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TISSUE- AND SUBUNIT-SPECIFIC REGULATION OF G-PROTEIN EXPRESSION BY HYPO- AND HYPERTHYROIDISM

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Abstract—Thyroid hormone status has profound effects on signal transduction in various tissues throughout the body. Therefore, we quantified the signal transducing G-proteins in the rat heart, cerebral cortex, vas deferens and liver by immunoblotting and pertussis toxin labeling in response to chemically induced hypothyroidism (treatment with propylthiouracil) and hyperthyroidism (treatment with triiodothyronine). Levels of the pertussis toxin (PTX) substrates $G_{i\alpha}$ and $G_{o\alpha}$ in the heart and vas deferens were inversely correlated with thyroid hormone levels, i.e. $G_{i\alpha}$ and $G_{o\alpha}$ were decreased or unchanged in hyperthyroid rats and increased in hypothyroid rats compared to control animals. The cerebral cortex and liver expression of PTX substrates $G_{i\alpha}$ and $G_{o\alpha}$ was not affected by changes in thyroid hormone. Regulation of $G_{s\alpha}$ protein was more complex in that $G_{s\alpha}$ was elevated in the hearts from both hypothyroid and hyperthyroid rats compared to control rats, while $G_{s\alpha}$ was unaffected in the other tissues tested. Expression of G-protein β-subunits was not affected by thyroid status in the heart, liver, or cerebral cortex. Our results suggest that tissue- and G-protein-specific factors are involved in the regulation of G-protein subunits by thyroid hormone. Moreover, cardiac expression of $G_{s\alpha}$ is upregulated by increases or decreases in the normal level of thyroid hormone.

Thyroid hormone has profound effects on cardiac, smooth muscle, metabolic and nervous system responses. It has long been recognized that thyroid hormones work at least partly by a permissive action on the functional responsiveness of G-protein-coupled receptors [1] which may occur at the site of the receptors, their G-proteins or their effector mechanisms [1]. On the functional and molecular level the regulatory effect of thyroid hormone is best characterized for α- and β-adrenoceptor function [2]. The overall picture suggests that thyroid hormone enhances β-adrenoceptor- and weakens α-adrenoceptor-mediated responses; however, such alterations are observed in some tissues but not in others [2], and the biochemical basis for these alterations appears to differ considerably among tissues (see below). Moreover, physiological increases in thyroid hormone levels regulate adrenoceptor responsiveness in some tissues while only excessive increases in thyroid hormone lead to detectable alterations in others [2].

Alterations in receptor number do not always reflect the observed functional changes in adrenergic receptor responsiveness due to altered thyroid hormone levels suggesting that alterations of post-receptor components of the signalling pathway may also be involved in the regulation of adrenergic receptor function by thyroid hormone. Indeed alterations of G-protein α- and β-subunit expression by thyroid hormone have been reported from several laboratories [3–10]. However, a systematic comparison of the effects of hypo- and hyperthyroidism on subunits of various G-proteins in multiple tissues is lacking. Therefore, we have compared the effects of hypo- and hyperthyroidism on the expression of the α-subunits of G_i, G_o and G_s and of their common β-subunits in cardiac tissue, a smooth muscle tissue (vas deferens), a metabolic tissue (liver) and a neuronal tissue (cerebral cortex) of the rat.

MATERIALS AND METHODS

Animal treatment. Adult male Wistar rats (250–400 g) were obtained from Lippische Versuchstierzucht (Extertal, F.R.G.) and (a) treated daily with 0.5 mg/kg triiodothyronine for 7 days (T_3‖), (b) treated by addition of 0.05% (w/v) 6-propyl-2-thiouracil to their drinking water for 6 weeks (PTU) or (c) remained untreated. In order to ascertain the effectiveness of our treatment we determined its effects on body weight, cardiac weight and on serum concentrations of triiodothyronine, thyroxine and thyroid-stimulating hormone (TSH) using specific commercially available radioimmunoassays. The results of these tests are given in Table 1. The animals were killed by decapitation and the heart, liver, vas deferens and cerebral cortex rapidly removed. The tissues were homogenized twice for 15 sec in an Ultra-turrax (Jahnke & Kunkel,

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‖ Abbreviations: PTU, rats treated with 6-propyl-2-thiouracil; PTX, pertussis toxin; T_3, rats treated with triiodothyronine; TSH, thyroid-stimulating hormone.

Table 1. Effects of thyroid status on physiological parameters

	Hypothyroid	Control	Hyperthyroid
Plasma triiodothyronine (ng/100 mL)	33 ± 2	81 ± 3	600 ± 63
Plasma thyroxine (μg/100 mL)	1.5 ± 0.0	6.1 ± 0.4	1.1 ± 0.0
Plasma TSH (μU/mL)	1.1 ± 0.1	<0.16	<0.16
Body weight (g)	352 ± 5	428 ± 6	383 ± 10
Heart weight (g)	0.70 ± 0.02	1.23 ± 0.04	1.47 ± 0.04

Data are means ± SEM of 9–11 animals.

All parameters were statistically significantly different in hypothyroid and hyperthyroid vs control animals ($P < 0.001$) except for plasma TSH in hyperthyroid animals. Note that TSH is already below the detection limit in control rats and thus further reductions could not be detected in hyperthyroid rats.

Staufen, F.R.G.) in 8–10 mL of a buffer (100 mM NaCl, 50 mM Tris–HCl, 2 mM EDTA at pH 7.4). The homogenates were centrifuged at 80,000 *g* for 20 min, resuspended in 50 mM Tris–HCl/2 mM EDTA at pH 7.4, and recentrifuged and resuspended twice more.

ADP-ribosylation experiments. Unless otherwise indicated cholate extracts of membranes (2% cholate for 1 hr at 4°, diluted 1:10) were used for all ADP-ribosylation experiments. Pertussis toxin (PTX) catalysed ADP-ribosylation was assessed under conditions yielding maximal ribosylation as described previously [11] with minor modifications. Briefly, cell membranes were incubated with [^{32}P]-NAD and PTX for 1 hr at 30°. The ribosylation reaction was stopped by addition of sample buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 125 mM Tris–HCl, pH 8.0) and subsequent boiling. The reaction products were separated by overnight electrophoresis on SDS gels containing 17% polyacrylamide, during which the dye front left the gel, in order to allow optimal resolution in the 30–50 kDa molecular mass range. Specific phosphorylation bands were identified on autoradiograms of the dried gels, and ^{32}P incorporation was quantified by cutting appropriate bands from the gels and counting them in a scintillation counter. ^{32}P incorporation in lanes which had been loaded with membranes which had been incubated with ^{32}P but without PTX served as control and was subtracted from the data obtained in the other lanes in order to obtain specific ^{32}P incorporation.

Protein dependency of ADP-ribosylation was assessed in each tissue of control rats. In the experiments comparing PTU and T_3 with control rats, gels were always loaded with protein amounts which were in the middle of the linear part of these protein dependency curves in order to ascertain that the assay could detect increases or decreases in ADP-ribosylation.

Western blotting analysis. G-protein subunits were assessed using quantitative western blotting as described by Burnette [12] with minor modifications. Briefly, membrane preparations without prior cholate extraction were diluted 4:1 with sample buffer, boiled for 5 min, and separated on SDS–polyacrylamide gels with 4% and 10% acrylamide in the stacking and running gel, respectively. The separated proteins were transferred to nitrocellulose membranes (Hybond ECL, Amersham, Braunschweig, F.R.G.) with an electric field at a voltage of 55 V overnight. Following the transfer the blots were washed for 90 min in TBS (20 mM Tris, 100 mM NaCl, pH 7.5) at room temperature in the presence of 2% non-fat dry milk. Subsequently the blots were washed twice for 10 min each in TTBS (TBS supplemented with 500 μL/L Tween-20) and then incubated overnight at 4° in 15 mL of TTBS containing 1% non-fat dry milk and a 1:500 dilution of the antisera. After removal of the antisera the blots were washed twice for 10 min each with TTBS and then incubated for 1 hr at room temperature in 100 mL of TTBS which had been supplemented with 1% non-fat dry milk and 70 μL [^{125}I]protein A solution (8.5 μCi/μg, 129 μCi/mL). Finally, the [^{125}I]-protein A was washed out four times for 10 min each with TTBS and the blots were used for autoradiography at −80°. Using the autoradiograms the molecular masses of the specific bands were identified and corresponding sections were cut from the blots and counted in a scintillation counter. In initial experiments we established for each antiserum the range of protein concentrations in which the amount of protein loaded correlated linearly with the observed immunostaining. For sample measurement an amount of protein was then loaded onto the gels which allowed detection of a doubling of staining within the linear range. In western blotting experiments for the detection of $G_{s\alpha}$, a lane with membranes from cyc$^-$ cells (which lack $G_{s\alpha}$) was included in each blot in order to test for specificity of band labeling.

Chemicals. Antisera against G_s were raised in rabbits against synthetic peptide corresponding to amino acids 28–42 common to all four forms of $G_{s\alpha}$; they were affinity purified on AffiGel 10 columns as described previously [13]. The antisera AS-7 (recognizing transducin, $G_{i\alpha 1}$ and $G_{i\alpha 2}$), GC/2 (recognizing $G_{o\alpha}$), SW/1 (recognizing common G-protein β-subunits), [^{32}P]NAD (sp. act. 30 Ci/mmol) and [^{125}I]protein A (sp. act. 8.5 μCi/μg) were obtained from New England Nuclear (Dreieich,

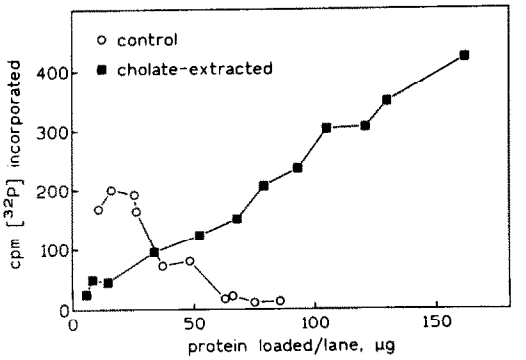

Fig. 1. Protein dependency of PTX catalysed ADP-ribosylation in rat cardiac membranes. Data are from a representative experiment in native membranes (open circles) and in cholate-extracted membranes diluted 10-fold prior to ADP-ribosylation (filled squares).

F.R.G.). PTX was from List (Campbell, CA, U.S.A.) and highly purified sodium cholate ("Ultrol") from Calbiochem (La Jolla, CA, U.S.A.). TSH was determined with a commercially available hypersensitive immunoradiometric assay (TSH-Irmaclone, Henning, Berlin, Germany) in which normal human plasma yields 0.3–0.4 μU/mL.

Data evaluation. All data are means ± SEM of N experiments. Statistical significance of differences between groups was assessed by one-way analysis of variance; if the variance among groups was significantly greater than that within groups, they were compared by *t*-tests with Bonferroni corrections for multiple comparisons. All statistical calculations were performed with the InStat program (GraphPAD Software, San Diego, CA, U.S.A.).

RESULTS

The effectiveness of our 6-propyl-2-thiouracil and triiodothyronine treatments is demonstrated by marked increases in plasma TSH and triiodothyronine levels, respectively (Table 1). It should be noted that both PTU and T_3 decreased body weight in our study despite their opposite effects on hormonal parameters.

In our initial experiments we defined assay conditions for PTX-catalysed ADP-ribosylation of membranes from various rat tissues. In native membranes from rat heart, PTX-catalysed incorporation of ^{32}P increased with loaded protein/lane only over a quite narrow range and further increases in membrane protein content were associated with a decrease in ^{32}P incorporation (Fig. 1). In contrast, PTX-catalysed ADP-ribosylation of 1:10 diluted cholate extracts of cardiac membranes was linear over a wide range of protein concentrations (Fig. 1); using smaller or greater dilutions of the cholate extracts yielded fewer detectable ribosylation products probably due to detergent interference with the ribosylation reaction and dilution, respectively. Similar data were obtained in membranes from the cerebral cortex, vas deferens and liver although the protein concentration yielding maximal ^{32}P incorporation differed somewhat among tissues (data not shown). These data indicate that an endogenous factor inhibiting ADP-ribosylation may be present in native membranes which appears to interfere less with ADP-ribosylation after cholate extraction; elimination of similar ADP-ribosylation inhibiting factors by cholate extraction has also been detected by other investigators [14]. Thus, all further ADP-ribosylation experiments were performed in cholate extracts.

Table 2. Effects of thyroid status on PTX substrates

Tissue	Hypothyroid	Control	Hyperthyroid
Heart	200 ± 8†	122 ± 4	102 ± 4
Vas deferens	112 ± 7*	94 ± 7	90 ± 4
Cerebral cortex	430 ± 24	429 ± 28	424 ± 21
Liver	68 ± 3	62 ± 4	68 ± 7

All data are from 8–11 animals (except for control hearts where N = 6) and expressed in fmol/mg protein.

* and † $P < 0.05$ and 0.001, respectively, vs control and hyperthyroid animals.

In rat cardiac membranes PTX catalysed the incorporation of [^{32}P]ribose into a single band with an apparent molecular mass of 39 kDa. The amount of PTX substrates was greatest in cardiac membranes from PTU and lowest in those from T_3 with intermediate values in control animals (122 ± 4 fmol/mg protein; Table 2). Similar data were obtained in membranes from rat vas deferens where PTX catalysed the incorporation of [^{32}P]ribose into a single band with an apparent molecular mass of 40 kDa (94 ± 7 fmol/mg protein; Table 2).

The effect of thyroid status on PTX substrates was quite different in the other two tissues. In rat cerebral cortex PTX catalysed the ADP-ribosylation of a triplet of bands with apparent molecular masses between 39 and 42 kDa with the major band having an apparent molecular mass of 39 kDa; all three bands were pooled for quantitative analysis. The amount of PTX substrates was similar in cerebral cortical membranes from all three groups (429 ± 28 fmol/mg protein in control animals; Table 2). In rat liver membranes PTX catalysed [^{32}P]ribose incorporation into a single band with an apparent molecular mass of 40 kDa. The amount of PTX substrates was similar in all three groups independent of their thyroid status (62 ± 4 fmol/mg protein in control animals; Table 2).

We next asked whether the above differences are caused by the presence of distinct G-proteins being PTX substrates in the various tissues or by differences in the tissue response to thyroid hormone. Thus, we quantified G-protein α-subunits in heart and cerebral cortex by immunoblotting with specific antisera directed against $G_{i\alpha}$ or $G_{o\alpha}$. It should be noted that the AS/7 antiserum used in the present study detects $G_{i\alpha1}$ and $G_{i\alpha2}$ but not $G_{i\alpha3}$, and thus our conclusions are limited to the former two and cannot necessarily be extrapolated to the latter. The $G_{i\alpha}$ antiserum recognized single bands with apparent molecular

Table 3. Effects of thyroid status on immunodetectable G-protein subunits

Tissue and G-protein subunit	Hypothyroid	Control	Hyperthyroid
$G_{i\alpha}$			
Heart	10,612 ± 4902*	8750 ± 1510	6387 ± 1336
Cerebral cortex	25,986 ± 3476	21,918 ± 2131	27,187 ± 3395
$G_{o\alpha}$			
Heart	2589 ± 574*	1977 ± 420	1216 ± 243
Cerebral cortex	38,034 ± 5132	44,759 ± 7202	39,994 ± 6204
$G_{s\alpha}$			
Heart (39 kDa)	8747 ± 693†	5140 ± 693	9007 ± 873†
Heart (48 kDa)	25,733 ± 3980	20,593 ± 3160	27,200 ± 4393
Vas deferens	35,313 ± 3433	33,660 ± 3127	33,653 ± 4233
Cerebral cortex	65,773 ± 8548	66,106 ± 8511	59,461 ± 3574
Liver	102,448 ± 9182	130,895 ± 15,069	126,836 ± 15,326
G_{β}			
Heart	17,328 ± 1084	15,061 ± 1750	20,745 ± 3242
Cerebral cortex	16,722 ± 1599	17,761 ± 2274	20,515 ± 2350
Liver	67,361 ± 6382	63,095 ± 3638	71,279 ± 5461

Data are means ± SEM of 7–9 animals and expressed as cpm [^{125}I]protein A specifically bound per mg of membrane protein.

* and † $P < 0.05$ and 0.01, respectively, compared to control animals.

Note that two forms of $G_{s\alpha}$ were detected which were analysed separately. Note also that the given data allow comparisons only between treatment groups; comparisons between tissues and G-protein subunits within one treatment group are not possible from these data since blotting efficiency and other factors may have differed between experiments.

masses of 39 and 40 kDa in cardiac and cerebral cortical membranes, respectively. Immunodetectable $G_{i\alpha}$ was highest in hearts from PTU and lowest in those from T_3 with those from control rats being intermediate (Table 3). Immunodetectable $G_{i\alpha}$ in the cerebral cortex, however, was similar in all three groups (Table 3). Similarly, immunodetectable $G_{o\alpha}$ was highest in hearts from PTU, intermediate in control animals and lowest in T_3 but did not differ among groups in the cerebral cortex (Table 3). Although PTX-catalysed ADP-ribosylation and immunoblotting detected similar alterations, it should be noted that the extent of these alterations appeared more pronounced in the ribosylation experiments, a phenomenon also noted by other investigators in adipocytes from hypothyroid rats [6].

The G_s antiserum recognized three bands in cardiac membranes; the lowest band had an apparent molecular mass of 39 kDa, the intermediate band of 48 kDa and the highest band of approximately 50 kDa. Among these bands, the 48 kDa band bound most [^{125}I]protein A but it was also faintly present in cyc$^-$ cells which lack $G_{s\alpha}$. The highest band was specific (not detected in cyc$^-$ cell membranes) but very faint and could not reliably be quantitated because it was too close to the intermediate band. The lowest band was specific (not detected in cyc$^-$ cell membranes) and of intermediate density. Binding of [^{125}I]protein A to this low molecular mass band was significantly increased in PTU as well as in T_3 compared to control animals (Table 3). Binding of [^{125}I]protein A to the intermediate band was also elevated in PTU and T_3 but this did not reach statistical significance. The G_s antiserum recognized a single band with an apparent molecular mass of 40 kDa in cerebral cortical membranes which did not differ in intensity among treatment groups (Table 3). In vas deferens membranes the G_s antiserum recognized a single band with apparent molecular mass of 45 kDa which did not differ in intensity among treatment groups (Table 3). In liver membranes, the G_s antiserum recognized three to four bands with apparent molecular masses of 39–52 kDa; the pooled specific binding of [^{125}I]protein A to these bands did not differ significantly among groups (Table 3).

The antiserum against common β-subunits recognized single bands with apparent molecular masses of 38 kDa in the heart and 35 kDa in the cerebral cortex and liver. The intensity of these bands was not significantly altered by either treatment in any tissue (Table 3).

DISCUSSION

Thyroid hormone exerts its physiological effects by binding to cytosolic receptors which upon agonist binding translocate to the nucleus to regulate the transcription of specific target genes [15]. Previous studies have shown that alterations of thyroid hormone levels affect the functional responsiveness of α and β-adrenoceptors and of other G-protein-coupled receptors [1, 2]. As shown in previous studies from our laboratory [16–18] and others [2, 19, 20] thyroid status-induced alterations of adrenoceptors are complex and occur in a tissue- and subtype-specific manner. For example, hyperthyroidism increases the number of β-adrenoceptors in the heart but not liver, whereas it decreases the number of α_1-adrenoceptors in the heart and liver but not in adipose tissue [2]. Even more specifically,

hypothyroidism decreases the density of α_{1B}-adrenoceptors in rat cerebral cortex without altering that of α_{1A}-adrenoceptors [18]. On the other hand, not all thyroid status-induced alterations in adrenoceptor responsiveness can be explained by changes in adrenoceptor number [2]. Since the functional responsiveness of adrenoceptors depends on their interaction with G-proteins and ultimately effector mechanisms including adenylate cyclase, phospholipases and ion channels, regulation of adrenoceptor responsiveness can potentially occur at each of these sites. Thus, evidence has also been presented for thyroid hormone-induced alterations of G-protein expression in adipocytes [3, 6, 21, 22] and in single reports in the heart [7] and in a synaptosomal preparation from certain brain areas [8]; many of these studies, however, have assessed effects of hypo- or hyperthyroidism only.

The present study extends the above observations in three ways. Firstly, we simultaneously determined thyroid status effects on G-protein expression in four rat tissues (heart, cerebral cortex, liver and vas deferens) to establish whether tissue-specific regulation exists similar to that observed for thyroid hormone-induced regulation of adrenoceptor number. Secondly, we compared thyroid hormone effects on the α-subunits of G_i, G_o and G_s, and on common G-protein β-subunits to investigate G-protein subunit specificity of such regulation similar to that observed for thyroid hormone effects on adrenoceptor subtype expression. Thirdly, we simultaneously compared euthyroid rats with hypo- and hyperthyroid animals to determine whether physiological thyroid hormone levels tonically regulate G-protein expression.

Our study has detected multiple examples of tissue-specific regulation of G-protein subunits by thyroid status. Induction of hypothyroidism increased PTX substrates in the heart and vas deferens but not in the cerebral cortex and liver of the rat; nor do we find changes in the lymphocytes of hypothyroid patients (Maisel and Michel, unpublished observations). In order to determine whether this represents true tissue specificity or merely reflects the fact that these tissues express different PTX substrates we determined the effects of thyroid status on immunodetectable $G_{i\alpha}$ (the major PTX substrate in the heart) and $G_{o\alpha}$ (the major PTX substrate in the brain). Our western blotting data suggest that hypothyroidism increases both $G_{i\alpha}$ and $G_{o\alpha}$ in the heart but not cerebral cortex. Hypothyroidism-induced upregulation of $G_{i\alpha}$ in rat heart has also been observed by other investigators [7]. A similar but distinct tissue dependency in the regulation of $G_{i\alpha1}$, $G_{i\alpha2}$ and $G_{o\alpha}$ by hypothyroidism has recently been observed by Orford *et al.* [8]: these investigators detected increased immunodetectable α-subunits for all three G-proteins in a synaptosomal preparation from rat cortex and striatum whereas some of these G-protein α-subunits remained unaltered in the medulla oblongata, hippocampus or cerebellum. We are uncertain why these investigators have detected increased $G_{i\alpha}$ and $G_{o\alpha}$ in the cerebral cortex of hypothyroid rats but speculate that this may be related to the use of a tissue homogenate (our study) vs a synaptosomal preparation [8]. Moreover, additional PTX-sensitive G-proteins such as $G_{i\alpha3}$ could undergo regulation which differs quantitatively or qualitatively from that of $G_{i\alpha1/2}$ and $G_{o\alpha}$ and thus might explain the quantitative differences observed between alterations of PTX substrates and immunodetectable G-protein α-subunits.

Thyroid status effects on immunodetectable $G_{s\alpha}$ were also tissue dependent, i.e. regulation occurred in rat (present study) and porcine heart [4] but not in the vas deferens, cerebral cortex and liver (present study) or in adipocytes [6, 22]. Finally, thyroid hormone effects on G_β expression also appear to be tissue specific since the amount of immunodetectable G_β remained unchanged in the heart, cerebral cortex and liver (our study) but is elevated in adipocytes from hypothyroid rats [3, 6]. Taken together, these data demonstrate that thyroid status regulates G-protein expression in a tissue-specific manner similar to that of adrenoceptors [2]. On the other hand, tissues such as liver are not unresponsive to thyroid hormone since for example hypothyroidism up-regulates protein kinase C and down-regulates protein kinase A in rat liver [23]. Why thyroid hormone fails to regulate G proteins in some tissues although they have thyroid hormone receptors remains unclear. It can be speculated, however, that intermediate and/or additional steps may be involved which participate in the regulation of G-proteins, adrenoceptors and possibly other proteins by thyroid hormone; these intermediate steps, however, remain to be defined.

Thyroid hormone effects on G-protein expression differ not only among tissues but also depend on the G-protein subunits under investigation. Thus, thyroid hormone reduces the expression of $G_{i\alpha}$, $G_{o\alpha}$ and G_β in all tissue where regulation has been observed including the heart, vas deferens (our study) and adipocytes [3, 6, 7, 21], and in synaptosomal preparations from certain brain areas [8]. In contrast, supra-physiological elevations of thyroid hormones can up-regulate immunodetectable $G_{s\alpha}$ in rat (present study) and porcine heart [4]. Thus, expression of some G-protein subunits is decreased by thyroid hormone while others can be increased. This picture resembles that found with adrenoceptor subtype regulation by thyroid hormone: thyroid hormone has been repeatedly found to up-regulate β-adrenoceptors [2] which couple to G_s and to down-regulate α_1-adrenoceptors [2] which at least in some tissues can couple to G_i [24]. However, such concomitant regulation of receptors and their G-proteins does not always occur. For example, hypothyroidism increases adipocyte G_i [3, 6, 21] but does not alter the number of G_i-coupled adenosine receptors in this tissue [21]. Additional examples of thyroid hormone-induced alterations of receptor responsiveness in the absence of changed receptor numbers or of altered receptor number not accompanied by similarly modulated responsiveness have been reviewed previously [2]. Thus, thyroid hormone regulates G-protein and adrenoceptor expression in a subtype-specific manner but receptors and G-protein subunits mediating the same physiological effect are not always regulated in the same way.

Finally, the simultaneous comparison of hypo-,

eu- and hyperthyroid animals allows conclusions not only on the effects of excessive addition or removal of thyroid hormone but also on the role of physiological thyroid hormone concentrations in the tonic regulation of G-proteins. Although removal of endogenous thyroid hormone by PTU increased $G_{i\alpha}$ and $G_{o\alpha}$ in the heart and vas deferens, supra-physiological thyroid hormone elevations in T_3 did not significantly lower these G-proteins subunits compared to euthyroid animals. Lack of significant alterations of G_i by hyperthyroidism has also been observed in rat heart by other investigators [7, 10] although a tendency towards decreased values was observed in all three studies. Marked increases in $G_{i\alpha}$ in hypothyroidism and minor non-significant decreases in hyperthyroidism have also been observed in adipocytes [3]. Thus, it appears that thyroid hormone within the physiological range already suppresses the expression of $G_{i\alpha}$ (and possibly $G_{o\alpha}$) almost maximally. On the other hand, up-regulation of $G_{s\alpha}$ by thyroid hormone in the hyperthyroid state was not accompanied by down-regulation in the hypothyroid state indicating that normal endogenous thyroid hormone levels do not exert a stimulatory effect on $G_{s\alpha}$ expression. In contrast, cardiac (but not vas deferens) $G_{s\alpha}$ was also elevated by hypothyroidism. We have no good explanation for this elevation but it should be noted that hypo- and hyperthyroidism also had similar effects on body weight, indicating a complex mode of action of thyroid hormone which may involve intermediate and/or additional factors as discussed above.

In conclusion, our study demonstrates that alterations in thyroid hormone may affect the expression of G-protein subunits in a manner which is specific for certain subunits and occurs only in some tissues; these regulatory effects of thyroid hormone may be stimulatory or inhibitory and may occur within or above the physiological range of thyroid hormone levels. Overall the regulation of G-protein subunits similar to the regulation of adrenoceptors appears to be complex and may involve the regulation of intermediate gene products. It should be considered as part of this complex regulatory pattern that alterations may not only occur at the level of transcription and translation but also at the level of protein half-life. Such differences in turnover rates might explain why hypothyroidism increases $G_{i\alpha}$ and $G_{o\alpha}$ in cortical synaptosomes [8] but not in a cortical homogenate (our study), since a synaptosomal preparation mainly consists of membranes which may be relatively far away from the site of protein formation at the cell body and thus have a distinct susceptibility to alterations of protein half-life.

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