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## Medical Intelligence

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### Natural inhibitors of cholinesterases: implications for adverse drug reactions

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**Purpose:** Acetylcholinesterase and butyrylcholinesterase are two closely related enzymes important in the metabolism of acetylcholine and anaesthetic drugs, including succinylcholine, mivacurium, and cocaine. The solanaceous glycoalkaloids (SGAs) are naturally occurring steroids in potatoes and related plants that inhibit both acetylcholinesterase and butyrylcholinesterase. There are many clinical examples of direct SGA toxicity due to cholinesterase inhibition. The aim of this study was to review the hypotheses that (1) SGAs may be the evolutionary driving force for atypical butyrylcholinesterase alleles and that (2) SGAs may adversely influence the actions of anaesthetic drugs that are metabolized by acetylcholinesterase and butyrylcholinesterase.

**Source:** The information was obtained by Medline search and consultation with experts in the study of SGAs and cholinesterases.

**Principal findings:** The SGAs inhibit both acetylcholinesterase and butyrylcholinesterase in numerous *in vitro* and *in vivo* experiments. Although accurate assays of SGA levels are difficult, published data indicate human serum SGA concentrations at least ten-fold lower than required to inhibit acetylcholinesterase and butyrylcholinesterase *in vitro*. However, we review evidence that suggests the dietary ingestion of SGAs can initiate a cholinergic syndrome in humans. This syndrome appears to occur at SGA levels lower than those which interfere with anaesthetic drug catabolism. The world distribution of solanaceous plants parallels the distribution of atypical alleles of butyrylcholinesterase and may explain the genetic diversity of the butyrylcholinesterase gene.

**Conclusion:** Correlative evidence suggests that dietary SGAs may be the driving force for atypical butyrylcholinesterase alleles. In addition, SGAs may influence the metabolism of anaesthetic drugs and this hypothesis warrants experimental investigation.

**Objectif :** L'acétylcholinestérase et la butyrylcholinestérase sont deux enzymes apparentés essentiels au métabolisme de l'acétylcholine et de certains produits utilisés en anesthésie comme la succinylcholine, le mivacurium et la cocaïne. Les glycoalcoïdes des solanacées (SGA) sont des stéroïdes naturels extraits de la pomme de terre et de plantes identiques qui inhibent à la fois l'acétylcholinestérase et la butyrylcholinestérase. On connaît plusieurs exemples de toxicité directe aux SGA causés par l'inhibition de la cholinestérase. Cette étude visait à revoir l'hypothèse selon laquelle 1) les SGA pourraient être le moteur évolutif des allèles de la butyrylcholinestérase et 2) les SGA pourraient avoir une influence défavorable sur les agents anesthésiques métabolisés par l'acétylcholinestérase et la butyrylcholinestérase.

**Source :** Information recueillie sur Medline et par consultation avec des experts en SGA et en cholinestérase.

**Principales constatations :** De nombreuses expériences *in vitro* et *in vivo* ont montré que les SGA inhibent à la fois l'acétylcholinestérase et la butyrylcholinestérase. Bien que le titrage précis de la concentration de SGA soit difficile, les données publiées indiquent que, chez l'homme, la concentration sérique de SGA est de dix fois inférieure à la concentration requise pour inhiber l'acétylcholinestérase et la butyrylcholinestérase *in vitro*. Cependant, nous avons trouvé des données suggérant que l'ingestion alimentaire de SGA peut induire un syndrome cholinergique chez l'humain. Ce syndrome survient à des concentrations inférieures à celles qui interviennent dans le métabolisme des produits anesthésiques. La distribution mondiale des solanacées est comparable à la distribution des allèles atypiques de la butyrylcholinestérase et peut expliquer la diversité génétique de la butyrylcholinestérase.

**Conclusion :** Comme le démontre la corrélation des données, les SGA alimentaires pourraient s'avérer le moteur évolutif des allèles atypiques de la butyrylcholinestérase. En outre, les SGA peuvent influencer le métabolisme des agents anesthésiques et cette hypothèse justifie une recherche expérimentale plus approfondie.

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**A**CETYLCHOLINESTERASE (AChE; E.C. 3.1.1.7) and butyrylcholinesterase (BuChE; acylcholine acylhydrolase; pseudocholinesterase; E.C. 3.1.1.8) are two closely related enzymes found in all vertebrate species. Acetylcholinesterase is vital to mammalian life, playing a crucial role in the neuromuscular junction by terminating cholinergic transmission. It is also a target for numerous inhibitors, such as neostigmine, which are important in medical therapy and toxicology. In contrast to AChE, the normal physiological function of BuChE remains a matter of speculation.<sup>1</sup> Of clinical importance to anaesthetists, BuChE hydrolyzes and limits the duration of action of the neuromuscular blocking agents succinylcholine and mivacurium, as well as ester local anaesthetics. Butyrylcholinesterase also hydrolyzes other compounds such as cocaine<sup>2</sup> and heroin.<sup>3</sup> Individuals who possess certain variant alleles for BuChE hydrolyze succinylcholine and mivacurium very slowly and develop prolonged apnea when these agents are used during anaesthesia.<sup>4-7</sup> Awareness of this untoward effect of succinylcholine led to the development of the dibucaine number assay, which predicts the presence of atypical BuChE alleles from differential inhibition of BuChE by the ester local anaesthetic dibucaine.<sup>8</sup>

The growing awareness of the role of BuChE in drug metabolism has led to interest in factors that alter its activity. A non-genetic factor that may influence BuChE activity is liver failure since BuChE is synthesized in the liver; in severe cases of hepatic dysfunction, response to succinylcholine can be prolonged.<sup>9</sup> Another clinically important drug interaction is that pancuronium and other myorelaxants inhibit BuChE, thus prolonging the action of drugs, such as mivacurium, dependent on BuChE-mediated catabolism (Figure 1).<sup>10</sup>

Depression in BuChE activity also appears to mediate adverse drug reactions to drugs other than the neuromuscular antagonists. For example, cocaine users who are suffering life-threatening adverse reactions have lower plasma BuChE concentrations than cocaine users presenting with less severe toxicity. There is also the suggestion that BuChE limits the adverse effects of maternal cocaine use on fetal development.<sup>11</sup> The human term placenta expresses BuChE, and the level and type of BuChE expression may be crucial in reducing fetal exposure to cocaine.<sup>12</sup>

Separate genes encode AChE and BuChE enzymes. In humans, the gene for AChE is located on chromosome 7, while the gene for BuChE is located on chromosome 3.<sup>13-16</sup> Although the DNA sequences for the AChE and BuChE genes differ considerably (i.e., the A, C, G, T sequences are different), the amino acid sequences of the enzymes encoded by the genes are

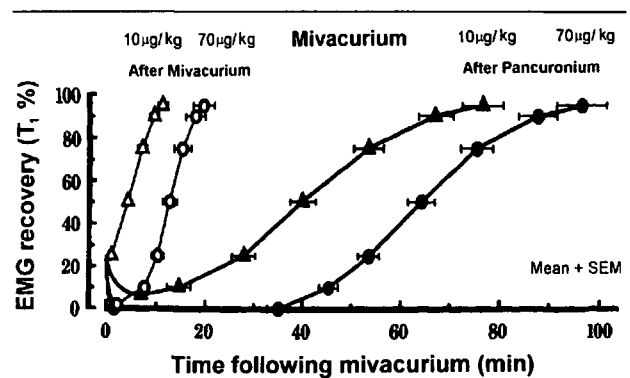


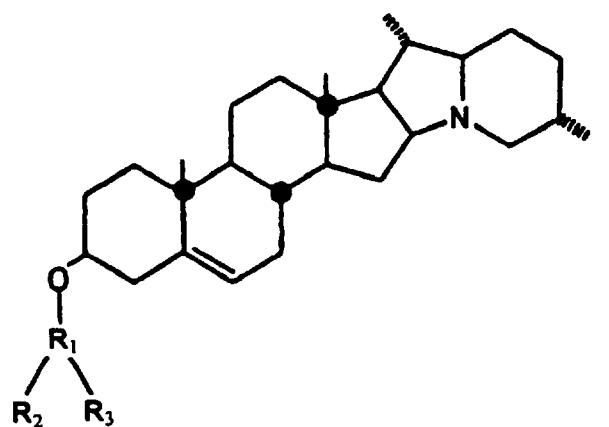
FIGURE 1 Spontaneous electromyography recovery of neuromuscular block after 10 (triangles) or 70 (circles),  $\mu\text{g}\cdot\text{kg}^{-1}$  mivacurium given at TI 25% during recovery from pancuronium (close symbols) or mivacurium block (open symbols).

Groups differed ( $P < 0.05$ ) from each other at every EMG recovery level other than mivacurium groups at 90% and 95% recovery levels. (Reprinted from *Erkola O, Rautoma P, Meretoja OA: Mivacurium when preceded by pancuronium becomes a long-acting muscle relaxant. Anesthesiology 1996; 84: 562-6* by kind permission of the authors and Lippincott-Raven Publishers).

>50% homologous. In addition, the BuChE and AChE genes share a similar intron-exon organization, i.e., the length, placement, and number of exons and introns comprising these genes are similar. The similarities of these genes implies that they arose from a common ancestral cholinesterase gene.

The evolution of proteins can also be inferred by comparison of DNA sequences of genes in different species. For the cholinesterases, the electric eel expresses two distinct cholinesterases (BuChE and AChE), while insects produce a single enzyme with mixed AChE/BuChE properties.<sup>17,18</sup> It is theorized that primitive life first developed a single cholinesterase gene and protein, that duplicated into two copies and, over time, mutated into two separate genes encoding different enzymes. Since all vertebrates contain two distinct cholinesterases, it is presumed that distinct BuChE and AChE genes and proteins actually evolved before the appearance of the first vertebrates.<sup>19</sup>

Genetic variation of the BuChE gene has been extensively explored.<sup>20</sup> More than 20 different naturally occurring BuChE mutants exist; some of these possess substantially different functional and pharmacological properties.<sup>21</sup> The most common of these is a point mutation that substitutes glycine for aspartate at position 70 (D70G), resulting in the so-called "atypical" allele.<sup>7</sup> The extensive variation of the gene for BuChE suggests a driving force and selective advantage for such variation.<sup>22</sup> Of possible importance in this regard is that nat-



**$\alpha$ -Solanine**  
**R<sub>1</sub> = D-galactose**  
**R<sub>2</sub> = D-glucose**  
**R<sub>3</sub> = L-rhamnose**

**$\alpha$ -Chaconine**  
**R<sub>1</sub> = D-glucose**  
**R<sub>2</sub> = L-rhamnose**  
**R<sub>3</sub> = L-rhamnose**

FIGURE 2 Molecular structures of the solanaceous glycoalkaloids  $\alpha$ -solanine and  $\alpha$ -chaconine. The steroidal backbone without the attached sugar moieties is the compound solanidine.

ural compounds found in potatoes and related plants inhibit both AChE and BuChE. Many of the BuChE allelic variants display different sensitivities to these natural inhibitors, and this may confer an evolutionary advantage against toxic dietary exposure.

A number of naturally occurring BuChE and AChE inhibitors have been discovered, including solanaceous glycoalkaloids (SGAs), organophosphates from cyanobacteria,<sup>23</sup> and the fungal antibiotic puromycin and related analogs.<sup>24</sup> The most common naturally occurring cholinesterase inhibitors, the SGAs, are found in plants of *Solanaceae* such as potato, eggplant, and tomatoes. The main SGAs in potatoes are  $\alpha$ -solanine and  $\alpha$ -chaconine, both triglycosides of solanidine, a steroidal alkaloid derived from cholesterol (Figure 2). Solanaceous glycoalkaloids have elicited concern about toxicity since among 5,000–10,000 identified plant toxins they alone inhibit *both* AChE and BuChE.<sup>25</sup> This review summarizes the effects of SGAs in humans and animals. The other natural inhibitors of AChE and BuChE are not considered since they have been less well studied.

Awareness of the action of SGAs on BuChE may be important for three reasons. First, depression of BuChE activity has implications for the use of anaesthetics and other drugs, since SGA depression of BuChE activity may influence drug toxicity. Second, inhibition of

esterases is important, not only with established drugs, such as succinylcholine and mivacurium, but also with newer drugs, such as remifentanyl, that are hydrolyzed by non-specific esterases.<sup>26,27</sup> Medicinal chemists have increasingly designed drugs that capitalize on enzymatic breakdown to insure reliable termination of action. The implicit assumption in this strategy is that degradatory capacity is increased by the sensitivity of the drug to these non-specific esterases, and thus the influence of inhibitors of AChE and BuChE is minimized. Although no important interactions of AChE or BuChE inhibitors with remifentanyl have yet been reported, and hepatic failure affects catabolism minimally,<sup>28</sup> the action of solanidine on nonspecific esterases has not been investigated. Given the trend to develop drugs with rapid catabolism by esterases, it is increasingly important to recognize and test for genetic and environmental influences that might alter the pharmacokinetic behaviour of such drugs. Third, the presence and evolutionary influence of natural BuChE inhibitors such as the SGAs may help to explain the extensive genetic variation for the BuChE gene. In particular, differential sensitivity to natural inhibitors by BuChE variants may provide an evolutionary selective advantage.

Solanaceous glycoalkaloid levels vary considerably among different plant species and vary with light exposure, mechanical damage, and the vegetable or fruit part (e.g., skin, tuber, green sprouts) (Table I). In potatoes, green shoots and skin often contain considerably higher levels of SGAs than other parts of the plant. Higher levels of SGAs generally impart a bitter, unpleasant taste.<sup>29</sup> Exposure to light, mechanical damage, and spoiling increase SGA levels; cooking does *not* alter levels.<sup>30</sup>

Solanaceous glycoalkaloids received scrutiny as a result of documented outbreaks of toxicity in humans and livestock after potato consumption. The long history of potato toxicity<sup>31,32</sup> includes some fatalities.<sup>33</sup> The clinical symptoms of potato toxicity in humans are fairly consistent throughout documented cases: acute gastrointestinal disturbances (abdominal pain, vomiting, and diarrhoea), progressing in severe cases to profound neurological symptoms (apathy, drowsiness, mental confusion, stupor, visual disturbances, dizziness, hallucinations, and trembling). Many of these symptoms mimic the clinical syndrome of massive cholinergic stimulation. Symptoms last approximately 2–24 hr after ingestion of potatoes.<sup>31</sup> The most recent major outbreak involved 78 British schoolchildren in which the most severely affected child recovered only after one week of hospitalization. Butyrylcholinesterase concentrations in 10 of 17 children analyzed were abnormally low *six days* after exposure. In all but one case, levels had returned to normal after four

TABLE I Glycoalkaloid Content of Various Food Products

Food Product	Total Glycoalkaloids (mg·kg <sup>-1</sup> )	Reference
Potato peel wedges, frozen	76–120	Bushway <i>et al.</i> , 1983 <sup>64</sup>
Potato peel slices, frozen	66–71	Bushway <i>et al.</i> , 1983 <sup>64</sup>
Potato chips, commercial	32–184 59–70 95–720 24–109	Davies & Blincow, 1984 <sup>65</sup> Jones & Fenwick, 1981 <sup>66</sup> Sizer <i>et al.</i> , 1980 <sup>67</sup> Friedman & Dao, 1992 <sup>68</sup>
French fries, fresh	0.8–8.4 19–58	Friedman & Dao, 1992 <sup>68</sup> Davies & Blincow, 1984 <sup>65</sup>
Potato wedges, fresh	44	Friedman & Dao, 1992 <sup>68</sup>
Potato skins, baked	31 52–63	Friedman & Dao, 1992 <sup>68</sup> Bushway <i>et al.</i> , 1983 <sup>64</sup>
Potato skins, fried	55–203 120–242	Friedman & Dao, 1992 <sup>68</sup> Bushway <i>et al.</i> , 1983 <sup>64</sup>
Eggplant, fresh	76–82	Jones & Fenwick, 1981 <sup>66</sup>
Green or red pepper, fresh	61–113	Jones & Fenwick, 1981 <sup>66</sup>

to five weeks.<sup>34</sup> This analysis suggests a substantial reservoir of SGAs after consumption of potatoes.

In addition to the clinical reports demonstrating toxic effects similar to those following inhibition of AChE, there are numerous animal and human experiments suggesting that *normal nutritional intake* of these natural inhibitors may result in levels that inhibit AChE and/or BuChE. This may then create direct toxic effects or, more insidiously, cause altered metabolism of compounds metabolized by BuChE. Morris and Lee<sup>32</sup> concluded that 2–5 mg SGA·kg<sup>-1</sup> body weight (BW) is toxic and that 3–6 mg·kg<sup>-1</sup> BW is potentially fatal. Subsequent research has supported this estimate and some investigators suggest that the toxic dose may be as low as 1 mg·kg<sup>-1</sup> BW. For example, in three studies using male volunteers, symptoms of diarrhoea, vomiting, and/or nausea occurred at SGA levels of 1.0–2.6 mg·kg<sup>-1</sup> BW.<sup>35</sup> The daily average amount of potatoes consumed by individuals in the United States, Great Britain, and Sweden is 167 g,<sup>36</sup> 140 g,<sup>37</sup> and 300 g,<sup>38</sup> respectively. Current agricultural guidelines recommend that potatoes for consumption contain no more than 200 mg·kg<sup>-1</sup> SGA.<sup>a</sup> Many potato products contain SGA levels close to this concentration (Table I). Individuals consuming a standard serving of potatoes can ingest as much as 20 to 60 mg SGA. This amount overlaps the potentially toxic range of SGA in small children and heavier consumption of foods containing SGAs would also reach the toxic range in adults. Although severe direct toxicity caused by SGAs is rare, the possibility that SGAs under typical dietary conditions may alter BuChE-

mediated metabolism has not been addressed experimentally. Given the wide range of commonly used drugs that appear to depend on BuChE for proper termination of action, the effects of SGA inhibition warrant careful study.

Animal studies also illustrate the toxicity of SGAs; however, no animal appears to be as sensitive to SGAs as humans. The oral lethal dose in sheep is 500 mg·kg<sup>-1</sup> BW with toxic effects noted at 225 mg·kg<sup>-1</sup> BW.<sup>39</sup> Rabbits fed greened potatoes (64 mg SGA per day) for 30 days showed a greater rate of death and weight loss than rabbits fed normal potatoes (22 mg SGA per day).<sup>40</sup> Administered orally, SGAs show poor bioavailability in rats and mice.<sup>41, 42</sup> In fact, oral administration must exceed 1000 mg·kg<sup>-1</sup> BW to produce toxic effects in mice. In contrast, parenteral administration of solanine at concentrations as low as 1–20 mg·kg<sup>-1</sup> BW is very toxic to mice, rats, and rabbits.<sup>41</sup> Hamsters seem to have a sensitivity to SGAs closest to that of humans. For this reason, some investigators suggest that hamsters are the preferred animal model for studying the toxic effects of SGAs.<sup>b</sup>

Solanaceous glycoalkaloids inhibit both BuChE and AChE in *in vitro* and *in vivo* studies (see below). Since symptoms of potato and SGA toxicity resemble the syndrome of massive cholinergic stimulation, inhibition of AChE at cholinergic synapses probably accounts for most of the direct toxic effects of SGAs described above. Pokrovskii first demonstrated anti-cholinesterase activity in potato extracts,<sup>43</sup> and other investigators subsequently confirmed the finding.<sup>44–46</sup> Table II and Figures 3 and 4 summarize the results of *in vitro* studies that have

<sup>a</sup> Friedman M, McDonald GM. Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. *Critical Rev. Plant Sciences* [in press]

<sup>b</sup> Friedman and McDonald

TABLE II Summary of In Vitro Studies that Assess Cholinesterase Inhibition by Solanaceous Glycoalkaloids

Compound	Source of ChE	Concentration ( $\mu\text{M}$ ) of SGA	Range of Inhibition	Reference
Potato peel extract	Plasma ChE, human*	1:100 dilution of extract	75–85% (normal) 30–45% (carrier) 15–20% (atypical)	Harris & Whittaker, 1959 <sup>46</sup>
$\alpha$ -solanine	Serum ChE, human*	2.88	84–87% (normal) 61–71% (carrier) 15–27% (atypical)	Harris & Whittaker, 1962 <sup>45</sup>
solanidine		3.14	78–82% (normal) 51–55% (carrier) 0–2% (atypical)	
solanidine	BuChE, human recombinant*	100	65% (normal) 14% (GS) 2% (GP)	Neville <i>et al.</i> , 1990 <sup>55</sup>
$\alpha$ -solanine	Serum ChE, rabbit Erythrocyte ChE, rabbit	20 mg·kg <sup>-1</sup> i.p. injection	11.8–55.2 % 0–38.4%	Patil <i>et al.</i> , 1972 <sup>47</sup>
$\alpha$ -solanine	Eel ChE	38	26.3%	Bushway <i>et al.</i> , 1987 <sup>69</sup>
solanidine		84	15.4%	
$\alpha$ -chaconine		39	26.8%	
7 other SGAs		31–82	4.2–23.3%	

GP refers to a mutant with aspartate-70 changed to glycine and serine-425 changed to proline. GS refers to a mutant with only aspartate-70 changed to glycine (the “atypical allele”).

\* Inhibition of plasma cholinesterase (BuChE) was determined *in vitro* on plasma from individuals who were presumed homozygous (normal phenotype), heterozygote (carrier), or homozygous abnormal (atypical). BuChE allele composition was based on dibucaine numbers and other assays.<sup>8</sup>

quantified inhibition of BuChE and AChE by SGAs. Clearly, the reported levels of inhibition vary considerably. However, SGAs, in sufficiently high concentrations, inhibit both BuChE and AChE *in vitro* as effectively as neostigmine.

In animals given SGAs, AChE inhibition has not been established definitively. Rabbits given 20 mg·kg<sup>-1</sup> BW solanine intraperitoneally died within 24 hr. Plasma BuChE and erythrocyte AChE inhibition ranged from 55.5% to 88.2% of control, with some animals showing inhibition up to four hours after dose.<sup>47</sup> Mice fed 1000–2000 mg·kg<sup>-1</sup> BW SGAs from *Solanum dimidiatum* (a wild plant known as potato weed which appears to cause a neurological condition known as “crazy cow syndrome” in grazing cattle) experienced a statistically significant (30%) inhibition of AChE.<sup>a</sup>

In humans, SGAs are sequestered in the body for long periods and may have long-term effects on BuChE and AChE activity. In one study, <10% of orally administered <sup>3</sup>H-labeled solanidine was excreted in 24 hr.<sup>48</sup> The advent of sensitive radioimmunoassay (RIA)<sup>49</sup> and high-performance liquid chromatography (HPLC)<sup>35</sup> techniques for quantifying SGA concentrations in human blood serum have provided additional support for accumulation of detectable levels of SGA from a normal diet. Radioimmunoassay methods have detected total potato SGA blood serum concentrations of

3.2–125 nM and 2.5–92.5 nM in healthy subjects from the United Kingdom and Sweden, respectively.<sup>50,51</sup> In another study, subjects who had eaten a meal of potatoes tailored to provide a load of 1 mg SGA·kg<sup>-1</sup> BW, exhibited serum levels of  $\alpha$ -solanine from 4.5–12.9 nM and of  $\alpha$ -chaconine from 7.3–25.1 nM, as assessed by HPLC. The alkaloid levels peaked at four to six hours, and then decreased with a mean  $t_{1/2}$  of 11 and 19 hr, respectively.<sup>35</sup> Dosing may also be important with SGAs. Larger single doses are more toxic than a series of smaller doses in animals, even when the total amount delivered by smaller dosing is much greater.<sup>52</sup> Oral absorption of SGAs increases with the size of individual doses.<sup>41,42</sup> The data above suggest that SGAs may be stored in the body for extended periods. Thus, chronic SGA consumption may result in a considerable SGA pool.

The key question for clinicians is whether normal dietary consumption of solanaceous plants can result in concentrations of SGA that produce clinically important effects. Although accurate measurement of SGAs is difficult, and different methods often provide conflicting results,<sup>b</sup> the weight of evidence suggests plasma SGA levels associated with normal animal dietary consumption may be lower than those required to inhibit AChE and BuChE *in vitro*. The pharmacokinetic studies above demonstrate submicromolar concentrations of SGAs in human plasma. However, only two *in vitro* studies cited

<sup>a</sup> Hueske KL. The neurological effects of *Solanum dimidiatum* in mice. PhD, Dissertation. Texas A&M University. 1991.

<sup>b</sup> Friedman and McDonald

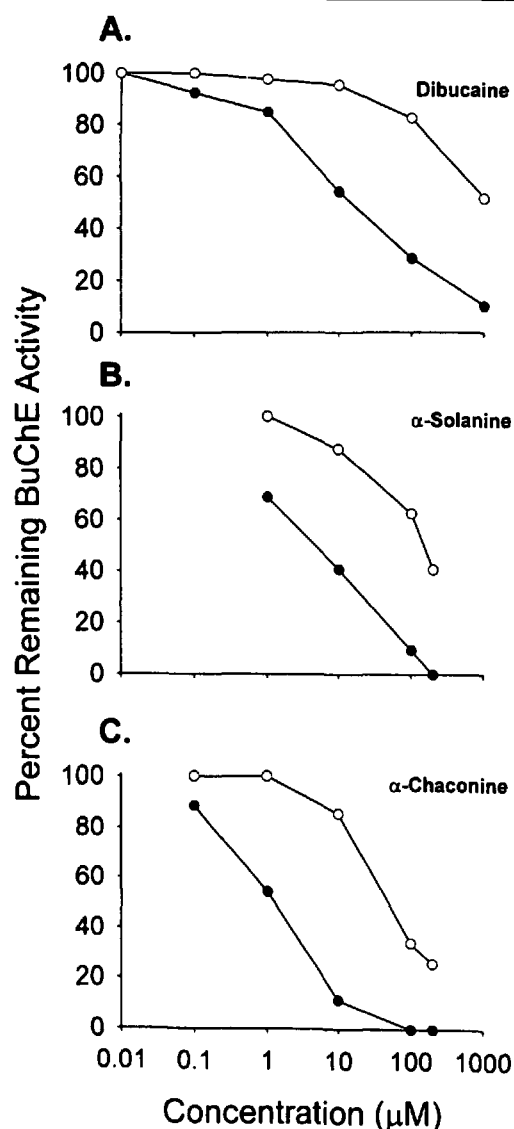


FIGURE 3 Solanaceous glycoalkaloids and dibucaine inhibit butyrylcholinesterase. The atypical butyrylcholinesterase enzyme is much less sensitive to this inhibition.

Semi-log plots showing the effects of the ester anaesthetic dibucaine (A) and the solanaceous glycoalkaloids  $\alpha$ -solanine (B) and  $\alpha$ -chaconine (C) on recombinant butyrylcholinesterases. Butyrylcholinesterase activity is assessed by ability to hydrolyze butyrylthiocholine. The effects of the inhibitors were assayed on normal butyrylcholinesterase (closed circles) and the naturally occurring "atypical" butyrylcholinesterase which contains an aspartate-70 to glycine mutation (open circles). Note that at all concentrations studied for the three compounds, there is far less inhibition of the atypical enzyme than the normal enzyme. The top panel illustrates the pharmacological basis for the dibucaine number assay (see introduction). The insensitivity of the atypical butyrylcholinesterase to the natural inhibitors  $\alpha$ -solanine and  $\alpha$ -chaconine may indicate that the atypical butyrylcholinesterase allele arose as an adaptive advantage in regions where solanaceous plants contributed to diet (Reprinted in adapted form from Neville LF, Gnatt A, Loewenstein Y, Seidman S, Ehrlich G, Soreq H: Intramolecular relationships in cholinesterases revealed by oocyte expression of site-directed and natural variants of human BCHE. *EMBO J* 1992; 11: 1641-9 by kind permission of Oxford University Press.)

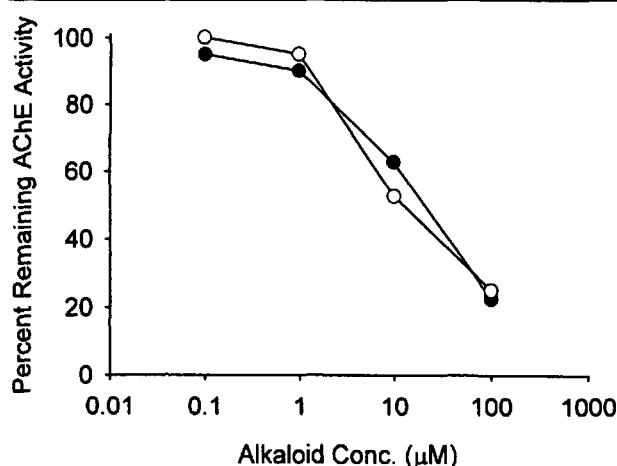


FIGURE 4 Solanaceous glycoalkaloids inhibit acetylcholinesterase. Semi-log plot showing the effect of the solanaceous glycoalkaloids  $\alpha$ -solanine (closed circles) and  $\alpha$ -chaconine (open circles) on inhibition of bovine acetylcholinesterase at pH 7. The ordinate indicates the percent of control acetylcholinesterase activity remaining (in this case ability to hydrolyze the substrate acetylthiocholine) of samples incubated with a given concentration of  $\alpha$ -solanine and  $\alpha$ -chaconine. (Reprinted in adapted form from Roddick JG: The acetylcholinesterase-inhibitory activity of steroidal glycoalkaloids and their aglycones. *Phytochemistry* 1989; 28: 2631-4 with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington OX5 1GB, UK.)

in Table II tested the effects of submicromolar SGA concentrations. In one of these,<sup>53</sup> 100 nM  $\alpha$ -solanine and  $\alpha$ -chaconine failed to inhibit bovine AChE; in the other, however, 100 nM  $\alpha$ -chaconine inhibited human recombinant BuChE by approximately 15%.<sup>22</sup> Thus, considerable inhibition of BuChE by nanomolar concentrations of SGAs may occur in humans.

Even though  $\alpha$ -solanine and  $\alpha$ -chaconine are the major SGAs in potatoes and related plants, other alkaloids exist in the same food products. How the SGAs interact with each other is unknown. Some experiments have described synergistic effects in the toxic actions of SGAs.<sup>52</sup> Although no synergy was observed in one study assessing inhibition of AChE *in vitro*,<sup>53</sup> the complex mixture of SGAs found in potatoes and other plants suggests that synergistic interaction is possible. The potential inhibition of cholinesterase activity by extracts of potatoes (Table II) may indicate synergistic effects.

The effects of SGAs on BuChE may be a rationale for the persistence of certain atypical BuChE alleles for thousands of years.<sup>54</sup> The sera of individuals who are dibucaine "resistant" (i.e., they are homozygous for the "atypical" BuChE allele) show markedly lower degrees

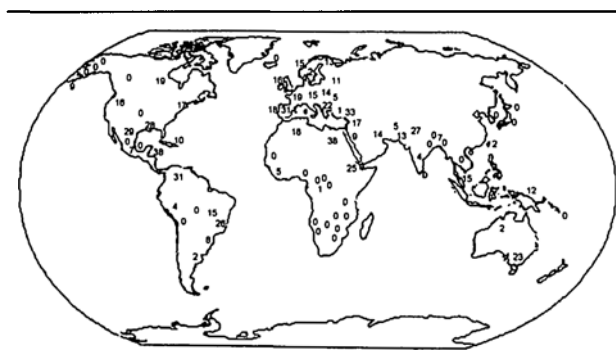


FIGURE 5 World map showing the frequency of the allele for the typical butyrylcholinesterase in different regions.

The numbers indicate number of atypical genes per thousand. Note the especially high frequency of the atypical allele in South and Central America, Europe, and some areas of the Middle East. Many of the areas with high atypical allele frequency are areas where foods containing high levels of solanaceous glycoalkaloids have been consumed for thousands of years. (Reprinted in adapted form from Whittaker M: Monographs in human genetics, Cholinesterase. Beckman L (Ed). Basel, Karger, 1986, pp. 47–50 with kind permission from S. Karger AG, P.O. Box CH-4009, Basel, Switzerland)

of cholinesterase inhibition after *in vitro* application of approximately 3  $\mu\text{M}$  solanine and solanidine.<sup>45</sup> Recombinant atypical BuChE is also resistant to SGA inhibition (Figure 3).<sup>55</sup> Variant BuChE alleles may confer a selective evolutionary advantage against exposure to natural glycoalkaloids.<sup>22,55</sup> An association between cultivation of the *Solanum* eggplant (*S. melongema*) and a high frequency of variant BuChE alleles in certain Middle Eastern ethnic groups has been proposed as one possible evolutionary advantage for BuChE genetic variation (Figure 5).<sup>54</sup> In addition, many ancestral species of the modern commercial potato have extremely high SGA levels (some greater than 1000  $\text{mg}\cdot\text{kg}^{-1}$ ), and among them, three wild species are known to have been consumed throughout history.<sup>56</sup> The potato appears to have been first cultivated in the Andes and was transported to Europe beginning in the 16th century.<sup>57</sup> Atypical BuChE alleles occur with high frequency in the Americas, Europe, and some middle East regions, with very low frequency in Africa and Asia (Figure 5).<sup>20</sup> This fact is consistent with the hypothesis that SGAs may be a driving force for the persistence of the mutant BuChE allele.

The above hypothesis depends upon vital physiological function for BuChE. Although crucial physiological roles of BuChE are not clear, recent research suggests an important role in cellular growth and development. For example, the BuChE gene is intensively expressed in bone marrow stem cells<sup>58</sup> and fetal tissue.<sup>59</sup> In addition,

the gene is amplified during haematopoiesis<sup>60</sup> and in ovarian carcinomas.<sup>61</sup> Also, its expression may be induced by exposure to certain toxins. For example, prolonged exposure of a family to organophosphate insecticides was shown to result in an abnormally high number of copies of the BuChE gene in germ cells (i.e., sperm or eggs).<sup>62</sup> In addition to the most common mutation of BuChE, which occurs at approximately 1:3500 in the population, there are mutations in BuChE that occur naturally which lack enzymatic activity entirely. Individuals who are homozygous for such mutant alleles, while rare (0.001% of homozygotes),<sup>20</sup> appear to be phenotypically normal.<sup>7</sup> Although this argues against a critical role for BuChE, compensatory mechanisms may operate in these rare individuals who completely lack catalytically active BuChE. Also, the effect of acute exposure to natural BuChE inhibitors at crucial periods of development may be harmful due to the insufficient activation of protective mechanisms.<sup>21</sup>

## Conclusion

Experimental evidence reveals that SGAs are able to inhibit AChE and BuChE both *in vivo* and *in vitro*. The documented cases of direct SGA toxicity resemble massive cholinergic stimulation as a result of AChE inhibition. The inhibition of BuChE may influence its reported role in growth and development. The origins of plants containing high SGA levels parallels the world distribution of the atypical BuChE allele, which is much less sensitive to SGA inhibition. Consequently, it is reasonable to hypothesize that resistance to SGA inhibition has been an evolutionary driving force for the high frequency of atypical alleles in some areas of the world.

It is not clear whether the SGAs may influence the pharmacokinetic behaviour of drugs dependent on BuChE for catabolism. There has recently been an increased appreciation that dietary intake can influence pharmacokinetics and drug disposition and metabolism. For instance, grapefruit juice markedly inhibits first-pass metabolism of many drugs, including felodipine and nifedipine.<sup>63</sup> The clinical syndromes of potato toxicity under a variety of conditions in humans and animals strongly suggest that SGAs from potatoes inhibit AChE and BuChE. *In vitro* data indicate that SGAs inhibit BuChE at least as potently as AChE. Thus it is reasonable to hypothesize that SGAs may alter the pharmacokinetics of drugs metabolised by BuChE, although conclusive experimental evidence on this is currently lacking.

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