Activation of β-adrenergoreceptors induces cardiomyocyte hypertrophy. In the present study, we examined isoproterenol-evoked intracellular signal transduction pathways leading to activation of extracellular signal-regulated kinases (ERKs) and cardiomyocyte hypertrophy. Inhibitors for cAMP and protein kinase A (PKA) abolished isoproterenol-evoked ERK activation, suggesting that Gs protein is involved in the activation. Inhibition of Gi protein by pertussis toxin, however, also suppressed isoproterenol-induced ERK activation. Overexpression of the G<sub>i</sub> subunit binding domain of the β-adrenergoreceptor kinase 1 and of COOH-terminal Src kinase, which inhibit functions of G<sub>i</sub> and the Src family tyrosine kinases, respectively, also inhibited isoproterenol-induced ERK activation. Overexpression of dominant-negative mutants of Ras and Raf-1 kinase and of the β-adrenergoreceptor mutant that lacks phosphorylation sites by PKA abolished isoproterenol-stimulated ERK activation. The isoproterenol-induced increase in protein synthesis was also suppressed by inhibitors for PKA, Gi<sub>i</sub>, tyrosine kinases, or Ras. These results suggest that isoproterenol induces ERK activation and cardiomyocyte hypertrophy through two different G proteins, Gs and Gi<sub>i</sub>. cAMP-dependent PKA activation through Gs may phosphorylate the β-adrenergoreceptor, leading to coupling of the receptor from G<sub>s</sub> to Gi<sub>i</sub>. Activation of Gi<sub>i</sub> activates ERKs through G<sub>i</sub>βγ, Src family tyrosine kinases, Ras, and Raf-1 kinase.

Cardiac hypertrophy is often associated with an increase in intracardiac sympathetic nerve activity and with elevated plasma catecholamines (1). Treatment of cardiac myocytes with catecholamines not only changes their functions such as beating rates and contractile activity but also induces typical hypertrophic responses (2–7). There are two major subtypes, α and β, in adrenoreceptors (ARs).<sup>3</sup> AR agonists such as norepinephrine (NE) (α and β), phenylephrine (PHE) (α), and isoproterenol (ISO) (β) have been reported to induce cardiomyocyte hypertrophy (2–7). Prolonged infusion of pressor doses of NE increases the mass of the myocardium and the thickness of the left ventricular wall, suggesting that NE has direct hypertrophic effects on cardiac myocytes without affecting afterload (2). We have reported that NE induces cardiomyocyte hypertrophy through both α- and β-ARs (7). It has been reported that PHE evokes hypertrophic responses in the cardiac myocytes of neonatal rats (5) and that expression of constitutively active α-AR induces cardiac hypertrophy in adult mice (8). Both in vivo and in vitro studies demonstrate that ISO also stimulates expression of proto-oncogenes in cardiomyocytes and induces cardiac hypertrophy (6, 9, 10).

Activation of each AR evokes specific intracellular signals (4, 11). It has been shown that stimulation of α<sub>1</sub>-AR activates phosphoinositide-specific phospholipase C via G<sub>s</sub> protein and hydrolyzes phosphoinositide 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol. Diacylglycerol activates protein kinase C (PKC) leading to activation of the Raf-1 kinase/extracellular signal-regulated protein kinase (ERK) cascade (12, 13). There was a report indicating that PHE activates ERKs and induces cardiomyocyte hypertrophy through the Ras-dependent pathway (5). Regarding β-AR-induced signaling pathways, stimulation of β-AR activates adenyl cyclase through a different G protein, Gi<sub>i</sub>. Activation of adenyl cyclase produces a second messenger cyclic adenosine monophosphate (cAMP), leading to activation of cAMP-dependent protein kinase (PKA) (14). PKA activation is important for the control of cell growth and differentiation. cAMP/PKA has been reported to have an inhibitory effect on the activation of ERKs stimulated by growth factors in many cell types such as Rat-1 cells, smooth muscle cells, Chinese hamster ovary cells, COS-7, and adipocytes (15–19). On the contrary, in some cell types such as PC12 cells, Swiss-3T3 cells, and 3T6 mouse lymphoma cells, cAMP activates ERKs and potentiates the effects of growth factors on differentiation and gene expression (20–24). In human endothelial cells, down-regulation of the α subunit of G<sub>s</sub> (G<sub>sα</sub>) abolishes β-AR-mediated ERK activation by ISO (25), suggesting that G<sub>sα</sub>-dependent cAMP elevation and PKA activation are responsible for the activation of ERKs. We and others have also demonstrated that β-AR agonists including ISO significantly activate ERKs and increase protein synthesis through cAMP/PKA in cardiac myocytes (6, 7). However, the
molecular mechanisms of β-AR agonist-induced ERK activation remain largely unknown. In the present study, we examined the molecular mechanism of ISO-evoked activation of ERKs in the cardiomyocytes of neonatal rats. We observed that ISO-induced activation of ERKs is dependent on both Gs/cAMP/PKA and Gβγ/Src/Ras signaling pathways. Quite recently, it has been reported that PKA activated through β-AR phosphorylates agonist-coupled β-AR, leading to a change of the receptor coupling from Gβγ to Gγ (26). We examined whether phosphorylation of β-AR is critical to ISO-induced activation of ERKs also in cardiac myocytes.

**EXPERIMENTAL PROCEDURES**

**Materials**—[γ-32P]ATP and [3H]phenylalanine were purchased from NEN Life Science Products. Dulbecco’s modified Eagle’s medium, fetal bovine serum, and gelatine were from Life Technologies, Inc. Pertussis toxin (PTX) was from List Biological Laboratories, Inc. Calphostin C was from BIOMOL. Rp-cAMP and H89 were from Biolg. Polyclonal antibodies against β1- or β2-AR, SHc, and Grb2 were from Santa Cruz Biotechnology Inc. Anti-hemagglutinin (HA) polyclonal antibody was purchased from Sigma. Gβγ subunit (D.N.Ras, and D.N.Raf-1 kinase (Ala-375), both of which are driven by the cytomegalovirus promoter, were provided and prepared as described previously (27, 28). Wild-type β2-AR (pRK5-β2-ARwt) and β2-AR mutant lacking phosphorylation sites for PKA (pDNA-β2-ARmut; point mutations of Ser residues at 261, 262, 345, and 346 to Ala) were kindly gifts from R. J. Lefkowitz and Y. Daaka (26).

**Assay of ERK Activity**—ERK activities were measured using myelin basic protein (MBP)-containing gel as described previously (27). In brief, cell lysates were electrophoresed on an SDS-polyacrylamide gel containing 0.5 mg/ml MBP. ERKs in the gel were denatured in guanidine HCl, renatured in Tris-HCl containing Trion X-100 and 2-mercaptoethanol, and incubated with [γ-32P]ATP. Phosphorylation activities of ERKs were assayed by subjecting to autoradiography. The activity of transfected HA-ERK2 was assayed as described previously (27). In brief, after transfection of HA-ERK2, cell lysates were incubated with an anti-HA polyclonal antibody. The immune complex was incubated with MBP and [γ-32P]ATP for 10 min at 30 °C. The sample was subjected to SDS-PAGE, and the phosphorylated MBP band was visualized by autoradiography.

**Phosphorylation of Shc and Its Association with Grb2**—Tyrosine phosphorylation of Shc and the association of Shc with Grb2 were examined by Western blot analysis as described previously (28). In brief, cell lysates were incubated with an anti-Shc or an anti-Grb2 antibody, and the immune complexes were subjected to SDS-PAGE and transferred to Immobilon-P membranes (Millipore). The membranes were immuno-blotted with an anti-phosphotyrosine antibody (PY20) or an anti-Shc antibody, and the immunoreactivity was detected using the enhanced chemiluminescence reaction (ECL) system (Amersham Pharmacia Biotech) according to the manufacturer’s directions.

**RESULTS**

**cAMP-dependent PKA Mediates ISO-induced Activation of ERKs**—We have demonstrated recently that β-AR stimulation by NE activates ERKs through cAMP/PKA-dependent pathways in cardiac myocytes (7). We therefore defined the role of cAMP/PKA in ISO-induced ERK activation. When cAMP was inhibited by a cAMP analog, Rp-cAMP (100 μM), ISO-evoked ERK activation was completely suppressed (see Fig. 1A). Pre-treatment with a PKA inhibitor, H89 (10 μM), also abolished ISO-induced activation of ERKs, whereas inhibition of PKC by calphostin C (1 μM) did not affect the ERK activation by ISO (Fig. 1A). On the other hand, PHE-induced activation of ERKs was suppressed by calphostin C but not by Rp-cAMP or H89 (Fig. 1B). These results suggest that cAMP-dependent PKA activation probably through Gs is required for ISO-induced activation of ERKs in cardiac myocytes.

**Gγ Protein Is Involved in ISO-induced Activation of ERKs**—We next examined the possibility that Gγ protein is involved in ISO-induced ERK activation because β-AR was reported to bind Gγ (35, 36), and activation of Gγ protein can activate ERKs in many cell types (37, 38). We preincubated cardiac myocytes with 100 ng/ml PTX and then treated them with ISO or PHE. Activation of ERKs by ISO was abolished by the PTX treatment for 24 h, whereas PTX had no effect on PHE-induced ERK activation (Fig. 2A). These results suggest that PTX-sensitive Gγ protein is responsible for ISO- and not PHE-induced activation of ERKs in cardiac myocytes.

It has been reported recently that stimulation of Gγ protein-coupled receptors activates ERKs by βγ subunits (Gβγ) but not by the α subunit (39–41). Therefore, the role of Gγ in ISO-induced ERK activation was examined by introducing HA-ERK2 and a minigene construct encoding βARK1495–689, which inhibits Gγ activity (29–32, 34), pulse labeling for 2 h with [3H]phenylalanyl-tRNA pool may be equilibrated after 5 min and remain so such that labeled amino acid accumulates asymptotically into protein. At the end of the labeling incubation, alanine is sufficient to achieve adequate equilibration of the specific activity of phenylalanyl-tRNA. Protein synthesis was determined by assessing the incorporation of labeled phenylalanine from the extracellular medium into the total trichloroacetic acid-precipitable cell protein (24 h) labeling of cultured cardiomyocytes is intrinsically nonlinear, 2 h before the harvest. It has been reported that continuous long term overexpression (29–32, 34), pulse labeling for 2 h with [3H]phenylalanyl-tRNA pool may be equilibrated after 5 min and remain so such that labeled amino acid accumulates asymptotically into protein.
Inhibition of G_i by PTX Does Not Affect Abundance of \( \beta \)-ARs—There is a possibility that blockade of G_i may lead to sustained stimulation of adenylyl cyclase and may induce sustained phosphorylation and desensitization (and perhaps down-regulation) of \( \beta \)-ARs (35). It has also been reported that...
PTX increases the sensitivity of β-AR to ISO (42). To test whether pretreatment of cardiac myocytes with PTX might affect the density of β-ARs on the cell membrane, we examined the amount of cell surface β-ARs, β₁-AR and β₂-AR, by Western blot analysis after long term treatment with PTX. As shown in Fig. 3, PTX did not affect the protein levels of β₁-AR and β₂-AR in membrane fractions. These results suggest that the inhibition of ISO-induced ERK activation by PTX is not caused by down-regulation of β-ARs.

Tyrosine Kinases Including Src Family Tyrosine Kinases Modulate ISO-induced Activation of ERKs—G_{βγ} has been reported to activate ERKs through non-receptor-type tyrosine...
kinases including Src family tyrosine kinases in many types of cells (28, 43). We examined whether tyrosine kinases are responsible for activation of ERKs induced by ISO in cardiac myocytes. Activation of ERKs by ISO was suppressed completely when cardiac myocytes were pretreated with a broad spectrum tyrosine kinase inhibitor, genistein, whereas the activation of ERKs by PHE was not affected by the same pretreatment (Fig. 4A). These suggest that tyrosine kinases are involved in ISO-induced activation of ERKs in cardiac myocytes, whereas PHE-induced activation of ERKs is not dependent on the tyrosine kinase pathway.

We examined further the role of Src family tyrosine kinases in ISO-induced ERK activation in cardiac myocytes. Csk has been reported to phosphorylate the tyrosine residue in the carboxyl terminus of Src family protein kinases and thereby inactivate their function (44, 45). We cotransfected Csk with ERK2 into cardiac myocytes and examined the activity of the transfected ERK2 after stimulation with ISO or PHE. Although cotransfection of Csk did not affect PHE-induced ERK2 activation, overexpression of Csk completely inhibited activation of ERK2 by ISO (Fig. 4B), suggesting that Src family tyrosine kinases are also involved in ISO-induced ERK activation in cardiac myocytes.

ISO Enhances Tyrosine Phosphorylation of Shc and Association of Shc with Grb2—Adapter proteins containing Src homology 2 domains such as Shc and Grb2 transduce activation of tyrosine kinases to the Ras/ERK pathway via the guanine nucleotide exchange factor Sos (46–48). We therefore examined whether Shc is activated by ISO in cardiac myocytes. ISO rapidly (within 30 s) increased levels of tyrosine phosphorylation of 52-kDa Shc (Fig. 5A). Phosphorylation levels decreased from 5 min and returned to the basal levels by 15 min. A faint band corresponding to 46-kDa Shc was also observed with long exposure (data not shown). Next, association of Grb2 with Shc was examined by immunoprecipitation with anti-Grb2 antibody and immunoblotting with an anti-Shc antibody. The intensities of the bands around 52 kDa and 46 kDa corresponding to Shc were enhanced by ISO stimulation (Fig. 5B), suggesting that the 52- and 46-kDa Shc form a complex with Grb2 after ISO stimulation.

Ras and Raf-1 Kinase Activation Is Essential to ISO-induced ERK Activation—Association of Grb2 with Shc usually results in the recruitment of a Ras activator Sos to the membrane fraction, leading to activation of Ras (46–48). The role of Ras was next analyzed in ISO-stimulated cardiac myocytes. ISO-induced activation of ERKs was suppressed by overexpression of D.N.Ras (Fig. 6A), suggesting that activation of Ras is required for ISO-induced activation of ERKs in cardiac myocytes. Activated Ras usually induces activation of ERKs through Raf-1 kinase and mitogen-activated protein kinase/ERK kinase (49). Activation of the transfected ERK2 by ISO or PHE was abolished by the overexpression of D.N.Raf-1 kinase (Fig. 6B), indicating that Raf-1 kinase is crucial for activation of ERKs by ISO and PHE in cardiac myocytes.

Activation of ERKs by ISO Requires PKA-dependent Phosphorylation of β-AR—Next we examined how both Gs/PKA- and Gi/Ras-dependent pathways are involved in ISO-induced activation of ERKs. Quite recently Daaka et al. (26) have reported that phosphorylation of β2-AR by PKA changes coupling of the receptor from Gs to Gi protein. We therefore examined this possibility by introducing the gene of β2-ARmut, which lacks phosphorylation sites for PKA, into cardiomyocytes. ISO-induced activation of ERK2 was completely suppressed by overexpression of β2-ARmut (Fig. 7). In contrast, overexpression of β2-ARwt enhanced the activation of ERK2 by ISO. These results suggest that PKA-dependent phosphorylation of β-AR is necessary to evoke signals from β-AR to ERKs in cardiac myocytes.

ISO-induced Protein Synthesis Also Occurs through Both Gs- and Gi-dependent Signal Transduction Pathways—ISO activates ERKs through both Gs/PKA- and Gi/Ras-dependent pathways. To determine whether cardiomyocyte hypertrophy is also induced through the same signal transduction pathways, we examined the effects of various inhibitory agents for signaling molecules on the ISO-induced increase in protein synthesis. We pretreated cardiac myocytes with PTX, genistein, manumycin (a Ras farnesyltransferase inhibitor), H89, or RpcAMP and stimulated the cells with ISO for 24 h. Although the pretreatment with these inhibitors alone did not affect the basal level of protein incorporation significantly (Fig. 8A), an ISO-induced increase in protein synthesis was suppressed significantly by all of these inhibitors (Fig. 8B), indicating that ISO enhanced protein synthesis through cAMP/PKA, Gs, tyrosine kinases, and Ras. These results suggest that the signal transduction pathway leading to activation of ERKs is also important for ISO-induced protein synthesis in cardiac myocytes.

DISCUSSION

Stimulation of β-AR usually activates an effector enzyme adenyl cyclase through Gs. The activation of adenyl cyclase induces an increase in cAMP levels, which in turn activates PKA (14, 19, 50, 51). In addition, a previous report indicated that down-regulation of Gs abolishes ISO-induced ERK activation in human endothelial cells (25), suggesting that Gs is required for activation of ERKs by β-AR. Our previous (7) and present studies also showed that ISO activates ERKs through cAMP/PKA in cardiac myocytes, supporting that Gs protein plays an essential role in the activation of ERKs. Many laboratories have reported that activation of cAMP-dependent PKA inhibits activation of the Raf-1 kinase/ERK cascade in various cell types such as Rat-1 cells, smooth muscle cells, Chinese hamster ovary cells, COS-7 cells, and adipocytes (16–19). In other cell types such as PC12 cells, S49 mouse lymphoma cells, and Swiss-3T3 cells, however, cAMP activates ERKs and potentiates the effects of growth factors on differentiation and gene expression (20–24). Taken together, cAMP-dependent PKA activation may have different effects on ERKs among cell types, and PKA may activate ERKs in cultured cardiac myocytes.

By examining the signal transduction pathway of ISO-induced activation of ERKs, we found that the Gs protein/Src/Ras pathway is also required for ISO-induced ERK activation. How do two different pathways, cAMP/PKA pathway and Gs/Src/Ras pathway, converge at the ERK cascade? Gs-coupled β2-AR was reported to activate simultaneously the pathway that leads to
Fig. 4. Role of tyrosine kinases including Src family tyrosine kinases in ISO-induced activation of ERKs in cardiac myocytes. Panel A, cardiac myocytes were pretreated with 30 μM genistein for 30 min and stimulated with 10 μM ISO or 10 μM PHE for 8 min. ERK activities were assayed as described under legend of Fig. 1. Panel B, HA-ERK2 was cotransfected into cardiac myocytes with Csk gene. 8 min after the addition of 10 μM ISO or 10 μM PHE, the transfected ERK2 activity was measured using MBP as described in the legend of Fig. 2B. A representative autoradiogram is shown. Relative kinase activities of 42-kDa ERK were determined by scanning each band with a densitometer. Activities were expressed relative to that obtained from unstimulated cardiomyocytes. Data are presented as the mean ± S.E. from three independent experiments. *p < 0.01 versus control.
functional inhibition of cAMP/PKA pathway via Gᵢ protein in cardiac cells (36). Moreover, it has been reported recently that PKA-induced phosphorylation of β-AR changes the coupling of the receptor from Gₛ to Gᵢ and activates ERKs through the Src/Ras pathway in HEK293 cells (26). We therefore examined the role of Gᵢ protein in ISO-evoked ERK activation and found that Gᵢ protein is also essential to ISO-induced activation of ERKs in cardiac myocytes. Although it has been reported that inhibition of Gᵢ protein may lead to sustained stimulation of adenylyl cyclase and down-regulation of β-ARs in some types of cell (35), Western blot analysis revealed that blocking of Gᵢ by PTX did not affect the abundance of β-ARs in the membrane fraction of cardiac myocytes, suggesting that in cardiac myocytes, the inhibitory effect of PTX is attributable to suppression
FIG. 6. Role of Ras and Raf-1 in ISO-induced activation of ERKs in cardiac myocytes. HA-ERK2 was cotransfected with D.N.Ras (panel A) or D.N. Raf-1 (panel B) into cardiac myocytes, and the cells were stimulated with 10 μM ISO or 10 μM PHE for 8 min. The activities of ERKs were assessed by measuring MBP phosphorylation as described in the legend of Fig. 2B. Representative autoradiograms are shown. Relative kinase activities of MBP were determined by scanning each band with a densitometer. Results are presented as the mean ± S.E. from three independent experiments. *p < 0.01 versus control.
of signaling from β-ARs to downstream but is not caused by
down-regulation of β-ARs.

It is necessary to define how G proteins are involved in the
ISO-induced ERK activation in cardiac myocytes. It has been
reported in COS-7 cells that activation of β-AR activates ERKs
through Gsbg, but not Gsa. Gsbg activates ERKs through a Ras-
dependent pathway, whereas Gsa inhibits activation of ERKs
via cAMP/PKA. The balance between these two opposite mech-
anisms of regulation may control the ERK response to
β-AR agonists (19). However, this model may not be applied to car-
diac myocytes because ISO-stimulated ERK activation is de-
pendent on cAMP/PKA, suggesting the importance of Gsa pro-
tein. It is also possible that ISO activates ERKs through both
Gs and Gi proteins independently. In the present study, how-
ever, because inhibition of cAMP/PKA or of Gi completely sup-
pressed ISO-induced ERK activation, it may be unlikely that
Gs and Gi proteins are involved independently. We introduced
β2-ARmut lacking phosphorylation sites for PKA with HA-
ERK2 into cardiac myocytes and showed that inhibition of
β2-AR phosphorylation inhibits ISO-induced activation of
ERKs. These results are consistent with the hypothesis that
activation of cAMP-dependent PKA phosphorylates the acti-
vated receptor, leading to the change in coupling of the receptor
from Gi to Gs protein.

Gsbg subunit derived from PTX-sensitive Gi protein regulates
many effectors within the cell (22, 41, 52, 53). Stimulation of
various receptors such as α2A-adrenergic, M2 muscarinic ace-
tylcholine, D2 dopamine, A1 adenosine, or angiotensin II type 1
receptors induces Ras-dependent ERK activation via the Gαi
subunit in COS-7 cells (53). By introducing the βARK1Δ635–689
polypeptide minigene, we showed in the present study that the
Gsbg subunit is required for ISO-induced ERK activation in
cardiac myocytes. There is a report showing that the Gβγ sub-
unit of Gs protein activates ERKs in COS-7 cells (19). Because
activation of adenylyl cyclase is usually mediated by the α
subunit rather than the Gβγ subunit of Gs (14, 24, 25, 50, 51) and
ISO activated ERKs through a cAMP/PKA-dependent pathway
in this study, ISO-induced activation of ERKs might be depend-
dent on the α subunit of Gi and on the Gβγ subunit of Gi in cardiac
myocytes.

The Gβγ subunit of Gi protein activates ERKs through tyro-
sine kinases including Src family tyrosine kinases (43, 54).
Because β-AR itself does not possess tyrosine kinase activity,
non-receptor-type tyrosine kinases may be responsible for ISO-
induced ERK activation. We therefore examined the involve-
ment of tyrosine kinases including Src family tyrosine kinases
in ISO-induced ERK activation in cardiac myocytes. Pretreat-
ment with a tyrosine kinase inhibitor or overexpression of Csk
strongly inhibited ISO-induced activation of ERKs in cardiac
myocytes. In contrast, PHE-induced activation of ERKs was
not dependent on the tyrosine kinase pathway. We have re-
ported that angiotensin II activates ERKs through PKC but not
through tyrosine kinases in cardiac myocytes (27). These re-
sults collectively suggest that tyrosine kinases including Src
family tyrosine kinases mediated Gi-coupled receptor-induced
ERK activation, whereas PKC but not tyrosine kinases play a

![Fig. 7. Role of PKA-dependent β-AR phosphorylation in ISO-induced ERK activation. β2-ARmut or β2-ARwt was cotransfected into cardiomyocytes with HA-ERK2. The cells were stimulated by ISO (10 μM) for 8 min. The transfected ERK2 activity was measured using MBP as described in the legend of Fig. 2B. Representative autoradiograms are shown.](image-url)
critical role in activation of ERKs by Gs-coupled receptors in cardiac myocytes.

Activation of tyrosine kinases leads to Ras activation through adaptor proteins such as Shc and Grb2 and the guanine exchange factor Sos (46–48). It has been demonstrated that Shc is tyrosine phosphorylated in response to angiotensin II in cardiac myocytes (55) and that Shc serves as a converging point of signaling pathways. Through adaptor proteins such as Shc and Grb2 and the guanine exchange factor Sos (46–48), it has been demonstrated that Sos binds to and activates Ras in response to growth factors, and that Sos is necessary for activation of ERKs in PC12 cells (24, 59). It has also been shown that cAMP activates ERKs independently of Raf-1 kinase (60). By contrast, we observed in the present study that Ras and Raf-1 kinase are necessary for ISO-induced ERK activation. Taken collectively, these findings suggest that ISO may activate ERKs through the signaling pathway consisting of Ras, Sos, Raf-1, and ERK in cardiac myocytes.

As reported previously, ERK activation is required for cell growth including cardiac hypertrophy (6, 7, 34, 61, 62). We also showed in the present study that the signal transduction pathway leading to activation of ERKs is important for ISO-induced protein synthesis in cardiac myocytes. The effect of isoproterenol on the net protein synthesis is small compared with severalfold increases in the activity of each signaling step. Although there are great differences between signaling steps and protein synthesis in many aspects such as the basal levels, the responsive ability to stimulation, and the methods of examination among them, it is also possible that the initiation of protein synthesis needs a much greater fold-increase in the activity of signaling molecules. Many previous studies also showed that signaling steps such as ERKs are activated more than severalfold by stimulation, whereas an increase in the protein synthesis rate is less than 50% (6, 7, 62, 63). Although it is unknown at present why there are big differences in the time course for protein synthesis and activation of signaling molecules, there are several possibilities. Protein synthesis may need activation of many signaling steps, or the initiation step of protein synthesis needs some time period after activation of molecules responsible to protein synthesis. Further studies are necessary to determine these possibilities.

In summary, the β-AR-evoked signal transduction pathways to activation of ERKs are different among cell types. In cardiac myocytes, ISO activates ERKs through the signal transduction pathway consisting of β-AR phosphorylation by Gs/cAMP-dependent PKA, Gβγ subunits derived from Gs, Src family tyrosine kinases, the formation of the Shc-Grb2-Sos complex, Ras, and Raf-1 kinase. We showed in this study that the phosphorylation of β-AR by PKA and the change of coupling of the receptor from Gs to Gγ play an important role in ISO-induced cardiomocyte hypertrophy.

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