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Influence of derivation on the lipophilicity and inhibitory actions of cardiac glycosides on myocardial Na^+-K^+-ATPase

Nduna Dzimiri, [1] Uwe Fricke & Wolfgang Klaus

Pharmakologisches Institut der Universität zu Köln, Gleueler Strasse 24, D-5000 Köln, F.R.G.

1 Lipophilicity and inhibitory actions on guinea-pig heart Na^+-K^+-ATPase of twenty-six digitalis and six strophanthus glycosides comprising the aglycones, mono-, bis-, tris-sugar, alkylated (acylated) tris-sugar, acyl steroid derivatives and three cardanolides were investigated.

2 Their octanol/water partition coefficients (P), reversed phase thin layer (r.t.l.c.) and reversed phase high performance liquid chromatography (r.h.p.l.c.) were determined and the viability of these methods as a measure of the lipophilicity of the cardiotonic steroids evaluated.

3 The influence of lipophilicity and so also structural changes on the inhibitory effects of the cardiac glycosides on myocardial Na^+-K^+-ATPase was then examined.

4 It is concluded that (a) r.t.l.c. and r.h.p.l.c. are just as effective as the conventional shake-flask method for estimation of the lipophilicity of cardiac glycosides and (b) the inhibitory potencies of cardiotonic steroids on the myocardial Na^+-K^+-ATPase increase with growing lipophilicity. The relationship between these two parameters is, however, governed by the influence of substitution or derivation of structural components on their inhibitory potencies on the myocardial Na^+-K^+-ATPase.

Introduction

The pharmacological effects of drugs or hormones are consequences of their interactions with specific molecular or macromolecular binding sites in the target tissue, i.e. their receptors. Both the pharmacokinetics (absorption, distribution, biotransformation and elimination) and pharmacodynamics (drug-receptor interaction) of the pharmacological agents are intimately related to the chemical structure, the physico-chemical properties of the drug itself and the biological systems involved. In particular, their lipophilic character, electronic properties (i.e. type of chemical bonds and charge distribution – inductive and resonance effects) and spatial (dimensional) configuration are postulated to be involved in drug-receptor interactions, mode and extent depending on whether the drug acts specifically i.e. interaction of defined structural regions of the drug with the complementary binding sites of the receptors, or non-specifically, i.e. interaction between the drug molecule and receptor depending mainly on the lipophilic nature of the drug (Eberlein, 1978).

Lipophilicity is an expression for the thermodynamical equilibrium which determines the distribution probability of a drug molecule between the mainly aqueous transport system and the lipophilic membrane barrier as well as the binding tendency of a drug molecule onto a lipophilic receptor counterpart. The hydrophobic (lipophilic) bond is assumed to provide probably the largest portion of the energy household of biological activities of many hormone- and drug-molecules (Eberlein, 1978). It is therefore a determinant factor for the structure-activity relationships of a large number of drug classes.

Several binary phase systems, i.e. water-saturated solvents and a solvent-saturated water phase, have been put up in order to resemble biological partition systems for the purpose of studying the partitioning behaviour of a number of drug-classes. Widest recognition has been accredited to the octanol/water (Tris-buffer/HCl, pH 7.4) system as most closely resembling physiological partition systems (Leo *et al.*, 1971; Hansch & Dunn, 1972; Smith *et al.*, 1975). However the conventional shake-flask method used to determine the partition coefficients is tedious, time-consuming and very susceptible to technical error, especially within the ranges of $10 > P < 1000$ (Cohnen *et al.*, 1978; Rogatti, 1983a). Consequently, alternative methods for establishing the lipophilic nature of

[1] Author for correspondence.

pharmacological agents have been sought for about two decades, among which the chromatographic behaviour of several drug classes, has been found to correlate very well with their partition coefficients (Henry *et al.*, 1967; Carlson *et al.*, 1975; McCall, 1975; Mirrles *et al.*, 1976; Yamana *et al.*, 1977; Hempelmann *et al.*, 1978; Miyake & Terada, 1978; Unger *et al.*, 1978; Baker *et al.*, 1980; Nahun & Horwath, 1980).

During the interaction between cardiac steroids and their only identified specific binding site, the Na^+ -K^+ -ATPase, the physico-chemical binding forces cited above, lipophilicity, electronic and hydrogen bond, are also postulated to be extensively involved. Accordingly, interactions are initiated by long-range electrostatic attractions between the lactone ring of the cardiac glycoside and the electrophilic sites on the receptor, resulting in a two-point attachment. This is reinforced by short-range interactions of the van der Waal's forces and/or hydrophobic type between the steroid nucleus and the receptor. The stability of this complex can be increased by interactions of sugar residues (hydrogen bond, hydrophobic bond) with the enzyme (Yoda & Yoda, 1977; Thomas *et al.*, 1980).

However, despite the importance accredited to hydrophobic binding forces with regard to the interactions of the cardiotonic steroids with their receptor, very little experimental evidence exists in the literature on the relationship between lipophilicity and pharmacodynamics of the cardiotonic steroids. Nevertheless, it can be deduced, that the lipophilic nature of cardiotonic steroids does exhibit at least some transient influence on their inhibitory actions on the myocardial Na^+ -K^+ -ATPase (Fricke *et al.*, 1981; Rogatti 1983a). In the present paper, this relationship has been investigated further with special regard to the interdependence between structural modifications, lipophilicity and the inhibitory potencies of the cardiotonic steroids.

Methods

Lipophilicity

Partition coefficients The partition coefficients of the cardiac glycosides were determined in octanol/water (20 mmol l^{-1} Tris/HCl-buffer, pH 7.4) at room temperature according to the shake-flask method of Hansch & Dunn (1972). The phases were first saturated against each other, the glycoside in question dissolved in octanol and shaken against the aqueous phase for 30 min. Drug concentrations were determined in the lipophilic phase by means of u.v.-spectrophotometry (Aminco DW2 UV/VIS) at 220/295 nm.

Reversed-phase thin layer chromatography (r.t.l.c.) After impregnating the pre-coated t.l.c.-plates (silicon-gel on gipsy, SIL G-25 HR, Macherey-Nagel, F.R.G.) with 5% *n*-octanol (Baker Chemicals, The Netherlands) in ether, the drugs were run for about 3 h until the mobile phase (methanol/water, 30:70) advanced 16 cm from the starting line. The developed plates were dried at about 100°C in a drying chamber and treated with modified Kedde (Kedde 1: 2.0 g dinitrobenzoic acid in 100 ml methanol; Kedde 2: 5.7 g potassium hydroxide in 100 ml methanol) and/or anis aldehyde reagent (5% anis aldehyde in 50% aqueous sulphuric acid solution, v/v). The r.t.l.c. values are expressed as R_m.

Reversed-phase high performance liquid chromatography (r.h.p.l.c.) The retentions (K'-values) of the cardiotonic steroids were determined using a Hewlett-Packard h.p.l.c. type 1084A with HP 79850A terminal equipped with a reversed-phase h.p.l.c. column 7991813 (20 cm long, internal radius 4.6 nm, size 2 μm). About 30–50 μl of the glycoside solutions (1×10^{-5} mol l^{-1} in distilled methanol) were injected as specimen. Elution was with acetonitrile/water (45:55) and detection at 220 nm.

The cardanolides were determined according to a slightly modified method of Rogatti (1983b): 10–50 μl of the stock solutions were injected into a h.p.l.c.-pump and reversed-phase h.p.l.c. column (Knaur F.R.G., column-filling LiChrosorb RP-18, column length 250 mm, particle size 10 μm). The eluent was air-sequestered through a 3 m long glass-coil, being simultaneously mixed with hydrochloric acid and dehydro-ascorbic acid reagent. The products were cooled again in a 3 m long coil, freed from air by means of sucking and detected fluorimetrically in a temperature-constant cell-holder at 490 nm. The intensity of the fluorescence was continuously detected and registered on a photomultiplier recorder. The respective parent cardenolides were used as the internal standards.

Influence of the cardiotonic steroids on guinea-pig heart Na^+ -K^+ -ATPase

Enzyme preparation Myocardial Na^+ -K^+ -ATPase was prepared according to the method developed by Fricke & Klaus (1974). About eight guinea-pig hearts were minced in a micromincer (Braun, F.R.G.) for 10 min in isotonic sucrose buffer (180 mmol l^{-1}) to give a 15% suspension. After sedimentation, the supernatant was filtered and applied to a sepharose 4B column (Pharmacia Fine Chemicals, Sweden) equilibrated with a KCl-buffer solution (KCl 500 mmol l^{-1}, imidazole hydrochloride Na_2.EDTA 1 mmol l^{-1}, pH 7.4). Ascending eluation of the enzyme was carried out at a flow of 0.7 ml min^{-1} maintained by means of a peristaltic pump. The fractions were funnelled together into a visking

dialysis membrane (Medicell International Ltd., U.K., dialysis tubing, visking size 3–20/32"), and dialysed three times, each time at least 6 h using first, twice 10 mmol l^{-1} and once 100 mmol l^{-1} imidazole/HCl buffer, pH 7.4. All steps were carried out at 4°C at which temperature the enzyme was also stored.

Determination of enzyme activities Enzyme activities were determined at 37°C in the presence of different concentrations of the test substances. Usually 8–12 μg protein was pre-incubated for 10 min in 100 mmol l^{-1} imidazole/HCl buffer, pH 7.4, containing Mg^{2+} 5 mmol l^{-1}, Na^+ 100 mmol l^{-1}, K^+ 5 mmol l^{-1} and Na_2.EDTA 1 mmol l^{-1}. The reaction was initiated by adding ATP, the final concentration being 2 mmol l^{-1}. After 20 min the inorganic phosphate was determined by the method of Eibl & Lands (1969), performing each assay in duplicate. Na^+ -K^+ -stimulated ATPase activity was calculated as the difference between the total and that of the Mg^{2+}, Na^+ -dependent activity. All results were corrected for blank values without enzyme.

Miscellaneous

The protein concentration of the enzyme preparation was determined according to the method of Lowry *et al.* (1951), using Labtrol (Merz & Dade, F.R.G.) as standard. The concentrations for half maximal inhibition of the Na^+ -K^+ -ATPase (ID_{50}) were calculated from the individual concentration-effect curves as proposed by Hafner *et al.* (1977). pD_2 values are the negative logarithm of the concentration of the respective cardiotonic steroids required for half maximal inhibition of the enzyme. All data were analysed by standard statistical methods (mean value and s.e.mean, regression analysis).

Materials

The cardiotonic steroids examined (Table 1) were purchased from Merck (F.R.G.) or Roth (F.R.G.). All other reagents (Analytic grade), unless otherwise stated, were obtained from Merck (F.R.G.).

Results

Lipophilicity

The partition coefficients (P) of the cardiotonic steroids increased in the following order: strophanthidin-< digoxigenin- <gitoxigenin- < digitoxigenin-derivatives. Within the same aglycone group of the digitalis glycosides, increasing lipophilicity was observed with growing sugar moieties and alkyl- (acyl-group, the influence of derivation of the C_{12}-OH position being comparatively more marked than that of its C_{16}-OH isomer. This augmentation of lipophilicity was also dependent on the type and size of the substituents. Among the strophanthus-derivatives, strophanthin-α was much more lipophlic than both strophanthin-β and -γ. Similar results were obtained for the R_m and K′ values of the glycosides (Table 1, Figure 1).

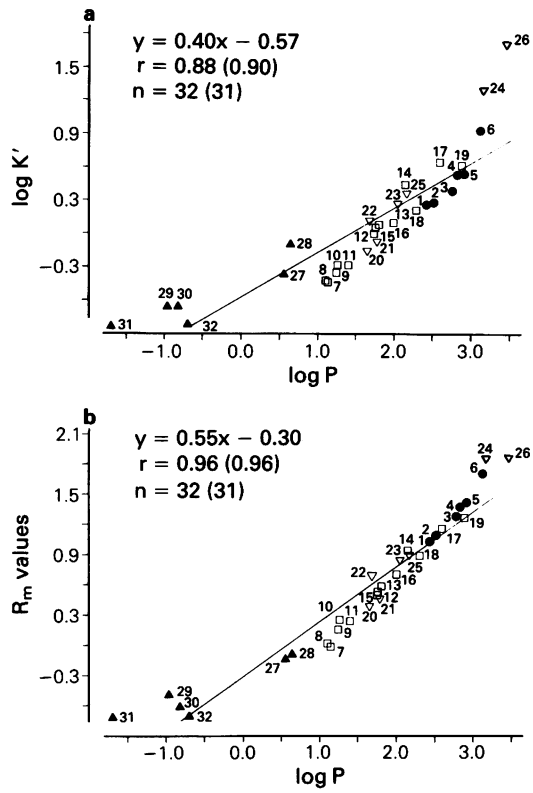

Figure 1 (a) Correlation of the partition coefficients (P) for the octanol/water partition system and the retentions (K′ values) for the acetonitrile/water r.h.p.l.c. elution system (45:55) of the cardiac glycosides examined. For code numbers given beside each point (in a and b) see Table 1. Plotted are the log P values against the log K′ values. The value in parentheses represents the regression coefficient excluding pentaacetylgitoxin (● = digitoxigenin-, ▽ = gitoxigenin-, □ = digoxigenin- and ▲ = strophanthus-derivatives). (b) Correlation of the partition coefficients (P) for the octanol/water partition system and the R_m values for the methanol/water r.t.l.c. mobile system (30:70) of the cardiac glycosides examined. Plotted are the log P values against the R_m values. The value in parentheses represents the regression coefficient excluding pentaacetylgitoxin (● = digitoxigenin-, ▽ = gitoxigenin-, □ = digoxigenin- and ▲ = strophanthus-derivatives).

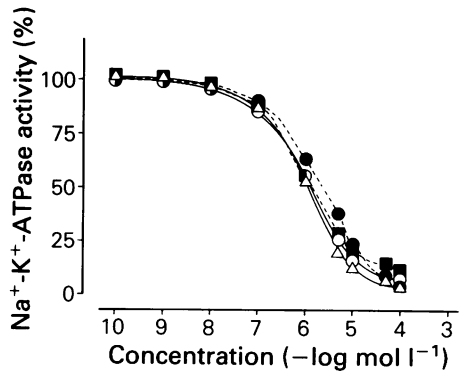

Figure 2 Influence of increasing concentrations of digoxin and its alkylated (acylated) sugar-derivatives on the activity of guinea-pig myocardial Na^+-K^+-ATPase. Plotted are the mean values of 8–12 individual determinations for digoxin (△), α-methyldigoxin (●), β-methyldigoxin (■) and α.β-dimethyldigoxin (○), the standard deviation of the mean being within 0.3–2.4%.

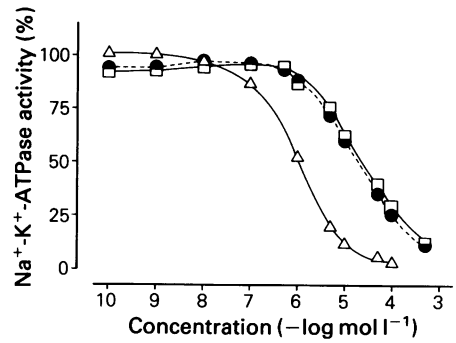

Figure 4 Influence of increasing concentrations of digoxin and its 12-acetyl derivatives on the activity of guinea-pig myocardial Na^+-K^+-ATPase. Plotted are the mean values of 8–12 individual determinations for digoxin (△), 12-acetyldigoxin (●) and 12-acetyl-β-methyldigoxin (□), the standard deviation of the mean being within 0.7–1.5%.

Inhibitory effects on the myocardial Na^+ -K^+ -ATPase

All of the cardiotonic steroids yielded concentration-dependent inhibitory effects on the myocardial Na^+ -K^+ -ATPase at concentrations above 10^{-7} mol l^{-1}. Below this concentration, some of the drugs showed activating effects. The ID_{50} values were employed as a measure of the potencies of the drugs. Accordingly, weakest inhibitory potencies were exhibited by strophanthus compounds and strongest by the digitoxigenin-derivatives. Derivatives of gitoxigenin and digoxigenin occupied an intermediate position, the former being generally more potent than the latter. The aglycones themselves were the least potent drugs, their mono-digitoxosides being about ten times as active. Non-alkylated glycosides of the same aglycone did not differ substantially from one another (Table 1).

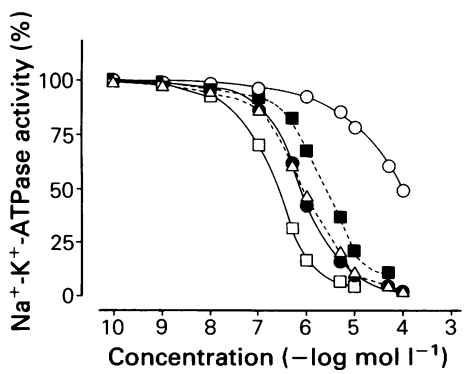

Figure 3 Influence of increasing concentrations of gitoxin and its alkylated (acylated) C_{16}-OH derivatives on the activity of guinea-pig myocardial Na^+-K^+-ATPase. Plotted are the mean values of 8–12 individual determinations for gitoxin (■), 16-formylgitoxin (●), pentaformylgitoxin (□), 16-acetylgitoxin (△) and pentaacetylgitoxin (○), the standard deviation of the mean being within 0.3–2.5%.

The alkyl (acyl) tris-digitoxosides showed little difference from their parent glycosides in their inhibitory potencies on myocardial Na^+ -K^+ -ATPase (Table 1, Figure 2). Alkylation (acylation) of the steroidal hydroxyl group either augmented (gitoxin) the inhibitory actions on guinea-pig Na^+-K^+-ATPase or diminshed (digoxin) this effect by more than 20 fold (Figures 3, 4). Hydrogenation of the lactone ring also resulted in weakening of the inhibitory effects. Furthermore, in comparison with the other glycosides studied, pentaacetylgitoxin showed by far the most highly lipophilic character while at the same time its inhibitory potency on the myocardial Na^+-K^+-ATPase remained virtually insignificant (Table 1, Figure 3).

Discussion

The correlations of the lipophilicity parameters studied demonstrate a very close relationship between the chromatographic behaviour and the partitioning properties of the steroids (Figure 1). It is therefore evident that, R_m- and K'-values are just as reliable as the partition coefficients themselves for estimation of

Table 1 The partition coefficients (P) in octanol/water system, R_m values for the reversed-phase thin layer chromatography (mobile phase: methanol/water 30:70), retentions (K') for the reversed-phase high pressure liquid chromatography (eluent acetonitrile/water 45:55) and the ID_{50} values of inhibitory effects of the cardiotonic steroids on the guinea-pig heart Na^+-K^+-ATPase

Cardenolides	*P*	R_m	*K'*	ID_{50} ($\times 10^{-6}$ mol l^{-1})
1 Digitoxigenin	270.0 ± 94.1	1.03	1.81	1.63 ± 0.06
2 Digitoxigenin-mono-digitoxoside	320.0 ± 56.5	1.09	1.89	0.54 ± 0.03
3 Digitoxigenin-bis-digitoxoside	580.0 ± 84.6	1.28	2.39	0.50 ± 0.03
4 Digitoxin	670.0 ± 105.0	1.37	3.34	0.31 ± 0.03
5 Dihydro-digitoxin	800.0 ± 112.0	1.42	3.40	32.40 ± 2.85
6 β-Methyldigitoxin	1300.0 ± 227.0	1.71	8.28	0.40 ± 0.03
7 Digoxigenin	13.5 ± 2.3	−0.02	0.36	10.70 ± 0.78
8 Digoxigenin-mono-digitoxoside	12.7 ± 0.6	0.01	0.37	1.13 ± 0.08
9 Digoxigenin-bis-digitoxoside	17.4 ± 2.0	0.15	0.44	1.06 ± 0.08
10 Digoxin	18.4 ± 2.0	0.25	0.50	0.90 ± 0.07
11 Dihydro-digoxin	25.0 ± 4.7	0.24	0.51	40.90 ± 3.35
12 α-Methyldigoxin	56.6 ± 9.8	0.54	1.13	2.23 ± 0.11
13 β-Methyldigoxin	63.3 ± 20.9	0.59	1.19	1.55 ± 0.06
14 α.β-Dimethyldigoxin	138.0 ± 12.1	0.94	2.76	1.28 ± 0.06
15 α-Acetyldigoxin	54.6 ± 7.8	0.50	0.99	2.50 ± 0.31
16 β-Acetyldigoxin	99.2 ± 4.1	0.70	1.25	1.60 ± 0.02
17 α.β-Diacetyldigoxin	392.0 ± 82.0	1.16	4.31	1.27 ± 0.02
18 12-Acetyldigoxin	195.0 ± 17.5	0.89	1.60	20.90 ± 0.98
19 12-Acetyl.β-methyldigoxin	746.0 ± 71.3	1.26	4.04	25.55 ± 1.70
20 Gitoxigenin	44.1 ± 1.8	0.38	0.67	21.37 ± 0.97
21 Gitoxigenin-mono-digitoxoside	60.1 ± 10.0	0.46	0.83	2.56 ± 0.21
22 Gitoxin	48.0 ± 11.2	0.68	1.28	2.55 ± 0.14
23 16-Formylgitoxin	110.9 ± 6.2	0.84	1.82	0.78 ± 0.01
24 Pentaformylgitoxin	1440.0 ± 118.3	1.85	8.99	0.23 ± 0.00
25 16-Acetylgitoxin	144.0 ± 32.8	0.89	2.27	0.85 ± 0.01
26 Pentaacetylgitoxin	2890.0 ± 668.0	1.86	49.45	112.00 ± 10.70
27 k-Strophanthidin	3.60 ± 0.5	−0.14	0.42	17.80 ± 0.81
28 k-Strophanthin-α	4.40 ± 0.3	−0.09	0.79	1.29 ± 0.14
29 k-Strophanthin-β	0.11 ± 0.01	−0.50	0.22	2.51 ± 0.12
30 k-Strophanthin-γ	0.15 ± 0.07	−0.62	0.21	2.47 ± 0.25
31 Ouabain (g-strophanthin)	0.02 ± 0.00	−0.73	0.14	2.28 ± 0.10
32 Dihydro-ouabain	0.20 ± 0.00	−0.71	0.15	45.50 ± 3.90

The standard deviations for the R_m- and K'- values were negligible (< 4%) and have therefore been omitted.

the lipophilicity of cardiotonic steroids. On account of the advantages presented by the two chromatographic methods over that of the partition coefficient by the shake-flask method (i.e. their easy methodology, reproducibility and minimal time-consumption) both the more economical r.t.l.c. and the much more reproducible, precise (but expensive) r.h.p.l.c. should be used rather than the shake-flask method for this purpose. The only advantage of the latter method over the other two is probably the fact that partition coefficients are absolute values, whereas the chromatographic behaviour can only be expressed in relative terms.

The trend exhibited by the three lipophilicity dimensions also suggests an intimate relationship between the lipophilicity and the chemical structure of the cardiac glycosides. This means that the augmentation of lipophilicity with growing carbon chain is reduced by increasing, for example, the polarity of constituent sugar moieties, or even more effectively by attaching hydrophilic groups, such as the hydroxyl group, onto the steroid nucleus itself. It can therefore be concluded that a simple and direct relationship exists between lipophilicity and the chemical structure of the cardiotonic steroids.

As mentioned previously, apart from the physicochemical forces, the chemical structures of pharmacological agents are also involved to a great extent in drug-receptor interactions. The relevance of structural properties for the activity of cardiac glycosides becomes very apparent on examining closely the linear regression analysis of correlation between lipophilicity

and the inhibitory potencies of the glycosides. Taking into consideration all the compounds examined, the analysis gives a non-significant relationship, although some general increasing tendency of the potencies with growing lipophilicity is obvious (c.f. Table 1). It is also quite evident that, according to the hierarchy established for both parameters, the pharmacodynamic behaviour of both the 12-acetyldigoxin-derivatives and the cardanolides are much weaker than would be expected from their lipophilicity values. The behaviour of pentaacetylgitoxin is unique. All of its three lipophilicity values and its ID_{50}-value varied outstandingly from the rest. Thus, accordingly, it is not only the most lipophilic in nature, but shows also the weakest inhibitory tendency of all the glycosides studied. Similar results have been obtained in a recent kinetic investigation. Its K_D value was also found to be comparatively much lower than the rest of the cardiac glycosides examined (Brown *et al.*, 1985).

If these compounds are excluded from the analysis, significant correlations of the inhibitory actions with the individual lipophilicity parameters are obtained, as illustrated by the correlation between pD_2 and the R_m values of the glycosides (Figure 5).

Thus, two essential points emerge from the above analysis. Firstly, the lipophilicity of cardiotonic steroids increases linearly with growing carbon chain and is reduced by attaching hydrophilic groups, such as the hydroxyl-group onto the steroid nucleus, or by polar sugar moieties (see below). Secondly, although a linear interdependence exists likewise between the inhibitory potencies and the lipophilic nature of the cardiac glycosides, this relationship appears inconceivable without taking into consideration the role played by some of the structural components of the glycosides. In order to elucidate this, a comparative analysis of the interdependence of these three components, i.e. chemical structure, lipophilicity and the inhibitory activities of the glycosides, is briefly discussed under the following aspects: (a) aglycone, (b) sugar moieties and (c) lactone ring.

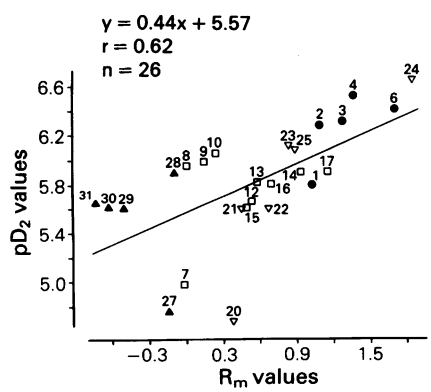

Figure 5 Correlation of lipophilicity and the inhibitory effects of cardiac glycosides on the myocardial Na^+-K^+-ATPase. Plotted are the pD_2 values against the R_m values. For code numbers beside each point see Table 1. (●) = digitoxigenin-; (▽) = gitoxigenin-; (□) = digoxigenin-; (▲) = strophanthus-derivatives.

Aglycone

Regarding digitoxigenin as the parent compound for the cardenolides studied, the following trends can be observed. Attaching a hydroxyl group at positions C_{12} (digoxin) or C_{16} (gitoxin) has a diminishing effect on both the lipophilicity and the inhibitory potencies. Much more conspicuous is the product of simultaneously adding a hydroxyl group at C_5 and the oxidation of C_{19}, resulting in strophanthidin, which is by far the most hydrophilic of the four aglycones. Its inhibitory activity is also very low.

The influence of steroid-derivation has been investigated at two positions, using the structural isomers, digoxin and gitoxin. Digoxin itself is less lipophilic but more potent than gitoxin. Acylation of the position C_{12}-OH (digoxin) almost abolishes whereas acylation of C_{16} (gitoxin) augments the inhibitory activity, despite the fact that lipophilicity is augmented in both cases (Figure 3, 4). These dissimilarities between the two isomers are probably a consequence of the difference in availability of the hydroxyl groups for the hydrogen bond induced by the structural conformation of the steroid nucleus. This means that the hydrogen bond forces of the hydroxyl groups render the compounds more hydrophilic than the parent aglycone, digitoxigenin and, assuming that the position C_{12} is more exposed to the complementary hydroxyl groups of the aqueous phase than the C_{16}, it would explain why digoxin is more hydrophilic than gitoxin.

Similarly, digoxin would undergo hydrogen bond interactions with the corresponding receptor more easily than gitoxin. The same arguments also explain why the inhibitory potencies of gitoxin-derivatives increase whereas those of digoxin-derivatives diminish with growing lipophilicity. Thus, whereas derivations of the sterically hindered C_{16}-OH increases lipophilicity and thereby probably acts synergistically in increasing the inhibitory activity, that of the exposed C_{12}-OH seems rather to be hindering this position from interacting with the receptor. In other words, attaching an alkyl group at C_{12}-OH would overwhelm its intrinsic enhancing potential towards the lipophilic binding forces of the steroid nucleus. For the sterically impeded C_{16}-OH, this counter effect is negligible.

Sugar moieties

While the moieties of the digitalis glycosides are composed of one type of sugar, i.e. digitoxose, those of the strophanthus glycosides studied comprise three different types, cymarose, rhamnose and glucose, whose polarities also increase in that order. From the points made previously, it is evident why the lipophilicity of the digitalis compound increases with growing sugar moieties and size of the substituents, whereas that of the strophanthus follow a different pattern altogether. Thus, increase in digitoxoside chain augments lipophilicity on the one hand, while attaching of more polar glucose to cymarin (strophantin-α) produces the less lipophilic strophanthin-β on the other. Furthermore, an additional glucose molecule (strophanthin-γ) has little influence.

The inhibitory effects of the mono-sugar derivatives on the myocardial Na^+ -K^+ -ATPase are about ten times as high as those of their respective aglycones. Whereas some slight increase in the activities takes place with growing digitoxoside moieties, addition of the glucose to cymarin produces a weakening effect. The same general trend has been established for the lipophilicity above.

Derivation of the sugar moieties was found to increase the lipophilicity of the compound. However, the inhibitory activities of the glycosides are slightly diminished. Again the hydrogen bond exerted by the hydroxyl group of the sugar moieties seems to be essential. Like the derivation of the steroid nucleus at C_{12}-OH, acylation of the hydroxyl groups of the sugar moieties weakens these forces but in a less marked manner. The slight increase in the activity of the sugar derivatives with growing size of the substituent is probably a result of augmenting lipophilicity.

Lactone ring

Hydration of the lactone ring is of little relevance for the lipophilicity of the cardiac glycosides, but has diminishing effects on their inhibitory potencies. This is further support for the theory that chemical structure plays a decisive role in the activity of the cardiotonic steroids.

The pharmacodynamics of cardiac glycosides are assumed to be embedded in the aglycone. Any structural change in the aglycone has been assumed in the past to be associated with weakening or diminishing of the inhibitory potencies of the cardiotonic steroids (Chen & Henderson, 1965; Saito *et al.*, 1970; Repke, 1972; Yoda & Yoda, 1977). Thus, the unsaturated lactone ring and the conformational structure of the steroid nucleus were regarded as the absolutely indispensible components. As mentioned previously, the cardiac Na^+-K^+-ATPase, is, as yet, the only identified receptor for the cardiotonic steroids. For the interaction between the cardiac glycosides and this enzyme, the model of Thomas *et al.* (1980) postulates that it is the binding of the aglycone itself (steroid nucleus and lactone ring) onto the enzyme that is essential for the activity of this class of drugs. The sugar moieties, in particular the one bound immediately to the aglycone, only serve to strengthen this bond. Although it is known today that the lactone ring itself, for example, is not absolutely essential (Jones & Middleton, 1970), the classical ideas are still proving to be a valuable basis for studying structure-activity relationships of the cardiac glycosides. From the present studies, it can be deduced that lipophilic binding forces are involved in the interaction between the glycosides and the Na^+ - K^+ -ATPase. This relationship is however subordinate to the influence of derivation or substitution of certain structural components of the glycosides.

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