EXPERIMENTAL RESULTS RELATING TO THE METABOLISM OF THE CARDIAC GLYCOSIDES

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The current state of investigations indicates that the essence of the metabolism of the cardiac glycosides in the living organism comprises six main processes: 1) the enzymatic hydrolysis of the sugar moieties of the glycosides [1-4]; 2) the epimerization of the aglycones at C_3 [5-6]; 3) hydroxylation [7, 8]; 4) the formation of conjugates with sulfuric and glucuronic acids; 5) reduction of the $-CHO$ group at C_{19} [9]; 6) deacetylation [10].

In view of the diversity of the metabolism of cardiac glycosides, we have studied the possible transformations of four pairs of these compounds differing in their genins, in the number and nature of the sugars that they contain, and in the substituents at C_{10} (Table 1). An investigation of the metabolism of erysimin was also performed.

The metabolism of the cardiac glycosides was studied at the stage of their passage through the liver and in the pathways of their excretion by the kidneys. In the bile and urine collected in the first 6 h after the administration of convallatoxin, securiside, erychroside, erysimin, and gofruside (in the first 3 h), paper chromatography showed the presence of the initial glycosides and of metabolites identified as convallatoxol, securisidol, erychrosol, and frugoside. During the same period in the bile and urine unchanged erysimin was found, together with three metabolites. One of them corresponded in its R_f value to helveticosol, the second was identified as the aglycone, and the third, highly polar and staying close to the starting line, we have not identified and have provisionally called it "metabolite M."

In the second 6 h (for gofruside, the second 3 h) only in the experiments with erychroside did we find two glycosides to be present – the initial glycoside and its metabolite erychrosol. Convallatoxin was found in the bile in trace amounts, and the intensity of the coloration of the spot showing the formation of convallatoxol was considerably greater. The kidneys excreted only convallatoxol. In the second set of samples, gofruside was present in the bile only in the form of traces, and frugoside was absent. We did not find the initial glycoside or its metabolite in the urine. Securiside was not found in the bile or the urine, either, while its metabolite was found both in the pathway of its passage through the liver and in the pathway of excretion by the kidneys. In the second checking period, neither the initial erysimin nor its metabolites could be determined either in the bile or in the urine.

Thus, the rates of conversion and excretion of the metabolites are similar for the two glycosides gofruside and erysimin. Convallatoxin and erychroside proved to be similar in the times of excretion of the metabolite and of the transformation of the main glycosides.

To show that the more polar metabolites formed are products of the reduction, and not of the oxidation, of the aldehyde group at C_{19}, we obtained samples of convallatoxin and erychroside oxidized by the method of W. Jacobs [11]. The results of the chromatography of the oxidized glycosides extracted from the biological media showed that the metabolites of convallatoxin and of erychroside appear at the same level as authentic samples of the forms of these glycosides reduced, and not oxidized, at C_{19}.

We performed further investigations with the same experimental arrangement to determine the metabolism of convallatoxol, erychrosol, securisidol, and frugoside, i.e., the forms of the corresponding glycosides reduced at C_{19}. It was found that at the stage of passage through the liver they underwent no changes in the first 6 h, and a single spot corresponding to the spot of an authentic sample of the initial glycoside

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TABLE 1

Glycoside	Genin	Substituent at C_{10}	Sugar component
Convallatoxin	Strophanthidin	$-CHO$	L-Rhamnose
Convallatoxol	Strophanthidol	$-CH_2OH$	L-Rhamnose
Erychroside	Strophanthidin	$-CHO$	D-Digitoxose D-Xylose
Erychrosol	Strophanthidol	$-CH_2OH$	D-Digitoxose D-Xylose
Securiside	Δ^4-Strophanthidin	$-CHO$	D-Xylose
Securisidol	Δ^4-Strophanthidol	$-CH_2OH$	D-Xylose
Gofruside	Corotoxigenin	$-CHO$	D-Allomethylose
Frugoside	Coroglaucigenin	$-CH_2OH$	D-Allomethylose
Erysimin	Strophanthidin	$-CHO$	D-Digitoxose

was found on the chromatogram. In the second six hours, no glycosides or their metabolites were found. It is possible that they were present in very small trace amounts which did not withstand isolation, purification, and determination.

To investigate the possible preformation of glycosides within the organism (with a prolonged residence time in the biological media studied), parallel experiments were performed with bile and urine collected from intact animals followed by the same treatment as was used in the previous experimental arrangement. No changes whatever in the glycosides due to contact with these media could be found.

The results obtained indicate that the most probable process for the transformation of the cardiac glycosides with an aldehyde group at C_{10} is their reduction with the formation of an alcohol group. This process imparts greater polarity to the metabolite, which affects the rate of excretion. However, together with the reduction of the sugar compoenent, its splitting off may take place, as was found in the experiment with erysimin. The formation of two other metabolites besides that produced by the reduction of the aldehyde group at C_{10} makes it possible to assume that the different processes of the transformation of the cardiac glycosides may take place both successively and in parallel.

A characteristic feature of the metabolism of gofruside – a high rate of transformation and of the excretion of the products obtained – is obviously responsible for its exceptionally brief cardiotonic effect.

A knowledge of the metabolism of a drug enables the most rational conditions for its use to be found.

EXPERIMENTAL

The glycosides were analyzed after appropriate purification and extraction from the biological media. The following solvent systems were used in the chromatographic investigations: 1) chloroform–tetrahydrofuran (1 : 1)/water; 2) benzene–butanol (1 : 1)/water; 3) benzene–butanol (2 : 1)/water; 4) toluene–butanol (1.5 : 1)/water; 5) methyl ethyl ketone–m-xylene (1 : 1)/formamide; 6) methyl ethyl ketone–benzene (1 : 1)/formamide; 7) ethyl acetate/water; 8) isoamyl alcohol/water.

In our investigations we used systems 1, 3, 7, and 8 for convallatoxin and convallatoxol, 1 and 5 for erychroside and erychrosol, 1 and 2 for gofruside and frugoside, 2, 3, and 4 for securiside and securisidol, and 1, 5, and 6 for erysimin.

The experiments were performed with 10 male albino rats weighing 250-300 g. The glycosides were administered intravenously in a dose of 1 mg/kg body weight of the animal. The urine and the bile were run off through polyethylene cannulas and were collected after 3, 6, 12, and 24 h. In the experiments on the metabolism at the stage or excretion by the kidneys, the animals were twice given an aqueous load (10 ml per rat). To collect the urine, the animals were kept in special cages from which the urine ran into receivers. The combined bile and urine were subjected to further treatment for the extraction and purification of the glycosides and possible metabolites by different methods for each substance studied. In addition to the experiments on rats, the changes in the glycosides studied were determined in control experiments with the addition of the cardenolides to bile and urinetaken from intact animals.

Convallatoxin. A. The bile collected after a predetermined interval of time was transferred to a separating funnel and made up with distilled water to 15 ml. To eliminate to the greatest possible extent components of the bile (pigments, etc.) capable of interfering in the chromatography of the glycosides, the aque-

ous solution of bile was treated twice with benzene–chloroform (1 : 4) in a shaking apparatus for 3-4 h. After the formation of layers, the benzene–chloroform fraction was run off and the aqueous phase containing the glycosides and possible metabolites was treated with mixtures of ethanol and chloroform (1 : 1), (1 : 2), and (1 : 3).

B. The urine collected in individual intervals of time was transferred to a separating funnel and treated with mixtures of ethanol and chloroform (1 : 1), (1 : 2), and (1 : 3) to extract the glycosides and possible metabolites. The combined and dried extracts were dissolved in 1.5 ml of ethanol–chloroform (1 : 3) and deposited on a column of alumina. The diameter of the column was 1.0 cm in all cases, and the weight of alumina used was 5-8 g. In all cases, purified commercial brands of alumina (Brockmann activities I and II) were used. The columns were first washed with 20-30 ml of chloroform for additional purification. The chloroform fraction was subjected to a qualitative reaction for the presence of the glycoside and its metabolites. The glycosides and the possible transformation products were eluted with mixtures of ethanol and chloroform (1 : 2) and (1 : 3) until glycosides could no longer be detected in the eluent. The combined extracts of the bile and the residues after the evaporation of the urine were dissolved in a small amount of ethanol–chloroform (1 : 3) for subsequent chromatography.

Erychroside and Erychrosol. A. In addition to the stage described for convallatoxin, the bile extract was purified on a column of alumina which was washed with 20-30 ml of chloroform. The glycosides and their metabolites were eluted with mixtures of ethanol and chloroform (1 : 1), (1 : 2), and (1 : 3).

B. The collected urine was sprinkled with an excess of zinc acetate to precipitate ballast substances; after 15-17 h it was filtered and the residue was washed thoroughly with ethanol–chloroform (1 : 2), the washings then being combined with the extracts. The clear solution of urine obtained after filtration treated by the method used for the bile.

Securiside and Securisidol. A. The purification of the bile differed from the method used for convallatoxin by the use of a mixture of benzene and chloroform (1 : 3). The glycosides were extracted from the bile in just the same way as in the experiments with convallatoxin. If emulsions difficult to separate were formed, the samples were centrifuged.

B. The urine was treated by the method described for erychroside.

Gofruside, Frugoside. A. The purification of the bile differed from the method used in the experiments with the other glycosides by the use of a mixture of benzene and chloroform (1 : 50). The glycosides were extracted with mixtures of ethanol and chloroform (1 : 2) and (1 : 3).

B. The collected urine was treated with zinc acetate. Further purification and extraction were performed by the method given for the bile.

Erysimin. A. The bile was placed in a separating funnel and treated with mixtures of ethanol and chloroform (1 : 2), (1 : 3), and (1 : 4) for 3-4 h in a shaking apparatus. The combined and dried extracts were deposited on an alumina filter and were eluted with the same mixtures of solvents under vacuum until the glycosides or metabolites had completely disappeared from the eluate. After drying, the residues were dissolved in a small amount of ethanol–chloroform for subsequent chromatography.

B. The collected urine was treated as described above and, after filtration, the washings were added to the subsequent extracts. The purified urine was extracted in a funnel with mixtures of ethanol and chloroform (1 : 2). (1 : 3), and (1 : 4) for 3-4 h. The preparation for chromatography was the same in all cases.

SUMMARY

1. The metabolism of nine cardenolides – convallatoxin, convallatoxol, erychroside, erychrosol, securiside, securisidol, gofruside, frugoside, and erysimin – has been studied at the stage of passage through the liver and in the pathways of excretion by the kidneys.

2. It has been found that the aldehyde at the C_{19} group of the cardiac glycosides studied is reduced to an alcohol group.

3. Erysimin differs in its metabolism from the other glycosides studied by the fact that in addition to the production of a form reduced at C_{19} it undergoes hydrolytic cleavage with the production of the genin. A third metabolite has not been identified and has provisionally been called metabolite M.

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