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Membrane-Bound and Cytosolic Forms of Heterotrimeric G Proteins in Young and Adult Rat Myocardium: Influence of Neonatal Hypo- and Hyperthyroidism

Jiri Novotny,[1]* Lenka Bouřová,[1] František Kolář,[2] and Petr Svoboda[1,3]

[1]Biochemistry of Membrane Receptors Group, Videnska 1083, Prague 4, Czech Republic
[2]Department of Developmental Cardiology, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, Prague 4, Czech Republic
[3]Department of Physiology, Faculty of Natural Sciences, Charles University, 128 00 Prague, Czech Republic

Abstract Membrane and cytosolic fractions prepared from ventricular myocardium of young (21-day-old) hypo- or hyperthyroid rats and adult (84-day-old) previously hypo- or hyperthyroid rats were analyzed by immunoblotting with specific anti-G-protein antibodies for the relative content of $G_s\alpha$, $G_i\alpha/G_o\alpha$, $G_q\alpha/G_{11}\alpha$, and $G\beta$. All tested G protein subunits were present not only in myocardial membranes but were at least partially distributed in the cytosol, except for $G_o\alpha 2$, and $G_{11}\alpha$. Cytosolic forms of the individual G proteins represented about 5–60% of total cellular amounts of these proteins. The long ($G_s\alpha$-L) isoform of $G_s\alpha$ prevailed over the short ($G_s\alpha$-S) isoform in both crude myocardial membranes and cytosol. The $G_s\alpha$-L/$G_s\alpha$-S ratio in membranes as well as in cytosol increased during maturation due to a substantial increase in $G_s\alpha$-L. Interestingly, whereas the amount of membrane-bound $G_i\alpha/G_o\alpha$ and $G_q\alpha/G_{11}\alpha$ proteins tend to lower during postnatal development, cytosolic forms of these G proteins mostly rise. Neonatal hypothyroidism reduced the amount of myocardial $G_s\alpha$ and increased that of $G_i\alpha/G_o\alpha$ proteins. By contrast, neonatal hyperthyroidism increased expression of $G_s\alpha$ and decreased that of $G_i\alpha$ and $G_{11}\alpha$ in young myocardium. Changes in G protein content induced by neonatal hypo- and hyperthyroidism in young rat myocardium were restored in adulthood. Alterations in the membrane-cytosol balance of G protein subunits associated with maturation or induced by altered thyroid status indicate physiological importance of cytosolic forms of these proteins in the rat myocardium. J. Cell. Biochem. 82: 215–224, 2001.

Key words: development; G proteins; young and adult rat myocardium; hypo- and hyperthyroidism; subcellular localization

Heterotrimeric G proteins (composed of α, β, and γ subunits) convey information from activated cell surface-bound receptors across the plasma membrane to appropriate effector molecules such as adenylyl cyclases, phospholipases, and ionic channels [Birnbaumer et al., 1990; Gautam et al., 1991; Neer, 1995; Helmreich and Hofmann, 1996; Hamm, 1998]. G proteins are known to reside predominantly in the plasma membrane where they fulfill their regulatory role in signal transduction. However, an increasing number of observations have been published which indicate that a portion of some G protein subunits might also exist in the cytosol, at least under certain conditions.

Several independent studies have demonstrated translocation of G protein α subunits from plasma membranes to the cytosol as a direct consequence of stimulation by agonists. Most attention has been so far paid to the stimulatory G protein (G_s) in this respect. Release of $G_s\alpha$ from the membranes after hormonal stimulation of S49 lymphoma cells has been observed [Ransnas and Insel, 1988, Ransnas et al., 1989, 1991, 1992]. Similar subcellular redistribution of $G_s\alpha$ was also found in mastocytoma cells after stimulation with the prostacyclin analog iloprost [Negishi et al., 1992]. In rat pituitary GH4C1 cells, $G_s\alpha$ was transferred from membranes to the cytosol after

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*Correspondence to: Jiri Novotny, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic.

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stimulation with vasoactive intestinal peptide [Yajima et al., 1998]. Recently, Witte et al. [1999] described isoproterenol-induced solubilization of $G_s\alpha$ from rat cardiac membranes. Soluble forms of other G protein α subunits have been also described. Cytosolic localization of pertussis toxin sensitive $G_i\alpha/G_o\alpha$ proteins was observed in neutrophils and adenomatous lactotrophs [Bokoch et al., 1988; Rudolph et al., 1989a,b; Volpp et al., 1989; Painson et al., 1994]. Takahashi and co-workers demonstrated dramatic solubilization of $G_i2\alpha$ in mastocytoma cells as a consequence of stimulation with thrombin [Takahashi et al., 1991a,b, 1992]. A partial translocation of membrane-bound $G_i\alpha$-2, $G_i\alpha$-3, and $G_o\alpha$ to the cytosol was reported after incubation of GH4C1 rat pituitary membranes with somatostatin [Yajima et al., 1993]. Soluble forms of $G_q\alpha/G_{11}\alpha$ proteins have been also detected. Prolonged treatment of transfected HEK-293 cells with thyrotropin-releasing hormone was shown to induce a dramatic shift of $G_q\alpha/G_{11}\alpha$ from plasma membranes to the cytosol [Svoboda et al., 1996; Drmota et al., 1998, 1999]. In addition, translocation of membrane-bound $G_q\alpha/G_{11}\alpha$ to the cytosol was observed after treatment of MDCK cells with bradykinin [Arthur et al., 1999].

Trimeric G proteins play an important regulatory role in modulation of heart function by hormones and it has been shown that postnatal maturation of myocardial transmembrane signaling, G proteins inclusive, is strongly influenced by thyroid status [Krawietz et al., 1982; Malbon et al., 1988; Bahouth, 1995; Novotny et al., 1999]. Except for $G_s\alpha$ and $G\beta$ there is currently no information about cytosolic forms of the other G protein subunits in heart muscle cells [Roth et al., 1992; Novotny et al., 1994; Kageyama, 1995; Muramoto et al., 1995]. Therefore here we decided to analyze the subcellular distribution of different isoforms of $G_s\alpha$, $G_i\alpha/G_o\alpha$, and $G_q\alpha/G_{11}\alpha$ during maturation of rat ventricular myocardium and, in parallel, to examine the presumed effect of neonatal hypo- and hyperthyroidism on these G proteins.

MATERIALS AND METHODS

Materials

L-triiodothyronine was purchased from Serva (Germany), sodium pentobarbital from Sanofi (France). All other chemicals were from Sigma (USA), and they were of the highest purity available.

Animal Model

Litters of newborn male Wistar rats (SPF strain) were rendered either hyperthyroid or hypothyroid as described previously [Kolář et al., 1992]. Hyperthyroidism (Hyper) was induced by daily subcutaneous injections of L-triiodothyronine (T_3) in a dose of 0.1 μg/g body weight on Days 2–21 postpartum. Hypothyroidism (Hypo) was induced by the inclusion of 0.05% 6-n-propyl-2-thiouracil (PTU) in the drinking water supplied to mothers also from Day 2–21. Control euthyroid (Con) animals received no treatment. On Day 21, the randomly selected part of the animals in each group was employed, while remaining rats were weaned on Day 28 and allowed to grow for additional 56 days. They were denoted as controls (Con), previously hyperthyroid (Hyper-Eu), and previously hypothyroid (Hypo-Eu). Mothers and weaned rats were maintained on standard diet ad libitum. At the age of either 21 days or 84 days, the animals were sacrificed, and the hearts were excised and trimmed of atria and large vessels. The ventricular tissue was rinsed in cold (+5°C) saline, weighted, frozen in liquid nitrogen and stored at −70°C until use. The investigation conformed with the 'Guide for the Care and Use of Laboratory Animals' published by the US National Institutes of Health (NIH Publication Number 85-23, revised 1996).

Preparation of Membrane and Cytosolic Fractions From Myocardial Homogenates

Three independent myocardial homogenates (each from three rat hearts) were prepared from nine animals in each experimental group. The rat ventricles were mixed with a homogenization buffer (20 mM Tris, 0.25 M sucrose and 1 mM EDTA; pH 7.4) and homogenized for 5 min on ice using a motor-driven homogenizer (Teflon-glass). The homogenates were then centrifuged at 600*g* for 5 min (4°C) in order to remove nuclei and particulate cellular debris. Protein concentration in clarified supernatants was adjusted to 2 mg/ml by addition of a homogenization buffer. The membrane and cytosolic fractions were obtained from these homogenates by centrifugation at 250,000*g* for 60 min (4°C). Final pellets were resuspended in the initial volume of a homogenization buffer and denoted "membranes". Supernatants

represented the cytosolic fraction. Thus obtained membrane and cytosolic fractions were stored in aliquots at −70°C until use.

Electrophoresis and Immunoblot Analysis

Samples (50 μl) of myocardial membrane and cytosolic fractions were solubilized in Laemmli buffer and loaded on standard (10% acrylamide/0.26% bis-acrylamide) or urea-(12.5% acrylamide/0.0625% bis-acrylamide containing 6 M urea)-polyacrylamide gels. SDS-PAGE was run at 30 mA for 15 h on a Hoefer SE-600 apparatus. The resolved proteins were subsequently transferred to nitrocellulose membrane (Schleicher and Schuell), blocked with 4% bovine serum albumine for 1 h and incubated with relevant G protein-specific primary antisera for at least 2 h at room temperature. Preparation of primary rabbit anti-$G_s\alpha$ ("CS3"), anti-$G_i\alpha$1,2 ("SG1"), anti-$G_i\alpha$3 ("I3B"), and anti-$G_q\alpha/G_{11}\alpha$ ("CQ") antisera has been previously described in detail [Mitchell et al., 1989; Mullaney et al., 1993; Svoboda et al., 1996]. Anti-$G_o\alpha$ and anti-Gβ were raised in rabbits using synthetic peptides corresponding to amino acids 22-35 of $G_o\alpha$ and the N-terminal decapeptide of Gβ1, respectively [Novotny et al., 1995; Bourova et al., 1999]. After three 10-min washes in TBS buffer (10 mM Tris, 150 mM NaCl; pH 8.0) containing 0.03% Tween 20, the secondary goat anti-rabbit IgG labelled with alkaline phosphatase was applied for 1 h. After another three 10-min washes in TBS-Tween, the blots were developed in TNM buffer (100 mM Tris-HCl, 100 mM NaCl, and 5 mM $MgCl_2$; pH 9.0) containing 5-bromo-4-chloro-3-indolyl phosphate (100 μg/ml) and nitroblue tetrazolium (200 μg/ml) as substrate [Novotny et al., 1995]. Guinea pig myocardial membranes rich in $G_s\alpha$ and rat brain microsomes highly abundant with $G_i\alpha/G_o\alpha$, $G_q\alpha/G_{11}\alpha$, and Gβ proteins were used as standards for reliable identification of these G protein subunits [Gierschik et al., 1986; Sethi et al., 1993]. The immunoblots were scanned (Astra 610P, UMAX) and quantitatively analyzed by the ImageQuant computer program.

Statistics

The relative amounts of myocardial G protein subunits were expressed as percent of the corresponding control levels detected at postnatal Day 21. All values are means ± SEM determined in three independent preparations. The effect of neonatal hypo- and hyperthyroidism on the content of myocardial G proteins was assessed by unpaired Student's *t*-test.

RESULTS

Altered Thyroid Status and Heart Weight

Heart growth was markedly affected by altered thyroid status. Whereas neonatal hypothyroidism reduced heart weight (by about 53%), neonatal hyperthyroidism raised heart weight (by about 31%) in young (21-day-old) rats. Heart weights of adult (84-day-old) previously hypo- or hyperthyroid rats were about 25% lower than those determined in age-matched controls. A more detailed analysis of weight and cardiac parameters on the same experimental model has been reported previously [Novotny et al., 1999].

$G_s\alpha$ Proteins

The long ($G_s\alpha$-L) and short ($G_s\alpha$-S) isoforms of the stimulatory G protein α subunit ($G_s\alpha$) were resolved by standard SDS-PAGE on 10% polyacrylamide gels and detected by immunoblotting with specific anti-$G_s\alpha$ antibodies (Fig. 1). First we analyzed postnatal changes of $G_s\alpha$ proteins in control euthyroid animals. The total amount of myocardial $G_s\alpha$ increased roughly by 150% between postnatal Day 21 and 84. Interestingly, this change was substantiated almost solely by the rise of $G_s\alpha$-L. The cytosolic fraction of young (21-day-old) as well as adult (84-day-old) rat hearts contained about one third of the total amount of $G_s\alpha$ and both $G_s\alpha$-L and $G_s\alpha$-S were identified in membranes as well as in the cytosol (Fig. 2). The ratio of $G_s\alpha$-L/$G_s\alpha$-S, however, differed markedly in myocardial membranes (6.8) and in the cytosol (4.1) of 21-day-old rats and changed to 21.2 in membranes and to 10.5 in the cytosol of 84-day-old animals. These findings strongly support the notion about unequal behavior and regulation of $G_s\alpha$ subforms under various physiological conditions [Novotny and Svoboda, 1998].

While analyzing the effect of altered thyroid status, the G protein levels in myocardial preparations from hypothyroid or hyperthyroid rats were compared with age-matched controls. Hypothyroidism in young (21-day-old) animals decreased the content of $G_s\alpha$ in both myocardial membranes and cytosol. This reduction was caused solely by a lower expression of $G_s\alpha$-L (by about 70%). By contrast, $G_s\alpha$ was overexpressed in hearts of young hyperthyroid rats. Interest-

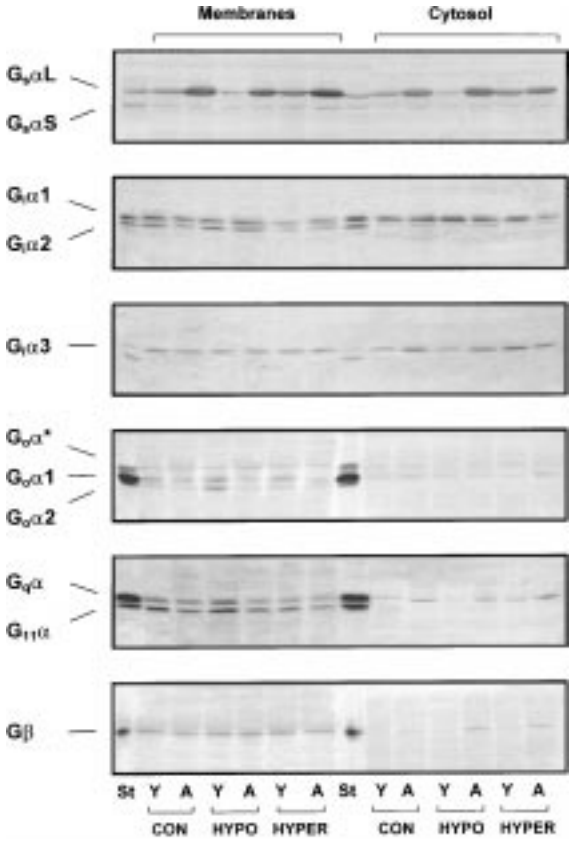

Fig. 1. Representative blot showing the membrane-cytosol distribution of $G_s\alpha$, $G_i\alpha/G_o\alpha$, $G_q\alpha/G_{11}\alpha$, and Gβ in myocardial preparations from young (21-day-old) and adult (84-day-old) rats. Membrane and cytosolic fractions were obtained by centrifugation of cell homogenates at 250 000*g* for 60 min. Resulting pellet (→ membranes) and supernatant (→ cytosol) (each 50 µl per lane) were subjected to SDS-PAGE and immunoblotted as described in Materials and Methods. Guinea pig myocardial membranes or rat brain microsomes were used as positive standards (Stand). CON-control untreated rats, HYPO-hypothyroid rats, HYPER-hyperthyroid rats.

ingly, no significant change was determined in the content of cytosolic forms of $G_s\alpha$, but $G_s\alpha$-L increased by about 60% in myocardial membranes (Fig. 3). In adult (84-day-old) rats, no statistically significant differences were found among myocardial $G_s\alpha$ levels in Hypo-Eu, Hyper-Eu, and Con groups.

$G_i\alpha/G_o\alpha$ Proteins

Individual isoforms of $G_i\alpha$ and $G_o\alpha$ proteins were only resolved by using urea-SDS-PAGE (12.5% polyacrylamide gels containing 6 M urea). Subsequent immunoblot analysis showed that the total amount of myocardial $G_i\alpha$ proteins slightly decreased between Day 21 and 84

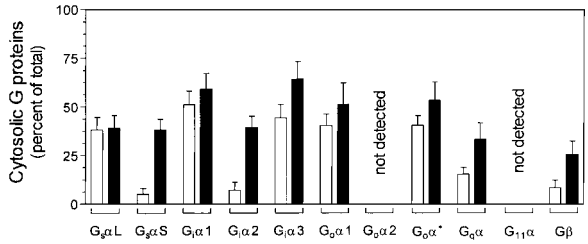

Fig. 2. Relative distribution of the cytosolic and membrane-bound forms of G protein subunits in control young (open bars) and adult (closed bars) rat myocardium. The proportion of individual types of cytosolic G proteins is expressed as percent (± SEM) of total cellular amount of the respective proteins.

(Fig. 4). Interestingly, very similar amounts of $G_i\alpha$ were found in the membrane and cytosol preparations (Fig. 2). Maturation was especially characterized by a decrease in myocardial membrane-bound $G_i\alpha 2$ (by about 40%) and parallel increase in cytosolic $G_i\alpha 2$ (by about 50%).

Myocardial $G_o\alpha 1$ isoform associated with membranes substantially decreased (by about 30%) during maturation, but the other membrane-bound $G_o\alpha$ isoforms remained unchanged. Cytosolic forms of $G_o\alpha$ represented less than one quarter of the total myocardial $G_o\alpha$ (Fig. 2). However, there was no detectable $G_o\alpha 2$ in the cytosol, the amount of cytosolic $G_o\alpha 1$ did

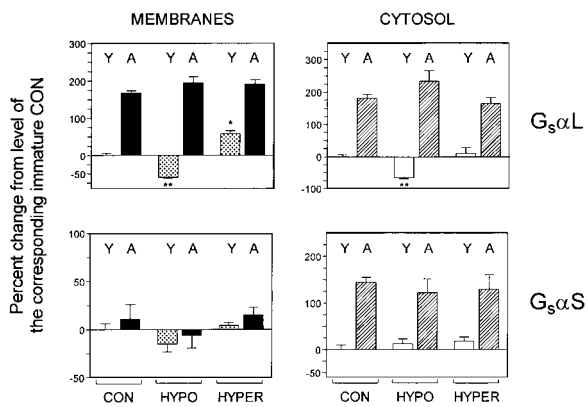

Fig. 3. Effect of maturation and neonatal hypo- or hyperthyroidism on the levels of membrane-bound and cytosolic forms of myocardial $G_s\alpha$-L and $G_s\alpha$-S proteins in young (Y) and adult (A) rats. Three independent myocardial preparations were analyzed using immunoblotting and the relative changes in the G protein levels quantitatively assessed by densitometric scanning. Data are expressed as percent (± SEM) of the values determined in young control animals. Effect of hypo- and hyperthyroidism was evaluated separately in young and adult animals and statistically significant differences are indicated by the asterisks ($^*P < 0.05$; $^{**}P < 0.01$).

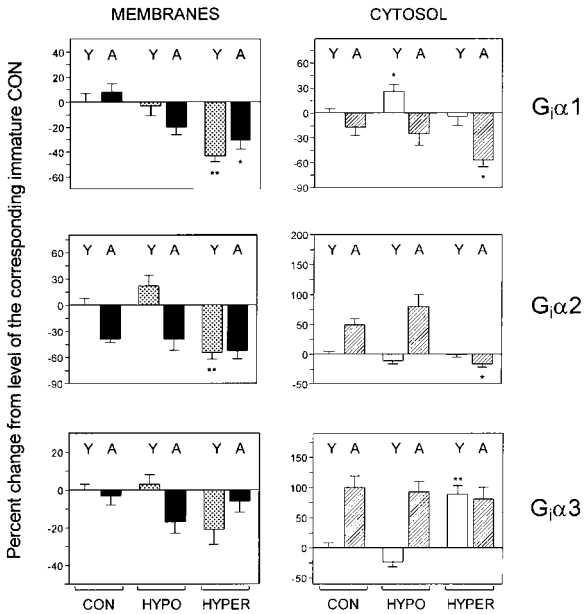

Fig. 4. Effect of maturation and neonatal hypo- or hyperthyroidism on the levels of membrane-bound and cytosolic forms of myocardial $G_i\alpha$ proteins in young (Y) and adult (A) rats. Three independent myocardial preparations were analyzed using immunoblotting and the relative changes in the G protein levels quantitatively assessed by densitometric scanning. Data are expressed as percent (± SEM) of the values determined in young control animals. Effect of hypo- and hyperthyroidism was evaluated separately in young and adult animals and statistically significant differences are indicated by the asterisks (*$P < 0.05$; **$P < 0.01$).

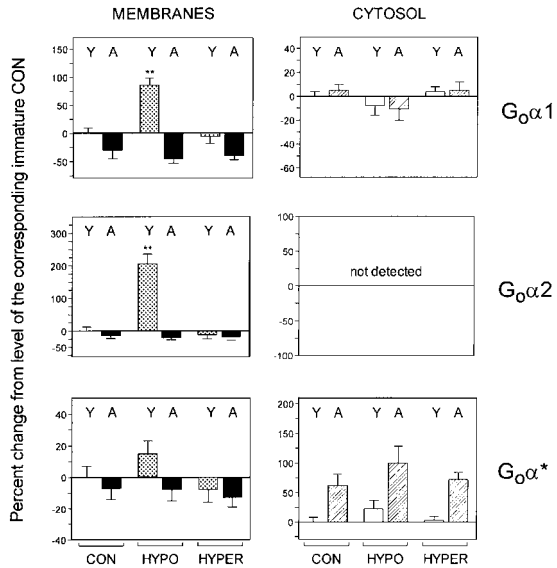

Fig. 5. Effect of maturation and neonatal hypo- or hyperthyroidism on the levels of membrane-bound and cytosolic forms of myocardial $G_o\alpha$ proteins in young (Y) and adult (A) rats. Three independent myocardial preparations were analyzed using immunoblotting and the relative changes in the G protein levels quantitatively assessed by densitometric scanning. Data are expressed as percent (± SEM) of the values determined in young control animals. Effect of hypo- and hyperthyroidism was evaluated separately in young and adult animals and statistically significant differences are indicated by the asterisks (*, $P < 0.05$; **, $P < 0.01$).

not change and $G_o\alpha*$ markedly increased during development (Fig. 5).

Neonatal hypothyroidism elevated the levels of cytosolic $G_i\alpha1$ (by about 25%), did not practically affect expression of the other forms of $G_i\alpha$ proteins and substantially increased the amount of membrane-bound $G_o\alpha1$ (by about 70%) and $G_o\alpha2$ (by about 200%). On the other hand, neonatal hyperthyroidism reduced expression of all membrane-bound $G_i\alpha$ proteins (by about 40%), increased cytosolic $G_i\alpha3$ (by about 60%), and did not change $G_o\alpha$ in young rat hearts (Figs. 4 and 5). The levels of myocardial $G_i\alpha/G_o\alpha$ proteins returned to normal in Hypo-Eu and Hyper-Eu animals, with the exception of $G_i\alpha1$, which remained lower in adult previously-hyperthyroid rats as compared to the corresponding control animals (Fig. 4).

$G_q\alpha/G_{11}\alpha$ Proteins

Sufficient resolution of $G_q\alpha$ and $G_{11}\alpha$ proteins was achieved only by using urea-SDS-PAGE (12.5% polyacrylamide gels containing 6 M urea). Quantitative immunoblot analysis revealed that both $G_q\alpha$ and $G_{11}\alpha$ were almost equally distributed in myocardial membranes and only a small amount of $G_q\alpha$ was present in the cytosol (Figs. 1 and 2). Maturation was accompanied by a mild decrease in the amount of membrane-bound $G_q\alpha/G_{11}\alpha$ proteins and by a substantial increase (by about 100%) of cytosolic $G_q\alpha$ (Fig. 6).

In young rats, neonatal hypothyroidism did not markedly change expression of $G_q\alpha/G_{11}\alpha$ proteins in myocardial membranes but caused almost total disappearance of cytosolic form of $G_q\alpha$. Neonatal hyperthyroidism was associated with a significant decrease in $G_{11}\alpha$ (Fig. 6). The content of myocardial $G_q\alpha/G_{11}\alpha$ proteins was not altered in Hypo-Eu and Hyper-Eu as compared to Con group.

Gβ Subunit

As expected, G protein β subunit was almost exclusively located in myocardial membrane fractions (Figs. 1 and 2). Nevertheless, a small

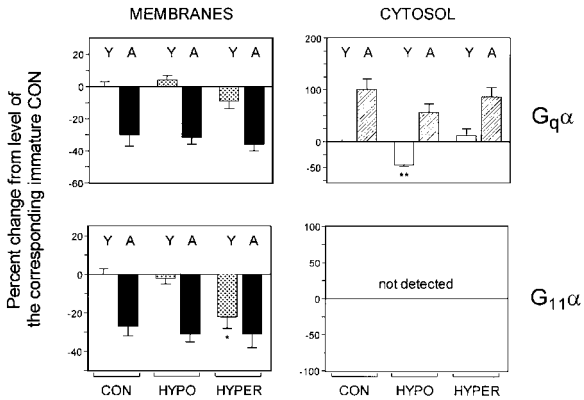

Fig. 6. Effect of maturation and neonatal hypo- or hyperthyroidism on the levels of membrane-bound and cytosolic forms of myocardial $G_q\alpha/G_{11}\alpha$ proteins in young (Y) and adult (A) rats. Three independent myocardial preparations were analyzed using immunoblotting and the relative changes in the G protein levels quantitatively assessed by densitometric scanning. Data are expressed as percent (± SEM) of the values determined in young control animals. Effect of hypo- and hyperthyroidism was evaluated separately in young and adult animals and statistically significant differences are indicated by the asterisks (*$P < 0.05$; **$P < 0.01$).

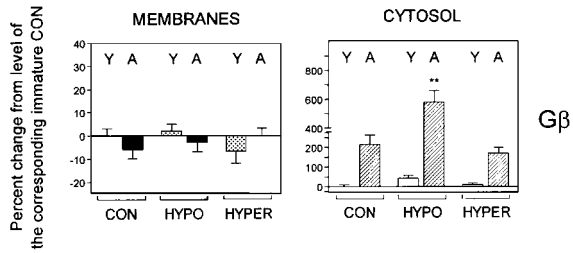

Fig. 7. Effect of maturation and neonatal hypo- or hyperthyroidism on the levels of membrane-bound and cytosolic forms of myocardial Gβ in young (Y) and adult (A) rats. Three independent myocardial preparations were analyzed using immunoblotting and the relative changes in the G protein levels quantitatively assessed by densitometric scanning. Data are expressed as percent (± SEM) of the values determined in young control animals. Effect of hypo- and hyperthyroidism was evaluated separately in young and adult animals and statistically significant differences are indicated by the asterisks (*$P < 0.05$; **$P < 0.01$).

amount (not more than 10%) of Gβ was detected in the cytosol, mainly in preparations from adult rats.

Whereas neonatal hyperthyroidism did not cause any significant change in the distribution of myocardial Gβ, hypothyroidism markedly elevated the level of Gβ in cytosolic preparations from adult rat hearts (Fig. 7).

DISCUSSION

The major aim of our present study was to analyze the cellular distribution of selected G protein subunits in young and adult rat ventricular myocardium. In parallel, we examined the effect of neonatal hypo- and hyperthyroidism on expression of myocardial G proteins. The individual G protein subunits were identified on Western blots by specific antisera.

Firstly we investigated distribution of the stimulatory and inhibitory G proteins, which are engaged in regulation of β-adrenergic signaling [Fleming et al., 1992]. Our analysis indicated that a substantial portion (roughly one third) of myocardial $G_s\alpha$ occurs in the cytosol, which is in accord with previously reported observations [Roth et al., 1992; Novotny et al., 1994, 1999]. The total amount of myocardial $G_s\alpha$ markedly increased during maturation and this rise was almost solely related to higher expression of $G_s\alpha$-L, which prevailed over $G_s\alpha$-S in both membranes and cytosol. The long and short isoforms of $G_s\alpha$ were not only expressed to different extent in young and adult myocardial tissue, but they differed in their membrane and cytosol localization as well. The ratio of $G_s\alpha$-L/$G_s\alpha$-S was much higher in myocardial membranes than in cytosol. These findings strongly support the notion about unequal behavior and regulation of $G_s\alpha$ subforms under various physiological conditions [Novotny and Svoboda, 1998]. Till date, rather controversial data have been published about regulation of expression of myocardial $G_s\alpha$ proteins during maturation. Kumar et al. [1994] reported that $G_s\alpha$-L prevailed in newborn and $G_s\alpha$-S in adult rabbits, but the total amount of myocardial $G_s\alpha$ did not significantly change. Similarly, no change was observed in the content of $G_s\alpha$ in the developing rat heart [Bartel et al., 1996]. On the contrary, an increase was detected in myocardial $G_s\alpha$ during early postnatal development, which was followed by a decrease in adulthood [Kojima et al., 1988]. The discrepancy between these and our present findings of elevated levels of $G_s\alpha$ (especially $G_s\alpha$-L) in adult rat myocardium most probably originate from a different kind of antibody used for immunodetection. In this context it is worth to mention that similar controversy exists in the literature dealing with changes of myocardial G proteins during adulthood and ageing where highly discrepant data

have been reported by different authors [Urasawa et al., 1991; Bazan et al., 1994; Miyamoto et al., 1994; Shu and Scarpace, 1994].

The decrease in $G_i\alpha$ protein which was determined during maturation of rat myocardium conforms well with earlier observations [Luetje et al., 1988; McMahon, 1989; Kumar et al., 1994; Bartel et al., 1996]. Interestingly, roughly one half of myocardial $G_i\alpha$ was present in the cytosol. Whereas the content of all myocardial membrane-bound $G_i\alpha$ proteins and cytosolic $G_i\alpha1$ decreased to different extent during development, the amounts of cytosolic $G_i\alpha2$ and $G_i\alpha3$ increased. Contrary to high levels of cytosolic $G_i\alpha$ proteins in rat hearts there were only weak immunological signals of $G_o\alpha1$ and $G_o\alpha^*$ in the cytosol and no detectable $G_o\alpha2$. Cytosolic forms of $G_o\alpha$ represented only minor part of the total myocardial $G_o\alpha$. Maturation was associated with a decrease in membrane-bound $G_o\alpha1$ and increase in cytosolic $G_o\alpha^*$.

The changes in $G_s\alpha$ and $G_i\alpha$ proteins determined during maturation might be well related to enhanced efficiency of myocardial β-adrenergic signaling in adulthood. Whereas neonatal hypothyroidism was previously shown to be associated with markedly diminished inotropic response to isoproterenol, neonatal hyperthyroidism did not significantly alter sensitivity to this β-adrenergic agonist [Novotny et al., 1999]. It is very difficult, however, to speculate about the possible functional consequences of changed $G_o\alpha$ levels in the developing rat myocardium because the role of $G_o\alpha$ in cellular signaling is not quite clear. As $G_o\alpha$ was identified on secretory granules of endocrine cardiomyocytes, it might be assumed that this G protein may have a function in regulated exocytosis of cardiac hormones [Wolf et al., 1998].

Until now, similarly as for $G_o\alpha$, there was only little information available about the behavior of $G_q\alpha/G_{11}\alpha$ proteins in the developing rat heart, and cytosolic localization of these proteins has not been described. A lower level of $G_q\alpha/G_{11}\alpha$ was previously detected in adult than in neonate myocardium of Sprague Dawley rats [Mochizuki et al., 1995] and content of myocardial $G_q\alpha$ was found to decrease between the 7th and 30th postnatal day in Wistar rats [Bartel et al., 1996]. Here we observed that there were quite comparable amounts of $G_q\alpha$ and $G_{11}\alpha$ in myocardial membranes and only small amount of $G_q\alpha$ was detected in the cytosol. Maturation was associated with a fall of membrane-bound $G_q\alpha/G_{11}\alpha$ and a marked increase in cytosolic $G_q\alpha$.

The G protein β and γ subunits, which form tightly-packed heterodimers under all but denaturing conditions, are considered as membrane-bound proteins [Sternweis, 1986]. There are some indications, however, that besides soluble transducin $\beta\gamma_t$ [Fung et al., 1981] certain amounts of other βγ subunits might occur in the cytosol as well. It has been shown that $G\beta_5$, which is totally soluble in the retina, is associated only up to 70% with cellular membranes in the brain [Watson et al., 1996]. Cytosolic forms of Gβ have been also described in rat cardiac ventricules [Kageyama, 1995; Muramoto et al., 1995]. Our present study confirmed that there might be some soluble Gβ in the rat heart, however, its amount does not exceed 10% of the total myocardial Gβ.

It has been shown that altered thyroid status can dramatically affect cardiac development and function when induced during the early postnatal period [Kolář et al., 1992; Heron et al., 1997]. Our previous study indicated that $G_s\alpha$ and $G_i\alpha$ proteins in the developing heart are controlled by thyroid hormones [Novotny et al., 1999]. Here we show that neonatal hypo- and hyperthyroidism not only change the total amounts of myocardial $G_s\alpha/G_i\alpha$ proteins but that the effect of altered thyroid status manifested even on the level of subcellular distribution of individual isoforms of these proteins. Neonatal hypothyroidism reduced the content of membrane-bound as well as cytosolic $G_s\alpha$-L, increased cytosolic $G_i\alpha1$ and did not change expression of the other isoforms of $G_i\alpha$ in young rats. On the other hand, neonatal hyperthyroidism increased $G_s\alpha$-L in myocardial membranes and $G_i\alpha3$ in the cytosol, but suppressed all membrane-bound $G_i\alpha$ isoforms. Interestingly, an inverse correlation between expression of myocardial $G_i\alpha$ proteins and thyroid hormone levels has also been observed on adult animals [Michel-Reher et al., 1993], and differential regulation of $G_s\alpha$ isoforms by perinatal hypothyroidism has been found in the developing rat brain [Wong et al., 1994]. By contrast, Levine co-workers reported that altered thyroid status induced in adult rats did not influence the relative amounts of myocardial $G_s\alpha$ [Levine et al., 1990]. Our present results are in line with previously observed increase in $G_s\alpha$ and decrease in $G_i\alpha$ expression in neonatal rat

ventricular myocytes affected by thyroid hormones [Bahouth, 1995].

It has been recently reported that $G_o\alpha$ gene expression in the brain of neonatal rats is affected by thyroid hormone deficiency during the developmental period. Perinatal hypothyroidism enhanced $G_o\alpha$ mRNA levels in various brain regions [Cai et al., 2000]. These data fit well to our present findings of substantially increased levels of membrane-bound $G_o\alpha 1$ and $G_o\alpha 2$ in the hearts of young hypothyroid rats. Interestingly, neonatal hyperthyroidism did not cause any changes in the content and distribution of myocardial $G_o\alpha$ proteins.

G proteins from the G_q/G_{11} family, which are involved in regulation of phosphoinositide signaling, are frequently implicated in adaptative and maladaptive responses of the heart [Dorn and Brown, 1999]. Receptor-mediated activation of Gq signaling is considered as a common pathway mediating cardiac hypertrophy and apoptotic heart failure [Adams et al., 1998]. Our present assessment of a possible influence of altered thyroid status on myocardial $G_q\alpha/G_{11}\alpha$ proteins is first of its sort. Neonatal hypothyroidism was found to be associated with no change in the content of membrane-bound $G_q\alpha/G_{11}\alpha$ and with depletion of cytosolic $G_q\alpha$ in young rat hearts. By contrast, neonatal hyperthyroidism significantly diminished the amount of myocardial $G_{11}\alpha$. Thus, altered thyroid status caused a specific disruption of the normal expression of $G_q\alpha/G_{11}\alpha$ proteins during early postnatal development of the heart. Physiological relevance of these changes is not clear. Interestingly, no changes or a mild transient increase in $G_q\alpha/G_{11}\alpha$ have previously been observed in the developing brain of hypothyroid immature rats [Wong et al., 1994; Leung et al., 1996].

It has been shown earlier that previously hypo- or hyperthyroid animals can quickly reestablish euthyroidism [Heron et al., 1997; Novotny et al., 1999]. When we tested the possible reversibility of changes induced by transiently altered thyroid status, newborn rats were treated with PTU or T_3 for three weeks and then allowed to recover until the age of 84 days. As described above, content and distribution of most tested myocardial G proteins markedly changed in young rats as a consequence of altered levels of thyroid hormones during early postnatal period. Interestingly, these changes were more or less completely settled to normal in adult previously hypo- or hyperthyroid animals.

In conclusion, besides confirming existence of cytosolic forms of $G_s\alpha$ protein in the heart, our present study showed that also $G_o\alpha$ and $G_q\alpha$ might occur in the cytosol. In addition, we observed that maturation was associated with differential expression and subcellular distribution of the individual isoforms of G protein subunits. Altered thyroid status in early postnatal period was found to strongly affect levels of myocardial G proteins. Interestingly, the changes in membrane-bound and cytosolic froms of some G proteins ($G_i\alpha$, $G_o\alpha$*, and $G_q\alpha$) had often clearly opposing character. These observations extend our knowledge about existence of cytosolic forms of trimeric G proteins and their specific regulation under various physiological conditions. It can be speculated that changes in the membrane-cytosol balance of G proteins might reflect actual need of the cell for these proteins and serve as a sort of adaptive compensatory mechanism regulating the efficacy of transmembrane signaling. Further investigations will be needed to elucidate the role of soluble forms of G proteins in cellular signaling.

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