Sedative-hypnotic Binding to 11β-hydroxylase

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ABSTRACT

Background: Etomidate potently suppresses adrenocortical steroid synthesis with potentially deleterious consequences by binding to 11β-hydroxylase and inhibiting its function. The authors hypothesized that other sedative-hypnotics currently in clinical use or under development (or their metabolites) might bind to the same site at clinically relevant concentrations. The authors tested this hypothesis by defining etomidate's affinity for this site and the potencies with which other sedative-hypnotics (and their metabolites) inhibit etomidate binding.

Methods: ^{3}H-etomidate's binding to adrenal membranes from Sprague-Dawley rats was characterized with a filtration assay, and its dissociation constant was defined using saturation and homologous ligand competition approaches. Half-inhibitory concentrations of sedative-hypnotics and metabolites were determined from the reduction in specific ^{3}H-etomidate binding measured in the presence of ranging sedative-hypnotic and metabolite concentrations.

Results: Saturation and homologous competition studies yielded ^{3}H-etomidate dissociation constants of 40 and 21 nM, respectively. Half-inhibitory concentrations of etomidate and cyclopropyl methoxycarbonyl metomidate (CPMM) differed significantly (26 vs. 143 nM, respectively; $P < 0.001$), and those of the carboxylic acid (CA) metabolites etomidate-CA and CPMM-CA were greater than or equal to 1,000× higher than their respective parent hypnotics. The half-inhibitory concentration of dexmedetomidine was 2.2 μM, whereas those of carboetomidate, ketamine, and propofol were greater than or equal to 50 μM.

Conclusion: Etomidate's in vitro dissociation constant for 11β-hydroxylase closely approximates its in vivo adrenocortical half-inhibitory concentration. CPMM produces less adrenocortical suppression than etomidate not only because it is metabolized faster but also because it binds to 11β-hydroxylase with lower affinity. Other sedative-hypnotics and metabolites bind to 11β-hydroxylase and inhibit etomidate binding only at suprahypnotic concentrations. **(ANESTHESIOLOGY 2016; 125:943-51)**

ETOMIDATE is an intravenous sedative-hypnotic agent that is highly valued for its ability to maintain hemodynamic stability even in elderly or critically ill patients.[1–3] Unfortunately, etomidate also suppresses adrenocortical function with potentially deleterious consequences, particularly in the critically ill.[4–7] Because this side effect occurs even at subhypnotic concentrations and etomidate's terminal elimination half-life is several hours, a single hypnotic dose of etomidate that produces only minutes of hypnosis can suppress adrenocortical function for many hours or even days.[8–11] Etomidate is believed to suppress adrenocortical function primarily by binding within a heme-containing cavity that forms the active site of 11β-hydroxylase, thus inhibiting the enzyme's function.[12–14]

Our laboratory has employed two distinct medicinal chemistry strategies—one pharmacokinetic and the other pharmacodynamic—to reduce etomidate's ability to suppress the adrenocortical function.[15,16] The pharmacokinetic strategy is to design analogs of etomidate that are rapidly hydrolyzed by nonspecific esterases to form a carboxylic acid (CA) metabolite with low affinity for 11β-hydroxylase.[17–19] Consequently, any adrenocortical suppression produced by the drug during administration would lift quickly after the administration had stopped. This "soft analog" approach is exemplified by cyclopropyl

> ### What We Already Know about This Topic
> - Etomidate inhibits adrenocortical function by binding to 11β-hydroxylase, a cytochrome P450 enzyme required for the biosynthesis of cortisol, corticosterone, and aldosterone
>
> ### What This Article Tells Us That Is New
> - The in vitro dissociation constant of etomidate for 11β-hydroxylase is approximately 30 nM, which is nearly two orders of magnitude lower than its hypnotic concentration
> - Cyclopropyl methoxycarbonyl metomidate produces less adrenocortical suppression than etomidate because it is metabolized faster and binds to 11β-hydroxylase with lower affinity
> - The carboxylic acid metabolites of etomidate and cyclopropyl methoxycarbonyl metomidate bind to 11β-hydroxylase with affinities that are at most 1% that of their parent hypnotics
> - Carboetomidate, dexmedetomidine, ketamine, and propofol do not suppress adrenocortical function at hypnotic concentrations because they bind to 11β-hydroxylase with very low affinities

methoxycarbonyl metomidate (CPMM), which is rapidly hydrolyzed to form CPMM-CA.[20,21] CPMM is currently undergoing clinical trials.

The pharmacodynamic strategy is to design analogs of etomidate that bind poorly to 11β-hydroxylase. This approach is exemplified by carboetomidate.[14,22] In carboetomidate, the basic nitrogen that is found in etomidate's imidazole ring and is hypothesized to be required for

high-affinity binding to 11β-hydroxylase has been replaced with a methylene group.[14] Although carboetomidate's binding affinity for 11β-hydroxylase has never been quantified, *in vitro* studies in an adrenocortical cell line revealed that it is three orders of magnitude less potent than etomidate at inhibiting cortisol synthesis and *in vivo* studies in animals showed that it does not suppress adrenocortical function when administered at hypnotic doses.[22,23]

The etomidate analogs that we have produced (and their metabolites) are, by definition, structurally similar to etomidate, and some have been shown to be capable of suppressing adrenocortical function.[21,24,25] This suggests that they may bind to 11β-hydroxylase at the same site as etomidate. Other hydrophobic sedative-hypnotics that are structurally distinct from etomidate (*e.g.*, dexmedetomidine, ketamine, and propofol) have similarly been shown to be capable of suppressing adrenocortical function, but their molecular sites of action are unknown.[26–29] We hypothesized that some of these drugs might also bind to 11β-hydroxylase at the same site as etomidate, particularly as this site is believed to be hydrophobic.[30] To test this hypothesis, we characterized the specific binding of ^{3}H-etomidate to its high-affinity site in adrenal membranes. We then quantified the abilities of etomidate (and its metabolite), the etomidate analogs CPMM (and its metabolite) and carboetomidate, as well as the intravenous sedative-hypnotics dexmedetomidine, ketamine, and propofol to bind to 11β-hydroxylase and displace ^{3}H-etomidate from that binding site.

Materials and Methods

Sedative-hypnotic Drugs and Metabolites

Figure 1 shows the structures of etomidate, the etomidate analogs CPMM and carboetomidate, the CA metabolites of etomidate and CPMM (etomidate-CA and CPMM-CA, respectively), and the clinically used sedative-hypnotic agents evaluated in this study. Etomidate was purchased from Bachem Americas (USA). Etomidate-CA was synthesized by base hydrolysis as previously described.[17] CPMM, CPMM-CA, and carboetomidate were synthesized by Aberjona Laboratories (USA) as previously described.[20,22,31] Dexmedetomidine, ketamine, and propofol were purchased from Sigma-Aldrich (USA). ^{3}H-etomidate was prepared from unlabeled ("cold") etomidate by Perkin-Elmer Life Sciences (USA) using a catalytic exchange reaction. The mass fragmentation pattern of ^{3}H-etomidate showed that all of the tritium was located on the imidazole ring. The specific activity of ^{3}H-etomidate was 11 Ci/mM.

Rat Adrenal Tissue Membrane Preparation

Adrenal glands from eight rats were purchased from Bioreclamation IVT (USA) and shipped frozen to our laboratory. After thawing, the adrenal glands were placed on a glass stand, cut into pieces, and then added to ice-cold buffer (HEPES, 10 mM; EDTA, 1 mM; leupeptin, 10 μg/ml; chymostatin, 10 μg/ml; pepstatin A, 10 μg/ml; aprotinin, 2 μg/ml; polymethanesulfonyl fluoride, 1 mM; and ethanol, 10 μl/ml). The solution was homogenized on ice first using an electric homogenizer (Junke & Kunkel, Germany) (six times for 5 s each) and then by hand using a glass and teflon homogenizer
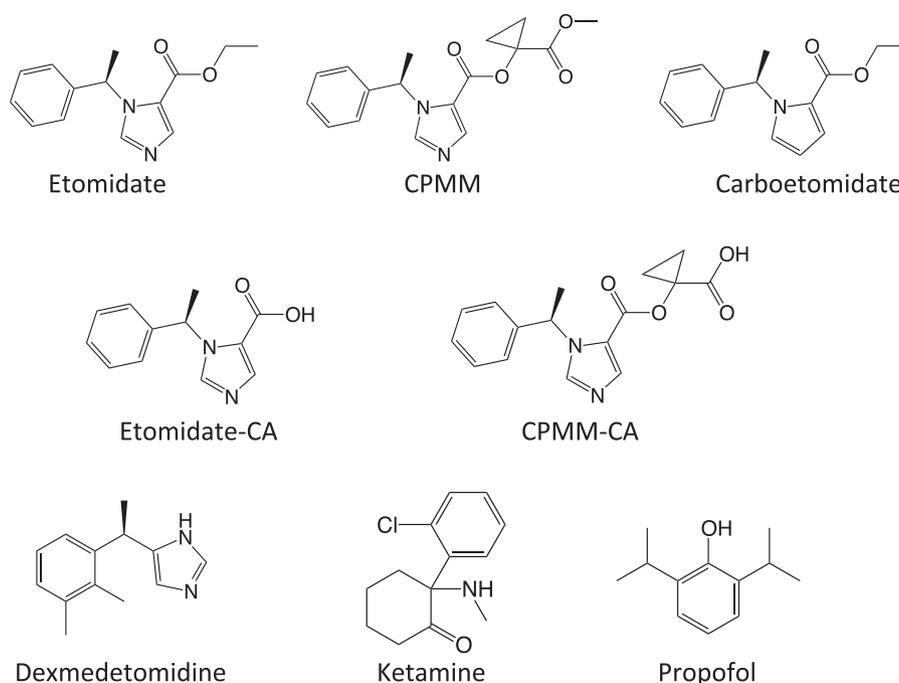
Fig. 1. Chemical structures of etomidate and the etomidate analogs cyclopropyl methoxycarbonyl (CPMM) and carboetomidate (*top*), carboxylic acid (CA) metabolites etomidate-CA and CPMM-CA (*middle*), and other clinical sedative-hypnotic agents (*bottom*).

(Kimble Chase Life Science and Research Products, USA) (20 passes). Diisopropyl fluorophosphate (Sigma-Aldrich; final concentration, 1 mM) was added to the homogenate, and the mixture was allowed to incubate for 60 min at room temperature to inactivate esterase activity. The mixture was then centrifuged (at 4°C, 43,100*g* for 30 min), and the pellet was washed. After a second centrifugation and washing cycle, the pellet was resuspended in 5 ml buffer by manual homogenization and passed through a 23-gauge needle three times. The final membrane preparation was aliquoted into 1-ml Eppendorf tubes and stored at –80°C until used. The protein concentration in these samples was quantified using a Pierce BCA Protein Assay Kit (ThermoFisher, USA). All binding experiments were performed in glass vials at room temperature, with the final concentration of protein adjusted to 0.07 mg/ml.

Association and Dissociation Kinetics of ^{3}H-etomidate with Rat Adrenal Membranes

For association rate studies, adrenal membranes (6.7 ml) were mixed with ^{3}H-etomidate (final concentration, 2 nM) by gentle vortexing. After the desired incubation time, a 0.5 ml aliquot was passed through a presoaked (with 0.5% polyethylenimine in water for 2 h) 25-mm GF/B glass fiber filter (Whatman, United Kingdom) under suction, and the filter was immediately washed twice with 5 ml of buffer. After drying under a heat lamp for 2 h, each filter was transferred to a scintillation vial. Liquiscint scintillation cocktail (National Diagnostics, USA) was added to the vial, and the radioactivity in the vial was quantified using a Packard Tri-Carb liquid scintillation counter (PerkinElmer, USA). The zero time point was defined in parallel experiments performed without membranes (*i.e.*, no ^{3}H-etomidate binding to membranes).

For dissociation rate studies, adrenal membranes were equilibrated (for 30 min) with ^{3}H-etomidate (final concentration, 2 nM). Excess cold etomidate (final concentration, 100 μM) was then added to the mixture with gentle vortexing. After the desired incubation time, a 0.5 ml aliquot was passed through a presoaked 25-mm GF/B glass fiber filter under suction, the filter was washed and dried, and the radioactivity was quantified as described in the previous paragraph for association rate studies. The zero time point was defined in parallel experiments performed without excess cold etomidate (*i.e.*, total ^{3}H-etomidate binding unperturbed by cold etomidate).

Equilibrium Binding of ^{3}H-etomidate to Rat Adrenal Membranes

Saturation Binding Experiments. Adrenal membranes were equilibrated (for 15 to 30 min) with a range of ^{3}H-etomidate concentrations along with cold etomidate (1:19 molar ratio of the two ligands; total volume, 0.5 ml). The purpose of adding cold etomidate along with ^{3}H-etomidate was to reduce the quantity of the latter required to achieve saturation binding (by 95%) and lower radiation

exposure.[32] Thus, the total etomidate concentration (*i.e.*, tritiated + cold) ranged from 0 to 256 nM, but the maximum ^{3}H-etomidate was only 12.8 nM. After equilibration, the mixture was passed through a presoaked 25-mm GF/B glass fiber filter under suction, the filter was washed and dried, and the radioactivity was quantified as described in the previous section for kinetic studies. Nonspecific binding was determined in parallel experiments performed in the presence of excess cold etomidate (final concentration, 100 μM).

Competitive Binding Experiments. Adrenal membranes were equilibrated (for 15 to 30 min) with 2 nM ^{3}H-etomidate along with ranging concentrations of the desired sedative-hypnotic agent or sedative-hypnotic metabolite (total volume, 0.5 ml). After equilibration, the mixture was passed through a presoaked 25-mm GF/B glass fiber filter under suction, the filter was washed and dried, and the radioactivity was quantified as described for kinetic studies.

Statistical Analysis

The association and dissociation half-times were defined by fitting the relationship between filter radioactivity and incubation time to a single exponential function. Etomidate's equilibrium dissociation constant (K_D) and the maximum concentration of binding sites in the sample (B_{max}) were determined from the total and nonspecific binding studies by nonlinear regression using the built-in function ("one site—total and nonspecific binding") in Prism 6 for Mac OS X (Graphpad, USA). We did not weight the data and used an extra sum-of-squares F test to compare one- *versus* two-site models. The one-site model was chosen over the two-site model as the *P* value was greater than 0.05. From homologous competition studies (*i.e.*, competition of 2 nM ^{3}H-etomidate binding by a range of cold etomidate concentrations), we defined etomidate's K_D and the B_{max} using the built-in function for equation 1 ("one site—homologous") in Prism 6 for Mac OS X:

$$^3\text{H-etomidate binding}$$
$$= \frac{B_{max} \times [^3\text{H-etomidate}]}{[^3\text{H-etomidate}] + [\text{cold etomidate}] + K_D} + \text{NS} \quad (1)$$

where ^{3}H-etomidate binding is the radioactivity in the washed filter (in counts per minute [CPM]), ^{3}H-etomidate is constant at 2 nM, and NS is the radioactivity in the filter at an infinite cold etomidate concentration (*i.e.*, nonspecific binding).

For homologous and nonhomologous competition studies, we calculated half-inhibitory concentrations (IC$_{50}$s) by nonlinear regression from equation 2 using the built-in function in Prism 6 for Mac OS X:

Normalized specific ^{3}H-etomidate binding

$$= \frac{100}{1+10^{(LogIC_{50} - log[\,competing\,ligand\,]) \times n_{Hill}}} \quad (2)$$

where normalized specific ^{3}H-etomidate binding is the cold etomidate-displaceable radioactivity in the washed filter (in CPM) normalized to a maximum value of 100% in the absence of competing ligand, and n_{Hill} is the Hill coefficient. Using the unweighted data, a statistical comparison between the IC_{50}s of etomidate and CPMM was performed using extra sum-of-squares F tests with Prism 6 for Mac OS X.

All data are reported as mean ± SD. Sample sizes (n = 3 experiments per data point for all studies) were chosen based on pilot studies. The result of all each fit is reported with the 95% CI. Hypothesis testing was two tailed, and a *P* value less than 0.05 was considered to be statistically significant. Statistical comparisons between binding models (one-site *vs.* two-site model) for saturation binding studies and statistical comparisons between the IC_{50}s of etomidate and CPMM for competition studies were performed using extra sum-of-squares F tests with Prism 6 for Mac OS X. No data were excluded in this study.

Results

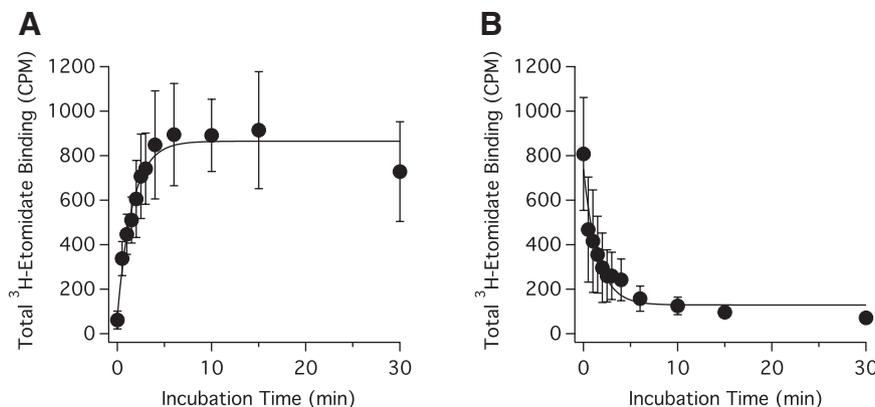### Association and Dissociation Kinetics of ^{3}H-etomidate with Rat Adrenal Membranes

To quantify the association rate of ^{3}H-etomidate with adrenal membranes, ^{3}H-etomidate (final concentration, 2 nM) was mixed with membranes. After the desired incubation time (ranging up to 30 min), the membrane-bound ^{3}H-etomidate fraction was collected by filtration. Figure 2A shows that bound ^{3}H-etomidate increased with incubation time before reaching a maximum value of approximately 900 CPM with

incubation times of 5 min or longer. These data were fit to a single exponential equation to yield an association half-time of 1.1 min (95% CI, 0.75 to 2.7).

To assess the dissociation rate of ^{3}H-etomidate from adrenal membranes, the membranes were preequilibrated (for 30 min) with 2 nM ^{3}H-etomidate. Excess cold etomidate (final concentration, 100 μM) was then added, and after the desired incubation time (ranging up to 30 min), the bound ^{3}H-etomidate fraction was collected by filtration. Figure 2B shows that bound ^{3}H-etomidate decreased with incubation time by approximately 90% from an initial value of 810 ± 250 to 71 ± 16 CPM by 30 min after adding excess cold etomidate (fig. 2B). These time-dependent data were fit to a single exponential equation to yield a dissociation half-time of 1.1 min (95% CI, 0.69 to 2.2). On the basis of the results of these experiments, we chose an equilibration time of 15 to 30 min.

Equilibrium Binding of ^{3}H-etomidate to Rat Adrenal Membranes

To define the binding affinity of ^{3}H-etomidate to adrenal membranes, we used a saturation binding assay. The membranes were equilibrated with a range of ^{3}H-etomidate concentrations, and the bound fraction was measured by filtration. Figure 3 shows the measured increase in total and nonspecific ^{3}H-etomidate binding to adrenal membranes with increasing concentrations of the isotopically diluted ^{3}H-etomidate. Also shown is the specific binding component, which was calculated as the difference between the total and nonspecific binding. A simultaneous fit of the total and nonspecific binding data to a one-site model yielded a K_D of 40 nM (95% CI, 30 to 51) and a B_{max} of 620 CPM (95% CI, 560 to 680) with a global r^2 value for the model of 0.9853 and an absolute sum of squares of 37,736. Analysis

Fig. 2. Association (*A*) and dissociation (*B*) kinetics of 2 nM ^{3}H-etomidate with adrenal membranes. (*A*) ^{3}H-etomidate was incubated with membranes for the indicated times before filtering. Each data point is the mean ± SD (n = 3) radioactivity measured in the washed filter. The curve is a fit of the data set to a single exponential equation to define the ^{3}H-etomidate association half-time (1.1 min). (*B*) ^{3}H-etomidate was equilibrated with membranes for 30 min. Excess cold etomidate (100 μM) was then added, and the mixture was filtered at the indicated incubation time after adding the cold etomidate. Each data point is the mean ± SD (n = 3) radioactivity measured in the washed filter. The curve is a fit of the data to a single exponential equation to define the ^{3}H-etomidate dissociation half-time (1.1 min). The final protein concentration was 0.07 mg/ml. Units of radioactivity are in counts per minute (CPM).
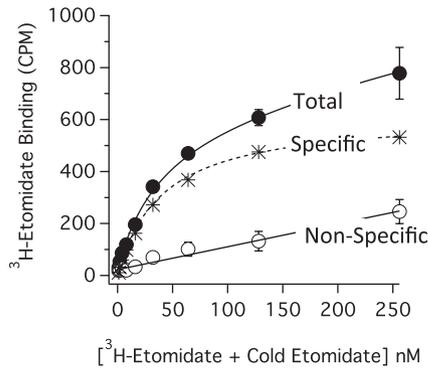
Fig. 3. Saturation binding of ³H-etomidate to adrenal membranes. Total ³H-etomidate binding was determined by equilibrating a range of ³H-etomidate concentrations (diluted with cold etomidate at a 1:19 molar ratio) with membranes. The mixture was then filtered, and radioactivity was measured in the washed filter. Nonspecific binding was determined in parallel binding experiments in the presence of excess cold etomidate (100 μM). Each data point is the mean ± SD (n = 3) radioactivity measured in the washed filter. Units of radioactivity are in counts per minute (CPM). The *solid curve* and *line* are simultaneous fits of the total and nonspecific binding data sets to a single-site binding model, yielding an equilibrium dissociation constant (K_D) and a maximum concentration of binding sites in the sample (B_{max}) of 40 nM and 620 CPM, respectively. Also shown is the specific binding, calculated at each concentration as total minus nonspecific binding (expressed as CPM). The *dashed curve* shows the calculated fit with K_D and B_{max} equal to 40 nM and 620 CPM, respectively. The final protein concentration was 0.07 mg/ml.

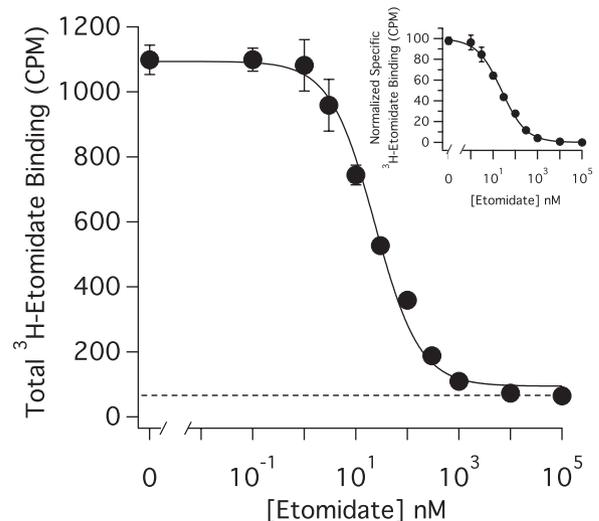**Fig. 4.** Displacement of ³H-etomidate by etomidate. ³H-etomidate (2 nM) and the desired concentration of cold etomidate were equilibrated with membranes. The mixture was then filtered, and radioactivity was measured in the washed filter. Each data point is the mean ± SD (n = 3) radioactivity measured in the washed filter. The *curve* is a fit of the data to a one-site homologous binding equation yielding an equilibrium dissociation constant (K_D) and maximum concentration of binding sites in the sample (B_{max}) of 21 nM and B_{max} of 11,700 counts per minute (CPM), respectively. The *dashed line* shows the binding not displaceable by 100 μM cold etomidate (*i.e.*, nonspecific binding). The *inset* shows the results replotted as normalized specific binding, and the *curve* is a fit of these data to a competitive binding equation yielding a half-inhibitory concentration (IC_{50}) and Hill coefficient of 26 nM and −0.82, respectively. The final protein concentration was 0.07 mg/ml.

of this data set using a two-site model did not significantly improve the fit ($P > 0.05$).

Homologous Competition of ³H-etomidate Binding to Rat Adrenal Membranes

We made a second determination of etomidate's K_D from competition studies between ³H-etomidate and cold etomidate. Figure 4 shows the progressive displacement of 2 nM ³H-etomidate from adrenal membranes with increasing concentrations of cold etomidate. A fit of the data to equation 1 yielded an etomidate K_D of 21 nM (95% CI, 17 to 27) and a B_{max} of 11,700 CPM (95% CI, 9,270 to 14,100) with a global r² value for the model of 0.9878 and an absolute sum of squares of 70,405. The inset of figure 4 shows the results of this experiment as normalized specific binding data. The IC_{50} and Hill coefficient for cold etomidate inhibition of specific ³H-etomidate binding were determined to be 26 nM (95% CI, 23 to 30) and −0.82 (95% CI, −0.90 to −0.74), respectively, from this normalized data using equation 2.

Competition of ³H-etomidate Binding to Rat Adrenal Membranes by Etomidate Analogs and Metabolites

Figure 5A shows the displacement of 2 nM ³H-etomidate from adrenal membranes with increasing concentrations of CPMM and carboetomidate, along with that of etomidate for comparison. The IC_{50} of CPMM was 143 nM (95% CI,

107 to 192) with a Hill coefficient of −0.55 (95% CI, −0.63 to −0.46). This IC_{50} value was significantly higher than that of etomidate ($P < 0.0001$). The IC_{50} of carboetomidate was 50 μM (95% CI, 13 to 184) with a Hill coefficient of −0.27 (95% CI, −0.34 to −0.20). Figure 5B shows the displacement of 2 nM ³H-etomidate from adrenal membranes with increasing concentrations of the metabolites etomidate-CA and CPMM-CA. Both metabolites displaced ³H-etomidate from adrenal membranes but only at relatively high concentrations with respective IC_{50}s of 1.1 mM (95% CI, 0.46 to 2.6) and 0.27 mM (95% CI, 0.19 to 0.40) with Hill coefficients of −0.32 (95% CI, −0.41 to −0.24) and −0.43 (−0.51 to −0.36).

Competition of ³H-etomidate Binding to Rat Adrenal Membranes by Clinical Sedative-hypnotic Agents

Figures 6, A to C show the displacement of 2 nM ³H-etomidate from adrenal membranes with increasing concentrations of dexmedetomidine, ketamine, and propofol, respectively. The respective IC_{50}s of dexmedetomidine and ketamine were 2.2 μM (95% CI, 1.3 to 3.7) and 79 μM (95% CI, 21 to 300), and their Hill coefficients were −0.33 (95% CI, −0.38 to −0.27) and −0.23 (95% CI, −0.29 to −0.17). An IC_{50} was not defined for propofol because we observed little
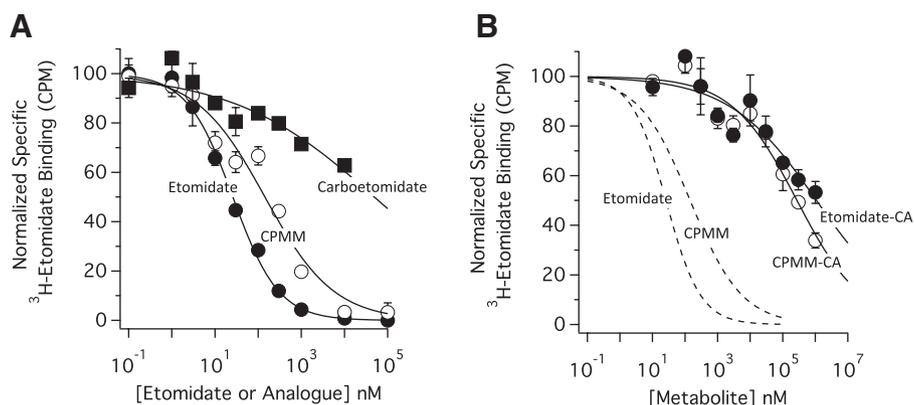
Fig. 5. Displacement of ^{3}H-etomidate by etomidate analogs (*A*) and metabolites (*B*). ^{3}H-etomidate (2 nM) and the desired concentration of etomidate analog or metabolite were equilibrated with membranes. The mixture was then filtered, and radioactivity was measured in the washed filter. Each data point is the mean ± SD (n = 3) radioactivity measured in the washed filter. The *curves* are fits of each data set to a competitive binding equation yielding half-inhibitory concentrations (IC$_{50}$s) of 143 nM (cyclopropyl methoxycarbonyl metomidate [CPMM]), 50 μM (carboetomidate), 1.1 mM (etomidate-CA), and 0.27 mM (CPMM-CA). (*A*) Data for etomidate are shown for comparison (IC$_{50}$: 26 nM). (*B*) The fits obtained for the parent sedative-hypnotics (*i.e.*, etomidate and CPMM) are shown for comparison. The final protein concentration was 0.07 mg/ml. CPM = counts per minute.
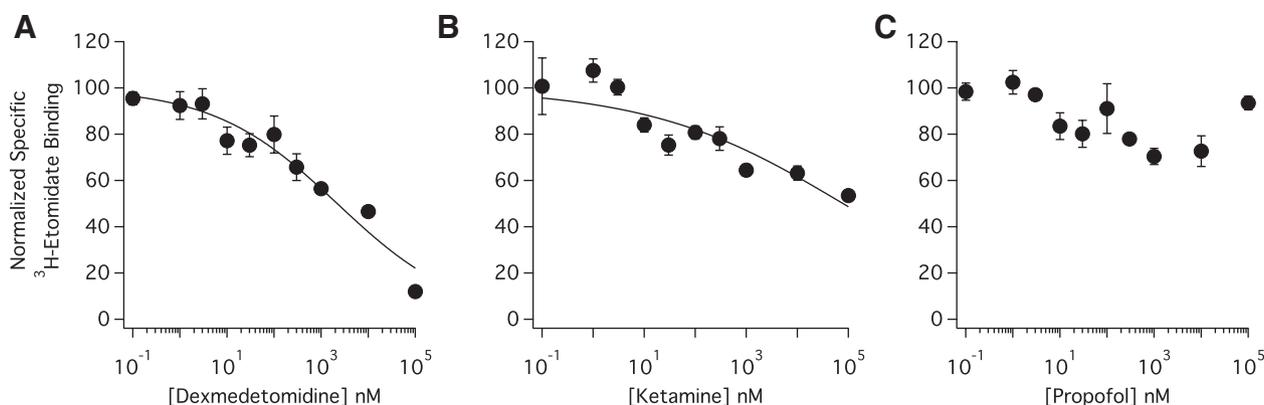
Fig. 6. Displacement of ^{3}H-etomidate by dexmedetomidine (*A*), ketamine (*B*), and propofol (*C*). ^{3}H-etomidate (2 nM) and the desired concentration of sedative-hypnotic were equilibrated with membranes. The mixture was then filtered, and radioactivity was measured in the washed filter. Each data point is the mean ± SD (n = 3) radioactivity measured in the washed filter. (*A* and *B*) The *curves* are fits of each data set to a competitive binding equation yielding half-inhibitory concentrations (IC$_{50}$s) of 2.2 μM for dexmedetomidine and 79 μM for ketamine. An IC$_{50}$ was not defined for propofol because we observed little concentration dependence, but the data set implies that it would be more than 100 μM. The final protein concentration was 0.07 mg/ml.

concentration dependence, but the data set implies that it would be more than 100 μM.

Discussion

Etomidate inhibits adrenocortical function primarily by binding to the adrenocortical enzyme 11β-hydroxylase, a cytochrome P450 enzyme required for the biosynthesis of cortisol, corticosterone, and aldosterone.[12,33] This inhibition is remarkably potent, occurring even at nanomolar etomidate concentrations.[14,34,35] Computational docking studies using homology models of 11β-hydroxylase and spectroscopic studies using heterologously expressed and purified enzyme indicate that etomidate's high inhibitory potency arises primarily from its ability to engage in a coordination bond with the heme iron located within the enzyme's active

site.[14] This site is believed to be hydrophobic, a feature that would also enhance the binding of etomidate and other hydrophobic drugs.[30]

In addition to etomidate, other experimental and clinical sedative-hypnotic agents have been reported to suppress adrenocortical steroid synthesis. During continuous infusion, CPMM reduced plasma corticosterone concentrations in adrenocorticotropin hormone–stimulated and endotoxin-treated rats; however, this reduction was less than that produced by etomidate, and corticosterone levels returned to control values faster with CPMM than with etomidate once the infusion was terminated.[21,24] Dexmedetomidine inhibited *in vitro* corticosterone production by rat adrenocortical cells and suppressed *in vivo* cortisol production in dogs.[26] However at equihypnotic doses, the magnitude of this suppression was less than that produced by etomidate. Although

several clinical studies have reported that dexmedetomidine administration reduces plasma cortisol levels in surgical patients, this seems to reflect a generalized depressant effect on the stress response to surgery rather than an etomidate-like direct action on adrenocortical function (*e.g.*, inhibition of 11β-hydroxylase).[36–38] Nevertheless, an anecdotal report describing clinically significant adrenocortical insufficiency (and a negative adrenocorticotropin hormone stimulation test) associated with long-term high-dose dexmedetomidine infusion suggests that direct effects on adrenocortical function—while rare—may occur in certain clinical circumstances.[27] Animal studies indicate that ketamine and propofol can reduce adrenocortical responsiveness to exogenously administered adrenocorticotropin hormone, consistent with a direct effect on adrenocortical steroid synthesis.[28,29] However, we are unaware of any clinical studies demonstrating a similar action in humans.

The present studies show that ^{3}H-etomidate binds to a site in adrenal membranes in a manner that is saturable, highly specific (*i.e.*, 90% etomidate displaceable when the ^{3}H-etomidate concentration is 2 nM), and of high affinity. We quantified etomidate's K_D for this site using two independent binding approaches: saturation binding and homologous ligand competition. The former uses increasing ^{3}H-etomidate concentrations to define the K_D, whereas the latter uses competition between a single low concentration of ^{3}H-etomidate and a range of concentrations of cold etomidate. The estimated K_D values using these different approaches were similar (40 and 21 nM, respectively) and within the range previously reported for etomidate inhibition of adrenocortical function *in vitro* and *in vivo*.[22,26,29,34,39,40] Taken together, we conclude that this saturable, highly specific, high-affinity etomidate binding site in adrenal membranes is located on 11β-hydroxylase.

We are unaware of any previous studies designed to define the K_D of etomidate for 11β-hydroxylase. However, using a similar approach to ours, Zolle *et al.*[41] estimated the K_D of an iodinated analog of the closely related hypnotic metomidate to be 11.6 ± 2.5 nM. This value is lower than our estimates for etomidate, suggesting that the iodine increases binding affinity perhaps by increasing the drug's hydrophobicity.

Similar to etomidate, CPMM inhibited ^{3}H-etomidate binding, but its IC_{50} was significantly (5.5-fold; $P < 0.001$) higher than that of etomidate. Thus, while these two drugs have similar hypnotic potencies (0.53 ± 0.17 and 0.69 ± 0.04 mg/kg, respectively, in rats), CPMM binds to 11β-hydroxylase with significantly lower affinity.[20] This finding explains why when hypnosis is maintained at equivalent depths by continuous infusion of these two drugs, CPMM produced less adrenocortical suppression than etomidate.[21,24] Thus, CPMM produces less adrenocortical suppression than etomidate for two distinct reasons. One reason is pharmacodynamic: during continuous infusions that achieve steady-state blood concentrations, CPMM binds to 11β-hydroxylase with lower affinity

compared with etomidate. The other reason is pharmacokinetic: after the infusion is stopped, CPMM is metabolized more quickly than etomidate to a metabolite whose affinity for 11β-hydroxylase is low.

The IC_{50}s of the CA metabolites etomidate-CA and CPMM-CA were greater than or equal to 1,000× higher than those of their respective parent sedative-hypnotics etomidate and CPMM. They were also higher than the maximal metabolite concentrations achieved after 2-h etomidate and CPMM infusions in dogs (1,680 ng/ml or 7.8 μM and 36,200 ng/ml or 121 μM, respectively).[29] This suggests that any adrenocortical suppression produced during such infusions was caused primarily by the parent sedative-hypnotic agent rather than from accumulated metabolite. This would explain why adrenocortical recovery rates after terminating infusions correlated with the rate of parent drug metabolism rather than the rate of metabolite excretion.[21,29] As the active site of 11β-hydroxylase is believed to be hydrophobic, it is highly likely that these metabolites bind poorly to the enzyme because they are charged at physiologic pH.

The IC_{50} of carboetomidate was 1,900-fold higher than that of etomidate, paralleling its 2,000-fold higher IC_{50} for suppressing *in vitro* cortisol synthesis by H295R adrenocortical cells.[22] Thus, our binding data are consistent with the supposition that carboetomidate is unable to suppress adrenocortical function at hypnotic concentrations because it binds to 11β-hydroxylase with very low affinity.

Similar to etomidate, dexmedetomidine contains a basic imidazole nitrogen that may coordinate with 11β-hydroxylase's heme iron and enhance binding to 11β-hydroxylase. Our studies showed that it inhibited ^{3}H-etomidate binding with an IC_{50} of 2.2 μM, a value that approximates its IC_{50} for inhibiting corticosterone synthesis by rat adrenocortical cells (1 μM) but is 2 to 3 orders of magnitude higher than the plasma concentrations achieved with typical sedative doses (1 to 6 ng/ml; 5 to 30 nM).[42,43] This suggests that with usual clinical doses, significant dexmedetomidine binding to the active site of 11β-hydroxylase is unlikely to occur. Ketamine and propofol were even less potent inhibitors of ^{3}H-etomidate binding with IC_{50}s of 79 and more than 100 μM, respectively. These values exceed their hypnotic concentrations by 1 to 2 orders of magnitude (ketamine) or more (propofol).[44,45]

A limitation of the current studies is that they do not distinguish between competitive and noncompetitive (allosteric) displacement of ^{3}H-etomidate from 11β-hydroxylase. Thus, it is possible that some fraction of the displacement that we measure could be caused by sedative-hypnotic or metabolite binding to sites that are distinct from the one that binds etomidate. Logically, noncompetitive interactions are most likely to contribute to low-affinity ligands whose structures are most distinct from that of etomidate (*i.e.*, ketamine and propofol). This possibility could be tested by varying the ^{3}H-etomidate concentration and determining whether IC_{50} values remained unchanged, a finding

that would be indicative of a noncompetitive interaction. However, this would not change our conclusion that these sedative-hypnotics bind negligibly to the etomidate binding site on 11β-hydroxylase (or to an allosteric site that inhibits etomidate binding) at clinically relevant concentrations.

In conclusion, etomidate's *in vitro* dissociation constant for 11β-hydroxylase is approximately 30 nM. This value is within the range previously reported for etomidate inhibition of adrenocortical function *in vitro* and *in vivo* but is approximately two orders of magnitude lower than its hypnotic concentration. CPMM produces less adrenocortical suppression than etomidate not only because it is metabolized faster but also because it binds to 11β-hydroxylase with lower affinity. The CA metabolites of etomidate and CPMM bind to this enzyme with affinities that are greater than or equal to 1,000× lower than their respective parent hypnotics, explaining why adrenocortical recovery rates correlate with parent drug metabolism rates rather than metabolite excretion rates. Other sedative-hypnotics may bind to 11β-hydroxylase and inhibit etomidate binding but only at concentrations that exceed those typically achieved during clinical use.

Research Support

Competing Interests

The Massachusetts General Hospital (Boston, Massachusetts) has published and submitted patent applications for certain etomidate analogs. Drs. Raines and Husain are inventors of this technology. They and their laboratories have received and may continue to receive income related to its development. This conflict is being managed by the Partners Healthcare Office for Interactions with Industry (Boston, Massachusetts). Mr. Pejo and Dr. Zhou declare no competing interests.

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