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Sugar moiety of cardiac glycosides is essential for the inhibitory action on the palytoxin-induced K^+ release from red blood cells

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Palytoxin (PTX), a highly toxic and sugar-containing substance isolated from *Palythoa tuberculosa*, caused K^+ release from rabbit red blood cells. Cardiac glycosides, such as ouabain, convallatoxin, cymarin, digoxin and digitoxin, inhibited the PTX-induced K^+ release. Their corresponding aglycones did not inhibit the K^+ release, but antagonized the inhibitory effect of the glycosides. All these cardiotonic steroids equally inhibited the activity of $(Na^+ + K^+)$-ATPase prepared from hog cerebral cortex. These results suggest that the sugar moiety of the cardiac glycosides is important for the inhibitory effect on the K^+ release induced by PTX and that the inhibition is not related to their inhibitory potency on the $(Na^+ + K^+)$-ATPase activity.

Palytoxin *Cardiac glycoside* *K^+ release* *Red blood cell* *$(Na^+ + K^+)$-ATPase*

1. INTRODUCTION

Palytoxin (PTX; $C_{129}H_{223}N_3O_{54}$), isolated from marine coelenterates of some zoanthid species (genus *Palythoa*), is the most potent animal toxin known to date [1]. The stereochemistry of PTX has recently been determined [2,3]. PTX is uniquely distinct from the molecules in terms of magnitude of molecular size, structural complexity, etc. It consists of a long partially unsaturated aliphatic chain, interspaced with 5 sugar moieties.

The biological actions of PTX have been extensively studied (for references see [4]). At concentrations of a few nanomolar, it causes depolarization of cardiac, smooth and skeletal muscles and nerve tissues. It also causes release of K^+ from red blood cells and smooth muscles. It has been reported that the effect of PTX is inhibited by ouabain in smooth muscles [5] and red blood cells [6,7]. Authors in [6,7] have suggested that PTX increases the ion permeability by altering the state of $(Na^+ + K^+)$-ATPase or its environment.

Here, to improve the understanding of the interaction of PTX with $(Na^+ + K^+)$-ATPase, we have investigated the effects of various cardiac glycosides and aglycones on the effects of PTX in red blood cells in relation to their inhibitory potency on $(Na^+ + K^+)$-ATPase activity.

2. MATERIALS AND METHODS

Red blood cells from rabbit heparinized (30 IU/ml) blood were washed 3 times in 5 vols physiological salt solution (PSS) (NaCl, 136.9 mM; $CaCl_2$, 1.0 mM; $MgCl_2$, 1.0 mM; glucose, 5.5 mM; Hepes, 10 mM; pH 7.3) by centrifugation at 3000–4000 rpm for 5 min. The buffy coat was removed by aspiration. Packed cells were resuspended in PSS (200000–300000 cells/ml) and stored at 4°C. Under these conditions, no apparent K^+ loss was observed for at least 5 h. The loss of K^+ after the addition of PTX at 37°C was measured using a K^+-selective electrode (Philips, IS 561 K) at 37°C. Maximum release of K^+ was achieved by adding saponin (10 μg/ml) for 10 min.

$(Na^+ + K^+)$-ATPase from hog cerebral cortex prepared as in [8] was purchased from Sigma. The specific activity of $(Na^+ + K^+)$-ATPase was

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0.6–0.7 μmol P_i released/mg protein per min at 37°C. The enzyme preparation was incubated in a solution containing NaCl (100 mM), KCl (20 mM), $MgCl_2$ (5 mM), ATP (5 mM) and Tris–HCl (50 mM) at pH 7.4. The final volume of the solution was 0.5 ml. The enzyme preparation was preincubated in the absence of ATP for 5 min and then the reaction was started by the addition of ATP. The amount of P_i liberated during the 15 min incubation was determined as in [9]. The activity of $(Na^+ + K^+)$-ATPase was calculated by the difference between the quantity of P_i liberated in the presence and absence of Na^+ and K^+.

PTX isolated from *Palythoa tuberculosa* was kindly donated by Dr Y. Hirata (Meijo University, Nagoya). The toxin was dissolved in distilled water to prepare a 10^{-4} M stock solution, which was kept frozen at −20°C. Other drugs used were ouabain (Merck), ouabagenin, convallatoxin, cymarin, strophanthidin, digoxin, digoxigenin (all from Sigma), digitoxin, digitoxigenin (both from Aldrich), saponin (ICN), melittin (Sigma), and nigericin (Calbiochem). The water-insoluble drugs were dissolved in ethanol, methanol or dimethylformamide, or a combination of these. The final volume of the organic solvents was less than 0.2%. The solvents alone did not affect the K^+ release. For the experiments on $(Na^+ + K^+)$-ATPase, water-insoluble drugs were dissolved in acetone or dimethylformamide and diluted with the incubation solution.

3. RESULTS AND DISCUSSION

PTX at concentrations above 10^{-10} M produced rapid loss of K^+ from rabbit red blood cells in a concentration-dependent manner (ED_{50} = 5 × 10^{-10} M). Ouabain (ouabagenin monorhamnoside) at a concentration as low as 10^{-7} M inhibited the PTX-induced K^+ release (fig.1). The K^+ release due to nigericin (10^{-6} M), saponin (10 μg/ml) and melittin (10^{-6} M) was not affected by ouabain (2 × 10^{-5} M), suggesting that the inhibition by ouabain is specifically directed against the action of PTX. Other monoglycosides, such as convallatoxin (strophanthidin monorhamnoside) and cymarin (strophanthidin monocymaroside) more potently inhibited the K^+ release due to PTX (10^{-9} M). Triglycosides, such as digoxin (digox-

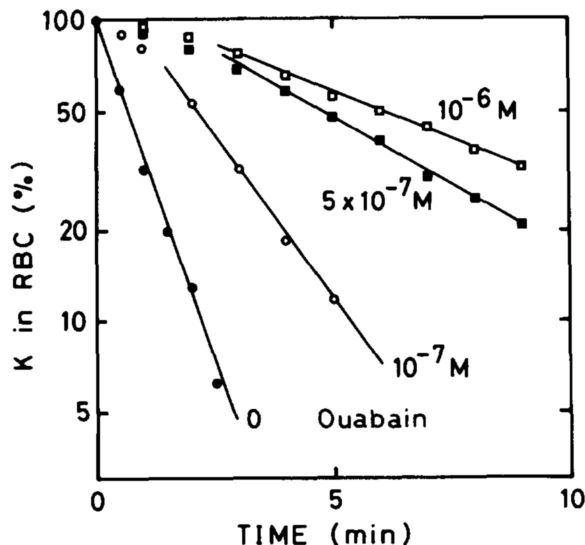

Fig.1. Inhibitory effect of various concentrations of ouabain on K^+ release induced by PTX. Red blood cells were incubated with PTX (2.5 × 10^{-9} M) and various concentrations of ouabain: (●) 0, (○) 10^{-7} M, (■) 5 × 10^{-7} M, (□) 10^{-6} M). Ordinate, K^+ remaining in red blood cells (%). Abscissa, time (min).

Table 1

Inhibitory effect of various cardiotonic steroids on K^+ release induced by PTX (A) and $(Na^+ + K^+)$-ATPase activity (B)

	(A) IC_{50} for K^+ release (M)	(B) IC_{50} for $(Na^+ + K^+)$-ATPase (M)
Ouabain	2.3 × 10^{-6}	7.6 × 10^{-7}
Convallatoxin	9.0 × 10^{-7}	8.4 × 10^{-7}
Cymarin	4.2 × 10^{-7}	3.4 × 10^{-7}
Digoxin	9.0 × 10^{-5}	5.9 × 10^{-7}
Digitoxin	8.8 × 10^{-5}	1.5 × 10^{-7}
Ouabagenin	*	n.d.
Strophanthidin	*	8.0 × 10^{-7}
Digoxigenin	*	3.4 × 10^{-7}
Digitoxigenin	*	2.1 × 10^{-7}

Red blood cells were incubated with PTX (10^{-9} M) and various concentrations of cardiotonic steroids for 30 min. $(Na^+ + K^+)$-ATPase was incubated with cardiotonic steroids and ATP (5 mM) for 15 min. Note that IC_{50} value for aglycones could not be calculated (*). n.d., not determined

igenin tridigitoxoside) and digitoxin (digitoxigenin tridigitoxoside), were less potent than ouabain. IC_{50} values of the cardiac glycosides for the action of PTX are summarized in table 1. In contrast, ouabagenin and strophanthidin at 10^{-5} M produced no inhibition of the PTX-induced K^+ release. Digoxigenin and digitoxigenin (10^{-5} M) also failed to inhibit the K^+ release. These results suggest that the sugar moiety of cardiac glycosides is important for the inhibitory effect on the PTX-induced K^+ release.

Fig.2 shows the effect of cymarin (10^{-6}–10^{-4} M) on PTX-induced K^+ release in the absence or presence of its aglycone, strophanthidin (10^{-5} M). In the presence of strophanthidin, the dose-response curve for the inhibitory effect of cymarin was shifted to the right. These results suggest that the binding of strophanthidin inhibits the binding of cymarin to the red blood cell membrane and thus the effect of cymarin is decreased.

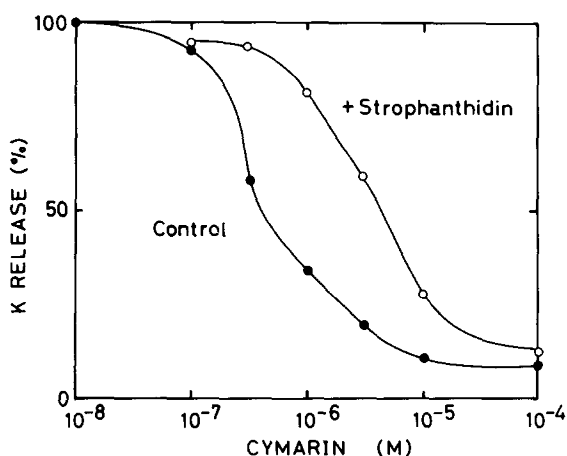

Fig.2. Antagonism by strophanthidin of the inhibitory effect of cymarin on PTX-induced K^+ release. Red blood cells were preincubated with various concentrations of cymarin (10^{-8}–10^{-4} M) and strophanthidin (10^{-5} M) for 5 min. PTX (10^{-9} M) was then added for 20 min to induce K^+ release. (●) Cymarin alone; (○) strophanthidin + cymarin. Ordinate, amount of K^+ release (%); abscissa, time (min).

Finally, the effect of these cardiotonic steroids on $(Na^+ + K^+)$-ATPase activity was investigated. Ouabain, convallatoxin, cymarin, digoxin and digitoxin, and their corresponding aglycones inhibited the activity of $(Na^+ + K^+)$-ATPase prepared from hog cerebral cortex with IC_{50} values of 10^{-7}–10^{-6} M (table 1). These results suggest that the inhibitory effect of cardiac glycosides on PTX-induced K^+ release is not related to their inhibition of the $(Na^+ + K^+)$-ATPase.

It has been suggested that the binding sites for PTX and ouabain are not identical but share some common sites [6]. One possibility is that the binding sites for PTX and ouabain overlap at the sugar binding site on $(Na^+ + K^+)$-ATPase or the neighbouring structure, since the PTX molecule also contains sugar moieties. The other possibility is that cardiac glycoside induces allosteric transitions in $(Na^+ + K^+)$-ATPase and decreases the binding affinity for PTX, and that the sugar moiety is essential for this transition.

REFERENCES

[1] Moore, R.E. and Sheuer, P.J. (1971) Science 172, 495–497.

[2] Moore, R.E., Baltolini, G., Brchi, J., Bothner-By, A.A., Dadok, J. and Ford, J. (1982) J. Am. Chem. Soc. 104, 3776–3779.

[3] Cha, J.K., Christ, W.J., Finan, J.M., Fujioka, H., Kishi, Y., Klein, L.L., Ko, S.S., Leder, J., McWhorter, M.M., Pfaff, K.P., Yonaga, M., Uemura, D. and Hirata, Y. (1982) J. Am. Chem. Soc. 104, 7369–7371.

[4] Ozaki, H., Nagase, H., Ito, K. and Urakawa, N. (1984) Jap. J. Pharmacol. 34, 57–66.

[5] Ishida, Y., Shibata, S., Satake, N., Habon, J. and Kitano, H. (1981) Blood Vessels 18, 216.

[6] Habermann, H. and Chhatwal, G.S. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 319, 101–107.

[7] Chhatwal, G.S., Hessler, H.J. and Habermann, H. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 323, 261–288.

[8] Nakao, T., Tashima, Y., Nagano, K. and Nakao, M. (1965) Biochem. Biophys. Res. Commun. 19, 755–758.

[9] Martin, J.B. and Doty, D.M. (1941) Anal. Chem. 21, 965–967.