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Functional regulation of GTP-binding protein coupled to insulin-like growth factor-I receptor by lithium during G_1 phase of the rat thyroid cell cycle

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The regulatory effects of lithium on the function of pertussis toxin-sensitive GTP-binding (G_i)-proteins located on the mitogenic pathway activated by insulin-like growth factor-I (IGF-I) in FRTL-5 cells were studied. Addition of GTP-γ-S to the thyroid stimulating hormone-primed cell membranes resulted in a decreased affinity of IGF-I receptor binding, and the dissociation constant (K_d) increased from 0.46 nM to 3.1 nM. Moreover, IGF-I stimulated GTP-γ-S binding to a 40-kDa protein, and pertussis toxin (PT) attenuated the stimulatory effect of IGF-I on the same protein. Lithium lowered the affinity of IGF-I receptor binding and the K_d (3.4 nM) was in the same range as that in the presence of GTP-γ-S. The inhibitory effect of lithium was markedly abolished by pretreatment with PT. Lithium attenuated the amounts of ADP-rebosylation of the 40-kDa protein by PT. In addition, lithium stimulated Ca^{2+} entry, similar to that by IGF-I, and induced cell proliferation via a PT-sensitive step. These findings suggest that lithium may be capable of modulating the function of G_i-proteins coupled to IGF-I receptors during the G_1 phase of the FRTL-5 cell cycle.

GTP-binding protein; Insulin-like growth factor-I (IGF-I) receptor; Lithium; Cell cycle; FRTL-5 cell

1. INTRODUCTION

Lithium, a monovalent cation, has been used therapeutically for the treatment of manic-depressive disease and has a variety of biological effects on many tissues [1,2]. Moreover, the mitogenic effect of lithium has been reported on thyroid cells and fibroblasts [3,4]. The modulation mechanism of cell proliferation induced by lithium, however, remains unknown.

The activation of GTP-binding protein (G-protein)-mediated pathways in the appropriate phase of the cell cycle might promote the changes required for cell proliferation. Many growth factors and hormones bind to specific receptors that are coupled to G-proteins. Insulin-like growth factor-I (IGF-I) is capable of opening specific Ca^{2+}-channels via pertussis toxin (PT)-sensitive G-proteins (G_i) but only in the G_1 phase of the cell cycle [5,6]. In addition, there is evidence that activating mutations of G-proteins can cause cell transformation [7]. On the other hand, lithium has the ability to affect G-protein-dependent phenomena such as receptor-activated inositol lipid metabolism and adenylate cyclase activity [8,9].

In this work, we examined whether the IGF-I receptor directly couples to G_i-proteins, and further studied the effect of lithium on the G_i-protein-coupled mitogenic system activated by IGF-I during the G_1 phase of the rat thyroid (FRTL-5) cell cycle.

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2. MATERIALS AND METHODS

2.1. *Materials*

[^{35}S]GTP-γ-S, [^{45}Ca]Cl_2 and [^{3}H]thymidine were purchased from DuPont-New England Nuclear, GTP-γ-S from Sigma Chemical Co., biosynthetic IGF-I, [^{125}I]IGF-I and [α-^{32}P]NAD from Amersham Co., pertussis toxin from Funakoshi Co. (Tokyo, Japan), and other materials and culture medium were obtained from commercial sources.

2.2. *Cell culture*

FRTL-5 cells were maintained in Coon's modified Ham's F-12 medium supplemented with 5% calf serum and six hormones (100 μU/ml thyroid stimulating hormone (TSH), 10 μg/ml insulin, 5 μg/ml transferrin, 10 ng/ml somatostatin, 10 nM cortisone, and 10 ng/ml glycyl-L-histidyl-L-lysine acetate) as described previously [10]. In preparation for experiments, quiescent cells were obtained as follows: cells were incubated for 3 days in culture medium containing the six hormones and then for 2 days in hormone-free basal medium consisting of Ham's F-12. TSH-primed (competent) cells were obtained as follows; quiescent cells were incubated for 6 h in Ham's F-12 medium containing 100 μU/ml TSH and 0.25% BSA, and then washed three times with PBS.

2.3. *IGF-I binding assay*

TSH-primed cells were detached with 1 mM EDTA in saline as described [11]. Cells were pelleted by centrifuging at 1,000 × g for 10 min, washed, resuspended in cold buffer containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.25 M sucrose, 4 mM iodoacetic acid, homogenized with a Dounce homogenizer, and centrifuged at 30,000 × g for 40 min. The resultant membrane pellet was resuspended in binding buffer containing 100 mM HEPES-NaOH (pH 7.5), 120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 15 mM CH_3COONa, 1 mM EDTA, 10

mM dextrose, and 0.5% BSA. Aliquots containing 20 μg of membrane protein were incubated with 10 pM iodinated IGF-I in the presence and absence of unlabeled IGF-I at 37°C for 1 h. The membranes were washed in several volumes of cold buffer, and bound radioactivity was measured by a γ-counter. Non-specific binding was determined in the presence of excess unlabeled IGF-I. Receptor number and affinity were estimated by Scatchard analysis according to the method described in [12].

2.4. *ADP-ribosylation of membranes by pertussis toxin (PT)*

Cell membranes were incubated in medium containing 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10 mM dithiothreithol, 2 mM $MgCl_2$, 10 mM thymidine, 1 mM ATP, 0.1 mM GTP, 10 μM [α-^{32}P]NAD, and 10 μg/ml PT at 30°C for 90 min. The reaction was terminated by the addition of 2 × SDS-PAGE sample buffer and immediate boiling. According to the method of Laemmli [13], proteins were electrophoresed in acrylamide gels with SDS. To quantify incorporated [^{32}P]ADP-ribose, a 40-kDa band was excised and radioactivity counted using a scintillation counter.

2.5. *GTP-γ-S binding assay*

Membranes from TSH-primed cells were incubated with or without IGF-I in buffer containing 20 mM HEPES-NaOH (pH 7.4), 100 μM EDTA, 125 μM $MgCl_2$, and 100 nM [^{35}S]GTP-γ-S at 37°C for 15 min as described [14]. Incubations were terminated by adding ice-cold buffer containing 100 mM Tris-HCl (pH 8.0), 25 mM $MgCl_2$, 100 mM NaCl, and 20 μM GTP. The GTP-γ-S binding to a 40-kDa protein was recovered by the gel filtration method [15], and radioactivity was counted in a liquid scintillator.

2.6. *Measurement of Ca^{2+} entry*

Ca^{2+} entry was determined by measuring ^{45}Ca uptake [16]. Cells were incubated for the indicated time in Ham's F-12 medium containing 0.25% BSA and with or without one or more of the following compounds; LiCl, IGF-I and PT. Cells were incubated at 37°C for 30, 60, 90 or 120 s, and the reaction was terminated by aspirating the labeled medium. Cells were washed five times with ice-cold phosphate-buffered saline containing 25 mM $MgCl_2$, and were lysed in 1 M NaOH, and the ^{45}Ca content of the lysate was determined by liquid scintillation spectrometry. The rate of Ca^{2+} entry was calculated by using a slope of the linear regression line of Ca^{2+} uptake [6].

2.7. *Measurement of cell proliferation*

Cell proliferation was assessed by [^{3}H]thymidine incorporation, with or without one or more of the following agents; LiCl, IGF-I and PT. The reaction was stopped by addition of 10% trichloroacetic acid and the radioactivity in acid-insoluble material was counted in a liquid scintillation spectrometer.

3. RESULTS

We studied the effect of GTP-γ-S on IGF-I binding to the receptor in membranes from TSH-primed FRTL-5 cells. The addition of GTP-γ-S altered the dissociation constant (K_d) of IGF-I receptor binding from 0.46 nM to 3.1 nM (Fig. 1). We further examined whether the IGF-I receptor was coupled to PT-sensitive G-proteins (G_i-proteins) in TSH-primed cells. Fig. 2 shows the effect of PT on IGF-I-induced stimulation of GTP-γ-S binding. IGF-I facilitated GTP-γ-S binding to a 40-kDa protein. When membranes were pretreated with PT, the GTP-γ-S binding to the 40-kDa protein by IGF-I was inhibited. These data demonstrate the direct coupling of the IGF-I receptor with G_i-proteins in TSH-primed cells.

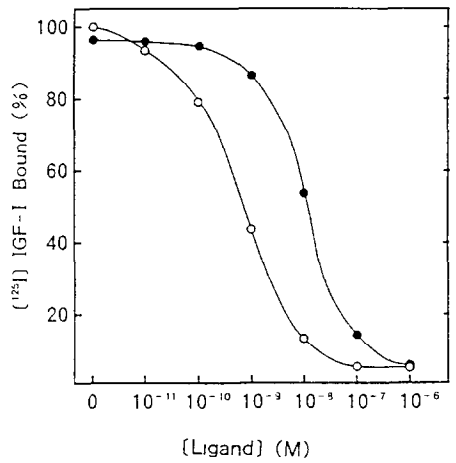

Fig. 1. Modulation of the IGF-I receptor binding by GTP-γ-S. Membranes of TSH-primed FRTL-5 cells were incubated with [^{125}I]IGF-I and increasing concentrations of unlabeled IGF-I in the presence (●) or absence (○) of 100 μM GTP-γ-S.

We next investigated the effect of lithium on G_i-proteins coupled to the IGF-I receptor. Treatment with lithium, at therapeutic concentrations, decreased the affinity of IGF-I receptor binding and increased the K_d value from 0.46 nM to 3.4 nM (Fig. 3A). The K_d value in the presence of 1 mM LiCl was in the same range as that in 100 μM GTP-γ-S. We observed the effect of PT on the lithium action. Pretreatment with PT abolished the effect of lithium on IGF-I receptor binding (Fig. 3B). Although the IGF-I receptor may be coupled to G_i-proteins, treatment with PT had no effect on the affinity of IGF-I receptor binding.

We examined the lithium action on ADP-ribosylation of G_i-proteins by PT. Fig. 4 shows that lithium decreased the amounts of ADP-ribosylation of G_i-proteins in a dose-dependent manner. Addition of 1 mM LiCl to the assay medium caused an about 20% decrease in ADP-ribosylation of G_i-proteins by PT compared with that in the absence of LiCl.

We next studied the effect of lithium on the function of G_i-proteins in mediating Ca^{2+} entry and cell cycle progression. As shown in Fig. 5, lithium, like IGF-I, markedly induced both sustained Ca^{2+} entry and DNA synthesis via the PT-sensitive pathway.

4. DISCUSSION

Conceivably, IGF-I receptors can be directly coupled to G_i-proteins in TSH-primed FRTL-5 cells. The following data support this conclusion; (i) the affinity of IGF-I binding to TSH-primed cell membranes was lowered by the addition of GTP-γ-S; (ii) IGF-I stimulates GTP-γ-S binding to the 40-kDa protein; (iii) treatment of membranes with PT attenuates the stimulatory effect of IGF-I on GTP-γ-S binding to the 40-kDa protein;

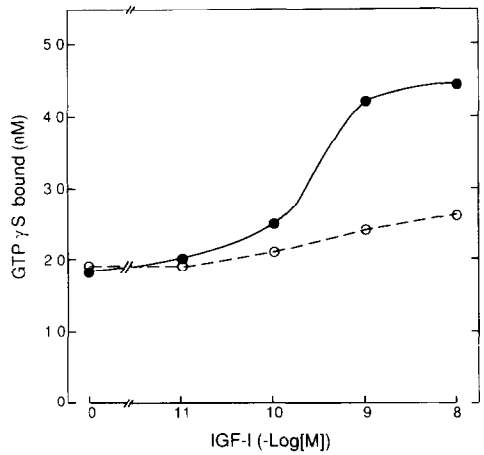

Fig. 2. Effect of PT on IGF-I-induced stimulation of GTP-γ-S binding. Membranes were pretreated with (○) or without (●) 1 ng/ml PT for 2 h and then stimulated with increasing concentrations of IGF-I.

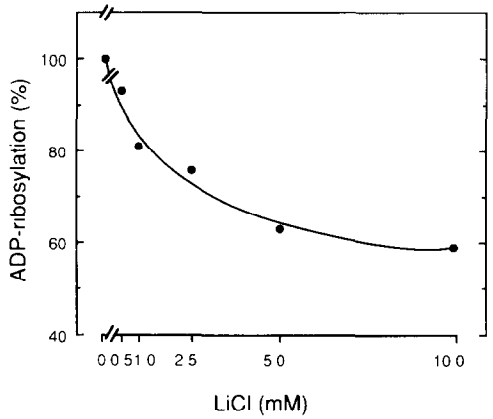

Fig. 4. Effect of lithium on ADP-ribosylation of the 40-kDa protein by PT. Membranes were incubated with PT (10 μg/ml) in the presence of increasing concentrations of LiCl for 2 h. The ADP-ribosylation is expressed as a percentage of the control value obtained with cells cultured in the absence of LiCl.

(iv) PT abolishes IGF-I-mediated stimulation of Ca^{2+} entry and cell cycle progression.

The present study provides evidence that one site of the action of lithium may be located on G_i-proteins with which the IGF-I receptor interacts in TSH-primed FRTL-5 cells. Lithium lowered the affinity of the IGF-I receptor in a PT-sensitive manner in TSH-primed cells. Moreover, lithium attenuated the amounts of ADP-ribosylation of the 40-kDa protein by PT. In TSH-primed cells, lithium stimulated Ca^{2+} entry, similar to that by IGF-I, and induced cell cycle progression via a PT-sensitive step. Taking these data together, lithium conceivably may open the Ca^{2+}-channel indirectly through functional modulation of G_i-proteins linked with the IGF-I receptor during the G_1 phase of the cell cycle. Further, Ca^{2+} entry stimulated by lithium, as well as by IGF-I, may be a mitogenic signal.

Recently, an involvement of G-proteins in the action of lithium has been reported. Some studies have demonstrated that chronic lithium treatment results in a damping of neurotransmitter agonist-stimulated inositol lipid hydrolysis and cAMP formation by perturbing G-protein-receptor coupling [8,9]. TSH-stimulated cAMP generation is known to be decreased by lithium [17]. Moreover, lithium attenuates either Gpp(NH)p- or forskolin-induced activation of adenylate cyclase [18]. Taken together, lithium may block the function of G-proteins. By contrast, lithium can activate the G_i-protein-associated Ca^{2+}-channel in FRTL-5 cells, thereby inducing a mitogenic signal. Therefore, lithium might

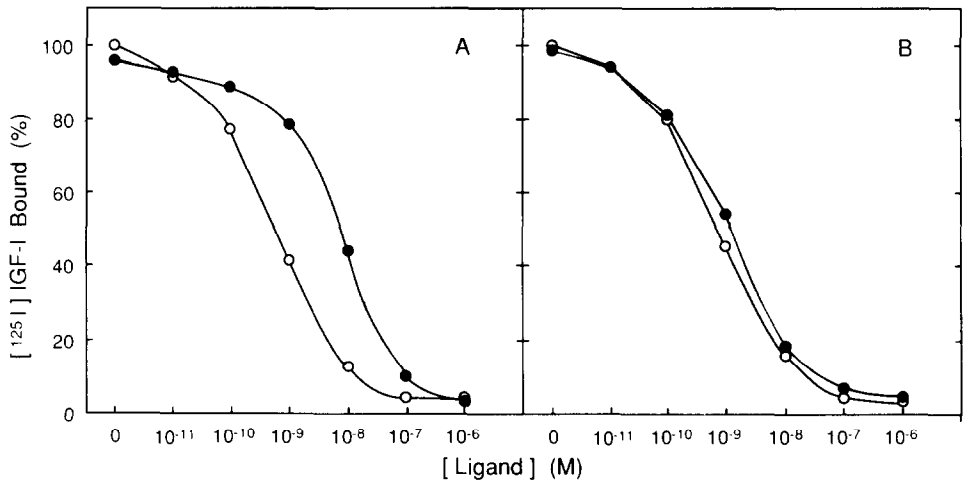

Fig. 3. Effect of lithium on IGF-I receptor binding. (A) Membranes of TSH-primed cells were incubated with [^{125}I]IGF-I and increasing concentrations of IGF-I in the presence (●) or absence (○) of 1 mM LiCl. (B) Abolition of the lithium effect on IGF-I receptor binding. Membranes were pretreated with PT (1 ng/ml) for 2 h. Then, membranes were incubated with [^{125}I]IGF-I and increasing concentrations of IGF-I in the presence (●) or absence (○) of 1 mM LiCl.

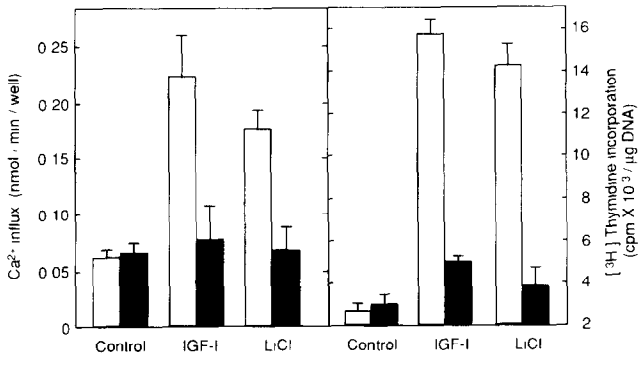

Fig. 5. Inhibitory effect of PT on lithium-stimulated Ca^{2+} entry and DNA synthesis. TSH-primed cells were pretreated with (filled columns) or without (open columns) 1 ng/ml PT for 2 h. Then, cells were incubated with 1 mM LiCl or 1 nM IGF-I for 15 min and 42 h for measurement for, respectively, (A) Ca^{2+} influx and (B) DNA synthesis. Columns and bars in A and B represent the mean ± S.D. for triplicate determinations.

have a dual action in the modulation of the function of G-proteins in mediating transmembrane signaling. With regard to the molecular basis of this action, it has been reported that lithium caused a conformational change of the G_i-protein trimer without any dissociation into its three subunits [19]. Presumably, lithium-induced conformational change may contribute to the alternation in the efficiency of IGF-I receptor interaction with G_i-proteins. Furthermore, it seems possible that lithium may activate the Ca^{2+}-channel-associated mitogenic system through the conformational change of G_i-proteins.

In summary, lithium regulates the function of G_i-proteins coupled to the IGF-I receptor during the G_1 phase of the rat thyroid cell cycle. In addition, lithium can stimulate the signal transduction pathway evoked by IGF-I via a modulation mechanism of G_i-protein function. This effect may be involved, at least in part, in the regulation of thyroid cell cycle progression by lithium.

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REFERENCES

[1] Berridge, M.J., Downes, C.P. and Hanley, M.R. (1989) Cell 59, 411–419.
[2] Maslanski, J.A., Leshko, L. and Busat, W.B. (1992) Science 256, 243–245.
[3] Tsuchiya, Y., Saji, M., Isozaki, O., Arai, M., Tsushima, T. and Shizume, K. (1990) Endocrinology 126, 460–465.
[4] Rybak, S.M. and Stockdale, F.E. (1981) Exp. Cell Res. 146, 263–270.
[5] Kojima, I., Matsunaga, H., Kurokawa, K., Ogata, E. and Nishimoto, I. (1988) J. Biol. Chem. 263, 16561–16567.
[6] Takada, K., Amino, N., Tada, H. and Miyai, K. (1990) J. Clin. Invest. 86, 1548–1555.
[7] Lyons, J., Landies, C.A., Harsh, G., Vallar, L., Grunewald, K., Feichtinger, H., Duh, Q.-Y., Clark, O.H., Kawasaki, E., Bourne, H.R. and McCormick, F. (1990) Science 249, 655–659.
[8] Kendall, D.A. and Nahorski, S.R. (1987) J. Pharmacol. Exp. Ther. 241, 1023–1027.
[9] Avissar, S., Schreiber, G., Danon, A. and Belmarker, R.H. (1988) Nature 331, 440–442.
[10] Ambesi-Impiombato, F.S., Parks, L.A.M. and Coon, H.G. (1980) Proc. Natl. Acad. Sci. USA 77, 3455–3459.
[11] Schumacher, R., Mosthaf, L., Schlessinger, J., Brandenburg, D. and Ullrich, A. (1991) J. Biol. Chem. 266, 19288–19295.
[12] Munson, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–239.
[13] Laemmli, U.K. (1970) Nature 227, 680–685.
[14] Ferguson, K.M., Higashijima, T., Smigel, M.D. and Gilman, A.G. (1986) J. Biol. Chem. 261, 7393–7399.
[15] Northup, J.K., Smigel, M.D. and Gilman, A.G. (1982) J. Biol. Chem. 257, 11416–11423.
[16] Muldoon, L.L., Rodland, K.D. and Magun, B.E. (1988) J. Biol. Chem. 263, 18834–18841.
[17] Mori, M., Tajima, K., Oda, Y., Matsui, I., Mashita, K. and Tarui, S. (1989) Endocrinology 124, 1365–1369.
[18] Newman, M.E. and Belmaker, R.H. (1987) Neuropharmacology 26, 211–217.
[19] Mattera, R., Codina, J., Sekura, R.D. and Birnbaumer, L. (1987) J. Biol. Chem. 262, 11247–11251.