Inhibition of Sodium Transport in Frog Skin by Cardiac Glycosides of Different Molecular Structure

The cardiac glycosides are the specific inhibitors of NaK-ATPase which is involved in active Na transport[1]. In view of the variety of therapeutic effects of cardiac glycosides and key role in their cardiotonic and natriuretic effect of the inhibition of Na transport[2,3], a study on the interrelation between their structure and their influence an the Na pump is of particular interest.

The present investigation was carried out on the frog skin in which Na transport is dependent only on the pump sensitive to strophanthine and is completely inhibited by the latter[4].

Materials and methods. The experiments were performed on the isolated abdominal skin on winter *Rana temporaria* (♂). The active Na transport was measured by the short circuit current method[5] by means of an automatically compensating device[6]. All the glycosides were added to Ringers solution from the corium side.

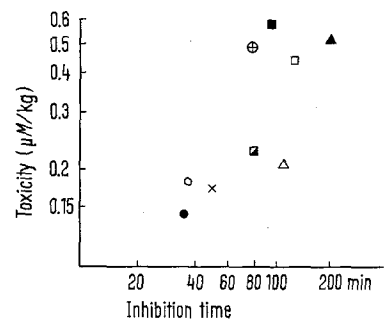
Results and discussion. A preliminary investigation of the inhibitory effect of cardiac glycosides in a concentration between 1×10^{-8}–$5\times10^{-5}M/l$ indicate that the dose $2.36\times10^{-6}M/l$ is the best for comparative evaluation of the effectiveness of preparations on Na transport; at this concentration all the preparations exhibit the inhibitory influence. The time period during which the initial Na transport decreased by 50% was used as an index of their inhibitory activity.

Among glycosides, K-strophanthin, K-strophanthosid and ouabain were found to be most effective, whereas sugarfree preparation, i.e. ouabagenine, exhibited the weakest effect on the sodium transport (Table).

The presence of acetyl radical in the molecule significantly increases the influence of a glycoside upon the Na transport, e.g., lanatoside C proved to be more active than desacetyl-lanatoside C and acetyldigitoxin acted more effectively than digitoxin (Table).

Higher activity of strophantine K and strophantoside K as compared with that of other preparations seems to depend on the presence of $-C\overset{O}{\underset{H}{}}$ group in position 19 of the molecule. Similar role of $-C\overset{O}{\underset{H}{}}$ group has been revealed in studies of the influence of glucosides on the Na transport in the frog muscle[7].

Since the cardiotonic effect of glycosides depends on their primary influence on the Na pump[2], it was of interest to compare the results obtained with the available data on the molar toxicity of the same preparations. In experiments on cats, MACHOVA[8] found positive correlation between the average lethal dose of glycosides and their effect on the active K transport in erythrocytes. A study of interrelation between the molar toxicity of preparations in cats[9] and inhibition of the Na pump in frog skin indicates the existence of positive correlation only for some of the preparations (Figure), especially for those



The relationship between the molar toxicity of the cardiac glycosides in cats and the inhibition of the sodium pump in frog skin. Ouabain (X); K-Strophantin (○); K-Strophantosid (●); Ouabagenin (▲); Lanatosid C (◪); Desacetyl-lanatosid C (△); Acetyl-digoxin (⊕); Acetyl-digitoxin (■); Digitoxin (□). Ordinate, the molar toxicity μM/kg, abscissa, the time to inhibite by 50% the Na transport by adding 2.36×10^{-6} M/l of the preparation.

[1] J. C. SKOU, Physiol. Rev. *45*, 596 (1965).
[2] F. Z. MEERSON, M. G. PSCHENNIKOVA, L. A. POGOSJAN, V. I. MASLYUK and N. V. KLYKOV, *Mechanism of Cardiotonic Action of Cardiac Glycosides* (Medicina, Moskow 1968), in Russian.
[3] J. A. NELSON and B. R. NECHAY, J. Pharmac. exp. Ther. *175*, 727 (1970).
[4] YU. V. NATOCHIN, Biofizika *11*, 626 (1966).
[5] H. H. USSING and K. ZERAHN, Acta physiol. scand. *23*, 110 (1951).
[6] V. V. IVANOV, YU. V. NATOCHIN, Fiziol. Zh. USSR *54*, 122 (1968).
[7] A. L. ABELES, J. gen. Physiol. *54*, 268 (1969).

The inhibition of active sodium transport by cardiac glycosides

Glycosides and aglycon	Modification of molecular structure					Sugar	R	Time for 50% inhibition of Na transport (min) M ± m
	5	11	12	14	19			
Ouabain	OH	OH	—	OH	CH_2OH	Rhamnose	—	51.0 ± 4.4
Ouabagenin	OH	OH	—	OH	CH_2OH	—	—	> 200
K-Strophanthin	OH	—	—	OH	$C\overset{O}{\underset{H}{}}$	Glucose-cymarose	—	38.8 ± 2.5
K-Strophanthosid	OH	—	—	OH	$C\overset{O}{\underset{H}{}}$	Glucose(2)-cymarose	—	38.2 ± 3.2
Lanatosid C	—	—	OH	OH	CH_3	Digitoxose (3)-glucose	CH_3CO	79.3 ± 13.5
Desacetyl-lanatosid C	—	—	OH	OH	CH_3	Digitoxose (3)-glucose	—	110.0 ± 9.1
Acetyldigoxin	—	—	OH	OH	CH_3	Digitoxose (3)	CH_3CO	76.7 ± 12.7
Digitoxin	—	—	OH	—	CH_3	Digitoxose (3)	—	125.0 ± 14.3
Acetyldigitoxin	—	—	OH	—	CH_3	Digitoxose (3)	CH_3CO	86.6 ± 5.9

used by MACHOVA. However, in a number of preparations this correlation was not observed (Figure).

These data imply that either glycoside = sensitive Na pumps are of different structure in cardiac muscle of cats and frog skin or the interaction of some glycosides with Na, K-ATPase in the whole organism differs from that in isolated biological membranes of frog skin.

ВЫВОДЫ. Сердечные гликозиды с $C\overset{O}{\underset{H}{<}}$ В 19-ОМ положении молекулы оказались наиболее эффективными ингибиторами натриевого насоса клеток кожи лягушки. Наличие ацетильного радикала увеличивало ингибирующую способность гликозида; агликон - оуабагенин оказался самым слабым инибитором.

Y. V. NATOCHIN and E. A. LAVROVA

Sechenov's Institute of Evolutionary Physiology and Biochemistry, Thorez pr. 52, Leningrad K-223 (USSR), 3 January 1972.

[8] J. MACHOVA, Experientia *16*, 553 (1960).
[9] G. BAUMGARTEN, *Die herzwirksamen Glykoside. Herkunft, Chemie und Grundlagen ihrer pharmakologischen und klinischen Wirkung* (Thieme, Leipzig 1963).
[10] We are indepted to Sandoz AG (Basel) for generous supplies of cardiac glycosides.

Effect of Pyruvate on the Acute Cyanide Poisoning in Mice

It is well known that, besides other collateral effects, e.g. block of the –SH groups, the acute toxicity of cyanide is due to its capacity to bind the terminal oxidase of the mitochondrial respiratory system[1].

At present, the most used antidotes to cyanide poisoning are: a) compounds able to produce methaemoglobin, such as sodium or amyl nitrite[2,3], *p*-aminopropiophenone and methylene blue[3] or b) thiosulfate[2,3], which is substrate for the enzyme rodhanase. The reason for employing the first groups of compounds is based on the fact that the competition between methaemoglobin and cytochrome oxidase for cyanide makes possible the formation of the non-toxic cyanmethaemoglobin. However, since the cytochrome oxidase-cyanide complex is much less dissociable than cyanmethaemoglobin[4], a high level of methaemoglobinemia is required. When thiosulfate is used, rodhanase catalyzes the following reaction: $CN^- + -S$ compounds $\rightleftharpoons CNS^-$[3]. The resulting thiocyanate is not toxic. The use of such antidote has also some limitations due to the presence of rodhanase almost exclusively in the tissues, so that its activity is restricted to the free cellular cyanide[3]. In addition, the rodhanase activity is counteracted by the activity of thiocyanate oxidase[3]. For a more detailed description of the present treatment of the acute cyanide intoxication, see DONE[3].

In a recent paper we presented evidence for the activity of pyruvate in removing quickly the inhibition of respiration induced by cyanide, leaving unaffected the integrity of oxidative phosphorylation of Ehrlich ascites cells in vitro[5]. The effect of this compound was attributed to its reaction with cyanide which leads to the formation of the non-toxic pyruvic-cyanhydrin. On this experimental basis and because of all the difficulties in the present therapy of acute cyanide intoxication, we thought it worth testing the effectiveness of sodium pyruvate on the cyanide poisoning in mice. The results presented here show that pyruvate is able to remove, even in vivo, the binding of cyanide to cytochrome oxidase. Moreover pyruvate, since is not toxic at the doses used and lowers significantly the lethality by cyanide, appears to be an antidote relatively more suitable than the others described above.

Materials and methods. Male albino mice of the Swiss strain weighing 23–25 g were used in all experiments. The animals were housed at random in stock cages, in groups of 12, and fed with a standard balanced diet and tap water ad libitum. The drugs were supplied to animals fasted 6 hours. NaCN, dissolved in bidistilled water, was given i.p.; sodium pyruvate, dissolved in sterile 0,9% NaCl, was injected i.v. 30 sec after NaCN. Groups of

[1] O. WARBURG, *Heavy Metal Prosthetic Groups and Enzyme Action* (Oxford University Press, London 1949).
[2] K. K. CHEN and C. L. ROSE, J. Am. med. Ass. *162*, 1154 (1956).
[3] A. K. DONE, Clin. Pharmac. Ther. *2*, 750 (1961).
[4] H. G. ALBAUM, J. TEPPERMAN and O. BODANSKY, J. biol. Chem. *163*, 641 (1946).
[5] A. CITTADINI, T. GALEOTTI and T. TERRANOVA, Experientia *27*, 633 (1971).

Effect of sodium pyruvate, injected i.v., on the mortality induced by cyanide in mice[c]

Treatment	% Mortality (cumulative values) $n = 12$[a] NaCN mg/Kg i.p.													LD_{50}	Limits (95% confidence)	PR[b]
	3.50	4.50	4.75	5.00	5.25	5.50	6.00	7.00	7.25	7.50	8.00	8.50	9.00			
Controls	25	41.7	67.7	91.7	100	100	100	100	100	—	—	—	—	4.785	(4.650–4.923)	
Sodium pyruvate 250 mg/kg i.v.	—	—	—	0	0	0	8.3	66.7	83.3	100	100	—	—	6.705	(6.487–6.931)	0.71
Sodium pyruvate 500 mg/kg i.v.	—	—	—	—	0	0	0	0	0	25	58.3	66.7	100	7.914	(7.565–8.280)	0.60

[a] Number of animals tested at each dose level. [b] LD_{50} NaCN/LD_{50} NaCN + sodium pyruvate. The slopes of all lines compared did not deviate significantly. [c] For the experimental conditions see materials and methods.