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Pharmacological Studies of Methoxycarbonyl Etomidate's Carboxylic Acid Metabolite

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Contribution: Dr. Raines designed the studies, interpreted the data, and wrote the manuscript.
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Abstract

Background—Methoxycarbonyl etomidate (MOC-etomidate) is a rapidly metabolized and ultra-short acting etomidate analog that does not produce prolonged adrenocortical suppression after bolus administration. Its metabolite (MOC-ECA) is a carboxylic acid whose pharmacology is undefined. We hypothesized that MOC-ECA possesses significantly lower pharmacological activity than MOC-etomidate, accounting for the latter's very brief duration of hypnotic action and inability to produce prolonged adrenocortical suppression after bolus administration. To test this hypothesis, we compared the potencies of MOC-ECA and MOC-etomidate in three biological assays.

Methods—The hypnotic potency of MOC-ECA was assessed in tadpoles using a loss-of-righting reflexes assay. The gamma-aminobutyric acid type A ($GABA_A$) receptor modulatory potencies of MOC-ECA and MOC-etomidate were compared by defining the concentrations of each required to directly activate $\alpha_1(L264T)\beta_2\gamma_{2L}$ $GABA_A$ receptors. The adrenocortical inhibitory potencies of MOC-ECA and MOC-etomidate were compared by defining the concentrations of each required to inhibit *in vitro* cortisol production by adrenocortical cells.

Results—MOC-ECA's EC_{50} for loss-of-righting reflexes in tadpoles was 2.8 ± 0.64 mM as compared to a previously reported value of 8 ± 2 μM for MOC-etomidate. EC_{50}s for direct activation of $GABA_A$ receptors were 3.5 ± 0.63 mM for MOC-ECA versus 10 ± 2.5 μM for MOC-etomidate. IC_{50} for inhibiting *in vitro* cortisol production by adrenocortical cells was 30 ± 7 μM for MOC-ECA versus 0.10 ± 0.02 μM for MOC-etomidate.

Conclusions—In all three biological assays, MOC-ECA's potency was approximately 300-fold lower than that of MOC-etomidate.

Introduction

Methoxycarbonyl etomidate (MOC-etomidate) is a rapidly metabolized and ultra-short acting etomidate analog that does not produce prolonged adrenocortical suppression in rats after bolus administration.[1] In common with remifentanil, esmolol, and remimazolam, it contains a metabolically-labile ester moiety that is rapidly hydrolyzed by esterases to form a carboxylic acid metabolite (Figure 1). The pharmacological activities of the carboxylic metabolites of remifentanil, esmolol, and remimazolam are orders of magnitude lower than those of their respective parent compounds (4600-fold, 300-fold to 1600-fold, and 300-fold, respectively).[2-6] However, the pharmacologic activity of MOC-etomidate's carboxylic acid metabolite (MOC-ECA) has not been assessed and its potency relative to MOC-etomidate is unknown. We hypothesized that this metabolite possesses significantly lower potency than the parent compound, accounting for the latter's very brief duration of hypnotic action and inability to produce prolonged adrenocortical suppression after bolus administration. This report describes the pharmacology of MOC-ECA and compares it to that of MOC-etomidate in three biological assays.

Methods

All animal studies were conducted with the approval of the Subcommittee on Research Animal Care at the Massachusetts General Hospital, Boston, Massachusetts. *Xenopus laevis* tadpoles (early pre-limb stage) and adult female Xenopus laevis frogs were purchased from Xenopus One (Ann Arbor, MI) and housed in our laboratory (tadpoles) or in the Massachusetts General Hospital Center for Comparative Medicine animal care facility

(frogs). MOC-etomidate was synthesized by Aberjonia Laboratories (Beverly, MA) using our previously published method.(1)MOC-ECA was synthesized in our laboratory as previously described and its highest aqueous concentration was limited in our studies to 10 mM to assure complete dissolution in aqueous solutions.[7]

Tadpole Loss-of-Righting Reflexes (LORR)

LORR in *Xenopus laevis* tadpoles was assessed as previously described.[1,8] In brief, groups of 5 tadpoles were placed in water containing the desired quantity of MOC-ECA and buffered with 2.5 mM Tris HCl (pH=7.4). Tadpoles were tipped every 5 minutes with a flame-polished pipette until the response stabilized. A tadpole was determined to have LORR if it failed to right itself within 5 seconds after being turned supine. At the end of each study, tadpoles were returned to fresh water to ensure reversibility. MOC-ECA's EC_{50} for LORR was then determined from the concentration-dependence of LORR using the quantal method of Waud.[9]

$GABA_A$ Receptor Electrophysiology

Stage 4 and 5 oocytes were obtained as previously described and injected with messenger RNA encoding the α_1(L264T), β_2, and γ_{2L} subunits of the human gamma-aminobutyric acid A($GABA_A$) receptor (~40 ng of messenger RNA total at a subunit ratio of 1:1:2).[1] We chose to use α_1(L264T)$\beta_2\gamma_{2L}$ rather than wild-type $GABA_A$ receptors to assess the relative potencies of MOC-etomidate and MOC-ECA on $GABA_A$ receptors because previous studies have shown that this mutant is very potently and effectively directly activated by anesthetics, which allows for more precise estimation of drug potency when drug solubility is limited.[10] After injection, 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=7.4) containing 50 U/ml of penicillin and 50 μg/ml of streptomycin at 17°C for at least 18 hours before electrophysiological experiments.

All electrophysiological recordings were performed using the whole cell two-electrode voltage-clamp technique with oocytes voltage clamped at −50 mV using a GeneClamp 500B amplifier (Molecular Devices, Sunnyvale, CA) and perfused with ND-96 buffer with 1 mM EGTA 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 (Molecular Devices) and driven by a Dell personal computer (Round Rock, TX). Current responses were recorded using Clampex 9.2 software (Molecular Devices) and processed using a Bessel (8-pole) low-pass filter with a cutoff at 50 Hz using Clampfit 9.2 software (Molecular Devices).

Currents were elicited by application of MOC-etomidate or MOC-ECA (each dissolved in ND-96 buffer with 1 mM EGTA). The recorded peak current amplitudes were normalized to control currents elicited with 1 mM GABA (also dissolved in ND-96 buffer with 1 mM EGTA)in the same oocyte. EC_{50}s for direct activation were calculated by fitting a plot of the normalized current amplitude versus MOC-etomidate or MOC-ECA concentration to a Hill equation.

Suppression of Cortisol Synthesis

Suppression of *in vitro* cortisol synthesis by MOC-etomidate and MOC-ECA was quantified as previously described using an *in vitro* adrenocortical cell (NCI-H295R; ATCC CRL-2128) assay.[8] Briefly, aliquots of cells (10^5 cells/well) were grown in 12-well culture plates with 2 ml of growth medium. After reaching near confluence, the growth medium was replaced with an assay medium containing 20 μM for skolin (to stimulate cortisol synthesis) and the desired concentration of either MOC-etomidate or MOC-ECA. After 48 hours, 1.2

ml of assay medium was collected from each well, centrifuged, and the cortisol concentration in the supernatant determined using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The cortisol concentrations in the supernatant of wells containing MOC-etomidate or MOC-ECA were normalized to those in control wells without MOC-etomidate or MOC-ECA. IC_{50}s for adrenocortical cell inhibition were then calculated by fitting a plot of the normalized cortisol concentration versus MOC-etomidate or MOC-ECA concentration to a Hill equation.

Statistical Analysis

All data are reported as mean ± SD unless otherwise noted. Curve fitting was performed using Igor Pro 6.1 (Wavemetrics, Lake Oswego, OR).

Results

The fraction of tadpoles that had LORR increased with MOC-ECA concentration and at the highest concentration studied (10 mM); 18 of 20 tadpoles had LORR (Figure 2). This LORR was reversible because all 52 tadpoles that had LORR in our studies recovered their righting reflexes when returned to water without MOC-ECA. From the MOC-ECA concentration-dependence of LORR, the EC_{50} for LORR was determined to be 2.8 ± 0.64 mM.

MOC-etomidate and MOC-ECA directly activated α_1(L264T)$\beta_2\gamma_{2L}$ $GABA_A$ receptors in a concentration-dependent manner (Figure 3). However, the concentration ranges over which this activation occurred differed between the two compounds by 2-3 orders of magnitude. With MOC-etomidate, this amplitude reached a plateau by 100 μM that approximated the amplitude elicited by 1 mM GABA. From the concentration-response relationship, we calculated an EC_{50} for direct activation of 10 ± 2.5 μM. With MOC-ECA, this amplitude failed to reach a plateau even at 10 mM, the highest concentration studied. From the MOC-ECA concentration-response relationship, we calculated an EC_{50} for direct activation of 3.5 ± 0.63 mM with the caveat that this value may somewhat overestimate the true potency of the metabolite because no clear plateau in the response was achieved.

MOC-etomidate and MOC-ECA also suppressed *in vitro* cortisol synthesis by human adrenocortical cells in a concentration-dependent manner. However as with direct activation, the concentration ranges over which this action occurred differed between the two compounds by 2-3 orders of magnitude (Figure 4). A fit of the two data sets to a Hill equation yielded IC_{50}s of 0.10 ± 0.02 μM for MOC-etomidate and 30 ± 7 μM for MOC-ECA.

Conclusions

MOC-ECA possesses pharmacological activity; however, in each of the three biological assays we performed, it was approximately 300-fold less potent than its parent compound MOC-etomidate (Table 1).This finding is consistent with our hypothesis that MOC-etomidate's very brief duration of hypnotic action and inability to produce prolonged adrenocortical suppression after bolus administration results from its rapid *in vivo* hydrolysis to a carboxylic acid metabolite with low pharmacological activity. Forming a metabolite with low potency is particularly important if MOC-etomidate is to be used as a continuous infusion because metabolite accumulation may occur, resulting in a persistent clinical effect if the metabolite possesses significant pharmacological activity. Our studies demonstrating that MOC-ECA is 2-3 orders of magnitude less potent than MOC-etomidate in three assays suggests that recovery after continuous infusions of MOC-etomidate should occur rapidly in humans. However, clinical trials (or computer simulations) will ultimately be required to gauge the importance of any pharmacological activity of MOC-ECA in

humans because the carboxylic acid metabolites of rapidly metabolized drugs may accumulate in renal failure. Under these circumstances, metabolites with even minimal activities might reach concentrations sufficient to produce significant pharmacological effects.

When assessed in a variety of assays, the carboxylic acid metabolites GI90291, ASL-8123, and CNS 7054 are similarly orders of magnitude less potent than their respective parent compounds remifentanil, esmolol, and remimazolam.[2-6] All four metabolites contain an identical carboxylic acid moiety whose pKa is 4.8.[12] Therefore at physiological pH, this moiety is uncharged (i.e., protonated) in 1/400 of all metabolite molecules. This value approximates the potency of MOC-ECA relative to MOC-etomidate, suggesting that MOC-ECA's pharmacological activity (and perhaps those of GI90291, ASL-8123, and CNS 7054) may arise principally from the uncharged, protonated fraction.

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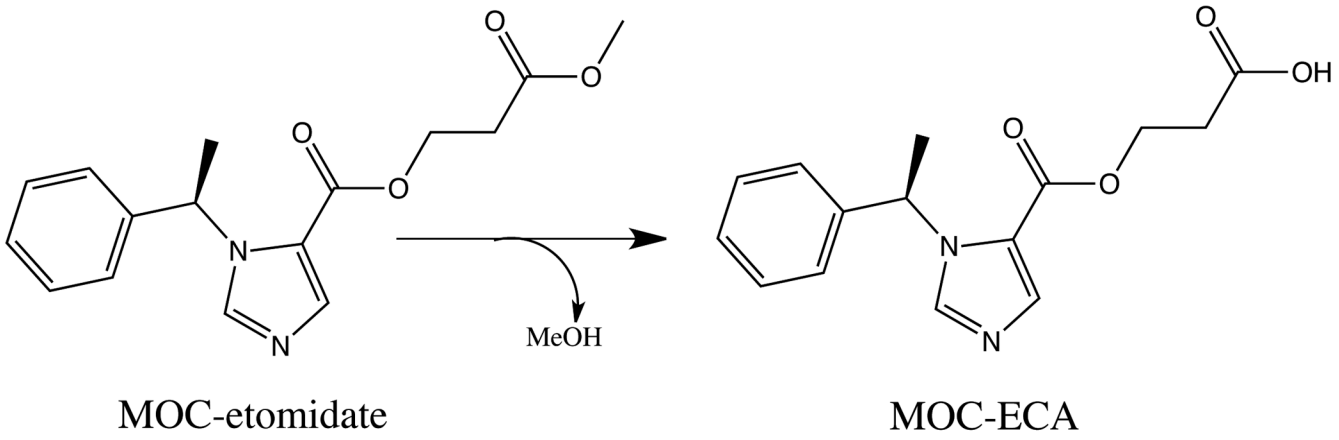

MOC-etomidate MOC-ECA

Figure 1.
Hydrolysis of methoxycarbonyl etomidate (MOC-etomidate) to its carboxylic acid metabolite (MOC-ECA).

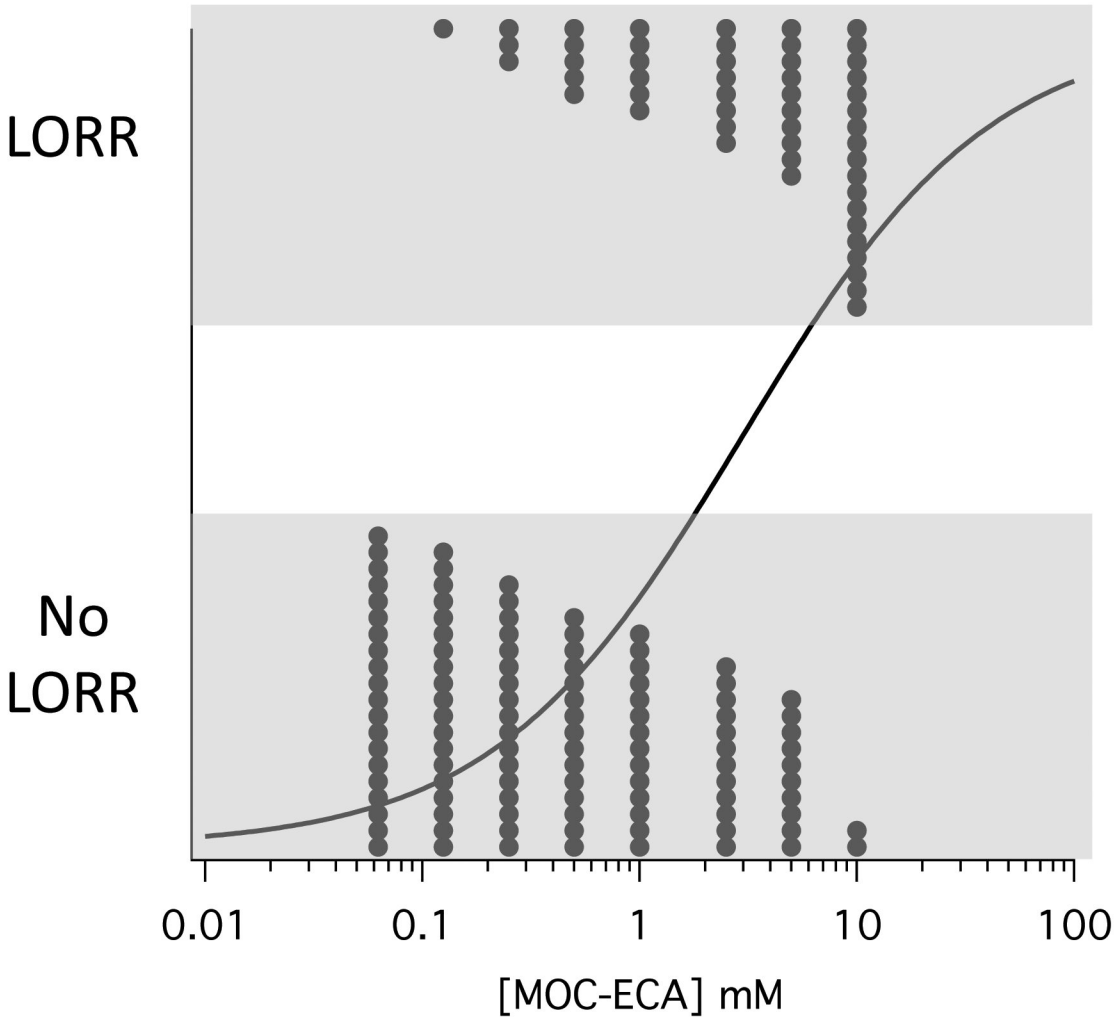

Figure 2.
Methoxycarbonyl etomidate metabolite (MOC-ECA) concentration-response curves for loss-of-righting reflexes (LORR) in tadpoles. Each symbol represents data from a single tadpole. The curve is a fit of the MOC-ECA concentration-mean response data yielding an EC_{50} of 2.8 ± 0.64 mM and a slope of 0.8 ± 0.16.

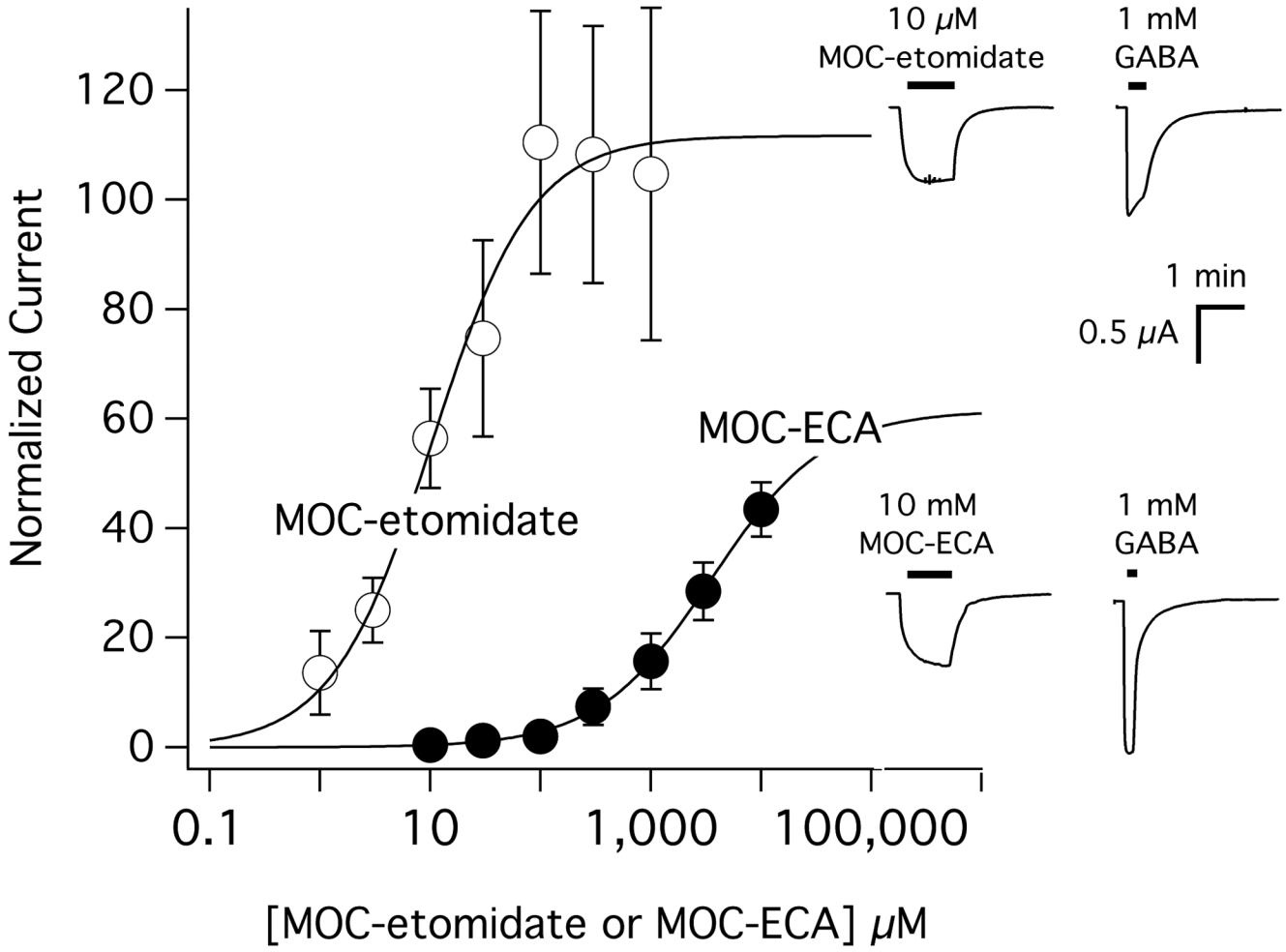

Figure 3.

Direct activation of α_1(L264T)$\beta_2\gamma_{2L}$ gamma-aminobutyric acid type A ($GABA_A$) receptors by methoxycarbonyl etomidate (MOC-etomidate) and its carboxylic acid metabolite (MOC-ECA). Current amplitudes were normalized to that elicited by 1 mM GABA in the same oocyte. Each data point is the mean normalized amplitude obtained in 4-6 oocytes. The curves are fits of the MOC-etomidate and MOC-ECA concentration-mean response data to a Hill equation yielding an EC_{50} of 10 ± 2.5 μM for MOC-etomidate and 3.5 ± 0.63 mM for MOC-ECA with respective maxima of 112 ± 6.2 and 61 ± 3.9 and slopes of 1.0 ± 0.19 and 0.84 ± 0.049. Upper inset shows representative current traces of activation by 10 μM MOC-etomidate and 1 mM GABA in the same oocyte. Lower inset shows representative current traces of activation by 10 mM MOC-ECA and 1 mM GABA in the same oocyte.

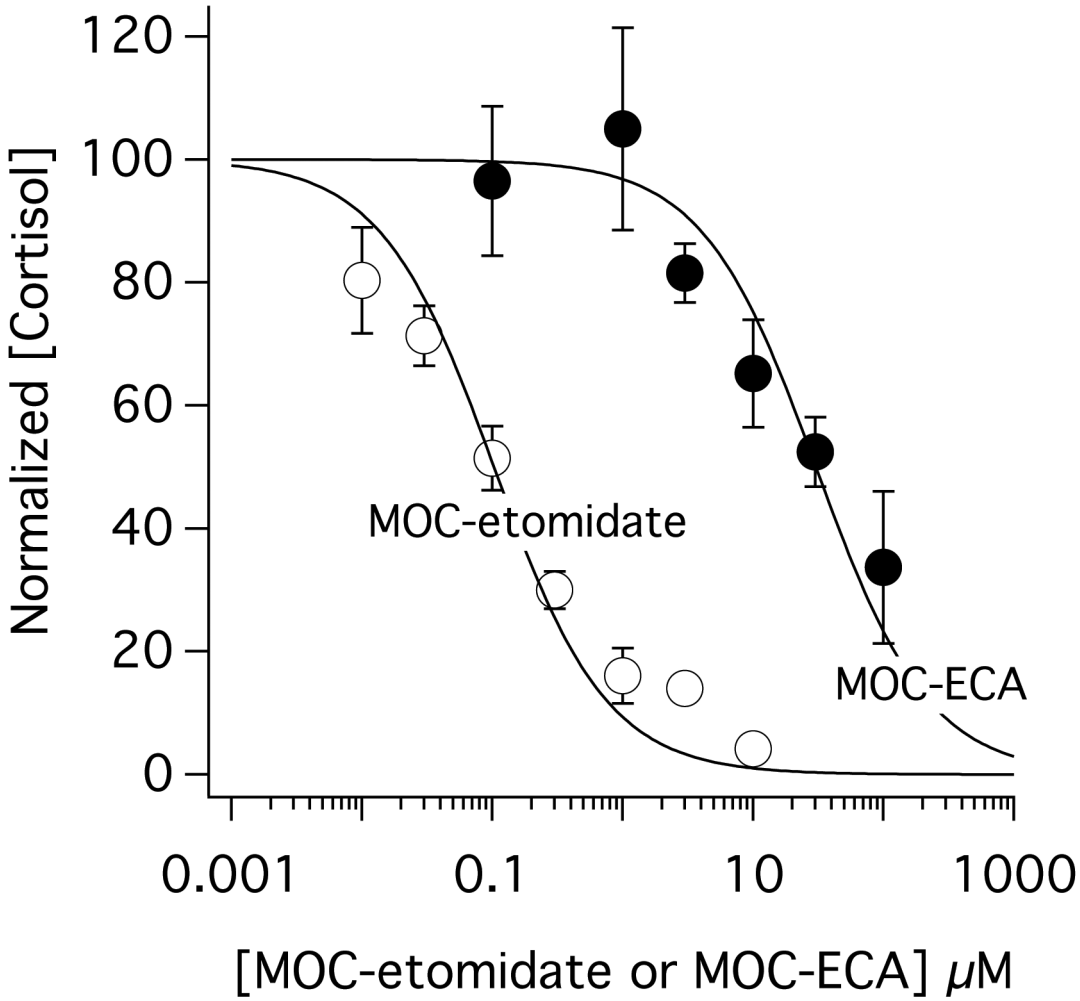

Figure 4.

Inhibition of *in vitro* cortisol synthesis by methoxycarbonyl etomidate (MOC-etomidate) or its carboxylic acid metabolite (MOC-ECA). Cortisol concentrations were normalized to that measured in the absence of MOC-etomidate or MOC-ECA. Each data point is the mean normalized concentration obtained from 3 wells. The curves are fits of the MOC-etomidate and MOC-ECA concentration-response data to a Hill equation (slope = -1). The calculated IC_{50}s were 0.10 ± 0.02 μM for MOC-etomidate and 30 ± 7 μM for MOC-ECA.

Table 1

Pharmacological activities of Etomidate, methoxycarbonyl (MOC)-etomidate, and MOC-ECA

Assay	Etomidate	MOC-etomidate	MOC-ECA	MOC-etomidate/MOC-ECA
Loss of righting reflexes in tadpoles (EC_{50})	2.3 ± 0.13 μM [a]	8 ± 2 μM[c]	2,800 ± 640 μM	1/350
Direct activation of $GABA_A$ receptors (EC_{50})	–	10 ± 2.5 μM	3,500 ± 630 μM	1/350
Inhibition of *in vitro* cortisol synthesis (IC_{50})	0.0013 ± 0.0002 μM [b]	0.10 ± 0.02 μM	30 ± 7 μM	1/300

[a] From Husain et al.[11]

[b] From Cotten et al.[8]

[c] From Cotten et al.[1]