Published in final edited form as:

Anesth Analg. 2016 April ; 122(4): 1008–1014. doi:10.1213/ANE.0000000000001146.

Distinct Hypnotic Recoveries After Infusions of Methoxycarbonyl Etomidate and Cyclopropyl Methoxycarbonyl Metomidate: The Role of the Metabolite

Ervin Pejo, BS,

Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts

Jifeng Liu, PhD,

Aberjona Laboratories, Woburn, Massachusetts

Xiangjie Lin, MSc, and

Aberjona Laboratories, Woburn, Massachusetts

Douglas E. Raines, MD

Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts

Abstract

Background—Methoxycarbonyl etomidate (MOC-etomidate) and cyclopropyl methoxycarbonyl metomidate (CPMM) are rapidly metabolized “soft” etomidate analogues. CPMM’s duration of

Corresponding Author: Douglas E. Raines, MD, Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114-2621, Telephone: 617 724-0343, draines@partners.org.

Disclosures

Name: Ervin Pejo, BS

Contribution: EP performed the experiments.

Attestation: EP approved the final manuscript and attests to the integrity of the original data and the analysis reported in this manuscript.

Conflicts of Interest: None.

Name: Jifeng Liu, PhD

Contribution: JL oversaw the synthesis and purification of CPMM-CA.

Attestation: JL approved the final manuscript.

Conflicts of Interest: None.

Name: Xiangjie Lin, M.Sc.

Contribution: XL synthesized and purified CPMM-CA.

Attestation: XL approved the final manuscript.

Conflicts of Interest: None.

Name: Douglas E. Raines, MD

Contribution: DER designed the experiments, analyzed the data, and wrote the manuscript.

Attestation: DER approved the final manuscript and attests to the integrity of the original data and the analysis reported in this manuscript. DER is the archival author.

Conflicts of Interest: DER is an inventor on patent applications submitted and held by the Massachusetts General Hospital. He, his department, his laboratory, and his institution could receive royalties relating to the development of cyclopropyl-methoxycarbonyl metomidate or related analogues. DER is a consultant for The Medicines Company, which has licensed technologies covered by those patents.

This manuscript was handled by: Ken B. Johnson, MD

See Disclosures for Author Conflicts of Interest.

Reprints will not be available from the authors.

hypnotic effect is context-insensitive whereas MOC-etomidate's is not. In the current study, we tested the hypothesis that CPMM's effect is context-insensitive because, unlike MOC-etomidate, its metabolite fails to reach physiologically important concentrations *in vivo* even with prolonged continuous infusion.

Methods—We compared the potencies with which MOC-etomidate and CPMM activate α_1 (L264T) $\beta_3\gamma_2$ gamma-aminobutyric acid type A receptors and induce loss-of-righting reflexes (i.e. produce hypnosis) in tadpoles with those of their metabolites (MOC-ECA and CPMM-CA, respectively). We measured metabolite concentrations in blood and cerebrospinal fluid of Sprague Dawley rats upon CPMM infusion and compared them to those achieved with MOC-etomidate infusion. We measured the rates with which brain tissue from Sprague Dawley rats metabolize MOC-etomidate and CPMM.

Results—Both analogues and their metabolites enhanced gamma-aminobutyric acid type A receptor function and induced loss-of-righting reflexes in a concentration-dependent manner. However in these 2 assays, CPMM-CA's potency relative to its parent hypnotic was approximately 1:4,900 and 1:1,900, respectively, whereas MOC-ECA's was only approximately 1:415 and 1:390, respectively. With 2-hr CPMM infusions, CPMM-CA reached respective concentrations in the blood and cerebrospinal fluid that were 2 and >3 orders of magnitude lower than that which produced hypnosis. CPMM was metabolized by brain tissue at a rate that is approximately 1/15th that of MOC-etomidate.

Conclusions—Hypnotic recovery after CPMM administration is context-insensitive because its metabolite does not accumulate to hypnotic levels in the central nervous system. This reflects the very large potency ratio between CPMM and its carboxylic acid metabolite CPMM-CA and the resistance of CPMM to metabolism by esterases present in the brain.

Introduction

A “soft” analogue is a congener of a longer-acting drug that contains a metabolically labile chemical moiety that was specifically built into its molecular structure to allow rapid and predictable one-step elimination.¹ Such drugs commonly used in anesthesia practice include remifentanyl and esmolol.^{2–4} Each of these soft drugs contains an ester moiety that is highly susceptible to nonspecific esterase hydrolysis and forms a carboxylic acid metabolite whose pharmacological activity is orders of magnitude lower than that of the parent drug.

Several years ago, our laboratory began a research program to develop a soft analogue of etomidate, a general anesthetic agent with relatively benign cardiovascular and respiratory side effects but whose use is limited to single bolus administration by its highly potent and prolonged suppression of adrenocortical steroid synthesis.^{5–12} Our primary goal was to create a drug that would retain etomidate's highly desirable properties, but whose effects on adrenocortical function would lift quickly after drug administration because the drug was rapidly eliminated. At the same time, it was hoped that recovery from the drug's hypnotic action would similarly be very rapid even after prolonged infusion.

Methoxycarbonyl etomidate (MOC-etomidate) was the first soft etomidate analogue (Figure 1A).⁵ Similar to remifentanyl and esmolol, it contains a metabolically labile ester moiety that is linked to the pharmacophore via a 2-carbon spacer. This spacer facilitates hydrolysis by

esterases by reducing steric hindrance around the ester. Preclinical studies in rats demonstrated that following single bolus administration or very brief (e.g. 5-min) continuous infusions, MOC-etomidate had an ultra-short duration of hypnotic action (~1 minute) and did not produce persistent adrenocortical suppression.^{5,6} However with longer infusions (e.g. 30-min) that required the administration of larger quantities of MOC-etomidate, hypnotic recovery slowed dramatically as MOC-etomidate's carboxylic acid metabolite (MOC-ECA) accumulated to levels in both blood and cerebrospinal fluid (CSF) that were sufficient to produce hypnosis.¹⁰

In an attempt to produce soft etomidate analogues devoid of such context-sensitivity, we developed a series of second generation soft etomidate analogues in which the chemical structure of the spacer varied.⁸ Of these new compounds, cyclopropyl methoxycarbonyl metomidate (CPMM) possessed the most promising pharmacology in animals (Figure 1B) because it exhibited the highest potency and produced hypnosis that reversed within several minutes after stopping continuous infusions lasting as long as 2 hours.^{7,8,11} CPMM is now undergoing trials in humans.

The purpose of the current study was to assess the pharmacology of CPMM's carboxylic acid metabolite (CPMM-CA). We synthesized CPMM-CA and then defined its potency for directly activating gamma-aminobutyric acid type A (GABA_A) receptors and producing hypnosis in tadpoles. We also measured the extent to which CPMM-CA accumulated in the blood and CSF with a 2-hr continuous CPMM infusion. We then compared these results with those obtained with MOC-etomidate and its metabolite. Our goal was to better understand why hypnotic recovery after CPMM infusion is context-insensitive whereas that after MOC-etomidate infusion is not. Our hypothesis was that hypnotic recovery after CPMM (as opposed to MOC-etomidate) infusion is context-insensitive because its metabolite fails to reach concentrations in either the blood or CSF that are sufficient to have an hypnotic effect.

Methods

Animals

All animal studies were conducted with the approval of the Subcommittee on Research Animal Care at the Massachusetts General Hospital, Boston, Massachusetts. *Xenopus laevis* tadpoles (early pre-limb stage) and adult female *Xenopus laevis* frogs were purchased from Xenopus One (Ann Arbor, MI) and housed in our laboratory (tadpoles) or in the Massachusetts General Hospital Center for Comparative Medicine animal care facility (frogs). Adult male Sprague-Dawley rats (330–430 g) were purchased from Charles River Laboratories (Wilmington, MA) and caged in the animal care facility of the Massachusetts General Hospital Center for Comparative Medicine. CPMM was administered through a 24 gauge venous catheter placed in a tail vein. Blood and CSF samples were taken from a femoral arterial catheter and an intracisternal cannula, respectively, implanted by the vendor prior to delivery.

Chemical Syntheses

MOC-etomidate, MOC-ECA, and CPMM were synthesized by Aberjona Laboratories (Woburn, MA) using our previously published methods.^{5,6,8} CPMM-CA was synthesized using the two-step scheme shown in Figure 2.

Step 1—To a solution of **1** (2.6g, 12 mol) in dichloromethane (100 mL) was added (COCl)₂ (1.6 mL) and dimethylformamide (4 drops) at 0°C drop wise. The reaction mixture was stirred at room temperature until completion of the reaction as monitored by high performance liquid chromatography (HPLC). The reaction mixture was concentrated and azeotroped 3 times with anhydrous toluene. The crude product **2** was then dried on a vacuum pump for 3 hrs for use in Step 2 without storage.

Step 2—To a solution of **3** (1.22g, 12 mmol) in dichloromethane (200 mL) was added triethylamine (6 mL) and 4-Dimethylaminopyridine (catalyst), followed by **2** from Step 1 (12 mmol) in dichloromethane (40 mL) at 0°C. The reaction mixture was stirred at 0°C for 2 hrs and then room temperature overnight. The reaction mixture was concentrated *in vacuo*. Tetrahydrofuran was added to the residue and acidified with diluted aqueous HCl to pH = 3. The mixture was concentrated and the residue was purified by preparative HPLC to yield the product **4** (1.1g; >98% purity).

MS (M+1 = 301), ¹H NMR (400 MHz, DMSO-d₆) δ 9.23 (s, 1H), 8.27 (s, 1H), 7.27–7.40 (m, 5H), 6.27 (q, J = 7.2 Hz, 1H), 1.90 (d, J = 7.2Hz, 3H), 1.41–1.44 (m, 2H), 1.11–1.31 (m, 2H).

GABA_A Receptor Electrophysiology

Oocytes were harvested from frogs as previously described and injected with messenger RNA encoding the α₁(L264T), β₃, and γ₂ subunits of the human GABA_A receptor (5 ng of messenger RNA total at a subunit ratio of 1:1:3).⁵ As previously reported, we incorporate the L264T ion channel mutation into the receptor because it stabilizes the receptor's open state, thus increasing anesthetic sensitivity and allowing more complete anesthetic concentration-response curves to be generated before reaching aqueous saturation at high anesthetic concentrations and without the potentially confounding effects of a co-administered agonist.^{13–15} Oocytes were then incubated in ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH=7.4) containing 0.05 mg/mL of gentamicin for at least 18 hours at 18°C before study.

Electrophysiological recordings were performed using the whole cell 2-electrode voltage-clamp technique as previously described.¹⁴ Peak current amplitudes elicited by a 30 second application of the desired compound were normalized to control currents elicited by 100 μM GABA in the same oocyte. For each compound, the concentration-mean peak current response data were fit to a Hill equation with minima and maxima constrained to 0% and 100%, respectively, to obtain the median effective concentration (EC₅₀) for direct activation of GABA_A receptors. From these data, a potency ratio was defined as the EC₅₀ of the metabolite relative to the EC₅₀ for the parent hypnotic. For each compound, the

concentration range studied was limited by its solubility in the buffer. Data reported for CPMM are from previously published studies by our laboratory using the same approach.¹²

Tadpole Loss of Righting Reflexes Assay

Loss-of-righting reflexes (LoRR) in *Xenopus laevis* tadpoles was assessed as previously described.^{5,16} In brief, groups of 5 tadpoles were placed in water buffered with 2.5 mM Tris HCl (pH=7.4) containing the desired concentration of CPMM-CA. Tadpoles were tipped every 5 minutes with a flame-polished pipette until the response stabilized (20 – 30 minutes). A tadpole was determined to have LoRR if it failed to right itself within 5 seconds after being turned supine. At the end of each study, tadpoles were returned to fresh water to ensure reversibility. The concentration-mean response data for LoRR were fit to a Hill equation with minima and maxima constrained to 0% and 100%, respectively, to obtain the EC₅₀ for LoRR. From these data, a potency ratio was defined as the EC₅₀ of the parent hypnotic relative to the EC₅₀ for the metabolite. The concentration range of CPMM-CA studied was limited by its solubility in the buffer. Data reported for MOC-etomidate, MOC-ECA, and CPMM, are from previously published studies by our laboratory using the same approach.^{5,12,15}

CPMM Infusion Protocol

CPMM was solubilized in saline (8 mg/ml) and infused IV for 2 hours using our previously published protocol.⁷ This protocol induces and maintains an electroencephalographic burst suppression ratio of 80% in the presence of 1% isoflurane and produces a steady-state anesthetic depth that is 1.3 times that required to produce immobility to a standard noxious stimulus in the absence of isoflurane. The total dose of CPMM delivered during these 2-hour infusions was 71 mg/kg. Comparative data reported for MOC-etomidate are from previously published 30-min infusion studies by our laboratory using a protocol that also induces and maintains an electroencephalographic burst suppression ratio of 80% in the presence of 1% isoflurane and produces a steady-state anesthetic depth that is 1.3 times that required to produce immobility to a standard noxious stimulus in the absence of isoflurane.¹⁰

Measurement of Blood and CSF CPMM-CA Concentrations

Before, during, and after CPMM infusion, blood samples (200 µl/sample) were intermittently collected through the femoral arterial catheter and immediately mixed with acetonitrile (200 µl). This blood was replaced with an equal volume of normal saline. The samples were then centrifuged and the resultant plasma was collected and stored at –20 °C until analyzed. After thawing, the CPMM-CA concentration in each sample was determined by HPLC using an Agilent Technologies 1200 system with a Poroshell 120, 4.6 × 50 mm, 2.7 µm C18 column (Santa Clara, CA) with the UV detector set to 242 nm. A linear gradient 20% to 90% acetonitrile in water over 30 minutes was used with a flow rate of 1 ml/min. Standards were made by dissolving the desired quantity of CPMM-CA in methanol.

Before, during, and after CPMM infusion, CSF samples (25 µl/sample) were collected through the intracisternal catheter, immediately mixed with acetonitrile (50 µl), and stored at –20 °C until analyzed. The concentration of CPMM-CA in CSF was assessed by HPLC as described above for blood.

A standard curve was constructed using 8 CPMM-EC concentrations ranging from 1 – 100 μM . The relationship between the peak area and the CPMM-EC concentration was linear with an $r^2 > 0.99$. The HPLC assay's lower limit of quantitation for CPMM-EC along with its precision and accuracy were determined using guidelines established by the International Conference on Harmonisation.¹⁷ Intra-day and inter-day precision and accuracy were assessed at 5, 10, and 20 μM CPMM-CA ($n = 27$ HPLC measurements over three days). At these three concentrations, the relative standard deviations (for both intra- and inter-day precision) were less than 5%, 3%, and 2%, respectively. The accuracy of all samples (both intra- and inter-day) were within 13% of their nominal values. The lower limit of quantitation was calculated from the standard deviation of the response and the slope of the calibration curve to be 0.7 μM .

Measurement of MOC-etomidate and CPMM In Vitro Metabolic Half-life in Brain Tissue

The metabolic half-lives of MOC-etomidate and CPMM were quantified using a previously described protocol.¹⁰ Pooled rat brain homogenate (20mg/mL in phosphate-buffered saline media pH 7.4) was purchased from Bioreclamation LLC (Hicksville, NY). MOC-etomidate or CPMM (120 μl from a 1 mM stock solution in saline) was added to 1.08 ml of the homogenate. After the desired incubation period at 37 $^{\circ}\text{C}$, a 100 μl aliquot was removed and the metabolism was stopped by mixing with 100 μl of acetonitrile. The samples were then centrifuged and the supernatant was collected and stored at -20°C until analyzed by HPLC as described above for *in vivo* studies. The metabolic half-lives of MOC-etomidate and CPMM in rat brain homogenate were calculated from the incubation time-dependent reduction in MOC-etomidate and CPMM concentrations assuming a first-order process and complete metabolism at infinite time.

Statistical Analysis

All data are reported as mean $\pm$ SD unless otherwise noted. In each study, the number of repetitions used to define the mean value of each data point (6 for oocyte studies, 5 for tadpole studies, and 3 for all other studies) was defined prior to initiating the study and was based upon the precision observed from previous studies from our laboratory. Curve fitting for concentration-response curves (GABA_A receptor and tadpole studies) was performed with Prism 6.0 (Graphpad, La Jolla, CA) and potency was quantified as an EC₅₀ and a 95% confidence interval (CI). The 95% confidence interval for each EC₅₀ was calculated using error propagation. Metabolic half-lives in rat brain homogenate were quantified (along with a 99% CI) and statistically compared using an extra sum-of-squares F test with Prism 6.0.

Results

Modulation of GABA_A Receptor Function

Both hypnotics (MOC-etomidate and CPMM) and their carboxylic acid metabolites (MOC-ECA and CPMM-CA, respectively) directly activated GABA_A receptors in a concentration-dependent manner (Figure 3). Figure 3A plots the relationship between the MOC-etomidate and MOC-ECA concentrations and the normalized peak current amplitude response. A fit of the data to a Hill equation yielded an EC₅₀ for direct activation of 20 μM (95% CI: 16 – 25 μM) for MOC-etomidate and 8,300 μM (95% CI: 6,280 – 11,100 μM) for MOC-ECA,

yielding a potency ratio between metabolite and parent hypnotic of 1:415 (95% CI: 1:260 – 1:665). Figure 3B plots the same relationship for CPMM and CPMM-CA. A fit of the data to a Hill equation yielded an EC_{50} for direct activation of 3.8 μM (95% CI: 2.8 – 5.0 μM) for CPMM and 18,800 μM (95% CI: 9,460 – 37,300 μM) for CPMM-CA, yielding a potency ratio between metabolite and parent hypnotic of 1:4,900 (95% CI: 1:1,540 – 1:16,340).

LoRR in Tadpoles

Both hypnotics and their carboxylic acid metabolites induced LoRR in tadpoles in a concentration-dependent manner (Figure 4) and without lethality. Figure 4A plots the relationship between the MOC-etomidate and MOC-ECA concentrations and the fraction of tadpoles that had LoRR. A fit of the data to a Hill equation yielded an EC_{50} for LoRR of 7.5 μM (95% CI: 5.2 – 10.7 μM) for MOC-etomidate and 2,900 μM (95% CI: 1,660 – 5,020 μM) for MOC-ECA, yielding a potency ratio between metabolite and parent hypnotic of 1:390 (95% CI: 1:103 – 1:1,445). Figure 4B plots the same relationship for CPMM and CPMM-CA. A fit of the data to a Hill equation yielded an EC_{50} for LoRR of 2.6 μM (95% CI: 2.1 – 3.1 μM) for CPMM and 5,000 μM (95% CI: 4,700 – 5,300 μM) for CPMM-CA, yielding a potency ratio between metabolite and parent hypnotic of 1:1,900 (95% CI: 1:1,330 – 1:2,890).

Metabolite Concentrations in Blood Upon Hypnotic Infusion

CPMM was continuously infused for 2 hours. The CPMM-CA concentration was 26 ± 10 μM measured in the first blood sample drawn 30 minutes after initiating that infusion and remained near that value (mean: 31 ± 6.8 μM , range 26 – 39 μM) throughout the 2-hr infusion period (Figure 5). Sixty minutes after the infusion ended, the CPMM-CA concentration in the blood decreased to 1.5 ± 0.3 μM . For comparison, Figure 5 also shows the blood concentrations of MOC-ECA measured in our previous study using a 30-min infusion of MOC-etomidate that achieved a similar hypnotic depth as judged by both behavioral and electroencephalographic metrics.¹⁰ By the end of the 30-min infusion, the metabolite concentration reached $1,170 \pm 48$ μM , a value that is approximately 45-fold higher than that reached upon infusing CPMM for the same duration of time and achieving the same anesthetic depth. After the MOC-etomidate infusion ended, the MOC-ECA concentration in the blood decreased to 820 ± 42 μM over the next 60 minutes and then to 480 ± 110 μM when measured 4 hours after the MOC-etomidate infusion stopped.

Metabolite Concentrations in CSF Upon Hypnotic Infusion

In our previous MOC-etomidate infusion studies in rats, we found that MOC-ECA poorly penetrated the blood-brain barrier but nevertheless reached a CSF concentration that was twice that present in the blood ($2,500 \pm 110$ μM).¹⁰ We concluded that most of this MOC-ECA was formed from MOC-etomidate that had diffused across the blood-brain barrier and was then metabolized *in situ* by esterases present in central nervous system tissues (e.g. brain). Once formed within the central nervous system, such MOC-ECA was trapped there for a prolonged period of time because it was charged at physiological pH. It was within this context that we measured CPMM-CA concentrations in the CSF during and after 2-hr CPMM infusions. Based on those studies with MOC-etomidate, we had expected that the

CPMM-CA concentration in the CSF would similarly be approximately twice that present in the blood (i.e. $\sim 50 \mu\text{M}$). However, we found no quantifiable CPMM-CA in the CSF at any time point during the study (data not shown). This implies that even with infusions of CPMM lasting 2 hours, the CPMM-CA concentration in the CSF was below our $0.7 \mu\text{M}$ limit of quantitation.

Metabolic Half-Lives in Brain Tissue: MOC-Etomidate Versus CPMM

The unexpectedly low levels of CPMM-CA in the CSF led us to hypothesize that CPMM is metabolized more slowly than MOC-etomidate by esterases within the central nervous system and, therefore, less of its metabolite accumulates there. To test this hypothesis, we compared the rates with which the 2 hypnotics were metabolized by rat brain tissue homogenate. Figure 6 shows that CPMM was metabolized significantly more slowly than MOC-etomidate with half-lives of 1,414 min (99% CI: 997 – 2,427 min) and 96 min (99% CI: 92 – 101 min), respectively.

Discussion

The primary goal of these studies was to characterize the pharmacology of CPMM-CA and to compare it to that of MOC-ECA so that we could better understand why hypnotic recovery after infusions of CPMM are context-insensitive whereas those after MOC-etomidate are not. Our studies showed that similar to MOC-ECA, CPMM-CA enhances GABA_A receptor function and produces LoRR in tadpoles in a concentration-dependent manner. However CPMM-CA's potency in these 2 assays relative to its parent hypnotic was approximately 1:4,900 and 1:1,900, respectively, whereas that for MOC-ECA was only approximately 1:415 and 1:390, respectively. Our studies also revealed that with 2-hr CPMM infusions, CPMM-CA reached respective concentrations in the blood and CSF that were 2 and >3 orders of magnitude lower than those which produce hypnosis (in tadpoles). These results may be contrasted with our previous studies showing that with just 30-min MOC-etomidate infusions, MOC-ECA reached concentrations in the blood and CSF that approximated those which produce hypnosis.¹⁰ Finally, we show that CPMM is metabolized significantly more slowly than MOC-etomidate by esterases found in the brain, which may explain why the concentration of CPMM-CA in the CSF is so much lower than that in the blood.

A key concept in the design of soft analogues is that the potency of the metabolite must be significantly lower than that of the parent drug to allow the pharmacological action to rapidly terminate once the parent drug has been metabolized.^{1,18,19} For soft drugs that are continually infused for a prolonged period of time, this potency ratio must be particularly large because their metabolites typically accumulate in the body during the infusion period and therefore could produce persistent pharmacological effects. In the cases of remifentanyl and esmolol, the metabolite:parent drug potency ratios have been estimated to be as high as 1:4,600 for the former and between 1:1,600 and 1:1,900 for the latter.^{20,21} Studies suggest that these potency ratios are sufficiently large to avoid a pharmacologically significant impact of the metabolite (GR90291 for remifentanyl and ASL-8123 for esmolol) even when its elimination is slowed by renal failure.^{22,23}

The potency ratio for CPMM:CPMM-CA is approximately an order of magnitude greater than that of MOC-etomidate:MOC-ECA in our assays, and approximates those of remifentanyl:GR90291 and esmolol:ASL-8123. Thus, it is possible to infuse CPMM (but not MOC-etomidate) at hypnotic doses for a prolonged length of time without metabolite concentrations reaching levels in either the blood or CSF that produce hypnosis. By analogy to remifentanyl and esmolol, we expect that this would be true even in the presence of renal failure because the CPMM:CPMM-CA potency ratio is so large.

Although CPMM and MOC-etomidate are metabolized in rat blood at similar rates (half-lives: 0.57 and 0.41 min, respectively), the present studies show that they are metabolized in rat brain tissue at rates that differ by 15-fold (half-lives: 1,414 and 96 min, respectively).⁸ Such substrate selectivity is not uncommon among esterases and may be leveraged to fine tune the pharmacology of soft drugs.^{24–26} This finding also suggests that the esterases responsible for metabolizing these drugs in the brain are different from those in the blood. We believe that CPMM's relatively low rate of metabolism in the brain (and presumably low blood-brain barrier permeability because it is a carboxylic acid) explains why CPMM-CA's concentration in the CSF was so much lower than in blood: CPMM-CA is poorly produced *in situ* by central nervous system tissue (e.g. brain) and little of the CPMM-CA that is produced in the periphery (e.g. by blood and the liver) crosses the blood-brain barrier to reach the CNS. This suggests that even had the potency ratio of CPMM:CPMM-CA been closer to that of MOC-etomidate:MOC-ECA, CPMM-CA concentrations in the CNS might still have been insufficient to have a hypnotic effect.

Although we did not characterize the elimination of CPMM-CA or MOC-ECA in any detail, we note that 60 minutes after stopping infusion of the parent hypnotic, the blood concentration of CPMM-CA decreased by 94% whereas that of MOC-ECA decreased by only 30%. Four hours after stopping the MOC-etomidate infusion, the blood concentration of MOC-ECA was still comparatively high having decreased by only 60%. The renal excretion of anionic compounds (including carboxylic acids) from the blood is saturable at high blood concentrations because it is dependent upon active tubular secretion.²⁷ Therefore, the relatively slow elimination of MOC-ECA (as compared to CPMM-CA) may be most simply explained by its nearly 2 order of magnitude higher blood concentration.

Conclusions

Hypnotic recovery after administration of CPMM, as opposed to MOC-etomidate, is context-insensitive because its carboxylic acid metabolite does not accumulate to hypnotic levels in the central nervous system even after prolonged infusion. This low level of accumulation reflects (1) the >1000-fold potency ratio between CPMM and its metabolite and (2) the relative resistance of CPMM to metabolism by esterases in the brain.

Acknowledgments

Funding: This research was supported by grant R01-GM087316 to DER and by the Department of Anesthesia, Critical Care & Pain Medicine, Massachusetts General Hospital.

References

1. Bodor N. Recent advances in retrometabolic drug design and targeting approaches. *Pharmazie*. 2000; 55:163–6. [PubMed: 10756533]
2. Glass PS, Hardman D, Kamiyama Y, Quill TJ, Marton G, Donn KH, Grosse CM, Hermann D. Preliminary pharmacokinetics and pharmacodynamics of an ultra-short-acting opioid: remifentanyl (GI87084B). *Anesth Analg*. 1993; 77:1031–40. [PubMed: 8105723]
3. Rosow C. Remifentanyl: A unique opioid analgesic. *Anesthesiology*. 1993; 79:875–6. [PubMed: 7902031]
4. Sum CY, Yacobi A, Kartzinel R, Stampfli H, Davis CS, Lai CM. Kinetics of esmolol, an ultra-short-acting beta blocker, and of its major metabolite. *Clin Pharmacol Ther*. 1983; 34:427–34. [PubMed: 6617063]
5. Cotten JF, Husain SS, Forman SA, Miller KW, Kelly EW, Nguyen HH, Raines DE. Methoxycarbonyl-etomidate: A novel rapidly metabolized and ultra-short-acting etomidate analogue that does not produce prolonged adrenocortical suppression. *Anesthesiology*. 2009; 111:240–9. [PubMed: 19625798]
6. Cotten JF, Le Ge R, Banacos N, Pejo E, Husain SS, Williams JH, Raines DE. Closed-loop continuous infusions of etomidate and etomidate analogs in rats: A comparative study of dosing and the impact on adrenocortical function. *Anesthesiology*. 2011; 115:764–73. [PubMed: 21572317]
7. Ge R, Pejo E, Husain SS, Cotten JF, Raines DE. Electroencephalographic and hypnotic recoveries after brief and prolonged infusions of etomidate and optimized soft etomidate analogs. *Anesthesiology*. 2012; 117:1037–43. [PubMed: 22929726]
8. Husain SS, Pejo E, Ge R, Raines DE. Modifying methoxycarbonyl etomidate inter-ester spacer optimizes in vitro metabolic stability and in vivo hypnotic potency and duration of action. *Anesthesiology*. 2012; 117:1027–36. [PubMed: 22929736]
9. Pejo E, Cotten JF, Kelly EW, Le Ge R, Cuny GD, Laha JK, Liu J, Lin XJ, Raines DE. In vivo and in vitro pharmacological studies of methoxycarbonyl-carboetomidate. *Anesth Analg*. 2012; 115:297–304. [PubMed: 21965364]
10. Pejo E, Ge R, Banacos N, Cotten JF, Husain SS, Raines DE. Electroencephalographic recovery, hypnotic emergence, and the effects of metabolite following continuous infusions of a rapidly metabolized etomidate analog in rats. *Anesthesiology*. 2012; 116:1057–65. [PubMed: 22417966]
11. Campagna JA, Pojasek K, Grayzel D, Randle J, Raines DE. Advancing novel anesthetics: Pharmacodynamic and pharmacokinetic studies of cyclopropyl-methoxycarbonyl metomidate in dogs. *Anesthesiology*. 2014; 121:1203–16. [PubMed: 25170571]
12. Ge R, Pejo E, Gallin H, Jeffrey S, Cotten JF, Raines DE. The pharmacology of cyclopropyl-methoxycarbonyl metomidate: A comparison with propofol. *Anesth Analg*. 2014; 118:563–7. [PubMed: 24557104]
13. Rusch D, Zhong H, Forman SA. Gating allosterism at a single class of etomidate sites on $\alpha 1\beta 2\gamma 2\delta$ GABAA receptors accounts for both direct activation and agonist modulation. *J Biol Chem*. 2004; 279:20982–92. [PubMed: 15016806]
14. Pejo E, Santer P, Jeffrey S, Gallin H, Husain SS, Raines DE. Analogues of etomidate: Modifications around etomidate's chiral carbon and the impact on in vitro and in vivo pharmacology. *Anesthesiology*. 2014; 121:290–301. [PubMed: 24777068]
15. Ge RL, Pejo E, Haburcak M, Husain SS, Forman SA, Raines DE. Pharmacological studies of methoxycarbonyl etomidate's carboxylic acid metabolite. *Anesth Analg*. 2012; 115:305–8. [PubMed: 22052979]
16. Cotten JF, Forman SA, Laha JK, Cuny GD, Husain SS, Miller KW, Nguyen HH, Kelly EW, Stewart D, Liu A, Raines DE. Carboetomidate: A pyrrole analog of etomidate designed not to suppress adrenocortical function. *Anesthesiology*. 2010; 112:637–44. [PubMed: 20179500]
17. Group IEW. Validation of Analytical Procedures: Text and Methodology Q2(R1) International Conference of Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 1994.
18. Bodor, N.; Buchwald, P. Retrometabolic drug design and targeting. Hoboken, NJ: John Wiley & Sons; 2012.

19. Stanczak A, Ferra A. Prodrugs and soft drugs. *Pharmacol Rep.* 2006; 58:599–613. [PubMed: 17085852]
20. Hoke JF, Cunningham F, James MK, Muir KT, Hoffman WE. Comparative pharmacokinetics and pharmacodynamics of remifentanyl, its principle metabolite (GR90291) and alfentanil in dogs. *J Pharmacol Exp Ther.* 1997; 281:226–32. [PubMed: 9103501]
21. Shaffer JE, Quon CY, Gorczynski RJ. Beta-adrenoreceptor antagonist potency and pharmacodynamics of ASL-8123, the primary acid metabolite of esmolol. *J Cardiovasc Pharmacol.* 1988; 11:187–92. [PubMed: 2452313]
22. Hoke JF, Shlugman D, Dershwitz M, Michalowski P, Malthouse-Dufore S, Connors PM, Martel D, Rosow CE, Muir KT, Rubin N, Glass PS. Pharmacokinetics and pharmacodynamics of remifentanyl in persons with renal failure compared with healthy volunteers. *Anesthesiology.* 1997; 87:533–41. [PubMed: 9316957]
23. Flaherty JF, Wong B, La Follette G, Warnock DG, Hulse JD, Gambertoglio JG. Pharmacokinetics of esmolol and ASL-8123 in renal failure. *Clin Pharmacol Ther.* 1989; 45:321–7. [PubMed: 2563962]
24. Buchwald P, Bodor N. Structure-based estimation of enzymatic hydrolysis rates and its application in computer-aided retrometabolic drug design. *Pharmazie.* 2000; 55:210–7. [PubMed: 10756543]
25. Buchwald P, Bodor N. Physicochemical aspects of the enzymatic hydrolysis of carboxylic esters. *Pharmazie.* 2002; 57:87–93. [PubMed: 11878195]
26. Buchwald P. Structure-metabolism relationships: Steric effects and the enzymatic hydrolysis of carboxylic esters. *Mini Rev Med Chem.* 2001; 1:101–11. [PubMed: 12369995]
27. van Ginneken CA, Russel FG. Saturable pharmacokinetics in the renal excretion of drugs. *Clin Pharmacokinet.* 1989; 16:38–54. [PubMed: 2650954]

Figure 1. Structures of parent hypnotics and their carboxylic acid metabolites. (A) Methoxycarbonyl etomidate (MOC-etomidate) and MOC-ECA. (B) Cyclopropyl methoxycarbonyl metomidate (CPMM) and CPMM-CA.

Figure 2.

Two-step synthesis of CPMM-CA. Et_3N is triethylamine. DCM is dichloromethane.

Figure 3.

Concentration-response relationships for direct activation of $\alpha_1(L264T)\beta_3\gamma_2$ gamma-aminobutyric acid (GABA) type A receptors by each metabolite and its parent hypnotic. (A) Methoxycarbonyl etomidate (MOC-etomidate) and MOC-ECA. MOC-ECA was 415-fold less potent than was MOC-etomidate. (B) Cyclopropyl methoxycarbonyl metomidate (CPMM) and CPMM-CA. CPMM-CA was approximately 4,900-fold less potent than was CPMM. Data for CPMM were taken from Reference 12. In both panels, each data point is the mean value ($\pm$ SD) derived from 6 oocyte experiments.

Figure 4.

Concentration-response relationships for loss-of-righting reflexes in tadpoles by each metabolite and its parent hypnotic. (A) Methoxycarbonyl etomidate (MOC-etomidate) and MOC-ECA. MOC-ECA was 390-fold less potent than was MOC-etomidate. (B) Cyclopropyl methoxycarbonyl metomidate (CPMM) and CPMM-CA. CPMM-CA was approximately 1,900-fold less potent than was CPMM. In both panels, each data point was obtained using 5 tadpoles. Data for MOC-etomidate and MOC-ECA were from Reference 15 and that for CPMM were from Reference 12.

Figure 5.

Blood metabolite concentrations with continuous infusions of parent hypnotic. MOC-etomidate was infused for 30 minutes and cyclopropyl methoxycarbonyl metomidate (CPMM) was infused for 120 minutes. All infusions were begun at Time = 0 minutes. Each data point is the mean value ($\pm$ SD) derived from 3 (CPMM infusions) or 4 (MOC-etomidate infusions) rat experiments. Data for MOC-etomidate infusions were from Reference 10.

Figure 6.

In vitro metabolism of methoxycarbonyl etomidate (MOC-etomidate) and cyclopropyl methoxycarbonyl metomidate (CPMM) in pooled brain tissue from rats. Each data point is the mean value ($\pm$ SD) from 3 experiments. The lines are nonlinear least squares fits of the data assuming a first-order process with the maxima and minima constrained to 100 and 0, respectively. The metabolic half-lives of MOC-etomidate and CPMM were determined to be 1,414 min (99% confidence interval: 997 – 2,427 min) and 96 min (99% confidence interval: 92 – 101 min), respectively. The same brain tissue homogenate lot was used for all data points.