Palytoxin Isolated from Marine Coelenterates

THE INHIBITORY ACTION ON (Na,K)-ATPase*

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Palytoxin (PTX), C₁₂H₂₃N₃O₆S, a highly toxic substance isolated from zoanthids of Palythoa tuberculosa, inhibited (Na,K)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) prepared from guinea pig heart and hog cerebral cortex in a dose-dependent manner at concentrations >10⁻⁸ M. In the presence of Na (100 mM) and K (20 mM), PTX showed potency nearly equal to that of ouabain. When the ATPase was activated by the various Na concentrations at a constant K concentration, both PTX and ouabain inhibited the ATPase activity noncompetitively. On the other hand, when K concentration was changed at a constant Na concentration, PTX caused a competitive inhibition in all ranges of K concentrations employed, whereas ouabain caused a competitive inhibition at low concentrations and a noncompetitive inhibition at high concentrations.

PTX¹ was reported to be an extremely poisonous substance from marine coelenterates of the genus Palythoa (1). It was isolated from the zoanthid "liru-make-o-Hana" (Palythoa toxica). The 50% lethal dose (LD₅₀) was 0.15 μg/kg in mice by intravenous injection. In monkeys, the value of LD₅₀ was reported to be extremely small, 0.08 μg/kg intravenously (2). Extracts from Palythoa caribaeorum and Palythoa mamilosa, which inhabit shallow waters off the shores of the Caribbean Islands, also caused the toxic response (3). Further, toxic Palythoa tuberculosa was found in tropical and subtropical coral reefs of Okinawa, Amami-Oshima, Marcus, and Tahiti islands (4). The toxicity is strong in eggs and also in other tissues of the female during maturation of ovary and, at the peak of toxicity, 1 g of egg is able to kill two tons of mice (4). Pigs have been reported to die after eating filefish, Altura scripta, which have fed on the toxic zoanthid (4).

The structure of PTX has recently been determined (5–7). PTX is one of the most complicated and largest naturally occurring molecules known (M, = 2660–2680) except for the naturally occurring polymers.

PTX was reported to have a powerful positive inotropic effect on heart muscle (8, 9). Cardiac glycosides such as ouabain are well known to cause a cardiotoxic action and have been beneficial for patients having cardiac failure. The mechanism of action of the glycosides is thought to be by the inhibition of (Na,K)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity (10, 11). Further, PTX was suggested to cause a contraction of intestinal smooth muscle, similar to ouabain (12). It has recently been reported that in the presence of ouabain the effect of PTX was inhibited in smooth muscles (13, 14) and erythrocytes (15), indicating some interaction between ouabain and PTX. Therefore, in the present experiment, the effect of PTX on the (Na,K)-ATPase activity was investigated.

MATERIALS AND METHODS

(Na,K)-ATPase was prepared from guinea pig heart as described by Pitts and Schwartz (16). (Na,K)-ATPase from hog cerebral cortex prepared by the method of Nakao et al. (17) was purchased from Sigma. Specific activities of (Na,K)-ATPase were 1.8–2.5 μmol of Pi/min for heart ATPase and 0.8–1.2 for the cerebral cortex at 37 °C. The enzyme preparation was incubated in a solution containing 100 mM NaCl, 20 mM KCI, 5 mM MgCl₂, 5 mM ATP, and 50 mM Tri-HCl at pH 7.4 (37 °C). The final volume of the solution was 0.5 ml. The enzyme preparation was preincubated in the absence of ATP for 10 min and then the reaction was started by addition of ATP. PTX or ouabain was added 5 min before the addition of ATP. The amount of Pᵢ liberated during the 15-min incubation was determined by the method of Martin and Doty (18).

Activity of (Na,K)-ATPase was calculated by the difference between the quantity of Pᵢ, liberated in the presence and absence of Na and K. In the absence of Na and K, Mg-activated ATPase activity was about 5% of total activity. Ouabain, 10⁻⁴ M, almost completely inhibited (Na,K)-ATPase activity in both heart and cerebral cortex preparations. Protein was determined by the method of Lowry et al. (19) with bovine serum albumin as the standard.

Ouabain and Tri-ATP was purchased from Merck and Sigma, respectively. PTX isolated and purified as described by Hirata et al. (20) was donated by Dr. Y. Hirata of the Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan. PTX (10⁻⁴ M) was stored in small amounts (0.2–0.5 ml) at −70 °C. Each small amount of solution was only once used for experiments since freezing and thawing reduced effectiveness of PTX by one third.

RESULTS

PTX and ouabain inhibited activities of (Na,K)-ATPases prepared from guinea pig heart and hog cerebral cortex (Fig. 1). The ID₅₀ (50% inhibition of the maximum activity stimulated by Na and K) of PTX was 3.1 × 10⁻⁸ M in the heart and 9.0 × 10⁻⁷ M in the cortex enzyme. ID₅₀ of ouabain was 2.4 × 10⁻⁶ M in the heart and 1.3 × 10⁻⁶ M in the cortex enzyme. PTX and ouabain at concentrations of 10⁻⁵ M did not affect basal Mg-activated ATPase activities of heart and cortex preparations.

The enzyme preparation of hog cerebral cortex was activated when external Na concentration was changed at a constant K concentration of 20 mM. A Hill plot (21) for the Na activation gave a Hill coefficient of about 1.5, indicating positive cooperative binding of Na to the enzyme. Therefore, the relative velocity (V) of the reaction was tentatively expressed as Vᵦ/Vₙ and an Eadie-Hofstee plot (22, 23) was employed to analyze the data (Fig. 2). PTX and ouabain caused parallel shifts of the plots of the lower value of Vᵦ/Vₙ, indicating they both had a noncompetitive inhibitory effect on the Na activation. Apparent Kᵢ values (dissociation constant of the inhibitor-enzyme complex) were calculated to be 7.1 μM for PTX and 4.3 μM for ouabain.

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* The abbreviation used is: PTX, palytoxin.
were the same as described in Fig. 1. When K concentration was changed at a constant Na concentration of 100 mM, the enzyme of the cortex was also activated in a concentration-dependent fashion. Hunter-Downs plots (24) were employed to examine inhibitory effects of PTX and ouabain (Fig. 3). Fig. 3a shows that PTX, 5 × 10⁻⁷ and 1 × 10⁻⁶ M, yields a straight line with an appreciable slope in the whole range of K concentrations employed, suggesting that PTX is competitive with K. On the other hand, Fig. 3b suggests that ouabain, 5 × 10⁻⁷ and 1 × 10⁻⁶ M, is competitive at low (<10 μM) concentrations of K but approaches noncompetitive inhibition at higher concentrations of K. Apparent Kᵰ values for competitive inhibition were 0.027 μM for PTX and 0.019 μM for ouabain.

**DISCUSSION**

Crude PTX from *P. caribaeorum* has previously been reported to cause inhibition of (Na,K)-ATPase of electroplax due to a contamination by 5-hydroxytryptamine (25, 26) and the purified PTX had no apparent effect on the ATPase activity of erythrocytes up to concentrations of 5 × 10⁻⁵ M (15). 5-Hydroxytryptamine inhibited the ATPase activity at concentrations more than 10⁻⁴ M with the ID₅₀ of about 10⁻³ M (26), which values are about 1000 times greater than those of PTX from *P. tuberculosa* in the present experiment. Furthermore, the purity of PTX used in these experiments was verified through various analyses including that by nuclear magnetic resonance (20, 27). Therefore, it is unlikely that the PTX used here contains 5-hydroxytryptamine. PTX was reported to be very unstable (1, 27). It was also reported that chemical modification of PTX to N-acetyl-PTX reduced the biological activity by one hundredth (28). Thus, the discrepancy between present and previous experiments might be attributed to the instability of PTX or to a small difference of the chemical structure of the preparations used.

The present work demonstrates that PTX inhibits the activity of (Na,K)-ATPase prepared from heart and cerebral cortex. In the presence of both Na and K, ID₅₀ values for PTX and ouabain were nearly equal, about 10⁻⁶ M. Both PTX and ouabain caused a noncompetitive inhibitory effect on the Na activation of the ATPase at a constant K concentration with Kᵰ values of about 5 μM. Furthermore, PTX and ouabain caused competitive inhibition of the K activation induced by low K concentration at a constant Na concentration with similar Kᵰ values of about 0.02 μM. Therefore, it is indicated that PTX inhibits the activity of (Na,K)-ATPase with nearly equal potency to that of ouabain, although the chemical structure is quite different.

When the K concentration was changed at a constant Na concentration, PTX caused a competitive inhibition in all ranges of K concentrations employed. On the other hand, ouabain caused a competitive inhibition at low (<10 μM) concentrations and a noncompetitive inhibition at high concentrations as reported previously (29, 30). The aglycone of ouabain, ouabagenin was also reported to cause the similar dual inhibitory effect on the K activation of the ATPase (31). Therefore, it is suggested that, compared with ouabain, PTX causes a simple competitive inhibition on the K-activated site of (Na,K)-ATPase.

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