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The Pyrrole Etomidate Analog Carboetomidate Potently Inhibits Human 5-HT_{3A} Receptor Function: Comparisons with Etomidate and Potential Implications for Emetogenesis

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Abstract

Background—5-Hydroxytryptamine type 3 (5-HT₃) receptors are excitatory ion channels belonging to the cys-loop family of ligand-gated ion channels. They are involved in nausea and vomiting and their antagonists are used clinically as antiemetic drugs. We previously reported the development of a novel pyrrole analog of etomidate (R)-ethyl 1-(1-phenylethyl)-1H-pyrrole-2-

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DISCLOSURES:

Name: Rooma Desai, PhD

Contribution: This author helped design the studies and conduct all the cell biology, electrophysiology and biochemistry experiments; analyze the data and prepare the manuscript.

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

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Conflicts of Interest: KWM is co-inventor on a patent application for carboetomidate submitted by the Massachusetts General Hospital. He, his department, his laboratory, and his institution could receive royalties relating to the development of carboetomidate or related analogs.

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Conflicts of Interest: DER is co-inventor on a patent application for carboetomidate submitted by the Massachusetts General Hospital. He, his department, his laboratory, and his institution could receive royalties relating to the development of carboetomidate or related analogs. DER is a consultant for and holds an equity position in Annovation BioPharma, a pharmaceutical company that seeks to develop technologies covered by that patent.

carboxylate (carboetomidate), which retains etomidate's desirable anesthetic and hemodynamic properties, but lacks its potent inhibitory effect on adrenocorticotrophic hormone-stimulated steroid synthesis. Also in contrast to etomidate, carboetomidate potently inhibits nicotinic acetylcholine receptors. As nicotinic acetylcholine and 5-HT₃ receptors are highly homologous, we hypothesized that carboetomidate might also potently inhibit 5-HT₃ receptors with potentially important implications for the drug's emetogenic activity. In the current studies, we investigated and compared modulation of 5-HT_{3A} receptors by carboetomidate and etomidate.

Methods—5-HT₃ receptors were heterologously expressed in human embryonic kidney cells. Drugs were applied with a multichannel superfusion pipette coupled to piezo-electric elements and currents were recorded from cells in either the whole-cell or excised outside-out patch configuration of patch clamp recordings.

Results—Carboetomidate and etomidate inhibited integrated 5-HT_{3A} receptor-mediated currents with respective half-inhibitory concentrations of 1.9 μ M (95% confidence intervals= 1.4 μ M to 2.7 μ M) and 25 μ M (95% confidence interval = 17 μ M to 37 μ M). These values may be compared to respective hypnotic concentrations of 5.4 μ M and 2.3 μ M. This inhibition reflected hypnotic effects on peak current amplitudes and desensitization rates. Half-inhibitory concentrations for reducing peak current amplitudes were 34 μ M (95% confidence interval = 24 μ M to 48 μ M) for carboetomidate and 171 μ M (95% confidence intervals = 128 μ M to 228 μ M) for etomidate. Half-inhibitory concentrations for reducing the desensitization time constant were 3.5 μ M (95% confidence interval = 2.4 μ M to 5.1 μ M) for carboetomidate and 36 μ M (95% confidence intervals = 21 μ M to 59 μ M) for etomidate.

Conclusions—In contrast to etomidate, carboetomidate inhibits 5-HT_{3A} receptor-mediated currents at hypnotic concentrations. This inhibition is primarily the result of enhancing the rate of desensitization. Because carboetomidate potently inhibits 5-HT_{3A} receptors, it may be less emetogenic than etomidate.

Introduction

Serotonin type 3 (5-HT₃) receptors belong to the cys-loop superfamily of ligand-gated ion channels.¹ They are expressed in the area postrema of the brain and at vagal afferences that serve as important mediators of nausea and vomiting.^{2,3} Antagonists of 5-HT₃ receptors (e.g., ondansetron) are often used clinically to prevent nausea and vomiting associated with chemotherapy and general anesthesia.⁴ Intravenous anesthetics (e.g., propofol, etomidate, and pentobarbital) can also inhibit 5-HT₃ receptor currents;⁵ however, this occurs only at supra-clinical concentrations. Logically, such inhibition is not clinically relevant. Conversely, most inhaled anesthetics (e.g., isoflurane, enflurane, desflurane, halothane, and chloroform) enhance 5-HT₃ receptor function,⁶ an effect that occurs at clinically relevant concentrations and may augment their emetogenic properties.

Etomidate is an IV sedative-hypnotic that is commonly used as an anesthetic induction drug in elderly and critically ill patients. It produces hypnosis by enhancing the function of gamma-aminobutyric acid A (GABA_A) receptors. Unfortunately, etomidate is emetogenic and suppresses adrenocortical steroid synthesis, characteristics that significantly reduce its utility.

We developed (R)-ethyl 1-(1-phenylethyl)-1H-pyrrole-2-carboxylate (carboetomidate) as a pyrrole analog of etomidate and showed that it retains etomidate's enhancing action on GABA_A receptors and ability to produce hypnosis, but lacks its potent inhibitory effect on adrenocorticotrophic hormone-stimulated steroid synthesis and enhancing effect on proinflammatory cytokine production in a model of sepsis.⁷⁻⁹ Our subsequent studies showed additional pharmacological differences between the two drugs as carboetomidate

inhibits neuronal nicotinic acetylcholine receptors at clinically relevant (i.e., hypnotic) concentrations whereas etomidate does not.¹⁰ As nicotinic acetylcholine and 5-HT₃ receptors are structurally and functionally highly homologous, we hypothesized that carboetomidate would also inhibit 5-HT₃ receptors at hypnotic concentrations. Such inhibition of 5-HT₃ receptors could have important clinical implications if it leads to a reduction in the emetogenic potential of carboetomidate relative to that of etomidate.

To test our hypothesis that carboetomidate inhibits 5-HT₃ receptors at hypnotic concentrations, we defined the effects of carboetomidate on 5-HT_{3A} receptors using whole-cell and excised outside-out configurations of the patch-clamp technique. For comparison, we also defined the effects of etomidate on this receptor. Our studies showed that while both hypnotics inhibit 5-HT_{3A} receptor function, carboetomidate is an order of magnitude more potent than etomidate and produces significant receptor inhibition at hypnotic concentrations.

Methods

IRB approval for these studies was not sought because neither animals nor humans were used.

Drugs and Solutions

5-HT was obtained from Sigma-Aldrich (St. Louis, MO). 5-HT solutions were freshly prepared daily in bath solution. Etomidate was purchased from Bachem Americas (Torrance, CA) and carboetomidate was custom synthesized (>95% purity) by Aberjona Laboratories (Beverly, MA) using our previously described method. One M stocks of both etomidate and carboetomidate were prepared in dimethyl sulfoxide (DMSO) and stored at -20°C in glass vials. Working solutions of both drugs were prepared fresh daily from DMSO stock solutions. For carboetomidate experiments, the highest final concentration of DMSO was 0.004%, which had no effect on 5-HT_{3A} currents. For etomidate experiments, the highest final concentration of DMSO was 0.1%, which inhibited 5-HT_{3A} currents peak current amplitudes by ~10%. As this DMSO concentration was achieved only in experiments using 1 mM etomidate, no attempt was made to correct for this additional modest level of inhibition.

Cell Culture and Preparation of Inducible Stable Cell Line Expressing 5-HT_{3A} Receptors

Human 5-HT_{3A} cDNA (accession # NM_000869) was sub-cloned into tetracycline-regulated expression vector pCDNA4/TO (Life Technologies, Grand Island, NY) using the BamH I and Not I sites. Previously generated human embryonic kidney 293 cells stably expressing tetracycline repressor protein (HEK293-TetR) cells¹¹ were grown in Dulbecco's modified eagles medium/F12 (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (heat-inactivated), 100 units/ml penicillin/streptomycin, and 5 µg/ml blasticidin, in a 5% CO₂ incubator at 37 °C. HEK293-TetR cells were seeded in a 25 cm² cell culture flask 24 h before transfection and maintained at 37°C until they were ~ 90% confluent. Cells were then transfected with 15 µg of 5-HT_{3A}-pCDNA4/TO plasmid DNA using Lipofectamine (Life Technologies, Grand Island, NY) according to manufacturers recommendations. Twenty-four hours post-transfection, zeocin (250 µg/mL; Life Technologies, Grand Island, NY) was added to the culture medium and cells grown for another two weeks to obtain zeocin-resistant cells. The zeocin-resistant cells were subjected to single-cell sorting using the analytic seven laser LSR flow cytometer (BD Biosciences, Durham, NC) at the Massachusetts General Hospital research flow cytometry core facility to sort single cell per well in a 96-well plate. Colonies from single cells were allowed to grow to confluence before testing them for 5-HT_{3A} receptor expression. After 24-hour induction

with tetracycline, single clones expressing high levels of 5-HT_{3A} channels were identified with whole-cell patch-clamp electrophysiology.

Membrane Isolation and Western Blotting

HEK 293 cells expressing 5-HT_{3A} channels (24 hours post-induction with tetracycline) were harvested by scraping and were washed twice in ice-cold phosphate-buffered saline (PBS). Crude membranes were isolated from harvested cells and Western blot was performed as previously described.¹² Blots were air-dried overnight and then blocked in PBS containing 10% nonfat milk and 0.05% Tween 20 (blocking buffer) for 1 h at room temperature with shaking. Blots were then incubated with 1 µg/ml anti-5-HT_{3A} antibody (goat polyclonal immunoglobulin [Ig]G; Tocris Bioscience, Minneapolis, MN) in the blotting buffer overnight at 4 °C. After three washes with blocking buffer, blots were incubated for 1 h with horseradish peroxidase-conjugated mouse anti-goat antibody, followed by extensive washes in PBS. Labeled proteins were detected by enhanced chemiluminescence (Figure 1). The predicted molecular weight of mature 5-HT_{3A} protein is 52.8 kDa. In Western blot analysis, anti-5-HT_{3A} antibody specifically recognized a ~50-kDa protein band in tetracycline-induced 5-HT_{3A}-HEK 293 cells, but not in control uninduced cells (Figure 1). In the tetracycline-induced lane, we also detected bands at ~100, ~200 and ~250 kDa, which may represent aggregates of channel protein. Based on the resolving power of our gel, our inducible cell line appears to express the full-length 5-HT_{3A} protein.

Electrophysiology

Whole-cell or excised outside-out configuration of patch clamp technique was used to record currents from HEK 293 cells expressing 5-HT_{3A} receptors. Cells were seeded on a glass coverslip and channel expression induced with tetracycline (2mg/ml) 24–36 hours before recordings. All experiments were performed at room temperature (20 – 22°C). The recording chamber was continuously perfused with the bath solution: (in mM) 150 NaCl, 0.5 CaCl₂, and 10 HEPES, pH 7.4 (pH adjusted with NaOH). The electrode solution contained (in mM) 134 KCl, 5 EGTA, 1 MgCl₂ and 10 HEPES, pH 7.4 (pH adjusted with KOH). The open pipette resistances ranged from 2–4 MΩ for whole-cell recordings, 4–8 MΩ for outside-out patches. Cells and patches were voltage clamped at –70 mV using the patch clamp amplifier (Axopatch 200B, Molecular Devices Corp., Sunnyvale, CA). Whole-cell membrane capacitance and series resistance were compensated electronically by more than 85 % with lag of 10 ms. Series resistance ranged from 1–5 MΩ and cell capacitances from 3.5–10 pF. 5-HT_{3A} receptors were activated with 5-HT delivered via a multichannel superfusion pipette coupled to piezo-electric elements that switched superfusion solution in under 1 ms. Currents were digitized at 2 kHz and filtered at 1 kHz and using pCLAMP v8.1 (Molecular Devices Corp., Sunnyvale, CA) for off-line analysis. Five to ten pulses of 5-HT were applied 120s apart until the peak currents stabilized before applying hypnotic drugs. During whole-cell recordings, the hypnotics were applied in equilibrium, by pre-incubating the cells for 5 minutes in bath solution containing hypnotic before its co-application with 100 µM 5-HT.

Data Analysis and Statistics

5-HT_{3A} Receptor Inhibition by Hypnotics and Recovery from Inhibition—

Control traces of nondesensitizing 5-HT_{3A} currents were obtained by applying a 30 s pulse of 1 µM 5-HT. Hypnotic effects were assessed by sequential application of agonist alone (for 10–15 s until peak amplitudes were reached), followed by agonist plus hypnotic for 10 s and then returned to agonist alone (10 s). To evaluate inhibition, percent changes in maximum peak current amplitude obtained during the first agonist alone pulse and minimum amplitude obtained during the agonist and hypnotic pulse from the same trace were

calculated. For assessing recovery from inhibition, the percent change in peak current amplitudes between the first and second 1 μ M 5-HT pulses were calculated.

Determination of Half-Inhibitory Hypnotic Concentrations (IC₅₀s) for reducing integrated currents and peak current amplitudes—Control currents were obtained by perfusing whole cells with bath solution containing 100 μ M 5-HT for 30 s. After a 2-minute recovery period, test currents were obtained by perfusing the same cell first with bath solution containing the desired hypnotic (for 5 minutes) and then with bath solution containing both hypnotic and 100 μ M 5-HT for 30 s. From each recorded current trace, the integrated current (i.e., the area under the curve) and peak current amplitude were calculated. To account for variable receptor expression in cells, integrated currents and peak current amplitudes obtained in the presence of hypnotic were normalized to the control values obtained in the absence of hypnotic in the same cell. Hypnotic concentration-normalized response curves were fit to a Hill equation in the form:

$$I_{norm} = \frac{1}{1 + 10^{\log[\text{Hypnotic}] - \log[\text{IC}_{50}]}}$$

where I_{norm} is the normalized integrated current (or peak current) obtained in the presence of hypnotic and the IC_{50} is the hypnotic concentration that reduces the integrated current (or peak current) by half.

Determination of time constants for desensitization and deactivation—Decay phase of currents were fitted with exponential functions using the Levenberg-Marquardt algorithm with least square minimization to determine time constants for deactivation and desensitization. The number of exponentials that best fit the decay of currents was determined using an F-test. Most decay components were best fit by two exponentials; however, some were fitted by more than two exponentials. To allow for comparisons, a weighted time constant $\Sigma\tau(A/\Sigma A_n)$ was determined. Current traces were analyzed using Clampfit version 9.0, (Molecular Devices).

All statistical analyses (curve fitting of dose-response relationships, 95% confidence interval determinations, and Student's *t* tests using Welch's method) were performed using Graphpad Prism v.5 software (Graphpad Software, Inc., San Diego, CA). For each experiment, a sample size was chosen based on an assessment of the magnitude and variability of the hypnotic effect observed in pilot experiments. Because the differences between carboetomidate and etomidate in measured integrated areas, peak amplitudes, and desensitization rates were large and consistent, we anticipated that statistical significance could be achieved with a relatively small sample size (i.e., $n=3-5$ cells). Hypnotic effects on deactivation rates measured in patches were smaller and less consistent. Therefore a larger sample size was used ($n=7$ patches). All data are expressed as mean $\pm$ SEM.

Results

Carboetomidate Inhibits 5-HT_{3A} Receptors At Hypnotic Concentrations Whereas Etomidate Does Not

As an initial assessment of the effects of carboetomidate and etomidate on 5-HT_{3A} receptor function, we activated 5-HT_{3A} receptors with 1 μ M 5-HT (a low agonist concentration that submaximally activates receptors and induces little receptor desensitization) and then co-applied either carboetomidate or etomidate (10 μ M). In Figure 2, the left panel shows a representative control current trace obtained upon application of 1 μ M 5-HT without hypnotic whereas the middle and right panels show the rapid but only partially reversible

inhibition produced by carboetomidate and etomidate when co-applied with 1 μM 5-HT for 10 s during the 30 s 1 μM 5-HT pulse. In these experiments, inhibition was greater and recovery was less with co-application of carboetomidate than with etomidate. Figures 2B show the results of multiple experiments ($n=3-5$) using this protocol and demonstrates that the differences between these two agents were highly statistically significant; inhibition was greater ($85.5 \pm 0.2\%$ versus $33 \pm 2\%$, $p < 0.0001$; left panel) and recovery less ($57 \pm 4\%$ versus $87.1 \pm 0.9\%$, $p < 0.0001$; right panel) with carboetomidate than with etomidate.

Figure 3A shows representative current traces obtained when 5-HT_{3A} receptors were pre-equilibrated for 5 min with either carboetomidate (left panel) or etomidate (right panel) and then activated for 30 s with 100 μM 5-HT, a concentration that induces maximal receptor activation followed by desensitization. These traces show that both hypnotics inhibit 5-HT_{3A} receptor-mediated currents. To quantify carboetomidate and etomidate potencies, IC₅₀s for inhibiting integrated currents were derived from studies using a range of hypnotic concentrations (Figure 3B, left panel). These studies showed that both hypnotics reduced integrated currents in a concentration-dependent manner. However, carboetomidate inhibited integrated currents significantly more potently than etomidate with respective IC₅₀s of 1.9 μM (95% confidence intervals = 1.4 μM to 2.7 μM) and 25 μM (95% confidence interval = 17 μM to 37 μM). These IC₅₀s are 35% and 1100% of the hypnotic EC₅₀s for carboetomidate and etomidate, respectively.

Because hypnotic effects on integrated currents clearly reflected the combined actions on peak current amplitudes and desensitization rates, we also individually characterized these actions. The middle and right panels in Figure 3B shows that both carboetomidate and etomidate produce concentration-dependent reductions in both peak currents and weighted time constants for desensitization. In both cases, carboetomidate acted more potently than etomidate. The calculated IC₅₀s for peak current inhibition were 34 μM (95% confidence interval = 24 μM to 48 μM) for carboetomidate and 171 μM (95% confidence intervals = 128 μM to 228 μM) for etomidate. The calculated IC₅₀s for reducing the desensitization time constant were 3.5 μM (95% confidence interval = 2.4 μM to 5.1 μM) for carboetomidate and 36 μM (95% confidence intervals = 21 μM to 59 μM) for etomidate.

Carboetomidate Accelerates 5-HT_{3A} Receptor Deactivation

We further investigated carboetomidate's effect on the 5-HT_{3A} receptor after very brief (4 ms) 5-HT exposure as might be encountered at a synapse. To achieve such a rapid change in agonist concentration, we used excised outside-out patches expressing 5-HT_{3A} receptors rather than whole cells. Figure 4A shows representative normalized current traces demonstrating the effects of carboetomidate on the rate of channel closure after a brief agonist pulse (i.e., deactivation) using such patches. In control traces (i.e., no carboetomidate), the deactivation time constant was 460 ± 130 ms ($n=7$). Carboetomidate significantly reduced the time constant (increased the deactivation rate) to 110 ± 25 ms (Figure 4B; $n=5$, Student's *t* test using Welch's method; $p=0.033$).

Discussion

Our studies show that carboetomidate and etomidate inhibit 5-HT_{3A} receptors by reducing the peak current amplitude and accelerating the rate of 5-HT-induced desensitization. However, because carboetomidate is an order of magnitude more potent an inhibitor of the 5-HT_{3A} receptor than is etomidate (1.9 μM versus 25 μM , respectively), it produces significant 5-HT_{3A} receptor inhibition at its hypnotic concentration of 5.4 μM .

The 5-HT_{3A} receptor belongs to a superfamily of homologous ligand-gated ion channels that also includes the GABA_A, glycine, and nicotinic acetylcholine receptors.¹³ Receptors in this

superfamily exist in a conformational equilibrium between resting, open, and desensitized states.^{14–16} Agonist binding shifts this equilibrium, producing the changes in ion permeability that define receptor function.^{14,15,17} In the absence of agonist, the resting state predominates. Because the ion channel in the resting state is closed, essentially no ions flow.¹⁵ The presence of agonist shifts the receptor equilibrium towards the open state.¹⁴ Ions flow through the open channels down their electrochemical gradients into neuronal cells, resulting in depolarization (in the case of 5-HT₃ and acetylcholine receptors) or hyperpolarization (in the case of GABA_A and glycine receptors). If agonist remains bound to receptors for a prolonged time, receptors isomerize to the desensitized state in a slowly reversible process called desensitization.¹⁵ Because the desensitized state does not allow ion flux, the desensitization process is an important negative feedback mechanism and its modulation a versatile mechanism for shaping synaptic transmission.¹⁸

A drug may inhibit the function of 5-HT₃ receptors by a variety of mechanisms.¹⁹ It may competitively inhibit 5-HT binding. It may also block the open channel. This blockade may reflect direct occlusion of the open state's ion channel or an allosteric effect in which the drug binds elsewhere on the open state and causes the channel to close. Because 5-HT₃ receptors open and close in response to agonist binding, drugs that change the rates with which receptors transition between states (i.e., their kinetics) may also produce inhibition. For example, a drug may inhibit receptor function by increasing the rates with which they isomerize to closed (resting and desensitized) states. Examples of drugs that inhibit 5-HT_{3A} receptors by increasing the 5-HT_{3A} receptor's desensitization rate include the antidepressants desipramine and imipramine.²⁰

The present studies with carboetomidate and etomidate indicate that these two hypnotics inhibit 5-HT_{3A} receptors by two similar, if not identical, mechanisms. They block open state receptors and accelerate the rates with which receptors desensitize with prolonged agonist exposure. However the potencies of these drugs differ significantly. Carboetomidate is 5-fold more potent a blocker of peak current amplitudes and 10-fold more potent an accelerator of desensitization than is etomidate. Consequently, carboetomidate inhibits integrated currents with an IC₅₀ of 1.9 μM, which is an order of magnitude lower than that of etomidate and a fraction of its own hypnotic EC₅₀ of 5.4 μM.

It is also notable that the relative potencies with which the two hypnotics block open state receptors and accelerate the rates with which receptors desensitize differ; compared to etomidate, carboetomidate is 13-fold more potent an accelerator of desensitization and 5-fold more potent a channel blocker. This relative potency difference suggests that these two receptor actions are modulated by hypnotic binding to different binding sites having different affinities for the two agents.

In addition to accelerating desensitization induced by prolonged (30 s) exposure to 100 μM 5-HT, we also found that carboetomidate accelerates the rate of deactivation after very brief (4 ms) 5-HT exposure. Interpreting this observation requires knowing what happens to receptors with brief agonist exposure. In the case of 5-HT_{3A} receptors, our previous studies indicate that 5-HT unbinds extremely slowly from open state receptors.¹⁵ Consequently, many open state receptors continue to desensitize even after terminating 5-HT application because 5-HT remains bound for many seconds after the agonist has been removed from the bath solution. This important link between desensitization and deactivation explains why 5-HT_{3A} receptor desensitization and deactivation occur on approximately the same time scale and predicts our observation that these two processes are similarly affected (accelerated) by carboetomidate.

We believe that the abilities of carboetomidate and etomidate to accelerate desensitization also explains the incomplete recoveries observed in experiments using 1 μ M 5-HT (Figure 2). Ordinarily, few receptors are desensitized in the presence of very low 5-HT concentrations because the rate with which receptors desensitize (which depends upon the 5-HT concentration) is much slower than the rate with which they recover from desensitization. However by accelerating the desensitization rate, carboetomidate and etomidate increase the fraction of receptors that are desensitized by 1 μ M 5-HT and thus reduce the fraction of receptors that rapidly recover their ability to open once hypnotic is removed.

The emetogenic potential of carboetomidate is not known because mammalian studies have been limited to rats, which are unable to vomit. Nausea and vomiting are mediated by the 5-HT₃ receptor and 5-HT₃ receptor inhibitors are commonly used for their prevention or treatment.^{2,3} With the caveat that emesis is a highly complex reflex involving multiple mechanisms, our results demonstrating that carboetomidate potently inhibits the 5-HT_{3A} receptor suggests that carboetomidate might be less emetogenic than etomidate in humans because it has an ondansetron-like action at hypnotic concentrations.^{21, 22} Future studies using non-rodent species and humans will be required to define carboetomidate's actual emetogenic activity.

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Figure 1. Expression of 5-HT_{3A} protein in HEK 293 inducible cell line

No signal was observed in uninduced HEK 293 cells control lane. Anti-5HT_{3A} immuno-reactive protein from tetracycline-induced HEK 293 cells expressing 5HT_{3A} cDNA has a molecular weight of ~50kDa, similar to its predicted molecular weight. 5-HT_{3A} protein aggregates were also observed at ~100, ~200 and ~250 kDa.

Figure 2. Carboetomidate and etomidate reversibly inhibit 5-HT_{3A} currents

(A) Representative current traces of 5-HT_{3A} currents elicited by 30 s application of 1 μ M 5-HT alone (left panel) and 10 s co-application of 10 μ M carboetomidate (middle panel) or etomidate (right panel) at midpoint during a 30 s application of 1 μ M 5-HT pulse. (B) Percent inhibition (left panel) and recovery (right panel) of peak current amplitudes after application of carboetomidate or etomidate on 1 μ M 5-HT elicited currents. Means $\pm$ SEM of $n=3-5$, *** $P<0.0001$, unpaired Students t -test.

Figure 3. Concentration-response curves for inhibition of 5-HT_{3A} currents by carboetomidate and etomidate

(A) Representative normalized current traces of inhibition of 5-HT_{3A} currents by different concentrations of carboetomidate (left) and etomidate (right). 5-HT_{3A} currents elicited by 100 μM 5-HT. (B) Normalized integrated current (left panel), normalized peak amplitudes (middle panel) and normalized time constants of desensitization (right panel) as a function of hypnotic concentration. The control time constants of desensitization were 8.7 ± 0.8 and 10.8 ± 0.8 s in the etomidate and carboetomidate groups, respectively. Data fit to Hill equation. Means $\pm$ SEM for 3–8 cells for each hypnotic concentration tested.

Figure 4. Carboetomidate enhances the 5-HT_{3A} current deactivation rates in outside-out patches (A) Representative normalized traces of 5-HT_{3A} currents elicited by a 4 ms pulse of 100 μ M 5-HT alone, or with 10 μ M carboetomidate during deactivation. (B) Mean $\pm$ SEM of deactivation time constants for control (closed square, n=7) and in the presence of carboetomidate (closed circle; n=5). *P < 0.043; unpaired Students *t*-test