Intracellular Ca^{2+}-calmodulin system involved in the palytoxin-induced K^+ release from rabbit erythrocytes

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Palytoxin (PTX) caused K^+ release from rabbit erythrocytes which was dependent on the concentrations of extracellular Ca^{2+} and PTX. In a Ca^{2+}-free solution, PTX still caused a slow K^+ release. An intracellular Ca^{2+} antagonist, TMB-8, an intracellular Ca^{2+} chelator, quin 2, and calmodulin inhibitors, prenylamine, W-7 and W-5, inhibited the PTX-induced K^+ release in a Ca^{2+}-free solution. These results suggest that the PTX-induced K^+ release is dependent on the process including intracellular Ca^{2+} and calmodulin.

Palytoxin *K^+ release* *Ca^{2+}* *Calmodulin*

1. INTRODUCTION

Palytoxin (PTX), isolated from marine coelenterates of the genus *Palythoa*, is one of the most poisonous animal toxins [1]. It has been reported that PTX increases K^+ efflux and Na^+ influx in erythrocytes, smooth muscle and pheochromocytoma cells [2–5]. In the preceding paper, we suggested that the PTX-induced K^+ release from erythrocytes is mediated by a Ca^{2+}-calmodulin system [6]. Since Chhatwal et al. [7] suggested that external Ca^{2+} is not essential for the PTX-induced K^+ release, we examined the role of intracellular Ca^{2+} on the PTX-induced K^+ release from rabbit erythrocytes using an intracellular Ca antagonist, TMB 8 [8], an intracellular Ca chelator, quin 2 [9], and calmodulin inhibitors, prenylamine [10], W-7 and W-5 [11].

2. MATERIALS AND METHODS

Rabbit erythrocytes were prepared as reported in [5]. Loss of K^+ from erythrocytes was continuously determined at 37°C using a K^+-selective electrode (Philips, IS 561 K). Since calmodulin inhibitors changed the sensitivity of the K^+-selective electrode, erythrocytes tested with these compounds were incubated with PTX for 18 min at 37°C, the reaction was stopped by a 2 min centrifugation at 650 × *g* at 25°C and the K^+ content of the supernatant was measured with a Hitachi type 208 flame photometer. The amount of maximum releasable K^+ was determined by adding saponin (10^{-5} g/ml) at the end of each experiment. Physiological salt solution (PSS) contained (mM): 136.9 NaCl, 1.0 $CaCl_2$, 1.0 $MgCl_2$, 5.5 glucose and 10 Hepes at pH 7.4. Ca^{2+}-free solution (below 5×10^{-7} M Ca^{2+}) was made by adding EGTA.

PTX isolated from *P. tuberculosa* (kindly donated by Dr Y. Hirata, Meijo University, Nagoya) was dissolved in a solution containing 0.1% bovine serum albumin and 2 mM Hepes (pH 7.0) at 10^{-4} M, stored at −20°C and diluted just before use. 2-[(2-Bis[carboxymethyl]amino-5-methylphenoxy)methyl]-6-methoxy-8-bis[carboxymethyl]aminoquinolinetetrakis[acetoxymethyl] ester (quin 2-AM, Sigma) and prenylamine (Hoechst) were dissolved in dimethyl sulfoxide (DMSO). Nigericin (Calbiochem) was dissolved in ethanol. 8-(*N,N*-Diethylamino)octyl 3,4,5-trimethoxybenzoate (TMB-8, generous gifts from Tanabe), *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfon-

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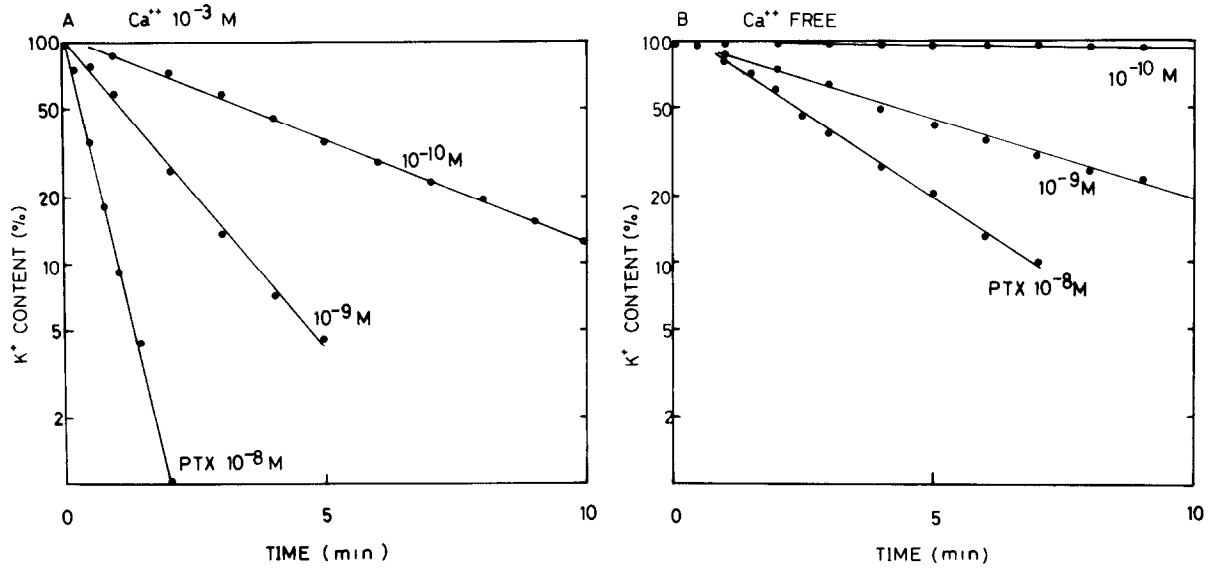

Fig.1. PTX-induced K^+ release in the presence or absence of extracellular Ca^{2+}. PTX was added after a 10 min preincubation period at 37°C in the presence of 10^{-3} M Ca^{2+} (A) or in the absence of Ca^{2+} (B). K^+ release was measured with a K^+-selective electrode. Abscissa, time (min); ordinate, K^+ content in erythrocytes (%). 100% represents the amount of total releasable K^+ measured by the addition of 10^{-5} g/ml saponin.

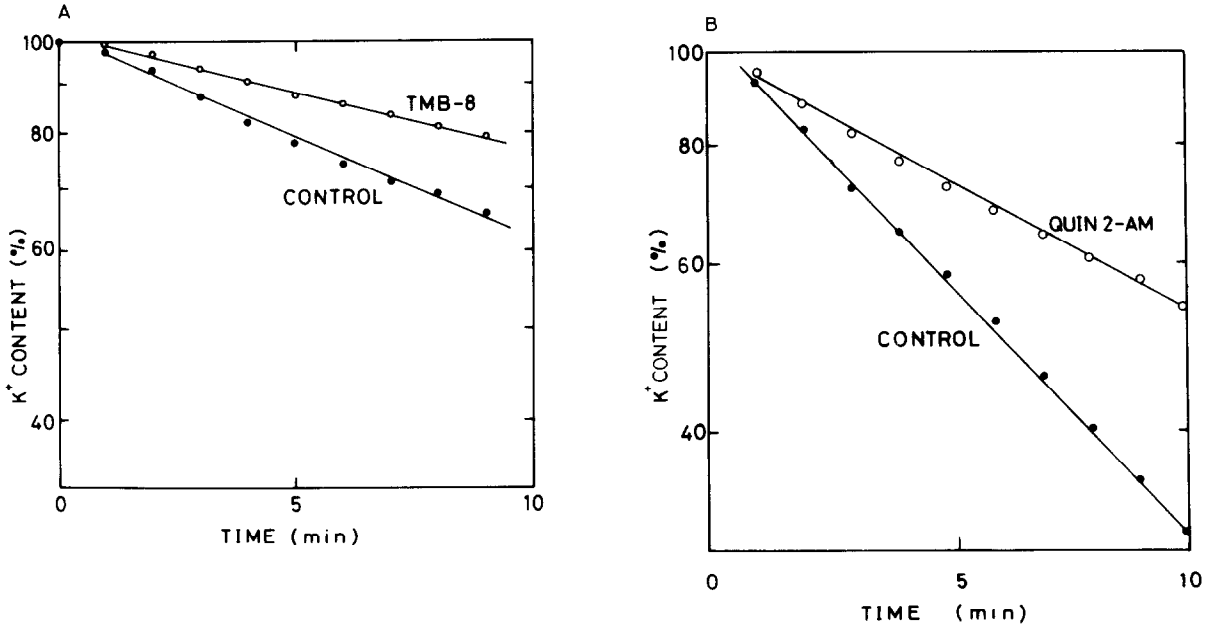

Fig.2. Effects of TMB-8 and quin 2-AM on the PTX-induced K^+ release in a Ca^{2+}-free solution. (A) 10^{-4} M TMB-8 was added 10 min before the application of 10^{-9} M PTX. (B) Erythrocytes were preincubated with a Ca^{2+}-free solution containing 10^{-4} M quin 2-AM at 37°C for 1 h, washed 3 times with a Ca^{2+}-free solution and then 10^{-9} M PTX was applied. In the control experiment, erythrocytes were treated with 1% DMSO alone. Abscissa, time (min); ordinate, K^+ content in erythrocytes (%).

amide (W-7), *N*-(6-aminohexyl)-1-naphthalenesulfonamide (W-5) (both from Rikaken) and saponin (ICN) were dissolved in PSS.

3. RESULTS

PTX caused K^+ release from erythrocytes in the presence of 10^{-3} M Ca^{2+} (fig.1A). The rate constants of K^+ release due to 10^{-10}, 10^{-9} and 10^{-8} M PTX were 0.21, 0.66 and 2.34 min^{-1}, respectively. The rate of K^+ release was markedly retarded in a Ca^{2+}-free solution (fig.1B); the rate constants due to 10^{-10}, 10^{-9} and 10^{-8} M PTX were 0.0075, 0.17 and 0.36 min^{-1}, respectively. In contrasts, the rates of K^+ release induced by saponin (10^{-5} g/ml) and nigericin (10^{-9} M) were independent of extracellular Ca^{2+} concentrations (not shown).

Fig.2 shows the effects of TMB-8 (10^{-4} M) and quin 2-AM (10^{-4} M) on the rate of K^+ release induced by PTX (10^{-9} M) in a Ca^{2+}-free solution. Although the control rate constants were slightly different, both TMB-8 and quin 2-AM decreased them to 56.8 and 51.7%, respectively.

Fig.3 shows the effects of prenylamine, W-7 and W-5 in a Ca^{2+}-free solution. The K^+ release due to PTX (10^{-9} M) was inhibited to 10.6% by prenylamine, 13.3% by W-7 and 66.6% by W-5.

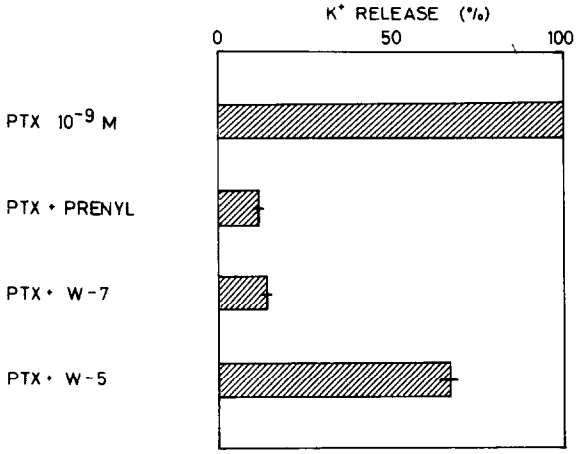

Fig.3. Inhibitory effects of prenylamine, W-7 and W-5 on the PTX-induced K^+ release in the absence of extracellular Ca^{2+}. Erythrocytes were preincubated for 10 min with a Ca^{2+}-free solution with or without 3×10^{-5} M prenylamine, 10^{-4} M W-7 or 10^{-4} M W-5. The amount of K^+ released during a 20 min PTX incubation period was measured by flame photometry. Mean ± SE of 4 experiments is shown.

4. DISCUSSION

In the present experiment, we confirmed the result by Chhatwal et al. [7] that the PTX-induced K^+ release is not completely inhibited in the absence of extracellular Ca^{2+}. However, this result does not necessarily indicate that Ca^{2+} is not involved in the effect of PTX since cellular bound Ca^{2+} in erythrocytes is resistant to the incubation with a Ca^{2+}-free solution [12]. In fact, the PTX-induced K^+ release in a Ca^{2+}-free solution was inhibited by an intracellular Ca antagonist, TMB-8, and an intracellular Ca^{2+} chelator, quin 2. Further, the calmodulin inhibitors, prenylamine and W-7, inhibited the PTX-induced K^+ release in the absence of extracellular Ca^{2+} whereas W-5, an analog of W-7 with lower affinity to calmodulin [11], showed smaller inhibition. Similar inhibitory potencies of these calmodulin inhibitors were reported in the presence of extracellular Ca^{2+} [6]. These results suggest that PTX mobilizes cellular bound Ca^{2+} and activates a Ca^{2+}-calmodulin system to induce K^+ release from rabbit erythrocytes.

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