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# *Cholinesterase Inhibition by Potato Glycoalkaloids Slows Mivacurium Metabolism*

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***Background:*** **The duration of action for many pharmaceutical agents is dependent on their breakdown by endogenous hydrolytic enzymes. Dietary factors that interact with these enzyme systems may alter drug efficacy and time course. Cholinesterases such as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) hydrolyze and inactivate several anesthetic drugs, including cocaine, heroin, esmolol, local ester anesthetics, and neuromuscular blocking drugs. Natural glycoalkaloid toxins produced by plants of the family Solanaceae, which includes potatoes and tomatoes, inhibit both AChE and BuChE. Here the authors assess the extent to which two solanaceous glycoalkaloids (SGAs), α-solanine and α-chaconine, can alter the effects of neuromuscular blocking drugs and cholinesterase inhibitors *in vivo* and *in vitro*.**

***Methods:*** **Inhibition of purified human AChE and BuChE by SGAs, neuromuscular blocking drugs, and cholinesterase inhibitors was assessed by an *in vitro* colorimetric cholinesterase assay. *In vivo* experiments were carried out using anesthetized rabbits to test whether SGAs affect recovery from mivacurium-induced paralysis.**

***Results:*** **SGAs inhibited human BuChE at concentrations similar to those found in serum of individuals who have eaten a standard serving of potatoes. Coapplication of SGAs (30–100 nM) with neuromuscular blocking drugs and cholinesterase inhibitors produced additive cholinesterase inhibition. SGA administration to anesthetized rabbits inhibited serum cholinesterase activity and mivacurium hydrolysis. In addition, SGA prolonged the time needed for recovery from mivacurium-induced paralysis (149 ± 12% of control; n = 12).**

***Conclusions:*** **These findings support the hypothesis that inhibition of endogenous enzyme systems by dietary factors can influence anesthetic drug metabolism and duration of action. Diet may contribute to the wide variation in recovery time from neuromuscular blockade seen in normal, healthy individuals. (Key words: Neuromuscular blocking drugs; rabbit; reversal agents; solanidine.)**

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ACETYLCHOLINESTERASE (AChE; E.C. 3.1.1.7) and butyrylcholinesterase (BuChE; acylcholine acylhydrolase; pseudocholinesterase; E.C. 3.1.1.8) are two closely related enzymes expressed by humans and other vertebrate species. AChE is responsible for terminating cholinergic transmission at the neuromuscular junction and in the central nervous system. This enzyme is also a target for numerous inhibitors that are important in medical therapy and toxicology (*e.g.,* reversal agents such as neostigmine, pyridostigmine and edrophonium). In contrast to AChE, the physiologic function of BuChE is still a matter of speculation.[1] Many drugs, including cocaine, heroin, esmolol, local ester anesthetics, cholinesterase inhibitors and neuromuscular blocking drugs, are hydrolyzed and inactivated by esterases.[2,3] The constitutive activity and ubiquitous expression of esterases provide an ideal mechanism for terminating the activity of a drug in a generally predictable time course. There is,

however, significant variability in the time needed for recovery from neuromuscular blockade by esterase-metabolized drugs. Although diseases and genetic abnormalities can prolong recovery time dramatically, the variation in healthy patients is surprisingly high even without these complications.[4] We hypothesized that dietary factors may contribute to this variability through the ingestion of compounds that interact with metabolic enzymes.

There are a number of naturally occurring BuChE and AChE inhibitors, including the solanaceous glycoalkaloids (SGAs), which are found in plants of the family Solanaceae such as potato, eggplant, and tomato.[5–7] The main SGAs in potatoes are $\alpha$-solanine and $\alpha$-chaconine, both of which are triglycosides of solanidine, a steroidal alkaloid derived from cholesterol. SGAs have elicited concern about toxicity because among 5,000–10,000 known plant toxins, they alone inhibit both AChE and BuChE.[8] Although SGAs are concentrated in potato sprouts, there are measurable levels in serum after ingestion of a standard serving of mashed potatoes; a serving of approximately 200–400 g results in serum SGA concentrations ranging from 1–100 nM.[9–10] The biological half-lives for $\alpha$-solanine and $\alpha$-chaconine are 11 and 19 h, respectively.[5] In this study, *in vitro* assays of purified human AChE and BuChE activity were carried out in the presence of neuromuscular blocking drugs and SGAs to test the possible additive effects of these compounds. Because BuChE is primarily responsible for metabolizing the muscle relaxant mivacurium, animal studies were carried out to determine whether SGA administration could influence cholinesterase activity and recovery from mivacurium-induced paralysis.

## Materials and Methods

### In vitro *Cholinesterase Activity Assays*

Human recombinant AChE (3-9 units/mg protein; Sigma, St. Louis, MO) was reconstituted in water, and aliquots were stored at −80°C. The BuChE was Human Serum Cholinesterase P Behring (Behringwerke, Marburg, Germany). Serum Cholinesterase P is a concentrate of highly purified enzyme; one vial contains BuChE activity equivalent to that in 500 ml fresh normal human plasma. Enzyme activity rates were measured using a spectrophotometric assay[11] modified for use on a computerized 96-well microtiter plate reader.[12,13] Final assay volumes were 200 $\mu$l, containing 170 $\mu$l of reaction solution (100 mM phosphate buffer, pH 7.4, 0.5 mM dithionitrobenzoic acid). For AChE activity measurements, each well contained 1 mM acetylthiocholine (as substrate) along with 1 U/ml AChE. For BuChE activity measurements, each well contained 1 U/ml BuChE and 1 mM butyrylthiocholine. Spectrophotometric readings were performed every 3–5 min for a period of 30–60 min using a Bio-Rad Model 550 96-well microtiter plate reader (Bio-Rad Laboratories, Hercules, CA). All enzyme assays were carried out at room temperature. For both enzymes, the accumulation of a yellow oxidation reaction product was assessed as an increase in the absorbance of 405 nm light; this increased absorbance is directly proportional to the cholinesterase hydrolysis rate.[11] The slope of the absorbance–time relation was determined for each well, and the data from replicates were averaged. Inhibition was determined as the percent decrease in the hydrolysis rate in the presence of inhibitor compared with control. These values were plotted versus inhibitor concentration. In some cases, the slope of the absorbance–time relation was converted to the rate of substrate hydrolysis using the following formula: Rate (moles/liter $\times$ min$^{-1}$) = $\triangle$ absorbance $\times$ min$^{-1}$/1.36 $\times$ $10^4$.[11] Inhibitors were allowed to preincubate with the enzyme for at least 30 min before loading of substrate and measurement of activity; parallel control reactions were incubated for similar times. We tested the cholinesterase inhibitory effects of six neuromuscular blocking drugs (atracurium, *cis*-atracurium, mivacurium, pancuronium, rocuronium, and vecuronium) at concentrations ranging from 30 nM to 1 mM for all compounds. Three reversal agents were tested (edrophonium, neostigmine, and pyridostigmine) at concentrations of 3 nM–1 mM. The two SGAs tested were $\alpha$-chaconine and $\alpha$-solanine at concentrations of 30 nM–100 $\mu$M. For each concentration–effect analysis, enzyme inhibition was determined for each decade concentration and three times each decade.

### In vivo *Animal Studies*

The techniques used in this study were approved by the Animal Care and Use Committee, University of California—Davis, Davis, California. Seventeen New Zealand white rabbits were used for data collection (weight range, 2.8–4.5 kg). Animals were not separated by gender in our studies because mivacurium sensitivity and recovery times did not differ between the seven male and 10 female animals used in our study. For the males *versus* females, we found 90.7 ± 3.4% *versus* 88.4 ± 4.1% ($P$ = 0.767) for the extent of blockade and 913 ± 86 s *versus* 873 ± 183 s ($P$ = 0.85) for 50% recovery

times for males and females, respectively, after infusion of 27 nmol/kg mivacurium. Anesthesia was induced with intramuscular ketamine (150 mg), and maintained with intravenous pentobarbital (15–50 mg). After tracheal intubation, ventilation was controlled by a mechanical ventilator (Small Animal Respirator Pump, Harvard Apparatus Co., West Warwick, RI). Vital signs were monitored using a pulse oximeter (Nellcor, St. Louis, MO) on the shaved tail, electrocardiogram using needle microelectrodes, end-expired carbon dioxide (Datex, Madison, WI), and a rectal temperature probe (YSI, Yellow Springs, OH). Body temperature was adjusted when necessary to 37–38°C by a heating pad. Catheters were placed in an ear vein and an artery for taking blood samples and administering drugs. Arterial blood pressure was transduced directly, and 1-ml arterial samples confirmed levels of carbon dioxide and oxygenation. Three-milliliter arterial samples were used for the mivacurium assay, and 3-ml arterial samples were used for cholinesterase determinations. After intubation, mivacurium (27 nmol/kg) was administered intravenously over a 30-s period. The time needed for recovery from mivacurium blockade was assessed by a supramaximal stimulation of the sciatic nerve at 2 Hz for 2 s, repeated every 12 s (train-of-four protocol).[14] Gastrocnemius muscle contractions were measured using a force pressure transducer (Grass FT 03; Grass Instrument Co., Quincy, MA) attached to the severed Achilles tendon. Time to 50% recovery was defined as the interval between the end of mivacurium administration and the recovery to one half the control twitch contraction amplitude during the train-of-four stimulation. SGA (12 $\mu$mol/kg) was infused for 20–60 min before subsequent mivacurium administrations. Each SGA treatment was preceded by atropine infusion (1.4 $\mu$mol/kg intravenously) to protect against autonomic reflex response. Control animals treated with atropine alone did not show differences in mivacurium recovery times. Mivacurium *ex vivo* degradation was blocked by drawing the arterial blood into a syringe containing echothiophate. Sampling protocol: After vital signs and twitch responses were stable, control samples were drawn for mivacurium and cholinesterase determinations. The first dose of mivacurium was given, then a mivacurium blood sample was drawn 5 min later. After recovery from mivacurium, solanine, chaconine, or saline was administered slowly over 1 h intravenously. Repeat mivacurium and cholinesterase samples were drawn, and mivacurium administration was repeated. The mivacurium administrations and sampling were repeated hourly until the animal deteriorated. Cholinesterase activity was assayed by the colorimetric method of Ellman *et al.*[11] modified for use with an automated microplate reader.

*Mivacurium Assay*

Determination of serum mivacurium levels was modified from Cook *et al.*[15] The assay included measurement of trans-trans, cis-trans, and cis-cis isomers. We have reported only the trans-trans results because these had the highest measured levels in our assays and showed the clearest change in the presence of SGA. Briefly, a high-performance liquid chromatography–fluorescence detection method was used in which plasma samples are extracted by solid-phase extraction using C-18 columns. Then an internal standard is added to the extract, and the extract is injected onto a C-18 analytical column. Fluorescence is measured using an excitation wavelength of 235 nm with an emission wavelength of 320 nm. Peak area ratios are determined for standards and samples to calculate the concentration of mivacurium in plasma samples.

*Drugs and Reagents*

All reagents were ordered from Sigma unless otherwise noted. $\alpha$-Solanine and $\alpha$-chaconine were both dissolved in 400 $\mu$l of 100 mm HCl and made up to 2 ml with double distilled $H_2O$ as previously described.[16] Stock solutions of both glycoalkaloids (10 mm) were stored at −20°C and dissolved to working concentrations on the day of the experiment. Other drugs included edrophonium bromide, neostigmine bromide, pyridostigmine bromide, atracurium besylate (Tracrium 10 mg/ml, Burroughs Wellcome Co., Research Triangle Park, NC), mivacurium chloride (Mivacron 2 mg/ml, Burroughs Wellcome), pancuronium bromide (1 mg/ml, Gensia Laboratories Ltd., Irvine, CA), *cis*atracurium besylate (Nimbex 2 mg/ml, Glaxo Wellcome Inc., Research Triangle Park, NC), vecuronium bromide (Marsham Pharmaceuticals Inc., Cherry Hill, NJ), and rocuronium bromide (Zemuron 10 mg/ml, Organon Inc., West Orange, NJ).

*Statistical Analyses and Curve Fitting*

All determinations of enzyme rates were carried out in triplicate, and each experiment was repeated a minimum of three times. All data were averaged and plotted as mean ± SEM. Relations between enzyme activity and inhibitor concentration were fit by a variant of the Hill equation (equation 1) to determine the inhibition parameters:

$$R/R_{max} = 1 - (X^n/(IC_{50}{}^n + X^n))$$

where R and $R_{max}$ are the rates of enzyme hydrolysis in the presence and absence of inhibitor, respectively; X is the inhibitor concentration; n is the Hill coefficient; and $IC_{50}$ is the concentration that produces half-maximal inhibition. Data from each experiment were fit independently, and the Hill number, $IC_{50}$, and maximal inhibition for each inhibitor were pooled and are presented as mean ± SEM in tables 1 and 2.

## Results

### In vitro *Inhibition of Acetylcholinesterase and Butyrylcholinesterase by Neuromuscular Blocking Drugs, Reversal Agents, and Solanaceous Glycoalkaloids*

Using purified human recombinant AChE and purified human plasma BuChE, we tested the inhibitory actions of the SGAs, α-solanine and α-chaconine, on AChE and BuChE activity. As shown in figure 1, both of the SGAs inhibit esterase activity but with much higher potency and efficacy for BuChE than AChE inhibition. Arrows in figure 1 denote the range of SGA concentrations that are present in serum of individuals after consumption of a standard serving of mashed potatoes (200–400 g).[9,10]

We then tested the effects of several drugs on BuChE and AChE activity, including six neuromuscular blocking drugs and three cholinesterase inhibitors. Tables 1 and 2 list the $IC_{50}$, Hill slope, and maximal inhibition values for inhibition of AChE and BuChE activity by the 11 compounds analyzed in this study. Clinically relevant plasma concentrations and $IC_{50}$ values from other studies are also listed for reference.

### *Interaction of Glycoalkaloids with Other Inhibitors of AChE and BuChE*

Next, we tested whether the inhibition of AChE or BuChE produced by the glycoalkaloids is additive, less than additive, or perhaps synergistic with that produced by the reversal agents or neuromuscular blocking drugs. These tests were designed to mimic the types of interactions that are possible *in vivo.* For AChE inhibition, we determined detailed concentration–response curves for atracurium, *cis*atracurium, edrophonium, neostigmine, pancuronium, pyridostigmine, and vecuronium in the presence or absence of 1 μm α-solanine or 1 μm α-chaconine. The SGA concentrations chosen were in the range of $IC_{10}$, to help resolve changes in the inhibitory effects of the other drugs. In all cases, analysis revealed that the inhibition produced by the glycoalkaloids was either additive or less than additive with the inhibition produced by the reversal agents or neuromuscular blocking drugs. In no case did we see evidence of synergism.

In a manner similar to that for BuChE inhibition, we determined concentration–response curves for atracurium, neostigmine, pancuronium, pyridostigmine, rocuronium, and vecuronium in the presence or absence

**Table 1. Acetylcholinesterase Inhibition by Neuromuscular Antagonists, Reversal Agents, and Potato Glycoalkaloids**

| Drug | $IC_{50}$ (μM) | Hill | Maximal Inhibition | Plasma Concentration (μM) [Reference] | $IC_{50}$ (μM), Other Studies [Reference] |
|---|---|---|---|---|---|
| Neuromuscular antagonists | | | | | |
| Atracurium | 13 ± 1.0 | 1.6 ± 0.2 | 58.2 ± 2.5% | 0.31–0.15 [26,27] | 340 [33] |
| *cis*-Atracurium | 8.4 ± 2.1 | 1.3 ± 0.2 | 66.7 ± 9% | 0.16–1.1 [28] | |
| Mivacurium | No activity | | | 0.0018–0.73 [2,3] | |
| Pancuronium | 39 ± 5.0 | 1.2 ± 0.1 | 94.6 ± 4.6% | 0.29–5.0 [26] | 0.15–0.30 [33–35] |
| Rocuronium | 74 ± 2.0 | 1.9 ± 0.1 | 49.1 ± 0.8% | 1.4–8.2 [29,30] | >100 [36] |
| Vecuronium | 7.8 ± 4.0 | 0.8 ± 0.2 | 83.1 ± 1.3% | 0.37–11.0 [26] | $6.6 \times 10^{-5}$ [33] |
| Reversal agents | | | | | |
| Edrophonium | 2.2 ± 0.1 | 1.3 ± 0.1 | 93.0 ± 1.5% | 60 [31] | 0.31–16.0 [37] |
| Neostigmine | 0.0086 ± .0009 | 1.0 ± 0.1 | 91.3 ± 2.2% | 0.0033–0.016 [32] | 0.047 [37] |
| Pyridostigmine | 0.12 ± 0.01 | 1.1 ± 0.1 | 96.0 ± 1.3% | 0.12–0.24 [32] | 0.71 [37] |
| Glycoalkaloids | | | | | |
| Chaconine | 17 ± 2.0 | 1.9 ± 0.03 | 67.3 ± 0.4% | 0.0032–0.13 [9,10] | 6.0 [21] |
| Solanine | 14 ± 1.0 | 1.5 ± 0.1 | 76.8 ± 2.7% | 0.0032–0.13 [9,10] | No inhibition [20]<br>37.0 [21] |

$IC_{50}$ = concentration that inhibits acetylcholinesterase to 50% of the maximal inhibition by the drug; Hill = the Hill coefficient from equation 1 (see Methods); Maximal inhibition = the percentage of control activity remaining at the highest inhibitor concentration, determined by the plateau of the concentration–effect relationship; Plasma concentration = for the neuromuscular antagonists and reversal agents, these are plasma concentrations achieved during paralysis or cholinesterase inhibition, respectively, in human subjects. The glycoalkaloid plasma concentrations are those found after a normal serving of potato (200–400 g).

**Table 2. Butyrylcholinesterase Inhibition by Neuromuscular Antagonists, Reversal Agents, and Potato Glycoalkaloids**

| Drug | $IC_{50}$ ($\mu$M) | Hill | Maximal Inhibition | Plasma Concentration ($\mu$M) [Reference] | $IC_{50}$ ($\mu$M) Other Studies [Reference] |
|---|---|---|---|---|---|
| Neuromuscular antagonists | | | | | |
| Atracurium | 57 ± 8 | 1.7 ± 0.2 | 56.1 ± 7.5% | 0.31–0.15 [26,27] | 120–330 [33] |
| *cis*-Atracurium | No activity | | | 0.16–1.1 [28] | |
| Mivacurium | No activity | | | 0.0018–0.73 [2,3] | |
| Pancuronium | 0.69 ± 0.05 | 1.0 ± 0.1 | 94.1 ± 1.8% | 0.29–5.0 [26] | 0.083–0.27 [33] |
| Rocuronium | 20 ± 9 | 0.9 ± 0.1 | 86.1 ± 10% | 1.4–8.2 [29,30] | |
| Vecuronium | 1.3 ± 0.3 | 0.8 ± 0.1 | 92.6 ± 4.3% | 0.37–11.0 [26] | 0.69–4.5 [33] |
| Reversal agents | | | | | |
| Edrophonium | >1000 | | | 60 [31] | 290–1300 [37] |
| Neostigmine | 0.14 ± 0.01 | 1.1 ± 0.1 | 96.5 ± 1.2% | 0.0033–0.016 [32] | 0.19 [37] |
| Pyridostigmine | 7.7 ± 0.4 | 1.1 ± 0.1 | 96.7 ± 2.2% | 0.12–0.24 [32] | 2.2 [37] |
| Glycoalkaloids | | | | | |
| Chaconine | 0.066 ± 0.001 | 1.1 ± 0.1 | 92.8 ± 2.1% | 0.0032–0.13 [9,10] | 1.2 [16] |
| Solanine | 0.17 ± 0.02 | 1.0 ± 0.1 | 91.5 ± 1.6% | 0.0032–0.13 [9,10] | 5.2 [16] |

$IC_{50}$ = the concentration that inhibits acetylcholinesterase to 50% of the maximal inhibition by the drug; Hill = the Hill coefficient from equation 1 (see Methods); Maximal inhibition = the percentage of control activity remaining at the highest inhibitor concentration, determined by the plateau of the concentration–effect relationship; Plasma concentration = for the neuromuscular antagonists and reversal agents, these are plasma concentrations achieved during paralysis or cholinesterase inhibition, respectively, in human subjects. The glycoalkaloid plasma concentrations are those found after a normal serving of potato (200–400 g).

of 10 nM $\alpha$-chaconine, 30 nM $\alpha$-chaconine, 30 nM $\alpha$-solanine, or 100 nM $\alpha$-solanine. These concentrations of glycoalkaloids were chosen because they produced measurable BuChE inhibition and are known to be present in human plasma after normal potato consumption.[9,10] As with AChE inhibition, the glycoalkaloids produced only additive or less than additive inhibition with the reversal agents or neuromuscular blocking drugs, with no synergistic effects noted at any glycoalkaloid concentration. Figure 2 shows representative data from the interaction studies, demonstrating that coapplication of nanomolar concentrations of $\alpha$-solanine or $\alpha$-chaconine have an additive effect on both neostigmine and pancuronium inhibition of BuChE.

### In vivo *Effect of SGAs on Recovery from Mivacurium Neuromuscular Blockade*

We tested the physiologic relevance of our *in vitro* observations by examining SGA effects in an animal model. To determine whether SGA administration can affect recovery from mivacurium-induced paralysis, a rabbit model was used because of the sensitivity of the species to mivacurium.[17] In addition, rabbit cholinesterases are reported to be sensitive to the toxic effects of both $\alpha$-solanine and $\alpha$-chaconine.[18] In our experiments, SGA administration was preceded by atropine infusion (0.4 mg intravenously) to protect against autonomic effects. Under these conditions, the rabbits maintained stable pulse, blood pressure, and body temperature throughout the experiments.

Infusion of mivacurium (12 nmol/kg) resulted in a highly consistent degree of paralysis among the animals tested (93 ± 1.7% of control twitch contraction), confirming the reproducibility of our measurements and the validity of this animal model. The influence of SGA ad

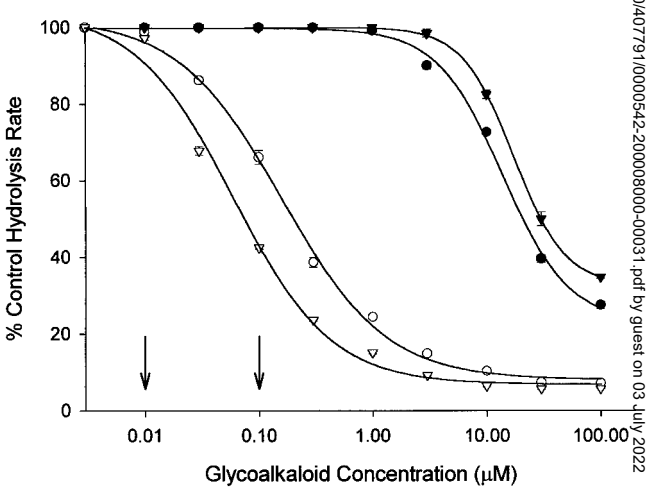


**Fig. 1. Solanaceous glycoalkaloids (SGAs) inhibit human acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The effects of varied SGA concentrations on the hydrolytic activity of human AChE (solid symbols) and BuChE (open symbols) are shown. Substrate hydrolysis activity was normalized and is presented as percentage of control. Inhibition by both $\alpha$-solanine (circles) and $\alpha$-chaconine (triangles) was concentration-dependent. Arrows denote the range of serum SGA levels after potato consumption.[9,10] Data points are mean ± SEM of at least five experiments for each concentration. Determinations within each experiment were carried out in triplicate. Error bars not visible are within symbol size.**

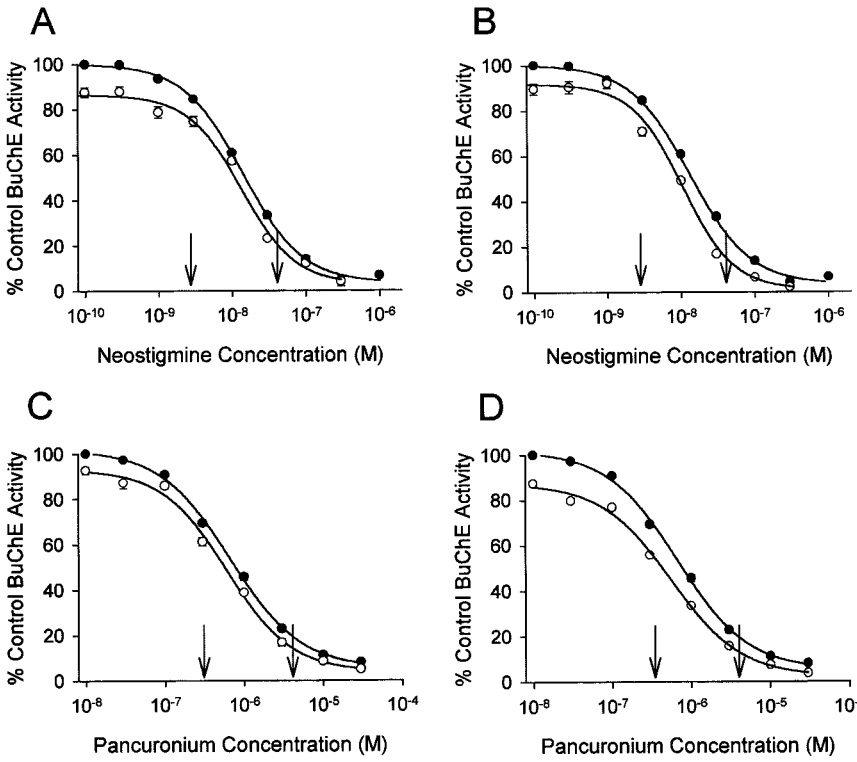


**Fig. 2. Coapplication of low concentrations of solanaceous glycoalkaloids (SGAs) with either neostigmine or pancuronium results in additive inhibition of butyrylcholinesterase (BuChE) activity. (*A* and *B*) Effect of neostigmine on BuChE activity in the absence (filled symbols) and presence of either 100 nM α-solanine (*A*, open symbols) or 30 nM α-chaconine (*B*, open symbols). (*C* and *D*) Effect of pancuronium on BuChE activity in the absence (filled symbols) and presence of either 100 nM α-solanine (*C*, open symbols) or 30 nM α-chaconine (*D*, open symbols). Data points are mean ± SEM of at least three experiments, with each determination carried out in triplicate. Arrows denote the range of clinically relevant serum concentrations (see table 2 for references). Error bars not visible are within symbol size.**

ministration on the recovery from mivacurium-induced paralysis was correlated with serum mivacurium levels and cholinesterase activity, which were measured from blood sampled at intervals throughout several trials. Figure 3A illustrates the relation between the measured mivacurium levels in arterial blood and the time needed for 50% recovery from paralysis (see Methods). Despite the anticipated variation among individual animals, the positive correlation indicates that the serum levels of mivacurium correlate with the pharmacodynamic endpoint of recovery from neuromuscular blockade ($P < 0.05$).

To test whether rabbit plasma cholinesterase activity was influenced by SGA administration in our model system, 12-μmol/kg doses of either α-solanine or α-chaconine were administered intravenously. As shown in figure 3B, the plasma cholinesterase activity decreased in all three of the SGA-treated animals that had measurable plasma cholinesterase.

In light of these findings, we hypothesized that the serum level of mivacurium would be higher after coadministration of mivacurium and SGAs than that measured after mivacurium administration alone. To test this, mivacurium assays were carried out on arterial blood samples collected in the presence and absence of SGAs (see Methods). As shown in figure 3C, mivacurium levels were elevated in those rabbits that received a coinjection of SGAs ($n = 3$; $P < 0.05$). These findings are consistent with the idea that inhibition of serum cholinesterases by SGAs can impede the breakdown of drugs hydrolyzed by cholinesterase, such as mivacurium.

To investigate SGA influence on the action of mivacurium, we tested the time needed for recovery from mivacurium-induced neuromuscular blockade. Anesthetized rabbits were given single doses of mivacurium (27 nmol/kg intravenously) and monitored by supramaximal stimulation of the sciatic nerve at 2 Hz for 2 s every 12 s for 1 h (see Methods). Figure 4A shows an example of the twitch contraction data from a rabbit tested after mivacurium infusion (solid symbols). After recovery, α-chaconine (12 μmol/kg intravenously) was administered, and 30 min later, the mivacurium paralysis protocol was repeated. As illustrated, the time needed for recovery was prolonged after SGA pretreatment. In control animals, successive mivacurium trials yielded slightly faster 50% recovery times (figs. 4B and 4C). After administration of either α-solanine or α-chaconine, there was a significant prolongation in recovery times (figs. 4B and 4C). Data shown in figure 4C were averaged from observations made during different time intervals after

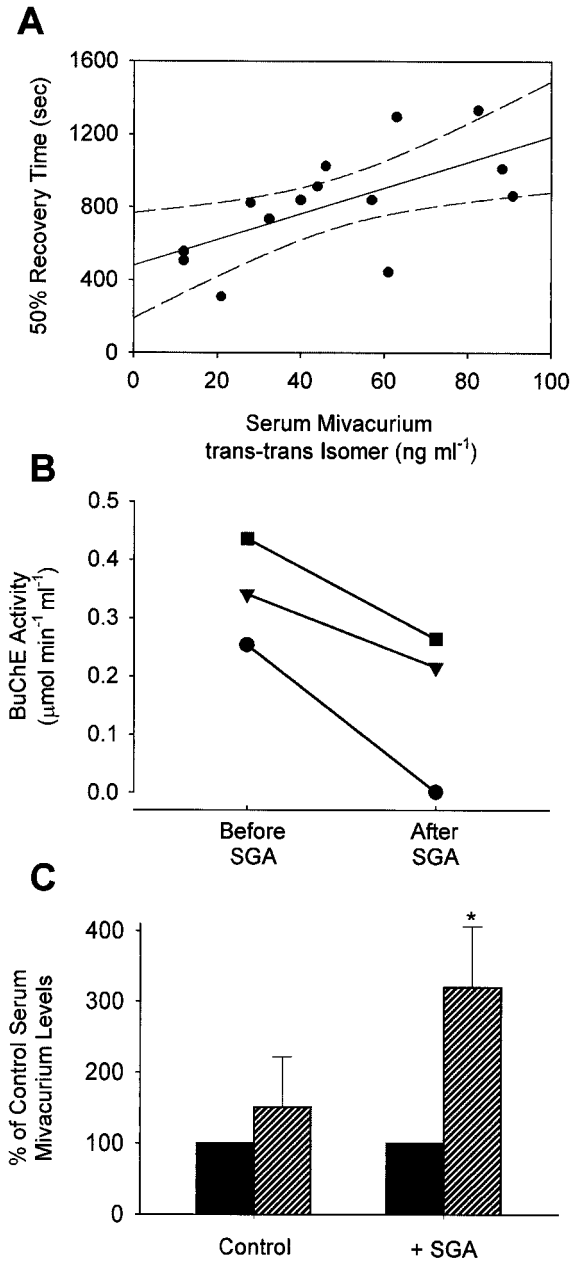


**Fig. 3. Serum mivacurium levels correlate with neuromuscular blockade. Solanaceous glycoalkaloid (SGA) administration inhibits serum cholinesterase and increases serum mivacurium levels. (*A*) Levels of the trans-trans isomer of mivacurium were determined in 14 rabbits. Each value is the mean of triplicate determinations. Mivacurium levels are plotted versus the time needed for 50% recovery of twitch amplitude after mivacurium administration. Animals with higher mivacurium levels showed longer recovery times ($r^2 = 0.39$; dashed line is 95% confidence interval; $P < 0.05$). (*B*) Three animals with measurable serum cholinesterase activity showed decreases after SGA administration. Blood samples from two other animals had basal cholinesterase activity near the lower limit of detection (not shown). (*C*) Mivacurium trans-trans isomer levels were determined in blood samples collected after two consecutive mivacurium administrations (10 min after each mivacurium infusion). All data were normalized to the serum levels after the first mivacurium administration. Striped bars indicate the percent change in mivacurium levels after the second administration under control conditions (*left*) and with coadministration of SGA (*right*). *$P < 0.05$ relative to initial determinations, paired *t* test; n = 7 animals).**

SGA administration ($P < 0.01$ at 200 and 300 min after SGA administration). These data illustrate that SGAs can alter mivacurium hydrolysis rates *in vivo*.

## Discussion

The use of purified human AChE and BuChE enabled the assessment of the sensitivity of these enzymes to inhibition by SGAs and of several neuromuscular blocking drugs and reversal agents. Physiologically relevant substrates, acetylthiocholine and butyrylthiocholine, were used for these experiments to assess the inhibitory effects of these compounds. Our findings are in close agreement with those of other laboratories; the enzyme inhibition correlates well with clinically relevant concentrations of these agents[19] (tables 1 and 2).

### *Acetylcholinesterase Inhibition*

The reversal agents edrophonium, neostigmine, and pyridostigmine produced potent and efficacious inhibition of the AChE hydrolysis rate, as reported previously and as expected from their clinical use as AChE inhibitors (table 1). In addition, atracurium and vecuronium also had $IC_{50}$ values for AChE activity within the clinically relevant plasma concentration range. Similar to results from other studies,[20,21] the glycoalkaloids α-solanine and α-chaconine had $IC_{50}$ values for AChE several orders of magnitude higher than plasma concentrations found in potato eaters[9,10] (table 1; fig. 1). The symptoms of glycoalkaloid poisoning strongly resemble those produced by inhibition of AChE.[5] This implies that in severe cases of SGA poisoning, micromolar concentrations of SGAs are present in serum, although assays of serum from these individuals have yet to be reported.

### *Butyrylcholinesterase Inhibition*

Of the neuromuscular blockers and reversal agents tested, pancuronium, vecuronium, and neostigmine in-

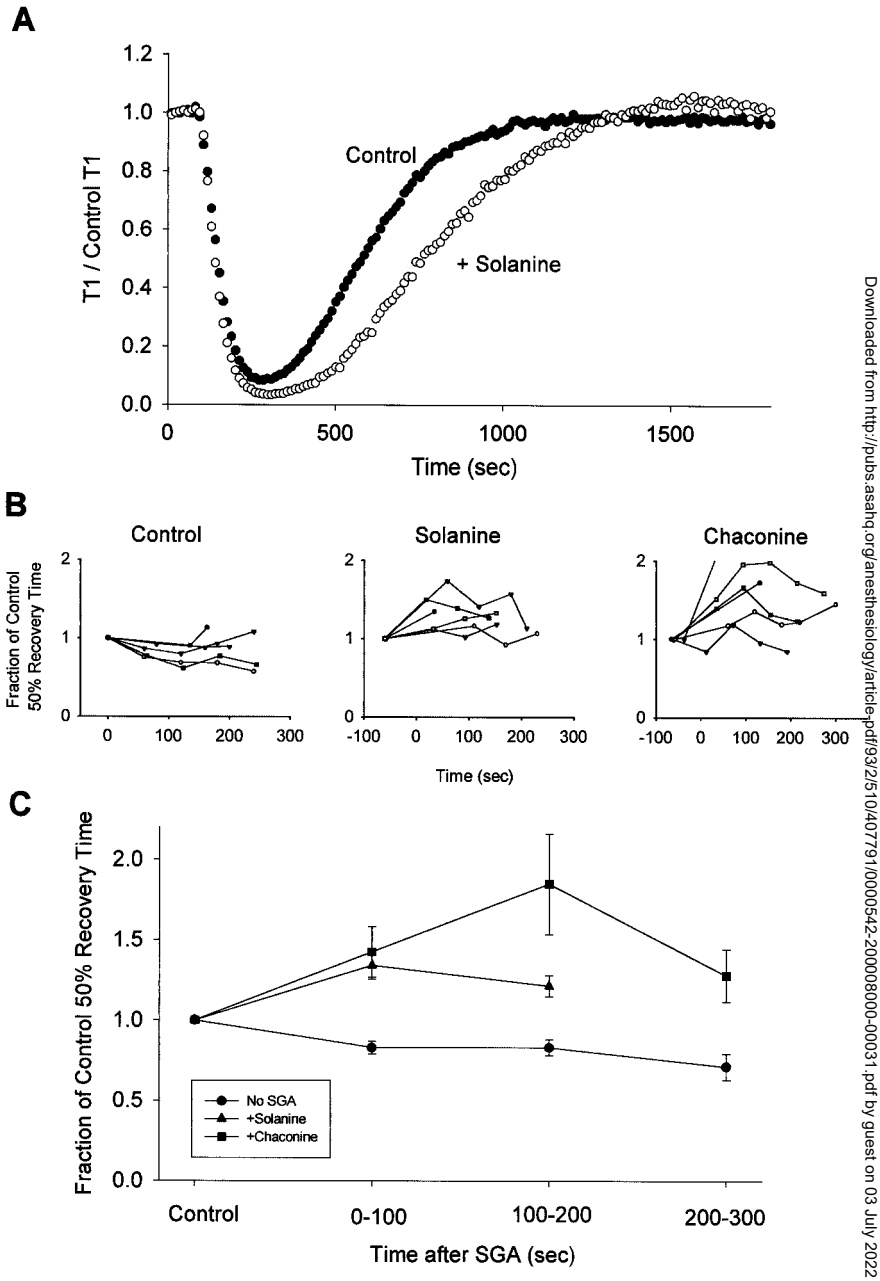


**Fig. 4. Solanaceous glycoalkaloid (SGA) administration prolongs the recovery time from mivacurium-induced paralysis. (*A*) Example mivacurium paralysis profiles illustrating twitch amplitude in a rabbit before (closed symbols) and after (open symbols) α-solanine administration (12 μmol/kg intravenously). Mivacurium adminstrations (27 nmol/kg intravenously) resulted in highly reproducible maximal paralysis levels. (*B*) Fifty percent recovery data from individual animals were normalized to the initial determination. Data from control (*left*), α-solanine–treated (*middle*), and α-chaconine–treated (*right*) animals are presented on equal scales for comparison. Both α-solanine and α-chaconine (12 μmol/kg intravenously) were infused over a 20–60-min period, ending at time 0. Data from one animal are not shown on this graph due to a large effect. (*C*) The 50% recovery times normalized to the initial recovery were pooled according to the time of the measurement relative to completion of SGA administration and averaged. Both α-solanine (triangles, n = 6 animals) and α-chaconine (squares, n = 6 animals) showed significantly longer recovery times relative to control (circles, n = 5 animals; $P < 0.05$ for less than 100-min group, $P < 0.01$ for 100–200-min and 200–300-min groups).**

duced significant BuChE inhibition at clinically relevant concentrations (table 2). Although mivacurium is rapidly hydrolyzed by BuChE,[2] we did not see an effect of mivacurium on BuChE activity rate at concentrations as high as 0.1 mM, even when applied only 15 s before spectrographic measurements. In this purified enzyme system, it is possible that mivacurium hydrolysis occurs on a time scale that is too rapid for our system to assay. The SGAs, α-solanine and α-chaconine, both inhibited BuChE activity substantially within the nanomolar concentration range (table 2; fig. 1). These data show that SGAs can inhibit human BuChE at concentrations that are found in serum of individuals who have eaten a normal serving of potatoes.[9,10] Further testing of the coadministration of SGAs with reversal agents and neuromuscular blockers indicated an additive effect on en-

zyme inhibition. Thus, SGA levels normally found in serum could significantly alter BuChE-mediated hydrolysis of drugs.

In vivo *Prolongation of Mivacurium-induced Paralysis by SGAs*

Investigation of SGA effects on mivacurium-induced paralysis provided direct assessment of the physiologic relevance of BuChE inhibition by SGAs. Treatment of rabbits with SGAs was found to inhibit serum cholinesterase activity, which is consistent with previous reports indicating that SGAs inhibit rabbit plasma cholinesterases, including BuChE.[18] Also consistent with SGA inhibition of BuChE was the observation that mivacurium blood levels after SGA administration were higher than those measured after mivacurium infusion alone. Concomitantly, SGA treatment prolonged mivacurium-induced paralysis. These findings are in line with previous observations in which nondepolarizing neuromuscular blocking drugs were found to inhibit cholinesterase and mivacurium breakdown.[22] Together, these findings suggest a level of causality consistent with the idea that ingestion of SGAs by normal individuals may contribute to the variability in the recovery from neuromuscular blockade.

*Implications for Drug Design*

The impetus for development of short-acting drugs that minimize paralysis times has led to the design of pharmaceutical agents that are degraded by endogenous enzymes. In the case of neuromuscular blocking drugs, the use of shorter-acting drugs has led to fewer respiratory complications.[23] Recent emphasis on ambulatory care provides additional motivation for rapid recovery from neuromuscular blockade. BuChE is a reasonable choice for the catalysis of neuromuscular blockers because of its robust expression (except in the case of liver failure), its constitutive activity, and the relative rarity of clinically relevant enzyme mutations described to date.[5] Although these properties reinforce the suitability of this enzyme system for drug metabolism, it is important to consider the effects of dietary constituents on enzyme activity.

There is precedent for the idea that naturally occurring compounds can alter drug metabolism. Components of grapefruit juice can alter drug metabolism by inhibiting the metabolic cytochrome P-450 isozyme CPY3A4. Normally, metabolism of terfenedine (Seldane) by this enzyme limits the bioavailability of this drug, protecting the patient from its potentially cardiotoxic effects.[24,25]

Therefore, the effects of pharmacological agents can be complicated by naturally occurring inhibitors of metabolic enzymes, a factor that may not be realized during clinical trials. Plants have adapted complex antipredation mechanisms, and many natural plant toxins are closely monitored by the food products industry. Although partial inhibition of an enzyme such as P-450 or BuChE may have little or no consequence in a normal, healthy individual, it is important to consider how small changes in activity may influence the time course and efficacy of drugs that are dependent upon hydrolysis by endogenous enzymes.

Coadministration of SGAs and neuromuscular blocking drugs alters drug potency, as shown by results from *in vitro* assays of purified human BuChE and from *in vivo* observations in our rabbit model system. Although conclusive evidence necessitates human studies, these results may explain the significant variation in the time needed for recovery from neuromuscular blockade in normal, healthy individuals. Our findings also support the hypothesis that naturally occurring dietary factors can influence drug metabolism.

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