Effect of Potato Glycoalkaloids α-Chaconine and α-Solanine on Sodium Active Transport in Frog Skin

James T. Blankemeyer,*,† Randall Atherton,† and Mendel Friedman‡

Department of Zoology, Oklahoma State University, Stillwater, Oklahoma 74078, and Food Safety and Health Research Unit, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

To test the effect of glycoalkaloids on sodium ion active transport, frog skin was exposed to the potato glycoalkaloids α-chaconine and α-solanine in a glass Ussing chamber. It was found that the short-circuit current (ISC), the measure of transepithelial active transport of sodium, decreased up to 30% at an α-chaconine concentration of 10 mg/L. α-Solanine had a similar but smaller effect, decreasing short-circuit current by 16%. The data suggest that (a) frog skin is a useful experimental model to evaluate effects of glycoalkaloids at the cellular level and (b) the mechanism of action of the two glycoalkaloids is to modify the active transport of sodium. The possible significance of these findings to food safety is discussed.

Keywords: *α-Chaconine; food safety; frog skin; glycoalkaloids; membrane potential; potatoes; short-circuit current; sodium ion transport; α-solanine*

INTRODUCTION

The Solanaceae and Lycopersicon families contain many plants important to man, including potatoes, eggplants, and tomatoes. Members of these families produce potentially toxic compounds during growth and after harvest, the most important of which are the structurally related steroidal glycoalkaloids (Aubert et al., 1989; Bushway and Ponnampalam, 1981; Friedman and Dao, 1992; Friedman et al., 1994).

Glycoalkaloids appear to be more toxic to man than to other animals (Harvey et al., 1985; Hellenaes et al., 1992; Morris and Lee, 1984; Renwick et al., 1984). Suggested modes and mechanisms of action of the glycoalkaloids include disruption of cell membranes and other adverse effects (Caldwell et al., 1991; Friedman, 1992; Hornfeldt, 1994; Keeler et al., 1991; Morris and Lee, 1984; Renwick et al., 1984; Roddick et al., 1992; Sharma et al., 1978).

In a previous study, Blankemeyer et al. (1992) found that the glycoalkaloids depolarized the membrane potential of frog embryos. Since the membrane potential of cells is the direct consequence of the active transport of ions, we hypothesized that the glycoalkaloids should also alter epithelial active transport of ions. We used frog skin as a model, since the frog skin actively transports sodium and mimics active transport in mammalian epithelia.

Isolated abdominal skin from frogs has been used as a model system for studying transepithelial active transport of sodium. *In vitro* experiments, mounting the frog skin on an Ussing chamber, show that the frog skin actively transports sodium from the pond side of the skin to the serosal side. The interstitial short-circuit current (ISC) correlates well with the measured net sodium flux (Ussing and Zerahn, 1950). Agents that inhibit sodium active transport in mammals act similarly on the frog skin. For example, ouabain and amiloride inhibit sodium active transport, whereas arginine vasopressin and aldosterone augment sodium active transport.

Recently, *in vitro* frog skin has been used to study the effect of organic toxicants on transepithelial active transport of sodium. Blankemeyer and Hefler (1990) showed that naphthalene increased sodium active transport of the frog skin and that a concentration of 4.4 mg/L induced a 50% increase in membrane current. This so-called EC_{50} value is defined as the concentration of a compound that induces a 50% change in the short-circuit membrane current. They also showed that the probable site of action of naphthalene was at the pond-side membrane of the frog skin. In a related study, Blankemeyer and Bowerman (1992) found that cyclic organic compounds decreased sodium active transport in a concentration-dependent manner.

In this study we used the two potato glycoalkaloids α-chaconine and α-solanine to test the hypothesis that the glycoalkaloids have a direct effect on active transport. Figure 1 shows the structures of alkaloids evaluated.

MATERIALS AND METHODS

α-Chaconine and α-solanine (purity > 95%) were obtained from Sigma Chemical Co. (St. Louis, MO). The two glycoalkaloids were also isolated from fresh sprouts of Russet potatoes purchased locally, as described in Friedman et al. (1993). They were purified by column chromatography to 99% or better, as determined by HPLC (Friedman and Levin, 1992). The commercial glycoalkaloids were used in exploratory studies and the prepared ones in the studies described below.

Solutions containing glycoalkaloids were freshly prepared in frog Ringer's solution (110 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, and 2.5 mM Tris buffer) adjusted to pH 5 with dilute HCl to solubilize the glycoalkaloids and then titrated to pH 8 with NaOH. The following experiments were performed to ensure adequate solubility of the glycoalkaloids in this procedure.

An excess of α-chaconine was added to 200 mL of the pH 5 frog Ringer's solution to form a cloudy suspension. This was allowed to stand for 48 h with occasional shaking. The solution was filtered through a Schleicher & Schuell 0.45 μm nylon filter. Aliquots (50 mL) were taken, and the initial pH of this saturated solution was determined and adjusted to higher

* Author to whom correspondence should be addressed.
† Oklahoma State University.
‡ U.S. Department of Agriculture.

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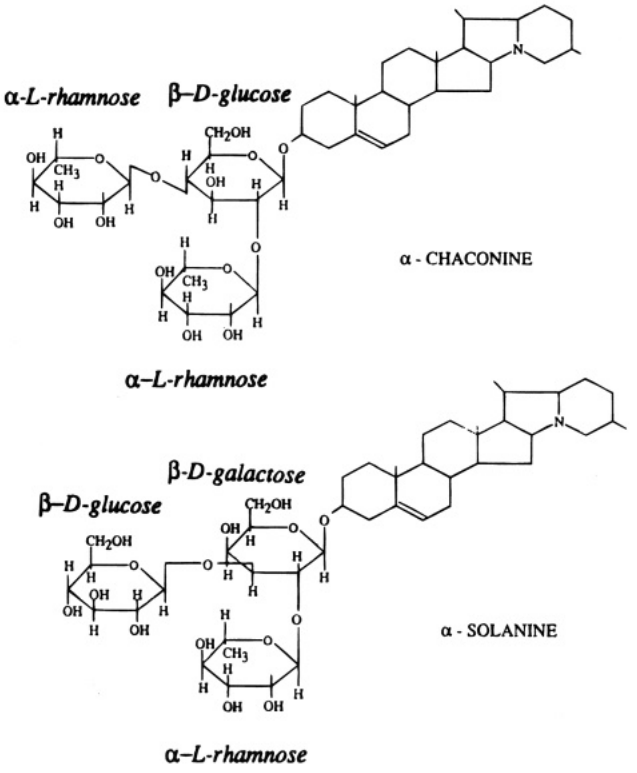

Figure 1. Structures of compounds evaluated in this study.

values with NaOH. These solutions were allowed to stand for 24 h with occasional shaking and then filtered as above. The α-chaconine content of the solutions was determined by HPLC (Friedman and Levin, 1992). An identical experiment was carried out with α-solanine. The following summarized results show that the solubilities of the two glycoalkaloids in pH 8 frog Ringer's solution are more than adequate for the levels used in this study: α-chaconine, pH 7.0 (initial) 142 mg/L, pH 7.5 125 mg/L, pH 8.0 53 mg/L; α-solanine, pH 6.8 (initial) 100 mg/L, pH 7.5 107 mg/L, pH 8.0 54 mg/L.

Frogs, *Rana pipiens*, were purchased from William Lemberger (Oshkosh, WI) and kept in tap water (pH 7, 20 °C, 146 mg/L hardness) until use. Generally, the frogs were used within 2 weeks of arrival. Frogs were anesthetized by injection of 10% urethane into the dorsal lymph sac and then euthanized by decapitation. All animal procedures were performed in strict concordance with the American Veterinary Medical Association accepted methods for euthanasia (Smith et al., 1986). The frog's abdominal skin was excised and placed in freshly prepared frog Ringer's solution adjusted to pH 8. The mucosal bath solution was Ringer's in which nitrate replaced the chloride.

A glass, modified Ussing chamber was used to mount the frog skin (Figure 2). The pond-side and serosal-side Ringer's solution, bathing each side of the frog skin respectively, was stirred and aerated by gas-lift pumps (aerate and stir). To avoid edge damage, the frog skin was tied onto the chamber lip with surgical thread.

In Figure 2, the operational amplifiers are depicted in the usual sense: inputs enter the base of the triangle, are processed electrically, and exit the vertex of the triangle. The potential difference (PD) was measured using 3% agar−frog Ringer's bridges. Each bridge electrical potential was conditioned by a voltage follower operational amplifier. Both conditioned voltage follower outputs were algebraically subtracted by a difference amplifier that produced the electrical potential difference (PD) of the frog skin. The Servo amplifier compared the PD to an externally set control voltage, 0 V for the short-circuit condition. If the PD differed from the control voltage, an error signal was output from the Servo amplifier to the voltage-to-current converter. The current, named the interstitial short-circuit current (ISC), was applied to the serosal Ag−AgCl electrode. The current was converted to a

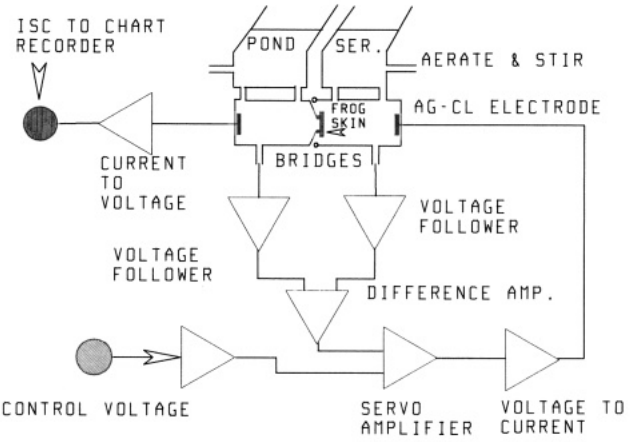

Figure 2. Schematic diagram of Ussing chamber and automatic voltage clamp used in this study. Frog skin is mounted in chamber bathed by pond- and serosal-side Ringer's solutions. Triangles represent operational amplifier blocks. Input to the automatic voltage clamp is a control voltage, usually 0 V. The output is a current, the short-circuit current (ISC), recorded on a chart recorder.

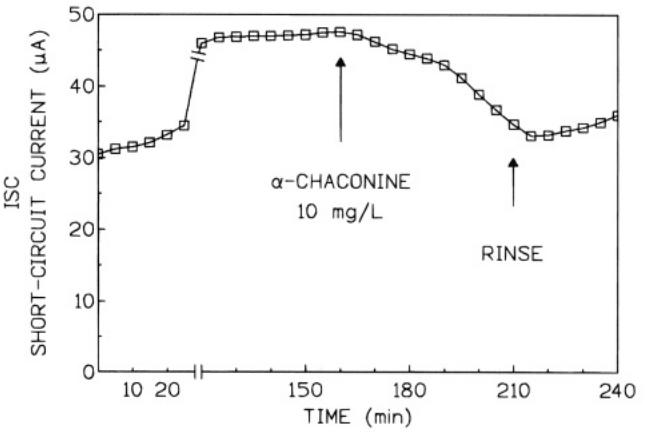

Figure 3. Time course representation of the effect of 10 mg/L α-chaconine on short-circuit current (ISC) of frog skin.

voltage by a current-to-voltage converter and output to a chart recorder. The ISC is the electron flow required to maintain the electrical potential difference at 0 V and is identical to the net ion flux through the frog skin. It was recorded from a digital panel meter on the automatic voltage clamp and on a chart recorder (Linear Instruments, Irvine, CA).

The effect of a test compound on the ISC was determined by using the 15 min period prior to glycoalkaloid treatment as a control period and then calculating the change in ISC during the 45 min glycoalkaloid exposure period as a percent of the control ISC. Data were collected from at least four frog skins for each glycoalkaloid concentration.

Statistical analysis was performed using Instat (Graphpad, Los Angeles, CA). Graphpad (Graphpad) was used to generate EC_{50} values from concentration−response data.

RESULTS

Figure 3 depicts results obtained with chamber-mounted frog skin during exposure to α-chaconine. The figure presents the current plotted versus time. At time zero, the frog skin was mounted on the chamber. Then the frog skin was "equilibrated" for 160 min. After 160 min, the mucosal (pond-side) nitrate−Ringer's solution was exchanged for a nitrate−Ringer's solution containing 10 mg/L α-chaconine. After the change to α-chaconine, the current decreased from a pretreatment value of 47 to 32 μA after 45 min, a 32% decrease from the control ISC. At 210 min, the α-chaconine bathing

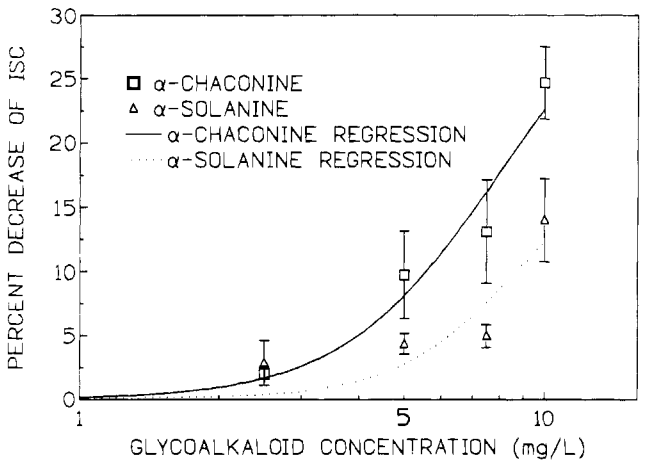

Figure 4. Effect of the potato glycoalkaloids, α-chaconine and α-solanine, on short-circuit current (ISC) of frog skin.

solution was replaced with control Ringer's solution. Once the α-chaconine solution was rinsed, the ISC began a slight recovery, returning to 73% of the control value. In no experiment did the ISC recover completely to control levels. Whether the residual activity is due to binding of the glycoalkaloid to cells is not known. This was not apparent from visual observation of the skin.

In a set of separate experiments, ISC increased slightly (mean of 12%, $n = 6$ frogs) when α-chaconine was added to the serosal bathing solution. The slight increase occurred within 5 min after change of the bathing solution from control Ringer's to α-chaconine chloride Ringer's. The increase was not significantly different from the control ISC using a two-tailed t-test ($p = 0.19$).

Figure 4 summarizes several time course experiments in which successively higher concentrations of α-chaconine and α-solanine were applied to frog skin. Concentrations up to 10 mg/L were plotted for both α-chaconine and α-solanine to compare trends.

The individual response of the frog skins to α-chaconine was highly variable, with percent decrease in ISC ranging from 20% to 32% at 10 mg/L α-chaconine. However, the concentration–response pattern was that increased concentration of α-chaconine produced a greater decline in ISC, indicating a decrease in net transport of sodium. Using nonlinear statistical regression and a sigmoid model, the concentration causing a 50% decrease (EC_{50}) was found to be 6 mg/L with a calculated 95% confidence interval of 4.7–7.6 mg/L. The solid line depicts the result of the nonlinear regression on the α-chaconine data.

Using the same protocol, the effect of increasing concentrations of α-solanine on ISC was also determined. Similarly, α-solanine was observed to reduce the ISC, but with a smaller percentage decrease. Using nonlinear statistical regression and a sigmoidal model, the determined EC_{50} was 7.7 mg/L with a calculated 95% confidence interval of 4.5–13.2 mg/L. Note that the calculated confidence interval extends to values higher than actually determined experimentally. The dashed line in Figure 4 depicts the nonlinear regression of the α-solanine data.

DISCUSSION

Previous studies have indicated that glycoalkaloids act on the membranes of the exposed cells or subcellular structures (Blankemeyer et al., 1992; Roddick et al., 1992). However, data in Figure 3 support a direct effect on active transport, decreasing the active transport of sodium in the frog skin model. The specificity for transport is suggested by the asymmetric response to the glycoalkaloids, which affect the pond side but not the serosal side. Figure 4 suggests that this process is concentration-dependent, with a concentration near 9–10 mg/L needed to induce a 50% change. Figure 4 illustrates that α-solanine has a similar effect on active transport of sodium but with a smaller decrease in current and thus a higher EC_{50} value.

This study suggests that the glycoalkaloids alter some specific element in the transport pathway. In frog skin, it is probably the entry channel for sodium in the pond-side membrane of the outermost cells, since the glycoalkaloids have no effect on the serosal-side membrane.

The results of this study can be interpreted as indicating an indirect membrane effect but more likely indicate a specific effect on active transport of sodium, since most (*ca.* 95–99%) of the short-circuit current is accounted for by Na^+ active transport. Although the EC_{50} concentrations are higher for the glycoalkaloids using frog skin than are the corresponding concentrations for teratogenicity and membrane disruption using *Xenopus* embryos, the effect on active transport in the frog skin suggests that a decrease in active transport probably causes the increasing membrane potential in the cell-health water-quality frog embryo assay (CHAWQ) (Blankemeyer et al., 1993) and embryo death in the frog embryo teratogenicity 96 h assay (FETAX) (Friedman et al., 1991, 1992; Rayburn et al., 1994). The glycoalkaloid-induced change in membrane potential of the early embryos is most easily explained by a slowing of active transport of some ion, probably sodium. The decrease in ion transport then results in a changing membrane potential as either a direct or indirect effect of the slowing of active transport.

This and earlier studies revealed that potato glycoalkaloids and other compounds disrupt sodium ion channels in frog skin and frog embryo cell membranes (Blankemeyer et al., 1992, 1993). Such disruption could cause the reported embryotoxic and teratogenic effects. This conclusion is reinforced by the recent report of Kupitz and Atlas (1993) that extracellular application of adenosine triphosphate (ATP) to defolliculated frog (*Xenopus laevis*) embryos activated a saturating inward current which was carried predominantly by sodium ions. The current was inhibited by amiloride, guanosine triphosphate (GTP), and related compounds. These compounds also inhibited *in vitro* fertilization of mature frog eggs and sperm. The authors conclude that an ATP-induced increase in sodium permeability mediates the fertilization process; *i.e.*, ATP receptors in mature eggs bind ATP from sperm and transmit a signal for egg activation leading to fertilization (Lipinsky et al., 1993).

Our studies imply that the inhibiting action described by Kupitz and Atlas (1993) for ATP receptor site antagonists may be mechanistically similar to the observed action of the potato glycoalkaloids on sodium-ion-generated currents in frog embryo and frog skin cells.

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