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Modulation of Lysophosphatidic Acid-Induced Cl^- Currents by Protein Kinases A and C in the *Xenopus* Oocyte

*Myung-Jun Kim, Yang-Soon Lee and Jin-Kwan Han**

Department of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk, 790–784, South Korea

ABSTRACT. The roles of protein kinase C (PKC) and protein kinase A (PKA) in the regulation of lysophosphatidic acid (LPA)-induced Cl^- currents in *Xenopus* oocytes were examined. PKC activation by phorbol 12-myristate 13-acetate (PMA) treatment completely blocked LPA-induced Cl^- currents by inhibiting inositol 1,4,5-trisphosphate (IP_3) elevation. This inhibitory effect of PMA on the LPA response was blocked by pretreatment of oocytes with staurosporine and 3-[*N*-(dimethylamino)propyl-3-indolyl]-4-[3-indolyl]maleimide (GF109203X), PKC inhibitors. In addition, treatment of oocytes with GF109203X enhanced the LPA response by increasing IP_3 production. Elevation of the intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) concentration by treating oocytes with either forskolin (FK) plus isobutylmethylxanthine (IBMX) or 2′-*O*-dibutyryl-cAMP (dB-cAMP) reduced LPA-induced Cl^- currents. The effect of activation of the cAMP pathway appears to be mediated by PKA, since treatment of oocytes with FK plus IBMX or dB-cAMP enhanced PKA activity. Furthermore, the inhibitory effect of dB-cAMP on the LPA response was blocked by treatment of oocytes with *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulframide-2HCl (H-89), a selective inhibitor of PKA. Both FK plus IBMX and dB-cAMP treatment reduced IP_3 generation in response to LPA stimulation. Inhibition of PKA activity with H-89 or Rp-cyclic 3′,5′-hydrogen phosphorothioate adenosine triethylammonium had no effect on LPA-induced Cl^- currents. Finally, inhibition of the LPA response by activation of PKA was independent of extracellular Ca^{2+}. These results demonstrate that both PKC and PKA play active roles in modulating the LPA-induced signaling pathway. BIOCHEM PHARMACOL **59**;3:241–247, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. lysophosphatidic acid; PKC; PKA; Cl^- current; Ca^{2+}; *Xenopus* oocyte

LPA† is an intercellular lipid mediator with a wide range of biological activities, including stimulation of cell proliferation, smooth-muscle contraction, platelet aggregation, membrane depolarization, cell-shape change, chemotaxis, and tumor cell invasion [1, 2]. LPA activates a specific G protein-coupled receptor to evoke multiple cellular responses, and recent work has identified at least three cDNAs encoding putative LPA receptors [3–5]. In *Xenopus* oocytes, LPA elicits Ca^{2+}-activated Cl^- currents [6–9]. Recently, using antisense oligonucleotide methodology, we demonstrated that the LPA-induced signaling pathway in *Xenopus* oocytes is transduced through G_q/G_{11} and novel *Xenopus* PLCs [9].

To further extend the understanding of the LPA-induced signal transduction pathway, we examined the effect of activation and inhibition of PKC and PKA on LPA-stimulated Cl^- currents in *Xenopus* oocytes. PKA and PKC have been known to be involved in the regulation of the signal transduction pathways of many G protein-coupled receptors [10]. Using pharmacological and electrophysiological approaches, we have found that activation of PKC by treatment of oocytes with PMA (100 nM, 10 min) completely abolished LPA-induced Cl^- currents. Treatment of oocytes with PMA abolished IP_3 production in response to LPA stimulation, suggesting that prevention of LPA-induced Cl^- currents by PKC activation occurs through inhibition of IP_3 generation. Activation of PKA in oocytes with FK plus IBMX, dB-cAMP, or by stimulation of heterologously expressed β_2-AR, which couples to adenylyl cyclase through the G_s protein, resulted in the attenuation of LPA-induced Cl^- currents. Inhibition of PKA by either *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfr-amide-2HCl (H-89) or Rp-cAMPS triethylamine had no effect on LPA-induced Cl^- currents. These results demon-

* Corresponding author: Dr. Jin-Kwan Han, Department of Life Science, Pohang University of Science and Technology, San 31 Hyoja-Dong, Pohang, Kyungbuk, 790–784, South Korea. Tel. (562) 279-2126; FAX (562) 279-2199; E-mail: jkh@postech.ac.kr

† *Abbreviations*: LPA, lysophosphatidic acid; cDNA, complementary DNA; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate; FK, forskolin; IBMX, isobutylmethylxanthine; cAMP, adenosine 3′,5′-cyclic monophosphate; dB-cAMP, 2′-*O*-dibutyryl-cAMP; Rp-cAMPS triethylamine, Rp-cyclic 3′,5′-hydrogen phosphorothioate adenosine triethylammonium; ISO, (±)-isoproterenol hydrochloride; β_2-AR, β_2-adrenoceptor; and IP_3, inositol 1,4,5-trisphosphate.

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strate that the LPA-induced signal transduction pathway in the *Xenopus* oocyte is modulated by the activities of both PKC and PKA.

MATERIALS AND METHODS

Materials

LPA (1-acyl-*sn*-glycerol-3-phosphate) was obtained from the Sigma Chemical Co. and Avanti Polar Lipids, Inc. Similar results were produced by LPA from these two sources. PMA, staurosporine, dB-cAMP, FK, 3-[*N*-(dimethylamino)propyl-3-indolyl]-4-[3-indolyl]maleimide (GF109203X), staurosporine, and IBMX were purchased from Sigma. Rp-cAMPS triethylamine and ISO were obtained from Research Biochemicals International. H-89 was purchased from Seikagaku. LPA was dissolved in aqueous 1% fatty acid-free BSA to a stock concentration of 1 mM. The stock solutions of phospholipids were kept at −20° in small aliquots. FK was prepared as a 10 mM stock solution in DMSO vehicle. PMA was solubilized in DMSO as a 1 mM stock solution, and stored at −20° as small aliquots. The bath concentration of 0.1 μM LPA was used throughout the experiment since this concentration elicited maximal current response.

Oocyte Preparation

Mature wild-type female *Xenopus laevis* purchased from Xenopus I were maintained at 18–24°. Individual oocytes at stage VI were dissected manually from their outer follicles using watchmaker's forceps and stored in Modified Barth Saline [MBS: 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 10 mM Na-HEPES (Sigma), pH 7.4] at 19° and were used within 1 day after isolation. Ca^{2+}-free OR_2 solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, 5 mM HEPES, 1 mM EGTA, pH 7.4) was used as the Ca^{2+}-free medium.

Voltage-Clamp Recording

Currents were recorded using the standard double electrode voltage-clamp technique, held at −70 mV. A single oocyte was placed in a recording chamber filled with 1.5 mL of MBS. Microelectrodes were pulled in one stage from capillary glass (Borosilicate Glass Capillaries with an inner filament, Cat. No. GT12, Warner Corp.) on a micropipette puller (model 700C; David Kopf Instruments), and the tips were broken to a diameter of ~10 μm. They were filled with 3 M KCl, and tip resistances were usually 1–5 MΩ. The cell was voltage clamped using a two-microelectrode voltage clamp amplifier (oocyte clamp OC725A; Warner Corp.), connected to a data acquisition system, MacLab/4e (AD Instruments Pty Ltd.) running on a Power Macintosh computer. Membrane currents were sampled at 4 Hz. Compounds were delivered in 15-μL aliquots over 1–2 sec using a hand-held micropipette positioned ~5 mm from the oocyte. Treatment with vehicle, 1% BSA (fatty acid-free) solution in water, or 0.6% DMSO for 1 hr did not induce any current responses. All experiments were performed at room temperature.

Microinjection of Oocytes

Microinjection was performed using a Nanoliter Injector (WPI). Ten nanograms of synthetic β_2-AR mRNA was injected into individual oocytes and cultured for 2 days in MBS containing 5 μg/mL of penicillin, 5 μg/mL of streptomycin, 400 μg/mL of BSA, and 2.5 mM pyruvate at 19°. Poor impalements caused leakage of the cytoplasm, and these oocytes were discarded.

IP_3 Measurements

Changes of IP_3 mass were measured by using a radioreceptor binding assay described elsewhere [11, 12] with some modifications. Briefly, groups of 30 oocytes were homogenized in 200 μL of 15% ice-cold trichloroacetic acid, followed by centrifugation at 10,000 *g* for 5 min at 4°. Supernatants were collected and extracted 7–8 times with 10 vol. of water-saturated diethyl ether, followed by neutralization to pH 7.5 with 200 mM Trizma base. For the receptor binding assay, 40 μL of cell extract was mixed with 40 μL of assay buffer (0.1 M Tris–HCl, pH 9.0, supplemented with 4 mM EDTA and 0.4% BSA). Forty microliters of the diluted [^{3}H]IP_3 (approx. 2000 cpm) and 40 μL of bovine adrenal microsomal protein (approx. 0.6 mg/40 μL) were incubated for 20 min on ice. After incubation, the reaction mixture was centrifuged at 10,000 *g* for 10 min at 4°, and the supernatant was discarded. The remaining pellet was suspended with 80 μL of ddH_2O, vortexed, and counted for radioactivity in a scintillation counter. All assays were conducted in triplicate. The IP_3 concentration in each sample was determined from the standard curve, which ranged from 0.15 to 5 pmol/40 μL.

cAMP Measurements and PKA Activity Assay

cAMP was determined using a [^{3}H]cyclic AMP assay kit (Amersham) according to the manufacturer's instructions. Briefly, the samples were extracted with 1 mL of ice-cold 70% ethanol containing 4 mM EDTA (pH 7.5), stored at room temperature for 5 min, and centrifuged at 4°. The supernatant was removed and lyophilized. The residues were dissolved with 150 μL of Tris–EDTA buffer (50 mM Tris, 4 mM EDTA, pH 7.4) and centrifuged to remove insoluble residues, and 50 μL of the supernatant was used directly in the assay.

PKA activity was determined using a Promega SignaTECT™ cAMP-Dependent Protein Kinase Assay System according to the manufacturer's instructions.

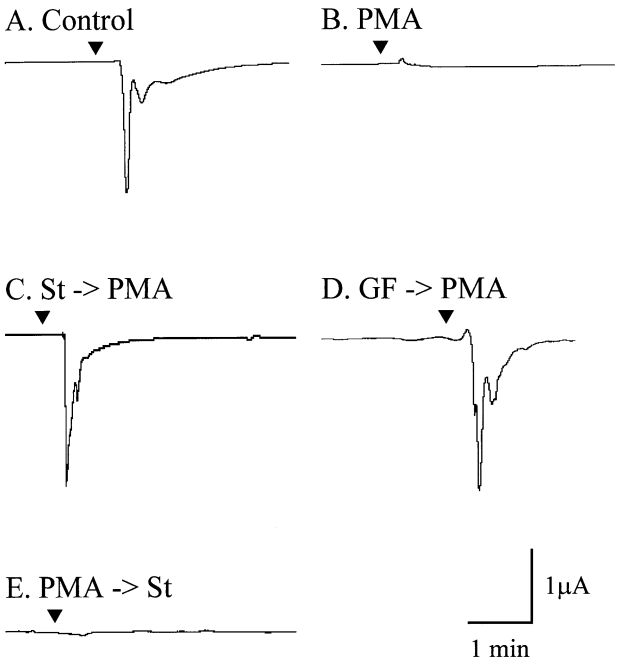

FIG. 1. Typical patterns of Cl^- currents induced by LPA under various conditions. (A) A normal response of an oocyte to 0.1 μM LPA. LPA (0.1 μM) was delivered in 15-μL aliquots over 1–2 sec by holding a hand-held pipette to the recording chamber, which was filled with 1.5 mL of MBS. (B) Effect of PMA treatment on the LPA current. Oocytes were treated with 100 nM PMA for 10 min before LPA application (N = 50). (C) Effect of pretreatment with staurosporine prior to PMA addition. Oocytes were incubated with 500 ng/mL of staurosporine for 1 hr, followed by 100 nM PMA for 10 min, and the LPA response was recorded (N = 35). (D) Effect of pretreatment with GF109203X prior to PMA addition. Oocytes were pretreated with 20 μM GF109203X for 1 hr prior to PMA addition. (E) Effect of staurosporine after PMA treatment. Oocytes were treated with 100 nM PMA for 10 min, followed by the addition of 500 ng/mL of staurosporine and incubation for 1 hr (N = 10). Black arrowheads represent the points when LPA was applied. Each trace is representative of at least three independent experiments. Abbreviations: St, staurosporine; GF, GF109203X.

Statistics

The results are expressed as means ± SEM. Student's *t*-test was used for comparing individual treatments with their respective control values. A probability of $P < 0.05$ was accepted as denoting a significant difference.

RESULTS

Inhibition of LPA-Induced Cl^- Currents by PKC Activation

In *Xenopus* oocytes, an application of 0.1 μM LPA triggered a characteristic inward Cl^- current (Fig. 1A). To determine the role of PKC activation in LPA-stimulated Cl^- currents, we treated oocytes with 100 nM PMA for 10 min prior to 0.1 μM LPA application. In these PMA-treated oocytes, the LPA-induced Cl^- currents were abolished completely (Fig. 1B). However, when oocytes were pretreated with PKC inhibitors, 500 ng/mL of staurosporine or 20 μM GF109203X, for 1 hr before PMA addition, the inhibitory effect of PMA on LPA-induced Cl^- currents was overcome, demonstrating that PMA acted through PKC activation (Fig. 1, C and D). Treatment of oocytes with staurosporine (Fig. 1E) or GF109203X (not shown) after PMA treatment did not rescue LPA-induced Cl^- currents.

Inhibitory Effect of PKA Activation on LPA Response

To examine the effect of activation of PKA on LPA-induced Cl^- currents in *Xenopus* oocytes, we attempted to elevate intracellular cAMP content by incubating oocytes with FK plus IBMX or dB-cAMP, a membrane-permeable cAMP analog. When oocytes were pretreated with 50 μM FK together with 500 μM IBMX for 1 hr, LPA-induced Cl^- currents were diminished substantially (to 45% of control) (Fig. 2A). Treatment of oocytes with 0.6% DMSO, the vehicle of FK and IBMX, for 1 hr had no effect on the LPA response. Similar inhibition of the LPA response (50% of control) was observed in oocytes exposed to 30 μM dB-cAMP for 1 hr (Fig. 2A). The inhibitory effect of dB-cAMP on LPA response was blocked by pretreatment of oocytes with 50 μM H-89, a specific inhibitor of PKA (Fig. 2A). A PKA activity assay revealed that both FK plus IBMX and dB-cAMP enhanced PKA activity in oocytes upon treatment (Fig. 2B). The basal activity of PKA in control oocytes was 1.36 pmol/min/μg. This is a slightly higher level than that reported by Cicirelli *et al.* [13].

To further assess the effect of PKA activators, oocytes were made to express β_2-AR and were stimulated with ISO for 5 min before LPA application. Ligand binding to the β_2-AR activated adenylyl cyclase through stimulatory G (G_s)-protein and activated PKA with an increased cAMP level (Fig. 2C). Expression of β_2-AR followed by exposure to ISO reduced Cl^- currents in response to LPA stimulation compared with those of water-injected control oocytes (Fig. 2C). The amount of decrease in peak currents was 50% of the control, which was similar to the change observed in oocytes treated with dB-cAMP or FK/IBMX for 1 hr (Fig. 2, B and C). These data indicate that PKA activation negatively regulated LPA-induced Cl^- currents in oocytes.

Enhancing Effect of Inhibition of PKC, but not PKA, on LPA Response

The data presented above suggested that the activation of PKC or PKA could regulate LPA response in a negative manner. Both PKC and PKA may inhibit LPA response via feedback regulation when they are activated as a downstream event of the LPA signaling pathway. It also may be possible that PKC and PKA negatively regulate the LPA pathway in its basal activity. To test this hypothesis, the effects of inhibition of PKC and PKA on the LPA response were examined. When oocytes were treated with 20 μM GF109203X for 3 hr, the LPA-induced Cl^- currents were

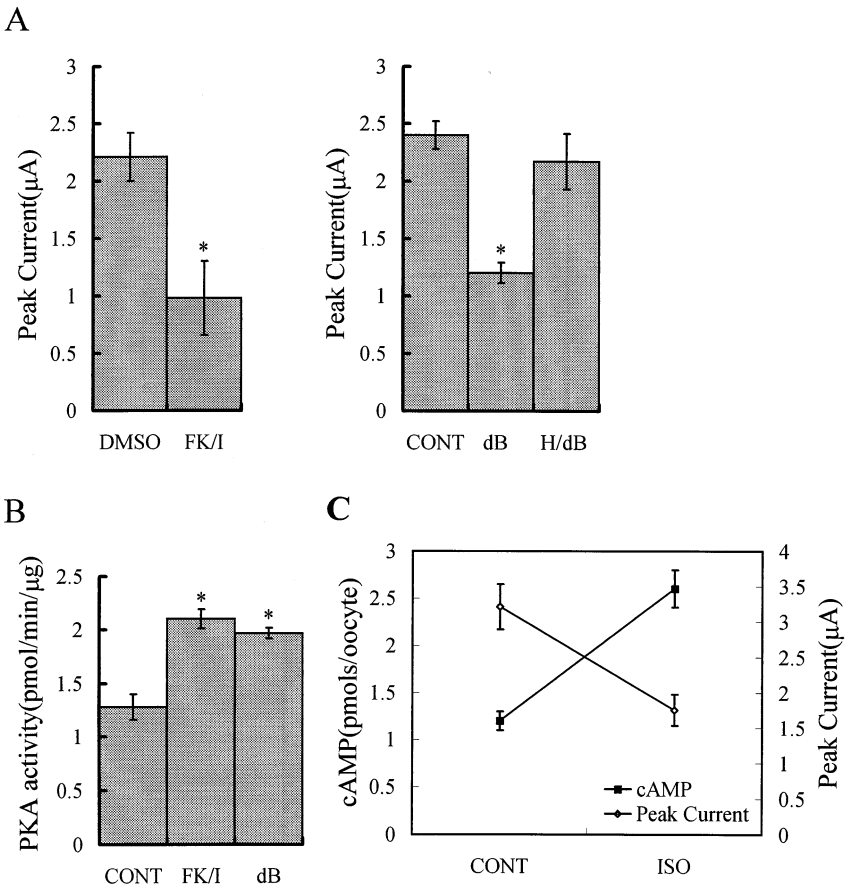

FIG. 2. Effects of PKA activation on LPA-evoked Cl^- currents in *Xenopus* oocytes. (A) Responses to 0.1 μM LPA of oocytes treated with FK plus IBMX or dB-cAMP. Oocytes were treated with 50 μM FK plus 500 μM IBMX (FK/I) or 30 μM dB-cAMP (dB) for 1 hr. Oocytes were incubated with 50 μM H-89 for 2 hr to inhibit PKA; then, 30 μM dB-cAMP was added, and the oocytes were further incubated for 1 hr (H/dB). Treatment of oocytes with 0.6% DMSO, the same concentration as the vehicle in the FK/I sample, for 1 hr was adopted as a control to determine the effect of the vehicle. Peaks of Cl^- currents elicited by LPA were measured and averaged. (B) PKA activities in the FK plus IBMX (FK/I) and dB-cAMP (dB)-treated samples were determined using a Promega assay kit. (C) Effect of acute activation of PKA by β_2-AR stimulation. Functional expression of β_2-AR in oocytes was examined (open diamonds). Ten nanograms of β_2-AR mRNA was injected into oocytes, and the oocytes were cultured for 2 days. Twenty-five oocytes were treated with 50 μM ISO for 5 min (ISO) and frozen in liquid nitrogen for cAMP measurement. Control oocytes (CONT) were not treated with ISO. cAMP contents were determined using an Amersham cAMP assay kit. When the peak Cl^- currents of β_2-AR-expressing oocytes were evaluated and averaged (filled squares), the average peak current was reduced in the ISO-treated sample. Key: (*) $P < 0.05$, compared with each control. Peak current data are expressed as means ± SEM of three independent experiments using 7–8 oocytes per condition from one animal. cAMP measurement and PKA activity data are means ± SEM of two determinations in three experiments from different animals.

enhanced greatly (Fig. 3A). A similar increment of LPA-induced Cl^- currents also was observed when voltage-clamp recording was done in Ca^{2+}-free OR_2 solution (Fig. 3A, OCa-OR2). Thus, the enhancing effect of GF109203X on LPA-induced peak currents was independent of extracellular Ca^{2+}. However, GF109203X treatment at the same concentration for 1 hr did not increase the LPA response, although this duration was sufficient to overcome the inhibitory effect of PMA (Fig. 1D). In addition, treatment of oocytes with 500 ng/mL of staurosporine for 1 hr also failed to potentiate the LPA response in spite of its ability to reverse the effect of PMA (Fig. 1C). We were not able to determine the effect of staurosporine further, since oocytes appeared to become weak when incubated for an extended period of time, as judged by the appearance of mottling of pigments on the animal pole.

Inhibition of PKA by treating oocytes with 50 μM H-89 or 40 μM Rp-cAMPS triethylamine for 1–3 hr had no effect on LPA-induced Cl^- currents (Fig. 3B). When PKA activity was assayed in an H-89-treated sample, it was reduced to 32% of basal activity (Fig. 3B). These data suggest that PKC may negatively regulate the LPA signaling pathway in its basal and activated state, whereas PKA may regulate the LPA pathway only when it is activated.

Effects of Modulations of PKA and PKC Activities on IP_3 Generation Induced by LPA

To test whether the effects of activation and inhibition of PKC on LPA-induced Cl^- currents were due to the changes in IP_3 generation, we directly measured IP_3 mass in the PMA- or GF109203X-treated oocytes. As shown in Fig.

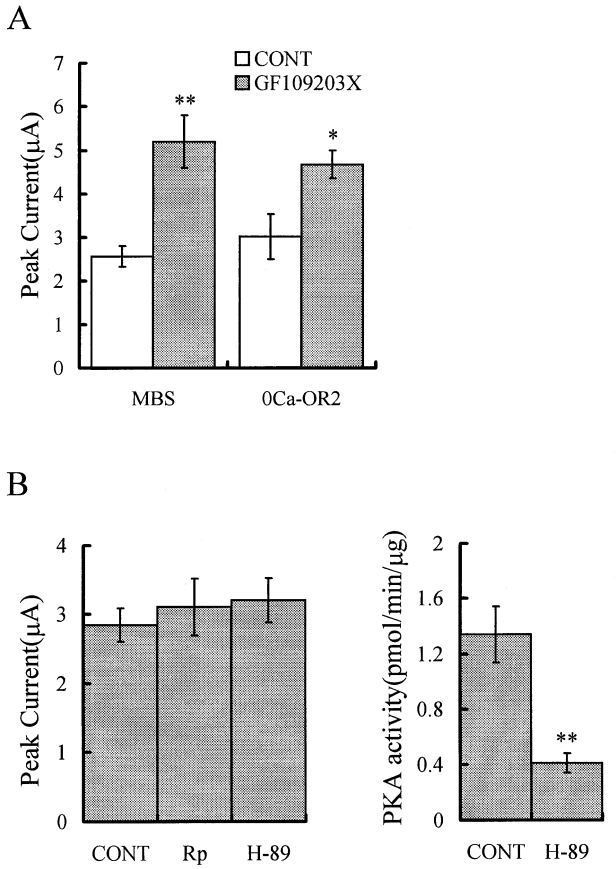

FIG. 3. Enhancement of LPA response by the inhibition of PKC. (A) Oocytes were incubated with 20 μM GF109203X for 3 hr, and LPA responses were recorded in MBS or Ca^{2+}-free OR_2 solution (OCa-OR2). (B) Oocytes were treated with 40 μM Rp-cAMPS triethylamine (Rp) or 50 μM H-89 (H-89) for 3 hr, followed by application of 0.1 μM LPA. PKA activity in the H-89-treated sample was determined using a Promega assay kit. Key: (*) $P < 0.05$ and (**) $P < 0.01$, compared with each control. Data are means ± SEM of three independent experiments. PKA activity data are means ± SEM of two determinations in three experiments from different animals.

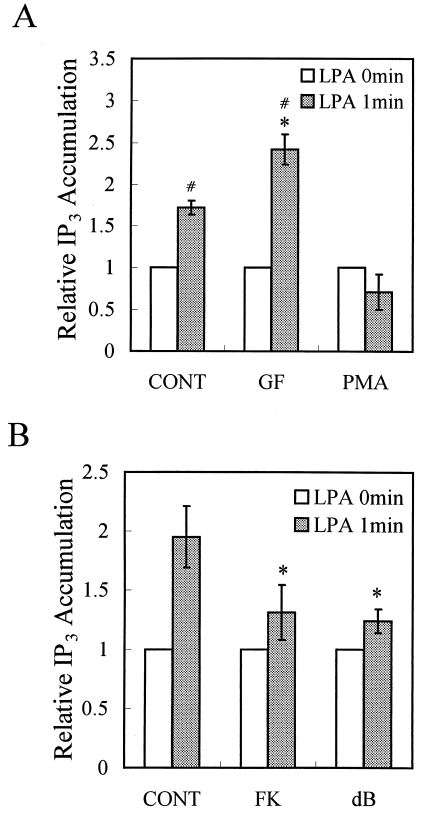

FIG. 4. Effects of activation or inhibition of PKA and PKC on intracellular IP_3 accumulation induced by LPA treatment. (A) Oocytes were pretreated with 100 nM PMA for 10 min (PMA) or 20 μM GF109203X for 3 hr (GF). Treated oocytes (30 cells) were sampled after a 1-min treatment with 0.1 μM LPA (gray bars) or no treatment with LPA (white bars). IP_3 was measured by the procedure described in Materials and Methods. (B) Oocytes were pretreated with 50 μM FK (FK) or 30 μM dB-cAMP (dB) for 1 hr, and 30 oocytes were sampled after a 1-min treatment with 0.1 μM LPA (gray bars) or without treatment (white bars). Key: (#) $P < 0.05$, compared with the LPA-untreated sample; and (*) $P < 0.05$, compared with the LPA-treated sample in the CONT group. The level of IP_3 of the LPA-treated sample was normalized (gray bars) to a value of 1.0 (white bars) representing that of the LPA-untreated sample. Data are means ± SEM of three determinations in three independent experiments from different animals.

4A, control oocytes, not treated with PMA or GF109203X, exhibited an increase in IP_3 mass within 1 min after LPA stimulation. The increase of IP_3 mass induced by LPA was potentiated in oocytes pretreated with 20 μM GF109203X for 3 hr (242 ± 18 vs 172 ± 8.5% in the GF109203X-untreated sample). However, treatment of oocytes with 100 nM PMA for 10 min blocked the IP_3 generation induced by LPA (71 ± 21% of the LPA-untreated sample). These findings suggest that the enhancement of the LPA response in GF109203X-treated oocytes resulted from increased IP_3 production, and prevention of LPA-induced Cl^- currents by PMA treatment occurred through the blockage of IP_3 generation.

The increase in IP_3 level induced by LPA also was suppressed in oocytes pretreated with FK and dB-cAMP (Fig. 4B). Treatment of oocytes with 50 μM FK or 30 μM dB-cAMP for 1 hr significantly reduced the IP_3 level in response to LPA stimulation (Fig. 4B). These results indicated that the reduced average peak current caused by the activation of PKA was due to the reduction of IP_3 production.

Effect of Extracellular Ca^{2+} on the Modulation of the LPA Response by PKC and PKA

To determine whether regulation of Ca^{2+} influx from the extracellular medium is another mechanism adopted by PKA and PKC to inhibit the LPA response, we recorded LPA-induced Cl^- currents in Ca^{2+}-free OR_2 solution. Reduction of the LPA response induced by PKA activation and blockage of it by PKC activation were still reproduced when LPA-induced Cl^- currents were recorded in Ca^{2+}-free OR_2 solution (Fig. 5). These data imply that reduction

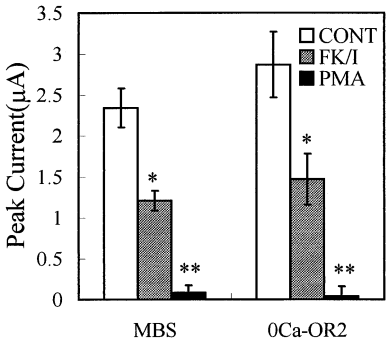

FIG. 5. Effect of extracellular Ca^{2+} on the modulation of LPA response by PKC and PKA. Oocytes were treated with 50 μM FK plus 500 μM IBMX (FK/I) for 1 hr (gray bars) or 100 nM PMA for 10 min (black bars). LPA responses were recorded in MBS or Ca^{2+}-free OR_2 solution (OCa-OR2). Key: (*) $P < 0.05$ and (**) $P < 0.01$, compared with each control. Data are means ± SEM of three independent experiments.

of Ca^{2+} influx from extracellular medium was not a mechanism used by PKA and PKC to inhibit the LPA response.

DISCUSSION

In *Xenopus* oocytes, LPA induces Ca^{2+}-activated Cl^- currents through receptor-mediated activation of the phosphoinositide signal transduction pathway. Previously, we have shown that the LPA-induced signaling pathway is transduced through G_q/G_{11} and an unknown PLC-β in *Xenopus* oocytes [9]. However, the regulation of intracellular signaling components in response to LPA is largely unknown. In the experiments presented here, we have demonstrated that both PKC and PKA modulated LPA-stimulated Cl^- currents in *Xenopus* oocytes.

The effect of PMA on inhibiting the LPA response is consistent with many previous findings that PKC activation regulates agonist-induced biological responses negatively [14, 15]. We also demonstrated that activation of PKC in oocytes blocked the elevation of the IP_3 level elicited by LPA treatment. This observation indicates that the molecular target of PKC in inhibiting LPA response could be located in any sites in the pathway from the LPA receptor to IP_3 generation. The inhibitory effect of PMA on LPA response was overcome by pretreatment of oocytes with GF109203X or staurosporine, PKC inhibitors, implying that PMA acted through PKC activation. Although we have not measured PKC activity, it has been reported that PMA activates, and GF109203X and staurosporine inhibit, the biological activities of *Xenopus* PKC at concentrations and durations of treatment similar to those used in this study [16, 17]. Since the LPA-induced signaling pathway involves G_q/G_{11} and PLC, PKC may be activated as a downstream event of the stimulated LPA signaling pathway. Upon activation, PKC blocks the LPA response, constituting feedback regulation. PKC also negatively regulates the LPA pathway in its basal activity. Thus, PKC may regulate the LPA signaling pathway before and after its stimulation.

PKA also modulates the LPA-induced signal transduction pathway in *Xenopus* oocytes. Unlike PKC, however, PKA does not affect the LPA signaling pathway in its basal activity. It seems necessary for PKA to be activated to regulate the LPA signaling pathway. Although the mechanism by which PKA is activated after stimulation of the LPA pathway is unclear, recent reports have shown that PKC activates some isoforms of adenylyl cyclase [18, 19]. Indeed, we found that the intracellular cAMP level was elevated in PMA-treated oocytes (data not shown). Thus, it is possible that PKC activation after stimulation of the LPA signaling pathway positively regulates adenylyl cyclase in *Xenopus* oocytes. Upon activation by increased cAMP, PKA negatively regulates the LPA-mediated signaling pathway through inhibition of IP_3 generation. One candidate target molecule of activated PKA in the LPA signal transduction pathway is PLC, since it has been demonstrated that PKA inhibits some isotypes of PLC [20].

In conclusion, we have shown in this report that both PKC and PKA negatively regulate LPA-induced Ca^{2+} current in *Xenopus* oocytes. Our results, however, do not reveal the molecular entities of PKA- and PKC-mediated phosphorylation. Further studies to demonstrate the molecular targets of PKA- and PKC-mediated phosphorylation and to determine the correlation between specific phosphorylations on target proteins and the regulation of the LPA signal transduction pathway by PKA and PKC are needed.

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