Electroencephalographic and Hypnotic Recoveries after Brief and Prolonged Infusions of Etomidate and Optimized Soft Etomidate Analogs

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ABSTRACT

Background: Methoxycarbonyl etomidate is the prototypical soft etomidate analog. Because it has relatively low potency and is extremely rapidly metabolized, large quantities must be infused to maintain hypnosis. Consequently with prolonged infusion, metabolite reaches sufficient concentrations to delay recovery. Dimethyl-methoxycarbonyl metomidate (DMMM) and cyclopropyl-methoxycarbonyl metomidate (CPMM) are methoxycarbonyl etomidate analogs with higher potencies and slower clearance. Because of these properties, we hypothesized that dosing would be lower and electroencephalographic and hypnotic recoveries would be faster—and less context-sensitive—with DMMM or CPMM versus methoxycarbonyl etomidate or etomidate.

Methods: Etomidate, DMMM, and CPMM where infused into rats (n = 6 per group) for either 5 min or 120 min. After infusion termination, electroencephalographic and hypnotic recovery times were measured. The immobilizing ED_{50} infusion rates were determined using a tail clamp assay.

Results: Upon terminating 5-min infusions, electroencephalographic and hypnotic recovery times were not different among hypnotics. However, upon terminating 120-min infusions, recovery times varied significantly with respective values (mean ± SD) 48 ± 13 min and 31 ± 6.5 min (etomidate), 17 ± 7.0 min and 14 ± 3.4 min (DMMM), and 4.5 ± 1.1 min and 4.2 ± 1.6 min (CPMM). The immobilizing ED_{50} infusion rates were (mean ± SD) 0.19 ± 0.03 mg·kg\(^{-1}\)·min\(^{-1}\) (etomidate), 0.60 ± 0.12 mg·kg\(^{-1}\)·min\(^{-1}\) (DMMM), and 0.89 ± 0.18 mg·kg\(^{-1}\)·min\(^{-1}\) (CPMM).

Conclusions: Electroencephalographic and hypnotic recoveries following prolonged infusions of DMMM and CPMM are faster than those following methoxycarbonyl etomidate or etomidate. In the case of CPMM infusion, recovery times are 4 min and context-insensitive.

METHOXYCARBONYL etomidate is the prototypical ultrarapidly metabolized etomidate analog (fig. 1). Similar to remifentanil, methoxycarbonyl etomidate is...
hydrolized by esterases to a carboxylic acid metabolite whose potency is orders of magnitude lower than that of the parent compound. However, unlike remifentanil, methoxycarbonyl etomidate has a duration of action that is markedly context-sensitive, as encephalographic and hypnotic recoveries following prolonged (e.g., 30-min) continuous infusions are 1 or 2 orders of magnitude slower than those following brief (e.g., 5-min) infusions or single boluses. This context sensitivity likely reflects the accumulation of methoxycarbonyl etomidate’s carboxylic acid metabolite in the central nervous system during prolonged continuous methoxycarbonyl etomidate infusion.

In the accompanying manuscript, we described the development of a family of methoxycarbonyl etomidate analogs whose members exhibit widely varying pharmacokinetic and pharmacodynamics properties. Among the 13 new compounds, dimethyl-methoxycarbonyl metomidate (DMMM) and cyclopropyl-methoxycarbonyl metomidate (CPMM) had the highest hypnotic potencies and clearances that were intermediate between those of methoxycarbonyl etomidate and etomidate. Based on these properties, we hypothesized that hypnosis could be maintained for prolonged periods of time with DMMM and CPMM using substantially lower infusion rates than with methoxycarbonyl etomidate, and that postinfusion recovery would be relatively rapid and less context-sensitive because less metabolite would be produced.

To test these hypotheses in a rat model, we used a closed-loop infusion system with electroencephalographic feedback to induce and maintain approximately equivalent hypnotic depths for either 5 or 120 min using etomidate, DMMM, and CPMM. We defined the infusion rates necessary to maintain an electroencephalographic burst suppression ratio (BSR) of 80% (in a background of 1% isoflurane) and the times required for the electroencephalogram to recover upon infusion termination. We also measured the times required for rats to regain their righting reflexes following 5 or 120 min infusions of etomidate, DMMM, and CPMM (in the absence of isoflurane) as a behavioral measure of recovery.

Finally, we determined ED$_{10}$ infusion rates of etomidate, DMMM, and CPMM infusion that maintain immobility in the rat to define the relative anesthetic potencies of these agents and assess the anesthetic depths achieved during our experiments.

### 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 (250–500 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed in the Massachusetts General Hospital Center for Comparative Medicine animal care facility. All intravenous drugs were administered through a femoral venous catheter preimplanted by the vendor before animal delivery to our animal care facility.

#### Hypnotic Drugs

Etomidate (2 mg/ml in 35% propylene glycol/water) was from Hospira (Lake Forest, IL). DMMM and CPMM were synthesized (more than 97% purity) by Aberjona Laboratories (Beverly, MA), using the previously described approach and solubilized at 5 mg/ml in 10% propylene glycol/normal saline. Isoflurane was purchased from Baxter (Deerfield, IL). Bupivacaine and heparin were from APP Pharmaceuticals (Schaumburg, IL).

#### Electroencephalographic Electrode Placement and Recording

Electroencephalographic electrodes were placed in each rat skull during 2 or 3% isoflurane anesthesia, as previously described. The electrodes were connected via a cable 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 Hypnotic Agents

We used the approach described in Rampil and Laster, Vijn and Sneyd, and Cotten et al. to estimate the BSR during each 6-s time epoch. 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. This suppression voltage window was defined individually in each rat as previously described.
Rats were then equilibrated with 1% inhaled isoflurane delivered through a tight-fitting nose cone for at least 45 min until the BSR stabilized before study. Closed-loop hypnotic infusion studies were done in a background of 1% inhaled isoflurane.

A KDS Model 200 Series infusion pump (KD Scientific, Holliston, MA) was used for continuous hypnotic 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 infusions, we used the algorithm described by Vijn and Sneyd in which the hypnotic infusion rate is increased or decreased every 6 s depending upon whether the BSR is below or above, respectively, the target value. The magnitude of the change in the infusion rate is dependent upon the difference between the current BSR measured in the rat and the BSR target. For all closed-loop experiments, we used a BSR target of 80%. To prevent overdosing, the algorithm was modified with a maximum infusion rate of 2 mg\(^{-1}\) · kg\(^{-1}\) · min\(^{-1}\) for etomidate and 3 mg\(^{-1}\) · kg\(^{-1}\) · min\(^{-1}\) for D MMM and CPMM. For each hypnotic, these maximal rates deliver a cumulative dose equivalent to four times the ED\(_{50}\) for loss-of-righting reflexes (as determined in single bolus studies) every minute. We also employed a minimum infusion rate of 0.1 mg\(^{-1}\) · kg\(^{-1}\) · min\(^{-1}\) for all hypnotics. The BSR was measured for 5 min before beginning closed-loop hypnotic infusion and then until the BSR recovered to the baseline value after infusion termination.

**Fitting and Analysis of Closed-loop Infusion Data to Define the 90% BSR Recovery Time**

Because the BSR increased from a preinfusion baseline to the 80% target in an approximately sigmoidal manner when hypnotic infusions were initiated and then decreased to a postinfusion baseline in a sigmoidal manner once hypnotic infusions were terminated, we analyzed the BSR data by fitting this entire infusion time-dependent change in the BSR to a biphasic sigmoidal equation using the analysis software Igor Pro 6.1 (Wavemetrics, Lake Oswego, OR). This biphasic equation was formed by the combination of two monotonically increasing sigmoidal functions among the six groups of rats receiving different hypnotics.

\[
BSR = \frac{M}{1 + \exp\left(-\frac{h - h_0}{r_1}\right)} \frac{M}{1 + \exp\left(-\frac{h - h_0}{r_1}\right)}
\]

In the equation, \(t\) is the time during the infusion experiment, \(h\) and \(h_0\) approximate the respective midpoints as the BSR rises and falls upon hypnotic infusion initiation and termination, \(r\) and \(r_1\) are the respective slopes of the rising and falling phases of the BSR, and \(M\), \(B\), and \(B_1\) together define the maximal and pre- and postinfusion baseline BSR values. From each fit, we calculated the 90% BSR recovery time, which was defined as the time from infusion termination until the time when the BSR fell 90% toward the postinfusion baseline value.

**Determination of Closed-loop Hypnotic Infusion Protocols**

For each 120-min closed-loop infusion experiment, we defined the average infusion rate required to maintain an 80% BSR during each 5-min epoch by recording the infusion rate every 6 s and binning this data into 24 different 5-min periods. For each study group (etomidate, D MMM, and CPMM), we then calculated the within-group average infusion rate during each 5-min period and fit the time-dependent change in infusion rate to an exponential equation using Igor Pro 6.1 in the form:

\[
y = A \exp(-\text{invTau} \cdot t) + y_0
\]

In the equation, \(y\) is the infusion rate at time \(t\), \(A + y_0\) is the initial infusion rate, \(y_0\) is the steady-state infusion rate after long infusion times, and invTau is the inverse time constant that defines the change in infusion rate over time.

**Recovery of Righting Reflexes after Hypnotic Infusions**

In individual rats, loss of righting was produced using a continuous infusion of the desired hypnotic (etomidate, D MMM, or CPMM) for either 5 or 120 min. To achieve approximately equivalent hypnotic depths, we used the infusion protocols defined by equation 2 using values of \(A\), \(y_0\), and invTau determined for each hypnotic in the closed-loop experiments. Approximately 3 min after the hypnotic infusion was begun, rats were turned supine. After the infusion was terminated, the recovery time (spontaneous righting onto all four legs) was measured with a stopwatch.

**Determination of Immobilizing ED\(_{50}\)S**

The immobilizing ED\(_{50}\) for each hypnotic was determined as generally described by Zhang et al. We chose an initial infusion rate for each hypnotic that was 30% of the steady-state infusion rate value determined in closed-loop studies (\(y_0\) in equation 2). This initial infusion rate was maintained for 40 min, then the tail was clamped with an alligator clip and the clip rotated 180° at 1 to 2 Hz for 1 min or until the rat made a purposeful response. If the rat responded, the infusion rate was increased by 20%, and after another 40-min equilibration period, the tail was clamped as before. This procedure was repeated every 40 min with escalating hypnotic infusion rates until the rat failed to respond. The ED\(_{50}\) infusion rate for immobility was then defined in that rat as the average of the highest rate that produced a response and the subsequent rate that did not.

**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. Statistical comparisons among the six groups of rats receiving different hypnotics...
Spacer-linked Etomidate Ester Infusions

for different durations of time were made using a one-way ANOVA followed by a Tukey post hoc test. \( P < 0.05 \) was considered statistically significant.

Results

BSR Recovery Following 5- and 120-min Closed-loop Infusions

In closed-loop experiments using rats preequilibrated with 1% isoflurane, the BSR reached the target value of 80% 3 or 4 min after initiating the infusion. Once the infusion was terminated, the BSR decreased to a postinfusion value that was similar to the preinfusion value. Figures 2A and B show the results of typical experiments in which a rat was infused with etomidate for either 5 min or 120 min, respectively. The respective 90% BSR recovery times for these experiments, calculated from the biphasic sigmoidal fit, were 9.0 min and 50.3 min. Figures 2C and D show the results of analogous experiments in which DMMM was infused. In this case, the calculated 90% BSR recovery times after 5-min and 120-min infusions were 6.3 min and 3.3 min, respectively.

Figure 3 summarizes the results of all closed-loop experiments to define the 90% BSR recovery times after infusing etomidate, dimethyl-methoxycarbonyl metomidate (DMMM), or cyclopropyl-methoxycarbonyl metomidate (CPMM). Each symbol represents recovery data obtained from a single rat experiment. The target BSR ratio was 80% for all experiments. Statistical differences among the six groups were determined using a one-way ANOVA followed by a Tukey post hoc test. All of the following were \( P < 0.001 \): etomidate (120-min infusion) versus etomidate (5-min infusion), etomidate (120-min infusion) versus DMMM (5-min infusion), etomidate (120-min infusion) versus CPMM (5-min infusion), and etomidate (120-min infusion) versus CPMM (120 min). The following was \( P < 0.05 \): DMMM (120-min infusion) versus CPMM (120-min infusion). Differences between all other groups were not statistically significant.
Fig. 4. Cumulative hypnotic doses administered by the closed-loop system to rats during 5- or 120 min closed-loop infusions of etomidate, dimethyl-methoxycarbonyl metomidate (DMMM), or cyclopropyl-methoxycarbonyl metomidate (CPMM). Each symbol represents recovery data obtained from a single rat experiment. The target burst suppression ratio was 80% for all experiments. Statistical differences among the six groups were determined using a one-way ANOVA followed by a Tukey post hoc test. All of the following were P < 0.001: etomidate (120-min infusion) versus DMMM (120-min infusion), etomidate (120 min infusion) versus CPMM (120-min infusion), etomidate (5-min infusion) versus DMMM (120-min infusion), etomidate (5-min infusion) versus CPMM (120-min infusion), CPMM (5-min infusion) versus DMMM (120-min infusion), CPMM (5-min infusion) versus CPMM (120-min infusion), DMMM (5-min infusion) versus DMMM (120-min infusion), and CPMM (120-min infusion) versus CPMM (5-min infusion). The following was P < 0.05: CPMM (120-min infusion) versus DMMM (120-min infusion). Differences between all other groups were not statistically significant.

For both DMMM and CPMM, recovery times did not vary significantly with infusion duration.

Fig. 5. Hypnotic infusion rate as a function of infusion time delivered during 120-min closed-loop infusions. The curve is a fit of each data set to an exponential equation in the form $y = A \exp(-\text{invTau} \cdot t) + y_0$, where $y$ is the infusion rate at time $t$, $A + y_0$ is the initial infusion rate, $y_0$ is the steady-state infusion rate after long infusion times, and invTau is the inverse time constant that defines the change in infusion rate over time. For each hypnotic, data points are average values obtained from six separate experiments. The inset table gives the values for each variable. CPMM = cyclopropyl-methoxycarbonyl metomidate; DMMM = dimethyl-methoxycarbonyl metomidate.

Hypnotic Recovery Following 5- and 120-min Infusions

To assess hypnotic recovery rates using a behavioral endpoint, we infused each hypnotic for either 5 or 120 min in the absence of isoflurane. We used the infusion rates that we had previously defined during the closed-loop studies (i.e., the exponential fit of the data shown in fig. 5) to induce and maintain approximately equivalent hypnotic depths. We then measured the time required for rats to recover their righting reflexes after the infusion was stopped (fig. 6). We found that all rats lost their righting reflexes approximately 3 min after initiating the hypnotic infusion. Upon terminating 5-min infusions, the recovery times did not vary significantly with the identity of the administered hypnotic and averaged 4.0 ± 0.8 min (etomidate), 3.3 ± 0.7 min (DMMM), and 4.2 ± 1.3 min (CPMM). However, upon terminating 120-min infusions, these recovery times varied significantly (P < 0.001) longer than after 5-min infusions.
Fig. 6. Times to righting after receiving 5- or 120-min infusions of etomidate, dimethyl-methoxycarbonyl metomidate (DMMM), or cyclopropyl-methoxycarbonyl metomidate (CPMM). The infusion rates for each hypnotic were defined from closed-loop infusion studies. Each symbol represents recovery data obtained from a single rat experiment. Statistical differences among groups were determined using a one-way ANOVA followed by a Tukey post hoc test. All of the following were \( P < 0.001 \): etomidate (120-min infusion) versus etomidate (5-min infusion), etomidate (120-min infusion) versus DMMM (5-min infusion), etomidate (120-min infusion) versus CPMM (5-min infusion), etomidate (120-min infusion) versus DMMM (120-min infusion), and etomidate (120-min infusion) versus CPMM (120-min infusion) versus etomidate (5-min infusion), DMMM (120-min infusion) versus DMMM (5-min infusion), DMMM (120-min infusion) versus CPMM (5-min infusion), and DMMM (120-min infusion) versus CPMM (120-min infusion). Differences between all other groups were not statistically significant.

However, for CPMM, recovery times following 5-min and 120-min infusions were identical.

**Etomidate, DMMM, and CPMM Immobilizing \( ED_{50} \)**

Continuous infusion of all three hypnotics produced immobilization at sufficiently high infusion rates. The average immobilizing \( ED_{50} \) \( (n = 5 \text{ rats/hypnotic}) \) were \( 0.19 \pm 0.03 \ \text{mg}^{-1} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) (etomidate), \( 0.60 \pm 0.12 \) (DMMM), and \( 0.89 \pm 0.18 \) (CPMM).

**Discussion**

Together with our previous studies, the current studies show that DMMM and CPMM maintain hypnosis with doses that are 1 or 2 orders of magnitude lower than methoxycarbonyl etomidate. They also show that following prolonged hypnotic infusion, electroencephalographic and hypnotic recovery times range from several minutes to several hours with CPMM less than DMMM less than etomidate less than methoxycarbonyl etomidate. In the case of CPMM, recovery times were independent of infusion duration.

Methoxycarbonyl etomidate, DMMM, and CPMM are members of a structurally related family of hypnotics that we have termed “spacer-linked etomidate esters” because each has a metabolically labile ester moiety that is linked to the etomidate backbone via a carbon spacer. Methoxycarbonyl etomidate is the prototypical member of this family, and its metabolically labile ester is linked to the etomidate backbone via a simple two-carbon spacer. When designing methoxycarbonyl etomidate, our objective was to minimize any steric hindrance that might inhibit esterase-catalyzed hydrolysis of the labile ester and thus slow recovery. In vitro studies demonstrated that the methoxycarbonyl etomidate half-life in rat blood is very short (20 s) and its in vivo duration of hypnotic action in rats following single bolus administration is extremely brief (1 or 2 min), even when given at several multiples of its hypnotic ED\(_{50}\) dose.?

In subsequent infusion studies, it became apparent that methoxycarbonyl etomidate dosing requirements were high and electroencephalographic and hypnotic recoveries upon infusion termination were remarkably context-sensitive. For example, electroencephalographic recovery following a single methoxycarbonyl etomidate bolus occurred within several minutes, whereas recovery following a 30-min methoxycarbonyl etomidate infusion occurred on the time-scale of hours. Analysis of metabolite levels in the cerebrospinal fluid revealed that with prolonged methoxycarbonyl etomidate infusion, the carboxylic acid metabolite of methoxycarbonyl etomidate reached millimolar concentrations. These are concentrations that produce significant hypnotic effects, which strongly suggested that the high context-sensitivity was the result of accumulated metabolite in the brain. Analysis of metabolite concentrations can logically be attributed to the large quantity of methoxycarbonyl etomidate that must be infused to maintain hypnosis for prolonged periods of time.

DMMM and CPMM are analogs of methoxycarbonyl etomidate that contain aliphatic groups (two methyl groups and a cyclopropyl group, respectively) designed to sterically protect the labile ester from enzymatic attack and increase metabolic stability relative to methoxycarbonyl etomidate. We hypothesized that this increased stability would reduce the DMMM and CPMM infusion rates necessary to maintain hypnosis (and thus the quantity of metabolite generated), resulting in less context-sensitivity than we had observed with methoxycarbonyl etomidate. During the initial characterization of DMMM and CPMM, we unexpectedly found that these agents also had hypnotic potencies that were nearly 8-fold higher than methoxycarbonyl etomidate, a property that would further reduce dosing requirements.

The current studies confirm our hypothesis, because hypnotic dosing requirements are significantly lower for DMMM and CPMM than previously shown for
methoxycarbonyl etomidate; the total (cumulative) doses of DMMM and CPMM required to maintain an 80% BSR for 120 min (107 ± 18 mg/kg and 143 ± 38 mg/kg, respectively) approximated the total dose of methoxycarbonyl etomidate that maintained an 80% BSR for just 2 or 3 min (all in a background of 1% isoflurane). In addition, electroencephalograpic recovery upon terminating DMMM or CPMM infusions did not show the marked context-sensitivity previously observed with methoxycarbonyl etomidate. With DMMM and CPMM (and etomidate), we also failed to see the slow phase of electroencephalographic recovery that was apparent after even brief methoxycarbonyl etomidate infusions and which was attributed to the slow clearance of metabolite from the brain. Although we did not measure metabolite concentrations in the present study, our results imply that metabolite failed to reach concentrations sufficient to significantly affect the BSR even after 120 min, because electroencephalographic recovery occurred over minutes, not hours.

The results of the righting reflexes studies closely paralleled those of the electroencephalographic studies. However, in the former studies, the difference in the recovery times following 120-min versus 5-min DMMM infusions reached statistical significance.

Our electroencephalographic studies utilized a high (80%) target BSR to more easily quantify recovery upon termination of hypnotic infusions. Although this degree of burst suppression is indicative of a relatively deep level of anesthesia, it was achieved in a background of 1% isoflurane.

This background allowed us to measure the full time-course of burst suppression induction and recovery without motion artifact. It was also expected to reduce the hypnotic infusion rates necessary to achieve such burst suppression toward a more clinically relevant range. This expectation was met as our immobility studies indicate that the steady-state infusion rates used in the electroencephalographic and hypnotic studies correspond to immobilizing ED₅₀ multiples of only 1.3, 1.4, and 1.3 for etomidate, DMMM, and CPMM, respectively.

In conclusion, DMMM and CPMM are sterically hindered analogs of methoxycarbonyl etomidate that maintain equivalent levels of hypnosis with significantly lower infusion rates than methoxycarbonyl etomidate. Encephalographic and hypnotic recoveries following DMMM and CPMM infusion occur without the marked context-sensitivity characteristic of methoxycarbonyl etomidate, which is attributed to metabolite accumulation in the brain. In the case of CPMM, encephalographic and hypnotic recoveries occur in approximately 4 min, independent of infusion duration.

References


Anesthesiology 2012; 117:1037–43