

Glycoalkaloids selectively permeabilize cholesterol containing biomembranes

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Abstract

The effects of the glycoalkaloids α -solanine, α -chaconine and α -tomatine on different cell types were studied in order to investigate the membrane action of these compounds. Hemolysis of erythrocytes was compared to 6-carboxyfluorescein leakage from both ghosts and erythrocyte lipid vesicles, whereas leakage of enzymes from mitochondria and the apical and baso-lateral side of Caco-2 cells was determined. Furthermore, the effects of glycoalkaloids on the gap-junctional communication between Caco-2 cells was studied. From these experiments, it was found that glycoalkaloids specifically induced membrane disruptive effects of cholesterol containing membranes as was previously reported in model membrane studies. In addition, α -chaconine was found to selectively decrease gap-junctional intercellular communication. Furthermore, the glycoalkaloids were more potent in permeabilizing the outer membrane of mitochondria compared to digitonin at the low concentrations used.

Keywords: Glycoalkaloid; Erythrocyte; Caco-2; Mitochondrion; Cholesterol; Permeabilization; Membrane disruption

1. Introduction

Glycoalkaloids are natural toxins occurring in night-shades like potato and tomato. These compounds are thought to be partially responsible for the natural protection of these plants against plagues and diseases. Toxicological effects of glycoalkaloids against fungi and insects but also higher organisms like humans have been reported [1,2]. The symptoms of human potato glycoalkaloid intoxication ranged from gastro-intestinal disturbances to increased heart-beat, hemolysis and neurotoxic effects [2]. Some glycoalkaloids have also been proposed to be teratogenic [3]. Although the toxicological effects of glycoalka-

loids have been investigated extensively, the mechanism(s) by which they cause the various symptoms have not been elucidated yet.

In the cultivated potato (*Solanum tuberosum* L), mainly two glycoalkaloids α -solanine and α -chaconine, are present, whereas in tomato (*Lycopersicon esculentum*) one glycoalkaloid, α -tomatine was found. They all consist of a six-ring steroidal aglycon with a nitrogen atom in the 6th ring, and a sugar moiety on the 3-OH position. Both potato glycoalkaloids consist of the same aglycon, but differ in their trisaccharide, whereas α -tomatine contains a different aglycon and a tetra-saccharide [8].

Next to inhibition of acetylcholine esterase [4], some reports indicated that these compounds strongly interfere with membrane structure and function [5–8]. Studies on plant cells [5], fungi [5] and mammalian cells [5,9] have shown that glycoalkaloids can induce leakage of cell contents. The leaked compounds ranged from ions [9] to large proteins [5] but the mechanism of the induced changes in barrier function remained unclear. Recently we investigated the effects of glycoalkaloids on model membranes

Abbreviations: AK, adenylate kinase; CF, 6-carboxyfluorescein; DMF, dimethylformamide; FUM, fumarase; GJIC, Gap-junctional intercellular communication; IM, mitochondrial inner membrane; LDH, lactate dehydrogenase; LUVETS, large unilamellar vesicles prepared by extrusion technique; MAO, monoamine oxidase; OM, mitochondrial outer membrane; PC, egg yolk phosphatidylcholine.

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and a detailed molecular model for the glycoalkaloid induced membrane disruption was proposed [10]. In this model the aglycon part of the glycoalkaloid inserts in the membrane and reversibly binds to sterol in a 1:1 ratio. Subsequently, when these glycoalkaloid/sterol complexes reach a certain density, co-operative sugar–sugar interactions between the sugar moieties of the glycoalkaloids will initiate the formation of an irreversible matrix of these complexes. This matrix formation causes a loss of barrier function of the bilayer. The type of complexes and the extent of membrane disruption strongly depended on the structure of both the glycoalkaloid and the sterol and the sterol concentration in the membrane.

This study was carried out to investigate whether this proposed model of glycoalkaloid induced membrane disruption is also valid for the effects of these compounds on biomembranes. Therefore, the effects of α -solanine, α -chaconine and α -tomatine on the barrier function of membranes of different cells were studied. The cell types studied included erythrocytes and intestinal epithelial cells which are both expected to be target cells of these compounds. It was found that glycoalkaloids indeed exerted disruptive effects on biomembranes in a similar fashion as was shown for model membranes. In addition, α -chaconine was found to selectively decrease intercellular gap-junctional communication between neighbouring cells. Furthermore, at low concentrations the glycoalkaloids were more potent in permeabilizing the outer membrane of mitochondria compared to the routinely used digitonin.

2. Materials and methods

2.1. Materials

α -Chaconine, α -solanine, α -tomatine, egg yolk phosphatidylcholine (PC) and lucifer yellow (LY) were obtained from Sigma (USA). Cholesterol, digitonin and dimethyl formamide (DMF) were obtained from Merck (Germany). 6-Carboxyfluorescein (CF, Eastman Kodak, USA) was purified by active carbon treatment, recrystallisation from water/ethanol (2:1, v/v) and Sephadex LH20 (Pharmacia) column chromatography [11]. The glycoalkaloids were dissolved in dimethylformamide and diluted in the different incubation solutions used. All reagents were analytically pure and solvents used were of analytical grade.

2.2. Hemolysis assay

Human erythrocytes were isolated from 10 ml outdated blood by centrifugation (10 min, $3000 \times g$, 4°C). After removal of the supernatant, the cells were resuspended in 10 ml 150 mM NaCl and centrifuged again. Subsequently, the cells were washed with physiological buffered saline (PBS, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 139 mM NaCl, 16

mM Na_2HPO_4 , pH 7.4) until free hemoglobine had been removed. The erythrocytes were diluted 2.5 times with PBS. To 2.92 ml PBS, 30 μl of glycoalkaloid in DMF or Triton X-100 (10%, w/v) were added. Then an aliquot of the erythrocyte suspension corresponding to 50 nmol phospholipid, determined after lipid extraction [12] and phospholipid determination [13], was added within 1 min and thoroughly mixed. After 5 min incubation at room temperature the mixture was centrifuged (15 min, $4000 \times g$, 15°C) and the released hemoglobine was measured at 577 nm. Addition of 30 μl DMF resulted in a minor lysis comparable to the blank, whereas addition of Triton X-100 resulted in maximal hemolysis. The release was expressed as the percentage hemolysis compared to maximal hemolysis.

2.3. CF leakage from erythrocyte ghosts

Right side out sealed ghosts were prepared according to the method of Schwach and Passow [14]. In order to obtain CF loaded ghosts, a suspension of erythrocytes isolated as described above was cooled to 0°C . One volume of this suspension was mixed with 10 volumes hemolysis medium (1.2 mM acetic acid, 4 mM MgSO_4 , pH 3.2) also cooled to 0°C . After an incubation of 5 min at 0°C , the pH was adjusted to 7.4 with 1 M Tris. The suspension was centrifuged ($16000 \times g$, 10 min, 0°C) and the supernatant removed. The pellet was diluted with one volume CF solution (80 mM CF, 20 mM Hepes, 170 mM NaCl, pH 7.4) and incubated 60 min at 37°C to cause resealing of ghosts [15]. Free CF was removed by gel-filtration over a Sephadex G-75 column (1×30 cm; Pharmacia), eluted with a 10 mM Hepes buffer (pH 7.4) containing 140 mM NaCl. An aliquot of the ghost suspension corresponding to 50 nmol phospholipid, determined as described above, was diluted with elution buffer to 1.98 ml. After 1 min mixing, 20 μl of a glycoalkaloid solution was added. Fluorescence was measured at room temperature on a Perkin Elmer LS-50 spectrofluorometer at an excitation of 430 nm monitoring CF release as an increase in emission intensity at 513 nm, due to quenching of CF fluorescence [15]. Total amount of CF loaded was determined by lysing the ghosts with 20 μl 10% (v/v) Triton X-100. Addition of 20 μl DMF only resulted in a minor CF leakage. CF leakage was monitored for 5 min and was expressed as the percentage CF leakage compared to the total amount loaded in the vesicles. In some experiments, the samples were not lysed but centrifuged (15°C , 15 min at $541000 \times g$) and the supernatant was tested for glycoalkaloids by adding another aliquot of ghosts.

2.4. Vesicle CF leakage assay

Human erythrocytes were isolated as described above and hemolysed by addition of an equal volume of H_2O . The hemolysate was extracted with 2-propanol and chloro-

form according to Rose and Oklander [16]. After evaporation of the solvent, lipids were dissolved in chloroform and non-soluble material was removed by centrifugation ($7000 \times g$, 15 min, 4°C). CF loaded unilamellar vesicles (LUVETS) were prepared by extrusion from this total lipid extract and CF leakage experiments were carried out according to Keukens et al [8]. After the leakage assay, some samples were not lysed but centrifuged and the glycoalkaloid activity of the supernatant was tested by addition of fresh vesicles.

2.5. Cell culture

Caco-2 cells, originating from a human colorectal carcinoma [17], were grown in 75 cm^2 flasks (Costar, USA) at 37°C in a humidified atmosphere of 5% CO_2 in air, using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 10 mM HEPES, 100 IU/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, fungizone and 1% non-essential amino acids (all Gibco). The medium was changed every other day until the cells reached 90% confluence. After the cells had formed a monolayer, the cells were detached from the flasks by incubating the monolayers with trypsin (0.25% in phosphate-buffered saline (PBS) at pH 7.4, containing 0.2% EDTA (Gibco) for 10 min at 37°C . For the different experiments cells were seeded either on polycarbonate filters (Transwell, $0.4 \mu\text{m}$ pore size), 24-transwell cell clusters (both Costar) or on 2.5 cm petridishes (Greiner, Germany). The integrity of the monolayer on the filters was checked by measuring possible diffusion of CF from the apical side to the baso-lateral side of the cells on the filter and only impermeable monolayers were used. In the other preparations the cells form a monolayer on the bottom of the container. After addition of the glycoalkaloids, the cells were incubated at 37°C . Lactate dehydrogenase (LDH) activity was determined in the apical and baso-lateral medium of the cells grown on filters and in the medium of cells grown in transwell plates and petri-dishes as described by Mitchell et al. [18].

2.6. Gap-junctional intercellular communication (GJIC)

After Caco-2 cells were grown to confluency in petri-dishes, GJIC was measured by the fluorescent dye transfer method using microinjection of Lucifer Yellow CH (Merck, Germany) and counting the number of fluorescent cells 10 min after injection [19]. After determination of the control GJIC, the cells in the same petri-dish were subsequently exposed to the tested concentrations of glycoalkaloids ($t = 0$) and GJIC was determined after 20 min. Approximately 20 to 35 cells in each petri-dish were injected and each experiment was performed in duplicate. The results are expressed as the percentage remaining GJIC compared to GJIC at $t = 0$. For the same incubations the LDH leakage from the cells was simultaneously determined as described above.

2.7. Mitochondria

Mitochondria were isolated from male Wistar liver cells by the procedure described by Hovius et al. [20]. Their concentration was determined by measuring the protein concentration using the colorimetric bicinchoninic acid method with bovine serum albumine as a standard (Pierce, USA). Mitochondrial intactness was determined by the latency of cytochrome-*c* oxidase [21] and was found to be 90%. Mitochondria (0.3 mg protein/ml) were incubated with glycoalkaloids or digitonin dissolved in DMF and after 15 min incubation the mixture was centrifuged ($8200 \times g$, 10 min). The supernatants and pellets were separated and kept on ice until enzyme activities were determined. Adenylate kinase (AK) activity was measured indirectly by following the NADH decrease at 340 nm (37°C , 3 min) [22], whereas monoamine oxidase (MAO) activity was measured by following the decrease of A_{360} , caused by the oxidation of kynuramine, for 10 min at 37°C [23]. Furthermore, fumarase (FUM) activity was assayed by monitoring the increase in A_{250} caused by the conversion of L-malate into fumarate [24].

3. Results

3.1. Erythrocytes

Erythrocytes were selected as a biomembrane test system because they consist of only one (cell) membrane, which contains approximately 50% cholesterol (molar), and they contain a high amount of hemoglobine which can be easily monitored. Fig. 1 demonstrates that all used glycoalkaloids were able to cause complete hemolysis which was very rapid and occurred within 1 min after addition (data not shown). The order of potency of the

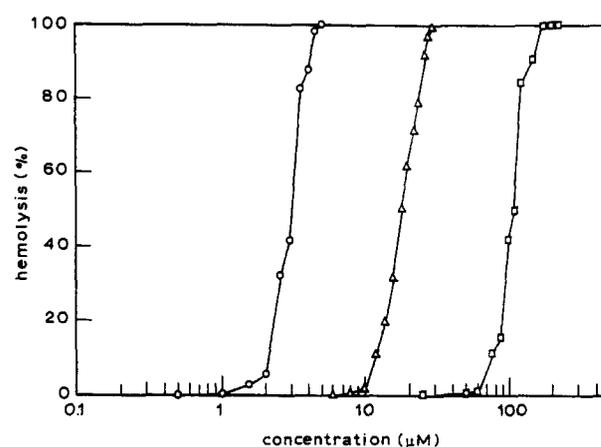


Fig. 1. Glycoalkaloid induced hemolysis of erythrocytes. Free hemoglobin concentrations were determined 10 min after incubation of erythrocytes (total lipid 100 nmol) with α -tomatine (○), α -chaconine (Δ) and α -solanine (\square). Each point represents the average of 8 replicates with a standard deviation of less than 5%.

Table 1

Regression analysis of data obtained after glycoalkaloid induced hemolysis of erythrocytes, or CF leakage from ghosts or vesicles

Glycoalkaloid	No effect level ¹ (μM)	50% effect (μM)
α -Tomatine		
Erythrocytes	1.9	2.9
Ghosts	1.9	4.8
Vesicles	4.1	13.4
α -Chaconine		
Erythrocytes	11.3	18.1
Ghosts	13.4	27.4
Vesicles	15.3	33.4
α -Solanine		
Erythrocytes	72.7	106.7
Ghosts	83.8	343.8
Vesicles	157.6	501

Linear regression was carried out on the steep part of the curves (see Figs. 1–3) and calculated values for the no effect level and 50% effect concentration are presented.

¹ No effect level: the highest concentration at which no effect is found.

glycoalkaloids was α -tomatine > α -chaconine > α -solanine. Addition of fresh erythrocytes to the supernatant did not result in additional hemolysis of these erythrocytes, illustrating that all glycoalkaloids were bound to the bilayers in the first incubation. From an analysis of the hemolysis curves concentrations causing 50% hemolysis as well as no effect levels can be obtained (Table 1). The no effect level is the highest concentration at which a compound does not exert an effect.

In order to study the leakage of a small molecule and to be able to make a comparison to model membrane studies, experiments were carried out using 6-carboxyfluorescein (CF, M_r 376) loaded right-side out resealed ghosts (Fig. 2). All glycoalkaloids were able to cause CF leakage and the order of potency was again α -tomatine > α -chaconine

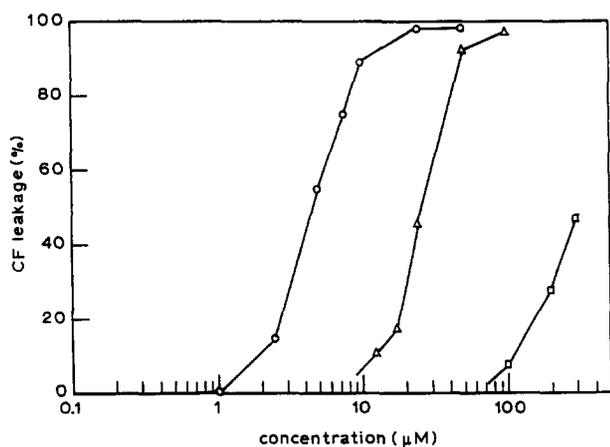


Fig. 2. Glycoalkaloid induced CF leakage from CF loaded ghosts. CF leakage was determined 5 min after addition of different amounts of α -tomatine (○), α -chaconine (Δ) or α -solanine (□) to the ghosts (total lipid 100 nmol). Each point represents the average of 5 replicates with a standard deviation of less than 4%.

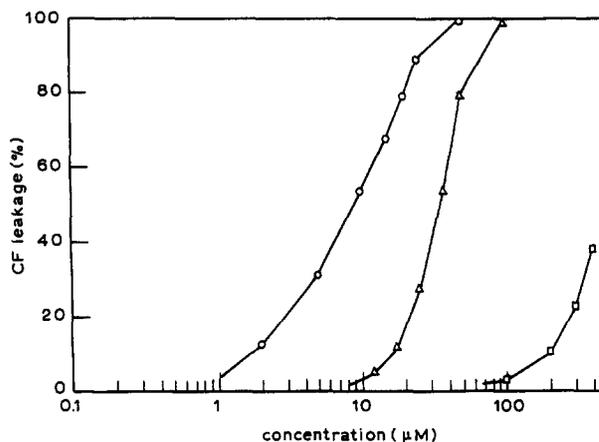


Fig. 3. Glycoalkaloid induced CF leakage from vesicles prepared of erythrocyte lipids. CF leakage was determined 5 min after addition of different amounts of α -tomatine (○), α -chaconine (Δ) or α -solanine (□) to the vesicles (total lipid 100 nmol). Each point represents the average of 5 replicates with a standard deviation of less than 2%.

> α -solanine. However, some differences between the CF and hemoglobine release curves were observed. Most importantly, CF leakage was induced less efficiently than hemoglobine release from the intact cells which was most clearly shown for α -solanine. This was furthermore illustrated by the increased 50% effect concentrations compared to the hemolysis experiments (Table 1). In contrast, the no effect levels of CF leakage from ghosts were similar to the hemoglobine release data from intact cells. The supernatant of the samples which were centrifuged after the leakage experiments, did not show any CF leakage from freshly added ghosts.

CF loaded LUVETS prepared from erythrocyte lipids were used to study the effect of the glycoalkaloids on a erythrocyte derived lipid vesicle system. Again all glycoalkaloids were able to induce CF leakage suggesting that the lipids are the primary target of the glycoalkaloids in red blood cell membranes (Fig. 3). The order of potency of the various glycoalkaloids was identical compared to the previous experiments. α -Solanine was not able to induce 100% CF leakage at the concentrations used. After centrifugation of the samples, the supernatant was unable to cause CF leakage from additional liposomes, illustrating the high affinity of the glycoalkaloid for the membranes. The no effect levels as well as the 50% effect concentrations were both increased for all glycoalkaloids compared to the ghost experiments (Table 1) demonstrating that the biomembrane is 1.5–3-fold more sensitive than the lipid vesicles.

To get insight in the relative affinities of glycoalkaloids for erythrocytes and derived LUVETS, a competition experiment was carried out using α -tomatine as test glycoalkaloid. Hemolysis was determined in the presence or absence of vesicles prepared of total erythrocyte lipids. The results clearly show that the hemolysis induced by a certain α -tomatine concentration decreased to the level

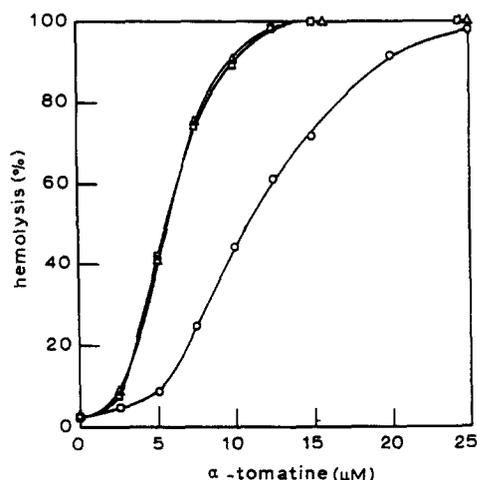


Fig. 4. α -Tomatine induced hemolysis of erythrocytes (total lipid 100 nmol) in the presence of PC vesicles (\square , total lipid 100 nmol), erythrocyte lipid vesicles (\circ , total lipid 100 nmol) or in the absence of vesicles (\triangle). Each point represents 6 replicates with a standard deviation of less than 5%.

obtained at half this concentration after addition of vesicles with an identical lipid concentration to the erythrocytes (Fig. 4). This demonstrates that this glycoalkaloid did not have a preference for either erythrocytes or vesicles prepared of erythrocyte lipids and supports the view that the glycoalkaloids primarily interact with the lipid part of this biomembrane. PC vesicles did not compete for α -tomatine induced hemolysis in accordance with the sterol requirement for the glycoalkaloid–lipid interaction [8].

Studies on model membranes have shown that a synergistic effect between the potato glycoalkaloids α -solanine and α -chaconine occurs, most likely due to the assembly of these molecules with cholesterol in membrane disruptive complexes [10,25]. To analyse possible synergism in the erythrocyte based systems, an α -solanine concentration was chosen from Figs. 1–3 at which this glycoalkaloid on its own did not cause an effect. In all systems a striking synergism was observed. An example is shown in Table 2. The presence of α -solanine greatly increased the membrane disruptive action of α -chaconine.

3.2. Caco-2 cells

Intestinal epithelia have two major functions: to absorb water, ions and nutrients present in the lumen, and to act as a barrier for separating the luminal content from underlying intestinal and vascular fluids. They form one of the first targets for glycoalkaloids after consumption. The effects of glycoalkaloids were tested on a human epithelial colon carcinoma cell line (Caco-2). These cells are polarized and contain an apical and baso-lateral side and form a continuous monolayer through tight-junctions. Furthermore, these cells are able to communicate with each other through gap-junctions. Firstly, membrane disruptive effects were determined by monitoring the leakage of the enzyme

Table 2

Synergism of the membrane disruptive action between α -solanine and α -chaconine on different membranes

Test system (parameter)	Effect (%)		
	α -solanine (12.5 μ M)	α -chaconine (12.5 μ M)	α -sol/ α -cha (25 μ M)
Erythrocytes (hemolysis)	0	11.5	84.5
Ghosts (CF leakage)	0.5	10.6	35.0
Lipid vesicles (CF leakage)	0.6	5.5	30.0

Hemolysis of erythrocytes and CF leakage from ghosts and vesicles was determined after addition of either α -solanine or α -chaconine or a 1:1 mixture of both. Each value represents the average of six replicates with a standard deviation of less than 5%.

lactate dehydrogenase (LDH, M_r 140 000) in the medium from cells grown on a solid surface of Transwell plates. α -Chaconine induced in a dose-dependent manner leakage of this enzyme from the cells at a relatively slow rate such that maximum release was observed after 45 min (Fig. 5). All glycoalkaloids tested induced substantial LDH leakage with an order of potency of α -tomatine > α -chaconine > α -solanine (Fig. 6). The 50% effect concentrations were 4, 12 and 70 μ M for α -tomatine, α -chaconine and α -solanine, respectively. The effective concentration ranges of the glycoalkaloids that induce LDH leakage from Caco-cells were similar to the concentration ranges necessary to induce hemolysis (compare Fig. 1), but the kinetics were much slower. Also some Ca^{2+} -leakage experiments were carried out which showed that leakage of this ion occurred within one min after incubation (data not shown) with glycoalkaloids which illustrated that LDH leakage is probably a secondary effect. Because these Ca^{2+} -leakage

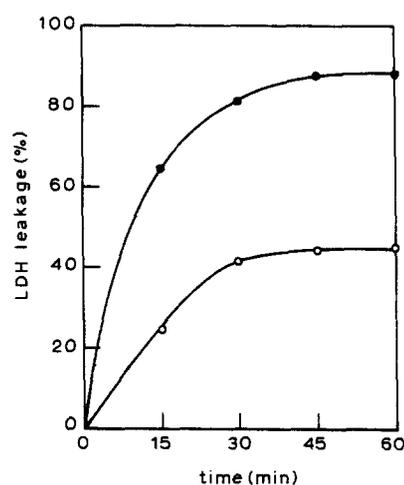


Fig. 5. Time dependency of α -chaconine induced LDH leakage from Caco-2 cells. Caco-2 cells were exposed to 10 (\circ) or 20 (\bullet) μ M α -chaconine and the amount of released LDH in the medium was determined. Each point represents the average of 6 replicates with a standard deviation of less than 5%.

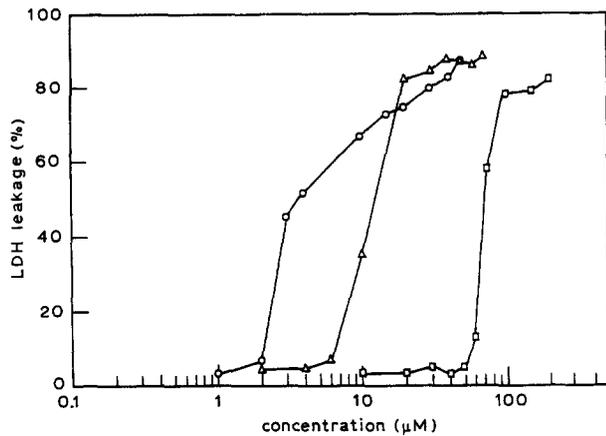


Fig. 6. Glycoalkaloid induced LDH leakage from Caco-2 cells. Caco-2 monolayers grown on a solid surface were apically exposed to different amounts of α -tomatine (o), α -chaconine (Δ) or α -solanine (\square) and LDH leakage was measured after 45 minutes. Each point represents the average of 6 replicates with a standard deviation of less than 5%.

effects could not be quantified in the method used, the LDH leakage assay was used.

The effects of glycoalkaloids on the baso-lateral and the apical side of a layer of Caco-2 cells was tested by growing a confluent monolayer of these cells on porous polycarbonate filters and subsequently exposing them to glycoalkaloids from either side of the filter. LDH release was determined in both the apical and the baso-lateral medium. Addition of α -chaconine from either side caused efficient LDH release from the cis side. Apical exposure appears to be more effective than baso-lateral exposure (Fig. 7). This was demonstrated by the additional trans release after apical exposure compared to the lack of trans

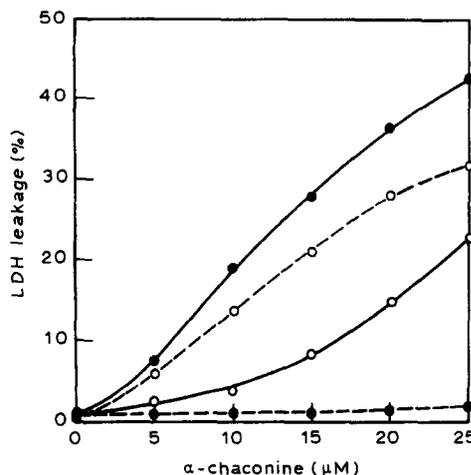


Fig. 7. Glycoalkaloid induced LDH leakage from polarized Caco-2 cells. Caco-2 cells were grown on filters and after the formation of a continuous monolayer they were exposed to α -chaconine on either the apical side (—) the baso-lateral side (---). LDH release on both the apical (●) and the baso-lateral (○) side were determined. Each point represents the average of 4 replicates with an standard deviation of less than 5%.

Table 3

Effect of glycoalkaloids on GJIC and LDH release of Caco-2 cells

Glycoalkaloid	Concentration (μ M)	GJIC (%) ¹	LDH release (%) ²
Blanc (DMF)	0	98	1.2
α -Solanine	15	87	1.8
	25	28	73.4
α -Chaconine	1	51	1.5
	5	49	1.4
	7.5	28	13.7
α -Tomatine	15	9	83.9
	5	77	14.5
	50	8	83.2

GJIC was determined by fluorescent dye transfer 20 min after exposure to glycoalkaloids. LDH was also determined after 20 min as described in Section 2.

¹ Percentage remaining GJIC compared to GJIC at $t = 0$ [27]. $n = 50$.

² $n = 6$.

leakage after baso-lateral exposure. α -Solanine and α -tomatine showed the same behaviour but at the expected different concentration ranges (data not shown). These results showed that the barrier function of the monolayer of Caco-2 cells was lost after apical exposure to glycoalkaloids.

In order to study the effect of glycoalkaloids on the intercellular communication between the cells, dye transfer was measured in a Caco-2 monolayer attached to the petri-dishes. The mean number of recipient cells after micro-injection of 1 cell with the dye was 27 under control conditions ($t = 0$, GJIC = 100%). Subsequently, the cells were exposed to different concentrations of the three glycoalkaloids and GJIC was determined. For all incubations LDH leakage was separately measured in the same system. The results showed that in general the GJIC decreased when LDH leakage occurred (Table 3). This effect will be caused by the leakage of fluorescent dye through the cell membrane instead of transport through the gap-junctions. Interestingly, in the 1–5 μ M range α -chaconine caused a significant decrease of GJIC whereas the LDH leakage was still very low, whereas α -solanine and α -tomatine only decreased GJIC when they also caused LHD leakage.

3.3. Mitochondria

Rat liver mitochondria were studied because they contain two membranes with very different sterol contents [26]. These organelles consist of an outer membrane (OM) and an inner membrane (IM) which contain approximately 9 and 1% cholesterol, respectively. Disruption of the outer membrane can be determined by measuring the activity of the inter membrane space localized adenylate kinase (AK), whereas possible disruption of the inner membrane can be determined by measuring the activity of the matrix localized enzyme fumarase. The effects of the glycoalkaloids were compared with the effects induced by digitonin, a

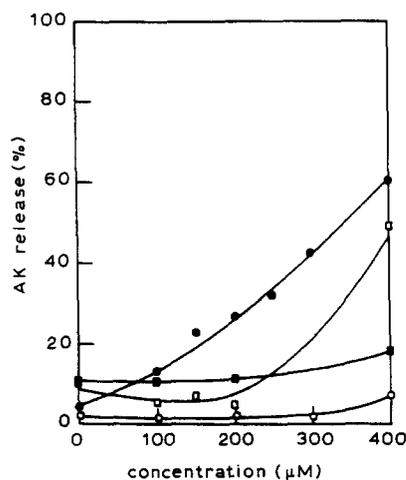


Fig. 8. Glycoalkaloid and digitonin induced release of adenylate kinase (AK) from rat liver mitochondria. Mitochondria were incubated with different amounts of α -tomatine (●), α -chaconine (□), α -solanine (■) or digitonin (○) and after 10 min incubation and subsequent centrifugation ($8200 \times g$, 10 min) the amount of released AK was determined. Each point represents the average of 4 replicates with a standard deviation of less than 5%.

saponin which is known to complex with sterols and which is commonly used for solubilisation and permeabilizing the outer membrane of mitochondria. The control release of AK varied between 0 and 10%. At the low concentrations used hardly any AK activity was detected in the supernatant after addition of digitonin or α -solanine. However, α -chaconine and α -tomatine induced up to 50 and 60% AK leakage, respectively (Fig. 8). No activity of monoamine oxidase, was detected (data not shown), indicating that the OM were not solubilized by either of the tested compounds at the concentrations used. Also, fumarase activity was not found in the supernatant which means that both digitonin and the glycoalkaloids were not able to disrupt the IM at the concentrations used.

4. Discussion

In this study the effects of the glycoalkaloids α -solanine, α -chaconine and α -tomatine on different biological membranes were studied. The main result is that the lipids of the biomembranes formed the major target for the glycoalkaloids and that the biomembranes became permeabilized, resulting in leakage of compounds ranging from CF to enzymes. Furthermore, α -chaconine was found to decrease GJIC between Caco-2 cells.

The mechanism of the action of glycoalkaloids on biomembranes reported in this study appears to be similar to the mechanism described in the model proposed for model membranes [10]. Three observations support the similarity of the models. Firstly, membrane permeabilization is related to the cholesterol content. This was illustrated by the selective disruption of the outer over the inner membrane of mitochondria. The much higher con-

centration of glycoalkaloids necessary to disrupt the outer membrane (9% cholesterol) compared to the membranes of erythrocytes (50%) and Caco-2 cells (31% apical membrane [27]) also demonstrates this cholesterol dependency. Furthermore, the competition experiments between erythrocytes and erythrocyte lipid vesicles or PC vesicles showed that glycoalkaloids have a high preferential affinity for cholesterol containing membranes. Secondly, a similar order of potency of the glycoalkaloids to induce membrane disruption was observed in the different systems. This relative order, α -tomatine > α -chaconine > α -solanine, was identical to that found for model membranes. And thirdly, α -solanine and α -chaconine showed synergism in their effect on biomembranes similar to that reported for lipid vesicles systems.

The effects of glycoalkaloids on erythrocytes, ghosts and erythrocyte lipid vesicles showed that glycoalkaloids exerted a similar membrane directed action causing leakage. Minor differences between the effects of the glycoalkaloids in the different test systems occurred. The 50% effect concentrations were higher in the ghost experiments compared to the hemolysis experiments. This difference could be due to an effect on ion-gradients after glycoalkaloid induced membrane disruption which subsequently causes fast lysis as a secondary effect. Up to 100% hemolysis of intact erythrocytes was induced by all three glycoalkaloids. Using CF loaded vesicles, a better comparison to the membrane effects on ghosts was made. The relatively small increase of the 50% effect concentration illustrates that the presence of proteins in the erythrocyte/ghost membrane does not have much consequences for glycoalkaloid induced effects. The difference between CF leakage from ghosts compared to LUVETS is possibly due to the much higher number of vesicles compared to ghosts. This means that more glycoalkaloid-molecules are necessary to disrupt all the vesicles compared to the lower number of ghosts. In all three systems it was shown that after the first glycoalkaloid induced leakage no membrane disruptive activity was present in the supernatant after centrifugation. This illustrated that the glycoalkaloids partition preferentially in the bilayer as was also shown in our previous study with model membranes [10].

LDH release from the Caco-2 cells was relatively slow and completed after 45 min whereas hemoglobine release from erythrocytes was completed within 1 min and CF leakage from ghosts after 5 min. Considering the fact that Ca^{2+} -leakage was observed within one min, and that LDH leakage is generally used as a parameter for cell death [18], it seems more likely that LDH leakage is a secondary effect of glycoalkaloid induced membrane disruption of Caco-2 cells. Another explanation could be that the epithelial cells need to be resistant against certain detergents like bile-acids. This protection might slow down the process of glycoalkaloid induced membrane disruption. The higher susceptibility of the apical membrane compared to the baso-lateral membrane can be explained by the much

higher amount of glycolipids in the apical membrane (37% vs. 19% [27]). Glycolipids were reported to have an enhancing effect on glycoalkaloid induced membrane disruption [10].

The effect of α -chaconine on GJIC is most probably due to the specific glycoalkaloid–cholesterol interaction. Gap-junctions were reported to be situated in cholesterol-rich plaques in the cell membrane [28]. Destabilisation of these plaques has an effect on GJIC [29]. Interestingly, α -chaconine induced a decrease of GJIC at low, non-disruptive concentrations. Because α -tomatine is much more effective compared to the other glycoalkaloids, exposure to this compound will probably always result in membrane disruption even at low concentrations where it might interfere with GJIC. α -Solanine, which was found to have a much lower affinity for cholesterol containing membranes, is probably not effective at non-disruptive concentrations.

Glycoalkaloids were more effective in permeabilizing the outer membrane of mitochondria compared to digitonin at the concentrations used. It can be concluded that the outer membrane was not solubilized but only permeabilized by the glycoalkaloids. Digitonin is used routinely for permeabilization of different cholesterol containing membranes [20]. Glycoalkaloids might form a useful tool in permeabilizing certain membranes. For instance, they could be used for specific permeabilization of plant membranes with low sterol contents because of the very high disruptive activity of these compounds on plant sterol containing membranes [8].

The glycoalkaloid induced membrane disruptive effect could well be causing the different symptoms after intoxication. Gastro-intestinal disturbances and hemolysis are most likely the result of membrane disruption. Neurotoxic effects are probably caused by inhibition of acetylcholine esterase but neuronal cells also contain high amounts of cholesterol making them susceptible to glycoalkaloids which then could disturb nerve impulses. Furthermore, heart cells are known to generate ion fluxes through gap-junctions in order to synchronize contractions. Thus, decreasing the GJIC would have an effect on these heart contractions. After a possible collapse of the barrier function of intestinal cells, glycoalkaloids are expected to enter the blood stream and reach other target cells. Not much is known about the uptake and possible hydrolysis of glycoalkaloids to give an indication of the amounts that enter the body. More studies on these factors need to be carried out to obtain a better insight into the effects and possible risks of high glycoalkaloid intake.

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