Effect of Potato Glycoalkaloids, α-Chaconine and α-Solanine, on Membrane Potential of Frog Embryos

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To demonstrate whether potato glycoalkaloids can alter the integrity of membranes of frog embryo, albino frog embryos were incubated with α-chaconine and α-solanine. Di-4-ANEPPS, an electrochromic fluorescent dye, was added to measure embryonic membrane potential. α-Chaconine increased the Di-4-ANEPPS fluorescence up to 1600% of control, α-solanine increased the fluorescence up to 400%, and solanidine had no effect. Increases in fluorescence, when plotted in a concentration–response format, produced EC_{50} values near published values for FETAX (frog embryo teratogenicity assay—*Xenopus*). Possible mechanisms and the significance of the fluorescence results to food safety are discussed.

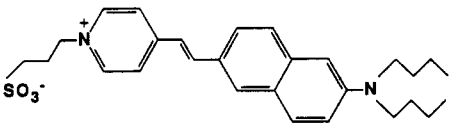
INTRODUCTION

Solanaceous plants, including such agronomically important crops as potatoes, synthesize secondary plant metabolites including glycoalkaloids. In commercial potato cultivars, the primary glycoalkaloids are α-chaconine and α-solanine. These compounds can have toxic effects in animals and humans (Jelinek et al., 1976; Munn et al., 1975). Thus, potatoes may represent a potential source of undesirable compounds, especially if improperly stored or processed (Morris and Lee, 1984).

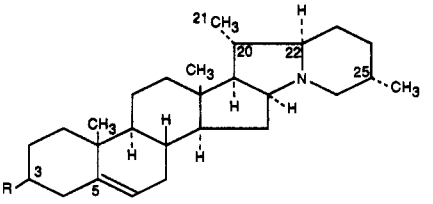
As part of a program designed to lower the potential toxicity of glycoalkaloids in potatoes, the toxicity of several potato alkaloids was tested with the frog embryo teratogenesis assay—*Xenopus* (FETAX). Using FETAX, α-chaconine was about 3 times more toxic than α-solanine (Friedman et al., 1991).

One proposed mechanism of action for the toxic action of the glycoalkaloids is disruption of membranes. Roddick and Rijenberg (1987) and Roddick et al. (1988) reported that potato and tomato glycoalkaloids disrupted liposome membranes. They also reported that mixtures of α-chaconine and α-solanine acted synergistically in lysing rabbit erythrocytes, red beet cells, and *Penicillum notatum* protoplasts. They speculated that the nature of the carbohydrate side chain affected the cell disruption process and that cell disruption appears not to be directly related to binding of the glycoalkaloids to membrane steroids.

The membrane potential of a cell is affected by the ionic concentrations inside and outside the cell and by the permeabilities of the carriers and ion pumps located in or near the cell membrane. If any are disrupted, the membrane potential across the cell will change. One way to measure the membrane potential of many cells is to use a fluorescent dye, usually termed an electrochromic dye, to measure the membrane potential of cells. Di-4-ANEPPS is a dialkylaminostyrl pyridiniumsulfonate dye with positive and negative charges (Figure 1) that is apparently interspersed in the membrane leaflet and that changes fluorescence directly in response to changes in membrane potential (Fluher et al., 1985).



di-4-ANEPPS



22αH, 25βH-SOLANID-5-EN-3β-R

Solanidine	R = OH
α-Solanine	R = O-galactose — glucose | rhamnose
α-Chaconine	R = O-glucose — rhamnose | rhamnose

Figure 1. Structures of fluorescent dye and potato glycoalkaloids used in this study.

Because alteration in a membrane pump or ion channels could explain alkaloid toxicity at the cellular level (Blankemeyer and Hefler, 1990), we examined the effect of glycoalkaloids on the membrane potential of embryos of the South African clawed frog, *Xenopus laevis*. We hope to establish whether the frog embryo can serve as a model and an index for mammalian toxicity studies.

The two glycoalkaloids evaluated in this study, α-chaconine and α-solanine, are found in approximately equal concentrations in the potato plant, *Solanum tuberosum*, and in several other *Solanum* and *Veratrum* species. The sugar side chain of α-chaconine has a branched *bis*(α-L-rhanmopyranosyl-β-D-glucopyranose) trisaccharide side chain. The sugar side chain of α-solanine is a branched

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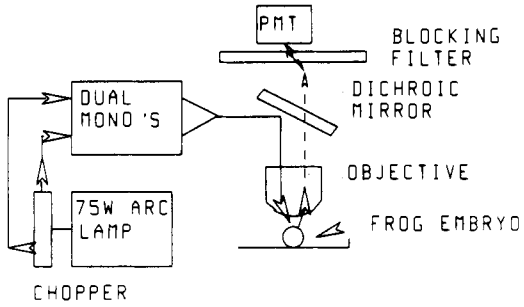

Figure 2. Schematic of the apparatus used for this experiment. Light from the 75-W xenon arc lamp was focused on the entrance slit of a monochromator set at 480 nm. Output from the monochromator was directed to a dichroic mirror that refracted wavelengths less than 500 nm and blocked excitation wavelengths greater than 500 nm. The refracted beam was directed onto an albino *Xenopus* embryo placed on an immersion slide in FETAX solution. The embryo, previously loaded with the styrl dye, Di-4-ANEPPS, fluoresced in the red around 600 nm. The fluorescent emission passed back through the objective and the dichroic mirror and through a blocking filter. The blocking filter, a high-pass filter, passed any wavelength greater than 580 nm. The fluorescent emission was captured by a photomultiplier tube (PMT) operating at 900 V in the photon-counting mode. The counts from the fluorescence were divided by 10, captured by a counter (Scientific Solutions) located in a personal computer, corrected for background, and stored for later analysis.

α-L-rhanmopyranosyl-β-D-glycopyranosyl-β-galactopyranose (Roddick et al., 1988). For comparison we also evaluated their common aglycon, solanidine, which lacks the carbohydrate side chain (Figure 1). Our results will show that the trisaccharide side chains have a strong influence on the Di-4-ANEPPS fluorescence and thus the membrane potential of frog embryos.

MATERIALS AND METHODS

Albino *X. laevis* frogs were purchased from Xenopus-I (Ann Arbor, MI). Breeding pairs were conditioned for 1 month to 6 weeks prior to usage. Four hours prior to mating, the frogs were injected with human chorionic gonadotropin (Sigma, St. Louis, MO). Following successful amplexus, eggs were collected, dejellied with 2% (w/v) cysteine adjusted to pH 8.1, and separated into viable and nonviable groups. Mid-to-late blastula to neurula stage embryos were selected under a dissecting microscope. Groups of 20 viable embryos were collected in covered plastic dishes. Embryos were maintained in FETAX solution which contained 10.8 mM NaCl, 1.2 mM $NaHCO_3$, 0.58 mM $MgSO_4$, 0.44 mM $CaSO_4$, 0.4 mM KCl, and 0.14 mM $CaCl_2$ and was at pH 8. α-Chaconine (95% purity) and α-solanine (95% purity) were purchased from Sigma and dissolved into FETAX solution as described previously (Friedman et al., 1991). Solanidine (99% purity) was purchased from Roth (Basel, Switzerland) and was dissolved in FETAX solution by first dissolving solanidine in 10 mL of DMSO and then dissolving the DMSO with solanidine in FETAX. The purity of the alkaloids was confirmed by high-performance liquid chromatography and thin-layer chromatography. Di-4-ANEPPS (99% purity) was purchased from Molecular Probes (Eugene, OR).

Each concentration of glycoalkaloid was tested with at least three groups of embryos. Each set of experimental embryos was compared against a control triplet of 20 embryos, cocultured with the experimental embryos. The test chemical, α-chaconine, α-solanine, or solanidine, was added to the Petri dish containing the embryos at the same time as the styryl dye Di-4-ANEPPS. The final concentration of Di-4-ANEPPS was nominally 10^{-6} M and was identically concentrated in control and experimental Petri dishes. After 30 min, the embryos were placed on an immersion slide and the fluorescence data collected.

Figure 2 shows the setup for measuring of fluorescence emanating from embryos loaded with Di-4-ANEPPS. A 75-W xenon arc lamp (USHIO) provided the light source. The collimated beam from the xenon lamp passed from a collimating and focusing mirror to a monochromator set to 490 nm. The 490-nm excitation passed through a dichroic mirror and was reflected through a microscope objective onto the embryo. Reflected light and fluorescent emission from the embryo returned through the objective. The reflected 490-nm light was filtered out by the dichroic mirror and a blocking filter (590-nm high pass). The intensity of fluorescent emission was measured by a photomultiplier tube operating at 900 V in photon-counting mode. The photon count was divided by 10 and collected via a module attached to a PC (Photon Technology Inc., Princeton, NJ). The emission data (in counts per second) were collected to disk and fitted by linear regression over the sample period (20 s). The calculated value for the midpoint in the time-based record was used as the emission intensity.

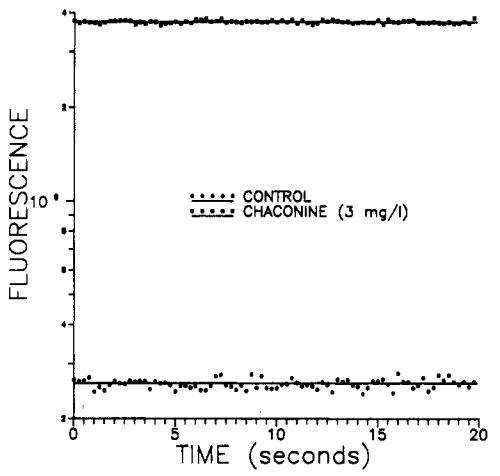

Figure 3. Plot of a time-course experiment comparing the effect of 3 mg/L α-chaconine (upper trace) to the control of no α-chaconine (lower trace) on the membrane potential of *Xenopus* embryos. Not all points are plotted. Both lines are flat over the 20-s sampling period. The fluorescence of the embryo exposed to 3 mg/L α-chaconine is markedly higher. The regression value for the α-chaconine trace was 3 739 120 photon counts/s, whereas the control trace was 259 000 photon counts/s. The ratio of these two traces was 14.4.

Statistical analysis of the data was performed with GraphPad (Los Angeles). EC_{50}'s were determined using the 50% point of the maximum effect of the glycoalkaloid on fluorescence. Curves were fitted by nonlinear regression techniques using iteration to find the best fit employing a sigmoidal model.

RESULTS

The structures of the compounds used in this study are illustrated in Figure 1. A typical trace of the fluorescent emission is displayed in Figure 3. As the figure shows, the Di-4-ANEPPS fluorescence (for the experimental and control) was nearly constant over the 20-s period. The ordinate is counts per second of the photons detected by the PMT and photon counter. These traces were retained on disk. Subsequent linear regression analysis produced values of 259 000 counts/s for the control trace and 3 739 120 counts/s for the 3 mg/L α-chaconine trace. The regression was obtained by fitting a linear equation to the data and then using the calculated counts at the midpoint of the data. For each concentration, the fluorescence was corrected for control by dividing the experimental counts by the control embryo counts. In Figure 3 the ratio was 14.4.

Figure 4 shows the results of an experiment wherein groups of 20 *Xenopus* embryos were used to obtain fluorescence ratios of experimental to control at various concentrations of α-chaconine. The embryos were exposed to α-chaconine for 30 min. The data are plotted as percent of control fluorescence on the ordinate (with error bars denoting standard error) and log α-chaconine concentra-

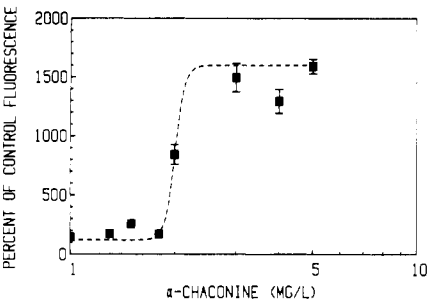

Figure 4. Plot of all α-chaconine data showing the effect of α-chaconine on Di-4-ANEPPS fluorescence in a concentration–response format. The concentration of α-chaconine is on the abscissa, and the fluorescence relative to the control is on the ordinate. All experimental values were corrected for background and normalized by dividing by the fluorescence of the control embryos. Each point (a mean of three) is represented as a central mark with error bars (SEM). The calculated EC_{50} was 2.03 ± 0.005 mg/L.

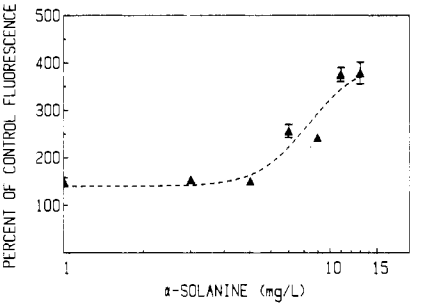

Figure 5. Plot of all α-solanine data showing the effect of α-solanine on Di-4-ANEPPS fluorescence in a concentration–response format. The concentration of α-solanine is on the abscissa, and the percent fluorescence of the control is on the ordinate. All experimental values were corrected for background and normalized by dividing by the fluorescence of the control embryos. Each point is the mean of the data ($n = 3$). Error bars represent standard error of the mean. The calculated EC_{50} was 8.3 ± 0.03 mg/L.

tion on the abscissa. The EC_{50} for fluorescence was 2.03 ± 0.005 mg/L. The maximum change in fluorescence was a 1600% increase over the control embryos. Each data point was significantly different from control fluorescence except the 1.3 mg/L point. Differences were judged to be statistically different at the $p > 0.05$ level. The increase in fluorescence determined for α-chaconine represents a decrease in membrane potential (hypopolarization) of some, most, or all of the cells in the embryo.

In a separate set of experiments, groups of 20 embryos were also incubated with various concentrations of α-solanine and a fixed concentration of Di-4-ANEPPS for 30 min. Figure 5 illustrates the result of those incubations in a concentration–response format. Each data point for each concentration of solanine was collected at least in triplicate as described above for α-chaconine. The error bars on the figure represent the standard error. All data points on the α-solanine figure were significantly different from control ($p > 0.05$). The Di-4-ANEPPS fluorescence increased in the α-solanine-treated embryos as did the fluorescence in the α-chaconine experiments. However, the maximum increase for solanine was 400% of the control fluorescence, much less than the α-chaconine increase. The EC_{50} for α-solanine was 8.3 ± 0.03 mg/L.

Solanidine, the aglycon of α-chaconine and α-solanine, was also tested at the solubility limit (10 mg/L) in FETAX solution. Solanidine had no effect on fluorescence of frog embryos using Di-4-ANEPPS when compared to control embryos in FETAX solution with DMSO.

DISCUSSION

FETAX was developed as an environmental bioindicator for the presence of toxicant in water (Dumont et al., 1982). FETAX has been useful in assessing the developmental, environmental, and genetic changes wrought by toxic substances (Bantle et al., 1990). FETAX uses 96-h endpoints of overall length, survival, and malformation to assess the effect of chemical compounds. A teratogenic index (TI) is calculated by dividing the LC_{50} (survival) concentration by the EC_{50} (malformation) concentration. TI is a measure of the teratogenicity of a chemical relative to its lethal concentration.

These data show that the membrane potential, as measured by the styryl dye Di-4-ANEPPS, changes rapidly and markedly upon administration of α-chaconine, showing an increase of up to 1600%. The EC_{50} of the 30-min α-chaconine exposure (2.03 mg/L) was close to the average EC_{50} of the full-scale 96-h FETAX assay (2.85 mg/L; Friedman et al., 1991). It is probable that the cellular event that we have observed in these young embryos is causal to the effects observed in the 96-h FETAX embryos.

The α-solanine data showed a similar increase in fluorescence when compared to that of control embryos, although the amount of the maximum increase was 400%, one-fourth of the α-chaconine effect. The EC_{50} for α-solanine in the 30-min exposure was 8.3 mg/L, close to the FETAX EC_{50} (Friedman et al., 1991 (9.65 mg/L)). Again, the EC_{50} for the α-solanine assay was close to the EC_{50} for the full-scale FETAX assay.

Since the EC_{50}/LC_{50} derived from the FETAX data was near the EC_{50} of the membrane potential change, we suggest that the effect of the glycoalkaloids on membrane potential (measured by fluorescence) correlates with the eventual malformation and/or death of the embryo. That is, the effect of the potato glycoalkaloids determined in the 96-h FETAX assay is predicted by the effect of the glycoalkaloids on the membrane potential of recently fertilized embryos. We speculate that the membrane potential assay is measuring a fundamental mechanism of action of the glycoalkaloids and that the mechanism of action affecting membrane potential is the same mechanism of action for the teratogenicity/lethality determined in the 96-h FETAX assay.

A striking result of this study is the 4-fold greater fluorescence intensity induced by α-chaconine compared to that induced by α-solanine. These two glycolalkaloids have the same aglycon but differ only in the nature of the carbohydrate attached to the 3-OH position of the aglycon (Figure 1). The three carbohydrate residues associated with α-chaconine are one glucose and two rhamnoses; the three carbohydrate residues associated with α-solanine are galactose, glucose, and rhamnose. In a previous study, Friedman et al. (1992) proposed that the carbohydrate residues influence the relative toxicities of the glycoalkaloids by participating in binding to sugar molecules associated with receptor sites of cell membranes. Supporting this suggestion is our negative result for solanidine, which has no carbohydrate moiety. However, the negative data for solanidine could be due to the marked difference in solubility between the aglycon (almost insoluble in water) and the glycoalkaloids (moderately soluble in water). The present results with the two glycosides and with solanidine are in agreement with the previous suggestion (effect of carbohydrate residues) since otherwise α-chaconine and α-solanine should not differ in toxicity since the steroid moiety is identical in each.

This study does not resolve the nature of the atomic and electronic interactions between the membrane, fluorescent dye, and glycoalkaloid. Di-4-ANEPPS measures the membrane potential of the cells of the developing embryo. Whether the change is directly on a surface receptor on the cells or through a secondary or tertiary messenger is not clear from our results. However, since the membrane potential change is associated with changes in ionic permeability of the membrane or with ionic activities on either side of the membrane, we conclude that (a) α-chaconine and α-solanine change the characteristics of ion channels, either passive, active, or both, in the developing frog embryo; (b) the fluorescence assay is potentially useful to evaluate the relative embryotoxicities of potato alkaloids and possibly other dietary ingredients; (c) the assay makes it possible to develop a better understanding of the molecular events and mechanisms governing the physiology and toxicology of the alkaloids. Such an understanding is needed to guide our efforts to develop safe foods.

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