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Cyclic AMP-induced desensitization of G-protein-regulated phospholipase C in turkey erythrocyte membranes

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Abstract

The interaction of the cyclic AMP and inositol lipid signalling systems was studied in turkey erythrocytes. Elevation of intracellular cyclic AMP concentrations by pretreatment of the cells with forskolin or 8-Br-cAMP resulted in a marked decrease in responsiveness of phospholipase C to G-protein activators in membranes prepared from treated cells. Decreases in responsiveness occurred with a $t_{1/2}$ of approximately 5 min and were reversible after transfer of desensitized cells to drug-free medium. Pretreatment of the cells with forskolin inhibited inositol phosphate formation in a concentration-dependent manner and addition of the phosphodiesterase inhibitor IBMX (3-isobutyl-1-methylxanthine) during pretreatment increased the capacity of forskolin to desensitize phospholipase C activity. IBMX also produced a similar potentiation of forskolin-stimulated accumulation of cyclic AMP in turkey erythrocytes. Isoproterenol pretreatment of the cells induced, like forskolin, partial inhibition of inositol phosphate generation in response to G-protein activators and to P_{2Y} purinoceptor and β-adrenoceptor agonists. The capacity of isoproterenol to induce desensitization of phospholipase C activity also was increased by the presence of IBMX during pretreatment of the cells. H8 (*N*-[2-(methylamino)ethyl]-5-isoquinoline-sulfonamide), an inhibitor of cyclic AMP-regulated protein kinase, completely prevented forskolin-induced desensitization but only partially blocked isoproterenol-induced desensitization. These results indicate that the cyclic AMP signalling cascade has a major inhibitory influence on receptor- and G-protein-activated inositol lipid signaling.

Keywords: Desensitization; Phospholipase C; G-protein; Adenylyl cyclase

1. Introduction

Agonist and drug-induced desensitization of receptor-regulated second messenger signalling responses is a prominent occurrence at most receptors. The molecular basis of this phenomenon has been delineated in the clearest detail for the β-adrenoceptor-regulated adenylyl cyclase (Lefkowitz et al., 1990; Palczewski and Benovic, 1991), but desensitization also occurs in other signalling pathways. For example, desensitization of the receptor-regulated inositol lipid signalling pathway has been broadly described (Menniti et al., 1990; Wojcikiewicz et al., 1993; Fisher, 1995) but relative to receptor-regulated adenylyl cyclase, little is known about its mechanisms of occurrence. This in part has been due to difficulty in reliably measuring receptor-coupled G-protein-regulated phospholipase C activity in cell-free systems.

It has become clear in recent years that many interactions occur between the known second messenger signalling pathways. For example, activation of protein kinase C has been shown to result in marked changes in receptor-regulated cyclic AMP synthesis (Bell et al., 1985; Sugden et al., 1985) and activity of adenylyl cyclase (Yoshimura and Cooper, 1993). Similarly, recent studies have suggested that elevation of intracellular cyclic AMP levels results in modification of responsiveness of the inositol lipid signalling cascade (Madison and Brown, 1988; McAtee and Dawson, 1989; Anwer et al., 1989; Teitelbaum and Strasheim, 1990; Kim et al., 1989; Wen et al., 1992). However, the details of this type of regulation have not been delineated.

The turkey erythrocyte has been developed as a model system to study receptor-coupled G protein-regulated phospholipase C (Harden et al., 1987). The avian erythrocyte G-protein-regulated phospholipase C has been purified

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(Morris et al., 1991a,b) and its cDNA cloned (Waldo et al., 1996), and the involved G-protein has been identified as G_{11} (Waldo et al., 1991; Maurice et al., 1993). We previously have reported that preincubation of turkey erythrocytes with P_{2Y} purinoceptor agonists results in desensitization of the inositol lipid signalling pathway (Martin and Harden, 1989; Galas and Harden, 1995). We now have utilized this system to assess at the membrane level the properties of cyclic AMP-induced desensitization of G-protein-regulated phospholipase C. We report that the cyclic AMP system has prominent negative regulatory effects on receptor-coupled G-protein-activated phospholipase C.

2. Materials and methods

2.1. Drugs

2-[^{3}H]*myo*-Inositol (15 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA), [2-^{3}H]adenine (27 Ci/mmol) was from Amersham (Airlington Heights, IL, USA), GTPγS (guanosine 5′-γ-thiotriphosphate) was from Boerhringer-Mannheim (Indianapolis, IN, USA). All other chemicals were obtained from Sigma (St. Louis, MO, USA).

2.2. Desensitization of turkey erythrocytes

Washed turkey erythrocytes from approximately 1 ml of packed cells were labelled overnight with 0.5 mCi of 2-[^{3}H]*myo*-inositol in a volume of 4.2 ml of inositol-free DMEM (Dulbecco's modified Eagle's medium) as previously described (Boyer et al., 1989). Labelled cells were washed with 50 ml of prewarmed MEM containing 20 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.4 (MEM/Hepes), centrifuged at 300 × *g* for 5 min, and resuspended in MEM/Hepes. Aliquots of 2 ml of the labelled cell suspension were incubated with or without testing agents at 30°C for the indicated times. Incubations were terminated by dilution of the cells in 20 ml ice-cold buffer, followed by centrifugation at 300 × *g* for 5 min. The pellet was resuspended in 25 ml of ice-cold lysis buffer (5 mM sodium phosphate, 5 mM $MgCl_2$, 1 mM EGTA, pH 7.4) and vigorously mixed for 20 s. The lysate was centrifuged at 12 000 × *g* for 5 min at 4°C. Erythrocyte ghosts were washed twice by resuspension in a large volume of lysis buffer, and by a final wash in lysis buffer without EGTA. The ghost pellets were resuspended in 20 mM Hepes, pH 7.0, just prior to use.

2.3. Phospholipase C assay

Phospholipase C activity was measured as previously described (Boyer et al., 1989). Briefly, ghost membranes were incubated for 5 min at 30°C in a final volume of 0.2 ml containing 0.424 mM $CaCl_2$ (final free concentration ≈ 1 μM), 0.91 mM $MgCl_2$, 2 mM EGTA, 115 mM KCl, 5 mM KH_2PO_4, 20 mM Hepes, pH 7.0, in the presence of the indicated drugs. The reaction was stopped by addition of 400 μl of 10% perchloric acid. The samples were neutralized with a mixture of 1.5 mM KOH, 75 mM Hepes, pH 7.0. After centrifugation at 300 × g for 5 min, the supernatants were applied to Dowex AG 1 × 8 columns and total inositol phosphates were isolated.

2.4. Phosphoinositide assay

[^{3}H]PtdIns (phosphatidylinositol), [^{3}H]PtdIns-(4)P (phosphatidylinositol-(4) phosphate) and [^{3}H]PtdIns-(4,5)P_2 (phosphatidylinositol-(4,5) phosphate) were quantitated as previously described (Harden et al., 1987).

2.5. Quantitation of phospholipase C catalytic activity

Maximal phospholipase C activity was measured using [^{3}H]PtdIns-(4)P substrate presented as a mixed phospholipid/detergent micelle. Assays contained 50 μM PtdIns-(4)P and 15 000–20 000 cpm of [^{3}H]PtdIns-(4)P. Lipids were dried under a stream of nitrogen and then sonicated in 10 mM Hepes, pH 7.4. Assays were carried out in a final volume of 100 μl containing 10 mM Hepes, pH 7.4, 120 mM KCl, 10 mM NaCl, 2 mM EGTA, 2.1 mM $CaCl_2$, 5.86 mM $MgSO_4$ and 0.5% sodium cholate. Incubations were at 30°C for 5 min and were terminated by the addition of 0.375 ml of $CHCl_3/CH_3OH/HCl$ (20:40:1) followed by 0.125 ml of $CHCl_3$ and 0.125 ml of 0.1 M HCl. Radioactivity released into the upper phase was quantified by liquid scintillation spectrometry.

2.6. Cyclic AMP accumulation

One milliliter of washed packed erythrocytes was resuspended in 5 ml of MEM/Hepes and labelled with 2-[^{3}H]adenine (10 mCi), for 2 h at 37°C. The cells then were washed twice in MEM/Hepes. Cells were resuspended in 50–60 ml of MEM/Hepes and aliquots of 1 ml were incubated in the presence of various agents for 10 min at 37°C. The reaction was stopped by centrifugation at 12 000 × *g* for 1 min. The supernatants were removed and 1 ml of 5% trichloracetic acid was added to the pellets. The samples were assayed for [^{3}H]cyclic AMP as modified (Meeker and Harden, 1982) from the original method of Salomon et al. (1974). Cyclic AMP accumulation is expressed as follows: Conversion (%) = ([^{3}H]cyclic AMP/([^{3}H]cyclic AMP + [[^{3}H]ATP)) × 100.

2.7. Statistical analysis

The data are expressed as the means ± S.E.M. Statistical differences were measured using Student's *t*-test. The level of significance was $P < 0.05$.

3. Results

The potential influence of the adenylyl cyclase signalling system on receptor- and G-protein-regulated phospholipase C was examined by preincubation of turkey erythrocytes with forskolin or a cell permeable analog of cyclic AMP. Cells were pre-labelled with [^{3}H]*myo*-inositol and then incubated for 20 min in the absence or presence of 10 μM forskolin + 100 μM IBMX (3-isobutyl-1-methylxanthine) or 100 μM 8-Br-cyclic AMP. Membranes were prepared and phospholipase C activity was measured in the presence of the G-protein activators, GTPγS or AlF_4^-, or the P_{2Y} purinoceptor agonist, ADPβS (adenosine 5′-[β-thio]diphosphate). Preincubation with either forskolin or 8-Br-cAMP induced a decrease of inositol phosphate response to all activators studied. The percent decrease of PLC activity observed after forskolin or 8-Br-cAMP pretreatment, respectively, were 44 ± 9.8% and 40 ± 10% for GTPγS-stimulated inositol phosphate accumulation, 35 ± 11% and 27 ± 12% for AlF_4^--stimulated activity and 40 ± 5.2% and 28 ± 7.3% for ADPβS + GTPγS-stimulated activity. The decrease in responsiveness to GTPγS in membranes from forskolin-treated cells was observed at all times of assay of phospholipase C activity (data not shown).

Forskolin- or cyclic AMP-induced decreases in the amount of polyphosphoinositide substrate potentially could explain the reduction in phospholipase C responsiveness in membranes from pretreated cells. However, forskolin pretreatment of turkey erythrocytes did not alter [^{3}H]PtdIns, [^{3}H]PtdIns-(4)P or [^{3}H]PtdIns-(4,5)P_2 levels (Table 1). As we have described previously in membranes from non-desensitized cells (Harden et al., 1987), GTPγS-induced increases in the formation of inositol phosphates were associated with a decrease in the amount of radioactivity in [^{3}H]PtdIns-(4)P and [^{3}H]PtdIns-(4,5)P_2, with no change in [^{3}H]PtdIns levels. The GTPγS-induced decrease in the radioactivity associated with [^{3}H]PtdIns-(4)P and [^{3}H]PtdIns-(4,5)P_2 was significantly lower in membranes from desensitized cells.

Although G-protein- or P_{2Y} purinoceptor-stimulated activity was markedly decreased in membranes from cells pretreated with forskolin plus IBMX, this effect occured with no measurable change in total phospholipase C activity associated with the membranes. That is, phospholipase C catalytic activity was measured in an assay using exogenous [^{3}H]PtdIns(4,5)P_2 substrate presented in a mixed cholate detergent/phospholipid micelle. No change in total enzyme activity was detected after preincubation of cells with forskolin plus IBMX (control = 51.6 ± 1 μmol/min and treated = 52.2 ± 2.2 μmol/min; each value represents the mean ± S.E.M. of three different experiments). Therefore, although P_{2Y} purinoceptor- or G-protein-regulated activity measured in membranes was markedly reduced by pretreatment of cells with an activator of adenylyl cyclase, no change in the total amount of phospholipase C activity associated with the membranes could be detected. Furthermore, no forskolin plus IBMX-induced change in phospholipase C levels could be detected in immunoblots using antisera that is specific for this phospholipase C (data not shown).

The time-course of forskolin-induced decrease in phospholipase C activity was determined (Fig. 1, left panel). Responsiveness to both GTPγS and AlF_4^- decreased rapidly in membranes obtained from erythrocytes incubated at 30°C with 10 μM forskolin in the presence of 100 μM IBMX. Desensitization occurred with a half time of about 5 min and was maximum (approximately 50% of control for GTPγS-stimulated activity and 70% of control for AlF_4^--stimulated activity) after approximately 30 min of preincubation with forskolin. Significant ($P < 0.01$) differences were noted between GTPγS- and AlF_4^--stimulated activities. Continued incubation for up to 1 h did not cause a further loss of responsiveness. Reversibility of the forskolin-induced loss of responsiveness of phospholipase C was examined by preincubating cells with forskolin + IBMX for 20 min, and then transferring these pretreated cells to drug-free medium and continuing the incubation at 30°C (Fig. 1, right panel). GTPγS-stimulated phospholi-

Table 1
Phosphoinositides in membranes from forskolin- or isoproterenol-pretreated turkey erythrocytes

		Control	Preincubation	
			10 μM forskolin + 100 μM IBMX	10 μM isoproterenol
IPs	Buffer	436 ± 29	461 ± 19	491 ± 8
	GTPγS	2570 ± 50	1422 ± 12	1306 ± 37
PtdIns(4,5)P_2	Buffer	14547 ± 1292	14493 ± 70	15387 ± 739
	GTPγS	12177 ± 261	13053 ± 143	13823 ± 320
PtdIns-4P	Buffer	9840 ± 740	9385 ± 412	9865 ± 368
	GTPγS	8767 ± 612	8863 ± 213	9442 ± 282
PtdIns	Buffer	81033 ± 9090	73646 ± 5051	75371 ± 4638
	GTPγS	81551 ± 7125	73665 ± 3903	75862 ± 5197

[^{3}H]Inositol-labelled turkey erythrocytes were incubated with buffer, 10 μM forskolin + 100 μM IBMX, or 10 μM isoproterenol for 20 min at 30°C. Membranes were prepared as described in Materials and methods. Phosphoinositides and inositol phosphates were measured after a 5 min incubation in the presence of vehicle or 100 μM GTPγS. The data (cpm) are the means ± S.E.M. of three different experiments.

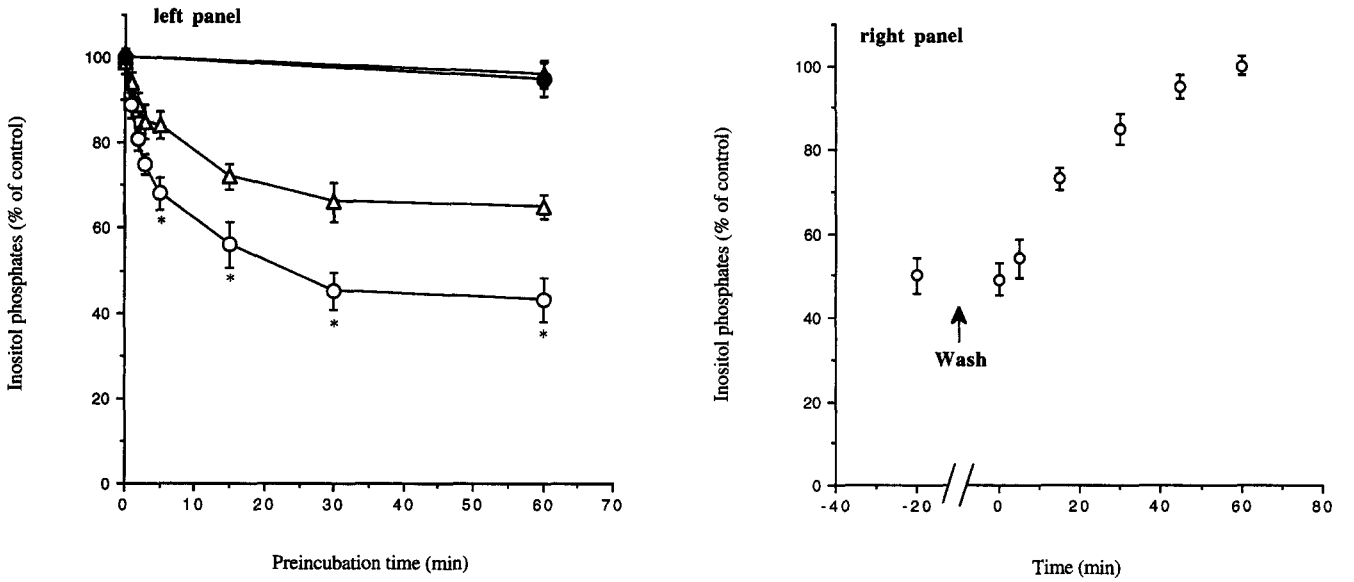

Fig. 1. (Left panel) Time-course of forskolin-induced loss of responsiveness. [^{3}H]Inositol-labelled turkey erythrocytes were incubated with buffer (closed symbols) or 10 μM forskolin plus 100 μM IBMX (open symbols) for the indicated times at 30°C. Membranes were prepared as described in Materials and methods. Inositol phosphate production was measured after 5 min incubation at 30°C with either AlF_4^- (Δ) or 100 μM GTPγS (O). * $P < 0.01$ statistically significant values compared to the corresponding values from membranes treated with AlF_4^- versus GTPγS. (Right panel) Time-course of recovery from forskolin-induced desensitization. [^{3}H]Inositol-labelled turkey erythrocytes were incubated with 10 μM forskolin plus 100 μM IBMX for 20 min and then rapidly washed with 100 ml of ice-cold MEM/Hepes buffer, centrifuged and resuspended in 30°C buffer. At the indicated times, membranes were prepared and inositol phosphate production was measured after 5 min incubation at 30°C with 100 μM GTPγS. The data are expressed as percent of the inositol phosphate response obtained for membranes from control cells prior to incubation. The data represent the means ± S.E.M. of three experiments with different membrane preparations.

pase C activity in membranes from these cells recovered with a half time of about 15 min and reached control levels after approximately 60 min of incubation of cells in drug-free medium.

Although the classical biochemical effect of forskolin is activation of adenylyl cyclase, other biological activities of this diterpene have been reported (McHugh and McGee, 1986; Wagoner and Pallotta, 1988). To verify whether forskolin-induced desensitization was mediated through its capacity to activate adenylyl cyclase and elevate cyclic AMP levels in turkey erythrocytes, we determined the effects of pretreatment of erythrocytes with forskolin in the absence versus presence of the phosphodiesterase inhibitor, IBMX (Fig. 2). Pretreatment of erythrocytes with various concentrations of forskolin induced a concentration-dependent decrease in GTPγS-stimulated phospholipase C activity. This concentration-effect relationship was markedly shifted to lower concentrations when IBMX was present during forskolin pretreatment ($P < 0.05$). The $K_{0.5}$ for induction of desensitization was approximately 3 μM in the absence of IBMX and 0.1 μM in the presence of this phosphodiesterase inhibitor. IBMX had no effect on the maximal degree of desensitization obtained after pretreatment with high concentrations of forskolin.

The effect of forskolin on cyclic AMP accumulation also was quantitated in the absence or presence of IBMX (data not shown). Forskolin induced a concentration-de-

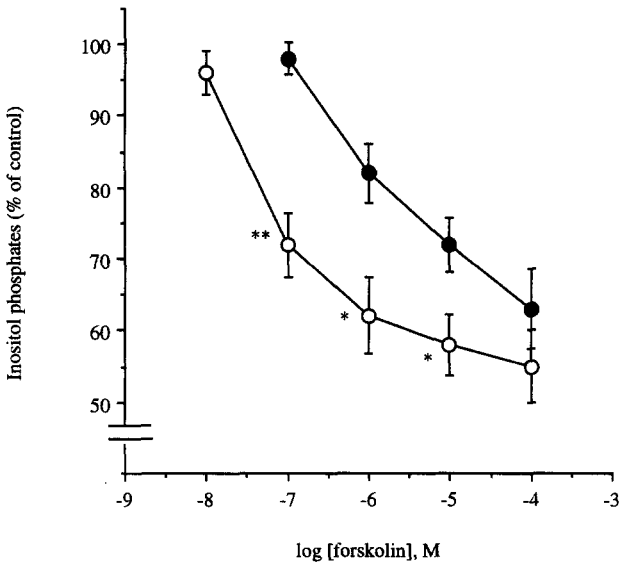

Fig. 2. Effect of IBMX on forskolin-induced loss of responsiveness. [^{3}H]Inositol-labelled turkey erythrocytes were incubated with various concentrations of forskolin (closed symbols) or forskolin plus 100 μM IBMX (open symbols) for 20 min at 30°C. Membranes were prepared as described in Materials and methods. Inositol phosphate production was measured after 5 min incubation with 100 μM GTPγS. The results are expressed as the percent of GTPγS-stimulated activity measured in membranes from control cells. Each value represents the mean ± S.E.M. of three different experiments. * $P < 0.05$; * * $P < 0.01$ statistically significant values compared to the corresponding values from membranes pretreated with forskolin only.

pendent accumulation of cyclic AMP in erythrocytes, and this effect was markedly potentiated in the presence of IBMX. This potentiation was observed as both a decrease in the $K_{0.5}$ for forskolin as well as an apparent increase in maximal effect. The difficulty in testing forskolin at concentrations higher than 100 μM made comparison of these activation curves problematic. Assuming that the same maximal level of accumulation would be obtained, the $K_{0.5}$ value for forskolin for elevation of cyclic AMP levels in the presence of IBMX was approximately 100-fold greater than the $K_{0.5}$ value for induction of desensitization under the corresponding condition.

Another means for elevation of cyclic AMP in turkey erythrocytes involves activation of the β-adrenoceptor. However, the avian erythrocyte β-adrenoceptor apparently differs from mammalian β-adrenoceptors since it has been reported to activate phospholipase C as well as adenylyl cyclase (Rooney et al., 1991; Vaziri and Downes, 1992). Thus, interpretation of effects induced by pretreatment of erythrocytes with isoproterenol are not straightforward. [^{3}H]Inositol labelled turkey erythrocytes were pretreated with buffer or with 10 μM isoproterenol and activation of phospholipase C was studied in membranes from control and isoproterenol-pretreated cells. AlF_4^--, GTPγS-, isoproterenol + GTPγS-, and ADPβS + GTPγS-stimulated activities all were decreased in membrane ghosts prepared from isoproterenol-preincubated cells (Table 2) ($P < 0.05$). The decreases in responsiveness were comparable to those observed after preincubation of cells with a maximally effective concentration of forskolin. As was described for membranes from forskolin-treated cells, the decrease in responsiveness to GTPγS in membranes obtained from isoproterenol-treated cells was observed at all times of assay of phospholipase C activity (data not shown).

To determine if isoproterenol-induced desensitization of PLC activity was mediated, at least in part, by activation of adenylyl cyclase, we examined the effects of preincubation of turkey erythrocytes with different concentrations of isoproterenol alone or in the presence of 100 μM IBMX. As shown in Fig. 3, a 10-min incubation of erythrocytes with various concentrations of isoproterenol in the presence of 100 μM IBMX markedly shifted the concentration-response curve for induction of desensitization to lower concentrations of isoproterenol ($P < 0.01$). The maximal extent of desensitization was not increased by IBMX. IBMX also increased the accumulation of cyclic

Table 2
Isoproterenol-induced desensitization of agonist-stimulated inositol phosphate formation

	Control (cpm)	10 μM isoproterenol (cpm)	
Buffer	559 ± 46	540 ± 2	
GTPγS	5178 ± 812	2500 ± 402	(43.6% ± 7.8)
AlF_4^-	1852 ± 241	1256 ± 154 [a]	(65.8% ± 7.7)
GTPγS + isoproterenol	3173 ± 672	1116 ± 183 [a]	(39.6% ± 7.6)
GTPγS + ADPβS	10346 ± 1452	6745 ± 965 [a]	(65.2% ± 7.2)

[^{3}H]Inositol-labelled turkey erythrocytes were incubated with buffer or 10 μM isoproterenol for 20 min at 30°C. Membranes were prepared as described in Materials and methods. The preparations were assayed for PLC activity for 5 min at 30°C in presence of buffer, 100 μM GTPγS, AlF_4^-, 100 μM isoproterenol + 10 μM GTPγS, or 100 μM ADPβS + 1 μM GTPγS. Each value (cpm) represents the mean ± S.E.M. of six different experiments. The values in parentheses express the percentage ± S.D. of IP accumulation after isoproterenol preincubation compared to control cells. [a] $P < 0.05$ statistically significant values compared to the corresponding values from membranes pretreated with buffer only.

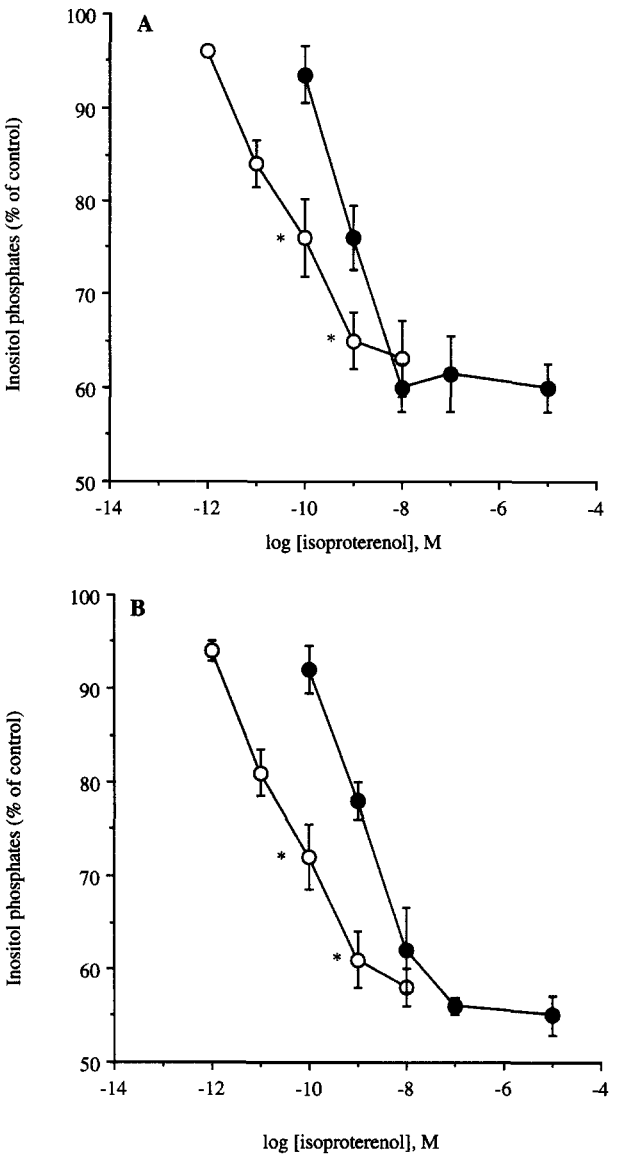

Fig. 3. Effect of IBMX on isoproterenol-induced loss of responsiveness. [^{3}H]Inositol-labelled turkey erythrocytes were incubated with the indicated concentrations of isoproterenol (closed symbols) or isoproterenol plus 100 μM IBMX (open symbols) for 20 min at 30°C. Membranes were prepared as described in Materials and methods. Inositol phosphate production was measured after 5 min incubation with either 100 μM GTPγS (A) or 100 μM isoproterenol + 10 μM GTPγS (B). The results are expressed as the percent of activity measured in membranes from control cells. Each value represents the mean ± S.E.M. of three different experiments. * $P < 0.01$ statistically significant values compared to the corresponding values from membranes pretreated with isoproterenol only.

AMP observed in the presence of isoproterenol without markedly changing the $K_{0.5}$ value for stimulation (data not shown). Using a radioimmunoassay, it was possible to detect two-fold increases of cyclic AMP concentration in turkey erythrocytes after stimulation by 10 nM isoproterenol in presence of 100 μM IBMX (data not shown).

The data presented thus far suggest that elevation of cyclic AMP results in desensitization of phospholipase C in turkey erythrocytes. To evaluate by another means the potential involvement of cyclic AMP, we tested the capacity of H8 (*N*-[2-(methylamino)ethyl]-5-isoquinoline-sulfonamide), a relatively selective inhibitor of cyclic AMP-regulated protein kinase (Hidaka et al., 1984), to prevent cAMP-induced desensitization. Turkey erythrocytes were incubated with either 10 μM forskolin + 100 μM IBMX or 10 μM isoproterenol in the presence of 100 μM H8 for 20 min at 30°C. Pretreatment with 100 μM H8 had no effect on phospholipase C activity in membranes from control cells. However, 100 μM H8 completely prevented forskolin-induced desensitization measured in membranes stimulated with 100 μM GTPγS or 100 μM isoproterenol + 10 μM GTPγS (Fig. 4A) ($P < 0.05$). Under the same conditions, H8 only partially prevented isoproterenol-induced desensitization of PLC measured in membranes stimulated by GTPγS (83 ± 4% of control)- or isoproterenol + GTPγS (77 ± 7% of control)-stimulated activities (Fig. 4B) ($P < 0.05$).

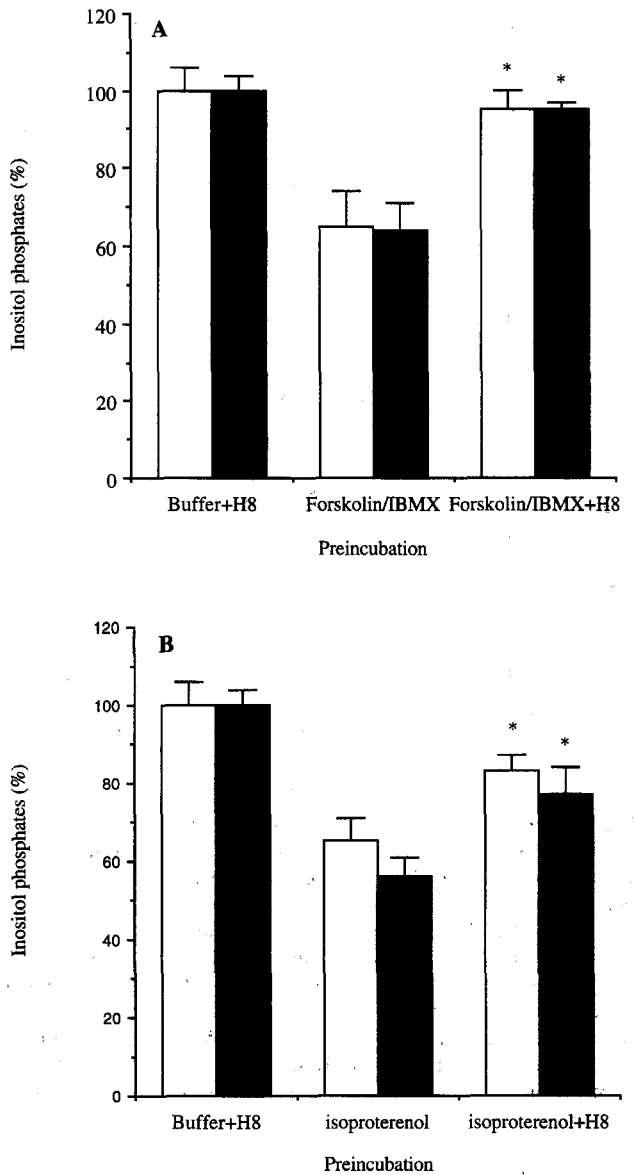

Fig. 4. Effect of a protein kinase A inhibitor on forskolin and isoproterenol-induced loss of responsiveness. [^{3}H]Inositol-labelled turkey erythrocytes were preincubated with 10 μM forskolin + 100 μM IBMX (A) or 10 μM isoproterenol (B), alone or in the presence of 100 μM H8 for 20 min at 30°C. Membranes were prepared as described in Materials and methods, and inositol phosphates production was measured after 5 min incubation with 100 μM GTPγS (□), or 100 μM isoproterenol + 10 μM GTPγS (■). The data are the means ± S.E.M. of three different experiments. * $P < 0.05$ statistically significant values compared to the corresponding values from membranes pretreated without H8.

4. Discussion

As is the case with other second messenger signalling systems, agonist and drug-induced desensitization of receptor-regulated phospholipase C has been widely reported (Menniti et al., 1990; Wojcikiewicz et al., 1993; Fisher, 1995). This phenomenon has been most clearly documented in cells or in membranes prepared from cells previously incubated with activators of phospholipase C-linked receptors (L'Allemain et al., 1986; Nakahata and Harden, 1987; Sugiya et al., 1987; Scwhwertschlag and Whorton, 1988; Hepler et al., 1988; Martin and Harden, 1989) or of protein kinase C (Labarca et al., 1984; Rittenhouse and Sasson, 1985; Orellana et al., 1985; Hepler et al., 1988) Although activators of other signalling systems, e.g. adenylyl cyclase, have been shown to result in modification of phospholipase C responsiveness (Madison and Brown, 1988; McAtee and Dawson, 1989; Anwer et al., 1989; Teitelbaum and Strasheim, 1990; Kim et al., 1989; Wen et al., 1992), these effects have not been consistently observed (Madison et al., 1988;, Alava et al., 1992; Blackmore and Exton, 1986; Godfrey et al., 1987; Pittner and Fain, 1989) and the properties of occurrence of heterologous modification of phospholipase C responsiveness have not been delineated in detail. Moreover, whereas remarkable progress has been made in understanding of the mechanisms of agonist-induced desensitization of adenylyl cyclase, essentially nothing is known of the corresponding mechanisms involved in desensitization of phospholipase C.

The preservation in turkey erythrocyte membranes of marked responses of phospholipase C to P_{2Y} purinoceptor agonists and guanine nucleotides make the turkey erythrocyte a good system to address mechanistic questions concerning the regulation of the inositol lipid signalling path-

way. As such, the work presented here provides the first detailed description of the properties of cyclic AMP-induced desensitization of a G-protein-regulated phospholipase C. Elevation of turkey erythrocyte cyclic AMP by any of three different means resulted in a marked desensitization of phospholipase C in membranes prepared from these cells. Forskolin-induced decreases in responsiveness were concentration- and time-dependent, reversible, and were augmented by the phosphodiesterase inhibitor, IBMX. The most parsimonious interpretation of these results is that cyclic AMP-dependent activation of cyclic AMP-dependent protein kinase results in modification of some component of the phospholipase C signalling system resulting in loss of hormone and guanine nucleotide responsiveness. Pharmacological prevention of forskolin-induced desensitization by the supposedly selective cyclic AMP-dependent protein kinase inhibitor, H8, is consistent with this conclusion. However, the true kinase specificity of H8 is essentially impossible to establish in intact cells, and cyclic AMP-dependent protein kinase could activate another kinase (or series of kinases) that ultimately are responsible for the heterologous desensitization that was observed.

Changes in either substrate availability or in inositol phosphate degradation are not involved in the cyclic AMP-induced decrement in responsiveness in turkey erythrocyte membranes, and our results point to a stable modification of G_{1l}, the G-protein-regulated phospholipase C, or both of these signalling components. The fact that a similar cyclic AMP-induced reduction in responsiveness occurred to all activators might suggest that it is a modification of the phospholipase C rather than $G\alpha_{1l}$ that accounts for desensitization. However, it is the capacity of the enzyme to be regulated by $G\alpha_{1l}$ that is the important issue, and further experiments clearly will be necessary to delineate whether it is $G\alpha_{1l}$ or the G-protein-regulated phospholipase C (or both) that is modified by cyclic AMP-dependent protein kinase (or another kinase).

Information on cyclic AMP-induced desensitization of phospholipase C in other target cells is very limited. Receptor- or drug-induced elevation of cyclic AMP has been shown to result in loss of responsiveness of phospholipase C in some systems (Madison and Brown, 1988; McAtee and Dawson, 1989; Anwer et al., 1989; Teitelbaum and Strasheim, 1990; Kim et al., 1989; Wen et al., 1992) but not in others (Alava et al., 1992). In addition, the cyclic AMP-induced loss of responsiveness in canine tracheal smooth muscle occurred for histamine but not muscarinic receptor-stimulated inositol phosphate accumulation (Madison and Brown, 1988) suggesting that modification occurred at the level of the receptor rather than G-protein or phospholipase C. Sanborn and coworkers have shown that elevation of cyclic AMP levels in rat myometrial cells results in a loss of G-protein- regulated phospholipase C activity in membranes prepared from these cells (Anwer et al., 1989). Further, a decrease in responsiveness was observed in membranes from control cells after treatment of these membranes with purified cyclic AMP-dependent protein kinase (Wen et al., 1992) supporting the idea that it is phosphorylation of a membrane protein that is responsible for cyclic AMP-induced desensitization. It is of interest that Rhee and coworkers have reported that one member of the Gq-regulated phospholipase C-β class of isoenzymes is a good substrate for phosphorylation by protein kinase C (Ryu et al., 1990) but was not phosphorylated in response to elevated cyclic AMP levels in C6Bu1 cells (Kim et al., 1989). The aforementioned preliminary results with the turkey erythrocyte phospholipase C suggest this may not be the case with the turkey erythrocyte G-protein-regulated enzyme.

Activation of the β-adrenoceptor was used in addition to forskolin and 8-Br-cyclic AMP as a means to elevate cyclic AMP levels and induce heterologous desensitization. As has been previously reported by Rooney and Thomas (Rooney et al., 1991) and by Vaziri and Downes (1992), not only does the turkey erythrocyte β-adrenoceptor activate adenylyl cyclase, but it also activates phospholipase C. Thus, desensitization induced by isoproterenol potentially could involve a cyclic AMP-mediated component, as well as effects secondary to activation of the inositol lipid signalling cascade.

In summary, elevation of cyclic AMP levels in turkey erythrocytes results in a marked desensitization of G-protein-regulated phospholipase C that is stable to cell lysis. The availability of a homogeneous cell preparation in which the identity of the component proteins of a receptor-regulated phospholipase C has been established should be useful in unambiguously delineating the molecular mechanisms whereby heterologous desensitization of phospholipase C can occur.

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