

# Methoxycarbonyl-etomidate

## A Novel Rapidly Metabolized and Ultra-short-acting Etomidate Analogue that Does Not Produce Prolonged Adrenocortical Suppression

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**Background:** Etomidate is a rapidly acting sedative-hypnotic that provides hemodynamic stability. It causes prolonged suppression of adrenocortical steroid synthesis; therefore, its clinical utility and safety are limited. The authors describe the results of studies to define the pharmacology of (R)-3-methoxy-3-oxopropyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate (MOC-etomidate), the first etomidate analogue designed to be susceptible to ultra-rapid metabolism.

**Methods:** The  $\gamma$ -aminobutyric acid type A receptor activities of MOC-etomidate and etomidate were compared by using electrophysiological techniques in human  $\alpha_1\beta_2\gamma_2$  receptors. MOC-etomidate's hypnotic concentration was determined in tadpoles by using a loss of righting reflex assay. Its *in vitro* metabolic half-life was measured in human liver S9 fraction, and the resulting metabolite was provisionally identified by using high-performance liquid chromatography/mass spectrometry techniques. The hypnotic and hemodynamic actions of MOC-etomidate, etomidate, and propofol were defined in rats. The abilities of MOC-etomidate and etomidate to inhibit corticosterone production were assessed in rats.

**Results:** MOC-etomidate potently enhanced  $\gamma$ -aminobutyric acid type A receptor function and produced loss of righting reflex in tadpoles. Metabolism in human liver S9 fraction was first-order, with an *in vitro* half-life of 4.4 min versus more than 40 min for etomidate. MOC-etomidate's only detectable metabolite was a car-

boxylic acid. In rats, MOC-etomidate produced rapid loss of righting reflex that was extremely brief and caused minimal hemodynamic changes. Unlike etomidate, MOC-etomidate produced no adrenocortical suppression 30 min after administration.

**Conclusions:** MOC-etomidate is an etomidate analogue that retains etomidate's important favorable pharmacological properties. However, it is rapidly metabolized, ultra-short-acting, and does not produce prolonged adrenocortical suppression after bolus administration.

ETOMIDATE is a rapidly acting imidazole-based intravenous (IV) sedative-hypnotic that is used to induce general anesthesia. In common with other IV induction agents, etomidate's hypnotic action in humans terminates after bolus delivery as it redistributes from the brain to other tissues and ultimately undergoes elimination by the liver with a half-life of several hours.<sup>1,2</sup> However, etomidate is distinguished from other induction agents by its ability to maintain hemodynamic stability, even in the setting of cardiovascular compromise.<sup>3–6</sup> It has consequently emerged as an agent of choice for use in critically ill patients.

Etomidate also potently inhibits 11 $\beta$ -hydroxylase, an enzyme in the biosynthetic pathway leading to adrenocortical steroid synthesis.<sup>7–10</sup> Etomidate's potency for inhibiting 11 $\beta$ -hydroxylase is at least 100-fold greater than its hypnotic potency.<sup>11</sup> Therefore, inhibition of steroid synthesis occurs even with subhypnotic doses of etomidate. At the doses necessary to produce hypnosis, etomidate causes adrenocortical suppression that can persist for more than 4 days after discontinuing a prolonged infusion, resulting in significantly increased mortality in critically ill patients.<sup>7,8,10</sup> Recent studies and reports of critically ill patients show that adrenocortical suppression after even a single induction dose of etomidate can last for 24 h or more, and several suggest that it increases morbidity and/or mortality.<sup>12–21</sup>

On the basis of our previous studies of etomidate analogues,<sup>22,23</sup> we hypothesized that analogues of etomidate could be designed that are metabolized quickly, providing etomidate's favorable pharmacological properties (e.g., rapid onset of action, high hypnotic potency, and hemodynamic stability) but also ultra-rapid recovery from both hypnosis and adrenal suppression. In this report, we describe the results of studies characterizing (R)-3-methoxy-3-oxopropyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate (MOC-etomidate), the first etomidate analogue designed to undergo ultra-rapid metabolism by esterases.

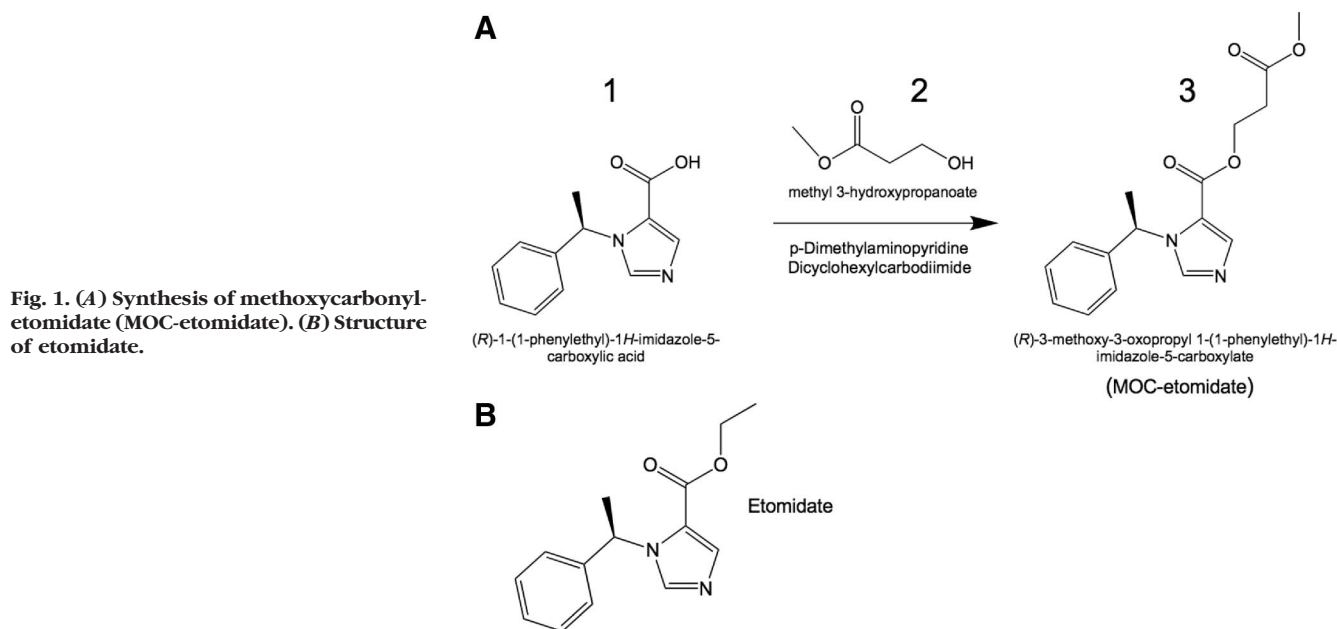
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## Materials and Methods

### Animals

All animal studies were conducted in accordance with rules and regulations of the Subcommittee on Research Animal Care at the Massachusetts General Hospital, Boston, Massachusetts. Early prelimb-bud stage *Xenopus laevis* tadpoles and adult female *Xenopus laevis* frogs were purchased from *Xenopus* One (Ann Arbor, MI) and maintained in our laboratory (tadpoles) or in the Massachusetts General Hospital Center for Comparative Medicine animal care facility (frogs). Adult male Sprague-Dawley rats (300–450 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed in the Massachusetts General Hospital Center for Comparative Medicine animal care facility.

All blood draws and all IV drug administrations used a lateral tail vein IV catheter (24 gauge, 19 mm) placed under brief (approximately 1–5 min) sevoflurane anesthesia delivered using an agent-specific variable bypass vaporizer with continuous gas monitoring. Animals were weighed immediately before IV catheter placement and were allowed to fully recover from sevoflurane exposure (at least 15 to 30 min) before any study. IV catheters were secured with tape and the tail was further secured with tape to a 1-inch by 6-inch rigid, acrylic support to prevent catheter dislodgement.

During all studies, rats were placed on a warming stage (Kent Scientific, Torrington, CT). Rectal temperatures were maintained between 36 and 38°C (BAT-12; Kent Scientific) as confirmed immediately upon recovery of righting reflexes and/or completion of measurements.

### Synthesis of MOC-Etomidate

**Synthesis of (R)-1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid (**1**).** A solution of (R)-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate ((R)-etomidate, 281

mg, 1.2 mmol) in methanol (5 ml) and 10% aqueous NaOH (1.7 ml) was refluxed for 30 min. After cooling, the solution was neutralized with 12 M HCl (0.351 ml). The mixture was dried by rotary evaporation, the residue was suspended in methanol-dichloromethane 1:4 v/v, and the sodium chloride was removed by filtration. (R)-1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid **1** was obtained by chromatography on a silica gel column, equilibrated with methanol-dichloromethane 1:4 v/v to give 220 mg (85% yield) of the product (fig. 1). <sup>1</sup>HNMR spectrum: (CD<sub>3</sub>OD) δ 9.30(d, 1H, imidazole CH), 8.23 (d, 1H, imidazole CH), 7.37 (m, 5H, phenyl), 6.64 (q, 1H, methine), 1.97 (d, 3H, methyl).

**Synthesis of Methyl-3-hydroxypropanoate (**2**).** The compound was prepared essentially as described by Bartlett and Rylander.<sup>24</sup> β-Propiolactone (4.36 g, 60.5 mmol) was added dropwise to a stirred solution of sodium methoxide (121 mg, 2.24 mmol) in anhydrous methanol (15 ml) at –78°C. The mixture was neutralized by adding an equivalent amount of HCl (2.24 ml of 1 M HCl). The mixture was filtered and rotary evaporated to remove methanol, and the oily residue was distilled at reduced pressure to obtain methyl-3-hydroxypropanoate **2** (2.72 g, 43% yield). <sup>1</sup>HNMR spectrum: (CDCl<sub>3</sub>) δ 3.88 (t, 2H, methylene), 3.73 (s, 3H, methyl), 2.59 (d, 2H, methylene).

**Synthesis of (R)-3-methoxy-3-oxopropyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate (MOC-etomidate, **3**).** To a mixture of (R)-1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid **1** (220 mg, 1 mmol) and methyl-3-hydroxypropanoate **2** (115 mg, 1.1 mmol) in anhydrous dichloromethane (3.5 ml) was added dicyclohexylcarbodiimide (139 mg, 1.1 mmol) and *p*-dimethylaminopyridine (134 mg, 1.1 mmol). The solution was stirred at room temperature for 48 h. The precipitate was removed by

filtration, and the clear solution was applied to a silica gel column equilibrated with dichloromethane. Elution with 10% ether in dichloromethane gave the product, which was further purified by preparative thin layer chromatography with hexane-ethyl acetate 1:1 v/v on 1-mm-thick silica gel plate. The oily product was treated with HCl in anhydrous ether to obtain white, crystalline (R)-3-methoxy-3-oxopropyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate.HCl (MOC-etomidate.hydrochloride; 198 mg, 59% yield). This product was pure as judged by high-performance liquid chromatography. <sup>1</sup>HNMR spectrum: (CDCl<sub>3</sub>) δ 8.92 (d, 1H, imidazole CH), 7.76 (d, 1H, imidazole CH), 7.36 (m, 5H, phenyl), 6.49 (q, 1H, methine), 4.60 (m, 2H, methylene), 3.73 (s, 3H, methyl), 2.76 (t, 2H, methylene), 2.01 (d, 3H, methyl).

### Tadpole LORR

Groups of five early prelimb-bud stage *Xenopus laevis* tadpoles were placed in 100 ml of oxygenated water buffered with 2.5 mM Tris HCl buffer (pH = 7.4) and containing a concentration of MOC-etomidate ranging from 0.1–128 μM. Tadpoles were manually tipped every 5 min with a flame-polished pipette until the response stabilized. Tadpoles were judged to have loss of righting reflex (LORR) if they failed to right themselves within 5 s after being turned supine. At the end of each study, tadpoles were returned to fresh water to assure reversibility of hypnotic action. The EC<sub>50</sub> for LORR was determined from the MOC-etomidate concentration-dependence of LORR using the method of Waud.<sup>25</sup>

### GABA<sub>A</sub> Receptor Electrophysiology

Adult female *Xenopus laevis* frogs were anesthetized with 0.2% tricaine (ethyl-m-aminobenzoate) and hypothermia. Ovary lobes were then excised through a small laparotomy incision and placed in OR-2 solution (82 mM NaCl, 2 mM KCl, 2 mM MgCl<sub>2</sub>, 5 mM HEPES, pH 7.5) containing collagenase 1A (1 mg/ml) for 3 h to separate oocytes from connective tissue.

Stage 4 and 5 oocytes were injected with messenger RNA encoding the α<sub>1</sub>, β<sub>2</sub>, and γ<sub>21</sub> subunits of the human γ-aminobutyric Acid Type A (GABA<sub>A</sub>) receptor (40 ng of messenger RNA total at a subunit ratio of 1:1:2). This messenger RNA was transcribed from complementary DNA encoding for GABA<sub>A</sub> receptor α<sub>1</sub>, β<sub>2</sub>, and γ<sub>21</sub> subunits by using the mMESSAGE mMACHINE High-Yield Capped RNA Transcription Kit (Ambion, Austin, Tx). Injected oocytes were incubated in ND-96 buffer solution (96 mM NaCl, 2 mM KCl, 1 mM CaCl<sub>2</sub>, 0.8 mM MgCl<sub>2</sub>, 10 mM HEPES, pH 7.5) containing 50 U/ml penicillin and 50 μg/ml streptomycin at 17°C for at least 18 h before electrophysiological experiments.

All electrophysiological recordings were performed using the whole cell two-electrode voltage-clamp technique. Oocytes were placed in a 0.04-ml recording chamber and impaled with capillary glass electrodes filled

with 3 M KCl and possessing open tip resistances less than 5 MΩ. Oocytes were then voltage-clamped at -50 mV by using a GeneClamp 500B amplifier (Axon Instruments, Union City, CA), and constantly perfused with ND-96 buffer at a rate of 4–6 ml/min. Buffer perfusion was controlled by using a six-channel valve controller (Warner Instruments, Hamden, CT) interfaced with a Digidata 1322A data acquisition system (Axon Instruments), and driven by a Dell personal computer (Round Rock, TX). Current responses were recorded by using Clampex 9.2 software (Axon Instruments), and they were processed using a Bessel (8-pole) low-pass filter with a cutoff at 50 Hz using Clampfit 9.2 software (Axon Instruments).

For each oocyte, the concentration of γ-aminobutyric acid (GABA) that produces 5–10% of the maximal current response (EC<sub>5–10</sub> GABA) was determined by measuring the peak current responses evoked by a range of GABA concentrations (in ND-96 buffer) and comparing them to the maximal peak current response evoked by 1 mM GABA. The effect of each hypnotic (*i.e.*, MOC-etomidate or etomidate) on EC<sub>5–10</sub> GABA-evoked currents was then assessed by first perfusing the oocyte with EC<sub>5–10</sub> GABA for 90 s and measuring the control peak evoked current. After a 5-min recovery period, the oocyte was perfused with drug for 90 s and then with EC<sub>5–10</sub> GABA plus hypnotic for 90 s and the peak evoked current was measured again. After another 5-min recovery period, the control experiment (*i.e.*, no drug) was repeated to assure reversibility. The peak current response in the presence of drug was then normalized to the average peak current response of the two control experiments. Drug-induced potentiation was quantified from the normalized current responses in the presence *versus* absence of hypnotic.

The effects of hypnotics on the GABA EC<sub>50</sub> for peak current activation were determined as described above, except that a wide range of GABA concentrations was used to evoke GABA<sub>A</sub> receptor current responses. All currents were normalized to that evoked by 1 mM GABA in the same oocyte in the absence of hypnotic. The EC<sub>50</sub> for peak current activation was then calculated from the GABA concentration-dependence of the normalized peak current response using a Hill equation.

### Metabolic Stability and Metabolite Identification

The metabolic stabilities of MOC-etomidate and etomidate were assessed *in vitro* by adding each (20 μM from a 10 mM ethanolic stock solution) to a 1-ml incubation mixture containing 0.3 mg of pooled liver S9 fraction with 1 mM nicotinamide adenine dinucleotide phosphate in phosphate buffer. After the desired time interval (5–40 min) at 37°C, 100-μl aliquots of the mixture were withdrawn, and metabolism stopped by vortexing with 200 μl of acetonitrile. After centrifugation (10,000g for 15 min), the supernatant was removed and evaporated



to dryness under vacuum. The drug was then reconstituted in H<sub>2</sub>O/acetonitrile (98/2%), and its relative levels were determined by liquid chromatography (LC)/mass spectrometry (MS) by using a Thermo Finnigan TSQ 7000 mass spectrometer (Thermo Finnigan, San Jose, CA) operating in electrospray ionization mode and interfaced with a Michrom BioResources Paradigm MS4 high-performance liquid chromatograph (Michrom Bioresources, Auburn, CA). The spectrometer was operating under the following conditions: polarity positive, capillary temperature 375°C, spray voltage 4.5 V, sheath gas (nitrogen) pressure 70 psi, and auxiliary gas flow 5 l/m. Argon was used as the collision gas at 2.0 mT. The MS was operated in the selected reaction monitoring mode using a MS/MS transition unique to the parent compound. The LC method used water (A) and acetonitrile (B) as the mobile phase, 0.1% formic acid as a mobile phase modifier, a rapid linear gradient from 2% A to 100% B in 3 min at 0.7  $\mu$ l/min, and a 2.1 mm  $\times$  20 mm MAGIC C18-AQ Bullet column from Michrom BioResources. The retention times of MOC-etomidate and its metabolite were 11.5 min and 9.4 min, respectively. *In vitro* half-life was calculated by curve fitting a plot of percent parent drug remaining values. Each percent parent drug remaining value was calculated from the ratio of parent compound signal (MS peak area) observed at each time point *versus* the time zero sample. Although this rapid *in vitro* approach is not useful for determining absolute concentrations in a given sample, it is satisfactory for estimating and comparing metabolic stability among related analogues. For metabolite identification, an aliquot obtained after 40 min of incubation with liver S9 fraction (same as above) was analyzed using high-performance LC/ion trap MS. The same ion source and LC devices were used for the profiling analysis. The LC method was modified for detailed analysis using a longer linear gradient (75% B in 24 min at 300  $\mu$ l/min) and longer column (150 mm). The ion trap MS (Thermo Finnigan LTQ equipped with an Ion Max Source) was operated in data-dependent MS/MS mode; whereby full and product ion spectra were obtained on all major components/ions observed throughout the LC run. The spectrometer was operating under the following conditions: polarity positive, capillary temperature 350°C, capillary voltage 40 V, spray voltage 4.00 kV, and tube lens voltage 100 V. Proposed structures were generated by comparing a given metabolite's product ion spectra with the product ion spectrum of the known parent compound. In addition, MS/MS spectra were observed on several source-generated fragments from each metabolite. These source fragments matched expected fragment ions produced in the corresponding product ion spectra of each parent compound and were used for elucidative purposes as well.

### *Rat LORR*

Rats were briefly restrained in a 3-inch-diameter, 9-inch-long acrylic chamber with a tail exit port. The desired dose of hypnotic was injected through a lateral tail vein catheter followed by an approximately 1-ml normal saline flush. Immediately after injection, rats were removed from the restraint device and turned supine. A rat was judged to have LORR if it failed to right itself (onto all four paws) within 5 s of drug administration. A stopwatch was used to measure the duration of LORR, which was defined as the time from drug injection until the animal spontaneously righted itself. The ED<sub>50</sub> for LORR upon bolus administration was determined from the dose-dependence of LORR by using the method of Waud.<sup>25</sup>

### *Rat Hemodynamics*

Femoral arterial catheters, tunneled between the scapulas, were preimplanted by the vendor (Charles River Laboratories). Animals were fully recovered from the placement procedure upon arrival. During housing and between studies, catheter patency was maintained with a heparin (500 U/ml) and hypertonic (25%) dextrose-locking solution, which was withdrawn before each use and replaced just after.

On the day of study and after weighing and lateral tail vein IV catheter placement, rats were restrained in the acrylic tube with a tail exit port and allowed to acclimate for approximately 15 to 20 min before data collection. The signal from the pressure transducer (TruWave, Edwards Lifesciences, Irvine, CA) was amplified by using a custom built amplifier (AD620 operational amplifier; Jameco Electronics, Belmont, CA) and digitized (1 kHz) using a USB-6009 data acquisition board (National Instruments, Austin, TX) without additional filtering. All data were acquired and analyzed by using LabView Software (version 8.5 for Macintosh OS X; National Instruments).

Data used for heart rate and blood pressure analysis were recorded for 5 min immediately before drug administration and for 15 min thereafter. All drugs were administered through the tail vein catheter followed by approximately 1-ml normal saline flush.

### *Rat Adrenocortical Suppression*

Methods for study of rat adrenal function were adapted and optimized from several previously published reports.<sup>26–28</sup> Immediately after weighing and IV catheter placement, dexamethasone (0.2 mg/kg IV; American Regent, Shirley, NY) was administered to each rat to inhibit endogenous adrenocorticotrophic hormone (ACTH) release, to suppress baseline corticosterone production, and to inhibit the variable stress response to restraint and handling. The IV tail vein catheter, used for both drug administration and blood draws, was heparin-locked after each use with 10 U/ml heparin to maintain patency; the heparin locking solution was wicked out of

the catheter before drug administration and blood draws to minimize rat and sample heparinization. All blood draws were approximately 0.3 ml in volume. All drugs administrations were followed by a 1 ml normal saline flush to assure complete drug delivery.

Two hours after dexamethasone treatment, blood was drawn (for baseline measurement of serum corticosterone concentration) and a second dose of dexamethasone (0.2 mg/kg) was administered along with either IV MOC-etomidate, etomidate, or vehicle (35% propylene glycol v/v in water) as a control. Fifteen minutes later, ACTH<sub>1-24</sub> (25 µg/kg; Sigma-Aldrich Chemical Co, St. Louis, MO) was given intravenously to stimulate corticosterone production. Fifteen minutes after ACTH<sub>1-24</sub> administration (*i.e.*, 30 min after drug or vehicle administration), a second blood sample was drawn to measure the ACTH<sub>1-24</sub>-stimulated serum corticosterone concentration. ACTH<sub>1-24</sub> was dissolved in 1 mg/ml deoxygenated water as stock, aliquoted, and frozen (−20°C); a fresh aliquot was thawed just before each use. Rats in all three groups (vehicle, etomidate, and MOC-etomidate) received the same volume of propylene glycol.

Blood samples were allowed to clot at room temperature (10 to 60 min) before centrifugation at 3,500g for 5 min. Serum was carefully expressed from any resulting superficial fibrin clot by using a clean pipette tip before a second centrifugation at 3,500g for 5 min. After the second centrifugation, the resultant straw-colored, clot-free serum layer was transferred to a fresh vial for a final, high-speed centrifugation (16,000g for 5 min) to pellet any contaminating red blood cells or particulates. The serum was transferred to a clean vial and promptly frozen (−20°C) pending corticosterone measurement within 1 to 2 days. After thawing and heat inactivation of corticosterone-binding globulins (65°C for 20 min), serum baseline and ACTH<sub>1-24</sub>-stimulated corticosterone concentrations were quantified by using an enzyme-linked immunosorbent assay (ELISA; Diagnostic Systems Laboratories, Webster, TX) and a 96-well plate reader (Molecular Devices, Sunnyvale, CA).

### Statistical Analysis

All data are reported as mean ± SD. Statistical analysis and curve fitting (using linear or nonlinear least squares regression) were done using either Prism v4.0 for Macintosh (GraphPad Software, Inc., LaJolla, CA) or Igor Pro 4.01 (Wavemetrics, Lake Oswego, OR). Metabolic half-life data were linearized by log transformation before analysis. Statistical significance indicates  $P < 0.05$  unless otherwise indicated. For multiple comparisons of physiologic data derived from rats, we performed a one-way analysis of variance (ANOVA) followed by either a Newman-Keuls or a Bonferroni post-test (which relies on an unpaired  $t$  test with a Bonferroni correction).

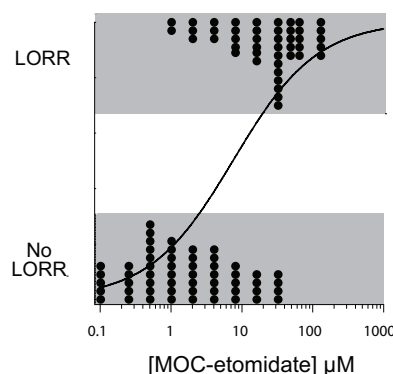


Fig. 2. Methoxycarbonyl-etomidate (MOC-etomidate) concentration response curve for loss of righting reflex (LORR) in tadpoles. Each data point represents the results from a single tadpole. The curve is a fit of the data set yielding an EC<sub>50</sub> of  $8 \pm 2$  µM. All tadpoles recovered when removed from methoxycarbonyl-etomidate and returned to fresh water.

## Results

### Loss of Righting Reflexes in Tadpoles by MOC-Etomidate

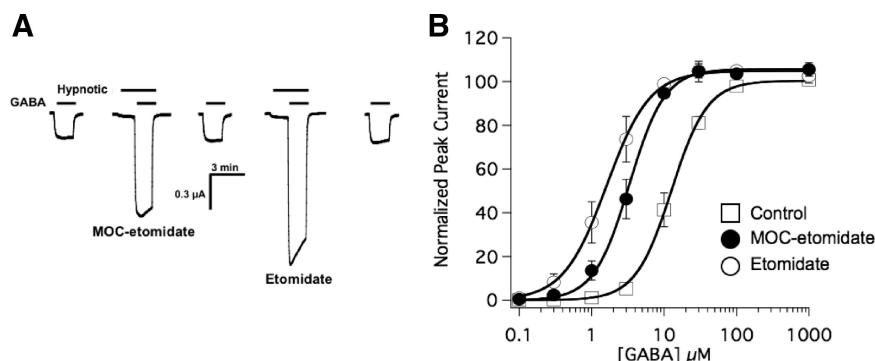
The MOC-etomidate concentration-response relationship for LORR in *Xenopus laevis* tadpoles ( $n = 100$ ) is shown in figure 2. The fraction of tadpoles that had LORR increased with MOC-etomidate concentration and at the highest MOC-etomidate concentrations studied (48–128 µM), all tadpoles had LORR. All tadpoles that had LORR recovered their righting reflexes when removed from MOC-etomidate and returned to fresh water. MOC-etomidate's EC<sub>50</sub> for LORR was  $8 \pm 2$  µM (mean ± SD), a value that is 3.5-fold higher than etomidate's previously reported EC<sub>50</sub> for LORR from this laboratory.<sup>22</sup>

### GABA<sub>A</sub> Receptor Modulation by MOC-etomidate and Etomidate

At the molecular level, there is compelling evidence that etomidate produces hypnosis by modulating the function of GABA<sub>A</sub> receptors containing  $\beta_2$  or  $\beta_3$  subunits.<sup>29–31</sup> To test whether MOC-etomidate acts by a similar mechanism, we defined its effects on human  $\alpha_1\beta_2\gamma_{2L}$  GABA<sub>A</sub> receptors. Figure 3A shows representative current traces recorded upon perfusing an oocyte expressing  $\alpha_1\beta_2\gamma_{2L}$  GABA<sub>A</sub> receptors with EC<sub>5–10</sub> GABA alone or along with either MOC-etomidate or etomidate at their respective EC<sub>50</sub>s for producing LORR in tadpoles. Both MOC-etomidate and etomidate significantly potentiated GABA-evoked currents. MOC-etomidate enhanced the peak current amplitudes of GABA-evoked currents by  $450 \pm 130\%$  ( $n = 6$  oocytes). In the same six oocytes, etomidate enhanced peak currents by  $660 \pm 240\%$ , a value that was not significantly different from that produced by MOC-etomidate (Student  $t$  test).

Although both drugs enhanced currents evoked by low GABA concentrations, they had relatively little effect on currents evoked by high GABA concentrations (fig.

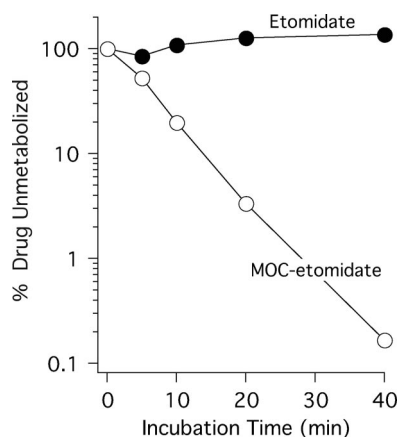
**Fig. 3.** Methoxycarbonyl-etomidate (MOC-etomidate) and etomidate modulation of human  $\alpha_1\beta_2\gamma_{2L}$   $\gamma$ -aminobutyric acid type A receptor function. **(A)** Representative traces showing enhancement of currents evoked by EC<sub>5-10</sub>  $\gamma$ -aminobutyric acid (GABA) by methoxycarbonyl-etomidate and etomidate. **(B)** GABA concentration-response curves for peak current activation in the absence (control) or presence of either methoxycarbonyl-etomidate or etomidate. Error bars indicate the SD. Both drugs shifted the GABA concentration-response curves leftward, reducing the GABA EC<sub>50</sub> from  $12.7 \pm 0.4 \mu\text{M}$  in the absence of drug to  $3.3 \pm 0.1 \mu\text{M}$  and  $1.6 \pm 0.1 \mu\text{M}$  in the presence of  $8 \mu\text{M}$  methoxycarbonyl-etomidate and  $2 \mu\text{M}$  etomidate, respectively ( $n = 3$  for each data point).



3B). This produced a statistically significant leftward shift in the GABA concentration-response curves, reducing the GABA EC<sub>50</sub> from  $12.7 \pm 0.4 \mu\text{M}$  in the absence of drug to  $3.3 \pm 0.1 \mu\text{M}$  and  $1.6 \pm 0.1 \mu\text{M}$  in the presence of  $8 \mu\text{M}$  MOC-etomidate and  $2 \mu\text{M}$  etomidate, respectively.

#### In Vitro Metabolism of MOC-etomidate and Etomidate

As the first step towards evaluating MOC-etomidate metabolism, we defined its stability in pooled human liver S9 fraction. Figure 4 plots the percentage of unmetabolized drug remaining as a function of incubation time in pooled human liver S9 fraction (+ nicotinamide adenine dinucleotide phosphate) on a semilogarithmic scale. Even after 40 min, we could detect no reduction in the concentration of etomidate, indicating that its metabolic half-life was much longer than 40 min. In contrast, MOC-etomidate was rapidly metabolized in human liver S9 fraction. The concentration of MOC-etomidate decreased as a first-order process reaching less than 1% of the original concentration by 40 min. From this data, the metabolic half-life of MOC-etomidate was determined to be approximately 4.4 min. In these studies, buspirone



**Fig. 4.** Metabolic stability of methoxycarbonyl-etomidate (MOC-etomidate) versus etomidate in pooled human liver S9 fraction. The metabolic half-life of methoxycarbonyl-etomidate was approximately 4.4 min. There was no detectable metabolism of etomidate during the 40-min incubation period.

was used as an internal standard to confirm metabolic activity in the liver fraction. Its metabolic half-life was approximately 15.4 min (data not shown).

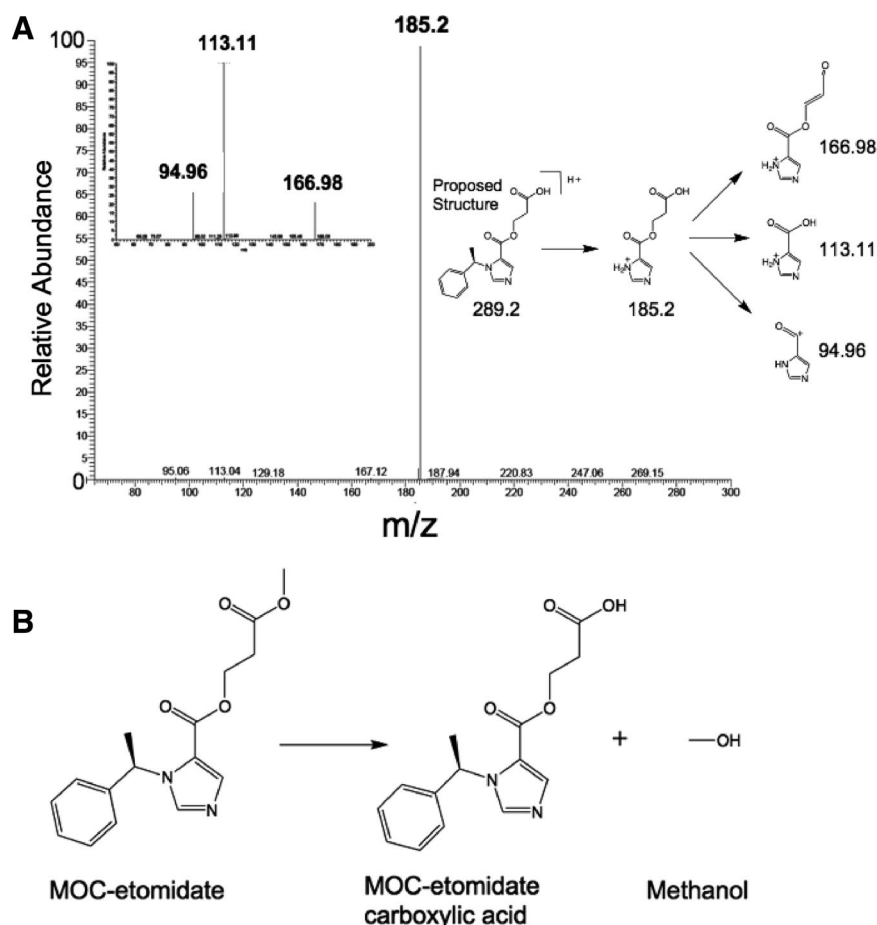
The structure of the metabolite formed after 40 min of incubation in pooled human liver S9 fraction (+ nicotinamide adenine dinucleotide phosphate) was analyzed by using high performance LC/ion trap MS. The ion chromatogram detected the presence of only one major metabolite. It had an  $m/z$  of 289.2, which is consistent with the carboxylic acid formed upon hydrolysis of MOC-etomidate's distal ester moiety. Figure 5A shows the MS/MS spectra of the major metabolite (main spectrum) and its major fragment ion (left inset spectrum). The right inset shows a possible fragmentation pathway supporting the proposed metabolite structure. On the basis of these results, we conclude that rapid metabolism of MOC-etomidate likely occurs exclusively *via* the designed pathway shown in figure 5B in which the distal ester moiety of MOC-etomidate is hydrolyzed to form the corresponding carboxylic acid along with methanol as the leaving group.

#### LORR in Rats by Propofol, Etomidate, and MOC-etomidate

The hypnotic potencies and durations of hypnotic action of MOC-etomidate were compared with those of etomidate and propofol in a rat model. Figure 6A shows the propofol, etomidate, and MOC-etomidate dose-response relationships for LORR in rats. The fraction of rats that had LORR increased with the dose. At the highest doses, all rats had LORR, and there was no obvious toxicity. From these data, the ED<sub>50</sub>s for LORR after bolus administration of etomidate, propofol, and MOC-etomidate were determined to be  $1.00 \pm 0.03 \text{ mg/kg}$  ( $n = 18$ ),  $4.1 \pm 0.3 \text{ mg/kg}$  ( $n = 20$ ), and  $5.2 \pm 1 \text{ mg/kg}$  ( $n = 20$ ), respectively.

At doses sufficient to produce LORR in rats, all three drugs produced LORR within several seconds of IV bolus administration. The duration of LORR increased approximately linearly with the logarithm of the dose (fig. 6B); however, the slope of this relationship, which depends on the drug's half-life in the brain,<sup>32,33</sup> was an order of





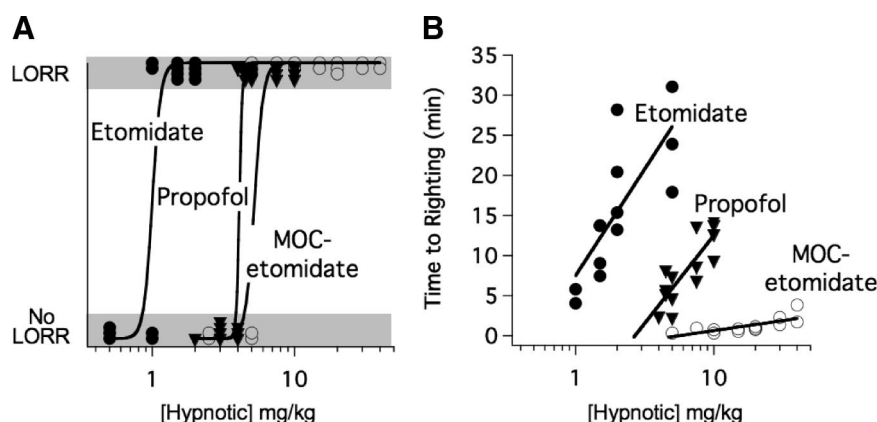
**Fig. 5.** Methoxycarbonyl-etomidate (MOC-etomidate) metabolite identification. **(A)** Mass spectrometry spectra of the major metabolite (main spectrum;  $m/z$  289.2) and its major fragment ion (left inset spectrum;  $m/z$  185.2). The metabolite produced a single major fragment ion at  $m/z$  185.2 with a neutral loss of  $m/z$  104, consistent with a conserved region of the parent compound. Subsequent mass spectrometry/mass spectrometry analysis of  $m/z$  185.2 produced three major ions at  $m/z$  94.96, 113.11, and 166.98. **Right inset** shows a possible fragmentation pathway supporting the proposed metabolite structure. **(B)** Metabolic pathway for methoxycarbonyl-etomidate upon incubation with human liver S9 fraction based on analysis of the metabolite's major fragment ion.

magnitude lower for MOC-etomidate ( $2.8 \pm 0.4$ ) than for etomidate ( $27 \pm 7$ ) or propofol ( $22 \pm 4$ ). The slopes for etomidate and propofol were not significantly different from one another.

#### *Hemodynamic Actions of Propofol, Etomidate, and MOC-etomidate in Rats*

Etomidate is often chosen for induction over other agents in the critically ill patient because it better preserves hemodynamic stability. To determine whether MOC-etomidate similarly preserves hemodynamic stability, we measured and compared the actions of propofol,

etomidate, MOC-etomidate, and vehicle (35% v/v propylene glycol in water) on heart rate and blood pressure in rats. To compare these drugs at equihypnotic doses, each was administered intravenously at twice its  $ED_{50}$  for LORR (*i.e.*, 2 mg/kg etomidate, 10 mg/kg MOC-etomidate, and 8 mg/kg propofol). The volume of propylene glycol administered was the same for vehicle, etomidate, and MOC-etomidate groups. After animal acclimatization, data were recorded for 5 min before (baseline) and for 15 min after drug/vehicle injection (fig. 7). Rats in each group had similar mean heart rates and blood pressure at baseline over the first 5 min ( $391 \pm 49$



**Fig. 6.** Dose-response curves for loss of righting reflex (LORR) and duration of LORR in rats. Each data point represents the results from a single rat. **(A)** Etomidate, propofol, and methoxycarbonyl-etomidate (MOC-etomidate) produced LORR with  $ED_{50}$ s of  $1.00 \pm 0.03$  mg/kg,  $4.1 \pm 0.3$  mg/kg, and  $5.2 \pm 1$  mg/kg, respectively. **(B)** For all three drugs, the duration of LORR increased linearly with the logarithm of the dose. The slope of this relationship was  $27 \pm 7$ ,  $22 \pm 4$ , and  $2.8 \pm 0.4$  for etomidate, propofol, and methoxycarbonyl-etomidate, respectively.

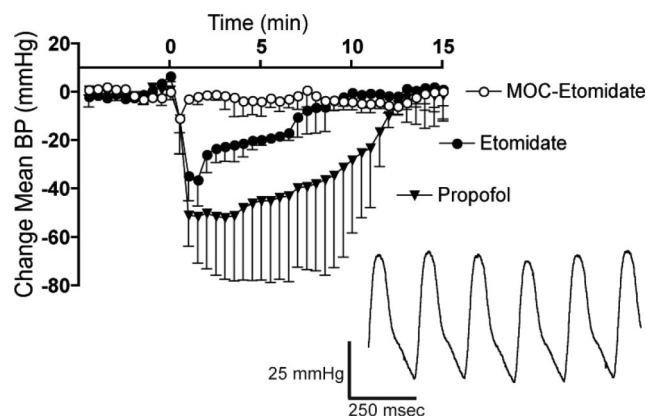


Fig. 7. The effects of methoxycarbonyl-etomidate (MOC-etomidate), propofol, and etomidate on mean blood pressure (BP) in rats. Drugs were given at doses equal to twice their respective  $ED_{50}$ s for loss of righting reflex. Drug was injected at time 0. Each data point represents the average ( $\pm$  SD) change in mean blood pressure from three rats during each 30-s epoch. The inset shows a representative arterial blood pressure trace before drug administration.

beats/min,  $118 \pm 9$  mm Hg). Vehicle caused no significant change in mean blood pressure relative to baseline ( $5 \pm 11$  mm Hg,  $n = 3$ , at 90 s); data not shown in figure 7 for clarity. However, MOC-etomidate, etomidate, and propofol ( $n = 3$  animals each) each caused a significant decrease in mean blood pressure relative to baseline and to each other in this rank order for both maximum magnitude ( $-11 \pm 15$  mm Hg,  $-36 \pm 11$  mm Hg, and  $-51 \pm 19$  mm Hg, respectively) and duration of significant effect (30 s, 6.5 min, and 7 min, respectively). For all groups, vehicle ( $36 \pm 14$  beats/min), MOC-etomidate ( $24 \pm 33$  beats/min), etomidate ( $49 \pm 67$  beats/min), and propofol ( $64 \pm 56$  beats/min), there was a small, transient, and variable increase in heart rate shortly after injection.

#### Adrenocortical Suppression after Administration of Etomidate and MOC-etomidate

To test whether MOC-etomidate produced prolonged adrenocortical suppression, we measured ACTH<sub>1-24</sub>-stimulated serum corticosterone concentrations in dexamethasone pretreated rats that had received MOC-etomidate, etomidate, or vehicle. Baseline serum corticosterone concentrations in rats ( $n = 12$ ) after dexamethasone administration averaged  $29 \pm 39$  ng/ml and were not significantly different among the three groups (vehicle, etomidate, and MOC-etomidate). Injection of ACTH<sub>1-24</sub> stimulated adrenocortical steroid production as all rats had significantly higher serum corticosterone concentrations fifteen minutes after ACTH<sub>1-24</sub> administration. However figure 8 shows that rats that had received etomidate fifteen minutes prior to ACTH<sub>1-24</sub> stimulation had significantly lower serum corticosterone concentrations than those that had received either vehicle or an equi-hypnotic dose of MOC-etomidate. In contrast, rats that had received MOC-etomidate had serum corticoste-

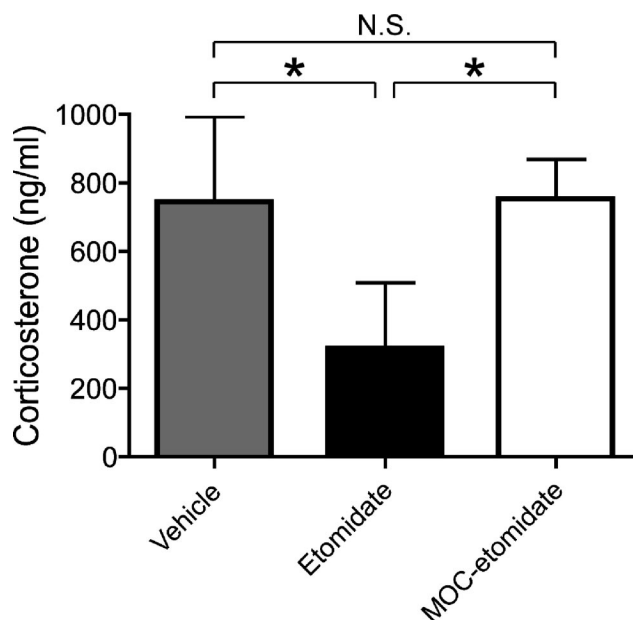


Fig. 8. Adrenocorticotrophic hormone<sub>1-24</sub>-stimulated serum corticosterone concentrations in rats 30 min after administration of vehicle, etomidate, or methoxycarbonyl-etomidate (MOC-etomidate). Drugs were given at doses equal to twice their respective  $ED_{50}$ s for loss of righting reflex. Four rats were studied in each group. Average serum corticosterone concentrations ( $\pm$  SD) were  $740 \pm 125$  ng/ml,  $320 \pm 97$  ng/ml, and  $750 \pm 58$  ng/ml after administration of vehicle, etomidate, and methoxycarbonyl-etomidate, respectively. \*  $P < 0.05$ . N.S. = no significant difference.

rone concentrations that were not different from those that had received only vehicle.

## Discussion

MOC-etomidate is a well-tolerated etomidate analogue that retains etomidate's important favorable pharmacological properties, including rapid onset of action, high hypnotic potency, and hemodynamic stability. Like etomidate, it potently enhances GABA<sub>A</sub> receptor function. However, in contrast to etomidate, MOC-etomidate is very rapidly metabolized, ultra-short-acting, and does not produce prolonged adrenocortical suppression after single IV bolus administration.

MOC-etomidate is a soft analogue of etomidate. A soft analogue is a derivative of a parent compound that is specifically designed to undergo rapid and predictable metabolism after exerting its therapeutic actions.<sup>34</sup> Commonly used soft analogues include the opioid remifentanyl and the  $\beta$ -blocker esmolol. Both of these compounds contain labile carboxylate ester moieties that are rapidly hydrolyzed to carboxylic acids by esterases found in various organs and/or blood. The elimination half-life of these two drugs in humans is 1–2 orders of magnitude shorter than their non-ester-containing analogues fentanyl and propranolol.<sup>35–40</sup> Etomidate also contains a carboxylate ester moiety that is hydrolyzed by liver esterases



to a carboxylic acid, but it is a poor substrate for these esterases as reflected by its several hour elimination half-life. Comparison of the structures of remifentanyl and esmolol with that of etomidate suggests two reasons for etomidate's slow rate of ester hydrolysis. *First*, the ester moiety in etomidate is attached directly to its imidazole ring, whereas the labile ester moieties in remifentanyl and esmolol are attached to ring structures *via* a spacer composed of two  $\text{CH}_2$  groups. This spacer may be critical because it reduces steric hindrance, allowing esterases freer access to the carbonyl group. In support of this, as esmolol's spacer is decreased in length, its rate of ester hydrolysis decreases.<sup>34</sup> *Second*, the electrons in etomidate's carbonyl group contribute to a  $\pi$  electron system that extends into the imidazole ring. This reduces the carbonyl carbon's partial positive charge, making it a poorer substrate for nucleophilic attack by esterases.

On the basis of this reasoning, we developed the strategy of adding a new ester moiety to etomidate that is both sterically unhindered and electronically isolated from the  $\pi$  electron systems in the imidazole ring to produce an etomidate analogue that would be rapidly metabolized. We expected that this ester moiety, like those in remifentanyl and esmolol, would be rapidly hydrolyzed by esterases present in various tissues and/or blood. This was confirmed by our *in vitro* metabolic studies of MOC-etomidate that showed that this moiety was rapidly metabolized to a carboxylic acid in S9 liver fraction, a commonly used *in vitro* drug biotransformation assay. Future work will need to define the specific *in vivo* site (e.g., blood, plasma, and/or liver) and to confirm the identity of *in vivo* metabolites. In addition, a more complete understanding of MOC-etomidate metabolism may suggest methods by which the duration of action of future related drugs might be further optimized through changes in drug structure (e.g., changes in spacer length or leaving group).

Our studies demonstrated that MOC-etomidate is a hypnotic in two species. It has a potency that is one-fourth to one-fifth of etomidate's potency and likely produces hypnosis *via* the same receptor mechanism. Our rat studies further demonstrated that MOC-etomidate is an ultra-short-acting hypnotic, even when given at large multiples of its  $\text{ED}_{50}$  for LORR. For example, when given at dose that is 4 times its  $\text{ED}_{50}$  for LORR (20 mg/kg), MOC-etomidate produced LORR in rats for only  $55 \pm 11$  s. In comparison, propofol and etomidate produced LORR for  $9.7 \pm 3.5$  min and  $24 \pm 7$  min, respectively, at approximately equihypnotic doses.

Recovery from IV bolus administration of propofol and etomidate is considered to reflect redistribution of drug from the brain to other tissues rather than metabolism. Therefore, the similar slopes in the relationship between the duration of LORR and the logarithm of the drug dose (fig. 6B) suggests that propofol and etomidate redistribute from the brain at similar rates. The much faster recov-

ery of righting reflexes and shallower slope of this relationship with MOC-etomidate suggests that ultra-rapid metabolism contributes significantly to the termination of MOC-etomidate's hypnotic action.

MOC-etomidate produced a correspondingly brief (30-s) reduction in blood pressure, suggesting that MOC-etomidate's hemodynamic effects also terminate upon metabolism. In addition, we found that the maximum magnitude of this reduction was significantly less after administration of MOC-etomidate than after administration of equihypnotic doses of etomidate or propofol. Thus, it is possible to modify etomidate's chemical structure while retaining its favorable hemodynamic effects.

In common with other hydrophobic imidazole-containing compounds, etomidate suppresses adrenocortical steroid production.<sup>9,10,41,42</sup> The primary mechanism underlying this suppression is inhibition of  $11\beta$ -hydroxylase, a critical enzyme in the biosynthetic pathway leading to adrenocortical synthesis of cortisol, corticosterone, and aldosterone.<sup>43</sup> It has been hypothesized that etomidate inhibits  $11\beta$ -hydroxylase by competing with steroid precursors at the enzyme's presumably hydrophobic catalytic site.<sup>44</sup> MOC-etomidate was designed to be rapidly metabolized by esterases to a highly polar carboxylic acid; therefore, we expected that MOC-etomidate would not produce prolonged adrenocortical suppression after administration. This expectation was realized as 30 minutes after administration, MOC-etomidate produced no reduction in the ACTH<sub>1-24</sub>-stimulated serum corticosterone concentration, whereas an equihypnotic dose of etomidate significantly reduced it. Our results also imply that any effect of MOC-etomidate's rapidly formed metabolite(s) on corticosterone synthesis is negligible after administration of a single IV dose; however, additional studies will be necessary to determine whether the metabolite could reach sufficiently high levels after repeat dosing or a continuous infusion to produce significant adrenocortical suppression. We also acknowledge the possibility that MOC-etomidate may spare adrenal function, at least in part, by binding to  $11\beta$ -hydroxylase with lower affinity than etomidate. This would open the possibility that MOC-etomidate or other etomidate analogues might be developed for continuous infusion, regardless of mode of metabolism, without suppressing adrenal function.

In our studies, we used rats as our experimental model to assess duration of action. Rats and other small animals usually metabolize drugs significantly faster than humans. For example, the elimination half-life of remifentanyl is less than 1 min in Sprague-Dawley rats as compared to 10 min (or longer) in humans.<sup>35,45-47</sup> Therefore, the duration of hypnosis produced by MOC-etomidate will almost certainly be longer in humans than in rats and probably be similar to those of remifentanyl and esmolol (5-10 min), the prototypical esterase-metabolized drugs after which MOC-etomidate was modeled.

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