etomidate etomidate infusion with values of 0.7 ± 0.3, 1.4 ± 0.4, and 1.2 ± 0.1 mM, for infusion durations of 5, 15, and 30 min, respectively, and then decreased over the next several hours.

**MOC-ECA and Methoxycarbonyl Etomidate Concentrations in the CSF with 30-min Intravenous Infusions of Methoxycarbonyl Etomidate or MOC-ECA**

Using separate groups of rats, we also tested whether MOC-ECA accumulates within the central nervous system (i.e.,
With continuous infusion of methoxycarbonyl etomidate. Because the preimplanted intracisternal catheters prevented our placing electroencephalographic electrodes, we could not use our closed-loop method for administering methoxycarbonyl etomidate. Instead, we used the average infusion protocol previously determined in rats without intracisternal catheters (shown in fig. 2A) to administer 540 mg/kg methoxycarbonyl etomidate over 30 min. Figure 4 shows that during continuous infusion of methoxycarbonyl etomidate, the concentration of MOC-ECA in the CSF progressively increased and reached a peak value of 2.5 ± 0.11 mM at the end of the 30-min infusion before decreasing to 0.7 ± 0.20 mM 4 h after the infusion was complete. Figure 4 also shows the results of analogous experiments with continuous infusions of MOC-ECA (540 mg/kg over 30 min using the protocol in fig. 2A). In these experiments, the concentration of MOC-ECA in the CSF also progressively increased; however, the peak MOC-ECA concentration was significantly lower and occurred 60 min later than with infusions of methoxycarbonyl etomidate.

We also measured methoxycarbonyl etomidate concentrations in the CSF during the continuous infusion experiments described in the previous paragraph. Figure 5 shows that with methoxycarbonyl etomidate infusion, the methoxycarbonyl etomidate concentration in the CSF reached a peak value of 24 ± 13 μM 5 min into the infusion (i.e., when the methoxycarbonyl etomidate infusion rate was greatest) before decreasing to values that approached our lower limit of quantitation and were not significantly different from zero. As expected, no methoxycarbonyl etomidate was detected in the CSF at any time during or after MOC-ECA infusion.

**In Vitro Determination of the Metabolic Half-life of Methoxycarbonyl Etomidate in Rat Brain Tissue and CSF**

To assess the ability of methoxycarbonyl etomidate to be metabolized within the central nervous system of the rat, we added the sedative-hypnotic to pooled brain tissue or CSF...
Discussion

The current studies in rats demonstrate that upon termination of continuous closed-loop infusions of methoxycarbonyl etomidate, the electroencephalographic BSR recovers in a biexponential manner with fast and slow components that have time constants that differ by more than 100-fold and amplitudes that vary inversely with infusion duration.

The fast component dominated when the methoxycarbonyl etomidate infusion was brief (i.e., 5 min) and was similar to the BSR recovery that we observed previously after administering single methoxycarbonyl etomidate boluses. Its time constant (~15 s) is one to two orders of magnitude faster than methoxycarbonyl etomidate’s in vitro metabolic half-life in rat brain tissue and CSF but similar to methoxycarbonyl etomidate’s in vitro metabolic half-life in rat blood (20 s). Based on these results, we conclude that the fast component of the BSR recovery reflects the rapid elimination of methoxycarbonyl etomidate from the brain as the hydrophobic drug diffuses across the blood-brain barrier and is metabolized by esterases in the blood and/or other peripheral tissues.

The slow component dominated when the methoxycarbonyl etomidate infusion was long (i.e., 30 min). By the end of such infusions, the concentration of MOC-ECA in the CSF reached 2.5 ± 0.11 mM, which approximates MOC-ECA’s EC50 for loss of righting reflexes in tadpoles of 2.8 ± 0.64 mM. In addition, the methoxycarbonyl etomidate infusion rate required to maintain an 80% BSR decreased by an order of magnitude over the course of such infusions.

These results suggest that with long methoxycarbonyl etomidate infusions, MOC-ECA reached sufficient concentrations in the brain to achieve (or at least contribute to) electroencephalographic burst suppression in our experiments. Upon discontinuing methoxycarbonyl etomidate infusion, the MOC-ECA concentration in the CSF decreased on approximately the same time scale (several hours) as the slow component of the BSR recovery (time constant ~1 h). Based on these results, we conclude that the slow component reflects the slow elimination of MOC-ECA from the brain.

We considered two possible mechanisms by which MOC-ECA might have reached such high concentrations in the CSF with prolonged methoxycarbonyl etomidate infusion. The first possibility is that methoxycarbonyl etomidate was metabolized in the periphery (e.g., blood) and the resulting MOC-ECA diffused across the blood-brain barrier into the CSF. The second possibility is that methoxycarbonyl etomidate was metabolized within the central nervous system, thus forming MOC-ECA in situ. Two lines of evidence favor the latter in situ mechanism as the major source of MOC-ECA in the CSF. First, the peak concentration of MOC-ECA in the CSF occurred 60 min after the MOC-ECA infusion ended, suggesting that MOC-ECA does not readily cross the blood-brain barrier. Slow penetration across the blood-brain barrier is expected for a charged compound and would explain our previous observation that MOC-ECA
failed to significantly increase the BSR during a 15-min intravenous infusion.2 Second, tissues from the rat central nervous system (i.e., brain tissue and CSF) not only metabolize methoxycarbonyl etomidate, but they do so on the approximate time scale that we observed accumulation of hypnotic concentrations of MOC-ECA in the CSF during continuous methoxycarbonyl etomidate infusion.

Figure 7 illustrates our current theory regarding the relationships between methoxycarbonyl etomidate infusion time, postinfusion recovery time, metabolite concentrations in the blood and CSF, and the rate of methoxycarbonyl etomidate metabolism in brain tissue and CSF. Panel A shows that with a single bolus or brief infusion, methoxycarbonyl etomidate rapidly equilibrates between the blood and brain to produce an increase in the BSR and loss-of-righting reflexes. Once administration has stopped, methoxycarbonyl etomidate concentrations in the blood decrease rapidly as the drug is hydrolyzed to MOC-ECA. Because equilibration between the blood and brain is very fast, methoxycarbonyl etomidate concentrations in the brain decrease in parallel with those in the blood. This results in rapid recovery of the BSR (i.e., the fast component) and the quick return of righting reflexes. Progressively longer methoxycarbonyl etomidate infusions lead to higher MOC-ECA concentrations in the brain because the charged metabolite is virtually trapped there after being formed in situ from methoxycarbonyl etomidate (fig. 7B). As MOC-ECA accumulates in the brain, it makes a progressively greater contribution to the burst suppression and reduces the infusion rate of methoxycarbonyl etomidate required to maintain the BSR at 80%. By the end of a 30-min methoxycarbonyl etomidate infusion, essentially all of the burst suppression (and hypnosis) in our rats is produced by accumulated metabolite alone. At this point, BSR recovery and the return of righting reflexes are slow because they are rate-limited by the slow transport of MOC-ECA out of the brain.

Within this theoretical construct, the relative amplitudes of the fast and slow components reflect the relative contributions made by methoxycarbonyl etomidate and MOC-ECA, respectively, to achieving our target BSR of 80%. Thus, with a single bolus or brief infusion of methoxycarbonyl etomidate that results in a BSR recovery that is largely composed of the fast component, methoxycarbonyl etomidate itself is responsible for most of the burst suppression. By the end of a 15-min methoxycarbonyl etomidate infusion when the fast and slow components have more similar amplitudes, methoxycarbonyl etomidate and MOC-ECA contribute more equally to achieving burst suppression. By the end of a 30-min methoxycarbonyl etomidate infusion, when only a slow component is observed, MOC-ECA is responsible for essentially all of the burst suppression. Our in vivo CSF results are consistent with this interpretation because the relative CSF concentrations of MOC-ECA measured after 5, 15, and 30 min of methoxycarbonyl etomidate infusion (0.14, 0.58, and 1, respectively) correlate reasonably well with the fraction of the BSR recovery that is represented by the slow component (0.26, 0.65, and 1, respectively) after 5-, 15-, and 30-min closed-loop continuous methoxycarbonyl etomidate infusions.

The current studies were performed in rats, which are typically (but not always) less sensitive than humans to sed-
ative-hypnotics and metabolize ester-containing drugs more rapidly.\(^1,15–18\) This suggests that MOC-ECA will not reach concentrations in humans that are as high as we observed in rats. However, at this point, we cannot rule out the possibility that pharmacologically significant MOC-ECA concentrations could be achieved. In particular, patients with renal failure may achieve steady-state metabolite concentrations with prolonged infusion of rapidly metabolized drugs that are orders of magnitude higher than those with normal renal function.\(^7,19\) Such patients are predicted to be the most vulnerable to the physiologic actions of MOC-ECA, which based on the current studies, includes prolonged hypnotic action and slow recovery of the electroencephalographic after infusion termination.

Other recently developed soft sedative-hypnotics include CNS7056 (remimazolam) and AZD3043.\(^20\) CNS7056 is a benzodiazepine that was developed as a shorter-acting alternative to midazolam, whereas AZD3043 is a propanidid analog intended for use as an anesthetic induction and/or maintenance agent. Both drugs have reached clinical trials, but it has been reported that development of AZD3043 has been delayed by formulation difficulties.\(^21\) Methoxycarbonyl etomidate is at an earlier stage of development, and our laboratory is working on the design of other soft estolate analogs that have somewhat longer hypnotic duration and/or higher hypnotic potency with the goal of reducing dosing requirements and any potential side effects related to metabolite accumulation.

In summary, MOC-ECA accumulates in rats with continuous methoxycarbonyl etomidate infusion and can reach sufficiently high concentrations in the brain to cause (or contribute to) electroencephalographic burst suppression with prolonged infusion. Because methoxycarbonyl etomidate and MOC-ECA are eliminated from the brain at very different rates, electroencephalographic recovery after termination of such infusions may occur as a biexponential process composed of rapid and slow components whose amplitude reflect the relative contributions that methoxycarbonyl etomidate and MOC-ECA make to achieving burst suppression. The time required for righting reflexes to recover similarly depends upon the duration of methoxycarbonyl etomidate infusion, suggesting that MOC-ECA concentrations in the brain may reach concentrations sufficient to produce hypnosis. Future studies in humans and human tissues will be necessary to determine the extent to which methoxycarbonyl etomidate is metabolized and accumulates in the human central nervous system and the role, if any, that accumulated metabolite plays in the recovery profile of methoxycarbonyl etomidate after prolonged continuous methoxycarbonyl etomidate infusion in man.

References