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EFFECTS OF PHOSPHOLIPASES C ON THE β-RECEPTOR–ADENYLATE CYCLASE SYSTEM OF CHICK ERYTHROCYTE MEMBRANES

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Abstract—The β-adrenergic receptor located in chick erythrocyte membranes was characterized using (−)-[^{3}H]-dihydroalprenolol ([^{3}H]-DHA) with rapid filtration techniques. The affinity of β-adrenergic antagonist, (−)-propranolol, was approximately 100-fold higher than that of (+)-propranolol. Catecholamines were bound with the receptor in the following order, (−)-isoproterenol > (−)-norepinephrine > (−)-epinephrine, suggested the binding site to be β_1-classification.

When the membrane preparation was treated with phosphatidylcholine-hydrolyzing phospholipase C (PCase) of *Clostridium perfringens* or phosphatidylinositol-specific phospholipase C (PIase) of *Bacillus thuringiensis*, [^{3}H]-DHA binding was decreased to the level of 66 or 86% of the control, respectively. The treatment with sphingomyelinase C (SMase) of *Bacillus cereus*, however, did not cause any appreciable reduction of [^{3}H]-DHA binding. Throughout these experiments, the equilibrium dissociation constant (K_D) of [^{3}H]-DHA was not influenced by phospholipases C.

The affinity of isoproterenol for β-receptor was decreased in the absence of GTP, but not altered in the presence of GTP by PIase action. Treatment with PCase or SMase, however, did not affect the affinity of isoproterenol for β-receptor.

Treatment with PIase inhibited basal, isoproterenol-stimulated and forskolin-stimulated adenylate cyclase activities. On the other hand, PCase treatment inhibited only isoproterenol-stimulated adenylate cyclase activity, but not basal and forskolin-stimulated activities.

These results suggest that membrane phospholipids, especially phosphatidylcholine (PC) and phosphatidylinositol (PI), are directly related to the receptor binding and that PI interacts with adenylate cyclase activity.

Phospholipases A_2, C and D are known to act on biomembranes, and hence are useful tools for elucidating the structure of the membrane and the function of phospholipids. Among these phospholipases, phospholipase C of bacterial origin is one of the most frequently used enzymes. Three kinds of bacterial phospholipases C have been reported [1]: phosphatidylcholine-hydrolyzing phospholipase C (EC 3.1.4.3, PCase*), which catalyzed the hydrolysis of phosphatidylcholine to diglyceride and phosphorylcholine, sphingomyelinase C (EC 3.1.4.12, SMase), which specifically hydrolyzed sphingomyelin into ceramide and phosphorylcholine, and phosphatidylinositol-specific phospholipase C (EC 3.1.4.10, PIase), which specifically hydrolyzed phosphatidylinositol into diglyceride and myoinositol-1,2-cyclic phosphate. The actions of these phospholipases C on erythrocyte membranes have been extensively investigated [2–9]. Taguchi and Ikezawa [2] reported that PCases from *Clostridium perfringens*, *Clostridium novyi* and *Pseudomonas aureofaciens* cause hemolysis of horse and sheep erythrocytes through the hydrolytic action on choline-containing phospholipids such as PC and SM. SMases from *Staphylococcus aureus* [3] and *Bacillus cereus* [4, 5] hydrolyze sphingomyelin on the outer surfaces of sheep and bovine erythrocytes, resulting in so-called "hot–cold hemolysis" of these cells. PIases from *Bacillus thuringiensis* [6, 7] and *S. aureus* [8, 9] evoke the release of acetylcholinesterase from mammalian erythrocytes as well as from *Torpedo* electric organ. These results indicate the specific roles of phospholipids in the maintenance of membrane functions.

There have been many reports investigating the effects of several phospholipids on the receptor–adenylate cyclase system of various erythrocytes or tissues by use of phospholipases or other methods [10–17]. According to McOsker *et al.* [10], PI incorporation into turkey erythrocytes inhibited adenylate cyclase activity by uncoupling β-adrenergic receptors from the remainder of the cyclase complex. Rodbell and his collaborators [11–13] examined the effects of phospholipase A_2 and phospholipase C on the glucagon–receptor complex. They reported that phospholipase A_2 treatment of rat liver plasma membrane resulted in concomitant losses of glucagon binding and of activation of cyclase by glucagon plus GTP, and that treatment with *B. cereus* phospholipase C which hydrolyzed PS and PI completely abolished glucagon-stimulated cyclase activity, whilst *Cl. perfringens* PCase was without effect.

* Abbreviations used: PCase, phosphatidylcholine-hydrolyzing phospholipase C; SMase, sphingomyelin-hydrolyzing phospholipase C; PIase, phosphatidylinositol-specific phospholipase C; PC, phosphatidylcholine; SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; [^{3}H]-DHA, (−)-[^{3}H]-dihydroalprenolol; DTT, dithiothreitol; K_D, equilibrium dissociation constant; B_{max}, total amount of binding site.

According to Low and Finean [14], the treatment of turkey erythrocytes with *S. aureus* PIase brought about the decrease in catecholamine-stimulated cyclase activity, but had no effect on either basal or fluoride-stimulated cyclase activity.

The purpose of the present work is to examine the effects of phospholipases C such as PCase, SMase and PIase on the β-receptor–adenylate cyclase system of chicken erythrocyte membranes, in order to investigate a link between membrane phospholipids and the receptor binding–adenylate cyclase system.

MATERIALS AND METHODS

Phospholipases C and chemicals. PCase from the culture medium of *Clostridium perfringens* type A (PB6K) was kindly supplied by Dr Yamakawa and Dr Ohsaka of the National Institute of Health in Tokyo. This enzyme preparation formed a single precipitation line with antitoxin in the double-diffusion method, and was more than 90% pure as indicated by SDS-disc electrophoresis. SMase was purified from the culture filtrate of *Bacillus cereus* IAM 1208 by the method of Tomita *et al.* [18]. PIase was purified from the culture filtrate of *Bacillus thuringiensis* IAM 12077 by the method of Ikezawa *et al.* [19]. Both SMase and PIase were purified to homogeneous state as revealed by polyacrylamide gel electrophoresis. The specific activities of PCase, SMase and PIase were 300, 512 and 480 units/mg, respectively. PC was purified from egg yolk and SM was from bovine brain, by silicic acid column chromatography according to the procedure described by Spanner [20]. PI was purified from an autolysate of baker's yeast by the method of Trevelyan [21] and by silicic acid column chromatography. (−)-[^{3}H] Dihydroalprenolol (88–97 Ci/mmole) was obtained from Amersham International plc (Amersham, U.K.). (+)-Propranolol and (−)-propranolol were kindly donated by ICI Pharmaceuticals. (−)-Isoproterenol hydrochloride, (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate and phentolamine were purchased from Sigma Chemical Company (London, U.K.). Glass-fiber filters (type GF/F) were purchased from Whatman (Maidstone, U.K.). YAMASA Cyclic AMP Assay Kit was purchased from Yamasa Shoyu K.K. All other chemicals used were of analytical reagent grade unless otherwise stated.

Protein determination. Protein was determined according to the method of Lowry *et al.* [22] with bovine serum albumin as a standard.

Membrane preparation. Membrane preparation from chick erythrocytes was prepared as described by Vauquelin *et al.* [23] with the following modifications. Chicken erythrocytes were washed three times with 150 mM NaCl in 10 mM Tris–HCl, pH 7.8, in order to remove platelets and white cells. Washed cells were lysed in 10 vol. of ice-cold buffer containing 2 mM $MgCl_2$ in 5 mM Tris–HCl, pH 7.8 (buffer A) for 10 min, and centrifuged at 1000 *g* for 15 min. The lysis procedure was repeated twice. The pellet was resuspended in buffer A, and was homogenized with a glass–Teflon homogenizer, thereafter layered over 35% sucrose (w/w) and centrifuged at 1200 *g* for 10 min. The upper layer was collected and the membranes were sedimented by centrifugation for 15 min at 30,000 *g*. The final pellet was suspended in buffer A as the membrane preparation for assay (3 mg of protein/ml).

Phospholipase C treatment. The membrane preparation was suspended at 1 mg protein/ml in buffer A and incubated for 30 min at 37° with PCase (1.7 μg/ml), SMase (1 μg/ml), PIase (1 μg/ml) and in the presence of 1 mM $CaCl_2$ when treated with PCase. At these concentrations, more than 90% of membrane phospholipids (PC, PE, SM and PI) were hydrolyzed specifically by PCase, SMase and PIase, respectively. In the control run (phospholipase C-untreated membranes), the glycerol–Tris buffer, pH 7.5, was added instead of phospholipase C. After incubation, the reaction mixture was placed in an ice-bath and the membranes were washed with buffer A by centrifugation at 30,000 *g* for 20 min.

Assay of adenylate cyclase activity. Adenylate cyclase assay was performed as described by Salomon's method [24] modified by McOsker *et al.* [10], in which unlabeled ATP was used. To the assay buffer containing 5 mM creatine phosphate, 50 units/ml creatine phosphokinase, 25 mM Tris–acetate buffer, pH 7.6, 5 mM acetate, 1 mM ATP, 1 mM DTT, 0.1 mg/ml BSA and 0.01 mM GTP, was added the membrane preparation (300 μg protein) and 1.3 mM 1-isobutyl-3-methylxanthine. The mixture (final volume, 200 μl) was incubated at 37° for 10 min. Incubations were terminated by addition of 50 μl of 0.1 N HCl. Then, cAMP was extracted and determined by the YAMASA Cyclic AMP Assay Kit.

Equilibrium binding of [^{3}H]-*DHA.* The membrane preparation (300 μg protein) was incubated with increasing concentration of [^{3}H]-DHA (1–100 nM) in buffer A for 10 min at 30° in a final volume of 200 μl. At the end of incubation, triplicate 50 μl aliquots were diluted in 4 ml of ice-cold buffer A, and filtered under reduced pressure through Whatman GF/F glass-fiber filters (2.5 cm dia.). Filters were washed rapidly with 10 ml of ice-cold buffer A. Then filters were placed in scintillation vials with 1 ml of 1 N HCl and 10 ml of scintillation fluid (PPO 4 g, dimethyl POPOP 0.1 g, Triton X-100 300 ml, toluene 700 ml), and the radioactivity was determined. Non-specific binding was determined in incubations which contained 1 μM (−)-propranolol and was less than 40% of total binding at 100 nM [^{3}H]-DHA used.

Displacement of [^{3}H]-*DHA by β-adrenergic agonist, antagonist and α-adrenergic agonist.* The membrane preparation (300 μg protein) was incubated in a final assay volume of 200 μl which contained 5 mM Tris–HCl, pH 7.8, 10 nM [^{3}H]-DHA and various concentrations of the agonist and the antagonist. The procedures of [^{3}H]-DHA binding were the same as those described above.

RESULTS

Characteristics of [^{3}H]-*DHA binding to chick erythrocyte membranes*

As shown in Fig. 1, β-adrenergic antagonists, (+), (−)-isomers of propranolol, competed with [^{3}H]-DHA binding to chick erythrocyte membranes. (−)-

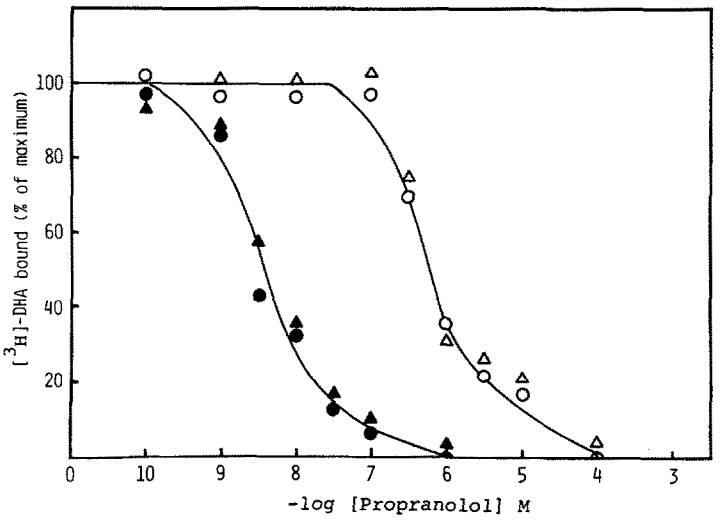

Fig. 1. Competition of propranolol against [^{3}H]-DHA binding. Chick erythrocyte membranes (300 μg of protein) were incubated at 37° with 10 nM [^{3}H]-DHA in the presence of varying concentrations of (−)-propranolol (●, ▲) or (+)-propranolol (○, △). After incubation for 30-min, the reaction mixtures were filtered and radioactivity in the glass-fiber filters was determined. Circle symbols stand for the untreated membranes and triangle symbols for the PIase-treated membranes. Values were the means for three experiments in triplicate.

Propranolol was 100 times as potent as its isomer, (+)-propranolol, showing that the [^{3}H]-DHA binding site was stereoselective (Table 2). As shown in Fig. 2 and Table 2, other adrenergic agonists competed with [^{3}H]-DHA binding to chick erythrocyte membranes in the following order of K_i: (−)-isoproterenol (96 ± 9 nM), (−)-norepinephrine (4800 ± 460 nM) and (−)-epinephrine (9600 ± 950 nM). The α-adrenergic antagonist, phentolamine, showed only weak affinity. These results indicated that the [^{3}H]-DHA binding site is a β-receptor belonging to β_1-classification.

Table 1. Binding parameters for [^{3}H]-DHA to phospholipases C-treated chick erythrocyte membranes

Membranes	Enzyme (μg/mg membrane protein)	B_{max} (fmol/mg protein)	K_D
Untreated		102 ± 6.3	9.3 ± 0.81
PIase treated	1.0	88 ± 4.1	10.7 ± 0.95
PCase-treated (+1 mMCa^{2+})	1.7	67 ± 5.2	11.2 ± 1.02
SMase-treated	1.0	97 ± 3.5	10.2 ± 0.89

Binding parameters were calculated from Scatchard plot of Fig. 3(b). B_{max} stands for the total amount of binding sites. K_D stands for the equilibrium dissociation constant. The values given were the means ± SEM for five experiments performed in triplicate.

Table 2. Effects of β-adrenergic agonists and antagonists on [^{3}H]-DHA binding to untreated and phospholipases C-treated chick erythrocyte membranes

Drugs	K_i (nM)			
	Untreated	PIase-treated	PCase-treated	SMase-treated
(−)-Propranolol	2.4 ± 0.15	2.1 ± 0.2	1.9 ± 0.2	2.3 ± 0.2
(+)-Propranolol	380 ± 42	370 ± 38	360 ± 35	410 ± 42
(−)-Isoproterenol	96 ± 9	480 ± 46	105 ± 10	98 ± 9
(−)-Isoproterenol + 100 μM GTP	970 ± 92	930 ± 89	960 ± 93	980 ± 97
(−)-Norepinephrine	4800 ± 460	N.D.	N.D.	N.D.
(−)-Epinephrine	9600 ± 950	N.D.	N.D.	N.D.

The values of K_i were calculated from Figs. 1, 2, 3 and 4, using the equation of Cheng and Prusoff [34]. Values were the mean ± SEM of three individual experiments performed in triplicate.

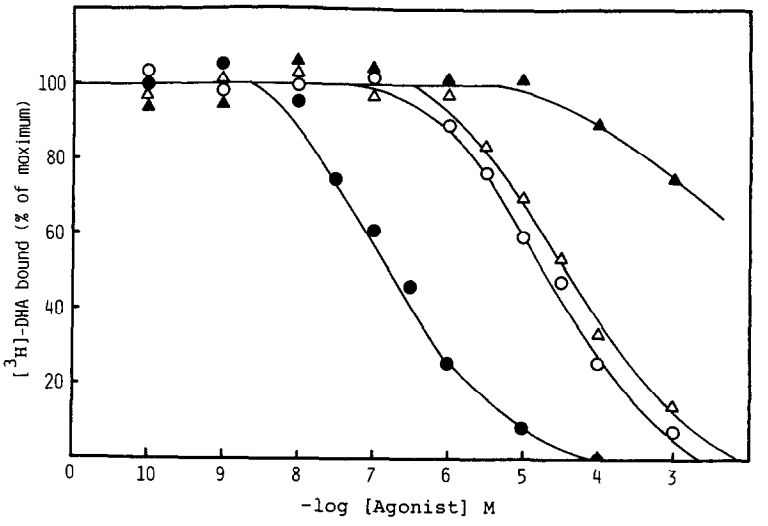

Fig. 2. Competition of β-adrenergic agonists or α-adrenergic antagonist against [^{3}H]-DHA binding. Chick erythrocyte membranes (300 μg of protein) were incubated at 37° with 10 nM [^{3}H]-DHA in the presence of varying concentrations of (−)-isoproterenol (●), (−)-norepinephrine (○), (−)-epinephrine (△) and phentolamine (▲). After incubation for 30-min, radioactivity was determined as described in Fig. 1. Values were the means for three experiments in triplicate.

Effects of phospholipases C on [^{3}H]*-DHA binding to the* β_1*-adrenergic receptors of chick erythrocyte membranes*

The specific binding of [^{3}H]-DHA to chick erythrocyte membranes was a saturable process. As shown in Fig. 3, specific binding reached a plateau upon increasing the free concentration of [^{3}H]-DHA. The maximal number of binding sites (B_{max}) and the equilibrium dissociation constant (K_D) were determined by Scatchard analysis [25] of the saturation binding data. B_{max} was found to be 102 fmol/mg of membrane protein and K_D was 9.3 nM (Table 1).

When chick erythrocyte membranes were incubated at 37° for 30 min with *Cl. perfringens* PCase, *B. cereus* SMase or *B. thuringiensis* PIase, B_{max} was decreased to the level of 65.7, 95.1 or 86.3% of the untreated membranes, respectively (Fig. 3 and Table 1), but K_D remained unchanged by the treatments with these phospholipases C. Ca^{2+}, added at 1 mM

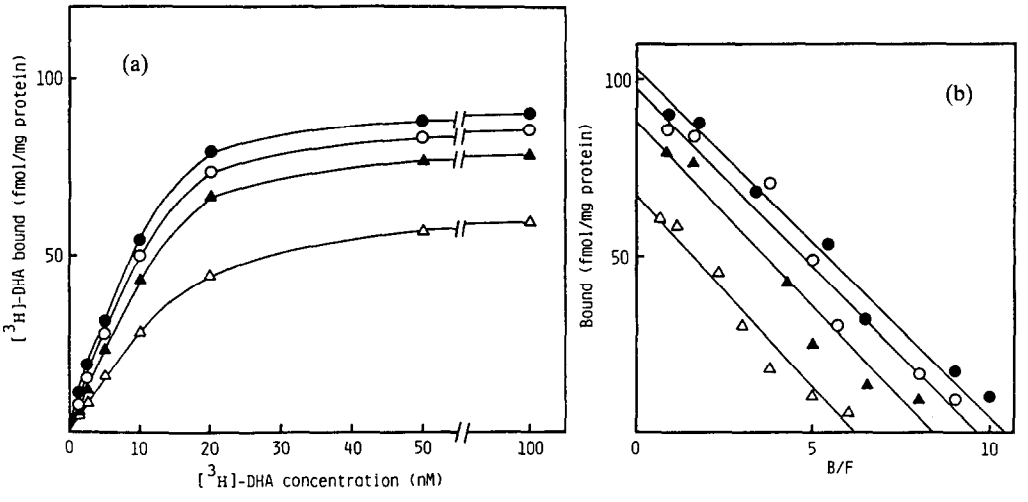

Fig. 3. (a) [^{3}H]-DHA-binding saturation curves of phospholipase C-treated chick erythrocyte membranes. Chick erythrocyte membranes were incubated at 37° with increasing concentrations of [^{3}H]-DHA ranging from 1 to 100 nM. After incubation, radioactivity was determined as described in Fig. 1. Non-specific binding was determined in incubations containing 1 μM (−)-propranolol. Values were the means for five experiments in triplicate. (b) Scatchard plot of the saturation-binding data in (a). ●, Untreated membranes; △, PCase-treated membranes; ○, SMase-treated membranes; ▲, PIase-treated membranes.

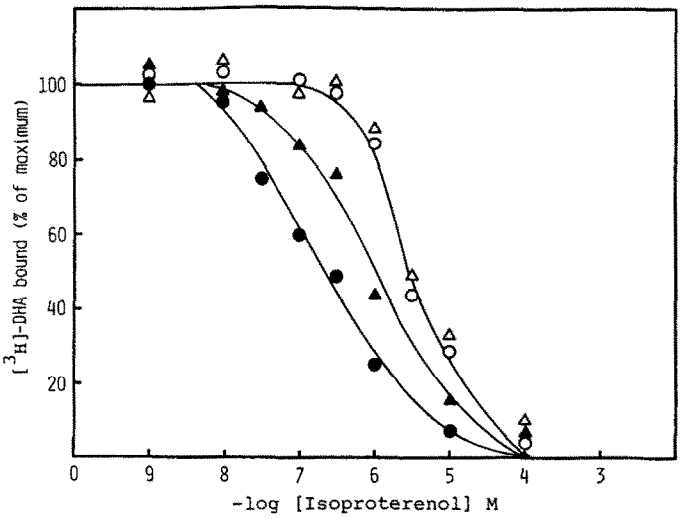

Fig. 4. Competition of isoproterenol against [^{3}H]-DHA binding to untreated or PIase-treated membranes. Chick erythrocyte membranes (300 μg of protein) were incubated at 37° with 10 nM [^{3}H]-DHA and the indicated concentrations of isoproterenol either in the presence (○, △) or absence (●, ▲) of 100 μM GTP. After incubation, radioactivity was determined as described in Fig. 1. Values were the means for three experiments in triplicate. Circle symbols stand for the untreated membranes and triangle symbols for the PIase-treated membranes.

in the treatment with PCase, was without effect on K_D or B_{max} values in contrast with control mixture containing 2 mM $MgCl_2$ in 5 mM Tris–HCl, pH 7.8. As reported previously [6, 7, 29, 30], *B. thuringiensis* PIase caused the release of alkaline phosphatase, 5′-nucleotidase, alkaline phosphodiesterase I and acetylcholinesterase from animal tissues, cell homogenates and erythrocytes. However, by the treatment with phospholipases C, [^{3}H]-DHA binding was not observed in the supernatant after centrifugation of the reaction mixture (data not shown).

Effects of phospholipases C on the binding of propranolol and isoproterenol to chick erythrocyte membranes

The treatment of chick erythrocyte membranes with PIase did not affect the affinity curves for (+), (−)-propranolol binding observed in Fig. 1 and Table 2. Also, as shown in Table 2, the treatment with PCase or SMase was without effect on K_i values for propranolol binding.

Figure 4 and Table 2 show that PIase treatment

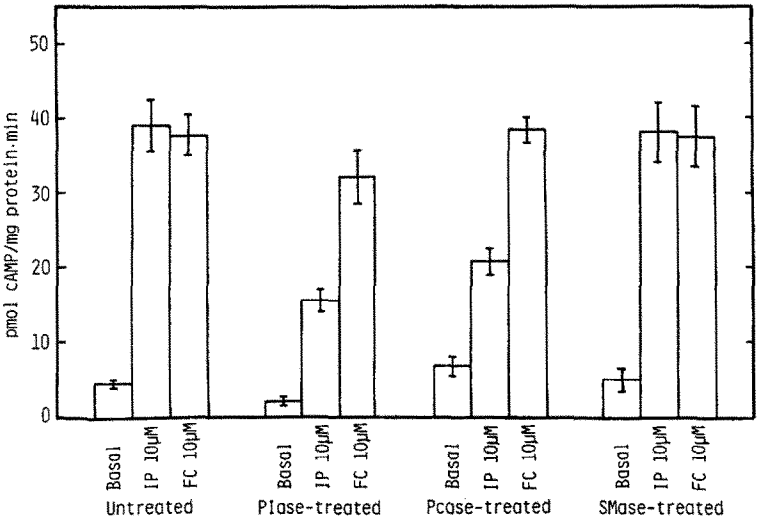

Fig. 5. Effects of phospholipases C on adenylate cyclase activity. Chick erythrocyte membranes (300 μg of protein) were incubated at 37° for 10 min with 1-isobutyl-3-methylxanthine and assay buffer, then for further 15 min and 10 μM isoproterenol or forskolin. After incubation, cAMP was extracted and determined by YAMASA Cyclic AMP Assay Kit. Values were the means for five experiments in triplicate.

did not change the K_i value for isoproterenol binding in the presence of 100 μM GTP. In the absence of GTP, however, PIase treatment increased K_i values five-fold. Treatments with PCase and SMase did not alter K_i values for isoproterenol binding in the absence or presence of GTP.

Effects of phospholipases C on adenylate cyclase activities

As shown in Fig. 5, basal, isoproterenol-stimulated and forskolin-stimulated adenylate cyclase activities of chick erythrocyte membranes (the left-hand columns) were 4.5 ± 0.4, 39.2 ± 3.5 and 37.8 ± 2.9 pmole cAMP/mg protein × min, respectively. When the membranes were treated with PIase, basal, isoproterenol- and forskolin-stimulated activities were decreased to 1.9 ± 0.2, 15.4 ± 1.4 and 32.1 ± 3.1 pmole cAMP/mg protein × min, respectively. When the membranes were treated with PCase, isoproterenol-stimulated activity was decreased to 21.3 ± 2.0 pmole cAMP/mg protein × min. However, basal and forskolin-stimulated activities remained unchanged by treatment with PCase. SMase did not significantly affect these adenylate cyclase activities.

Figure 6 shows the dose–response curves for isoproterenol-stimulated adenylate cyclase activity in the untreated, PIase-treated and PCase-treated chick erythrocyte membranes. Maximal cAMP accumulation was significantly decreased by PCase and PIase treatments (Fig. 6a). As shown in Fig. 6b, the response to isoproterenol for each curve in Fig. 6a was plotted as a percentage of the maximal stimulation. There was no appreciable difference in the concentrations of isoproterenol required for half-maximal stimulation of cAMP accumulation between the untreated (ED_{50}: 0.15 ± 0.01 μM) and PCase-treated membranes (ED_{50}: 0.20 ± 0.01 μM). However, in PIase-treated membranes, ED_{50} of isoproterenol (0.98 ± 0.08 μM) became slightly higher. These results indicated that the number of receptors capable of stimulating adenylate cyclase activity was decreased in PCase-treated membranes, and that PIase might alter the receptor affinity or the catalytic subunit of adenylate cyclase.

DISCUSSION

There have been many reports concerning the role of membrane phospholipids on the receptor–adenylate cyclase system. Panagia *et al.* [16] have revealed the PIase action on the adenylate cyclase activity of rat heart sarcolemma. They reported that complete hydrolysis of PI in sarcolemma was associated with a marked loss of the basal adenylate cyclase activity. Rubalcava and Rodbell [12] have reported that treatment with *B. cereus* phospholipase C (probably a mixture of PCase and PIase) hydrolyzing PS and PI completely inhibited glucagon-stimulated adenylate cyclase activity, but that the *Cl. perfringens* PCase had no effect. Also, Low and Finean [14] reported that treatment of turkey erythrocyte membranes with *S. aureus* PIase decreased catecholamine-stimulated adenylate cyclase activity, but was without effect on either basal or fluoride-stimulated activity. These results indicate that negatively charged phospholipids such as PI and PS are necessary in the maintenance of adenylate cyclase activity.

In the present work, we have tried to find the phospholipid(s) playing an important role in receptor binding and adenylate cyclase activity in the chick

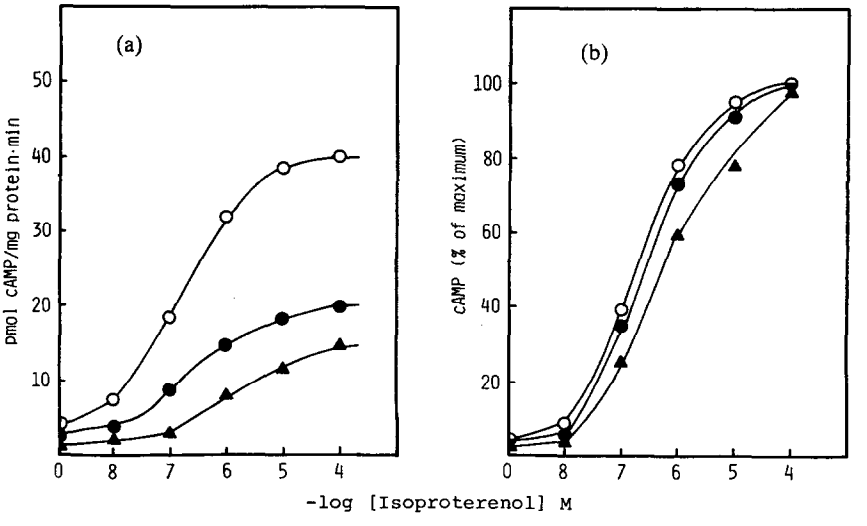

Fig. 6. Dose–response curves for isoproterenol stimulation of adenylate cyclase activity. (a) Chick erythrocyte membranes (300 μg of protein) were incubated at 37° for 10 min with 1-isobutyl-3-methylxanthine and assay buffer, then for further 15 min with varying concentrations of isoproterenol. After incubation, cAMP was extracted and determined by YAMASA Cyclic AMP Assay Kit. (b) The response to isoproterenol in each curve (Fig. 6(a)) was replotted as a percentage of the maximal stimulation. ○, Untreated membrane; ●, PCase-treated membrane; ▲, PIase-treated membrane. Values were the means for three experiments in triplicate.

erythrocyte membranes. [^{3}H]-DHA binding site of chick erythrocytes proved to be a β_1-classification. However, Dickinson and Naorski [35] reported that chick erythrocytes possess a homogeneous population of receptors and do not strictly correspond either to β_1- or β_2-adrenergic receptor, and so in this experiment, chick erythrocyte β-receptor might be an atypical β_1-adrenergic receptor.

The decrease in specific [^{3}H]-DHA binding by the treatment with PCase or PIase was consistent with the finding of Limbird and Lefkowitz [15], who reported that the digestion of frog erythrocyte membranes with *Cl. perfringens* PCase caused a dose-dependent decline in the receptor-binding capacity without altering receptor affinity. PIase evoked the release of ectoenzymes such as alkaline phosphatase, 5′-nucleotidase, acetylcholinesterase and alkaline phosphodiesterase I from several animal tissues and erythrocytes [7, 8, 26–33], but, from the present study, β-receptor in the chick erythrocytes was not released by PIase. Therefore, the decrease in B_{max} of β-receptor caused by PCase or PIase might be due to conformational change of β-receptor without alteration of the binding affinity.

The values of K_D for [^{3}H]-DHA and of K_i obtained in the present study are higher than those reported by Dickinson and Naorski [35]. These differences are probably due to the differences in the method of membrane preparation and in the chicken species. If anything, the values of K_D and K_i obtained by the authors are comparable to those of intact turkey erythrocyte β-receptor [36].

When chick erythrocyte membranes were treated with PCase, isoproterenol-stimulated adenylate cyclase activity was decreased without altering basal and forskolin-stimulated activities. These results suggested that the decrease in isoproterenol-stimulated adenylate cyclase activity was due to the decrease in [^{3}H]-DHA binding site by PCase action. In contrast, treatment with PIase caused the decrease in the basal, isoproterenol- and forskolin-stimulated adenylate cyclase activities. According to Zilversmit's group [10], the decrease in isoproterenol-stimulated adenylate cyclase activity induced by incorporation of PI into turkey erythrocytes was due to the decrease in the relative percentage of high-affinity binding site. The decrease in isoproterenol-stimulated adenylate cyclase activity observed in the present work also may be due to the decrease in the relative percentage of high-affinity binding site. However, a direct correlation might exist between membrane PI and catalytic unit of adenylate cyclase, since basal and forskolin-stimulated adenylate cyclase activities were significantly decreased by PIase action.

In the present study, we used three different phospholipases C in the homogeneous state as indicated by polyacrylamide gel electrophoresis [18, 19]. *Cl. perfringens* PCase catalyzes the hydrolysis of PC, SM and PE [2, 37], SMase, the hydrolysis of SM and lyso PC [38], and PIase the hydrolysis of PI and lyso PI [39]. Therefore, the results in this study were brought about by the specific action of each of three phospholipases C. In particular, the effects of PIase action on adenylate cyclase activities must be specific, since PIase hydrolyzes membranous PI, which is a minor constituent of the phospholipids in the erythrocyte membrane. In addition to these observations, the decrease in adenylate cyclase activity by the action of PIase is not due to the splitting products from PI, i.e. diglycerides and phosphorylated bases. For instance, diglycerides were formed much more abundantly by the action of PCase than by PIase, in that the membranes were enriched in PC and PE whereas the content of PI was much lower. Nevertheless, the action of PCase toward adenylate cyclase activity in the erythrocyte membrane was weaker than that of PIase.

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