Biochemical Pharmacology, Vol. 58, pp. 1497–1500, 1999.



ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00230-0

**SHORT COMMUNICATION**

# Translocation of G-Protein β3 Subunit from the Cytosol Pool to the Membrane Pool by β1-Adrenergic Receptor Stimulation in Perfused Rat Hearts

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**ABSTRACT.** To elucidate the intracellular function and localization of the heterotrimeric G-protein β3 subunit (Gβ3) in the heart, we studied the effects of subtype-specific β-adrenergic receptor (β-AR) stimulation on Gβ3 localization using isoform-specific antibodies. The amount of Gβ3 in the cytosol dramatically decreased in hearts perfused with isoproterenol (ISO) alone or ISO with ICI 118551, a β2-AR antagonist. Propranolol or CGP 20712A, a β1-AR antagonist, blocked the ISO-induced decrease in the Gβ3 content of the cytosol. In contrast, Gβ3 content of the membrane fraction significantly increased in hearts perfused with ISO alone or ISO with ICI 118551. We conclude that stimulation of the β1-AR induces isoform-specific translocation of Gβ3 from the cytosol to the membrane fraction in rat hearts. BIOCHEM PHARMACOL **58**;9:1497–1500, 1999. © 1999 Elsevier Science Inc.

**KEY WORDS.** heterotrimeric G-proteins; β subunits; translocation; cytosolic fraction; β1-adrenergic receptor; intracellular localization

Heterotrimeric G-proteins act as signal transducers by coupling membrane-bound receptors to intracellular effectors and related proteins. The heterotrimeric G-proteins consist of three subunits: α, β, and γ [1]. Of these three subunits, more than 16 α (Gα¶), 5 β (Gβ), and 11 γ (Gγ) isoforms have been characterized. Therefore, over 300 unique combinations for the G-protein trimers are possible. The assembly of the β and γ isoforms is selective [2], and the βγ dimer (Gβγ) has been shown to be capable of interacting with a variety of proteins [1]. Gβ3 cDNA was first isolated by Levine *et al.* from human and bovine retinal cDNA libraries. The expression of Gβ3 mRNA has been reported in the human and bovine retinas and human tumor cell lines [3]. The mouse Gβ3 gene, *Gnb-3*, is located on chromosome 6 near the *Raf-1* gene [4]. Fung *et al.*, Peng *et al.*, and Lee *et al.* have generated specific antisera against retinal Gβ3 protein and characterized the expression of Gβ3 in the retina [5–7]. We have previously described the cytosolic localization of Gβ3 in the rat heart [8]. In addition, others have isolated Gβ3 homolog cDNA from the rat heart [9]. However, it has not yet been confirmed whether the Gβ3 protein identified by isoform-specific antibodies [8] corresponds to the product of the gene cloned by Ray and Robishaw [9].

The intracellular function of Gβ3 has not been fully determined. The signal transduction pathway involving Gβ3 is represented by the muscarinic receptor in rat pituitary GH3 cells in which Gβ3 forms a heterotrimer with $G\alpha_{o1}$ and $G\gamma_4$ [10]. One study has reported a mutation in the gene encoding Gβ3 which results in hyperactive sodium proton exchanger and human hypertension [11]. G-proteins usually localize just inside the plasma membrane because of myristoylation and palmitoylation of Gα's and farnesylation or geranylgeranylation of Gγ's [1]. Therefore, the cytosolic distribution of Gβ3 represents an unusual subcellular localization for one of the G-protein subunits [5, 6, 8]. Ong *et al.* cloned the $G\gamma_8$ cDNA, the product of which localizes with $G\beta_3$ and presumably forms dimers with retinal $G\beta_3$ [12]. $G\gamma_8$, like $G\gamma_1$, is farnesylated at a CIIS motif site in the C-terminus portion [12]. However, a Gγ isoform which forms a dimer with Gβ3 in the heart has not been identified. In addition, no report has addressed lipid modification of the Gβ3 subunit. Therefore, complete characterization of the soluble form of Gβ3 remains to be performed.

Several proteins have been reported to translocate from

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¶ *Abbreviations:* β-AR, β-adrenergic receptor; ISO, isoproterenol; Gα, G-protein α subunit; Gβ, G-protein β subunit; and Gγ, G-protein γ subunit.

Received 6 May 1998; accepted 20 May 1999.

the cytosol to the plasma membrane or cytoskeleton, including GLUT4 [13], phospholipase C-γ 1 [14], c-Src [15], and Raf-1 [16]. The mechanism responsible for the translocation of these proteins from the cytosol to cellular membranes is not known. G-proteins have also been detected in association with intracellular membranes such as those of secretory granules, endoplasmic reticulum, endosomes, and the trans-Golgi network [17]. In contrast to the relatively well-characterized interaction between G-proteins and other proteins in the plasma membrane, virtually nothing is known regarding the functional significance of G-proteins detected in association with intracellular membranes and the soluble cytosolic fraction. Therefore, we examined the subcellular distribution of Gβ3 after β-adrenergic receptor stimulation in perfused rat hearts to determine the functional significance of soluble Gβ3.

## MATERIALS AND METHODS

### *Heart Perfusions*

Hearts were removed from male Sprague–Dawley rats weighing 200 to 250 g (9 weeks old), and perfused according to the method described below. Krebs–Henseleit bicarbonate buffer (119 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$, and 10 mM glucose, pH 7.6, 37°) equilibrated with 95% $O_2$/5% $CO_2$ was used as the perfusion medium. Hearts were initially perfused by the Langendorff technique [18]. After 10 min of retrograde perfusion, the hearts were perfused for 30 min using the working heart technique with a left atrial filling pressure of 9 mm Hg and a hydrostatic aortic afterload pressure of 60 mm Hg. Hearts were perfused with the same buffer or buffer containing $10^{-5}$ M ISO (Sigma Chemical Co.) [18]. In experiments using ISO and different β-AR antagonists, the hearts were first perfused for 10 min using the Langendorff technique followed by perfusion with the β-AR antagonists alone (10 μM propranolol, 10 μM CGP20712A or 10 μM ICI118551; Novartis Pharmaceutical Co.) for 5 min. The perfusion was then continued with ISO and the β-AR antagonist for 30 min. At the end of the perfusion, hearts were immediately frozen with clamps chilled in liquid nitrogen and stored at −80° until use. At least five hearts were perfused under the same experimental conditions.

### *Preparation of Membrane and Cytosolic Fractions*

Hearts were homogenized with a Polytron homogenizer for 30 sec in 10 volumes of 10 mM Tris–HCl (pH 7.5) containing 1 mM EDTA, 0.25 M sucrose, and 50 U/mL aprotinin. The homogenate was centrifuged at 1000 *g* for 10 min and the resulting supernatant was centrifuged twice at 105,000 *g* for 60 min. This final supernatant was used as the cytosolic fraction for immunoblotting. The pellet was resuspended in 50 volumes of the same buffer and recentrifuged at 105,000 *g* for 60 min. The final pellet was resuspended in an appropriate volume of 10 mM Tris–HCl (pH 7.5) containing 1 mM EDTA and 50 U/mL aprotinin, and used as the membrane fraction. Both fractions were stored at −80° until use or immediately boiled for 2 min in Laemmli buffer [19] and stored at −20° until use.

### *Immunoblotting*

One hundred eighty micrograms of membrane protein and 60 to 120 μg of cytosolic protein were resolved on an 11% polyacrylamide gel containing sodium dodecyl sulfate [19] and transferred onto a polyvinylidine difluoride (PVDF) membrane (ProBlott, PE Applied Biosystems). Protein concentration was determined with a DC Protein Assay Kit (Bio-Rad) using bovine serum albumin as a standard. The specificities of polyclonal antisera raised against Gβ1, Gβ2, and Gβ3 were evaluated by slot-blot analysis and immunoprecipitation of the three isoforms synthesized by *in vitro* translation [7]. Each of these antisera was found to be specific for the immunizing peptide and the complete synthetic polypeptide, and demonstrated little or no cross-reactivity with the other two proteins. The polyvinylidine difluoride (PVDF) membrane was incubated for 8 hr with an appropriate primary antiserum in a solution containing 20 mM Tris–HCl, 0.5 M NaCl, pH 7.5 and 0.05% (v/v) Tween-20 (TTBS). After washing the membrane three times with TTBS for 5 to 10 min at room temperature, the membrane was incubated with biotinylated goat anti-rabbit immunoglobulin G (Amplified Alkaline Phosphatase Goat Anti-Rabbit Immun-Blot Kit, Bio-Rad) or anti-rabbit immunoglobulin G (γ-chain-specific) alkaline phosphatase conjugate (Sigma) for 1 hr. After the membrane was washed as described above, it was incubated with streptavidin-biotinylated alkaline phosphatase complex for 1 hr, washed again, and then incubated with 100 mL of color development buffer (100 mM $NaHCO_3$, 1 mM $MgCl_2$, pH 9.8, nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate) for at least 5 min. Development was stopped by immersing the membrane in water for 10 min.

## RESULTS AND DISCUSSION

The effects of subtype-specific β-AR stimulation on the subcellular localization of Gβ3 were investigated in perfused rat hearts. Perfusion for 30 min in the presence of 10 μM ISO decreased the cytosolic Gβ3 content and increased the amount of Gβ3 in the membrane fraction (Fig. 1, A and B). Perfusion for 10 min in the presence of 10 μM ISO decreased the cytosolic Gβ3 content and increased the amount of Gβ3 in the membrane fraction to a lesser extent (data not shown). To determine whether changes in the distribution of Gβ3 between the two fractions are mediated through subtype-specific β-AR stimulation, perfusions were performed in the presence of both ISO and various β-AR antagonists (Fig. 1, A and B). Propranolol, a non-selective β-AR antagonist, and CGP20712A 10 μM, a β1-AR antagonist, completely blocked changes in the distribution of Gβ3 between the two fractions caused by ISO (Fig. 1, A

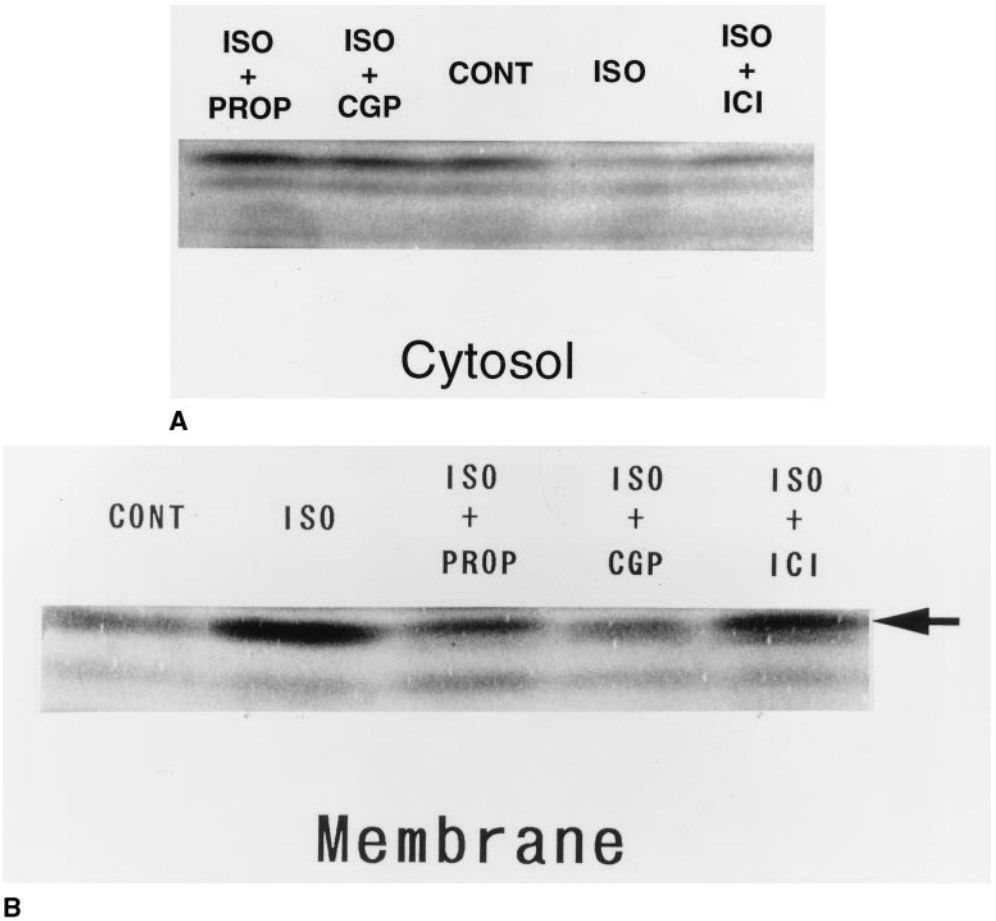


**FIG. 1. Immunoblot analysis with an anti-β3-specific antibody of cytosolic (A) and membrane (B) proteins obtained from rat hearts perfused with ISO and various β-AR antagonists. ISO, 10 μM ISO; PROP, 10 μM propranolol; CGP, 10 μM CGP20712A; ICI, 10 μM ICI118551.**

and B). In contrast, 10 μM ICI118551, a β2-AR antagonist, did not significantly inhibit the ISO-induced changes in the amount of Gβ3 in either fraction (Fig. 1, A and B). The change in Gβ3 content was determined by densitometry in several independent experiments. ISO alone (10 μM) and 10 μM ISO in the presence of 10 μM ICI118551 decreased the content of Gβ3 in the cytosol pool, reaching 11 ± 1% and 18 ± 2% of the control value (N = 5). ISO alone (10 μM) and 10 μM ISO in the presence of 10 μM ICI118551 increased the content of Gβ3 in the membrane pool, reaching 340 ± 8% and 300 ± 9% of the control value (N = 5). In contrast, 10 μM ISO in the presence of either propranolol or CGP20712A did not change the content of Gβ3 in either pool. We performed experiments using several different concentrations of ISO (Fig. 2) and concluded that ISO-induced changes in the distribution of Gβ3 are dose-dependent. Based on our findings, β1-, but not β2-AR stimulation induces changes in the amount of Gβ3 in both fractions. In contrast, the contents of Gβ1 and Gβ2 did not change in either the cytosolic or membrane fractions of hearts perfused with ISO or epinephrine alone (Fig. 3).

The mammalian heart has at least two different subtypes of β-AR, β1 and β2, and may also have β3-AR [20, 21].

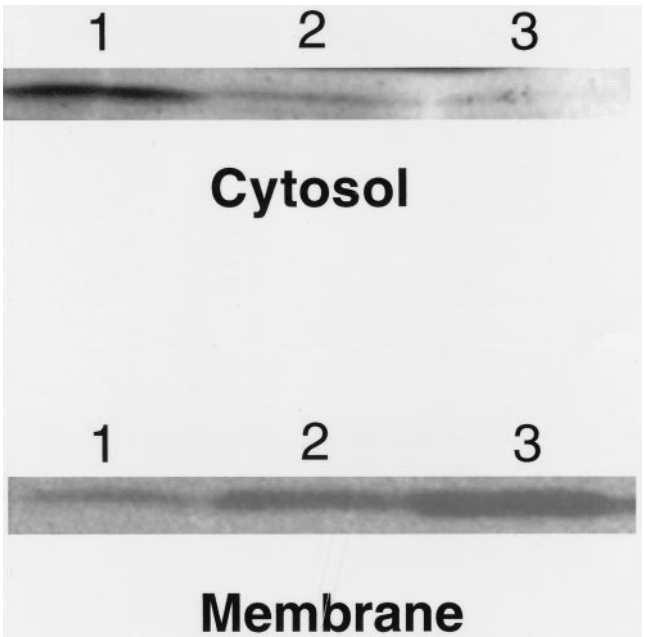


**FIG. 2. Immunoblot analysis with an anti-β3-specific antibody of cytosolic and membrane proteins obtained from rat hearts perfused without (lane 1) and with 2.5 μM ISO (lane 2) or 10 μM ISO (lane 3).**

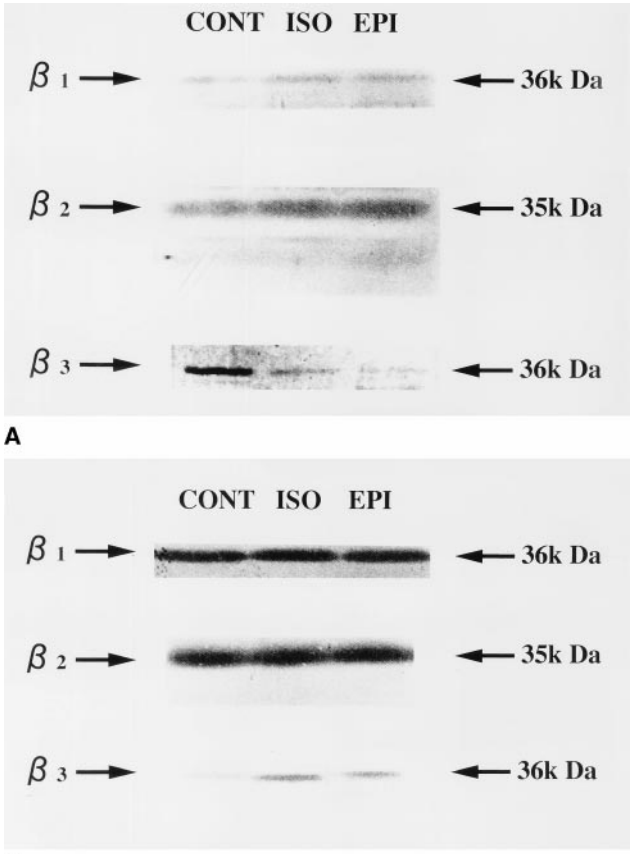


**FIG. 3. Immunoblot analysis with anti-β1-, anti-β2-, and anti-β3-specific antibodies of cytosolic (A) and membrane (B) proteins obtained from rat hearts perfused without (CONT) and with 10 μM ISO (ISO) or 10 μM epinephrine (EPI).**

However, the dominant β-AR in the heart are thought to be the β1 and β2 receptors. It has been shown that β1- and β2-AR are differentially regulated under various physiologic conditions and disease states [20]. Our results demonstrated translocation of Gβ3 from the cytosol to cellular membranes. To the best of our knowledge, no report has described the translocation of Gα or Gβγ in this direction.

*We are grateful to Ms. Fuyuko Kanda for technical and secretarial assistance. We would like to thank Dr. Bernard K.-K. Fung for the gift of isoform-specific antisera, and Tanabe Seiyaku Co. for reagents. This study was supported by a Grant-in-Aid for Scientific Research and a Grant-in-Aid for Exploratory Research (08877107) from the Ministry of Education, Science, and Culture of Japan, grants from the Ministry of Health and Welfare and the Akiyama Foundation of Japan, and a Japanese grant from the Study Group of Molecular Cardiology.*

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