Effect of Hypothyroidism on G Protein-Coupled Receptor Kinase 2 Expression Levels in Rat Liver, Lung, and Heart*

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ABSTRACT

GRK2 is a member of the G protein-coupled receptor kinase family that phosphorylates the activated form of β-adrenergic and other G protein-coupled receptors and plays an important role in their desensitization and modulation. Alterations in thyroid hormone levels have been reported to lead to important changes in adrenergic receptor responsiveness and signaling in a variety of tissues. In this context, we have explored the effects of experimental hypothyroidism on GRK2 protein levels in rat heart, lung, and liver using a specific antibody. Hypothyroid animals show significant up-regulation (~50% increase compared with controls) in GRK2 levels in heart and lung at 60 days after birth, whereas a 50% reduction is detected in the liver at this stage. These alterations are selective, as β-adrenergic receptors or other G protein-coupled receptor regulatory proteins, such as G protein-coupled receptor kinase 5 or β-arrestin-1, display a different pattern of expression changes in the hypothyroid animals. The reported changes in GRK2 levels and in the receptor/kinase ratio predict alterations in adrenergic receptor desensitization and signal transduction efficacy consistent with those observed in thyroid disorders, thus suggesting a relevant role for the modulation of GRK2 expression in this physiopathological condition. (Endocrinology 142: 987–991, 2001)

The adrenergic system and thyroid hormones interact physiologically in a coordinated manner. In a variety of tissues, thyroid hormones enhance the β-adrenergic receptor (βAR)-mediated actions of catecholamines by increasing the accumulation of cAMP by mechanisms acting at both the receptor and postreceptor levels as well as by enhancing the transcriptional effects of cAMP. Hypothyroidism severely reduces the β-adrenergic response in most tissues, contributing to the development of cardiovascular dysfunction, altered metabolic responses, and decreased thermogenesis (1–4). Thyroid hormone deficit appears to promote such impaired responsiveness or sensitivity to catecholamines by reducing the number of βAR and increasing the number of α-adrenergic receptors. However, additional mechanisms are needed to explain the overall effect on hypothyroidism on adrenergic signal transduction (4). Receptors for catecholamines belong to the G protein-coupled receptor (GPCR) family. G protein-coupled receptor kinases (GRKs) are a family of serine-threonine kinases that specifically phosphorylate the agonist-occupied form of GPCR. This is followed by binding to the phosphorylated receptor of members of a second family of proteins, termed β-arrestins, leading to receptor uncoupling from heterotrimeric G proteins and signal shut off. This process is known as desensitization, a general feature of GPCR signaling that involves a loss of receptor responsiveness after acute or sustained activation. GRK2 is one of the more abundant and broadly expressed GRKs that has been shown to participate in the regulation of adrenergic receptors and many other GPCRs (5–7). Interestingly, changes in the expression levels of GRK2 have been suggested to underlie alterations in adrenergic receptor signaling and desensitization in several physiopathological situations, such as heart failure, hypertension, experimental models of cardiac hypertrophy, or neonatal stress (7–13). In this context, we have explored the consequences of experimentally induced hypothyroidism on the protein expression levels of GRK2 at two developmental stages in several rat tissues (liver, heart, and lung) markedly affected by thyroid hormone deficit. We have also explored the expression levels of β-adrenergic receptors; of GRK5, a member of the GRK family that modulates a variety of GPCR in the cardiovascular system (5, 6); and of β-arrestin-1, a ubiquitous member of the β-arrestin regulatory family. Our data indicate that hypothyroidism promotes significant and specific alterations in GRK2 levels in these tissues that may contribute to the previously reported changes in adrenergic receptor signaling in such pathological situations.

Materials and Methods Animal treatment

Wistar rats raised in our animal facilities were used, and the rules of the European Community Council (directive of November 24, 1986,
SDS-polyacrylamide gels, blotted to a nitrocellulose membrane, and euthyroid and hypothyroid rats, as indicated in five or six animals) were obtained at two different postnatal ages from extracts from the indicated rat tissues (three independent pools of and hypothyroid rats at different stages of postnatal development.

**Determination of GRK2, GRK5, and β-arrestin-1 protein levels**

Rat tissues were homogenized using a Polytron device (Brinkmann Instruments, Inc., Westbury, NY) in 4 vol 20 mM Tris-HCl (pH 7.5), 5 mM EDTA, 5 mM EGTA, and protein inhibitors (buffer A). The homogenate was centrifuged (1500 × g, 5 min, 4 C) to obtain a crude postnuclear supernatant. Aliquots of these lysates containing 100 µg protein were resolved in 7.5% or 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes for 60–75 min in 10 mM NaHCO₃, 3 mM Na₂CO₃ (pH 10), and 20% methanol using a Transblot cell (Bio-Rad Laboratories, Inc., Richmond, CA). The filters were blocked with 10 mM Tris-HCl (pH 7.5)/150 mM NaCl and 5% fat-free dried milk. GRK2 protein was detected with AbFP1, a polyclonal antibody raised against recombinant GRK2 protein (13) that displays cross-reactivity with GRK5 (C-20), Santa Cruz Biotechnology, Inc., Santa Cruz, CA; dilution, 1:100] as well as a polyclonal antibody (Ab9) raised against recombinant GRK2 protein (13) that displays cross-reactivity specifically against this arrestin isoform (17, 18). Blots were developed using a chemiluminescent method (ECL, Amersham Pharmacia Biotech, Arlington Heights, IL) after incubation with a goat antirabbit antibody conjugated to peroxidase.

**Radioligand binding assay of β-adrenergic receptors**

Rat tissues were homogenized with a Polytron device (three times, 30 sec each time) in 10 vol buffer A. The supernatant of a low speed centrifugation (2,000 × g, 4 min, 4 C) was centrifuged at 45,000 × g for 20 min at 4 C to obtain a plasma membrane pellet. Membranes were washed three times in buffer A and finally resuspended in the same buffer at concentrations of 3–10 mg protein/ml. Total βAR number was determined as previously reported (13), using 5 nM [3H]dihydroalprenolol (Amersham Pharmacia Biotech) and 100 µM (-)propanolol to define nonspecific binding. β₁AR and β₂AR levels were estimated using 1 µM alprenolol as a specific β₁AR competitor. At least three different tissue samples from pools of three or four control or hypothyroid rats were employed for each binding determination; determinations were performed in triplicate.

**Results and Discussion**

To test the effect of hypothyroidism on GRK2 expression levels, we performed immunoblot analysis using extracts from selected rat tissues of normal and hypothyroid animals obtained at two different postnatal ages and a specific kinase antibody. In the heart, the normal developmental pattern of probed with a specific antibody raised against GRK2 (AbFP1; dilution, 1:1000) as indicated in Materials and Methods, followed by densitometric analysis of the specific GRK2 bands. Expression levels in control conditions at each postnatal age are taken as 100%. Data are the mean ± SEM of six determinations. **P < 0.01; ***P < 0.0001 (compared with levels under euthyroid conditions at the corresponding age). □. Control condition; ■, hypothyroid condition. Representative immunoblots are shown below.
GRK2 expression is characterized by high levels at birth, followed by a sustained diminution until adulthood (Penela, P., unpublished data). Comparison of GRK2 immunoreactivity in the hearts of control and hypothyroid animals indicates that thyroid hormone deficiency significantly increases (~1.5-fold compared with euthyroid animals) the expression of GRK2 at 60 days after birth, whereas the GRK2 expression levels attained on postnatal day 5 were similar in control and hypothyroid rats (Fig. 1). A significant increase in GRK5 protein levels was also observed in the heart of hypothyroid animals at 60 days after birth (Fig. 2), whereas the expression of the uncoupling protein β-arrestin-1 was not altered at 5 or 60 days of postnatal development in the treated animals (Fig. 3).

Alterations in the cellular complement of GRK2 and in the receptor/kinase ratio have been reported to affect GPCR desensitization and signaling (5, 6). Namely, changes in the balance between β-adrenergic receptors and GRK2 levels have been proved relevant in cardiac receptor signaling. The positive chronotropic and inotropic effects triggered by catecholamines on cardiac function are modulated by kinase levels; they are decreased or enhanced in transgenic animals overexpressing either GRK2 or a GRK2 inhibitory construct, respectively (19). Moreover, the extent of contractility and sensitivity to β-agonists was inversely related to GRK2 activity in genetically modified mice with different levels of kinase expression (20), and, interestingly, reduction of GRK2 activity in a genetic model of murine heart failure can restore normal cardiac function (11). The extent of change in GRK2 levels in hypothyroid rat heart is in the same range as that observed in other physiopathological situations, such as in human heart failure (9), in cerebral cortex or locus coeruleus after opiate treatment (16), or in circulating lymphocytes of hypertensive patients (10), thus suggesting that such changes might be physiologically relevant.

Increased GRK2 levels, as detected in the hearts of hypothyroid rats, would be predicted to impair β-adrenergic receptor signaling. Interestingly, we found that both β1AR and β2AR levels are decreased at 60 days after birth in the hypothyroid rat heart (β1AR, 32.2 ± 2.3 fmol/mg protein in control animals and 15.8 ± 6.5 fmol/mg protein in treated animals; β2AR, 12.1 ± 6.4 fmol/mg protein in control rats and 7.6 ± 3.6 fmol/mg protein in hypothyroid animals; mean ± SEM of three to five independent experiments). It has been previously reported that βARs are markedly down-regulated in hypothyroid conditions (21–23), and that the sensitivity of cardiac cells to β-agonist stimulation is decreased, particularly at high concentrations of agonist (21). Thus, hypothyroidism appears to simultaneously promote a reduction in βAR density and an increase in GRK2 levels, leading to marked alterations in the receptor/GRK2 ratio and therefore to increased desensitization and impaired signaling of the remaining receptors. This situation is similar to that taking place in heart failure patients, where βAR reduction and increased GRK2 levels promote marked receptor desensitization and increased sympathetic tone (9, 24). In fact, similar alterations in catecholamine signaling and an increase in cardiac efferent sympathetic activity have also been reported as a consequence of T3 deficiency (3). The fact that GRK5, a member of the GRK family involved in the modulation of GPCR present in cardiovascular cells (5, 6), also displays increased levels of expression in the hypothyroid rat heart would further lead to a decreased signaling mediated by β-adrenergic and other GPCR in these circumstances. In summary, our data strongly suggest that the increased levels of GRKs in hypothyroid heart would contribute to the impairment of adrenergic responsiveness and cardiac function reported in such pathological situations.

Similar changes in GRK2 levels are also detected in the}

Fig. 2. Protein levels of GRK5 in adult heart and lung of euthyroid and hypothyroid rats. Tissue samples were obtained as described in Fig. 1, and proteins (75 μg/lane) were resolved by electrophoresis on 7.5% SDS-polyacrylamide gels. GRK5 expression was investigated by immunoblot as indicated in Materials and Methods, followed by densitometric analysis. The expression level in control conditions was taken as 100%. Data are the mean ± SEM of four to seven determinations. **, P < 0.01 compared with control levels. Representative immunoblots are shown. □, Control condition; ■, hypothyroid condition.
lung of hypothyroid rats at 60 days after birth (Fig. 1). At this postnatal stage we do not detect significant changes in βAR levels in the hypothyroid rat lung (data not shown), although a previous study reported diminished βAR expression in the hypothyroid rats at 28 days of age (25). Overall, these data indicate that in the adult rat lung, T3 deficiency modifies the normal pulmonary balance between βARs and their regulatory protein GRK2. Given that the expression level of this kinase has been correlated to the extent of β-agonist-promoted desensitization in different pulmonary cell types (26), our findings suggest that the observed changes in GRK2 levels may indeed result in alterations of receptor function that may contribute to the impairments of surfactant release and smooth muscle relaxation commonly associated with thyroid disorders (4).

A different effect of thyroid hormone deficit on GRK2 levels is observed in the liver. In this tissue, GRK2 levels at birth are markedly high compared with adult values in control animals (13) (Penela, P., unpublished observations), and no GRK5 immunoreactivity is detected (not shown). Hypothyroidism appears to promote a further decrease in GRK2 immunoreactivity at 60 days of life (Fig. 1). At this stage, a significant, approximately 2-fold reduction in GRK2 expression is observed in hypothyroid rats. Such a decrease in GRK2 levels would be predicted to relieve GPCR desensitization, thus increasing responsiveness to agonists. Consistently, hypothyroidism has been shown to potentiate, rather than reduce, catecholamine action in this tissue compared with that in heart or lung. This effect was ascribed to a specific increase in βAR number that would result in a preferential β-agonist stimulation of gluconeogenesis and glycogenolysis (27–29), although there are controversial data on the effect of hypothyroidism in rat liver βARs depending on the rat strain and the methods of induction of hypothyroidism and radioligand binding employed (27, 30). In our hands, β1AR levels were decreased (~3-fold) and β2AR number was increased (~3.5-fold) in the hypothyroid rat liver (data not shown), consistent with the important role of β2AR in mediating the actions of adrenaline in the liver under certain physiological conditions (31, 32). Hypothyroidism also promotes an improvement in βAR coupling that cannot be ascribed to changes in adenylyl cyclase or Gs protein (7, 33, 34) and that could be explained by our reported changes in GRK2 expression. Interestingly, signaling through other GPCRs that play an essential role in hepatic metabolism, such as receptors for vasopressin or angiotensin II, is also altered in hypothyroid animals (35). Agonist stimulation of these receptors leads to a second messenger response similar to that of euthyroid controls despite the reduced number of receptors in such a condition, thus suggesting a better coupling of receptors to the transduction machinery, which would be favored by reduced GRK2 levels.

The observed changes in β-arrestin-1 expression in lung and liver are opposed to those found for GRK2 levels in these tissues, confirming an independent regulation of the expres-

Fig. 3. Effects of hypothyroidism on β-arrestin-1 protein levels in rat heart, lung, and liver at different stages of postnatal development. Samples were obtained as described in Fig. 1, and proteins (100 μg/lane) were resolved by electrophoresis on 10% SDS-polyacrylamide gels. β-Arrestin-1 levels were analyzed with a specific polyclonal antibody (Ab186; dilution, 1:600) as detailed in Materials and Methods. Expression levels in control conditions at each postnatal age are taken as 100%. Data are the mean ± SEM of at least six determinations. **, P < 0.01 compared with levels under control conditions at the corresponding age. □, Control condition; ■, hypothyroid condition. Representative immunoblots are shown below.
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