Modifying Methoxycarbonyl Etomidate Inter-Ester Spacer Optimizes *In Vitro* Metabolic Stability and *In Vivo* Hypnotic Potency and Duration of Action

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ABSTRACT

Background: Methoxycarbonyl etomidate is the prototypical very rapidly metabolized etomidate analog. Initial studies suggest that it may be too short acting for many clinical uses. We hypothesized that its duration of action could be lengthened and clinical utility broadened by incorporating specific aliphatic groups into the molecule to sterically protect its ester moiety from esterase-catalyzed hydrolysis. To test this hypothesis, we developed a series of methoxycarbonyl etomidate analogs (spacer-linked etomidate esters) containing various aliphatic-protecting groups and spacer lengths.

Methods: Spacer-linked etomidate esters were synthesized and their hypnotic potencies and durations of action following bolus administration were measured in rats using a loss-of-righting reflexes assay. Octanol:water partition coefficients and metabolic half-lives in pooled rat blood were determined chromatographically.

Results: All spacer-linked etomidate esters produced hypnosis rapidly and in a dose-dependent manner. ED_{50}s for loss of righting reflexes ranged from 0.69 ± 0.04 mg/kg for cyclopropyl-methoxycarbonyl metomidate to 11.1 ± 0.8 mg/kg for methoxycarbonyl metomidate. The slope of a plot of the duration of loss of righting reflexes *versus* the logarithm of the dose ranged 12-fold among spacer-linked etomidate esters, implying widely varying brain clearance rates. The *in vitro* metabolic half-lives of these compounds in rat blood varied by more than two orders of magnitude and were diastereometrically selective.

Conclusions: We created 13 new analogs of methoxycarbonyl etomidate and identified two that have significantly higher potency and potentially address the too-brief duration of action for methoxycarbonyl etomidate. This work may provide a blueprint for optimizing the pharmacological properties of other soft drugs.

What We Already Know about This Topic

- Methoxycarbonyl etomidate is a soft drug because it has an ester group, linked to the more resistant ester group of etomidate by a two-carbon spacer, that is rapidly hydrolyzed by esterases
- Methoxycarbonyl etomidate is metabolized so rapidly and has such low potency that it must be infused rapidly to maintain hypnosis, producing high metabolite concentrations

What This Article Tells Us That Is New

- Dimethyl-methoxycarbonyl metomidate (DMMM) and cyclopropyl-methoxycarbonyl metomidate (CPMM) have hypnotic potencies in rats, durations of action after bolus administration to rats, and half-lives in rat blood that are intermediate between those of etomidate and methoxycarbonyl etomidate

SOFT drugs are specifically designed to be rapidly metabolized, and are thus short-acting. A common design feature is the presence of an ester group that is highly susceptible to hydrolysis by esterases. Remifentanil and esmolol are examples of soft drugs commonly used by anesthesiologists, whereas remimazolam (an analog of midazolam) and AZD3043 (an analog of the hypnotic propanidid) are not

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Address correspondence to Dr. Raines: Department of Anesthesia and Critical Care, Massachusetts General Hospital, 55 Fruit Street, GRB444, Boston, Massachusetts 02114. draines@partners.org. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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yet approved for clinical use, but are in the development pipeline and have reached human trials.[1–5] Pharmaceutical development in anesthesiology has gravitated toward soft drugs because they allow one to quickly respond to rapidly changing clinical needs, which is a significant advantage in the dynamic operating room environment.[6]

A key feature of all soft drugs is that their metabolic stabilities and durations of action must fall within an optimal range to be most clinically useful.[7] A drug that is too rapidly metabolized and short-acting will require the administration of impractically large quantities to maintain a therapeutic effect and may generate metabolite concentrations sufficient to produce undesirable side effects when given for a prolonged period of time.[8] Conversely, a drug that is too slowly metabolized and long-acting will have pharmacokinetic properties that are not meaningfully different from the metabolically stable "hard" drug from which it was derived. Unfortunately, design strategies for controlling the metabolism of soft drugs to optimize their durations of action and improve their clinical utilities are not well established.

We recently developed methoxycarbonyl etomidate as the prototypical member of a new class of "spacer-linked etomidate esters" to test the hypothesis that soft analogs of etomidate could be designed that produce hypnosis without prolonged adrenocortical suppression (fig. 1).[9] We showed that it is rapidly hydrolyzed in rat blood and human liver s9 fraction and produces hypnosis and adrenocortical suppression of extremely short duration when administered to rats as an intravenous bolus.[8,9] Although rats typically hydrolyze ester-containing drugs faster than large animals, subsequent studies in sheep and dogs indicated that the duration of action for methoxycarbonyl etomidate is similar across species (verbal communication, John Randle, Ph.D., Annovation BioPharma, Inc., Cambridge, MA, October 2011). This suggested that methoxycarbonyl etomidate would be too short-acting for many clinical uses and led us to consider how its duration of action could be lengthened.

Previous studies have shown that esterase-catalyzed hydrolysis of ester moieties is hindered by nearby substituents that protect the ester by steric, electronic, inductive, or, in the case of chiral substituents, by chiral effects.[10,11] This led us to hypothesize that the hydrolysis rate of methoxycarbonyl etomidate could be attenuated and its duration of hypnotic action beneficially lengthened using this molecular design strategy. If this hypothesis were correct, we predicted that it would be possible to develop a family of spacer-linked etomidate esters with members having widely varying pharmacokinetic properties, allowing the identification of compounds with the most promising characteristics for further testing and development. More broadly, our work could provide a blueprint for tailoring the pharmacological properties of other soft drugs to achieve specific clinical needs. To test our hypothesis, we synthesized spacer-linked etomidate esters containing various aliphatic groups appended to the carbon spacer linking the labile ester moiety to the etomidate backbone. We then assessed the impact of these substituent groups on *in vitro* metabolic stability in rat blood and *in vivo* hypnotic potency and duration of action in rats.

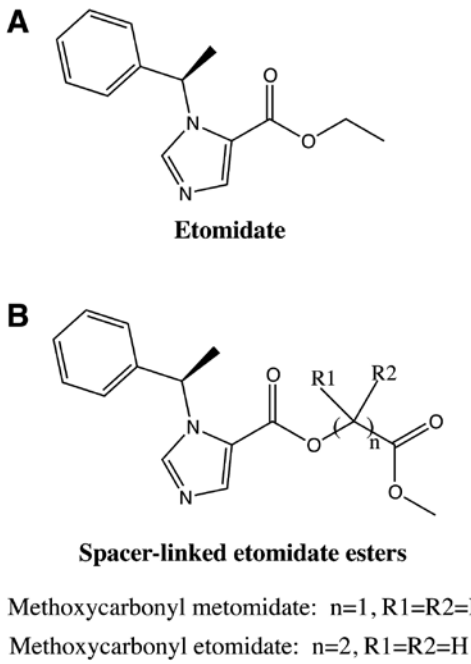


Fig. 1. Structures of (*A*) etomidate and (*B*) spacer-linked etomidate esters. The number of CH_2 groups in the spacer is n.

Materials and Methods

Animals

All studies were conducted in accordance with rules and regulations of the Subcommittee on Research Animal Care at the Massachusetts General Hospital, Boston, Massachusetts. Adult male Sprague-Dawley rats (230–350 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed in the Massachusetts General Hospital Center for Comparative Medicine animal care facility. All drugs were administered through a femoral venous catheter preimplanted by the vendor before animal delivery to our animal care facility.

Hypnotic Drugs

Etomidate was purchased from Bachem (Torrance, CA). Spacer-linked etomidate esters and the (*R*)-enantiomer of metomidate were synthesized (more than 97% purity) either within our laboratory or by Aberjona Laboratories (Beverly, MA), essentially using the approach described previously.[9]

Step 1: Synthesis of (*R*)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic Acid (Hydrolyzed Etomidate). (*R*)-ethyl-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate.HCl [(*R*)-etomidate HCl] in methanol and 10% aqueous NaOH was refluxed for 30 min. After cooling, the solution was neutralized with 12

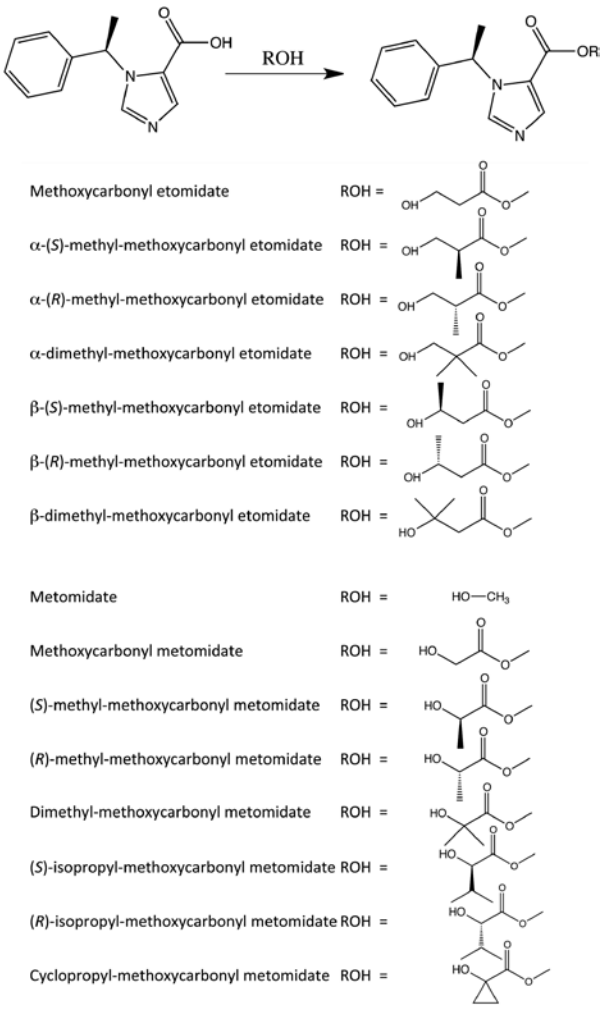


Fig. 2. Each spacer-linked etomidate ester and metomidate was synthesized by coupling the desired alcohol with (*R*)-1-(1-phenyethyl)-1*H*-imidazole-5-carboxylic acid. The *top box* contains the alcohols used to synthesize etomidate analogs, whereas the *bottom box* shows alcohols used to synthesize metomidate and metomidate analogs.

M HCl. The mixture was dried by rotary evaporation, the residue suspended in methanol-dichloromethane 1:4 v/v, and the sodium chloride removed by filtration. (*R*)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid was obtained by chromatography on a silica gel column equilibrated with methanol-dichloromethane 1:4 V/V.

Step 2: Synthesis of Spacer-linked Etomidate Esters and Metomidate. Each spacer-linked etomidate ester (and metomidate) was synthesized by esterification of (*R*)-1-(1-phenyethyl)-1*H*-imidazole-5-carboxylic acid with the appropriate alcohol (fig. 2), using the coupling agent 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride and the nucleophilic catalyst 4-(dimethyamino)pyridine. Except for methyl 3-hydroxy-3-methylbutanoate, which we synthesized, all alcohols were available commercially. The final product was then purified on a silica gel column and the purity confirmed by high-performance liquid chromatography.

The general procedure that was used for the synthesis of all spacer-linked etomidate esters (and metomidate) is exemplified by the following method used for the preparation of dimethyl-methoxycarbonyl metomidate: A stirred solution of (*R*)-1-(1-phenyethyl)-1H-imidazole-5-carboxylic acid (325 mg, 1.5 mM) and methyl 2-hydroxyisobutyrate (195 mg, 1.65 mM) in anhydrous dichloromethane (3 ml) was mixed with 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride (316 mg, 1.65 mM) and dimethylaminopyridine (37 mg, 0.3 mM). The mixture was stirred at room temperature for 18 h. The product was purified by chromatography on a column of silica gel with dichloromethane/ethyl acetate 8:2 as the eluent to yield viscous, oily dimethyl-methoxycarbonyl metomidate (316 mg, 48% yield). 1H nuclear magnetic resonance spectrum: (CDCl3) δ 7.80 (s, 1H, imidazole CH), 7.72 (s, 1H, imidazole CH), 7.73 (m, 3H, phenyl), 7.15 (m, 2H, phenyl), 6.28 (q, 1H, methine), 3.60 (s, 3H, ester methyl), 1.85 (d, 3H, methyl), 1.60 (6H, methyl).

Synthesis of Methyl 3-hydroxy-3-methylbutanoate. A solution of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (633 mg, 3.3 mM) in methanol (1.2 ml, 30 mM) and anhydrous dichloromethane (10.8 ml) was mixed with 3-hydroxy-3-methyl-butyric acid (354 mg, 3.0 mM), dimethylaminopyridine (184 mg, 1.5 mM), and dimethylaminopyridine hydrochloride (238 mg, 1.5 mM). The mixture was stirred at room temperature for 18 h. The reaction mixture was purified by chromatography on a column of silca gel with dichloromethane/ethyl acetate 8:2 as the eluent to yield (168 mg, 42%) methyl 3-hydroxy-3-methylbutanoate. 1H nuclear magnetic resonance spectrum: (CDCl3) δ 1H, 3.72 (s, 3H, ester methyl), 2.51 (s, 2H, methylene), 1.29 (6H, methyl).

Nomenclature. We used a nomenclature system for these new compounds that is based upon four criteria (fig. 3). First, the length of the carbon spacer linking the labile ester to the etomidate backbone. Etomidate analogs have two methylene groups in this spacer, whereas metomidate analogs have only one. Second, the identity of the aliphatic group or groups (*i.e.*, methyl, dimethyl, isopropyl, or cyclopropyl). Third, the specific location of the aliphatic group on the carbon spacer (for etomidate analogs). The carbon immediately adjacent to the metabolically labile ester was defined as the α carbon, whereas the more distant one was the β carbon. Finally, the entaniomeric configuration of the new chiral center (*R* or *S*) that results from the addition of the new aliphatic group. It should be noted that the entaniomeric configuration of the existing chiral center of etomidate is not modified by our syntheses and remains in the *R* configuration for all of our ester-linked etomidate esters; our ester-linked etomidate esters were made from enantiomerically pure *R*-etomidate. For simplicity, our nomenclature system does not reference this existing chiral center. For the convenience of the reader, we have also given each spacer-linked etomidate ester a number (table 1).

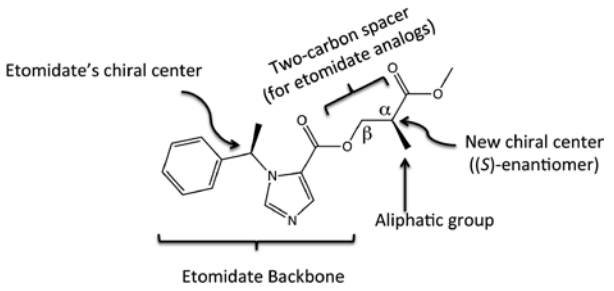


Fig. 3. Nomenclature for spacer-linked etomidate esters. All etomidate esters contain the etomidate backbone with the chiral carbon in the (*R*)-entantiomeric form. This chiral center is not modified by our syntheses and thus remains in the *R* configuration for all of our ester-linked etomidate esters, and is not specifically referenced in our nomenclature system. Etomidate analogs have a two-carbon spacer between the etomidate backbone and the labile ester moiety (shown), whereas metomidate analogs have a one-carbon spacer. An aliphatic group may be located either immediately adjacent to the labile ester moiety (on the α carbon) or one carbon away from that ester (on the β carbon). The enantiomeric configuration of the aliphatic group is defined as either *R* or *S*.

Measurement of In Vivo *Hypnotic Potency and Duration of Action*

The hypnotic potencies of all compounds were assessed in rats using a loss-of-righting reflexes (LORR) assay.[9] Briefly, the desired dose of hypnotic in dimethyl sulfoxide or saline vehicle was rapidly injected through the femoral venous catheter followed by a 1-ml normal saline flush. Immediately after injection, rats were turned supine. A rat was judged to have LORR if it failed to right itself (onto all four paws) after 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. For each compound, the ED_{50} for LORR was determined from a data set of at least 15 separate doses using the method of Waud.[12]

Measurement of In Vitro *Metabolic Half-lives in Rat Blood*

On the day of study, whole blood was drawn from the femoral venous catheters of three Sprague-Dawley rats (1–2 ml/rat), immediately anticoagulated with heparin (30 U), pooled, and stored on ice. A 1.35-ml aliquot of blood was warmed at 37°C for 5 min and hypnotic (from a 40 mM stock in dimethyl sulfoxide stock solution) was added to a final concentration of 200 μM. After the desired incubation time, a 150 μl sample was removed and the metabolic reaction was quenched with 150 μl acetonitrile (Sigma-Aldrich, St. Louis, MO). Zero time-point samples were prepared by adding 150 μl acetonitrile to 150 μl blood before adding hypnotic (from a 4 mM stock in dimethyl sulfoxide stock solution). The quenched samples were centrifuged and the resultant supernatant separated and stored at −20°C until analyzed. Hypnotic concentrations in thawed samples were determined by high performance liquid chromatography using a Varian Prostar system with a 4.6 × 250 mm Proto 300 C18 column (Nest Group, Southborough, MA) with the UV detector set at 240 nm. A linear gradient 20–45% acetonitrile in water with 0.05% trifluoroacetic acid (Thermo Scientific, Rockford, IL) for more than 20 min was used with a flow rate of 1 ml/min. The lower limit of quantitation of this assay is 3 μM and the accuracy within 15% at 10 μM. Metabolic half-lives were quantified from the incubation time-dependent change in drug concentration.

Octanol:Water Partition Coefficients

One mg of each hypnotic was added to 10 ml of water buffered with 10 mM Tris (pH 7.4) and 1 ml of octanol. The mixture was stirred overnight and then centrifuged to fully separate the organic and aqueous phases. The relative hypnotic concentration in each phase (*i.e.*, the partition coefficient) was determined by high-performance liquid chromatography as described for blood.

Statistical Analysis

Unless indicated otherwise, data are reported as mean ± SD. Statistical analyses were done using Prism v5.0 for the Macintosh (GraphPad Software, Inc., LaJolla, CA) or Igor Pro 6.1 (Wavemetrics, Lake Oswego, OR).

Results

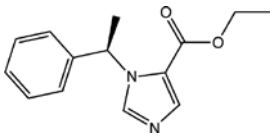
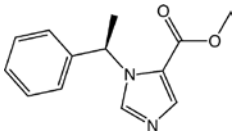
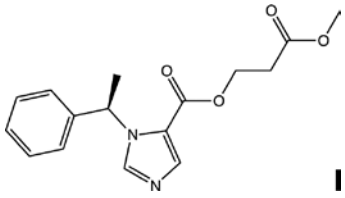
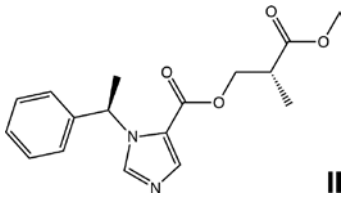
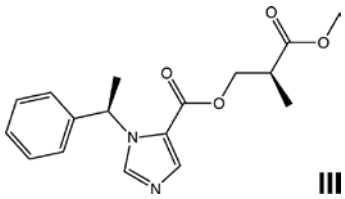
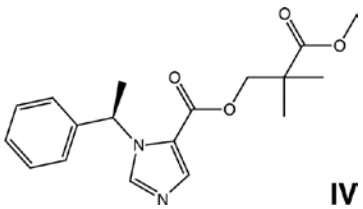
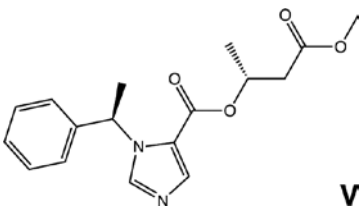
Hypnotic Activity of Spacer-linked Etomidate Esters

When administered as an intravenous bolus to rats, all spacer-linked etomidate esters produced rapid, dose-dependent LORR. Figure 4A and table 1 show that the ED_{50}s for LORR ranged from 0.69 ± 0.04 mg/kg for cyclopropyl-methoxycarbonyl metomidate (table 1, structure XIV) to 11.1 ± 0.8 for methoxycarbonyl metomidate (table 1, structure VIII). Two rats died during our studies after receiving a spacer-linked etomidate ester. One had received a 20 mg/kg dose of dimethyl-methoxycarbonyl metomidate (table 1, structure XI), which was subsequently determined to be 28-fold higher than the ED_{50} for LORR. The other rat died after receiving a 20 mg/kg dose of (*R*)-isopropyl-methoxycarbonyl metomidate (table 1, structure XII).

For the four compounds that exist as diastereometric pairs–α-methyl-methoxycarbonyl etomidate (table 1, structures II and III), β-methyl-methoxycarbonyl etomidate (table 1, structures V and VI), methyl-methoxycarbonyl metomidate (table 1, structures IX and X), and isopropyl-methoxycarbonyl metomidate (table 1, structures XII and XIII) – the hypnotic potency was higher (ED_{50} for LORR was lower) for the (*S*)-form *versus* the (*R*)-form (table 1). This potency difference was larger for the two metomidate analogs (methyl-methoxycarbonyl metomidate and isopropyl-methoxycarbonyl metomidate *R*/*S* ED_{50} ratios were 2.7 and 3.0, respectively) *versus* the two etomidate analogs (α-methyl-methoxycarbonyl etomidate and β-methyl-methoxycarbonyl etomidate *R*/*S* ED_{50} ratios were 1.7 and 1.2, respectively).

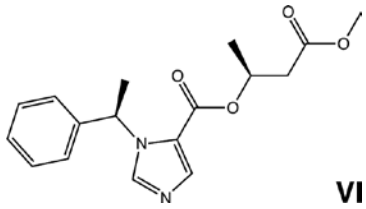
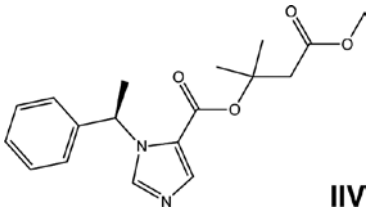
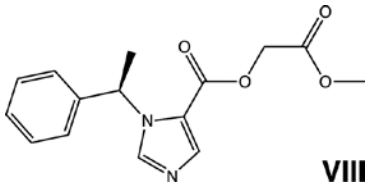
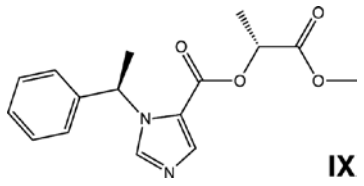
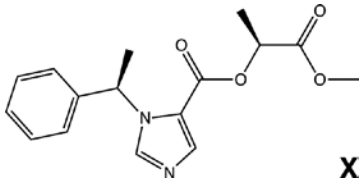
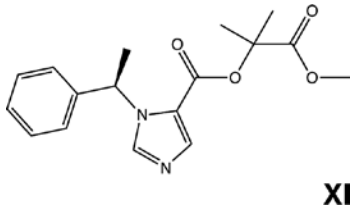
For representative spacer-linked etomidate esters, figure 4B plots the duration of LORR as a function of dose on a

Table 1. Pharmacodynamic and Pharmacokinetic Properties of Etomidate, Metomidate, and Etomidate Esters

Structure Number	Name	ED_{50} ± SD, mg/kg	Slope of Duration *vs.* Log Dose ± SD, min/log mg/kg	Blood Half-life, min (95% CI)	Octanol: Water Partition (Coefficient ± SD)
	Etomidate	0.53 ± 0.17	24.6 ± 4.7	99 (81–126)	800 ± 180*
	Metomidate	0.73 ± 0.50	33.0 ± 3.9	143 (124–170)	380 ± 48
 I	Methoxycarbonyl etomidate	5.3 ± 1.5	1.0 ± 0.3	0.41 (0.31 to 0.60)	190 ± 25
 II	α-(*R*)-methyl-methoxycarbonyl etomidate	5.2 ± 0.5	2.6 ± 0.3	0.19 (0.17 to 0.22)	670 ± 120
 III	α-(*S*)-methyl-methoxycarbonyl etomidate	3.1 ± 0.4	2.4 ± 0.3	0.76 (0.58 to 1.0)	530 ± 170
 IV	α-dimethyl-methoxycarbonyl etomidate	2.4 ± 1	9.8 ± 1.5	2.6 (1.9–4.2)	2,240 ± 150
 V	β-(*R*)-methyl-methoxycarbonyl etomidate	3.5 ± 0.6	2.9 ± 0.8	0.91 (0.76 to 1.1)	500 ± 24

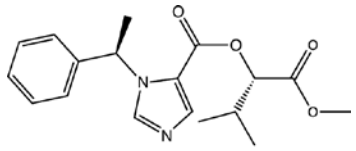
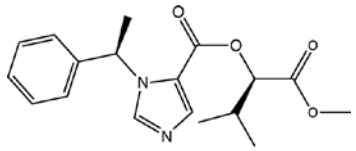
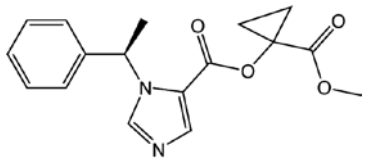
(*continued*)

Table 1. (continued)

Structure Number	Name	ED_{50} ± SD, mg/kg	Slope of Duration vs. Log Dose ± SD, min/log mg/kg	Blood Half-life, min (95% CI)	Octanol: Water Partition (Coefficient ± SD)
 VI	β-(*S*)-methyl-methoxycarbonyl etomidate	2.9 ± 0.3	2.0 ± 0.5	0.72 (0.56 to 0.98)	484 ± 12
 IIV	β-dimethyl-methoxycarbonyl etomidate	1.9 ± 0.3	11.9 ± 0.6	23.4 (19.6–28.9)	1,580 ± 40
 VIII	Methoxycarbonyl metomidate	11.1 ± 0.8	1.6 ± 0.4	<0.03	159 ± 15
 IX	(*R*)-methyl-methoxycarbonyl metomidate	9.6 ± 1.9	4.6 ± 0.7	<0.03	380 ± 15
 X	(*S*)-methyl-methoxycarbonyl metomidate	3.5 ± 0.4	1.9 ± 0.2	0.14 (0.08 to 0.62)	330 ± 16
 XI	Dimethyl-methoxycarbonyl metomidate	0.72 ± 0.16	9.6 ± 0.8	8.7 (7.4–10.7)	660 ± 110

(*continued*)

Table 1. (Continued)

Structure Number	Name	ED_{50} ± SD, mg/kg	Slope of Duration vs. Log Dose ± SD, min/log mg/kg	Blood Half-life, min (95% CI)	Octanol: Water Partition (Coefficient ± SD)
 XII	(*R*)-isopropyl-methoxycarbonyl metomidate	3.6 ± 0.8	6.6 ± 1.3	0.15 (0.15 to 0.16)	3,830 ± 310
 XIII	(*S*)-isopropyl-methoxycarbonyl metomidate	1.2 ± 0.19	12.1 ± 1.1	15.5 (11.6–23.1)	2,860 ± 67
 XIV	Cyclopropyl-methoxycarbonyl metomidate	0.69 ± 0.04	6.9 ± 0.5	0.57 (0.49 to 0.68)	420 ± 11

* From Pejo *et al.*[8]

semilogarithmic scale. It demonstrates that the duration of LORR increased approximately linearly with the logarithm of the dose. The slope of this relationship, which is inversely related to the rate of drug clearance from the brain, ranged from 1.0 ± 0.3 for methoxycarbonyl etomidate (table 1, structure I) to 12.1 ± 1.1 min/log mg/kg for (*S*)-isopropyl-methoxycarbonyl metomidate (table 1, structure XIII).[13,14] For comparison, this plot also shows the same relationship for etomidate, which had a slope of 24 ± 4.7 min/log mg/kg.

In Vitro *Metabolic Half-life of Spacer-linked Etomidate Esters in Rat Blood*

To assess the susceptibility of each spacer-linked etomidate ester to metabolism, we added each compound to rat blood and measured the incubation time-dependent reduction in drug concentration. For representative compounds, figure 5 shows that the concentration of each drug decreased with incubation time in an approximately first-order manner. We could calculate metabolic half-lives for 12 of the 14 spacer-linked etomidate esters. Their half-lives ranged from 0.14 min (95% CI: 0.08 to 0.62 min) for (*S*)-methyl-methoxycarbonyl metomidate (table 1, structure X) to 15.5 min (95% CI: 11.6–23.1 min) for (*S*)-isopropyl-methoxycarbonyl metomidate (table 1, structure XIII). However in the cases of methoxycarbonyl metomidate (table 1, structure VIII) and (*R*)-methyl-methoxycarbonyl metomidate (table 1, structure IX), metabolism was so fast that their concentrations in blood could not be quantified using our high-performance liquid chromatography technique after 10 s, our shortest incubation time. Based on our lower limit of quantification, this indicates a metabolic half-life that is less than 2 s. For comparison, figure 5 also shows metabolism data for etomidate and metomidate, which had calculated metabolic half-lives of 99 min (95% CI: 81–126 min) and 143 min (95% CI: 124–170 min), respectively.

With the exception of β-methyl-methoxycarbonyl etomidate (table 1, structures V and VI), the rate of metabolism in blood was diastereometrically selective, with the (*R*)-form metabolized more quickly than the (*S*)-form (table 1). This selectivity was at least 4-fold and ranged to 100-fold for isopropyl-methoxycarbonyl metomidate (table 1, structures XII and XIII).

Discussion

The current studies show that introducing aliphatic groups onto methoxycarbonyl etomidate spacer modifies *in vitro* rate of metabolism in rat blood and *in vivo* duration of action and hypnotic potency in rats for the drug. Furthermore, if the aliphatic group is placed immediately adjacent to the ester moiety carbonyl group, the effects on *in vitro* metabolism and *in vivo* potency are diastereometrically selective, as each (*R*)-form is metabolized in blood more quickly and has

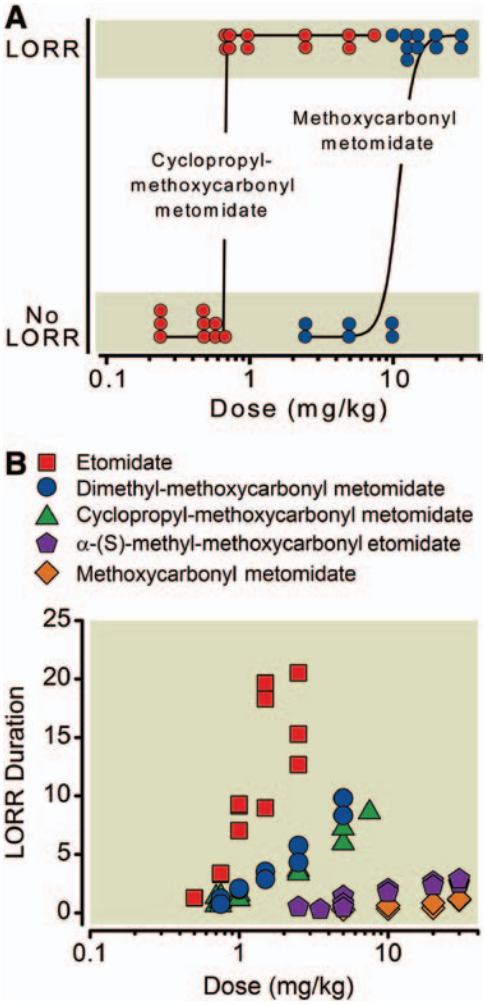


Fig. 4. Loss-of-righting reflexes (LORR) in rats. (*A*) Cyclopropyl-methoxycarbonyl metomidate and methoxycarbonyl metomidate dose-response curves for LORR. Each point represents data obtained from a single rat experiment. The curves are fits of each data set using the method of Waud, yielding ED_{50}s of 0.69 ± 0.04 mg/kg for cyclopropyl-methoxycarbonyl metomidate and 11.1 ± 0.8 mg/kg for methoxycarbonyl metomidate. (*B*) For representative spacer-linked etomidate esters, a semilogarithmic plot of the duration of LORR as a function of dose. Each point represents data obtained from a single rat experiment.

a hypnotic potency that is lower than the corresponding (*S*)-form.

The structures of the spacer-linked etomidate esters described in this study are based on that of methoxycarbonyl etomidate, a soft analog of etomidate that contains a metabolically labile ester moiety that is linked to the existing ester of etomidate *via* a simple two-carbon spacer.[9] This metabolically labile ester is distinct from the existing ester moiety on etomidate, which is attached directly to the imidazole ring and is a relatively poor substrate for esterase-catalyzed hydrolysis, as evidenced by the long (more than 1 h) *in vitro* metabolic half-life of etomidate in rat blood and human s9 liver fraction, and several-hour *in vivo* terminal elimination half-life in humans.[9,15,16]

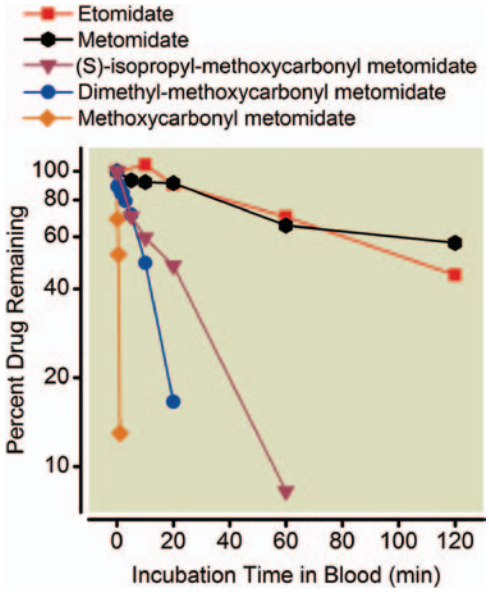


Fig. 5. Stability of spacer-linked etomidate esters, etomidate, and metomidate in pooled rat blood. The metabolic half-lives calculated from this data were 99 min (etomidate), 143 min (metomidate), 15.5 min ([*S*]-isopropyl-methoxycarbonyl metomidate), 8.7 min (dimethyl-methoxycarbonyl metomidate), and 0.41 min (methoxycarbonyl etomidate). For clarity, etomidate and metomidate data points obtained with incubation times longer than 120 min are not shown.

In Vitro *Metabolism in Rat Blood*

We chose to measure the metabolic stabilities of our spacer-linked etomidate esters in rat blood because it is easy to acquire, has high esterase activity, metabolizes methoxycarbonyl etomidate very rapidly *in vitro*, and is thought to be an important (but not the exclusive) site of methoxycarbonyl etomidate metabolism *in vivo*.[8,17,18] The strategy that we employed in this study to reduce the rate of ester hydrolysis and prolong the duration of hypnotic action was to introduce steric hindrance by adding various aliphatic groups onto the two-carbon spacer of methoxycarbonyl etomidate. This strategy was based on previous studies showing that the presence of bulky chemical groups near metabolically labile ester moieties may slow the rate of ester hydrolysis.[10,11,19,20] In some cases we also shortened the length of the spacer from two carbons to one, forming metomidate rather than etomidate analogs, and found that this accelerated metabolism in rat blood. For example, the metabolic half-life of methoxycarbonyl etomidate (table 1, structure I) in rat blood was 20 s, whereas the half-life of methoxycarbonyl metomidate (table 1, structure VIII) was less than 2 s. Similarly, the metabolic half-lives of the (*R*)- and (*S*)-forms of α-methyl-methoxycarbonyl etomidate (table 1, structures II and III) were at least 4-fold longer than the respective (*R*)- and (*S*)-forms of methyl-methoxycarbonyl metomidate (table 1, structures IX and X). This was contrary to our expectation, because the shorter spacer was predicted to introduce greater steric hindrance because it brings the labile ester closer to the rigid imidazole ring. However, the shorter

spacer also reduces (to a single carbon) the distance between the carbonyl group of the labile ester moiety and the oxygen of the stable ester (fig. 1B, where n = 1). Such proximity may allow this oxygen atom, which is electronegative, to more effectively withdraw electron density from the carbonyl carbon and thus promote nucleophilic attack by esterases. This mechanism is thought to explain why a similarly located chlorine atom – which is also electronegative – increases by 40-fold the rate of esterase-catalyzed acetate ester hydrolysis.[21]

We also found that for three of the four diastereometric pairs, the (*R*)-form was metabolized in rat blood significantly more quickly than the (*S*)-form. In the case of isopropyl-methoxycarbonyl metomidate (table 1, structures XII and XIII), the difference in metabolism rate was 100-fold. The only diastereometric pair that failed to demonstrate high selectivity was β-methyl-methoxycarbonyl etomidate (table 1, structures V and VI). This was also the only pair in which the aliphatic group is not located immediately adjacent to the labile ester moiety, suggesting that metabolism in blood is most stereoselective when the chiral center is closest to the labile ester. The stereoselective metabolism in blood that we observe with our spacer-linked etomidate esters is reminiscent of, but larger than, that previously reported for esmolol.[22] In those studies, the blood from different species (*e.g.*, rats and dogs) exhibited differential selectivity for the two esmolol enantiomers, and human blood exhibited no selectivity at all.

In Vivo *Hypnotic Potency in Rats*

Our data suggest that hypnotic potency may be diastereometrically selective, because all (*R*)-forms of our spacer-linked etomidate esters had lower hypnotic potencies than their respective (*S*)-forms (table 1). We do not know whether this results from their lower intrinsic potencies (*i.e.*, potencies at the γ-aminobutyric acid receptor) or their faster metabolism. The latter could be important if ultra-rapid metabolism in blood lowers the concentration of drug that reaches the brain following bolus injection.

Unexpectedly, two compounds, dimethyl-methoxy-carbonyl metomidate (table 1, structure XI) and cyclopropyl-methoxycarbonyl metomidate (table 1, structure XIV), had hypnotic potencies that were nearly an order of magnitude higher than that of methoxycarbonyl etomidate and similar to that of etomidate. When combined with their longer durations of action, this potency advantage can be predicted to dramatically reduce the infusion rate required to maintain hypnosis *versus* methoxycarbonyl etomidate and thus the quantity of metabolite that is produced with prolonged administration.

In conclusion, we have developed a series of spacer-linked etomidate esters with varying aliphatic substituents and spacer lengths with the goal of identifying new hypnotics with optimized durations of action. Our studies show these substituents significantly affect the pharmacokinetic and pharmacodynamic properties of these compounds. We believe that this approach may be a generalizable strategy for optimizing the pharmacological properties of other soft drugs (*e.g.*, AZD3043) that may be too short-acting or insufficiently potent for clinical use.[5,23] At this stage, we believe that dimethyl-methoxycarbonyl metomidate (table 1, structure XI) and cyclopropyl-methoxycarbonyl metomidate (table 1, structure XIV) are the most promising spacer-linked etomidate esters for further testing and development because their hypnotic potencies are highest and their durations of action are intermediate between those of methoxycarbonyl etomidate and etomidate. Future studies in both rats and larger animals will be necessary to more fully evaluate the potential clinical utility of these new hypnotics.

References

1. Kilpatrick GJ, McIntyre MS, Cox RF, Stafford JA, Pacofsky GJ, Lovell GG, Wiard RP, Feldman PL, Collins H, Waszczak BL, Tilbrook GS: CNS 7056: A novel ultra-short-acting Benzodiazepine. Anesthesiology 2007; 107:60–6
2. Upton RN, Somogyi AA, Martinez AM, Colvill J, Grant C: Pharmacokinetics and pharmacodynamics of the short-acting sedative CNS 7056 in sheep. Br J Anaesth 2010; 105:798–809
3. Wiltshire HR, Kilpatrick GJ, Tilbrook GS, Borkett KM: A placebo and midazolam-controlled phase I single ascending dose study evaluating the safety, pharmacokinetics, and pharmacodynamics of remimazolam (CNS 7056). Part II: Population pharmacokinetic and pharmacodynamic modeling and simulation. Anesth Analg 2012; 115:284–96
4. Antonik LJ, Goldwater DR, Kilpatrick GJ, Tilbrook GS, Borkett KM: A placebo- and midazolam-controlled, phase I, single ascending-dose study evaluating the safety, pharmacokinetics, and pharmacodynamics of remimazolam (CNS 7056): Part I. Safety, efficacy, and basic pharmacokinetics. Anesth Analg 2012; 115:274–83
5. Sneyd JR, Rigby-Jones AE: New drugs and technologies, intravenous anaesthesia is on the move (again). Br J Anaesth 2010; 105:246–54
6. Egan TD: Is anesthesiology going soft?: Trends in fragile pharmacology. Anesthesiology 2009; 111:229–30
7. Bodor N, Buchwald P: Soft drug design: General principles and recent applications. Med Res Rev 2000; 20:58–101
8. 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
9. 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
10. Buchwald P, Bodor N: Quantitative structure-metabolism relationships: Steric and nonsteric effects in the enzymatic hydrolysis of noncongener carboxylic esters. J Med Chem 1999; 42:5160–8
11. Buchwald P, Bodor N: Physicochemical aspects of the enzymatic hydrolysis of carboxylic esters. Pharmazie 2002; 57:87–93
12. Waud DR: On biological assays involving quantal responses. J Pharmacol Exp Ther 1972; 183:577–607
13. Liao M, Sonner JM, Husain SS, Miller KW, Jurd R, Rudolph U, Eger EI 2nd: R (+) etomidate and the photoactivable R (+) azietomidate have comparable anesthetic activity in wild-type mice and comparably decreased activity in mice with a N265M point mutation in the γ-aminobutyric acid receptor β3 subunit. Anesth Analg 2005;101:131–5

14. Shafer SL: Principles of pharmacokinetics and pharmacodynamics. In: Longnecker DE, Tinker JH, Morgan GE, eds. Principles and Practice of Anesthesiology. St. Louis: Mosby–Year Book; 1997:1159–210
15. Pejo E, Cotton JF, Kelly EW, Le Ge R, Cuny GD, Laha JK, Liu J, Je Lin X, Raines DE: *In vivo* and *in vitro* pharmacological studies of methoxycarbonyl-carboetomidate. Anesth Analg 2012; 115:297–304
16. Van Hamme MJ, Ghoneim MM, Ambre JJ: Pharmacokinetics of etomidate, a new intravenous anesthetic. ANESTHESIOLOGY 1978; 49:274–7
17. Minagawa T, Kohno Y, Suwa T, Tsuji A: Species differences in hydrolysis of isocarbacyclin methyl ester (TEI-9090) by blood esterases. Biochem Pharmacol 1995; 49:1361–5
18. Wang S, Yang J, Peng Y, Zou Y, Xiang H, Yao H, Chen J, Bi D, Yao J: Species-dependent plasma metabolism of the ester compound daidzein 7,4´di-succinic acid mon-ester-O-ethoxy (DZ5). Pharmazie 2007; 62:574–6
19. Kam ST, Matier WL, Mai KX, Barcelon-Yang C, Borgman RJ, O'Donnell JP, Stampfli HF, Sum CY, Anderson WG, Gorczynski RJ, Lee RJ: [(Arylcarbonyl)oxy]propanolamines. 1. Novel ⊠-blockers with ultrashort duration of action. J Med Chem 1984; 27:1007–16
20. Buchwald P: Structure-metabolism relationships: Steric effects and the enzymatic hydrolysis of carboxylic esters. Mini Rev Med Chem 2001; 1:101–11
21. Laumen K, Schneider MP: A highly selective ester hydrolase from Pseudomonas Sp. for the enzymatic preparation of enantiomerically pure secondary alcohols chiral auxiliaries in organic synthesis. J Chem Soc Chem Commun 1988; 598–600
22. Quon CY, Mai K, Patil G, Stampfli HF: Species differences in the stereoselective hydrolysis of esmolol by blood esterases. Drug Metab Dispos 1988; 16:425–8
23. Sneyd JR: Recent advances in intravenous anaesthesia. Br J Anaesth 2004; 93:725–36

ANESTHESIOLOGY REFLECTIONS FROM THE WOOD-LIBRARY MUSEUM

Edmund Andrews' "Oxygen Mixture"

After earning his A.B. and his M.D. in Michigan and then medically serving Generals Grant and Sherman during the Civil War, Edmund Andrews (1824–1904) spent most of his surgical career in Chicago. Yet like Horace Wells and Gardner Q. Colton, Andrews was actually a Vermont native. However, unlike those nitrous oxide pioneers, he avoided hypoxic gas mixtures by supplementing his laughing gas with oxygen. Anesthesiologists salute Andrews for publishing in *The Chicago Medical Examiner* that with nitrous oxide "the best proportion of oxygen will be … one-fifth by volume," an "oxygen mixture" for anesthetizing patients "for the longest as well as for the shortest surgical operations…." (Copyright © the American Society of Anesthesiologists, Inc.)

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