ELSEVIER

European Journal of Pharmacology 341 (1998) 317–322

ejp

Suppression of phospholipase C blocks G_i-mediated inhibition of adenylyl cyclase activity

Guo-Huang Fan, Tian-Hua Zhou, Wen-Bo Zhang, Gang Pei *

Shanghai Institute of Cell Biology, Chinese Academy of Sciences, 320 Yue Yang Road, Shanghai 200031, PR China

Received 7 August 1997; revised 24 October 1997; accepted 28 October 1997

Abstract

The potential effect of inhibition of phospholipase C on the response of G_i-coupled receptors was investigated in neuroblastoma × glioma hybrid (NG108-15) cells. The phospholipase C specific inhibitor 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione (U73122), which did not affect basal and forskolin-stimulated adenylyl cyclase activities, time- and dose-dependently blocked δ-opioid receptor-mediated inhibition of adenylyl cyclase activity, the EC_{50} (0.5 μM) of which was consistent with that for inhibition of bradykinin-dependent phospholipase C activation (EC_{50} = 1 μM). U73122 treatment also blocked functional responses of m4 muscarinic receptor and α_2-adrenoceptor in NG108-15 cells and three opioid receptors (μ, δ and opioid receptor-like receptor (ORL_1)) in human neuroblastoma SK-N-SH cells. 1-[6-((17β-3-Methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-2,5-pyrrolidinedione (U73343), an inactive analog of U73122, did not show any effect, which suggests that the blockade by U73122 of G_i-coupled receptor-mediated signaling is probably mediated through inhibition of phospholipase C, although a possible direct modification of G proteins can not be excluded. Furthermore, treatment with U73122 but not U73343 blocked the GTP-induced inhibition of adenylyl cyclase, indicating blockade at the level of G_i proteins. © 1998 Elsevier Science B.V.

Keywords: Phospholipase C; G_i protein; Adenylyl cyclase; Phospholipase C inhibitor; G protein-coupled receptor

1. Introduction

Heterotrimeric guanine nucleotide regulating proteins (G proteins) relay information from cell surface receptors to intracellular effectors, and either α or βγ subunits of G proteins can independently transmit signals (Clapham and Neer, 1993; Murthy et al., 1996; Murthy and Makhlouf, 1996). With high fidelity, each G protein transduces the signal from a certain subset of receptors to a restricted array of effectors, including adenylyl cyclase and inositol-phospholipid-specific phospholipase C. In the case of adenylyl cyclase, two different kinds of G proteins are involved, namely G_s, which stimulates adenylyl cyclase and G_i, which inhibits adenylyl cyclase. In the case of phospholipase C, some of its isoforms such as phospholipase Cβ are stimulated by the α subunit of G_q and βγ subunits of G proteins. All four well-characterized forms of phospholipase Cβ are regulated by the α subunit of the G_q class while three of them (phospholipase Cβ1–3) are controlled by βγ subunits (Smrcka et al., 1991; Taylor et al., 1991; Lee et al., 1992).

Besides the high specificity, another prominent characteristic of G protein-mediated signaling is the cross-regulation between the different signaling pathways. For instance, stimulation of the G_s-mediated adenylyl cyclase pathway inhibits activation of phospholipase C mediated by the G_q-coupled receptor (Morris et al., 1991). It has also been shown that activation of cAMP dependent protein kinase after stimulation of G_s-coupled receptors strongly inhibits phospholipase Cβ2 activity induced by the βγ subunits of G proteins (Liu and Simon, 1996). Such cross-regulation among different signaling pathways is probably important to induce diverse cellular responses and to fine-tune the cell functions.

Although it has been shown that activation of G protein/adenylyl cyclase pathway can positively or negatively regulate the G protein/phospholipase C system, it is not clear whether the G protein/phospholipase C system can cross-regulate the G protein/adenylyl cyclase cascade. In the present study, we investigated the potential effect of inhibition of phospholipase C on the adenylyl cyclase

* Corresponding author. Tel.: +86-21-64716049; fax: +86-21-64718563; e-mail: gangpei@sunm.shcnc.ac.cn.

activity mediated by G proteins, using the specific phospholipase C inhibitor, 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione (U73122) (Wu et al., 1992; Yule and Williams, 1992) and its inactive structural analog, 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-2,5-pyrrolidinedione (U73343) (Wu et al., 1992). Our results demonstrated that suppression of phospholipase C blocked G_i-mediated inhibition of adenylyl cyclase activity.

2. Materials and methods

2.1. Materials

[D-Pen^2,D-Pen^5]enkephalin (DPDPE); [D-Ala^2,*N*-MePhe^4,Gly-ol^5]enkephalin (DAMGO), carbachol and 5-bromo-6-[2-imidazolin-2-ylamino]-quinoline (UK14304) were from Sigma Chemical Co. (St. Louis, MO). Nociceptin was from Tocris Cookson (Bristol, UK). Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 and fetal calf serum were from Gibco (Grand Island, NY).

2.2. Cell cultures

NG108-15 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine and HAT (0.1 mM hypoxanthine, 10 μM aminopterin and 16 μM thymidine) as described previously (Cai et al., 1996). Human neuroblastoma SK-N-SH cells (American Type Culture Collection) were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine as described previously (Cheng et al., 1997).

2.3. Assay for inositol phosphate formation

Cells were labelled with 0.75 ml of DMEM containing *myo*-[^{3}H]inositol (2.5 μCi/ml) and 5% (v/v) fetal calf serum. At 24 h after labeling, the cells were rinsed with 2 ml of assay medium (20 mM HEPES-buffered DMEM with 20 mM LiCl) followed by incubation at 37°C for 1 h in 1 ml of assay medium with the indicated drugs. inositol phosphates production was estimated by determining the ratio of [^{3}H]inositol phosphates to [^{3}H]inositol plus [^{3}H]inositol phosphates as described previously (Conklin et al., 1992).

2.4. Cyclic AMP assay

Cells ($\sim 1 \times 10^5$ cells/sample) were incubated at 37°C for 10 min with 10 μM forskolin, 500 μM 1-methyl-3-isobutylxanthine (IBMX) and test reagents. The reaction was terminated with 1 N perchloric acid and neutralized with 2 N K_2CO_3. The cAMP levels were determined by radioimmunoassay as described previously (Cai et al., 1997). Values were calculated as $100 \times$ [cAMP(For + agonist) − cAMP(basal)]/[cAMP(For) − cAMP(basal)]. cAMP(For + agonist) represents cAMP accumulation in the presence of forskolin and agonist, cAMP(basal) represents cAMP accumulation in the absence of forskolin and agonist and cAMP(For) represents cAMP accumulation in the presence of forskolin alone.

2.5. Statistical analysis

Each experimental point was performed in triplicate and at least three experiments were carried out. Data are expressed as means ± S.E. of all determinations. When not shown, error bars are smaller than symbols. Statistical significance of the experimental results was assessed by using Student's *t*-test on STATVIEW program. $P < 0.05$ was accepted as denoting statistical significance.

3. Results

3.1. Blockade by U73122 of bradykinin-mediated activation of phospholipase C

Bradykinin, a specific agonist of the bradykinin receptor which couples to the G_q family of G proteins, has been reported to stimulate the metabolism of inositol phospholipids in NG108-15 cells by activation of phospholipase C (Nishizuka, 1984). After treatment of the cells with bradykinin (10 μM) together with U73122, a specific phospholipase C inhibitor, the stimulation of inositol phosphates production by bradykinin was dose-dependently blocked by U73122 with an EC_{50} of approximately 1 μM (Fig. 1).

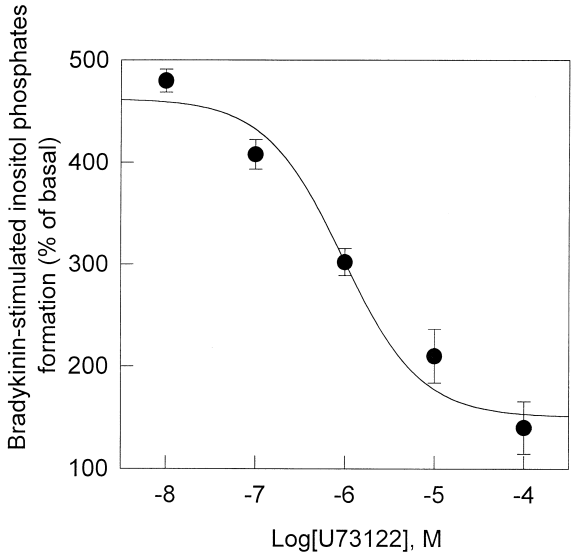

Fig. 1. Blockade by U73122 of bradykinin-stimulated inositol phosphates formation. Cells were labeled with myo-[^{3}H]inositol. inositol phosphates production was measured after exposure to bradykinin (10 μM) in the absence or presence of increasing concentrations of U73122 as indicated.

3.2. Reversal of δ-opioid receptor-mediated inhibition of adenylyl cyclase by U73122 in NG108-15 cells

The δ-opioid receptor is functionally coupled to the inhibitory G protein (G_i) and, thus, negatively regulates adenylyl cyclase in NG108-15 cells (Law et al., 1983). After coadministration of DPDPE, a selective δ-opioid receptor agonist, with U73122 (reported $IC_{50} = 1–2$ μM), the inhibition of cAMP accumulation by DPDPE was potently blocked. The effect of U73122 was time-dependent and maximal inhibition was reached after 10 min incubation (Fig. 2A). Therefore, a 10 min of incubation time was used in all the experiments performed thereafter. Fig. 2B shows the concentration–response curve of U73122

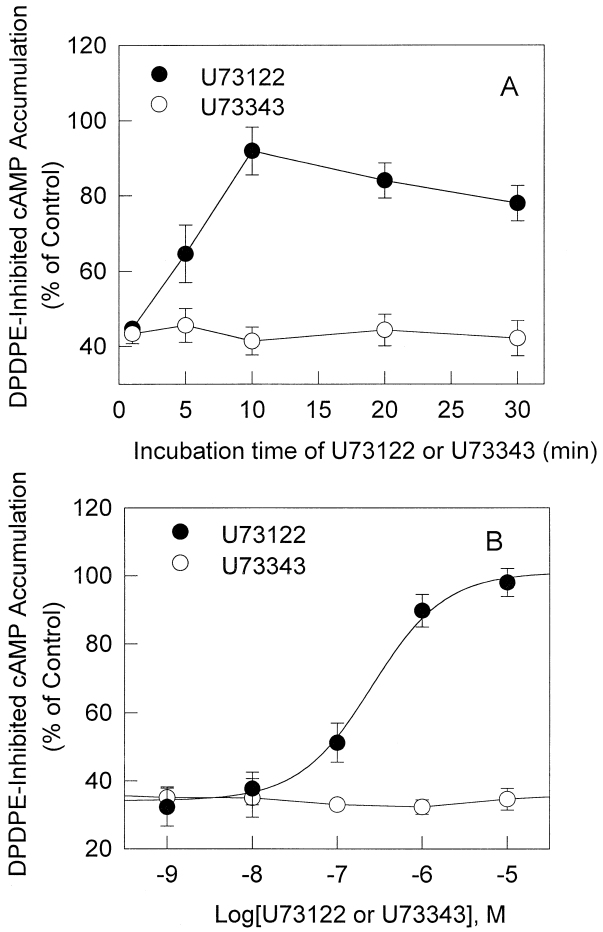

Fig. 2. Time- and dose-dependent blockade by the phospholipase C inhibitor U73122 of δ-opioid receptor-mediated inhibition of adenylyl cyclase in NG108-15 cells. (A) Time-dependent effects of U73122 on DPDPE inhibition of forskolin-stimulated cAMP accumulation. Cells were incubated at 37°C with forskolin (10 μM), IBMX (500 μM) and DPDPE (1 μM) for 10 min in the presence of U73122 (10 μM) or U73343 (10 μM) for different times indicated. The reaction was terminated with 1 N perchloric acid and cAMP concentration was measured by radioimmunoassay. (B) Dose-dependent effect of U73122 on DPDPE inhibition of cAMP accumulation. Cells were incubated at 37°C for 10 min with forskolin (10 μM), IBMX (500 μM) and DPDPE (0.1 μM) in the absence or presence of different concentrations of U73122 or U73343. The reaction was terminated and cAMP concentration was measured by radioimmunoassay. Values were calculated as described in Section 2.

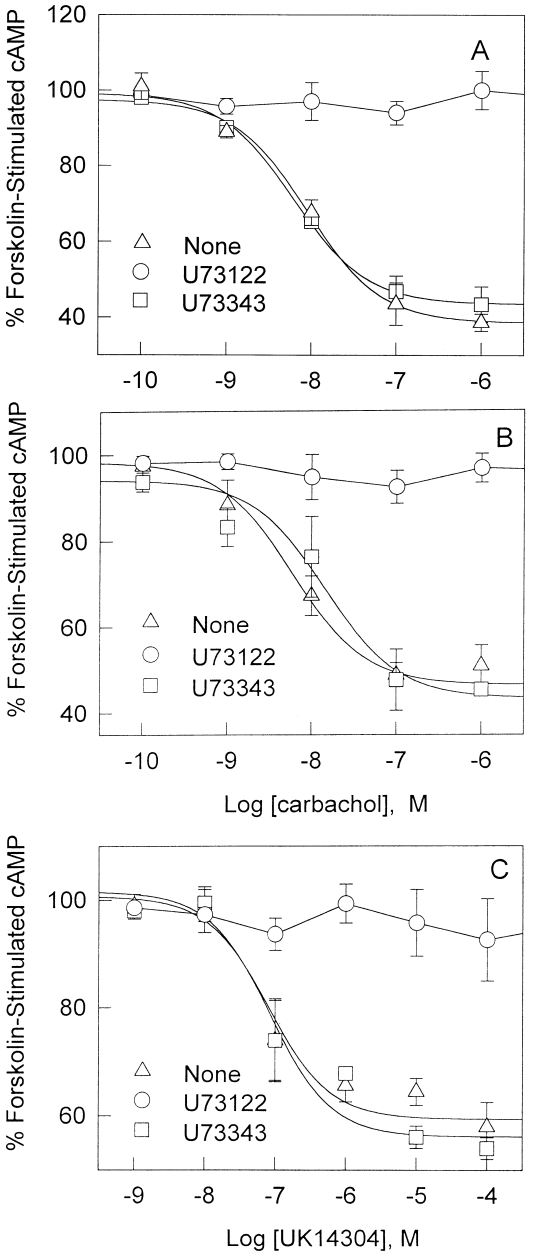

Fig. 3. Blockade by U73122 of inhibition of cAMP accumulation by DPDPE (panel A), carbachol (panel B), or UK14304 (panel C) in NG108-15 cells. Cells were incubated at 37°C for 10 min with forskolin (10 μM), IBMX (500 μM) and DPDPE (10^{-10}–10^{-6} M) or carbachol (10^{-10}–10^{-6} M) or UK14304 (10^{-9}–10^{-4} M) in the absence or presence of U73122 (10 μM) or U73343 (10 μM). cAMP levels were determined and calculated as described Section 2.

on DPDPE-induced attenuation of cAMP accumulation. Half-maximal inhibition was achieved at approximately 0.5 μM, which agreed with that for inhibition of agonist-dependent phospholipase C activation. The effect of U73122 was not mimicked by U73343, an inactive structural analog of U73122, suggesting the effect of U73122 was possibly mediated through inhibition of phospholipase C, though a possible direct modification could not be excluded.

3.3. Blockade of G_i-coupled receptor-mediated inhibition of adenylyl cyclase activity by U73122 in NG108-15 cells

In addition to δ-opioid receptor, there are other G_i protein-coupled receptors such as m4 muscarinic receptor and α_2-adrenoceptor expressed in NG108-15 cells (McClue and Milligan, 1990; Strassheim and Malbon, 1994). To test if the inhibition of adenylyl cyclase activity by these G_i-coupled receptors was also affected by phospholipase C inhibitor, NG108-15 cells were challenged with U73122 together with either the muscarinic receptor agonist carbachol (10^{-10}–10^{-6} M) or the α_2-adrenoceptor agonist UK14304 (10^{-9}–10^{-4} M). Interestingly, the inhibition by both carbachol and UK14304 of forskolin-stimulated cAMP accumulation was potently blocked by U73122 treatment but not by U73343 treatment (Fig. 3B and C).

3.4. Effect of U73122 on G_i-coupled receptor function in SK-N-SH cells

To exclude the possibility that the effect of phospholipase C inhibitor on G_i-coupled receptor function is a characteristic limited to NG108-15 cells, human neuroblastoma cell line SK-N-SH cells were tested for an effect of U73122 on G_i-coupled δ, μ and ORL_1 receptors (Zhang et al., 1996; Cheng et al., 1997). Treatment of the cells with U73122 (10 μM) together with 0.1 μM DPDPE, 0.1 μM DAMGO (a specific μ-opioid receptor agonist), or 0.1 μM nociceptin (a specific ORL_1 receptor agonist) potently blocked the inhibition of adenylyl cyclase activity induced by these agonists (Fig. 4). In contrast, treatment with U73343 (10 μM) displayed no apparent effect (Fig. 4).

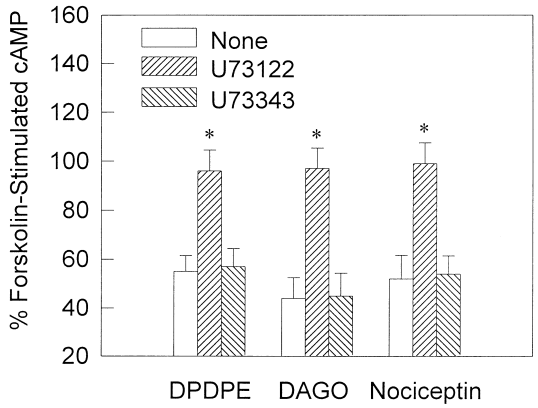

Fig. 4. Blockade by U73122 of μ, δ and ORL_1 receptor-mediated inhibition of adenylyl cyclase activity in SK-N-SH cells. Cells were incubated at 37°C for 10 min with forskolin (10 μM), IBMX (500 μM), DPDPE (0.1 μM) or DAGO (0.1 μM) or nociceptin (0.1 μM) in the absence or presence of U73122 (10 μM) or U73343 (10 μM). cAMP levels were determined and calculated as described in Section 2. $^*P <$ 0.05 in comparison with control.

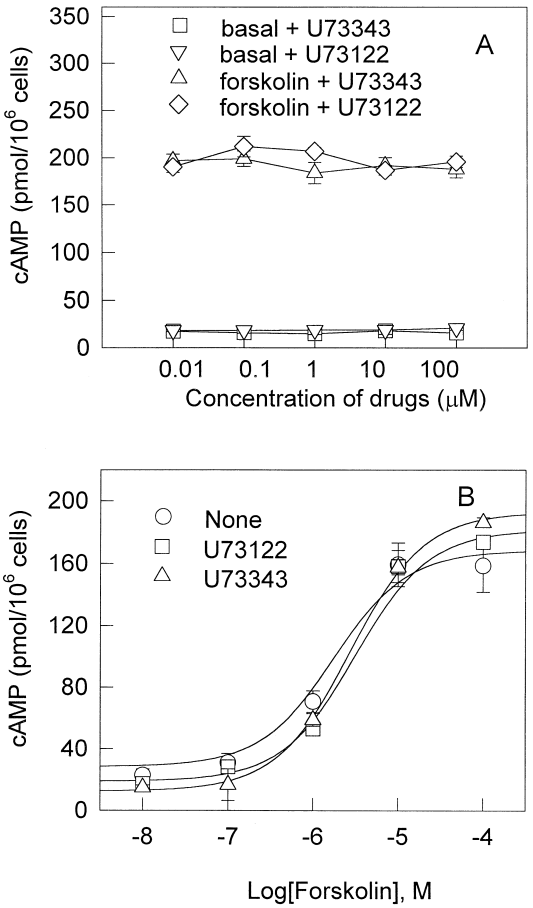

Fig. 5. Lack of effect of U73122 on basal and forskolin-stimulated adenylyl cyclase activity in NG108-15 cells. (A) Cells were treated at 37°C for 10 min without or with forskolin (10 μM), and IBMX (500 μM) in the absence or presence of different concentrations of U73122 (10^{-8}–10^{-4} M) or U73343 (10^{-8}–10^{-4} M). (B) Cells were treated at 37°C for 10 min with forskolin (10^{-8}–10^{-4} M) and IBMX (500 μM) in the absence or presence of 10 μM U73122 or 10 μM U73343. cAMP levels were determined by radioimmunoassay as described in Section 2.

3.5. Lack of effect of U73122 on basal and forskolin-stimulated adenylyl cyclase activity in NG108-15 cells

The above results raise the question whether the inhibition of phospholipase C by U73122 is mediated by its effect on adenylyl cyclase. NG108-15 cells were treated without or with forskolin in the presence of U73122 or U73343 and then cAMP concentrations were measured. As shown in Fig. 5A, basal and forskolin-stimulated cAMP levels were not altered by different concentrations of U73122 or U73343. The concentration-dependent stimulation of cAMP accumulation by forskolin was also unchanged after treatment with either 10 μM U73122 or 10 μM U73343 (Fig. 5B). These results, taken together, indicate that inhibition of phospholipase C did not affect the basal and forskolin-stimulated activity of adenylyl cyclase.

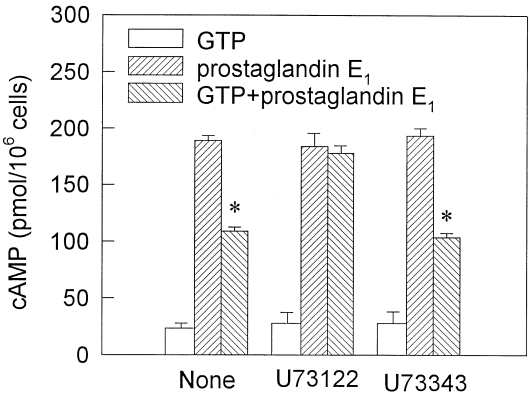

Fig. 6. Inhibition of adenylyl cyclase by GTP was blocked in broken cell preparations from U73122 treated cells. Cells were homogenized in phosphate buffered saline and the homogenates were incubated at 37°C for 10 min with IBMX (500 μM), GTP (0.1 mM), or prostaglandin E_1 (10 μM), or GTP (0.1 mM)+prostaglandin E_1 (10 μM) in the absence or presence of either U73122 (10 μM) or U73343 (10 μM). cAMP levels were determined by radioimmunoassay. $^{*}P < 0.05$ in comparison with prostaglandin E_1 treated cells.

3.6. Blockade of GTP-induced inhibition of adenylyl cyclase by U73122

In order to assess the potential effects of phospholipase C inhibition on the activity of G_i proteins, we assayed adenylyl cyclase activity in cell homogenates prepared from control, U73343- or U73122-treated NG108-15 cells. Cells were treated for 10 min with 10 μM U73122 or 10 μM U73343, immediately homogenized and then assayed for adenylyl cyclase activity. In this assay, cyclic AMP accumulation in response to stimulation by prostaglandin E_1 (10 μM) was not altered by inhibition of phospholipase C. High concentrations (0.1 mM) of GTP generally activate inhibitory adenylyl cyclase (Strassheim and Malbon, 1994), possibly due to an effect of phospholipase Cβ and/or an effect of G_i. It was shown that GTP inhibition of prostaglandin E_1-stimulated cyclic AMP accumulation was significantly blocked by treatment with U73122 but not by U73343 treatment (Fig. 6). Our data suggest that G proteins are probably affected by the inhibition of phospholipase C.

4. Discussion

The present study demonstrated that the specific phospholipase C inhibitor U73122 dose- and time-dependently blocked the δ-opioid receptor-mediated inhibition of adenylyl cyclase activity in NG108-15 cells. The inhibition of adenylyl cyclase by two other G_i-coupled receptors, m4 muscarinic receptor and α_2-adrenoceptor, was also blocked by U73122. In addition, the δ, μ and ORL_1 receptor-induced reduction of cAMP levels was completely reversed by U73122 in SK-N-SH cells. The fact that treatment with U73343, an inactive structural analog of U73122, did not show any effect under the same conditions, and that the EC_{50} for U73122 to inhibit the DPDPE response was similar to that to inhibit phospholipase C activation in NG108-15 cells suggested that the observed effect of U73122 was likely mediated through inhibition of phospholipase C, though a possible covalent modification by the compound could not be excluded. Taken together, our results demonstrate that the phospholipase C inhibitor U73122 blocked G_i-mediated inhibition of adenylyl cyclase activity.

It has been established that stimulation of phospholipase C leads to generation of inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol followed by mobilization of Ca^{2+} from intracellular stores and activation of protein kinase C. But it seems unlikely that the blockade of the G_i/adenylyl cyclase system by inhibition of phospholipase C was due to suppression of Ca^{2+} mobilization or protein kinase C, since removal of intracellular Ca^{2+} with the Ca^{2+} chelator did not affect the functional responses of opioid receptors (data not shown), and nor did acute blockade of protein kinase C change opioid receptor-mediated inhibition of adenylyl cyclase, as has been demonstrated in our previous study (Cai et al., 1997). On the contrary, activation rather than inhibition of protein kinase C has been shown to attenuate inhibition of adenylyl cyclase mediated by opioid and other G_i-coupled receptors (Strassheim and Malbon, 1994). This suggests that inhibition of phospholipase C itself cross-regulates the G_i/adenylyl cyclase cascade.

The blockade by U73122 of G_i-coupled receptor signaling could occur at the level of the receptor, G protein, or adenylyl cyclase. U73122 treatment, which significantly blocked δ-opioid receptor-mediated signaling, did not affect agonist binding to the receptor (data not shown). In addition, basal and forskolin-stimulated adenylyl cyclase activity was unchanged in response to the phospholipase C inhibitor treatment. Further evidence that the GTP-induced inhibition of adenylyl cyclase activity was blocked by U73122 but not U73343 and that opioid-stimulated activation of G proteins was blocked by U73122 but not by U73343 (data not shown) indicated the blockade was at the level of G_i proteins.

Although the present study shows that the phospholipase C inhibitor U73122 blocks G_i-mediated signaling, the underlying mechanisms remain unclear. In addition to the potential direct modification of G proteins by the compound, one possibility could be that inhibition of phospholipase C, which possesses a pleckstrin homology domain and binds to $\beta\gamma$ subunits of G proteins (Harlan et al., 1995; Luttrell et al., 1995), may indirectly affect the activity of G_i proteins by interfering with the dissociation of $\beta\gamma$ subunits from the α subunit. Another possibility could be that phospholipase C acts as a guanine nucleotide release protein, which catalyses the release of bound GDP and promotes the reactivation of G proteins (Bourne et al.,

1991) and thus inhibition of phospholipase C would prevent GDP release and the subsequent activation of G proteins.

Acknowledgements

We thank Dr. Lan Ma, Dr. Li-Guang Lou, Ya-Lan Wu and Li-Zhen Jiang for technical support and helpful discussion. This work was supported by the research grants from the National Science Foundation of China (39630130 and 39625010), Chinese Academy of Sciences and German Max-Planck Society.

References

Bourne, H.R., Sanders, D.A., McCormick, F., 1991. The GTPase superfamily: Conserved structure and molecular mechanism. Nature 349, 117–126.

Cai, Y.-C., Zhang, Y., Wu, Y.-L., Pei, G., 1996. δ-opioid receptor in neuronal cells undergoes acute and homologous desensitization. Biochem. Biophys. Res. Commun. 219, 342–347.

Cai, Y.-C., Ma, L., Fan, G.-H., Zhao, J., Jiang, L.-Z., Pei, G., 1997. Activation of *N*-methyl-D-aspartate receptor attenuates acute responsiveness of δ-opioid receptors. Mol. Pharmacol. 51, 583–587.

Cheng, Z.-J., Fan, G.-H., Zhao, J., Zhang, Z., Wu, Y.-L., Jiang, L.-Z., Zhu, Y., Pei, G., Ma, L., 1997. Endogenous opioid receptor-like receptor in human neuroblastoma SK-N-SH cells: Activation of inhibitory G protein and homologous desensitization. Neuroreport 8, 1913–1918.

Clapham, D.E., Neer, E.J., 1993. New roles for G-protein βγ-dimers in transmembrane signalling. Nature 365, 403–406.

Conklin, B.R., Chabre, O., Wong, Y.H., Federman, A.D., Bourne, H.R., 1992. Recombinant $G_{q\alpha}$: Mutational activation and coupling to receptors and phospholipase C. J. Biol. Chem. 267, 31–34.

Harlan, J.E., Yoon, H.S., Hajduk, P.J., Fesik, S.W., 1995. Structural characterization of the interaction between a pleckstrin homology domain and phosphatidylinositol 4,5-bisphosphate. J. Biol. Chem. 34, 9859–9864.

Law, P.Y., Hom, D.S., Loh, H.H., 1983. Opiate regulation of adenosine 3′,5′-cyclic monophosphate level in neuroblastoma×glioma (NG108-15) hybrid cells: Relationship between receptor occupancy and effect. Mol. Pharmacol. 23, 26–35.

Lee, H.L., Park, D., Wu, D., Rhee, S.G., Simon, M.I., 1992. Members of the $G_{q\alpha}$ subunit gene family activate phospholipase Cβ isozymes. J. Biol. Chem. 267, 16044–16047.

Liu, M., Simon, M.I., 1996. Regulation by cAMP-dependent protein kinase of a G-protein-mediated phospholipase C. Nature 382, 83–87.

Luttrell, L.M., Hawes, B.E., Touhara, K., Van-Biesen, T., Koch, W.T., Lefkowitz, R.T., 1995. Effect of cellular expression of pleckstrin homology domains on G_i-coupled receptor signaling. J. Biol. Chem. 270, 12984–12989.

McClue, S.J., Milligan, G., 1990. The α_2B adrenergic receptor of undifferentiated neuroblastoma×glioma hybrid NE108-15 cells, interacts directly with the guanine nucleotide binding protein, $G_{i\alpha 2}$. FEBS Lett. 269, 430–434.

Morris, G.M., Hadcock, J.R., Malbon, C.C., 1991. Cross-regulation between G-protein-coupled receptors. J. Biol. Chem. 266, 2233–2238.

Murthy, K.S., Makhlouf, G.M., 1996. Opioid μ, δ, and κ receptor-induced activation of phospholipase C-β3 and inhibition of adenylyl cyclase is mediated by G_{i2} and G_o in smooth muscle. Mol. Pharmacol. 50, 870–877.

Murthy, K.S., Coy, D.H., Makhlouf, G.M., 1996. Somatostatin receptor-mediated signaling in smooth muscle. J. Biol. Chem. 271, 23458–23463.

Nishizuka, Y., 1984. The role of protein kinase C in cell surface signal transduction and tumao promotion. Nature 308, 693–698.

Smrcka, A.V., Hepler, J.R., Brown, K.O., Sternweis, P.C., 1991. Regulation of polyphosphoinositide-specific phospholipase C activity by purified G_q. Science 251, 804–807.

Strassheim, D., Malbon, C.C., 1994. Phosphorylation of $G_{i\alpha 2}$ attenuates inhibitory adenylyl cyclase in neuroblastoma×glioma hybrid (NG108-15) cells. J. Biol. Chem. 269, 14307–14313.

Taylor, S.J., Chae, H.Z., Rhee, S.G., Exton, J.C., 1991. Activation of the β_1 isozyme of phospholipase C by α subunits of the G_q class of G proteins. Nature 350, 516–518.

Wu, H., James-Krache, M.R., Halenda, S.P., 1992. Direct relationship between intracellular calcium mobilization and phospholipase D activation in prostaglandin E-stimulated human erythroleukemia cells. Biochemistery 31, 3370–3377.

Yule, D.I., Williams, J.A., 1992. U73122 inhibits Ca^{2+} oscillations in response to cholecystokinin and carbachol but not to JMV-180 in rat pancreatic acinar cells. J. Biol. Chem. 267, 13830–13835.

Zhang, L., Yu, Y., Mackin, S., Weight, F.F., Uhl, G.R., 1996. Differential μ-opioid receptor phosphorylation and dephosphorylation induced by agonists and phorbol esters. J. Biol. Chem. 271, 11449–11454.