Carboetomidate

A Pyrrole Analog of Etomidate Designed Not to Suppress Adrenocortical Function

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ABSTRACT

Background: Etomidate is a sedative hypnotic that is often used in critically ill patients because it provides superior hemodynamic stability. However, it also binds with high affinity to 11β-hydroxylase, potently suppressing the synthesis of steroids by the adrenal gland that are necessary for survival. The authors report the results of studies to define the pharmacology of (*R*)-ethyl 1-(1-phenylethyl)-1H-pyrrole-2-carboxylate (carboetomidate), a pyrrole analog of etomidate specifically designed not to bind with high affinity to 11β-hydroxylase.

Methods: The hypnotic potency of carboetomidate was defined in tadpoles and rats using loss of righting reflex assays. Its ability to enhance wild-type $\alpha_1\beta_2\gamma_{2l}$ and etomidate-insensitive mutant $\alpha_1\beta_2$M286Wγ_{2l} human γ-aminobutyric acid type A receptor activities was assessed using electrophysiologic techniques. Its potency for inhibiting *in vitro* cortisol synthesis was defined using a human adrenocortical cell assay. Its effects on *in vivo* hemodynamic and adrenocortical function were defined in rats.

Results: Carboetomidate was a potent hypnotic in tadpoles and rats. It increased currents mediated by wild-type but not etomidate-insensitive mutant γ-aminobutyric acid type A receptors. Carboetomidate was a three orders of magnitude less-potent inhibitor of *in vitro* cortisol synthesis by adrenocortical cells than was etomidate. In rats, carboetomidate caused minimal hemodynamic changes and did not suppress adrenocortical function at hypnotic doses.

Conclusions: Carboetomidate is an etomidate analog that retains many beneficial properties of etomidate, but it is dramatically less potent as an inhibitor of adrenocortical steroid synthesis. Carboetomidate is a promising new sedative hypnotic for potential use in critically ill patients in whom adrenocortical suppression is undesirable.

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What We Already Know about This Topic

❖ Etomidate produces less cardiovascular depression than any other anesthetic induction agent, but interferes with steroid biosynthesis

What This Article Tells Us That Is New

❖ Carboetomidate was synthesized to affect γ-aminobutyric acid (GABA) receptors, but not the steroid synthetic enzyme affected by etomidate

❖ Carboetomidate produced hypnosis in tadpoles and rats, was much less potent than etomidate to inhibit steroid synthesis, and produced minimal cardiovascular depression in rats

ETOMIDATE is an intravenous (IV) sedative hypnotic that is used to induce general anesthesia and is distinguished from other agents by its minimal effects on cardiovascular func-

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tion.[1–4] Consequently, it is often used in patients who are elderly or critically ill. Etomidate contains an imidazole ring and, in common with many other imidazole-containing drugs, it suppresses the synthesis of adrenocortical steroids.[5–11] This suppression occurs even with administration of subhypnotic etomidate doses and is extremely long lasting.[12,13] Such "chemical adrenalectomy" precludes etomidate administration by continuous infusion to maintain anesthesia in the operating room (or sedation in the intensive care unit) and has raised serious concerns regarding the administration of even a single bolus for anesthetic induction in critically ill patients.[14–19]

This led us to search for solutions to the problem of etomidate-induced adrenocortical suppression. In a previous study, we tested a pharmacokinetic strategy for reducing the duration of adrenocortical suppression after bolus administration. We synthesized an analog of etomidate (methoxycarbonyl-etomidate) designed to be rapidly metabolized by esterases and demonstrated that it does not produce prolonged adrenocortical suppression in rats after bolus administration.[20]

We have also considered pharmacodynamic strategies for reducing etomidate-induced adrenocortical suppression. Etomidate suppresses adrenocortical steroid synthesis by inhibiting 11β-hydroxylase, a cytochrome P450 enzyme, that is required for the synthesis of cortisol, corticosterone, and aldosterone.[21] X-ray crystallographic studies of other imidazole-containing drugs to cytochrome P450 enzymes indicate that high-affinity binding occurs because the basic nitrogen in the imidazole ring of drug coordinates with the heme iron in the active site of enzyme; cytochrome P450 enzymes (including 11β-hydroxylase) contain heme prosthetic groups at their active sites.[22–24] Although 11β-hydroxylase has not yet been crystallized nor its interaction with etomidate precisely defined, homology modeling studies suggest that high affinity binding of etomidate to 11β-hydroxylase also involves coordination between the basic nitrogen of the drug and the heme iron of the enzyme (fig. 1A).[25] This led us to hypothesize that high affinity binding to 11β-hydroxylase (and thus adrenolytic activity) could be "designed out" of etomidate without disrupting potent anesthetic and γ-aminobutyric acid type A (GABA$_A$) receptor

activities by replacing this nitrogen with other chemical groups that cannot coordinate with heme iron. On the basis of this hypothesis, we have designed and synthesized (Appendix) (R)-ethyl 1-(1-phenylethyl)-1H-pyrrole-2-carboxylate (carboetomidate) as the lead compound in a new class of pyrrole-based sedative hypnotic analogs of etomidate, which was designed not to inhibit adrenocortical function at pharmacologically relevant doses (fig. 1B).

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* 1 (Ann Arbor, MI). Tadpoles were maintained in our laboratory, and frogs were maintained in the Massachusetts General Hospital Center for Comparative Medicine animal care facility. Adult male Sprague-Dawley rats (300–500 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed in the Massachusetts General Hospital Center for Comparative Medicine animal care facility.

Blood draws and IV drug administrations used a lateral tail vein IV catheter (24 gauge, 19 mm) placed under brief (approximately 1–5 min) sevoflurane or isoflurane 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 inhaled anesthetic exposure before study. In all studies, rats were placed on a warming stage (Kent Scientific, Torrington, CT) that was shown in our previous studies to maintain rectal temperatures between 36° and 38°C in anesthetized rats.[20]

Loss of Righting Reflex

Tadpoles. 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 carboetomidate ranging from 1 to 40 μM. Tadpoles were tipped manually 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 ensure reversibility of hypnotic action. The EC$_{50}$ for LORR was determined from the carboetomidate concentration dependence of LORR using the method of Waud.[26]

Rats. Rats were briefly restrained in a 3-inch diameter, 9-inch long acrylic chamber with a tail exit port. The desired dose of carboetomidate in dimethyl sulfoxide (DMSO; typically at 40 mg/ml) was injected through a lateral tail vein catheter followed by an approximately 1-ml normal saline flush. After injection, rats were removed from the restraint device and turned supine.
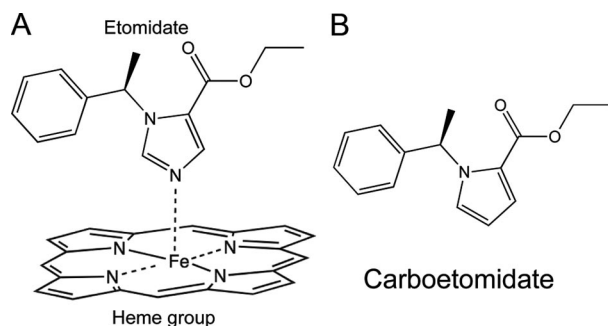

Fig. 1. (*A*) Hypothesized attractive interaction between the basic nitrogen in the imidazole ring of etomidate and the heme iron at the active site of 11β-hydroxylase. (*B*) Structure of carboetomidate.

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 carboetomidate injection until the animal spontaneously righted itself. The ED_{50} for LORR on bolus administration was determined from the dose dependence of LORR using the method of Waud.[26]

Onset time for LORR was determined separately by injecting rats with 28 mg/kg of carboetomidate (40 mg/ml in DMSO) or 4 mg/kg of etomidate (5.7 mg/ml in DMSO) through a lateral tail vein catheter followed by an approximately 1-ml normal saline flush. After injection, rats were immediately removed from the restraint device and repeatedly turned supine until they no longer spontaneously righted. The onset time was defined as the time from injection until LORR occurred.

GABA$_A$ 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$_2$, 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_1, β_2 (or β_2M286W), and γ_{2l} subunits of the human GABA$_A$ receptor ($\sim$40 ng of messenger RNA total at a subunit ratio of 1:1:2). This messenger RNA was transcribed from complementary DNA encoding for GABA$_A$ receptor α_1, β_2 (or β_2M286W), and γ_{2l} subunits 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$_2$, 0.8 mM MgCl$_2$, 10 mM HEPES, pH 7.5) containing 50 U/ml of penicillin and 50 μg/ml of streptomycin at 17°C for at least 18 h before electrophysiologic experiments.

All electrophysiologic 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 using a GeneClamp 500B amplifier (Axon Instruments, Union City, CA) and perfused with ND-96 buffer at a rate of $4-6$ ml/min. Buffer perfusion was controlled 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 using Clampex 9.2 software (Axon Instruments) and 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 GABA that produces 5–10% of the maximal current response (EC$_{5-10}$ GABA) was determined by measuring the peak current responses evoked by a range of GABA concentrations (in ND-96 buffer) and comparing them with the maximal peak current response evoked by 1 mM GABA. The effect of carboetomidate on EC$_{5-10}$ GABA-evoked currents was then assessed by first perfusing the oocyte with EC$_{5-10}$ GABA for 90 s and then measuring the control peak evoked current. After a 5-min recovery period, the oocyte was perfused with carboetomidate for 90 s and then with EC$_{5-10}$ GABA plus carboetomidate for 90 s, and the peak evoked current was measured again. After a 15-min recovery period, the control experiment (*i.e.*, no carboetomidate) was repeated to test for reversibility. A longer recovery period after carboetomidate exposure was used to facilitate washout of the drug. The peak current response in the presence of carboetomidate was then normalized to the average peak current response of the two control experiments. Carboetomidate-induced potentiation was quantified from the normalized current responses in the presence *versus* absence of carboetomidate.

Rat Hemodynamics

The effects of hypnotics on rat hemodynamics were defined as described previously.[20] Femoral arterial catheters, tunneled between the scapulas, were preimplanted by the vendor (Charles River Laboratories). Animals were fully recovered from the placement procedure on 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, 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 10 to 20 min before data collection. The signal from the pressure transducer (TruWave, Edwards Lifesciences, Irvine, CA) was amplified 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 using LabView Software (version 8.5 for Macintosh OS X; National Instruments).

Data used for blood pressure analysis were recorded for 5 min immediately before hypnotic administration and for 15 min thereafter. Carboetomidate (40 mg/ml) dissolved in DMSO, etomidate (5.7 mg/ml) dissolved in DMSO, or DMSO vehicle alone as a control was administered through the tail vein catheter followed by approximately 1-ml normal saline flush.

Inhibition of In Vitro Cortisol Synthesis

In vitro cortisol synthesis was measured using the human adrenocortical cell line H295R (NCI-H295R; ATCC CRL-2128). Aliquots of 10^5 cells per well were grown in 12-well culture plates with 2 ml of growth medium (Dulbecco's Modified Eagle Medium/F12 supplemented with 1% insu-

lin, transferrin, selenium, and linoleic acid, 2.5% NuSerum, and Pen/Strep). When cells reached near confluence (typically 48–72 h), the growth medium was replaced with assay medium (Dulbecco's Modified Eagle Medium/F12 supplemented with 0.1% insulin, transferring, selenium containing antibiotics and 20 μM forskolin) that contained etomidate or carboetomdiate. After 48 h, 1.2 ml of assay medium was collected from each well, centrifuged to pellet any cells or debris, and the cortisol concentration in the supernatant was quantified by enzyme-linked immunosorbent assay using commercially available 96-well kits based on horseradish peroxidase-conjugated cortisol in a competitive antibody binding assay (R&D Systems, Minneapolis, MN, KGE008).

Rat Adrenocortical Suppression

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 adrenocorticotropic hormone (ACTH) release, to suppress baseline corticosterone production, and to inhibit the variable stress response to restraint and handling. 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 IV carboetomidate, etomidate, or DMSO vehicle as a control. The concentrations of carboetomidate and etomidate in DMSO were 40 and 5.7 mg/ml, respectively. Immediately after hypnotic or vehicle administration, $ACTH_{1-24}$ (25 μg/kg; Sigma-Aldrich Chemical Co, St. Louis, MO) was given intravenously to stimulate corticosterone production. Fifteen minutes later, a second blood sample was drawn to measure the $ACTH_{1-24}$-stimulated serum corticosterone concentration. $ACTH_{1-24}$ was dissolved in 1 mg/ml of deoxygenated water as stock, aliquoted, and frozen ($-20°C$); a fresh aliquot was thawed just before each use. Rats in all three groups (carboetomidate, etomidate, and vehicle) received the same volume of DMSO (350 μl/kg).

Corticosterone concentrations in blood serum were determined as reported previously.[20] Blood samples were allowed to clot at room temperature (10–60 min) before centrifugation at 3,500g for 5 min. Serum was carefully expressed from any resulting superficial fibrin clot using a clean pipette tip before a second centrifugation at 3,500g for 5 min. After the second centrifugation, the resultant 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. After thawing and heat inactivation of corticosterone-binding globulins (65°C for 20 min), serum baseline and $ACTH_{1-24}$-stimulated corticosterone concentrations were quantified using an enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, Webster, TX) and a 96-well plate reader (Molecular Devices, Sunnyvale, CA).
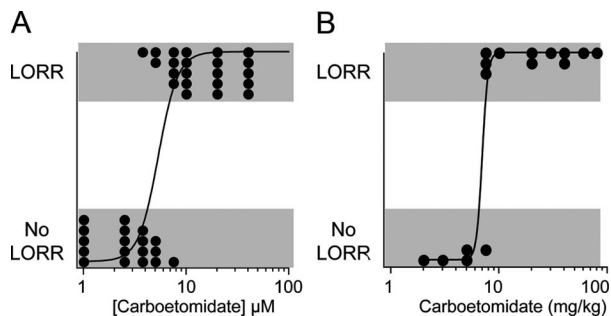

Fig. 2. (*A*) Carboetomidate concentration-response curve for loss of righting reflex (LORR) in tadpoles. Each data point represents the result from a single tadpole. The *curve* is a fit of the data set using the method of Waud,[26] yielding an EC_{50} of 5.4 ± 0.5 μM. (*B*) Carboetomidate dose–response curve for LORR in rats. Each data point represents the results from a single rat. The *curve* is a fit of the data set using the method of Waud,[26] yielding an ED_{50} of 7 ± 2 mg/kg.

Statistical Analysis

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

Results

Loss of Righting Reflexes in Tadpoles and Rats by Carboetomidate

Figure 2A shows the carboetomidate concentration-response relationship for LORR in *Xenopus laevis* tadpoles. The fraction of tadpoles that had LORR increased with carboetomidate concentration, and at the highest concentrations studied (10–40 μM), all tadpoles had LORR. All tadpoles that had LORR recovered their righting reflexes when removed from carboetomidate and returned to fresh water. From the concentration-response data, anesthetic EC_{50} of carboetomidate was determined to be 5.4 ± 0.5 μM.

IV administration of carboetomidate also produced LORR in Sprague-Dawley rats. Figure 2B shows the carboetomidate dose-response relationship for LORR in rats after IV bolus administration. 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_{50} for LORR was determined to be 7 ± 2 mg/kg. We noted that the onset time for LORR with carboetomidate was slower than the time we had previously observed with etomidate.[20] We quantified this difference using equihypnotic doses of carboetomidate and etomidate (28 and 4 mg/kg, respectively; 4 × ED_{50} for LORR).[20] The onset time for LORR with carboeto-
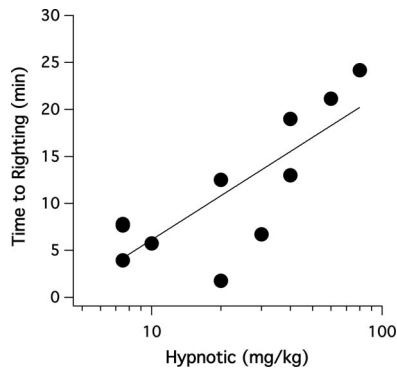
Fig. 3. The duration of loss of righting reflex in rats increased with the logarithm of the carboetomidate dose. The slope of this relationship was 16 ± 4 min/(log mg/kg) (r^2 = 0.64).

midate was 33 ± 22 s (n = 10; range 10–63 s) when compared with 4.5 ± 0.6 s (n = 4; range 4–5 s) with etomidate.

Figure 3 shows the relationship between the carboetomidate dose and the duration of LORR after IV bolus administration to Sprague-Dawley rats. The duration of LORR increased approximately linearly with the logarithm of the dose with a slope of 16 ± 4 min/(log mg/kg).

Modulation by Carboetomidate of Wild-type $\alpha_1\beta_2\gamma_{2l}$ and Etomidate-insensitive $\alpha_1\beta_2M286W\gamma_{2l}$ GABA$_A$ Receptors

Figure 4 shows representative electrophysiologic traces elicited by EC$_{5-10}$ GABA in the absence or presence of 10 μM carboetomidate. Figure 4A shows traces that were mediated by wild-type $\alpha_1\beta_2\gamma_{2l}$ GABA$_A$ receptors, whereas Figure 4B shows traces that were mediated by etomidate-
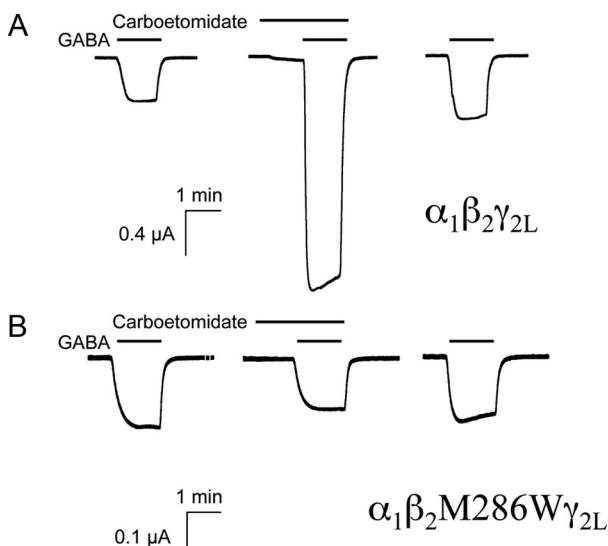

Fig. 4. Carboetomidate modulation of human γ-aminobutyric acid (GABA) type A receptor function. (*A*) Representative traces showing reversible enhancement by 10 μM carboetomidate of currents mediated by wild-type receptors when evoked by EC$_{5-10}$ γ-aminobutyric acid. (*B*) Representative traces showing minimal enhancement by 10 μM carboetomidate of currents mediated by etomidate-insensitive mutant receptors when evoked by EC$_{5-10}$ γ-aminobutyric acid.
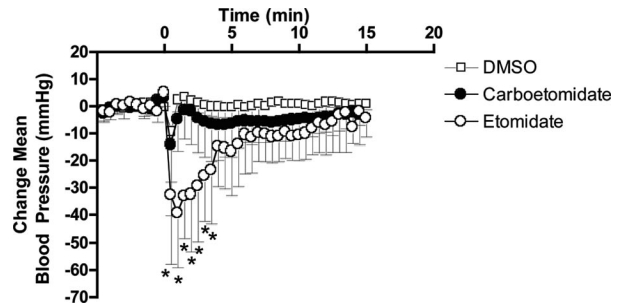

Fig. 5. The effects of 14 mg/kg of carboetomidate (n = 7), 2 mg/kg of etomidate (n = 6), and dimethyl sulfoxide (DMSO) vehicle alone (n = 4) on mean blood pressure in rats. Hypnotics were given at doses equal to twice their respective ED$_{50}$s for loss of righting reflex in DMSO vehicle. All rats received the same quantity of DMSO vehicle (350 μl/kg). Hypnotic in DMSO vehicle or DMSO vehicle alone was injected at time 0. Each data point represents the average (±SD) change in mean blood pressure during each 30-s epoch. * P < 0.05 *versus* DMSO vehicle alone.

insensitive mutant $\alpha_1\beta_2M286W\gamma_{2l}$ GABA$_A$ receptors. Carboetomidate significantly enhanced currents mediated by wild-type receptors (390 ± 80%) but did not enhance currents mediated by etomidate-insensitive mutant receptors (−9 ± 16%).

Hemodynamic Actions of Carboetomidate Versus Etomidate in Rats

Figure 5 shows the effects of 14 mg/kg of carboetomidate (n = 7), 2 mg/kg of etomidate (n = 6), and DMSO vehicle (n = 4) on mean arterial blood pressure in rats. For carboetomdiate and etomidate, these are equihypnotic doses. During the study period, the effect of carboetomidate on mean blood pressure was not significantly greater than that of DMSO vehicle alone (P > 0.05 by two-way ANOVA). However, at times from 30 to 210 s after administration, etomidate significantly reduced mean blood pressure relative to vehicle. Baseline mean blood pressures for vehicle, carboetomidate, and etomidate groups were similar (P = 0.15 by ANOVA) at 114 ± 5, 116 ± 9, and 127 ± 17 mmHg, respectively.

Inhibition by Carboetomidate and Etomidate of Human Adrenocortical Carcinoma Cell Cortisol Synthesis

Figure 6 shows the carboetomidate and etomidate concentration-response curves for the inhibition of cortisol synthesis by human adrenocortical cells. Although both hypnotics inhibited cortisol synthesis in a concentration-dependent manner, the concentration ranges over which this inhibition occurred differed by three orders of magnitude. The half maximal inhibitory concentration for carboetomidate and etomidate were calculated from these data sets to be 2.6 ± 1.5 μM and 1.3 ± 0.2 nM, respectively.

Adrenocortical Suppression in Rats on Administration of Carboetomidate versus Etomidate

Serum corticosterone concentrations were measured in dexamethasone-pretreated rats before (baseline) and 15 min after
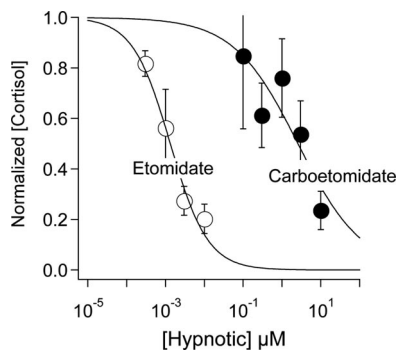
Fig. 6. The effects of carboetomidate and etomidate on cortisol synthesis by human adrenocortical carcinoma cells. The curves are fits of each data set to a Hill equation yielding half maximal inhibitory concentrations of 2.6 ± 1.5 μM and 1.3 ± 0.2 nM for carboetomidate and etomidate, respectively. Each data point is the average ($\pm$SD) of three measurements. The control cortisol concentration was 0.86 ± 0.20 ng/ml.

they received $ACTH_{1-24}$ and carboetomidate, etomidate, or DMSO vehicle control. Baseline serum corticosterone concentrations in rats (n = 12) averaged 39 ± 49 ng/ml and were not significantly different among the three groups (carboetomidate, etomidate, and control). Administration of $ACTH_{1-24}$ stimulated adrenocortical steroid production in all three groups. However, figure 7 shows that serum corticosterone concentrations varied among the three groups 15 min after $ACTH_{1-24}$ administration. Rats given carboetomidate had serum corticosterone concentrations that were significantly higher than those given an equihypnotic dose of etomidate and not significantly different from those given vehicle.

Discussion

Carboetomidate is a pyrrole analog of etomidate that retains the hypnotic action of etomidate, $GABA_A$ receptor modulatory activity, and hemodynamic stability. However, it is a three orders of magnitude less-potent inhibitor of adrenocortical cortisol synthesis than etomidate, and unlike etomidate, it does not suppress adrenocortical function in rats at hypnotic doses.
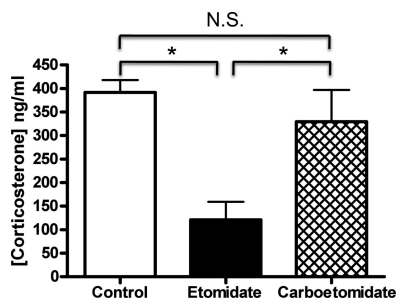

Fig. 7. Adrenocorticotropic hormone$_{1-24}$-stimulated serum corticosterone concentrations in rats 15 min after administration of vehicle, 2 mg/kg of etomidate, or 14 mg/kg of carboetomidate. Hypnotics were given at doses equal to twice their respective ED_{50}s for loss of righting reflex. Four rats were studied in each group. Average serum corticosterone concentrations ($\pm$SD) were 390 ± 50 ng/ml, 120 ± 80 ng/ml, and 330 ± 70 ng/ml after administration of dimethyl sulfoxide vehicle (control), etomidate, and carboetomidate, respectively. $* P < 0.05$. N.S. = no significant difference.

Etomidate suppresses adrenocortical function primarily by inhibiting 11β-hydroxylase (CYP11B1), a member of the cytochrome P450 superfamily of enzymes. 11β-Hydroxylase is required for the synthesis of cortisol, corticosterone, and aldosterone. This suppression occurs at very low etomidate concentrations, which is thought to reflect a very high affinity of etomidate to the active site of the enzyme.[11,25] We designed carboetomidate based on the hypothesis that etomidate binds with high affinity to 11β-hydroxylase because the basic nitrogen in its imidazole ring coordinates with the heme iron of the active site. This interaction has been observed in the binding of other imidazole-containing drugs to various cytochrome P450 enzymes using crystallographic techniques. For example, the inhibitor 4-(4-chlorophenyl)imidazole binds to the active site of enzyme 2B4 in a single orientation with the basic nitrogen of its imidazole ring coordinated to the enzyme's heme iron at a bond distance of 2.14 Å.[22] This binding triggers a conformational transition in which the enzyme closes tightly around the bound ligand. Similarly, imidazole-containing antifungal agents bind within the active sites of CYP130 and CYP121 where they form a coordination bond between basic nitrogen and the heme iron of the enzyme.[23,24] Evidence of such coordination has also been found using spectroscopic techniques, as the heme group serves as a chromophore that undergoes a characteristic spectral shift when a coordination bond is formed with an imidazole-containing inhibitor.[23,27–29] Although the interactions of etomidate with 11β-hydroxylase have not been defined experimentally using crystallographic or spectroscopic techniques, *in silico* homology modeling suggests that coordination between the basic nitrogen of hypnotic and the heme iron of enzyme also contributes to high-affinity binding.[25]

We used an adrenocortical carcinoma cell assay to compare the inhibitory potencies of carboetomidate and etomidate. This assay has been used previously to compare the potencies with which drugs inhibit the synthesis of adrenocortical steroids.[30–32] Our studies showed that carboetomidate is a three orders of magnitude less-potent inhibitor of cortisol synthesis than etomidate. This is consistent with an important role of the basic nitrogen of hypnotic in stabilizing binding to the enzyme and provides strong evidence that high-affinity binding of etomidate to 11β-hydroxylase can be designed out of etomidate by replacing this nitrogen with other chemical groups (in this case, CH) that cannot coordinate with heme iron. As a consequence of its low adrenocortical inhibitory potency, carboetomidate failed to inhibit $ACTH_{1-24}$-stimulated production of corticosterone in rats when given as a bolus at a hypnotic dose.

Although carboetomidate is a three orders of magnitude less-potent inhibitor of *in vitro* cortisol synthesis than etomidate, it is only modestly less potent as a hypnotic. It has one-third and one-seventh the hypnotic potency of etomidate in tadpoles[33] and rats,[20] respectively. These results provide proof-of-principle that one may alter anesthetic structure to dramatically reduce the potency for producing an undesirable side effect without greatly impacting hypnotic potency.
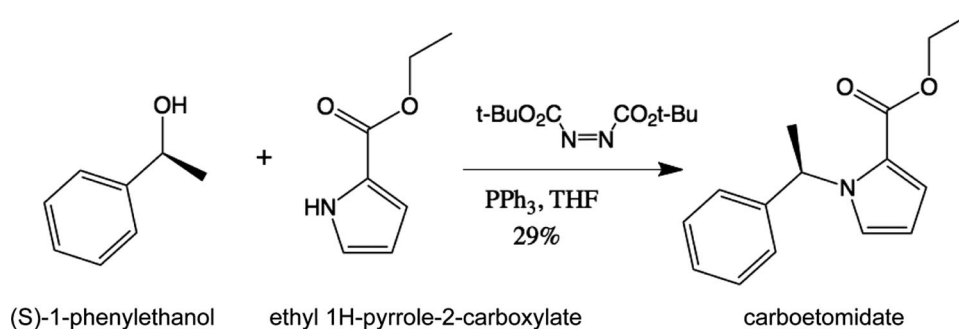
Fig. 8. Synthesis of carboetomidate.

In common with etomidate, carboetomidate significantly enhances the function of wild-type $\alpha_1\beta_2\gamma_{2l}$ GABA$_A$ receptors.[34] Thus, it seems likely that carboetomidate also produces hypnosis *via* actions on the GABA$_A$ receptor. Previous electrophysiologic studies of the GABA$_A$ receptor have shown that a mutation in the β subunit at the putative etomidate binding site (M286W) nearly completely abolishes etomidate enhancement.[35] Our studies show that this mutation also abolishes enhancement by carboetomidate, suggesting that carboetomidate modulates GABA$_A$ receptor function by binding to the same site on the GABA$_A$ receptor as etomidate.

The magnitude of potentiation that we observed with 10 μM carboetomidate ($390 \pm 80\%$) in the current study is no greater than that observed in our previous study with 4 μM etomidate ($660 \pm 240\%$). This implies that carboetomidate is less potent and/or efficacious at the GABA$_A$ receptor than is etomidate. This may explain why a higher concentration (in our tadpole assay) and dose (in our rat assay) of carboetomidate than etomidate is needed to produce LORR. It also indicates that the basic nitrogen in the imidazole ring of etomidate contributes modestly to etomidate's action on GABA$_A$ receptors.

The onset of LORR was slower with carboetomidate than with etomidate. The reason for this is unclear. However, as both hypnotics potentiate the GABA$_A$ receptor, it seems likely that onset is delayed because carboetomidate reaches its site of action in the brain more slowly than etomidate.

Carboetomidate was conceived as a pharmacodynamic solution to the problem of etomidate-induced adrenocortical suppression because it was designed not to bind to 11β-hydroxylase with high affinity. We recently described the results of studies on methoxycarbonyl etomidate, an etomidate analog developed as a pharmacokinetic solution to this same problem.[20] Methoxycarbonyl etomidate is very rapidly metabolized by esterases and, therefore, produces hypnosis of extremely short duration and does not cause prolonged suppression of adrenocortical function after administration. In contrast, carboetomidate produces hypnosis that is similar in duration to etomidate and propofol (when given at equivalent multiples of their respective ED$_{50}$ values for LORR) without inhibiting the steroid synthesis at a hypnotic dose.

Appendix

Synthesis of (R)-Ethyl 1-(1-phenylethyl)-1H-pyrrole-2-carboxylate (Carboetomidate)

Following a procedure described for Mitsunobu alkylation of imidazoles,[11] a solution of (*S*)-1-phenylethanol (135 mg, 1.10 mmol, >99% entaniomeric ratio) in dry tetrahydrofuran (2 ml) was added dropwise to a stirred solution of ethyl 1H-pyrrole-2-carboxylate (140 mg, 1.00 mmol) and triphenylphosphine (340 mg, 1.30 mmol) in dry tetrahydrofuran (THF) (3 ml) in an argon atmosphere at room temperature (fig. 8). Then a solution of di-tert-butyl azodicarboxylate (304 mg, 1.32 mmol) in dry THF (2 ml) was added, and the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was concentrated under reduced pressure. The residue was mixed with diethyl ether (5 ml) and stirred for 2 h. The residue (Ph$_3$PO and hydrazo ester) was collected and washed with diethyl ether (3×2 ml). The filtrate was evaporated under reduced pressure to yield a residue, which was purified by flash chromatography (hexanes/CH$_2$Cl$_2$ = 7:3) on silica gel to give a colorless viscous liquid (71 mg, 29% yield): IR (KBr, cm^{-1}): 737, 1106, 1231, 1700, 2980, 3328; ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.30 (t, J = 7.5 Hz, 3H), 1.80 (d, J = 7.0 Hz, 3H), 4.17–4.28 (m, 2H), 6.17 (dd, J = 4.0, 3.0 Hz, 1H), 6.60 (q, J = 7.0 Hz, 1H), 6.98–7.01 (m, 2H), 7.12–7.14 (m, 2H), 7.20–7.25 (m, 1H), 7.28–7.32 (m, 2H); ^{13}C NMR (500 MHz, CDCl$_3$): δ 14.6, 22.3, 55.5, 60.0, 108.5, 118.5, 122.6, 125.5, 126.4, 127.5, 128.7, 143.3, 161.4; LC-MS observed 244.10, calculated 244.10 for C$_{15}$H$_{18}$NO$_2$ (M + H); Analytical calculation for C, 74.05; H, 7.04; N, 5.76. Found: C, 74.25; H, 6.94; N, 5.66. The final product was determined to be essentially enantiomerically pure (>99% ee) by chiral high-pressure liquid chromatography analysis using a AD-H column, isocratic eluent of hexane and isopropanol (97:3), a flow rate of 1 ml/min and detection at l = 220 nm. See figure, Supplemental Digital Content 1, which shows the analysis of enantiomerically pure carboetomidate produced as described above using (*S*)-1-phenylethanol, http://links.lww.com/ALN/A570. For comparison, the figure also shows the analysis of racemic carboetomidate produced in a similar manner using racemic 1-phenylethanol.

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