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Toxicologic and Inhibitory Receptor Actions of the Etomidate Analog ABP-700 and Its Metabolite CPM-Acid

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- The investigational etomidate analog ABP-700 causes involuntary muscle movements in humans at anesthetic doses and seizures in dogs at 10-fold higher toxicologic doses
- The mechanism of seizures in dogs, and their relationship to involuntary muscle movements in humans, is unknown

What This Article Tells Us That Is New

- Toxicologic studies in dogs using supratherapeutic ABP-700 doses caused involuntary muscle movements and seizures, but these were temporally and electroencephalographically distinct, suggesting different underlying mechanisms
- Events occurred at ABP-700 and metabolite concentrations one and two orders of magnitude higher, respectively, than those found in humans
- Electrophysiologic studies of the principal metabolite of ABP-700 in oocyte-expressed γ-aminobutyric acid type A receptors showed inhibition at the high supratherapeutic concentrations achieved in the dogs, and such inhibition may explain seizure activity
- Proepileptiform effects of ABP-700 in dogs may not be relevant to humans at therapeutic doses

ABSTRACT

Background: The etomidate analog ABP-700 produces involuntary muscle movements that could be manifestations of seizures. To define the relationship (if any) between involuntary muscle movements and seizures, electroencephalographic studies were performed in Beagle dogs receiving supra-therapeutic (~10× clinical) ABP-700 doses. γ-aminobutyric acid type A ($GABA_A$) and glycine receptor studies were undertaken to test receptor inhibition as the potential mechanism for ABP-700 seizures.

Methods: ABP-700 was administered to 14 dogs (6 mg/kg bolus followed by a 2-h infusion at 1 mg · kg^{-1} · min^{-1}, 1.5 mg · kg^{-1} · min^{-1}, or 2.3 mg · kg^{-1} · min^{-1}). Involuntary muscle movements were documented, electroencephalograph was recorded, and plasma ABP-700 and CPM-acid concentrations were measured during and after ABP-700 administration. The concentration-dependent modulatory actions of ABP-700 and CPM-acid were defined in oocyte-expressed $\alpha_1\beta_3\gamma_{2L}$ $GABA_A$ and $\alpha_1\beta$ glycine receptors (n = 5 oocytes/concentration) using electrophysiologic techniques.

Results: ABP-700 produced both involuntary muscle movements (14 of 14 dogs) and seizures (5 of 14 dogs). However, these phenomena were temporally and electroencephalographically distinct. Mean peak plasma concentrations were (from lowest to highest dosed groups) 35 μM, 45 μM, and 102 μM (ABP-700) and 282 μM, 478 μM, and 1,110 μM (CPM-acid). ABP-700 and CPM-acid concentration–$GABA_A$ receptor response curves defined using 6 μM γ-aminobutyric acid exhibited potentiation at low and/or intermediate concentrations and inhibition at high ones. The half-maximal inhibitory concentrations of ABP-700 and CPM-acid defined using 1 mM γ-aminobutyric acid were 770 μM (95% CI, 590 to 1,010 μM) and 1,450 μM (95% CI, 1,340 to 1,560 μM), respectively. CPM-acid similarly inhibited glycine receptors activated by 1 mM glycine with a half-maximal inhibitory concentration of 1,290 μM (95% CI, 1,240 to 1,330 μM).

Conclusions: High dose ABP-700 infusions produce involuntary muscle movements and seizures in Beagle dogs *via* distinct mechanisms. CPM-acid inhibits both $GABA_A$ and glycine receptors at the high (~100× clinical) plasma concentrations achieved during the dog studies, providing a plausible mechanism for the seizures.

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ABP-700 is a "soft" analog of etomidate (fig. 1).[1–4] Similar to other soft drugs such as remifentanil and esmolol, it contains a metabolically-labile ester moiety that is rapidly hydrolyzed by esterases to a carboxylic acid metabolite (CPM-acid). In developing this and other soft etomidate analogs, our primary goal was to retain the potent sedative-hypnotic activity and benign cardiorespiratory actions of etomidate, while eliminating the prolonged suppression of adrenocortical steroid biosynthesis that plagues etomidate administration. A secondary goal was to produce an agent that would allow rapid anesthetic recovery. Subsequent human studies have confirmed that at clinically relevant doses, ABP-700 minimally affects respiratory and cardiovascular function.[4,5] However, unlike etomidate, it does not suppress steroid biosynthesis and has a fast recovery profile even after prolonged infusion.[4,6,7]

With ABP-700 administration, involuntary muscle movements are commonly observed.[4–6] Although such movements are also commonly observed with etomidate administration and not associated with seizures, the corporate licensee of ABP-700 (The Medicines Company, USA) undertook studies in Beagle dogs to determine whether there was any electroencephalographic seizure activity

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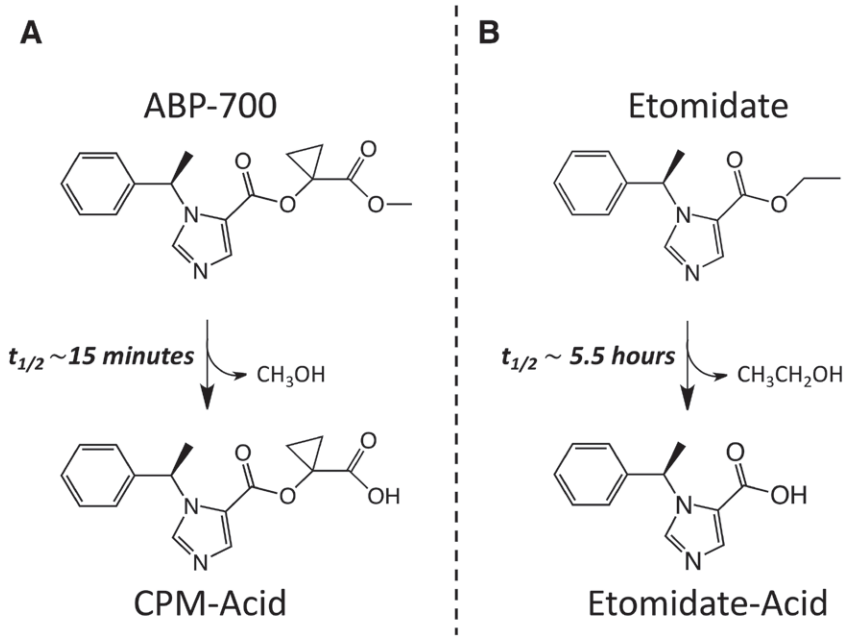

Fig. 1. Chemical structures of (*A*) ABP-700 and its principal metabolite CPM-acid and (*B*) etomidate and its principal metabolite etomidate-acid. The elimination half-lives ($t_{1/2}$) of ABP-700 and etomidate in humans are ~15 min and 5.5 h, respectively.[3,4]

during these movements. During those toxicologic studies—which were performed at the direction of the United States Food and Drug Administration with the goal of achieving ABP-700 exposure levels that are 10× higher than the maximum associated with clinical doses—electroencephalographically-confirmed convulsive seizures were sometimes observed. Although no seizures or postictal behaviors were reported in clinical studies, establishing the likely mechanism(s) for these seizures in Beagle dogs and assessing the potential of ABP-700 for causing seizures in humans is critical for the future development of ABP-700 as a therapeutic drug.[4,5] More broadly, defining the cause of these seizures may be important for guiding the design and development of other novel sedative-hypnotic agents that act by similar receptor mechanisms.

Many general anesthetics are widely believed to produce their behavioral effects—at least in part—by acting on γ-aminobutyric acid type A ($GABA_A$) receptors in the central nervous system (CNS).[8] Within the clinically relevant concentration range, these agents enhance ("potentiate") $GABA_A$ receptor function in a concentration-dependent manner leading to a reduction in CNS excitability that manifests as sedation or hypnosis. This receptor action also accounts for the anti-convulsant activity of many general anesthetic agents.[9,10] Conversely, at lethally high concentrations and similar to classic convulsants such as pentylenetetrazol and picrotoxin, general anesthetics commonly inhibit $GABA_A$ receptor function.[11–14] For propofol and etomidate, this inhibitory action is typically evident at concentrations greater than or equal to 30 μM, which is more than 10-fold higher than their respective sedative-hypnotic concentrations.[11,14,15] This inhibitory action presumably results from anesthetic binding to a low affinity site(s) on the $GABA_A$ receptor that is distinct from those responsible for potentiation. Consequently, concentration-response curves that define anesthetic actions on $GABA_A$ receptors are typically bell-shaped, exhibiting potentiation at clinically-relevant anesthetic concentrations and inhibition at lethally high ones.[11,16]

In the current article, we report the results of the dog toxicology studies along with *in vitro* studies aimed at characterizing the $GABA_A$ and glycine receptor modulatory actions of ABP-700 and CPM-acid. Our goal in undertaking the receptor studies was to test the hypothesis that ABP-700 or its metabolite CPM-acid produced seizures in these dogs because it had reached concentrations sufficient to inhibit the function of these inhibitory receptors, which is a well-established mechanism for producing seizures.[17,18]

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Materials and Methods

Sources of Drugs and Chemicals

ABP-700 for Beagle dog studies was obtained from KABS Laboratories (Canada) as a sterile formulation at 20 mg/ml in 10% sulfobutylether-β-cyclodextrin (pH 2.5) and stored frozen (−20°C) before being brought to room temperature within 6 h of administration. ABP-700 and CPM-acid for $GABA_A$ receptor studies were synthesized by Aberjona Laboratories (USA) using our previously described methods.[1,19] Etomidate-acid was synthesized as previously described.[20] Other chemicals were purchased from Sigma-Aldrich (USA).

Toxicologic Studies of ABP-700 in Beagle Dogs

All Beagle dog studies were performed by CiToxLAB North America (Canada) and conducted in accordance with their institutional animal care and use committee and the principles outlined in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. Dogs (n = 14 dogs; 7 males and 7 females; age: 8 to 9 months at the time of ABP-700 dosing) were purchased from Marshall BioResources (USA). Dogs were individually housed in stainless steel cages equipped for telemetry data acquisition and day/night vision cameras for continuous video monitoring. Room temperature was maintained at 21° ± 3°C with 12-h light-dark cycles. At least 26 days before ABP-700 dosing, dogs were anesthetized with propofol and isoflurane and surgically instrumented for telemetric monitoring of electroencephalography and electromyography, intraarterial blood pressure, heart rate, body temperature, and physical activity generally as previously described.[21] Sterile transmitters (D70-EEE and M10; Data Science International, USA) were inserted between the internal abdominal oblique muscle and the aponeurosis of the transverses abdominis and secured with nonabsorbable suture material. The electroencephalographic and electromyographic leads were tunneled to a dorso-cervical neck incision. Femoral venous and intraarterial catheters were also inserted at this time. Electroencephalographic electrodes were attached to the cranium to monitor two standard bipolar derivations (C_Z-O_Z and C_4-O_2) and the electromyographic electrodes were placed in the temporalis muscle. Both electroencephalographic and electromyographic signals were continuously recorded and analyzed beginning at least 30 min before ABP-700 administration and ending at least 24 h after administration was complete using Dataquest ART software and NeuroScore software with the seizure detection module (Data Sciences International, USA). Electroencephalographic interpretation was completed by a board certified veterinary neurologist who included a description of the involuntary movements and possible electroencephalographic correlates in a report to the corporate sponsor. That interpretation was peer-reviewed by an independent neuroscientist. A seizure was defined as an electroencephalographic pattern lasting greater than or equal to 10 s and satisfying either of the following criteria: (1) repetitive generalized or focal spikes, sharp-waves, spike-and-wave, or sharp-and-slow wave complexes at greater than or equal to 3 Hz; or (2) sequential rhythmic, periodic, or quasiperiodic waves at greater than or equal to 1 Hz and unequivocal evolution in frequency (gradual increases/decreases by greater than or equal to 1 Hz), morphology, or location.[22] Heart rate, intraarterial blood pressure, and body temperature were acquired and analyzed using DSI Ponemah 6.30 software (Data Sciences International).

Before study, dogs were fasted for 12 to 24 h. Each dog was premedicated with glycopyrrolate (0.02 mg/kg intramuscularly) and then received an ABP-700 bolus (6 mg/kg) immediately followed by a 2-h ABP-700 infusion at 1 mg · kg^{-1} · min^{-1} (total dose: 126 mg/kg; n = 4 dogs), 1.5 mg · kg^{-1} · min^{-1} (total dose: 186 mg/kg; n = 6 dogs), or 2.3 mg · kg^{-1} · min^{-1} (total dose: 282 mg/kg; n = 4 dogs). When sufficiently anesthetized, dogs were placed on a heating pad to maintain core body temperature and endotracheally intubated. Ventilation was mechanically assisted with oxygen (ventilation rate: 7 to 20 breaths/min, inspiratory pressure: 18 to 25 cm H_2O). Hemoglobin oxygen saturation, end-tidal carbon dioxide (target: 25 to 55 mmHg), heart rate, and body temperature (target: 37.2° to 38.8°C) were monitored continuously and maintained at target values. Involuntary muscle movements were identified from video recordings and their time, duration, severity (mild, moderate, or severe), and body locations were noted. The severity of each discrete episode of involuntary muscle movement was judged using two criteria: (1) Were there full muscle contractions affecting at least two different body parts? and (2) Did the movements last more than 3 min? Each individual episode was graded as slight, moderate, or severe if it met neither, one, or both criteria, respectively. When necessary to control severe involuntary muscle movements that risked interfering with electroencephalographic signal acquisition and interpretation (and thus seizure detection) during ABP-700 infusion, rocuronium (0.1 to 2.0 mg/kg, IV) was administered. At the end of ABP-700 infusions, dogs were extubated as they emerged from anesthesia and exhibited adequate respiratory function. Midazolam (0.2 mg/kg, IV) and phenytoin (0.2 mg/kg, IV) were administered after the ABP-700 infusion when needed to control repeated episodes of clonic and/or tonic convulsions.

Blood samples (0.5 ml each) were drawn 5, 15, 30, 45, 60, 90, and 115 min after the start of the ABP-700 infusion and then 2, 10, 20, 60, 120, 240, and 480 min and 24 h after the 2-h ABP-700 infusion was completed. These samples were immediately transferred into pre-chilled tubes containing K_2EDTA and 16 μl NaF, and then centrifuged for 10 min under refrigeration (4°C; 1,500 g) within 30 min of collection. ABP-700 and CPM-acid concentrations were analyzed by Envigo CRS (USA) using a validated liquid chromatography/tandem mass spectroscopic protocol that

had lower limits of quantitation of 1.00 and 20.0 ng/ml for ABP-700 and CPM-acid, respectively. A noncompartmental toxicokinetic analysis of the plasma concentration *versus* time data was performed by CiToxLAB North America using Phoenix WinNonlin 6.3 software (Certara, USA) to define toxicokinetic parameters.

$GABA_A$ Receptor Electrophysiology

Oocytes were harvested from adult female *Xenopus laevis* frogs using procedures that were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee (Boston, Massachusetts) and are in accordance with the principles outlined in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health (Bethesda, Maryland).[20] For $GABA_A$ receptor studies, oocytes were injected with 1 ng of messenger RNA (mRNA) encoding the α_1, β_3, and γ_{2L} subunits of the human $GABA_A$ receptor at a subunit ratio of 1:1:3. For glycine receptor studies, oocytes were injected with 1 ng of mRNA encoding the α_1 and β subunits of the human glycine receptor at a ratio of 1:1. After injection, oocytes were incubated for 18 to 48 h in ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, pH=7.4) containing 0.05 mg/mL of gentamicin and then studied using the whole cell two-electrode voltage-clamp technique.[23]

Potentiation of 6 μM GABA-evoked $GABA_A$ Receptor–mediated Currents by ABP-700 and CPM-Acid

Each oocyte was perfused with ND96 buffer containing 6 μM γ-aminobutyric acid (GABA) alone for 20 s followed immediately by 6 μM GABA plus test compound (ABP-700 or CPM-acid) at the desired concentration for 60 s and the current response was recorded. This GABA concentration was chosen because it produces a current whose peak amplitude is ~5% of that produced by 1 mM GABA (commonly termed an EC_5 GABA concentration). To account for variable receptor expression among oocytes, all peak current responses were normalized to the peak current response evoked by 1 mM GABA measured in the same oocyte.

Inhibition of Maximally-activated $GABA_A$ and Glycine Receptor–mediated Currents by ABP-700, CPM-Acid, and Etomidate-acid

To maximally activate receptors, each oocyte was perfused for 70 s with ND96 buffer containing 1 mM GABA (for $GABA_A$ receptor experiments) or 1 mM glycine (for glycine receptor experiments). Ten seconds into this activation period, the test compound (ABP-700, CPM-acid, or etomidate-acid) was added for 30 s. The inhibitory effect of the test compound on currents was then quantified from the recorded electrophysiologic traces as the reduction in current amplitude at the end of test compound application. To account for desensitization during test compound application, an interpolated straight line was fit between the pre- and posttest compound phases of the current recording period. That line was then used as the baseline against which the effect of the test compound was quantified.

Voltage-dependence of $GABA_A$ Receptor Inhibition by ABP-700 and CPM-Acid

Oocytes expressing $GABA_A$ receptors were voltage-clamped at the desired transmembrane potential ranging from −110 mV to +10 mV. Receptors were then maximally activated by perfusing each oocyte with 1 mM GABA for 70 s. Ten seconds into this activation period, ABP-700 or CPM-acid was added for 30 s. The inhibitory effect of the test compound on currents was then quantified from the recorded electrophysiologic traces as the reduction in current amplitude at the end of test compound application as described in the previous section.

Impact of CPM-acid on $GABA_A$ and Glycine Receptor Agonist Concentration-response Curves

Each oocyte expressing either $GABA_A$ receptors or glycine receptors was perfused with ND96 buffer containing CPM-acid for 30 s, followed immediately by CPM-acid plus the desired concentration of receptor agonist (GABA or glycine, respectively) for 15 s and the current response was recorded. Control studies were performed in the absence of CPM-acid. The peak current response obtained in the absence or presence of CPM-acid was normalized to the peak current response evoked by 1 mM agonist (without CPM-acid) measured in the same oocyte.

Data Analysis

Concentration-response curves for potentiation were fit using Prism 6.0h software (GraphPad, USA) using its built-in four-parameter equation for stimulation (equation 1):

$$\text{Normalized Peak Current Amplitude} = \text{Minimum} + \frac{\text{Maximum}-\text{Minimum}}{1+10^{(\text{LogEC}_{50}-[\text{test compound}])*n}}$$

where minimum is the normalized peak current amplitude in the absence of test compound (ABP-700, CPM-acid, or GABA), maximum is the normalized peak current amplitude at infinitely high test compound concentrations, [test compound] is the test compound concentration, EC_{50} is the test compound concentration that evokes a peak current amplitude that is half way between the maximum and minimum values, and n is the slope of the relationship.

Concentration-response curves for inhibition of 1 mM GABA-evoked and 1 mM glycine-evoked currents were similarly fit using Prism 6.0h software using its built-in four-parameter equation for inhibition (equation 2):

$$\text{Normalized Peak Current Amplitude} = \text{Minimum} + \frac{\text{Maximum} - \text{Minimum}}{1 + 10^{(\text{LogIC}_{50} - [\text{test compound}]) * n}}$$

where maximum is the normalized peak current amplitude in the absence of test compound (ABP-700, CPM-acid, or etomidate-acid), minimum is the normalized peak current amplitude at infinitely high test compound concentrations, [test compound] is the test compound concentration, half-maximal inhibitory concentration is the test compound concentration that evokes a peak current amplitude that is half way between the maximum and minimum values, and n is the slope of the relationship.

Statistical Analysis

In Beagle dog studies, each individual data point represents a single measurement in a dog unless otherwise indicated. In receptor studies, individual data points are reported as the mean ± SD of five separate oocyte experiments whose results were normally distributed as assessed using the Kolmogorov–Smirnov test with the Dallal-Wilkinson-Lilliefor corrected *P* value. The extra sum-of-squares F test was used to test whether the agonist EC_{50} or the at maximum peak current at infinitely high agonist concentrations differed significantly in the presence *versus* the absence of CPM-acid. The uncertainties in fitted parameters are reported as 95% CI. There was no lost or missing data. To avoid output saturation, oocytes producing 1 mM GABA-evoked peak currents greater than 10 μA were discarded. Fitting and statistical tests were performed with GraphPad Prism 6.0h (USA). No *a priori* statistical power calculations were conducted and the sample size was based on previous experience.[24,25] Statistical significance was assumed for $P < 0.05$.

Results

Toxicologic Studies of ABP-700 in Beagle Dogs

ABP-700 induced and maintained anesthesia in all 14 Beagle dogs at a depth sufficient to allow endotracheal intubation without a paralytic agent within 5 to 15 min of initiating ABP-700 administration. Involuntary muscle movements were observed in all 14 dogs during 2-h ABP-700 infusions and in 9 of the dogs after the infusions were complete. These movements ranged from slight tongue movements to severe whole body shaking. The total number of recorded episodes of involuntary muscle movements in the 14 dogs was 321 (157 in males and 164 in females), with 278 episodes occurring during ABP-700 infusions and 43 episodes occurring afterward. Only one of the episodes of involuntary muscle movements (0.36%) recorded during ABP-700 infusions coincided with electroencephalographic evidence of seizure activity. This occurred in a dog from the 186 mg/kg group that had a seizure 107.5 min after the start of an ABP-700 infusion (*i.e.*, 12.5 min before the end of the 2-h infusion and after receiving 167 mg/kg of ABP-700). The percentages of all episodes of involuntary muscle movement characterized as mild, moderate, and severe were 80%, 14%, and 6%, respectively. There was no clear correlation between the ABP-700 dose and the number or severity of these episodes (data not shown). The total dose of rocuronium administered to a dog during ABP-700 infusion to control severe movements ranged from 1.6 to 4.8 mg/kg (126 mg/kg group), 1.6 to 5.6 mg/kg (186 mg/kg group), and 3.2 to 5.6 mg/kg (282 mg/kg group), respectively. Only one dog did not receive any rocuronium because all of its involuntary muscle movements were slight, and no dog received rocuronium during the final 20 min of ABP-700 infusion. Dogs began to right themselves 17 to 58 min after the conclusion of the 2-h ABP-700 infusions.

Heart rate remained within normal values for this species throughout ABP-700 administration (data not shown). However, figure 2 shows that mean blood pressures progressively decreased in all dogs during ABP-700 infusions from 115 ± 10 mmHg (mean value in all three dosage groups during the one hour prior to beginning the ABP-700 infusions ± SD) to mean minimum values of 88 ± 13 mmHg (126 mg/kg group), 76 ± 9 mmHg (186 mg/kg group), and 65 ± 6 mmHg (282 mg/kg group) by the end of the 2-h ABP-700 infusions. Mean blood pressures returned to baseline values within an hour of completing ABP-700 infusions, paralleling recovery from anesthesia.

Spikes, isolated sharp waves, and repeated sharp waves were identified in the electroencephalographic recordings of all dogs during ABP-700 infusions. Tonic and/or clonic convulsions associated with electroencephalographically-confirmed seizures were observed in 5 of the 14 dogs. A representative electroencephalographic recording of one such seizure is shown in figure 3A to 3C. By group, there were two dogs (one male and one female) in both the 126 mg/kg and 186 mg/kg groups, and one dog (female) in the 282 mg/kg group that had seizures. In one dog from each of the three groups, the first seizure was preceded by premonitory signs including salivation, chewing, excessive vocalization, tremors, head shaking, and/or muscle twitching or contractions. Seizures first occurred 6.5 and 152 min (126 mg/kg group), 81 min (186 mg/kg group), and 743 min (282 mg/kg group) after completion of ABP-700 infusion (fig. 3D). As noted in the first paragraph of the Results section, one dog (186 mg/kg group) had its first (and only) seizure 12.5 min before the end of the 2-h ABP-700 infusion. Of the dogs that had seizures, two dogs had just one seizure and two dogs had three seizures. The fifth dog, which was in the 186 mg/kg group, had a total of 64 seizures over the 24 h that followed ABP-700 infusion. Thus, 71 out of 72 of the electroencephalographic-confirmed seizures occurred during the post-infusion period. These seizures were self-limiting, lasting 10 to 226 s. However, two dogs received midazolam and phenytoin to

control convulsive behavior in the post ABP-700 infusion period that the *post hoc* electroencephalographic review determined were not due to seizures.

For each of the three dosage groups, figure 4A and 4B plot the time-dependent changes in plasma ABP-700 and CPM-acid concentrations, respectively. They reveal that the plasma concentrations of both ABP-700 and CPM-acid generally increased during ABP-700 infusions. Plasma concentrations of ABP-700 and CPM-acid were dose-dependent and achieved peak values near the end of the infusions. After completion of the infusions, plasma ABP-700 and CPM-acid concentrations decreased by 90% on approximate timescales of 10 min and 2 h, respectively. For each of the five dogs that had seizures, figure 4A and 4B also indicate the time of its first seizure. Two dogs (one in each of the 126 mg/kg and 186 mg/kg groups) had their first seizures within minutes of the end of the ABP-700 infusion (6.5 min after and 12.5 min before, respectively) when the plasma concentrations of ABP-700 and CPM-acid were both near their maximum values. The other three dogs had their first seizure 1.4, 2.5, and 12.4 h after both ABP-700 and CPM-acid had reached their peak plasma concentrations.

Table 1 shows the results of noncompartmental analysis of the toxicokinetic data from the three groups for both ABP-700 (table 1A) and CPM-acid (table 1B). The toxicokinetic parameters suggest no large differences between male *versus* female dogs. The mean (combined male and female data) maximum measured plasma concentrations of ABP-700 were 11,025 ng/ml (35 μM), 14,000 ng/ml (45 μM), and 32,180 ng/ml (102 μM) in the 126 mg/kg, 186 mg/kg, and 282 mg/kg groups, respectively. The mean maximum measured plasma concentrations of CPM-acid were an order of magnitude higher than those of ABP-700 with values of 84,180 ng/ml (282 μM), 142,840 ng/ml (478 μM), and 331,750 ng/ml (1,110 μM) in the 126 mg/kg, 186 mg/kg, and 282 mg/kg groups, respectively.

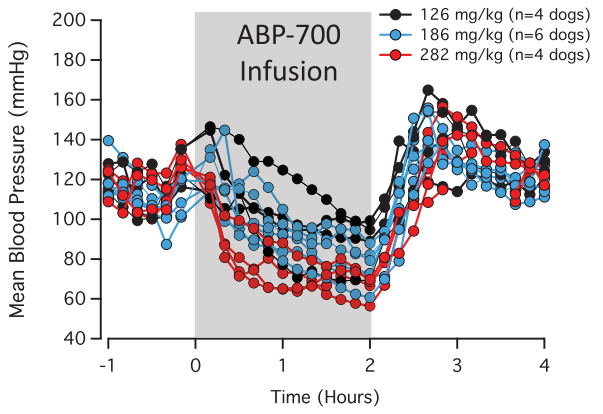

Fig. 2. Mean intraarterial blood pressures recorded in Beagle dogs. Blood pressure data is shown for all 14 dogs beginning 1 h prior to ABP-700 administration and ending 2 h after the 2-h infusion was completed. The gray bar highlights the time period when ABP-700 was administered. Dogs received a 6 mg/kg bolus of ABP-700 at time 0, followed by a 2-h continuous ABP-700 infusion of 1 mg · kg^{-1} · min^{-1} (126 mg/kg total dose; *black symbols*), 1.5 mg · kg^{-1} · min^{-1} (186 mg/kg total dose; *blue symbols*) or 2.3 mg · kg^{-1} · min^{-1} (282 mg/kg total dose; *red symbols*).

Potentiation of 6 μM GABA-evoked $GABA_A$ Receptor-mediated Currents by ABP-700 and CPM-Acid

We characterized the effects of ABP-700 and CPM-acid over a range of concentrations on currents mediated by $\alpha_1\beta_3\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus* oocytes and evoked by 6 μM GABA. We chose to use this low, submaximally-activating GABA concentration for our initial receptor studies because such concentrations commonly allow one to detect the $GABA_A$ receptor potentiating and inhibiting actions of test compounds in a single electrophysiologic trace.[13] Figure 5A shows representative electrophysiologic traces obtained during ABP-700 studies and demonstrates that the sedative-hypnotic modified 6 μM GABA-evoked

Table 1A: Mean Plasma ABP-700 Toxicokinetic Parameters Following Administration of ABP-700

	Total Dose	126 mg/kg		186 mg/kg		282 mg/kg	
	Sex	M	F	M	F	M	F
	Number Animals	2	2	3	3	2	2
C_{max}	(ng/mL)	10,500	11,550	13,770	14,230	26,550	37,800
T_{max}	(h)	1.708	1.916	1.916	1.500	1.708	1.708
$AUC_{(last)}$	(h*ng/mL)	17,780	19,570	23,040	24,290	48,150	60,360
Initial t½	(h)	0.15	0.22	0.17	0.12	0.18	0.17
Terminal t½	(h)	1.28	NC	1.30	1.25	0.80	0.86

NC: Not calculated due to non-qualifying parameters (R^2 adjusted ≤ 0.9000)

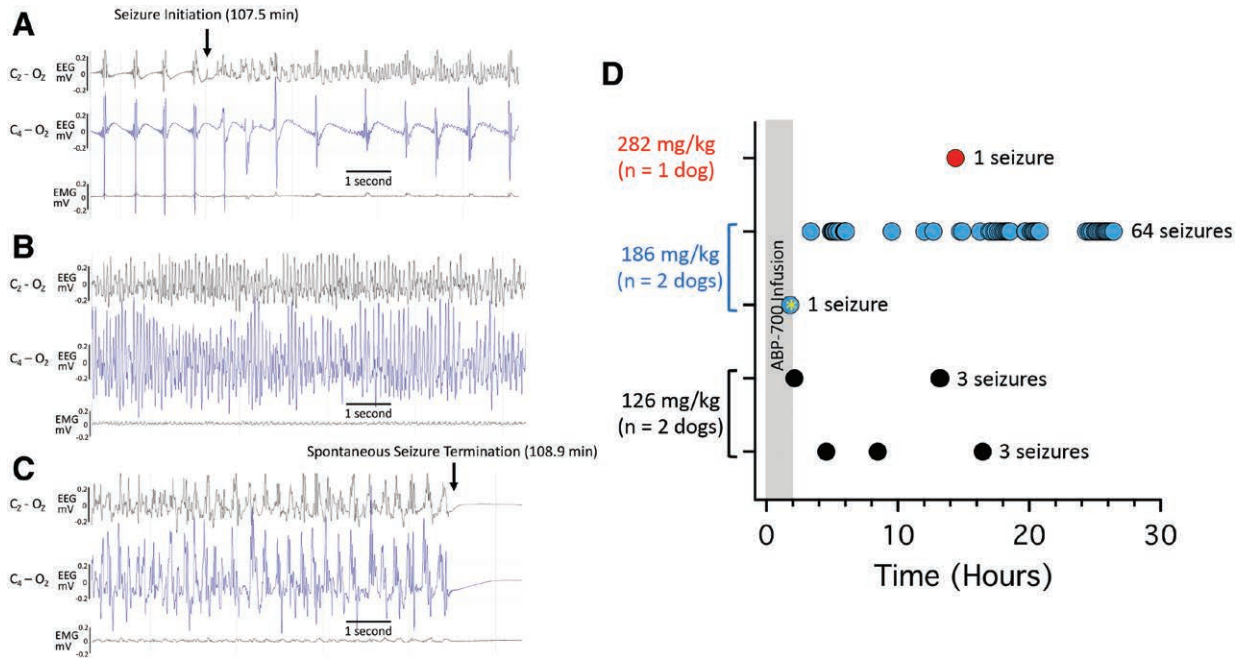

Fig. 3. Electroencephalographic and electromyographic signals recorded from a dog (in the 186 mg/kg group) that had a seizure after receiving a 6 mg/kg ABP-700 bolus immediately followed by a 2-h ABP-700 infusion at 1.5 mg · kg^{-1} · min^{-1} (*A*) upon initiation of the seizure 107.5 min after beginning ABP-700 administration (*i.e.,* after receiving 167 mg/kg of ABP-700), (*B*) during the seizure, and (*C*) upon spontaneous termination of the seizure 1.4 min after it began. (*D*) Timing of all seizures in Beagle dogs. Five dogs had electroencephalographically-confirmed seizures. Two dogs received 126 mg/kg ABP-700 (*black symbols*), two dogs received 186 mg/kg ABP-700 (*blue symbols*), and one dog received 282 mg/kg ABP-700 (*red symbols*). The only seizure that occurred during ABP-700 administration is highlighted by a *yellow star*. In one dog in the 126 mg/kg group, two seizures occurred within 6 min of one another (T = 13.15 and 13.24 h). Consequently, their symbols largely overlap. The number of seizures that each dog had during the study period is also numerically indicated. The *gray bar* highlights the 2-h time period when ABP-700 was administered.

currents in a concentration-dependent manner. Within the clinically-relevant ABP-700 concentration range (less than or equal to10 μM), ABP-700 potentiated GABA-evoked currents. The magnitude of this potentiation progressively increased with ABP-700 concentration before reaching a plateau. However, at high ABP-700 concentrations (greater than or equal to 100 μM), electrophysiologic traces also exhibited "surge" currents upon ABP-700 and GABA washout and the peak current amplitude of the GABA response began to decrease with ABP-700 concentration. Together, these phenomena are indicative of $GABA_A$ receptor inhibition.[13,26] Figure 5B shows analogous electrophysiologic traces obtained during studies of CPM-acid. At equivalent concentrations, CPM-acid produced consistently

Table 1B: Mean Plasma CPM-Acid Toxicokinetic Parameters Following Administration of ABP-700

	Total Dose	126 mg/kg		186 mg/kg		282 mg/kg	
	Sex	M	F	M	F	M	F
	Number Animals	2	2	3	3	2	2
C_{max}	(ng/mL)	85,850	82,500	133,000	152,670	350,500	313,000
T_{max}	(h)	1.98	1.92	2.03	2.03	2.03	2.11
$AUC_{(last)}$	(h*ng/mL)	207,520	194,570	325,550	390,300	921,460	814,160
Initial t½	(h)	0.55	0.58	0.57	0.62	0.66	0.61
Terminal t½	(h)	4.63	4.61	5.59	3.48	5.62	5.09

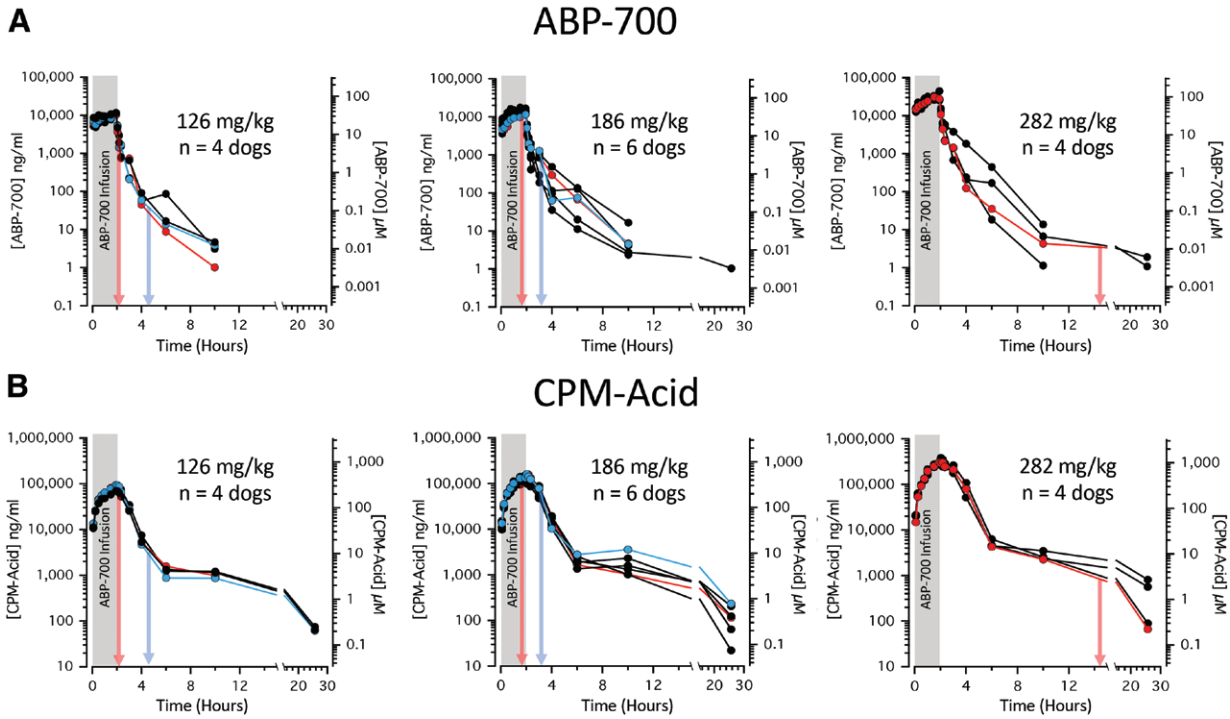

Fig. 4. Toxicokinetics of (*A*) ABP-700 and (*B*) its principal metabolite CPM-acid in Beagle dogs. T = 0 is the time of ABP-700 bolus administration (6 mg/kg), which was immediately followed by a 2-h ABP-700 infusion at 1 mg · kg^{-1} · min^{-1} (126 mg/kg total dose), 1.5 mg · kg^{-1} · min^{-1} (186 mg/kg total dose), or 2.3 mg · kg^{-1} · min^{-1} (282 mg/kg total dose). The left and right vertical axes report concentrations in units of ng/ml and μM, respectively. In each, individual datasets obtained using a single dog are represented by connected symbols. Data from dogs that had electroencephalographically confirmed seizures are shown as red and blue data points. The time when each of the five dogs had their first seizure is also indicated in each panel by an arrow, which is color-coded to the dog. In each panel, the *gray bar* highlights the 2-h time period when ABP-700 was administered. Values below the lower limits of quantitation (1.00 ng/ml and 20.0 ng/ml for ABP-700 and CPM-acid, respectively) were not plotted.

less potentiation of 6 μM GABA-evoked currents than ABP-700, although surge currents were still observable in electrophysiologic traces at the very high CPM-acid concentrations where potentiation occurred. Figure 5C plots the relationship between the mean peak current amplitude of the GABA response and the concentration of ABP-700 or CPM-acid. For both ABP-700 and CPM-acid, this relationship was bell-shaped with the peak current amplitude first increasing before decreasing with concentration. A fit of the rising portions of the ABP-700 and CPM-acid concentration-response relationships to equation 1 yielded respective EC_{50}s for current potentiation of 2.3 μM (95% CI, 1.6 to 3.3 μM) and 350 μM (95% CI, 120 to 970 μM) and respective maximal peak current amplitudes that were 96% (95% CI, 87 to 105%) and 28% (95% CI, 15 to 41%) of that produced by 1 mM GABA.

Inhibition of 1 mM GABA-Evoked $GABA_A$ Receptor-mediated Currents by ABP-700 and CPM-Acid

To better quantify the $GABA_A$ receptor inhibitory potencies of ABP-700 and CPM-acid, we defined their actions on $GABA_A$ receptor–mediated currents evoked by 1 mM GABA. Under these high agonist conditions, the confounding effect of simultaneous potentiation on the inhibitory action is greatly reduced because most (~85%) $GABA_A$ receptors will already have been opened by the agonist prior to the addition of ABP-700 or CPM-acid.[27–29] This receptor-saturating GABA concentration is also physiologically relevant because it approximates that achieved at synapses.[30] Figure 6A and 6B show representative electrophysiologic traces obtained during such studies. They show that at an intermediate concentration (30 μM), ABP-700 modestly potentiated 1 mM GABA-evoked currents whereas CPM-acid had no measurable effect. At high concentrations, both ABP-700 and CPM-acid inhibited currents in a concentration-dependent manner. Figure 6C plots the relationship between the mean amplitude of the current trace recorded during ABP-700 or CPM-acid application and the ABP-700 or CPM-acid concentration. It shows that ABP-700 modestly potentiated 1 mM GABA-evoked currents when applied at low to intermediate concentrations (3 to 30 μM) but inhibited them at high concentrations (greater than or

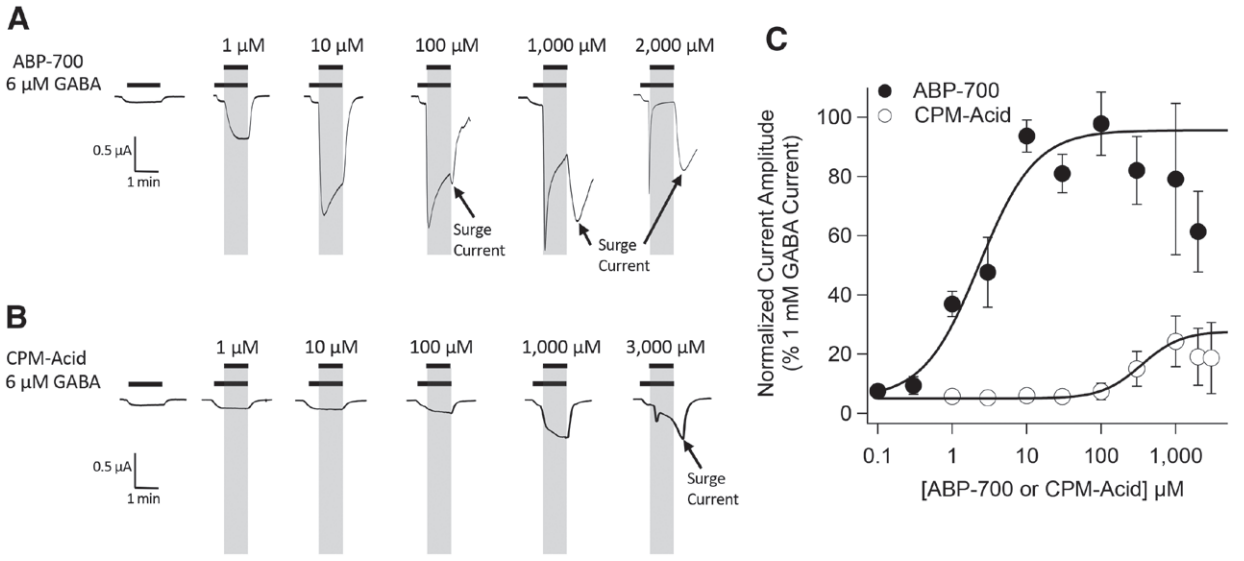

Fig. 5. Potentiation by ABP-700 and CPM-acid of peak electrophysiologic currents evoked by 6 μM γ-aminobutyric acid (GABA) and mediated by $\alpha_1\beta_3\gamma_{2L}$ γ-aminobutyric acid type A ($GABA_A$) receptors. Representative electrophysiologic traces recorded upon perfusing oocytes expressing $GABA_A$ receptors with 6 μM GABA alone for 20 s followed immediately by 6 μM GABA plus either (*A*) ABP-700 or (*B*) CPM-acid at the indicated concentrations for 60 s. Surge currents, which are indicated by the *arrows* and indicate relief from inhibition, were not measured when defining peak current amplitudes because they occur upon agonist and ABP-700/ CPM-acid washout. The *gray bars* highlight the time periods when ABP-700 or CPM-acid was applied. For each compound, all traces were obtained from the same oocyte. (*C*) ABP-700 and CPM-acid concentration-response curves for potentiation of peak currents evoked by 6 μM GABA. Each symbol is the mean ± SD derived from five different oocytes. The curves are nonlinear least square fits of the rising portions of the two datasets to equation 1 (see Data Analysis section). As this concentration of GABA is equivalent to a concentration that produces a current whose peak amplitude is ~5% of that produced by 1 mM GABA, the minimum was constrained to 5%. For ABP-700 and CPM-acid, the respective EC_{50}s for potentiation were 2.3 μM (95% CI, 1.6 to 3.3 μM) and 350 μM (95% CI, 120 to 970 μM), the respective maximal peak current amplitudes were 96% (95% CI, 87 to 105%) and 28% (95% CI, 15 to 41%) of that produced by 1 mM GABA, and the respective slopes were 1.2 (95% CI, 0.8 to 1.6) and 1.6 (95% CI, 0 to 3).

equal to 300 μM). In contrast, the only measurable action of CPM-acid was to inhibit 1 mM GABA-evoked currents. A fit of the two datasets to equation 2 yielded half-maximal inhibitory concentrations for current inhibition of 770 μM (95% CI, 590 to 1010 μM) for ABP-700 and 1,450 μM (95% CI, 1,340 to 1,560 μM) for CPM-acid.

Voltage-dependence of $GABA_A$ Receptor Inhibition by ABP-700 and CPM-Acid

To determine whether this receptor inhibition was voltage-dependent, we quantified the magnitude of inhibition produced by ABP-700 and CPM-acid at different membrane potentials in receptors activated with 1 mM GABA. Figure 7A and 7B respectively plot the relationship between the fractional inhibition produced by ABP-700 and CPM-acid (at their approximate respective half-maximal inhibitory concentrations of 770 μM and 1,500 μM) as a function of membrane potential. These figures demonstrate that $GABA_A$ receptor inhibition by ABP-700 was voltage-independent as the slope of this relationship (−0.056; 95% CI, −0.15 to 0.04) was not statistically significantly different from zero ($P = 0.247$). In contrast, the magnitude of receptor inhibition produced by CPM-acid was voltage-dependent, increasing as the membrane potential was varied from −110 mV to +10 mV. The slope of this relationship was −0.24 (95% CI, −0.38 to −0.09) which was statistically significantly non-zero ($P = 0.002$).

Impact of CPM-Acid on the GABA Concentration—Response Curve

We sought to further define the mechanism of CPM-acid inhibition of $GABA_A$ receptors by assessing its impact on the GABA concentration-response relationship. We chose to focus on CPM-acid rather than ABP-700 because only the former reached plasma concentrations in the dog toxicology studies that were sufficient to measurably inhibit $GABA_A$ receptor function. In addition, almost all of the seizures in dogs occurred in the minutes to hours after terminating the 2-h high-dose ABP-700 infusions suggesting that the rapidly formed (and relatively slowly eliminated) metabolite, rather than the parent compound itself, was the causative agent. Finally, animal studies indicate that as is typical for sedative-hypnotic agents, ABP-700 is an anti-convulsant rather than a convulsant.[31] Figure 8 plots the relationship between the GABA concentration and the

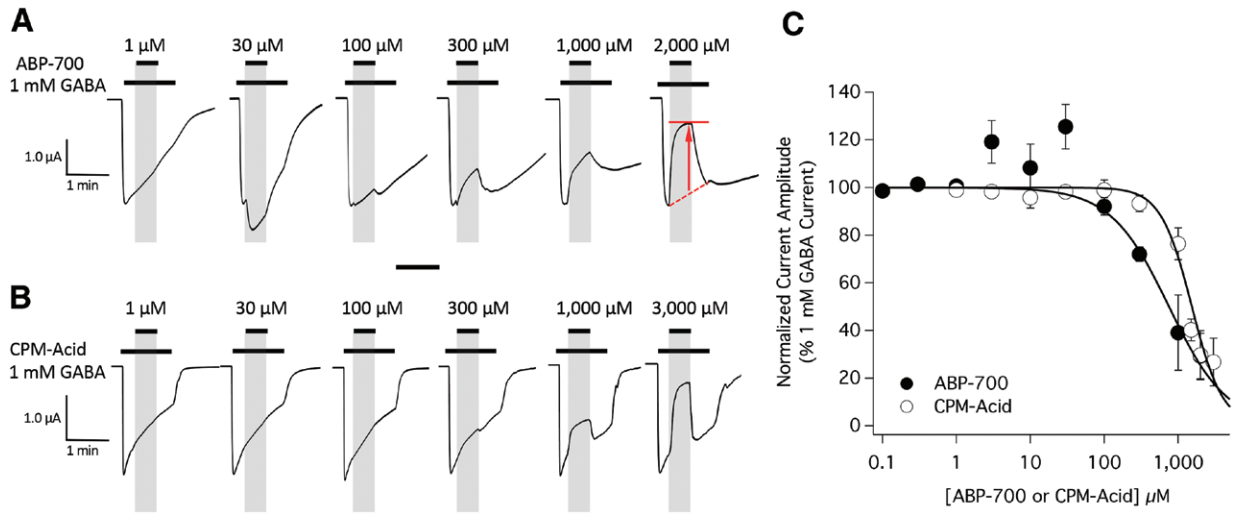

Fig. 6. Inhibition by ABP-700 and CPM-acid of electrophysiologic currents evoked by 1 mM γ-aminobutyric acid (GABA) and mediated by $\alpha_1\beta_3\gamma_{2L}$ γ-aminobutyric acid type A ($GABA_A$) receptors. (*A* and *B*) Representative electrophysiologic traces recorded upon perfusing oocytes expressing $\alpha_1\beta_3\gamma_{2L}$ $GABA_A$ receptors with 1 mM GABA alone for 70 s. Ten seconds into this activation period, (*A*) ABP-700 or (*B*) CPM-acid was added for 30 s. The *gray bars* highlight the periods when ABP-700 or CPM-acid was applied. For each compound, all traces were obtained from the same oocyte. As an example of how this inhibition was quantified, the trace exemplifying the inhibitory action of 2000 μM ABP-700 has several overlays. The *dotted red line* shows the interpolated line used as the baseline against which the effect of ABP-700 was quantified, the *solid red line* shows the point of maximum inhibition at the end of ABP-700 application, and the intervening *red arrow* quantifies the inhibitory effect of 2000 μM ABP-700 on the GABA-activated current. (*C*) ABP-700 and CPM-acid concentration response curves for inhibition of currents evoked by 1 mM GABA. Each symbol is the mean ± SD derived from five different oocytes. The curves are nonlinear least square fits of the two datasets to equation 2 (see Data Analysis section) with the maximum and minimum values constrained to 100% and 0%, respectively. For ABP-700 and CPM-acid, the respective half-maximal inhibitory concentrations for inhibition were 770 μM (95% CI, 590 to 1010 μM) and 1,450 μM (95% CI, 1,340 to 1,560 μM) with respective slopes of –1.3 (95% CI, –1.7 to –0.8) and –2.0 (95% CI, –2.4 to –1.6).

amplitude of the peak current response in the absence and presence of 1,500 μM CPM-acid. A fit of the two datasets to equation 1 yielded essentially identical EC_{50}s of 44 μM (95% CI, 35 to 54 μM) in the absence of CPM-acid and 45 μM (95% CI, 32 to 62 μM) in the presence of CPM-acid ($P = 0.9148$). However, the maximal peak current amplitudes were statistically significantly different with respective values of 104% (95% CI, 99 to 108%) and 64% (95% CI, 60 to 69%) of that produced by 1 mM GABA ($P < 0.0001$).

CPM-Acid Also Inhibits the Glycine Receptor

To further evaluate the ways in which CPM-acid might produce seizures in dogs, we assessed whether it could also inhibit the glycine receptor. This is another important inhibitory neurotransmitter receptor in the CNS that is functionally and structurally similar to the $GABA_A$ receptor, may colocalize at the same neuronal synapses in the brain and spinal cord as the $GABA_A$ receptor thus providing potential redundancy when $GABA_A$ receptors are inhibited, and whose inhibition can also produce seizures.[32–35] Figure 9A shows representative electrophysiologic current traces demonstrating the inhibitory effect of CPM-acid on electrophysiologic currents evoked by 1 mM glycine and mediated by $\alpha_1\beta$ glycine receptors. Similar to its effects on $GABA_A$ receptors, CPM-acid inhibited glycine receptors in a concentration-dependent manner. Figure 9B plots the relationship between the mean amplitude of the current trace during CPM-acid application and the CPM-acid concentration. A fit of the dataset to equation 2 yielded a half-maximal inhibitory concentration for current inhibition of 1,290 μM (95% CI, 1,240 to 1,330 μM).

We then studied the effects of CPM-acid on the glycine concentration–response curve for current activation. Figure 9C plots the relationship between the glycine concentration and the amplitude of the peak current response in the absence and presence of 1,300 μM CPM-acid, an approximate half-maximal inhibitory concentration in the glycine receptor. A fit of the two datasets to equation 1 yielded EC_{50}s of 115 μM (95% CI, 100 to 133 μM) in the absence of CPM-acid and 260 μM (95% CI, 200 to 340 μM) in the presence of CPM-acid ($P < 0.0001$) and respective maximal peak current amplitudes of 98% (95% CI, 94 to 102%) and 75% (95% CI, 69 to 81%) of that produced by 1 mM glycine ($P < 0.0001$).

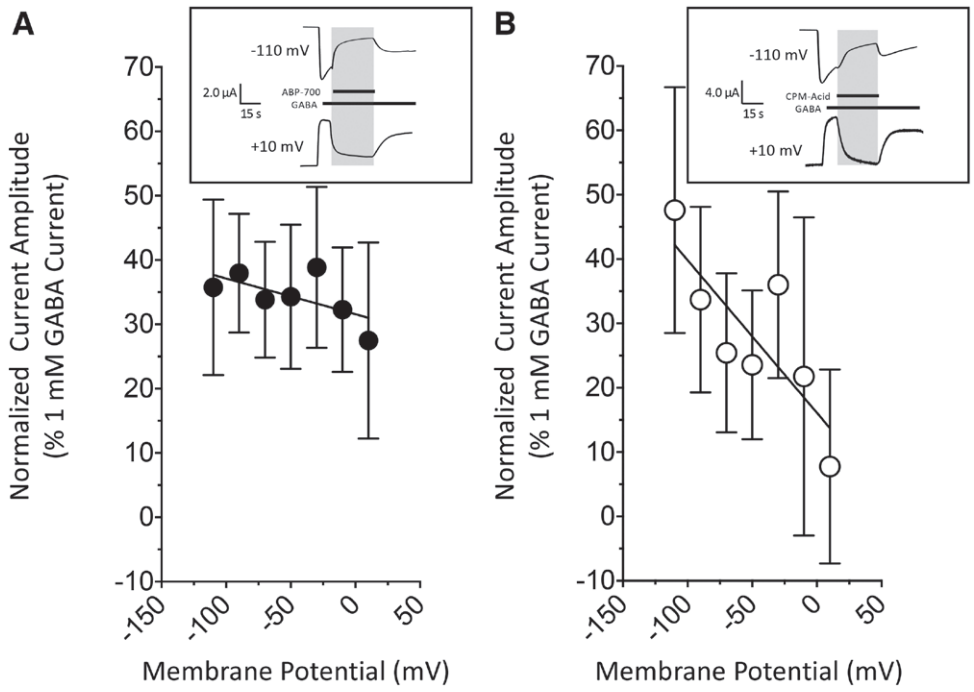

Fig. 7. Voltage-dependence of γ-aminobutyric acid type A ($GABA_A$) receptor inhibition by (*A*) ABP-700 and (*B*) CPM-acid. Currents were activated with 1 mM γ-aminobutyric acid (GABA). Each symbol is the mean ± SD derived from five different oocytes. In each panel, the line is a linear least square fit of the data. For ABP-700, the slope of this relationship was −0.056 (95% CI, −0.15 to 0.04) whereas for CPM-acid it was −0.24 (95% CI, −0.38 to −0.09). For CPM-acid (but not ABP-700), this slope was significantly different from zero ($P = 0.002$). Insets show the impact of the drug on electrophysiologic traces obtained with oocytes voltage-clamped at either −110 mV or +10 mV as indicated in the figure. In these insets, the amplitudes of all traces were normalized to the peak amplitude produced by 1 mM GABA alone to facilitate visual comparisons and the *gray bars* highlight the periods when ABP-700 or CPM-acid was applied.

$GABA_A$ and Glycine Receptor Function Is Also Inhibited by Etomidate-acid

The structural similarity between CPM-acid and etomidate-acid (fig. 1) suggested to us that the latter may also inhibit receptor function at high concentrations. To test this, we defined the ability of etomidate-acid to inhibit $GABA_A$ and glycine receptor function using agonist concentrations of 1 mM GABA and 1 mM glycine, respectively. Figure 10 plots the relationship between the mean amplitude of the current trace during etomidate-acid application and the etomidate-acid concentration. A fit of the two datasets to equation 2 yielded etomidate-acid half-maximal inhibitory concentrations for current inhibition of 7,050 μM (95% CI, 6,300 to 7,890 μM) in the $GABA_A$ receptor and 2,930 μM (95% CI, 2,710 to 3,170 μM) in the glycine receptor.

Discussion

The purpose of the Beagle dog toxicologic studies was to determine whether the involuntary muscle movements that commonly occur during ABP-700 administration are manifestations of seizures. They utilized supra-therapeutic ABP-700 doses and achieved plasma concentrations of ABP-700 and CPM-acid that were on the order of 10× and 100× higher, respectively, than those typically reached during Phase 1 studies in humans.[4] Involuntary muscle movements were observed in all 14 dogs and seizures in 5 dogs. However, these two phenomena were temporally and electroencephalographically distinct. The large majority of involuntary muscle movements (278 episodes, 87% of all recorded episodes) occurred during ABP-700 infusion and essentially all of these (99.6%) occurred in the absence of electroencephalographic evidence of seizure activity. Because rocuronium was given intermittently during (but not after) ABP-700 infusion to eliminate involuntary muscle movements when they risked interfering with electroencephalographic signal acquisition and interpretation, this incidence of involuntary muscle movements almost certainly underestimates that which would have occurred during ABP-700 infusion had the paralytic not been used. In contrast, most seizures (*i.e.*, 80% of first seizures and 99% of all seizures) occurred in the minutes to hours after the 2-h ABP-700 infusions were complete. Only one seizure occurred during ABP-700 infusion, and this occurred during the final 12.5 min of the 2-h infusion. These results indicate that the involuntary muscle movements caused by ABP-700 administration are

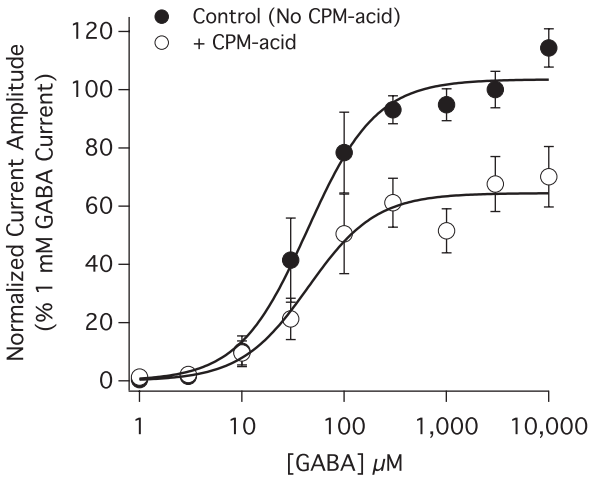

Fig. 8. Impact of 1,500 μM CPM-acid on the γ-aminobutyric acid (GABA) concentration-response relationship. Each symbol is the mean ± SD derived from five different oocytes. The curves are nonlinear least square fits of the two datasets to equation 1 (see Data Analysis section), with the minimum constrained to 0% (*i.e.*, no current in the absence of GABA). These fits yielded GABA EC_{50}s of 44 μM (95% CI, 35 to 54 μM) in the absence of CPM-acid and 45 μM (95% CI, 32 to 62 μM) in the presence of CPM-acid ($P = 0.9148$). The maximal peak current amplitudes were 104% (95% CI, 99 to 108%) in the absence of CPM-acid and 64% (95% CI, 60 to 69%) in the presence of CPM-acid ($P < 0.0001$) with identical slopes of 1.3 (95% CIs, 1.0 to 1.6 and 0.8 to 1.8, respective).

not of epileptic origin and strongly suggest that the underlying mechanisms responsible for ABP-700-induced involuntary muscle movements and seizures are different.

Electroencephalographic spikes and sharp waves were also recorded in the electroencephalographs of dogs during ABP-700 infusion. These are phenomena that are also frequently found in the electroencephalographs of nonepileptic patients receiving etomidate, sevoflurane, or high doses of opiates.[36–40] In epileptic patients, numerous sedative-hypnotic agents that act *via* a variety of different mechanisms produce (or increase the frequency of) spikes and/or sharp waves, including dexmedetomidine, opiates (alfentanil, remifentanil, and fentanyl), methohexital, clonidine, enflurane, and diphenhydramine.[41–48] The clinical significance of this pharmacologic action is not completely clear; although, it has been used as a tool to help locate epileptiform foci in the brain during neurosurgical resection.[42,49]

Involuntary muscle movements are also seen upon administration of other anesthetic induction agents, particularly etomidate where the incidence has been reported to be as high as 70 to 87% in unpremedicated patients.[50–52] Although the etiology of etomidate-induced involuntary muscle movements is unknown, it has been hypothesized to result from either a disequilibrium of the drug and/or differential sensitivity to the drug among its various CNS effect sites, and is not associated with seizures.[37,53,54] A recent study by our group showed that when the structure of etomidate is modified to abolish its potent and efficacious $GABA_A$ receptor positive modulatory activity, the ability of the drug to produce myoclonic muscle movements (in rats) is also eliminated.[55] This supports an important role for $GABA_A$ receptor positive modulation in mediating this side effect.

What, then, caused the seizures in Beagle dogs during the high dose toxicology studies, and why did they sometimes occur hours after the 2-h ABP-700 infusions were complete? We hypothesized that the seizures were caused by inhibition of $GABA_A$ receptors—perhaps together with glycine receptors—by either ABP-700 itself or its principal metabolite, CPM-acid. This hypothesis was based upon the results of previous studies demonstrating that anesthetics and anesthetic-like compounds commonly inhibit $GABA_A$ receptors at very high (typically lethal) concentrations along with the data generated by the industry sponsor and reported in this article revealing that ABP-700 and, in particular, CPM-acid reached very high concentrations in these dogs by the end of these high-dose infusions.[11–13,56] It was also informed by numerous previous studies showing that inhibition of GABAergic neurotransmission (either chemically or resulting from genetic mutations) causes seizures.[57,58]

The results of the current studies support this hypothesis for CPM-acid (but not ABP-700) as they reveal that within the plasma concentration range achieved in these dogs, the metabolite inhibits $GABA_A$ receptors. Studies utilizing a range of GABA concentrations showed that CPM-acid reduces the maximal peak current response obtained at high agonist concentrations without affecting the agonist EC_{50}. Such insurmountable antagonism implies that CPM-acid inhibits $GABA_A$ receptors in a noncompetitive manner, a molecular mechanism it shares with the convulsant picrotoxin.[59,60] The magnitude of this inhibition was voltage-dependent, suggesting direct blockade of the ion channel by the negatively charged metabolite.

In addition, our studies showed that at high concentrations, CPM-acid also inhibits glycine receptors. Similar to its effect on $GABA_A$ receptors, CPM-acid reduced the maximal peak current response obtained at high agonist concentrations. However, it also increased the agonist EC_{50} of the receptor, suggesting that there may be multiple CPM-acid inhibitory mechanisms (*i.e.*, competitive and noncompetitive) with this receptor. Such multiple mechanisms may explain why inhibition of glycine receptors by CPM-acid is characterized by such a steep concentration-response relationship (slope: −5.5).

We also found that at high concentrations, the principal metabolite of etomidate (*i.e.*, etomidate-acid) similarly inhibits both $GABA_A$ and glycine receptors. However, etomidate-acid is formed *in vivo* at a much slower rate than

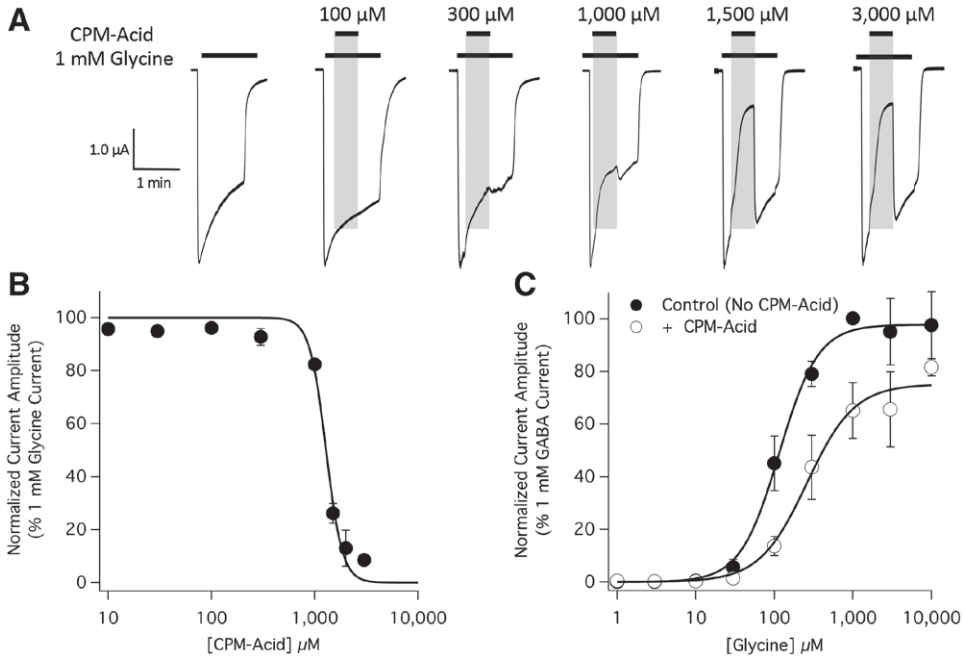

Fig. 9. Current inhibition by CPM-acid of electrophysiologic currents evoked by 1 mM glycine and mediated by $\alpha_1\beta$ glycine receptors. (*A*) Representative electrophysiologic traces recorded upon perfusing oocytes expressing glycine receptors with 1 mM glycine alone for 70 s. Ten seconds into this activation period, CPM-acid was added for 30 s. The *gray bars* highlight the periods when CPM-acid was applied. All traces were obtained from the same oocyte. (*B*) CPM-acid concentration response curves for inhibition of currents evoked by 1 mM glycine. Each symbol is the mean ± SD derived from five different oocytes. The curves are nonlinear least square fits of the dataset to equation 2 (see Data Analysis section) with the maximum and minimum values constrained to 100% and 0%, respectively. This fit yielded a half-maximal inhibitory concentration of 1,290 μM (95% CI, 1,240 to 1,330 μM) with a slope of −5.5 (95% CI, −6.4 to −4.6). (*C*) Impact of 1,300 μM CPM-acid on the glycine concentration-response relationship. Each symbol is the mean ± SD derived from five different oocytes. The curves are nonlinear least square fits of the two datasets to equation 1 (see Data Analysis section) with the minimum constrained to 0%. These fits yielded glycine EC_{50}s of 115 μM (95% CI, 100 to 133 μM) in the absence of CPM-acid and 260 μM (95% CI, 200 to 340 μM) in the presence of CPM-acid, respective maximal peak current amplitudes of 98% (95% CI, 94 to 102%) and 75% (95% CI, 69 to 81%), and slopes of 1.8 (95% CI, 1.4 to 2.2) and 1.4 (95% CI, 1.0 to 1.9).

CPM-acid. Consequently, when their respective parent drugs are administered to dogs at rates that achieve similar levels of sedation/hypnosis, etomidate-acid reaches plasma concentrations that are only one-twentieth that of CPM-acid.[6] Such concentrations would be far below those required to inhibit either $GABA_A$ or glycine receptors and cause seizures.

In spite of the frequency with which high-dose ABP-700 infusions produced seizures in the 14 Beagle dogs (36%), no seizures or postictal behaviors were observed and reported in Phase 1 clinical studies involving 90 humans who received ABP-700 as boluses or continuous infusions lasting as long as 30 min.[4,5] In addition, an independent analysis of raw frontal electroencephalographic waveforms from bispectral index monitoring recorded from 52 of these volunteers and a subsequent review of such waveforms from an additional 29 volunteers who received fentanyl premedication prior to ABP-700 administration found no evidence of seizures during or after ABP-700 administration even during episodes of severe involuntary muscle movements (Report to The Medicines Company and Addendum, Brad J. Kolls, M.D., M.M.Ci. and David S. Warner, M.D., September 15, 2015). In these Phase 1 clinical studies, typical peak plasma CPM-acid concentrations reached 1,500 ng/ml (5 μM) and the highest concentration measured at any time in any individual was only ~3,000 ng/ml[4] (Michel Struys, M.D., Ph.D., F.R.C.A., the Department of Anesthesiology,University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; written communication, April 3, 2018). Such concentrations are just 1% of the peak values achieved in the Beagle dog studies and far below those necessary to inhibit either $GABA_A$ or glycine receptors and produce seizures *via* these receptor mechanisms (fig. 11).

An additional potential contributing factor is that Beagle dogs may be relatively susceptible to seizures. A study utilizing a colony of more than 1,200 Beagle dogs determined that 5.7% of all dogs experienced clinically observable

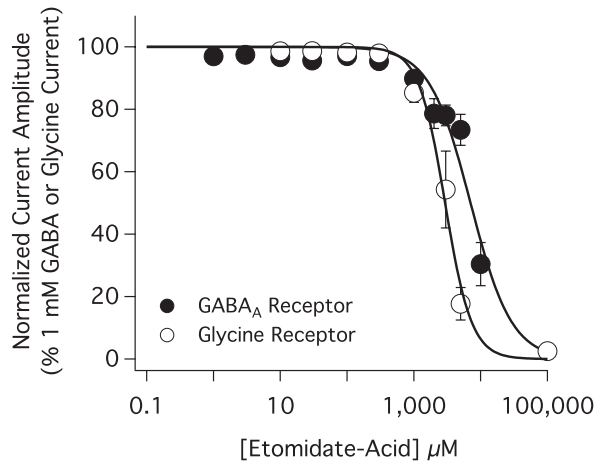

Fig. 10. Current inhibition by etomidate-acid of electrophysiologic currents evoked by 1 mM γ-aminobutyric acid (GABA) or glycine and mediated by $\alpha_1\beta_3\gamma_{2L}$ γ-aminobutyric acid type A ($GABA_A$) or $\alpha_1\beta$ glycine receptors, respectively. Each symbol is the mean ± SD derived from five different oocytes. The curves are nonlinear least square fits of the dataset to equation 2 (see Data Analysis section) with the maximum and minimum values constrained to 100% and 0%, respectively. This fit yielded half-maximal inhibitory concentrations of 7,050 μM (95% CI, 6,300 to 7,890 μM) in the $GABA_A$ receptor and 2,930 μM (95% CI, 2,710 to 3,170 μM) in the glycine receptor with respective slopes of −1.4 (95% CI, −1.7 to −1.2) and -2.3 (95% CI, −2.8 to −1.9).

spontaneous convulsive seizures resembling epilepsy.[61] This is approximately an order of magnitude higher than the prevalence of epilepsy in humans.[62,63] In that Beagle study, an analysis of the pedigree data along with the seizure rates showed a nonrandom incidence that led the authors to conclude that there was a genetic basis for this seizure-susceptibility. Such genetic susceptibility differences may explain why some of our Beagle dogs seized while others did not. Finally (and perhaps most relevantly), Beagle dogs may be relatively sensitive to $GABA_A$ receptor–inhibiting compounds as they convulse with lower doses of pentylenetetrazol than Sprague–Dawley rats or cynomolgus monkeys, two other preclinical models commonly used during drug development.[21] Such high sensitivity is mirrored in *in vitro* studies as hippocampal slices from Beagle dogs are more sensitive to pentylenetetrazol-induced changes in population spike area and number than slices from rats, minipigs, and cynomolgus monkeys.[64]

The U.S. Food and Drug Administration has issued nonbinding guidance for establishing the safety of drug metabolites that provides useful context for the present studies.[65] It advocates for the safety testing of drug metabolites prior to initiating large scale human trials if such metabolites reach plasma concentrations that are greater than 10% of total parent drug-related exposure at steady-state. Such concentrations are routinely achieved when administering soft drugs because they are specifically designed to be metabolized rapidly.[66,67] Safety testing may include the use of animal models, provided that the chosen model produces adequate metabolite exposure levels. Such levels are defined as being at least as high as those encountered in humans. In the current dog studies, however, plasma metabolite concentrations reached levels that are 100× greater than those achieved in human studies, so high that they inhibited both $GABA_A$ and glycine receptors. Thus, it seems highly unlikely that inhibition of these receptors with resultant seizure production is relevant for ABP-700 administration to humans, particularly when such administration is brief.

A limitation to this study is that we cannot directly relate the concentrations of CPM-acid that we found inhibit $GABA_A$ and glycine receptors with those present in the CNS of the Beagle dogs because the latter were not measured. We used peak venous plasma concentrations as a surrogate, but peak CNS concentrations may have been higher and occurred later in time. In particular, if ABP-700 was metabolized within the CNS of these dogs, then CPM-acid—which is negatively charged at physiologic pH—could have become trapped within their CNS by the blood brain barrier. With prolonged ABP-700 infusions, this could have led to peak CPM-acid concentrations in the CNS that were even higher than those measured in peripheral venous blood. This is a phenomenon that we previously observed in rats that received 30-min infusions of methoxycarbonyl etomidate, the lead compound for the development of ABP-700 and other soft etomidate analogs.[7] In that study, peak metabolite concentrations measured in the cerebrospinal fluid were twice those measured in peripheral blood. Such trapping would cause CPM-acid to accumulate within the CNS during ABP-700 infusion, followed by its slow release from the CNS into the peripheral blood after the ABP-700 infusion was complete. Such a release might explain the slow component of the biphasic decay evident in the CPM-acid toxicokinetic plot that has a half-life of ~5 h (fig. 4B). The resulting disequilibrium between CPM-acid concentrations in the CNS and plasma could explain why seizures sometimes occurred in dogs hours after plasma CPM-acid concentrations had reached their maximum values. A similar disequilibrium has been described for tranexamic acid, a carboxylic acid antifibrinolytic that also inhibits $GABA_A$ and glycine receptors, has poor blood–brain barrier permeability, and produces seizures approximately 5 to 10 h after administration has ceased and blood concentrations have peaked.[33,68,69] Such a large disequilibrium cannot occur with ABP-700 because it is uncharged, highly hydrophobic, and crosses the blood–brain barrier readily to allow rapid anesthetic induction and emergence.

In summary, high doses of ABP-700 produced both involuntary muscle movements and seizures in Beagle dogs. However, these two phenomena were temporally and electroencephalographically distinct. These results indicate that ABP-700–induced involuntary muscle movements are not of epileptic origin and strongly suggest that the underlying

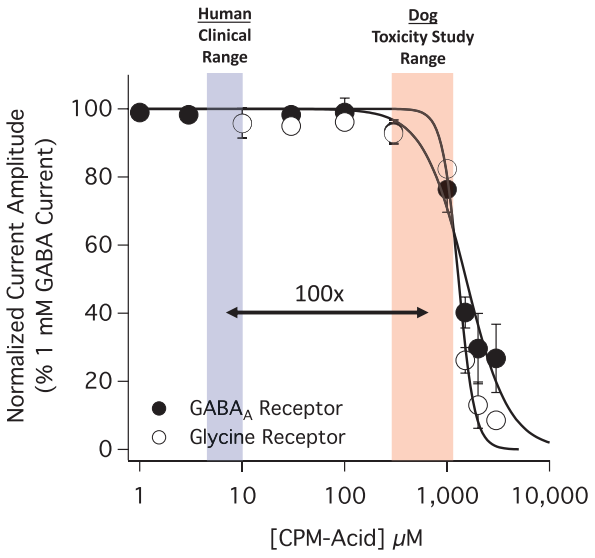

Fig. 11. Peak plasma concentration ranges of CPM-acid achieved in human clinical studies (*blue bar*) and Beagle dog toxicology studies (*red bar*) superimposed on CPM-acid concentration response curves for inhibition of currents evoked by 1 mM γ-aminobutyric acid (GABA) or glycine and mediated by γ-aminobutyric acid type A ($GABA_A$) receptors or glycine receptors, respectively. Peak CPM-acid plasma concentrations were 100× higher in the Beagle dog studies than in the human clinical trials.

mechanisms responsible for ABP-700-induced involuntary muscle movements and seizures are different. The principal metabolite of ABP-700 (*i.e.*, CPM-acid) reached plasma concentrations during these toxicology studies that are sufficient to inhibit the function of both $GABA_A$ and glycine receptors. Such inhibition provides a plausible mechanism for the seizures that occurred in this seizure-prone animal model. It occurs at CPM-acid concentrations that are approximately 100× higher than those achieved in clinical studies of ABP-700 and during which no seizures were observed, strongly suggesting that these actions are irrelevant to the administration of therapeutic ABP-700 doses to humans.

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Competing Interests

Dr. Raines is the lead inventor of ABP-700. He, his department, his laboratory, and his institution could receive royalties relating to the development of ABP-700 or related analogs. Dr. Raines was the scientific founder of Annovation BioPharma (Cambirdge, Massachusetts) and previously served on the Scientific Advisory Boards of Annovation BioPharma and The Medicines Company (Parsippany, New Jersey). The other authors declare no competing interests.

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

References

1. Husain SS, Pejo E, Ge R, Raines DE: Modifying methoxycarbonyl etomidate inter-ester spacer optimizes *in vitro* metabolic stability and *in vivo* hypnotic potency and duration of action. ANESTHESIOLOGY 2012; 117:1027–36
2. Santer P, Pejo E, Feng Y, Chao W, Raines DE: Cyclopropyl-methoxycarbonyl metomidate: Studies in a lipopolysaccharide inflammatory model of sepsis. ANESTHESIOLOGY 2015; 123:368–76
3. Hebron BS, Edbrooke DL, Newby DM, Mather SJ: Pharmacokinetics of etomidate associated with prolonged i.v. infusion. Br J Anaesth 1983; 55:281–7
4. Struys MMRF, Valk BI, Eleveld DJ, Absalom AR, Meyer P, Meier S, den Daas I, Chou T, van Amsterdam K, Campagna JA, Sweeney SP: A Phase 1, single-center, double-blind, placebo-controlled study in healthy subjects to assess the safety, tolerability, clinical effects, and pharmacokinetics-pharmacodynamics of intravenous cyclopropyl-methoxycarbonylmetomidate (cyclopropyl-methoxycarbonyl metomidate) after a single ascending bolus dose. ANESTHESIOLOGY 2017; 127:20–35
5. Valk BI, Absalom AR, Meyer P, Meier S, den Daas I, van Amsterdam K, Campagna JA, Sweeney SP, Struys

MMRF: Safety and clinical effect of i.v. infusion of cyclopropyl-methoxycarbonyl etomidate (cyclopropyl-methoxycarbonyl metomidate), a soft analogue of etomidate, in healthy subjects. Br J Anaesth 2018; 120:1401–11
6. Campagna JA, Pojasek K, Grayzel D, Randle J, Raines DE: Advancing novel anesthetics: pharmacodynamic and pharmacokinetic studies of cyclopropyl-methoxycarbonyl metomidate in dogs. Anesthesiology 2014; 121:1203–16
7. Ge R, Pejo E, Husain SS, Cotten JF, Raines DE: Electroencephalographic and hypnotic recoveries after brief and prolonged infusions of etomidate and optimized soft etomidate analogs. Anesthesiology 2012; 117:1037–43
8. Brohan J, Goudra BG: The role of GABA receptor agonists in anesthesia and sedation. CNS Drugs 2017; 31:845–56
9. Greenfield LJ Jr: Molecular mechanisms of antiseizure drug activity at GABAA receptors. Seizure 2013; 22:589–600
10. Palma E, Ruffolo G, Cifelli P, Roseti C, Vliet EAV, Aronica E: Modulation of $GABA_A$ receptors in the treatment of epilepsy. Curr Pharm Des 2017; 23:5563–8
11. Pistis M, Belelli D, Peters JA, Lambert JJ: The interaction of general anaesthetics with recombinant $GABA_A$ and glycine receptors expressed in Xenopus laevis oocytes: A comparative study. Br J Pharmacol 1997; 122:1707–19
12. Hapfelmeier G, Schneck H, Kochs E: Sevoflurane potentiates and blocks GABA-induced currents through recombinant alpha1beta2gamma2 $GABA_A$ receptors: Implications for an enhanced GABAergic transmission. Eur J Anaesthesiol 2001; 18:377–83
13. Davies PA, Kirkness EF, Hales TG: Modulation by general anaesthetics of rat $GABA_A$ receptors comprised of alpha 1 beta 3 and beta 3 subunits expressed in human embryonic kidney 293 cells. Br J Pharmacol 1997; 120:899–909
14. Hill-Venning C, Belelli D, Peters JA, Lambert JJ: Subunit-dependent interaction of the general anaesthetic etomidate with the gamma-aminobutyric acid type A receptor. Br J Pharmacol 1997; 120:749–56
15. Tomlin SL, Jenkins A, Lieb WR, Franks NP: Stereoselective effects of etomidate optical isomers on gamma-aminobutyric acid type A receptors and animals. Anesthesiology 1998; 88:708–17
16. Belelli D, Lambert JJ, Peters JA, Wafford K, Whiting PJ: The interaction of the general anesthetic etomidate with the gamma-aminobutyric acid type A receptor is influenced by a single amino acid. Proc Natl Acad Sci U S A 1997; 94:11031–6
17. Treiman DM: GABAergic mechanisms in epilepsy. Epilepsia 2001; 42 Suppl 3:8–12
18. Löscher W: Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 2011; 20:359–68
19. Pejo E, Liu J, Lin X, Raines DE: Distinct hypnotic recoveries after infusions of methoxycarbonyl etomidate and cyclopropyl methoxycarbonyl metomidate: The role of the metabolite. Anesth Analg 2016; 122:1008–14
20. Cotten JF, Husain SS, Forman SA, Miller KW, Kelly EW, Nguyen HH, Raines DE: Methoxycarbonyl-etomidate: A novel rapidly metabolized and ultra-short-acting etomidate analogue that does not produce prolonged adrenocortical suppression. Anesthesiology 2009; 111:240–9
21. Bassett L, Troncy E, Pouliot M, Paquette D, Ascah A, Authier S: Telemetry video-electroencephalography (EEG) in rats, dogs and non-human primates: Methods in follow-up safety pharmacology seizure liability assessments. J Pharmacol Toxicol Methods 2014; 70:230–40
22. Chong DJ, Hirsch LJ: Which EEG patterns warrant treatment in the critically ill? Reviewing the evidence for treatment of periodic epileptiform discharges and related patterns. J Clin Neurophysiol 2005; 22:79–91
23. Pejo E, Cotten JF, Kelly EW, Le Ge R, Cuny GD, Laha JK, Liu J, Lin XJ, Raines DE: *In vivo* and *in vitro* pharmacological studies of methoxycarbonyl-carboetomidate. Anesth Analg 2012; 115:297–304
24. Ge RL, Pejo E, Haburcak M, Husain SS, Forman SA, Raines DE: Pharmacological studies of methoxycarbonyl etomidate's carboxylic acid metabolite. Anesth Analg 2012; 115:305–8
25. Pejo E, Santer P, Jeffrey S, Gallin H, Husain SS, Raines DE: Analogues of etomidate: modifications around etomidate's chiral carbon and the impact on *in vitro* and *in vivo* pharmacology. Anesthesiology 2014; 121:290–301
26. Adodra S, Hales TG: Potentiation, activation and blockade of $GABA_A$ receptors of clonal murine hypothalamic GT1-7 neurones by propofol. Br J Pharmacol 1995; 115:953–60
27. Steinbach JH, Akk G: Modulation of GABA(A) receptor channel gating by pentobarbital. J Physiol 2001; 537(Pt 3):715–33
28. Rüsch D, Zhong H, Forman SA: Gating allosterism at a single class of etomidate sites on alpha1beta2gamma2L $GABA_A$ receptors accounts for both direct activation and agonist modulation. J Biol Chem 2004; 279:20982–92
29. Ziemba AM, Forman SA: Correction for inhibition leads to an allosteric co-agonist model for pentobarbital modulation and activation of $\alpha1\beta3\gamma2L$ GABAA receptors. PLoS One 2016; 11:e0154031
30. Barberis A, Petrini EM, Mozrzymas JW: Impact of synaptic neurotransmitter concentration time course on the kinetics and pharmacological modulation of

inhibitory synaptic currents. Front Cell Neurosci 2011; 5:6
31. Marota JJ, Lehoux D, Zerler B, Campagna J: The novel soft-etomidate analogue cyclopropyl-methoxycarbonyl metomidate increases seizure threshold in rodents. Annual meeting of the American Society of Anesthesiologists, 2016, pp A1106
32. Burgos CF, Yévenes GE, Aguayo LG: Structure and pharmacologic modulation of inhibitory glycine receptors. Mol Pharmacol 2016; 90:318–25
33. Lecker I, Wang DS, Romaschin AD, Peterson M, Mazer CD, Orser BA: Tranexamic acid concentrations associated with human seizures inhibit glycine receptors. J Clin Invest 2012; 122:4654–66
34. Kehne JH, Kane JM, Miller FP, Ketteler HJ, Braun DL, Senyah Y, Chaney SF, Abdallah A, Dudley MW, Ogden AM: MDL 27,531 selectively reverses strychnine-induced seizures in mice. Br J Pharmacol 1992; 106:910–6
35. Moore LA, Trussell LO: Corelease of Inhibitory Neurotransmitters in the Mouse Auditory Midbrain. J Neurosci 2017; 37:9453–64
36. Milde LN, Milde JH, Michenfelder JD: Cerebral functional, metabolic, and hemodynamic effects of etomidate in dogs. Anesthesiology 1985; 63:371–7
37. Reddy RV, Moorthy SS, Dierdorf SF, Deitch RD Jr, Link L: Excitatory effects and electroencephalographic correlation of etomidate, thiopental, methohexital, and propofol. Anesth Analg 1993; 77:1008–11
38. Sonkajärvi E, Alahuhta S, Suominen K, Hakalax N, Vakkuri A, Löppönen H, Ohtonen P, Jäntti V: Topographic electroencephalogram in children during mask induction of anaesthesia with sevoflurane. Acta Anaesthesiol Scand 2009; 53:77–84
39. Schultz B, Otto C, Schultz A, Osthaus WA, Krauss T, Dieck T, Sander B, Rahe-Meyer N, Raymondos K: Incidence of epileptiform EEG activity in children during mask induction of anaesthesia with brief administration of 8% sevoflurane. PLoS One 2012; 7:e40903
40. Kearse LA Jr, Koski G, Husain MV, Philbin DM, McPeck K: Epileptiform activity during opioid anesthesia. Electroencephalogr Clin Neurophysiol 1993; 87:374–9
41. Chaitanya G, Arivazhagan A, Sinha S, Reddy KR, Thennarasu K, Bharath RD, Rao MB, Chandramouli BA, Satishchandra P: Dexmedetomidine anesthesia enhances spike generation during intra-operative electrocorticography: A promising adjunct for epilepsy surgery. Epilepsy Res 2015; 109:65–71
42. McGuire G, El-Beheiry H, Manninen P, Lozano A, Wennberg R: Activation of electrocorticographic activity with remifentanil and alfentanil during neurosurgical excision of epileptogenic focus. Br J Anaesth 2003; 91:651–5
43. Grønlykke L, Knudsen ML, Høgenhaven H, Moltke FB, Madsen FF, Kjaer TW: Remifentanil-induced spike activity as a diagnostic tool in epilepsy surgery. Acta Neurol Scand 2008; 117:90–3
44. Tempelhoff R, Modica PA, Bernardo KL, Edwards I: Fentanyl-induced electrocorticographic seizures in patients with complex partial epilepsy. J Neurosurg 1992; 77:201–8
45. Kirchberger K, Schmitt H, Hummel C, Peinemann A, Pauli E, Kettenmann B, Stefan H: Clonidine- and methohexital-induced epileptiform discharges detected by magnetoencephalography (MEG) in patients with localization-related epilepsies. Epilepsia 1998; 39:1104–12
46. Ito BM, Sato S, Kufta CV, Tran D: Effect of isoflurane and enflurane on the electrocorticogram of epileptic patients. Neurology 1988; 38:924–8
47. Diaz-Guerrero R, Feinstein R, Gottlieb JS: EEG findings following intravenous injection of diphenhydramine hydrochloride (benadryl). Electroencephalogr Clin Neurophysiol 1956; 8:299–306
48. Okuma T, Kuba K, Matsushita T, Nakao T, Fujii S, Shimoda Y: Study on 14 and 6 per second positive spikes during nocturnal sleep. Electroencephalogr Clin Neurophysiol 1968; 25:140–9
49. Manninen PH, Burke SJ, Wennberg R, Lozano AM, El Beheiry H: Intraoperative localization of an epileptogenic focus with alfentanil and fentanyl. Anesth Analg 1999; 88:1101–6
50. Guler A, Satilmis T, Akinci SB, Celebioglu B, Kanbak M: Magnesium sulfate pretreatment reduces myoclonus after etomidate. Anesth Analg 2005; 101:705–9, table of contents
51. Kelsaka E, Karakaya D, Sarihasan B, Baris S: Remifentanil pretreatment reduces myoclonus after etomidate. J Clin Anesth 2006; 18:83–6
52. Nyman Y, von Hofsten K, Ritzmo C, Eksborg S, Lönnqvist PA: Effect of a small priming dose on myoclonic movements after intravenous anaesthesia induction with Etomidate-Lipuro in children. Br J Anaesth 2011; 107:225–8
53. Modica PA, Tempelhoff R, White PF: Pro- and anticonvulsant effects of anesthetics (Part II). Anesth Analg 1990; 70:433–44
54. Doenicke AW, Roizen MF, Kugler J, Kroll H, Foss J, Ostwald P: Reducing myoclonus after etomidate. Anesthesiology 1999; 90:113–9
55. McGrath M, Ma C, Raines DE: Dimethoxy-etomidate: A nonhypnotic etomidate analog that potently inhibits steroidogenesis. J Pharmacol Exp Ther 2018; 364:229–37
56. Cenani A, Brosnan RJ, Knych HK: *In vitro* and *in vivo* GABAA receptor interaction of the propanidid metabolite 4-(2-[diethylamino]-2-oxoethoxy)-3-methoxy-benzeneacetic acid. Pharmacology 2019; 103:10–6

57. Roberts E: Failure of GABAergic inhibition: A key to local and global seizures. Adv Neurol 1986; 44:319–41
58. Hirose S: Mutant GABA(A) receptor subunits in genetic (idiopathic) epilepsy. Prog Brain Res 2014; 213:55–85
59. Akaike N, Hattori K, Oomura Y, Carpenter DO: Bicuculline and picrotoxin block gamma-aminobutyric acid-gated Cl- conductance by different mechanisms. Experientia 1985; 41:70–1
60. Krishek BJ, Moss SJ, Smart TG: A functional comparison of the antagonists bicuculline and picrotoxin at recombinant GABAA receptors. Neuropharmacology 1996; 35:1289–98
61. Bielfelt SW, Redman HC, McClellan RO: Sire- and sex-related differences in rates of epileptiform seizures in a purebred beagle dog colony. Am J Vet Res 1971; 32:2039–48
62. Bell GS, Sander JW: The epidemiology of epilepsy: The size of the problem. Seizure 2002; 11 Suppl A:306–14; quiz 315–6
63. Oka E, Ohtsuka Y, Yoshinaga H, Murakami N, Kobayashi K, Ogino T: Prevalence of childhood epilepsy and distribution of epileptic syndromes: A population-based survey in Okayama, Japan. Epilepsia 2006; 47:626–30
64. Accardi MV, Huang H, Authier S: Seizure liability assessments using the hippocampal tissue slice: Comparison of non-clinical species. J Pharmacol Toxicol Methods 2018; 93:59–68
65. Safety testing of drug metabolites: Guidance for industry, https://www.fda.gov/downloads/Drugs/.../Guidances/ucm079266.pdf, Revision 1 edition, 2016, pp 1–14
66. Sum CY, Yacobi A, Kartzinel R, Stampfli H, Davis CS, Lai CM: Kinetics of esmolol, an ultra-short-acting beta blocker, and of its major metabolite. Clin Pharmacol Ther 1983; 34:427–34
67. Westmoreland CL, Hoke JF, Sebel PS, Hug CC Jr, Muir KT: Pharmacokinetics of remifentanil (GI87084B) and its major metabolite (GI90291) in patients undergoing elective inpatient surgery. Anesthesiology 1993; 79:893–903
68. Kratzer S, Irl H, Mattusch C, Bürge M, Kurz J, Kochs E, Eder M, Rammes G, Haseneder R: Tranexamic acid impairs γ-aminobutyric acid receptor type A-mediated synaptic transmission in the murine amygdala: A potential mechanism for drug-induced seizures? Anesthesiology 2014; 120:639–49
69. Gertler R, Wiesner G, Tassani-Prell P, Martin K, Gruber M: Measurement of tranexamic acid serum concentrations in a 7-month-old infant with clinical seizures after open heart surgery. Pediatr Neurol 2014; 51:e1–2