Volume 334, number 1, 143–148 FEBS 13231 November 1993

GTP-binding protein-activator sequences in the insulin receptor

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Received 25 June 1993

Some functions of the insulin receptor (insR) are assumed to be mediated by pertussis toxin-sensitive G_i/G_o proteins. Here we have located G-protein-activator domains in the cytoplasmic region of the human insR. We searched the sequence of insR and found three candidate regions at residues 1039–1061, 1147–1168 and 1325–1345, referred to as ISRP1, ISRP2 and ISRP3, respectively. Among them, the G_i/G_o-activating function was observed only in peptide ISRP3. ISRP1 specifically activated G_s, whereas ISRP2 had no effect on G proteins. ISRP2 and ISRP3 contained five of six autophosphorylated tyrosine residues in insR. After tyrosine phosphorylation, ISRP2 showed specific G_i-activating function, and ISRP3 potentiated its ability and became capable of activating G proteins generally. This is the first study that specifies G-protein-activator domains in insR and describes their modification by autophosphorylation.

Insulin receptor; G-protein-activator sequence; Autophosphorylation

1. INTRODUCTION

Insulin is a polypeptide playing a key role in a variety of metabolic processes, including glucose uptake in various cells and tissues. It exerts biological actions by binding to a specific receptor located on the surface of target cells [1,2]. Insulin also elicits mitogenic actions through this receptor in several kinds of cells. The insR (insulin receptor) is a membrane-bound glycoprotein, consisting of two α chains (each of $\approx$ 135 kDa) and two β chains (each of $\approx$ 90 kDa). The α chain, located extracellularly, binds insulin, and the β chain, a major portion of which is located intracellularly, possesses an insulin-dependent tyrosine kinase function. Evidence has accumulated that insR-mediated tyrosine phosphorylation conveys incoming information from insulin binding to specific subsets of intracellular effector proteins [3]. Although IRS1 of 185 kDa appears to be the only established substrate of insR kinase [4], several signaling pathways, such as the *ras* p21-c-*fos* expression system [5], the MAP kinase-mediated system [6], and the PI-3 kinase system [7], have been identified as specific targets of insR kinase. On the other hand, however, the physiological role of tyrosine phosphorylation triggered by the insulin/insR complex has been poorly defined, although its significance in insulin actions is clear.

In addition to tyrosine phosphorylation, G-protein-mediated signal transduction is another candidate for insulin-triggered signaling pathways of insR. G proteins are guanine nucleotide-binding regulatory proteins that transmit signals from receptors to intracellular effectors such as enzymes and ion channels [8]. The GDP-bound form of G proteins is inactive for effector activation, and the GTP-bound form is active. In the unstimulated basal state, G proteins bind GDP. Receptors activate G proteins by stimulating the GDP/GTP exchange of G proteins. It has been reported that pertussis toxin inhibits insulin-stimulated metabolic action as well as mitogenic action in intact cells [9–14]. Pertussis toxin ADP-ribosylates G_t, G_i, and G_o and uncouples them from receptors [15]. Therefore, those reports suggest that pertussis toxin-sensitive G proteins might be involved in insR-triggered signals. Yet, this does not directly imply that insR interacts with G proteins. The possibility remains that insR modulates other G-coupled receptor function via intracellular phosphorylation or via secretion of extracellular ligands, and thereby activates G-protein systems. In fact, it is reported that insR-induced phosphorylation alters the function of βAR (β-adrenergic receptor) [16].

However, in vitro studies have so far indicated that insR directly couples to G proteins. Heyworth et al. [17] reported that insulin attenuates ADP-ribosylation of a cholera toxin substrate, referred to as G_{ins}, in hepatocyte membranes. Lutterel et al. [18] found that insulin promotes guanine nucleotide-binding to the membranes of BC_3-H-1 myocytes, where they had earlier reported that pertussis toxin inhibits DNA synthesis and glucose

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uptake promoted by insulin [10]. Similar findings have been reported using adipocytes [11–13,19]. It is also reported that in hepatocyte membranes, insulin attenuates the pertussis-toxin sensitivity of a 40-kDa G_i-like protein [20,21], suggesting that the insulin-bound insR activates the pertussis-toxin-sensitive G protein. Most recently, Jo et al. [22] reported that insR is directly associated with two proteins of 40 kDa and 67 kDa that bind GTP. Therefore, it is very important to address the question of whether insR possesses a mechanism whereby it is able to interact with G proteins directly.

While receptors that couple to G proteins usually consist of a seven-transmembrane-domain structure [23], it has been proven that such a configuration is not essential for G-protein coupling. The IGF-IIR (insulin-like growth factor II receptor), a receptor with a single transmembrane domain, is demonstrated to couple directly to G_{i2} in a manner similar to that of conventional G-protein-coupled receptors [24,25]. We [26] discovered that IGF-IIR has a G_{i2}-activator domain at residues 2410–2423, which is RVGLVRGEKARKGK in the cytoplasmic region of IGF-IIR. This region activates G_{i2} directly in the same manner as G-coupled receptors and is responsible for the G_i-coupling function of IGF-IIR in phospholipid vesicles.

Based on the structure-function relationship of the IGF-IIR peptide, the structural characteristics for the G_{i2}-activating function were defined [26,27]. These characteristics served as the basis for identifying the G_s- and G_i-activator regions in β-adrenergic, muscarinic cholinergic, and α_2-adrenergic receptors [28–30]. In the present study, we searched for regions satisfying these characteristics and found G-protein-activator regions in insR. We also noted that these functional domains were modified by tyrosine phosphorylation.

2. EXPERIMENTAL

2.1. *Materials*

The receptor polypeptides used in this study were synthesized by the solid phase method and purified to near homogeneity by high-performance liquid chromatography, as described previously [26]. The lyophilized synthetic polypeptides were dissolved in distilled water. The G proteins, which were trimeric forms purified from bovine brain and purified to homogeneity, were provided by Dr. Toshiaki Katada (Tokyo Institute of Technology, Japan) [31].

2.2. *GTPγS binding assay*

GTPγS binding to G proteins was assayed at 37°C in the presence of 20 μM Mg^{2+} and 60 nM [^{35}S]GTPγS (DuPont-New England Nuclear), as described previously [26,29]. GTPγS binding to polypeptides was negligible. The total amount of G proteins was measured as maximal GTPγS at room temperature. Binding of GTPγS to G proteins obeyed the law of first-order kinetics according to the equation $\ln[(B_1-B)/B_T] = -k_{app}t$, where B is the binding at time t and B_T is the total binding observable at an infinite time. Thus, the apparent first-order rate constant for GTPγS binding (k_{app}), which is equal to the slope of the tangent to the GTPγS-binding curve at time 0 and represents the actual GTPγS binding rate, was calculated from this equation. The value of B was at 2 min for G_o, 4 min for G_{i1}, 5 min for G_{i2}, and 10 min for G_s.

2.3. *Phosphorylation of peptides*

The insulin receptor peptides ISRP2 and ISRP3 were phosphorylated by $p43^{v\text{-}abl}$ protein-tyrosine kinase (Oncogene Science) in buffer (50 mM HEPES/NaOH (pH 7.5), 0.1 mM EDTA, 0.015% polyoxyethylene 23 laruryl ether, 0.1 mM ATP, 10 mM $MgCl_2$) at 30°C for 30 min. Reaction was stopped by adding 10% phosphoric acid solution. Phosphorylated peptides were purified by reverse phase HPLC using Cosmosil 5C18-P column with eluates of 10 60% gradient concentrations of CH_3CN, as described previously [28]. After lyophilization and immediately before experiments began, they were dissolved in distilled water. Experiments with 2 μCi [γ-^{32}P]ATP showed that ≈ 3 and 2 mol of phosphates were incorporated into one mol of ISRP2 and ISRP3, respectively. This suggests that all of the tyrosine residues contained in each peptide were phosphorylated. The phosphorylation efficiency (phosphopeptide/original peptide) was estimated to be 0.8% for ISRP2 and 1.6% for ISRP3, respectively.

3. RESULTS AND DISCUSSION

The G_i-activator sequence first identified in native receptors is RVGLVRGEKARKGK, located at residues 2410–2423 of human IGF-IIR [26]. This sequence requires the following two structural characteristics for its G_i-activating function: (i) at least two basic residues at the N-terminal side and (ii) the C-terminal consensus structure, *B-B-X-B* or *B-B-X-X-B* (*B* = basic residue or aromatic residues, *X* = non-basic, non-aromatic residues). In this study, we searched the primary sequence

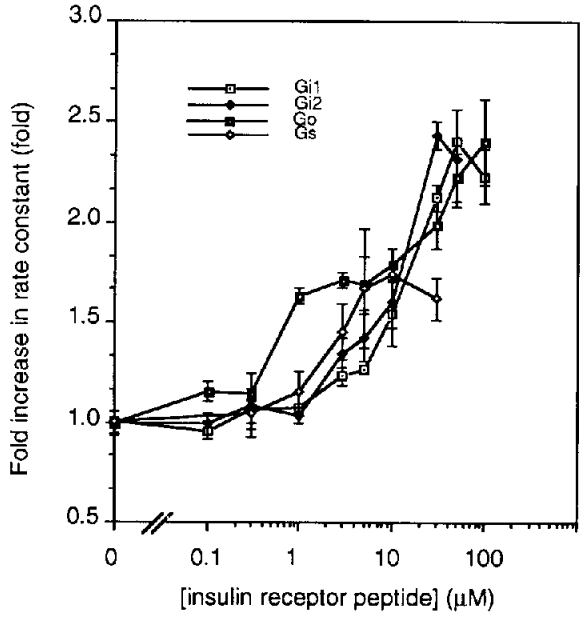

Fig. 1. Effect of the insulin receptor peptide, ISRP1 on various G-proteins. By incubating G-proteins (G_{i1}, □; G_{i2}, ♦; G_o, ■; G_s, ◇) with peptide ISRP1 (RERIEFLNEASVMKGFTCHHVVR, residues 1039–1061 of human insulin receptor). GTPγS binding was assayed under the conditions described in section 2. The GTPγS binding rate was assessed as the rate constant, k_{app}, and the extent of stimulation was expressed as the degree of stimulation of the rate constant relative to the basal rate. The method used for calclation is described in section 2. The basal values of k_{app} were 0.113 ± 0.05 for G_{i1}, 0.102 ± 0.05 for G_{i2}, 0.222 ± 0.05 for G_o, and 0.028 ± 0.001 for G_s (min^{-1}, mean ± S.E.M., n = 3) at these experiments.

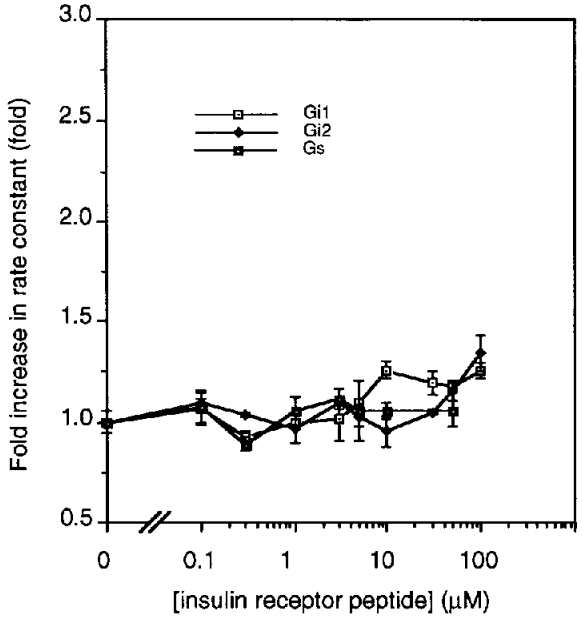

Fig. 2. Effect of the insulin receptor peptide, ISRP2 on various G-proteins. By incubating G proteins (G_{i1}, □; G_{i2}, ♦; G_s, ■) with peptide ISRP2 (KIGDFGMTRDIYETDYYRKGGK, residues 1147–1168 of human insulin receptor), GTPγS binding was assessed as in the figure legend 1. The basal values of k_{app} were essentially the same as those in the Fig. 1 experiment.

of human insR within 26 residues that satisfy these structural characteristics, as we did in our previous study [28–30]. Three criteria-satisfying regions were found in human insR at residues 1039–1061, 1147–1168, and 1325–1345 (numbering according to Ebinal et al. [1]). They are referred to as ISRP1, ISRP2, and ISRP3, respectively.

We chemically synthesized ISRP1, ISRP2, and ISRP3. ISRP1 showed remarkable promotion of the rate of GTPγS binding to G_s (Fig. 1). This peptide had little effect on other G proteins, G_{i1}, G_{i2}, or G_o. The EC_{50} of G_s-activating function of ISRP1 was 4 μM, and the saturated effect on G_s was observed at ≈ 10 μM. When its concentration exceeded 30 μM, the effect of the peptide was attenuated, which was attributed to denaturation of the G protein after strong stimulation, as described [32]. Although the Arg^{259}–Lys^{273} region of βAR has a higher potency in the activation of G_s, it also activates G_i [28]. The Arg^{131}–Arg^{148} region in the second intracellular loop of α_2AR has been the most selective activator for G_s, but with 10-times lower potency than ISRP1 [29]. Thus, at present, ISRP1 is the most potent, G_s-selective G-protein-activator peptide. In contrast, ISRP2 had negligible action on GTPγS binding to any G protein tested (Fig. 2), whereas it appeared to have a very weak but significant function to activate G_i.

ISRP3 showed a strong activity to activate G_i and G_o, and weak action on G_s (Fig. 3). The rate constant of GTPγS binding to G_o was saturated once by 1 μM ISRP3, further accelerated by ≥ 10 μM of the peptide, and reached another saturation at 100 μM. Although our G_o consisted of a single band in silver staining (data not shown), this may suggest that our G_o preparation contained two different isoforms of G_o, each of which responded differently to ISRP3 peptide.

These results demonstrate that insR possesses at least two G-protein-activator domains in its intracellular portion. The ISRP1 domain at residues 1039–1061 has a specific function to activate G_s, and the ISRP3 domain at residues 1325–1345 has a G_i/G_o-activating function. The inability of the ISRP2 domain at residues 1147–1168 to activate G proteins provides evidence that not all regions possessing similar structure can activate G proteins, showing the action specificity of both ISRP1 and ISRP3. Although extensive studies have suggested the functional linkage of insR with G proteins, especially G_i proteins, this is the first report identifying the G-protein-activator domains in insR.

Pertussis toxin-sensitive G proteins, which belong to the G_i/G_o subclass, are the most likely G proteins to be linked with insR functions. This toxin attenuates some of the insulin effects, including glucose uptake [9–14]. In hepatocyte membranes, insulin causes a change in a 40-kDa G_i-like protein [20,21], which can be recognized by reduction in pertussis toxin-catalyzed ADP-ribosylation. Insulin promotes the binding of GTP analogs to myocyte and adipocyte membranes [18,19]. InsR is directly associated with a G_i protein of 40 kDa [22]. Another line of evidence comes from the altered function

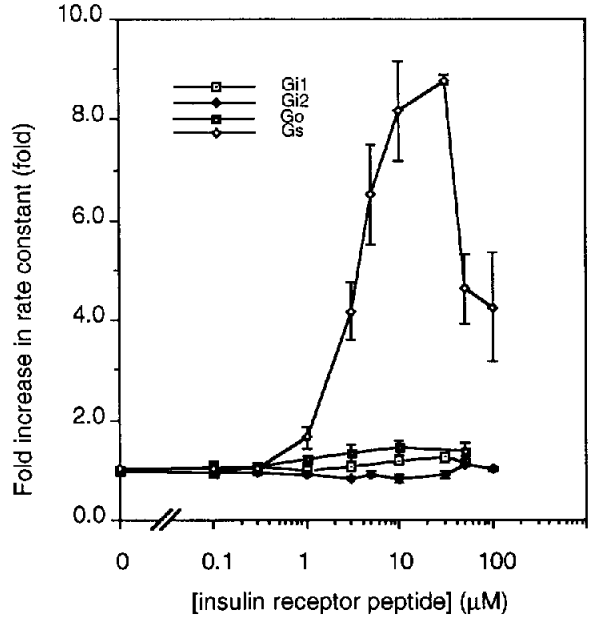

Fig. 3. Effect of the insulin receptor peptide, ISRP3 on various G-proteins. By incubating G proteins (G_{i1}, □; G_{i2}, ♦; G_o, ■; G_s, ◇) with peptide ISRP3 (KRSYEEHIPYTHMNGGKKNGR, residues 1325–1345 of human insulin receptor), GTPγS binding was assessed as in the Fig. 1 legend. The basal values of k_{app} were essentially the same as those in the Fig. 1 experiment.

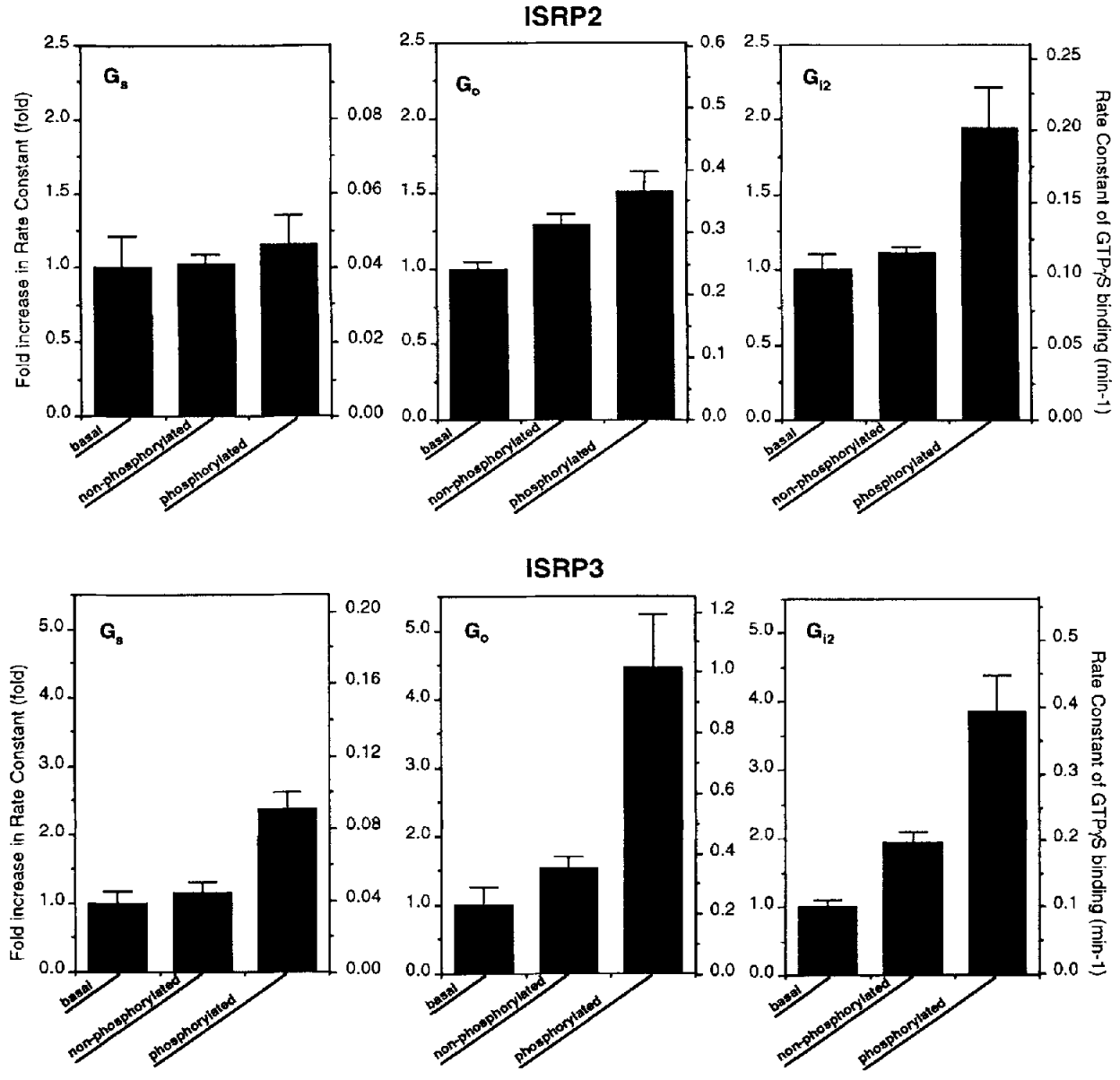

Fig. 4. Modulation by phosphorylation of the effects of ISRP2 and ISRP3 on G-proteins. By incubating G-proteins (G_s, left; G_o, middle; G_{i2}, right) with 10 μM of ISRP2 or phosphorylated ISRP2, GTPγS binding was assayed. The GTPγS binding rate was assessed as the rate constant k_{app} (shown in the right vertical axis) and the extent of stimulation was expressed as the degree of stimulation of the rate constant relative to the basal rate (shown in the left vertical axis) as described in the Fig. 1 legend.

of G_i in diabetes, where abolished effects of insulin on G_i-like proteins have been documented [33]. It has also been reported that the G_i function is impaired in liver membranes of animals with experimentally induced diabetes [34–37]. These suggest that some metabolic effects of insR may be mediated by G_i-like proteins. These findings are consistent with the present result, which shows that insR possesses a region (the ISRP3 region) that encodes a strong G_i/G_o-activating function.

In contrast, the data indicating that this receptor contains a region (the ISRP1 region) that encodes G_s-activating function were unexpected. There has been no evidence that insR activates G_s. It is very unlikely that insR stimulates adenylate cyclase, whereas this receptor lowers cAMP via cAMP phosphodiesterase stimulation [38]. However, there is still a possibility that insR activates G_s or G_s-like proteins. In liver membranes, insulin activates PPM-PDE (peripheral plasma membrane cAMP phosphodiesterase), which is identical to the enzyme stimulated by cholera toxin [39]. Cholera toxin is known to act on G_s and keep it in an active state [8]. Therefore, this provides a view that insR may activate G_s. However, this view has been controversial, for the following reasons [38]. First, insulin does not increase

cAMP levels in hepatocytes. Second, glucagon cannot activate PPM-PDE, despite the fact that this hormone activates adenylate cyclase. Third, dibutyryl cyclic AMP fails to activate PPM-PDE.

One simple interpretation would be that insulin regulation of PPM-PDE is mediated by a unique cholera toxin-sensitive G protein, which is similar but distinct from G_s. On the other hand, there is another interpretation, which refers to compartmentalization of G_s. Jouneaux et al. [40] reported that calcitonin, parathyroid hormone, and glucagon each inhibit calcium pump activity in liver membranes, whereas these hormones do not stimulate adenylate cyclase. In contrast, isoproterenol does not inhibit the calcium pump, whereas it activates adenylate cyclase. Genetically translated $G_s\alpha$ showed both functions, decreasing calcium pump activity and increasing adenylate cyclase activity. Based on these findings, Jouneaux et al. concluded that receptor-induced G_s signal is compartmentalized in liver membranes. This hypothesis makes it possible to speculate that PPM-PDE is exclusively stimulated by G_s compartmentalized for insR. If insR couples to G_s, it is highly likely that the ISRP1 region is responsible for this function of insR, because ISRP1 is the most potent, G_s-selective G-protein-activator peptide. Even if insR couples to G_s-like proteins other than G_s, the G-protein-activating function of ISRP1, as assessed with purified G_s, would represent this activity.

ISRP2 and ISRP3 contain almost all important Tyr residues, except Tyr^{960}, that receive autophosphorylation (Tyr^{1158}, Tyr^{1162}, Tyr^{1163} in ISRP2, and Tyr^{1328}, Tyr^{1334} in ISRP3). This prompted us to examine the effect of tyrosine phosphorylation on the G-protein-activator activity of ISRPs (Fig. 4). After tyrosine phosphorylation of ISRP2, the function of this peptide to activate G_i was enhanced; its activity at 10 μM was promoted from 1.1-fold stimulation to 1.9-fold stimulation. The G_s-activating activity was as low as that of non-phosphorylated ISRP2. The G_o-stimulating activity was slightly enhanced (from 1.3-fold stimulation to 1.5-fold stimulation). Therefore, ISRP2 peptide acquired the ability to activate G_i by tyrosine phosphorylation. In contrast, after phosphorylation, the G-protein-activating functions of ISRP3 were enhanced for all of G_s, G_i and G_o. The G_{i2}-activating function was ≈ 2 times enhanced (from 1.9-fold stimulation to 3.9-fold stimulation) and the G_o-activating one was ≈ 3 times enhanced (from 1.5-fold stimulation to 4.5-fold stimulation). It is most remarkable that ISRP3 became capable of activating G_s only after phosphorylation. Phosphorylation thus conferred on ISRP2 the ability to activate G_i, and endowed ISRP3 with the ability to activate multiple classes of G proteins. These results, based on a non-receptor tyrosine kinase, may be preliminary. However, our findings open the novel possibility that tyrosine autophosphorylation does play a role in amplifying the functions of the subdomains in insR.

Pertussis toxin inhibits insulin-induced diacylglycerol production [10]. Whereas hydrolysis of PIP_2 is not activated by insulin, hydrolysis of PI-glycan, which results in diacylglycerol production, is stimulated by insulin in an ATP-Mn^{2+}-dependent manner [41], suggesting that this action requires insR kinase activity. Therefore, it is likely that G_i-like proteins mediate the InsR-induced PI-glycan hydrolysis, which is supported by insR kinase. This is consistent with the present finding that the G_i-activating function of the G-protein-activator domains in insR is enhanced when they undergo phosphorylation.

The notion that tyrosine autophosphorylation amplifies the functions of G-protein-activator domains of insR itself provides a new insight into additional roles of insR kinase in insR signaling pathways. It has been speculated that insR transmits insulin signals by phosphorylating downstream molecules. However, it is suggested here that insR kinase may also play a role in enhancing the G-protein-activating function of insR subdomains. If such an enhancement by phosphorylation is essential for sufficient signal generation by those domains in insR, kinase-deficient insR should result in an inability to generate its own signals through those domains. Such concept would reconcile the apparently conflicting data, one showing the involvement of insR kinase activity in glucose uptake [3] and another showing the involvement of pertussis toxin-sensitive G proteins in the same output of insR [10].

For another example, it is reported that insulin treatment of cells rapidly desensitizes βAR from adenylate cyclase without down-regulation of βAR through insR kinase activity [16]. The conventional view would suggest that insR phosphorylates the βAR-G_s-adenylate cyclase-protein kinase A system, and attenuates βAR response. However, the data outlined here provide another possibility: insR may potentiate its own G_i-activating activity through its kinase function, resulting in rapid antagonism against βAR-induced stimulation of adenylate cyclase.

The fact that insR encodes domains whose function is different from tyrosine kinase would lead us to use caution in interpreting the results gained from receptor mutagenesis. Some of the mutations nullifying the insR kinase may also perturb the domains on the insR β chain which have specific functions to interact with G proteins. This logic is also applicable to insR mutations found in patients with genetic resistance to insulin. For example, the Met^{1153} of insR is mutated to Ile in patients with such resistance [42]. This mutation is known to reduce tyrosine kinase activity [42], whereas this Met residue is also included in the ISRP2 region. The possibility must therefore be considered that this mutation may also cause abnormality in insR–G-protein interactions.

As yet, functional reconstitution of purified insR with purified G proteins has not been accomplished. This is

in part because it is difficult to incorporate $\alpha_2\beta_2$ of $\alpha\beta$ forms of insR into phospholipid vesicles. On the other hand, methods to antagonize the action of G_i/G_o proteins more specifically than is possible with pertussis toxin have recently have developed, including the use of antibodies [43] and anti-sense RNAs [44] specific against G-protein-subunits. Furthermore, co-immunoprecipitation of receptors and G proteins by anti-receptor or anti-G-protein antibodies has proven to be useful in demonstrating receptor coupling with specific G proteins [45–47]. Using these advanced techniques, insR-G-protein coupling and its physiological significance may soon be clarified.

Acknowledgements: We especially thank Toshiaki Katada (Tokyo Institute of Technology) for his gift of G-proteins; Sigetaka Asano, Yoshiomi Tamai and Yumi Tamai for indispensible support; Yoshito Kaziro for discussion about this work. The technical assistance of Yasuko Homma, Misaki Nagashima, Lorraine Duda, and Dovie R. Wylie is greatly acknowledged. This work was supported in part by grants from the Ministry of Education, Science, and Culture of Japan, the Mochida Memorial Foundation for Medical and Pharmaceutical Research, the Arima Memorial Foundation for Medical Research, the Kudo Science Foundation, and the Uehara Foundation. Toshimi O. was a recipient of a fellowship from Tokyo Medical College. Takashi O. was supported by fellowships from the Byotai-Taisha Foundation and the Mochida Memorial Foundation.

REFERENCES

[1] Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J.-H., Masiarz, F., Kan, Y.W., Goldfine, I.D., Roth, R.A. and Rutter, W.J. (1985) Cell 40, 747–758.
[2] Ullrich, A., Bell, J.R., Chen, E.Y., Herrera, R., Petruzzelli, L.M., Dull, T.J., Gray, A., Coussens, L., Liao, Y.-C., Tsubokawa, M., Mason, A., Seeburg, P.H., Grunfeld, C., Rosen, O.M. and Ramachandran, J. (1985) Nature 313, 756 761.
[3] Ebina, T., Araki, E., Taira, M., Shimada, F., Mori, M., Craik, C.S., Siddle, K., Piesce, S.B., Roth, R.A. and Rutter, W.J. (1987) Proc. Natl. Acad. Sci. USA 84, 704–708.
[4] Sun, X.J., Rothenberg, P., Kahn, C.R., Backer, J.M., Araki, E., Wilden, P.A., Cahill, D.A., Goldstein, B.J. and White, M.F. (1992) Nature 352, 73–77.
[5] Medema, R.H., Wubbolts, R. and Bos, J.L. (1991) Mol. Cell. Biol. 11, 5963–5967.
[6] Sturgill, T.W., Ray, L.B., Erickson, E. and Maller, J.L. (1988) Nature 334, 715–718.
[7] Endemann, G., Yonezawa, K. and Roth, R.A. (1990) J. Biol. Chem. 265, 396–400.
[8] Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615–650.
[9] Heyworth, C.M., Grey, A.M., Wilson, S.R., Hanski, E. and Houslay, M.D. (1986) Biochem. J. 235, 145 149.
[10] Luttrel, L.M., Hewlett, E.L., Romero, G. and Rogol, A.D. (1988) J. Biol. Chem. 263, 6134–6141.
[11] Vila, M.C., Milligan, G., Standaert, M.L. and Farese, R.V. (1990) Biochemistry 29, 8735–8740.
[12] Goren, H.J., Northup, J.K. and Hollenberg, M.D. (1985) Can. J. Physiol. Pharmacol. 63, 1017–1022.
[13] Mills, I. and Fain, J.M. (1985) Biochem. Biophys. Res. Commun. 130, 1059 1065.
[14] Ciaraldi, T.P. and Maisel, A. (1989) Biochem. J. 264, 389–396.
[15] Ui, M., Katada, T., Murayama, T., Kurose, H., Yajima, M., Tamura, M., Nakamura, T. and Nogimori, K. (1984) Adv. Cyclic Nucleotide Protein Phosphorylation Res. 17, 145–151.
[16] Bahouth, S.W. and Lopes, S. (1992) Life Sci. 51, 271–276.
[17] Heyworth, C.M., Whetton, A.D., Wong, S., Martin, B.R. and Houslay, M.D. (1985) Biochem. J. 228, 593–603.
[18] Luttrel, L., Kilgour, E., Larner, J. and Romero, G. (1990) J. Biol. Chem. 265, 16873–16879.
[19] Kellerer, M., Obermaier-Kusser, B., Pröfrock, A., Schleicher, E., Seffer, E., Mushack, J., Ermel, B. and Häring, H.-U. (1991) Biochem. J. 276, 103 108.
[20] Rothenberg, P.L. and Kahn, C.R. (1988) J. Biol. Chem. 263, 15546–15552.
[21] Pyne, N.J., Heyworth, C.M., Balfour, N.W. and Houslay, M.D. (1989) Biochem. Biophys. Res. Commun. 165, 251–256.
[22] Jo, H., Cha, B.Y., Davis, H.W. and McDonald, J.M. (1992) Endocrinology 131, 2855–2862.
[23] O'Dowd, B.F. and Lefkowitz, R.J. (1991) Encyclopedia of Human Biology 1, 81–92.
[24] Nishimoto, I., Murayama, Y., Katada, T., Ui, M. and Ogata, E. (1989) J. Biol. Chem. 264, 14029–14038.
[25] Murayama, Y., Okamoto, T., Ogata, E., Asano, T., Iiri, T., Katada, T., Ui, M., Grubb, J.H., Sly, W.S. and Nishimoto, I. (1990) J. Biol. Chem. 265, 17456–17462.
[26] Okamoto, T., Katada, T., Murayama, Y., Ui, M., Ogata, E. and Nishimoto, I. (1990) Cell 62, 709–717.
[27] Okamoto, T., Ohkuni, Y., Ogata, E. and Nishimoto, I. (1991) Biochem. Biophys. Res. Commun. 179, 10–16.
[28] Okamoto, T., Murayama, Y., Hayashi, Y., Inagaki, M., Ogata, E. and Nishimoto, I. (1991) Cell 67, 723–730.
[29] Okamoto, T. and Nishimoto, I. (1992) J. Biol. Chem. 267, 8342–8346.
[30] Ikezu, T., Okamoto, T., Ogata, E. and Nishimoto, I. (1992) FEBS Lett. 311, 29–32.
[31] Katada, T., Oinuma, M., Kusakabe, K. and Ui, M. (1987) FEBS Lett. 213, 353–358.
[32] Higashijima, T., Uzu, S., Nakajima, T. and Ross, E.M. (1988) J. Biol. Chem. 263, 6491–6494.
[33] Gawler, D.J., Milligan, G., Spiegel, A.M., Unson, C.G. and Houslay, M.D. (1987) Nature 327, 229–232.
[34] Lynch, C.J., Blackmore, P.F., Johnson, E.H., Wange, R.L., Krone, P.K. and Exton, J.H. (1989) J. Clin. Invest. 83, 2050 2062.
[35] Green, A. and Johnson, J.L. (1990) Diabetes 40, 88 94.
[36] Bushfield, M., Griffiths, S.L., Murphy, G.L., Pyne, N.J., Knowler, J.T., Milligan, G., Parker, P.J., Mollner, S. and Houslay, M.D. (1990) Biochem. J. 271, 365–372.
[37] Allard, W.J., Vicario, P.P., Saperstein, R., Slater, E.E. and Strout, H.V. (1991) Endocrinology 129, 169–175.
[38] Houslay, M.D. (1990) in: G-proteins (R. Iyengar and L. Birnbaumer eds.) Academic Press, 1990, pp. 521–553.
[39] Heyworth, C.M., Wallace, A.V. and Houslay, M.D. (1983) Biochem. J. 214, 99–110.
[40] Jouneaux, C., Audigier, Y., Goldsmith, P., Pecker, F. and Lotersztajn, S. (1993) J. Biol. Chem. 268, 2368–2372.
[41] Suzuki, S., Toyota, T., Tamura, S., Kikuchi, K., Tsuiki, S., Huang, L., Villar-Palasi, C., Larner, J. and Goto, Y. (1987) J. Biol. Chem. 262, 3199–3204.
[42] Cama, A., Sierra, M.D.L.L., Ottini, L., Kadowaki, T., Gorden, P., Imperato-McGinley, J. and Taylor, S. (1991) J. Clin. Endocrinol. Metab. 73, 894–901.
[43] Spiegel, A.M. (1988) in: ADP-Ribosylating Toxins and G Proteins (Moss, J. and Vaughan, M. eds.) pp. 207–224, American Society for Microbiology, Washington, DC.
[44] Kleuss, C., Hescheler, J., Ewel, C., Rosenthal, W., Schultz, G. and Wittig, B. (1991) Nature 353, 43 48.
[45] Murray-Whelan, R. and Schlegel, W. (1992) J. Biol. Chem. 267, 2960–2965.
[46] Okuma, Y. and Reisine, T. (1992) J. Biol. Chem. 267, 14826–14831.
[47] Nishimoto, I., Okamoto, T., Matsuura, Y., Okamoto, T., Murayama, Y. and Ogata, E. (1993) Nature 362, 75–79.