Targeted expression of activated Q227L Gαs in vivo

XI-PING HUANG,1 XIAOSONG SONG,1 HSIENT-YU WANG,2 AND CRAIG C. MALBON1

1Department of Molecular Pharmacology and 2Department of Physiology and Biophysics, Diabetes and Metabolic Diseases Research Program, University Medical Center, State University of New York at Stony Brook, Stony Brook, New York 11794

Received 17 July 2001; accepted in final form 22 March 2002

Huang, Xi-Ping, Xiaosong Song, Hsien-Yu Wang, and Craig C. Malbon. Targeted expression of activated Q227L Gαs in vivo. Am J Physiol Cell Physiol 283: C386–C395, 2002; 10.1152/ajpcell.00320.2001.—We report the creation of transgenic mice with an inducible, tissue-targeted expression of a constitutively active mutant form (Q227L) of Gαs. Mice expressing activated Gαs in fat tissue, liver, and skeletal muscle displayed normal body mass and blunted glucose metabolism. cAMP accumulation in adipose tissue was increased in the basal state, but far less than would be expected. Marked adaptation to elevated cAMP levels occurred, leading to an increase in total cAMP-specific phosphodiesterase activity, a 50% decline in cAMP-dependent protein kinase (protein kinase A) activity, and an increased expression of Gβγ. The reduction in kinase activity coincided with >50% increase in the expression of R1α and R1α regulatory subunits of protein kinase A, with no change in the amount of catalytic subunit. These data demonstrate the existence of adaptive responses of protein kinase A, phosphodiesterase, and Gβγ in tissues expressing constitutively active Gαs that may act to rectify the impact of increased cAMP accumulation.

A MOST INTRIGUING EXAMPLE of gain-of-function mutations in G protein α-subunits causing human disease is the case of McCune-Albright syndrome (MAS) (11). MAS is a sporadic disease typified by precocious puberty, monostotic or polyostotic fibrous dysplasia, café au lait pigmentation, and several endocrinopathies (23). Hyperthyroidism, Cushing syndrome, hyperparathyroidism, acromegaly, and hepatomegaly are frequently observed in patients with this syndrome. The hyperactivity of the endocrine tissues appears to result from activating mutations (e.g., Arg201) in Gαs that generate gain of function (23). The expression of activated Gαs is not uniform in MAS but rather is a complex pattern reflecting the occurrence of a mutation in the multicell developing embryo. This feature of the disease generates a mosaic of expression that reflects the fate map of the cell in which the mutation arises. Patients with MAS are true chimera, the severity of the disease manifested largely by the spectrum of organs and tissues in which activated Gαs occurs. Recent studies suggest that a severe form of the syndrome may be the cause of early death in childhood, especially when the activated G protein is expressed in tissues such as the liver, heart, and gastrointestinal tract, nonclassic targets of MAS (18, 19).

The impact of gain-of-function mutations of Gαs on signaling has been examined in a variety of cell types in culture, including neuroblastoma × glioma hybrid NG108-15 cells (14), mouse NIH 3T3-L1 (4), Swiss 3T3 cells (27), rat pituitary GH3 cells (6), and FRTL-5 rat thyroid cells (15). Gαs is known to regulate adenyl cyclase activity, Ca2+ channels, and apoptosis (17, 24). Study of the stoichiometry of Gαs protein-coupled receptors, Gαs, and adenyl cyclase suggests that Gαs is in molar excess of receptor and effector (16). Spatial compartmentation and oligomerization of elements in the receptor > Gαs > effector cascade may well negate the simple stoichiometry (16), because loss of ~50% of the Gαs complement in Albright hereditary osteodystrophy leads to reduced signaling to adenyl cyclase in humans (10, 21). These data suggest that probing the functions of Gαs in vivo may best be approached by targeted expression of a constitutively activated form of Gαs rather than wild-type Gαs. Expression of wild-type Gαs may contribute to endogenous levels of this G protein that may already be in excess of G protein-coupled receptors, whereas expression of Q227L Gαs leads to a situation of chronic activation of Gαs-regulated effectors. Overexpression of wild-type Gαs in hearts of transgenic mice has been accomplished with a rat α-myosin heavy chain promoter (2). mRNA levels for Gαs in the transgenic mice increased nearly 40-fold and Gαs expression in the heart increased less than threefold, whereas there was little evidence by histopathological evaluation of the myocardium for lesions in the young adult (4–7 mo old) animals (2). These transgenic mice do develop increased cardiac contractility in response to β-adrenergic stimulation and with aging cardiomyopathy (3).

Targeted expression of constitutively activated mutant forms of Gαs in transgenic mice may provide a useful model for study of Gαs function in specific tissues. The physiological mechanism(s) by which changes in G protein subunit expression are regulated and the extent to which cells adapt to changes in...
signaling in response to mutations in G protein α-subunits remain to be established and will be essential to our understanding of G protein signaling in human diseases. Creation of mice with tissue-specific expression of an activated Goα may provide insights into the adaptive mechanisms that arise to ameliorate the increase in cAMP levels.

**EXPERIMENTAL PROCEDURES**

**Construction of pPEPCK-Q227L Goα plasmid.** The cDNA encoding the Q227L mutant of Goα was engineered into convenient restriction sites of the pPEPCKQ205L Goα vector as previously reported (1). The coding sequence of a constitutively activated Goα (Q227L) and 3′-untranslated region (212 bp) was employed to replace the phosphoenolpyruvate carboxykinase (PEPCK) coding sequence, remaining under the control of PEPCK promoter.

**Transfection and screening of FTO-2B cells.** The calcium phosphate precipitation method was used in transfection of FTO-2B cells, which are normally grown in DMEM containing 10% FBS at 37°C under humidified 95% air-5% CO2 (12). The procedures used in the transfection, selection, and induction of the stably transfected FTO-2B clones with 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) were described previously (1).

**Creation of transgenic mice with conditional, tissue-specific expression of Q227L Goα.** The Q227L Goα and PEPCK promoter (3.7 kb) were excised by XhoI and NotI from the pPEPCKQ227L Goα construct and isolated by low-melting agarose gel electrophoresis. The constructs were injected into preimplantation embryos, which were transplanted into pseudo-pregnant mice (C57Black6) in the University Transgenic Mouse Facility at the State University of New York at Stony Brook. Mouse tail DNAs were isolated with a DNeasy Tissue Kit according to the manufacturer’s protocol and used for PCR amplification. The primers used were GACATCATC-CAGCGCATGCATC (PT1) and CATCGGGATTACATCTGC-CTGTA (PT2), which on amplification yield a 574-bp fragment specific for the PEPCK-Q227L Goα construct. The amplification products were applied to electrophoresis on 1.5% agarose gels and made visible in ethidium bromide. Transgenic mice were mated pseudo-pregnant mice (C57Black6) in the University Transgenic Mouse Facility under ultraviolet irradiation. Transgenic mice were mated with wild-type C57Black6 mice purchased from Taconic (Germantown, NY) for five generations. Glucose tolerance tests and insulin sensitivity tests were performed as previously described (13). Wild-type control mice were littermates of the transgenic animals. The target age for analysis was 4 wk for study of FTO-2B cells, which are normally grown in DMEM containing 5% FBS at 37°C under humidified 95% air-5% CO2 (12). The procedures used in the transfection, selection, and induction of the stably transfected FTO-2B clones with 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) were described previously (1).

**Phosphodiesterase activity assays.** Phosphodiesterase activity was determined in the absence of the PKA activator, cAMP, and 3′-untranslated region (212 bp) was employed to replace the phosphoenolpyruvate carboxykinase (PEPCK) coding sequence, remaining under the control of PEPCK promoter.

**Experimental procedures.** Construction of pPEPCK-Q227L Goα plasmid. The cDNA encoding the Q227L mutant of Goα was engineered into convenient restriction sites of the pPEPCKQ205L Goα vector as previously reported (1). The coding sequence of a constitutively activated Goα (Q227L) and 3′-untranslated region (212 bp) was employed to replace the phosphoenolpyruvate carboxykinase (PEPCK) coding sequence, remaining under the control of PEPCK promoter.

**Transfection and screening of FTO-2B cells.** The calcium phosphate precipitation method was used in transfection of FTO-2B cells, which are normally grown in DMEM containing 10% FBS at 37°C under humidified 95% air-5% CO2 (12). The procedures used in the transfection, selection, and induction of the stably transfected FTO-2B clones with 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) were described previously (1).

**Creation of transgenic mice with conditional, tissue-specific expression of Q227L Goα.** The Q227L Goα and PEPCK promoter (3.7 kb) were excised by XhoI and NotI from the pPEPCKQ227L Goα construct and isolated by low-melting agarose gel electrophoresis. The constructs were injected into preimplantation embryos, which were transplanted into pseudo-pregnant mice (C57Black6) in the University Transgenic Mouse Facility at the State University of New York at Stony Brook. Mouse tail DNAs were isolated with a DNeasy Tissue Kit according to the manufacturer’s protocol and used for PCR amplification. The primers used were GACATCATC-CAGCGCATGCATC (PT1) and CATCGGGATTACATCTGC-CTGTA (PT2), which on amplification yield a 574-bp fragment specific for the PEPCK-Q227L Goα construct. The amplification products were applied to electrophoresis on 1.5% agarose gels and made visible in ethidium bromide under ultraviolet irradiation. Transgenic mice were mated with wild-type C57Black6 mice purchased from Taconic (Germantown, NY) for five generations. Glucose tolerance tests and insulin sensitivity tests were performed as previously described (13). Wild-type control mice were littermates of the transgenic animals. The target age for analysis was 4 wk for study of FTO-2B cells, which are normally grown in DMEM containing 5% FBS at 37°C under humidified 95% air-5% CO2 (12). The procedures used in the transfection, selection, and induction of the stably transfected FTO-2B clones with 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) were described previously (1).

**Expression of constitutively active Goα, in vivo**

GCT AGA CTC GAC ATG GGC T3’ (S3m), specific only for the PEPCK-Q227L Goα transgene, was used to differentiate mRNAs of the mutant and wild-type forms. The PCR using primers S1m and S2m produces a 400-bp product from endogenous Goα and Q227L Goα, whereas the PCR using primers S3m and S2m produces a 551-bp product from PEPCK-Q227L Goα transgene only.

**Isolation of adipocytes.** Fat tissues were collected, minced, and digested with collagenase type 4 (1 mg/ml) in Krebs-Ringer Buffer [KRB; in mM: 120 NaCl, 4.8 KCl, 2.6 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1 sodium phosphate buffer (pH 7.4), 2.5 d-glucose, and 5 HEPES] containing 3% bovine serum albumin (BSA) at 37°C on a shaker (100–150 rpm) for 30 min. The adipocytes were washed three times with KRB and transferred into KRB containing 5% BSA, 0.1 mM Ro-20-1724 (unless otherwise noted), and adenosine deaminase for cAMP production assays.

**Adipocyte cAMP production assays.** Assays were conducted in 1.5-ml test tubes in triplicate sets. The β-adrenergic agonist isoproterenol and/or the diterpene forskolin were preincubated with the fat cell buffer containing Ro-20-1724 and adenosine deaminase; this aliquot was added to the incubation tubes first. The KRB buffer minus cells served as basal control. The reaction was started by addition of adipocytes (10,000–50,000/tube) and incubated at 37°C for 15 min on a shaker (300 rpm). To stop the reaction, the tubes were returned to an ice bath and 300 μl of prechilled (−20°C) ethanol was added to each well. The assay of the cAMP was as described elsewhere (20).

**Protein kinase A assays.** The assay is based on the phosphorylation of a synthetic peptide substrate (kemptide: Leu-Arg-Ala-Ser-Leu-Gly) by cAMP-dependent protein kinase (protein kinase A; PKA) in the presence of [γ-32P]ATP. The enzyme activity of PKA was determined with a PKA assay kit in triplicate in a final volume of 40 μl according to the manufacturer’s protocol (20). The reaction contained 50 mM Tris (pH 7.5), 100 mM MgCl2, and 100 μM ATP supplemented with [γ-32P]ATP, 0.25 mg/ml BSA, and 50 μM PKA substrate peptide kemptide, “Basal” PKA activities were determined in the absence of the PKA activator, cAMP, and defined as the PKA activity sensitive to inhibition by PKA inhibitor (PKI). PKI, a 17-amino acid peptide derived from the PKI sequence (1 μM final), was included to determine PKA-specific protein kinase activity. “Total” PKA activities were determined in the presence of 10 μM cAMP.

Briefly, fat tissues taken from mice were homogenized with a glass homogenizer fitted with a glass pestle and extraction buffer (in mM: 5 EDTA and 50 Tris, pH 7.5). The whole homogenate was cleared of cell debris by 2,000 g centrifugation for 15 min at 4°C, and the supernatant was recovered for PKA activity assay. The reaction was carried out at 30°C for 5 min. Aliquots (20 μl) were spotted onto phosphocellulose paper disks. The disks were washed three times with phosphoric acid (1% vol/vol) for 5 min and then rinsed once with acetone. The radioactivity of 32P contained in the disk papers was then counted by liquid scintillation counter. The PKA activity was defined as picomoles of [32P]phosphate transferred to kemptide substrate per minute per milligram of protein.

**Phosphodiesterase activity assays.** Fat tissues were collected and homogenized with a glass homogenizer in TMK buffer (in mM: 40 Tris, 5 MgCl2, and 30 KCl, pH 8.0) containing proteinase inhibitors [5 μg/ml aprotinin, 5 μg/ml leupeptin, and 200 μM phenylmethylsulfonyl fluoride (PMSF)] as described. After 2,000 g centrifugation, supernatants were collected for total phosphodiesterase (PDE) activity measurement. Briefly, PDE activity was determined in
RESULTS

The expression of the Q227L constitutively activated Goαs was directed by use of the promoter for the PEPCCK gene (Fig. 1A). The expression vector harboring Q227L Goαs was first screened for expression in FTO-2B rat hepatoma cells, cells that enable induced expression of the PEPCCK gene. FTO-2B clones stably transfected with the pPEPCCK-Q227L Goαs construct should display induction of the Q227L Goαs in response to the positive regulator of the PEPCCK gene, cAMP (Fig. 1B). Treatment of the clones with CPT-cAMP (25 μM) resulted in a robust expression of the transgene, as evidenced by RT-PCR amplification of the mRNA. The promoter was not found to be “leaky,” i.e., the transgene mRNA was not detected in these clones in the absence of added CPT-cAMP. Expression of the Q227L Goαs at the protein level in the FTO-2B clones induced with 25 μM CPT-cAMP was demonstrated by using immunoblotting with an antibody specific for Goαs (Fig. 1C). Neither the wild-type FTO-2B cells nor the clones stably transfected with the pPEPCCK-Q227L Goαs plasmid but not treated with CPT-cAMP, in contrast, displayed increased expression of immunoreactive Goαs. Quantification of the blots from several independent clones revealed a 40–50% increase in the total amount of Goαs (endogenous Goαs + expressed Q227L Goαs), indicative of significant expression of the mutant Goαs over that of wild-type Goαs.

The linearized Xhol-NotI fragment (3.7 kb) was injected into preimplantation single-cell embryos to generate transgenic mice. Transgenic mice were identified at 4 wk of age by PCR amplification of tail DNA (Fig. 2A). Three founder lines were identified and propagated. The bulk of the studies were performed with mice that were 4 mo of age, unless otherwise noted. RT-PCR amplification was performed with primers common to all forms of Goαs as well as with primers that would hybridize only with Q227L Goαs DNA (Fig. 2B). Expression of the mRNA encoding the Q227L Goαs was observed in the fat and liver target tissues, but not in kidney, of the transgenic but not the wild-type (nontransgenic littermates) mice. Examination at the level of protein expression in these transgenic mice revealed expression of increased immunoreactive Goαs in tissues that are targeted by the PEPCCK promoter, i.e., fat, liver, and skeletal muscle (Fig. 2C). The amount of mutant Goαs expressed in vivo, equivalent to the differences in total immunoreactive Goαs between the transgenic and the wild-type mice, was similar to that expressed in the stably transfected FTO-2B cells when challenged with cAMP (Fig. 1C). Tissues of the transgenic mice that are not targeted by the PEPCCK promoter used in these studies, such as kidney, spleen, and brain (not shown), displayed no apparent increase in the amount of immunoreactive Goαs (Fig. 2C). The nontargeted kidney and spleen tissues expressed the same amount of Goαs in transgenic mice as in mice of the same age and sex as the transgenic counterparts (Fig. 2C) or nontransgenic littermates (not shown). We examined the expression of immunoreactive Goαs in fat...
tissue of transgenic Q227L \(\alpha_s\) mice and wild-type mice at 4 and 7 mo of age (Fig. 2D). The increased expression of immunoreactive \(\alpha_s\) attributed to the expression of Q227L \(\alpha_s\) was maintained up to 7 mo of age. The expression of \(\alpha_{i2}\) was also examined, because it has been shown that increased levels of cAMP provoke increased expression of \(\alpha_{i2}\) (5). Expression of \(\alpha_{i2}\) was found to be increased in fat tissue of the transgenic mice at both 4 and 7 mo of age (Fig. 2D). The expression of \(\alpha_{i2}\) in the liver, in contrast, was not found to be enhanced in the transgenic mice at 4 mo of age and only slightly increased in liver from 7-mo-old Q227L \(\alpha_s\) mice compared with wild-type mice (Fig. 2E).

The breeding and macroscopic phenotype of the Q227L \(\alpha_s\) mice were found to be unremarkable, with growth curves for both male and female transgenic mice tracking identically with their nontransgenic counterparts (not shown). Necropsy data suggested some mixed inflammatory cell infiltrates in the liver and skeletal muscle but no routine significant lesions. Alterations in the expression of \(\alpha_s\) such as observed in Albright hereditary osteodystrophy and in hemizygous/heterozygous \(\alpha_s\) knockout mice, have been shown to lead to changes in insulin action and glucose metabolism. Increased insulin sensitivity has been reported in such \(\alpha_s\) knockout mice (25). We examined the glucose metabolism of the Q227L \(\alpha_s\) mice. In glucose tolerance tests, the Q227L \(\alpha_s\) mice demonstrated a markedly suppressed ability to rectify blood glucose levels in response to a bolus administration of glucose (Fig. 3). The Q227L \(\alpha_s\) mice required an additional >2–3 h after bolus administration of glucose to achieve the blood glucose levels of their nontransgenic counterparts. Insulin sensitivity curves derived from studies with fasted Q227L \(\alpha_s\) vs. control littermates performed over a range of insulin concentrations (0.75–6.0 IU/kg) were not significantly different (not shown).

Biochemical analysis of the impact of Q227L \(\alpha_s\) expression on transgenic mice was performed with white fat cells isolated from the endometrial fat pads of transgenic and control mice. The levels of intracellular cAMP were found to be increased, but only by 25–30%

---

*Fig. 1. Expression of the pPEPCK Q227L \(\alpha_s\) construct in FTO-2B rat hepatoma cells leads to cAMP-inducible expression of the Q227L \(\alpha_s\). A: schematic of the pPEPCK-Q227L \(\alpha_s\) vector. B: inducible expression of Q227L \(\alpha_s\) in stably transfected FTO-2B cells: analysis by reverse transcription-polymerase chain reaction (RT-PCR) amplification of Q227L \(\alpha_s\). pPEPCK-Q227L \(\alpha_s\)-transfected FTO-2B cells were treated with the cAMP analog 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (CPT-cAMP; 25 \(\mu\)M) for 72 h, and RT-PCR was performed on total RNA. DNA samples from nontransfected FTO-2B cells served as negative control, and PEPCK-Q227L \(\alpha_s\) plasmid served as positive control. Amplification products were applied to electrophoresis on 1.5% agarose gels and made visible in ethidium bromide under ultraviolet irradiation. N, negative control; P, positive control; MK, 100-bp markers. C: inducible expression of Q227L \(\alpha_s\) in transfected FTO-2B cells. pPEPCK-Q227L \(\alpha_s\)-transfected FTO-2B cells were treated with CPT-cAMP (25 \(\mu\)M) for 4 days. Cells were harvested, and membrane proteins (50 \(\mu\)g protein/lane) were subjected to electrophoresis on a SDS-PAGE and then immunoblotting (IB). Resolved membrane proteins were incubated with a polyclonal rabbit \(\alpha_s\) antibody (CM129) and a second goat antirabbit IgG coupled with horseradish peroxidase. Bands were visualized with enhanced chemiluminescence (ECL) methods and quantified with imaging densitometry. Transgenic mice expressing Q227L \(\alpha_s\) (Q227L) or their nontransgenic wild-type controls (WT) were used. Data represent means ± SE from 4 experiments. A representative of blots is shown. L, lower-mobility "long form" of \(\alpha_s\); S, higher-mobility "short form." *P < 0.05 (paired t-test).
Fig. 2. Expression of the pPEPCK Q227L Goα construct in transgenic mice leads to inducible, tissue-specific expression of Q227L Goα and Goα2. A: mouse tail samples (~1 cm long) were collected at age of ~4 wk, and genomic DNAs were isolated and amplified by PCR. The PCR products were applied to electrophoresis on 1.5% agarose. Positive identification of 5 mice harboring the Q227L Goα transgene is observed (lanes 1–5). M, 100-bp markers; N, negative control with genomic DNA from C57B6 wild type mouse; P, positive control with pPEPCK-Q227L Goα plasmid as a template. B: RT-PCR amplification of mRNA encoding total Goα (endogenous + Q227L Goα) as well as mRNA of the Q227L Goα only. Samples from fat, liver, and kidney of 4-mo-old Q227L Goα transgenic (TG) mice and their WT counterparts were subjected to extraction of RNA. Total RNA was subjected to RT-PCR and amplified with primers that detect total Goα, mRNA and primers unique to Q227L Goα mRNA. PCR products and detection were as in A. C: expression of Q227LGoα in tissues targeted and not targeted by the PEPCK gene promoter: analysis by immunoblotting with antibodies to Goα. Tissues were excised from 4-mo-old mice. Fat tissue (30 μg protein/lane), liver (100 μg protein/lane), and muscle (100 μg protein/lane), all targeted tissues for the PEPCK Goα mRNA. PCR products and detection were as in A. C: expression of Q227LGoα in tissues targeted and not targeted by the PEPCK gene promoter: analysis by immunoblotting with antibodies to Goα. Tissues were excised from 4-mo-old mice. Fat tissue (30 μg protein/lane), liver (100 μg protein/lane), and muscle (100 μg protein/lane), all targeted tissues for the PEPCK Goα mRNA. PCR products and detection were as in A. C: expression of Q227LGoα in tissues targeted and not targeted by the PEPCK gene promoter: analysis by immunoblotting with antibodies to Goα. Tissues were excised from 4-mo-old mice. Fat tissue (30 μg protein/lane), liver (100 μg protein/lane), and muscle (100 μg protein/lane), all targeted tissues for the PEPCK Goα mRNA. PCR products and detection were as in A. C: expression of Q227LGoα in tissues targeted and not targeted by the PEPCK gene promoter: analysis by immunoblotting with antibodies to Goα. Tissues were excised from 4-mo-old mice. Fat tissue (30 μg protein/lane), liver (100 μg protein/lane), and muscle (100 μg protein/lane), all targeted tissues for the PEPCK Goα mRNA. PCR products and detection were as in A. C: expression of Q227LGoα in tissues targeted and not targeted by the PEPCK gene promoter: analysis by immunoblotting with antibodies to Goα. Tissues were excised from 4-mo-old mice. Fat tissue (30 μg protein/lane), liver (100 μg protein/lane), and muscle (100 μg protein/lane), all targeted tissues for the PEPCK Goα mRNA. PCR products and detection were as in A.
n
d
mined over the next 3 h. Results displayed are mean intraperitoneal (ip) bolus of glucose, and blood glucose was deter-

glucose tolerance test. The 4-mo-old mice were administered an

collagenase, and cAMP accumulation was measured in the acutely

mice. Fat pads of control WT and TG mice were digested with

basal intracellular cAMP levels. Tissue was excised from 4-mo-old

Q227L mice and WT littermates of the same age and sex were subjected to a
genic mice leads to impaired glucose tolerance. Q227L mice and

prepared adipocytes. Isolated adipocytes were incubated in the ab-

between the cAMP responses of the Q227L G

of the fat cells from these mice. These relationships

by the magnitude in the increase in basal cAMP levels

constitutively active G

expected increased PKA activity, we

were surprised

Fig. 3. Expression of the pPEPCK Q227L Gαs, construct in trans-
genic mice leads to impaired glucose tolerance. Q227L mice and

and control WT littermates of the same age and sex were subjected to a
glucose tolerance test. The 4-mo-old mice were administered an

intraperitoneal (ip) bolus of glucose, and blood glucose was deter-
determined over the next 3 h. Results displayed are mean ± SE values for

WT (n = 5) and Q227L (n = 8) mice.

(Fig. 4). In view of the level of expression of Q227L Gαs

and its constitutively active nature, we were surprised

by the magnitude in the increase in basal cAMP levels

of the fat cells from these mice. These relationships

between the cAMP responses of the Q227L Gαs mice vs. their nontransgenic littermates were the same in

the absence (not shown) or presence (Fig. 4) of 0.1 mM

RO20-1724, an inhibitor of cAMP-specific PDE activity.

Furthermore, the cAMP response to stimulation

with either a range of β-adrenergic agonist (isoproterenol)

or the plant diterpene forskolin was not signific-
antly different although it was routinely greater in

Q227L Gαs compared with control mice. These data

suggested that some adaptive mechanism(s) must be

operating to nullify the output of the expression of the

constitutively active Q227L Gαs in vivo. The increased

expression of the antagonistic heterotrimeric G protein

Gα12 (Fig. 2, D and E) likely plays some role in damp-

ening the signaling of Gαs to adenyl cyclase.

There is ample literature to demonstrate that elevat-

ing intracellular cAMP levels can provoke increases in

PDEs that metabolize the cyclic nucleotide. We com-

pared the bulk cAMP-specific PDE activity of fat cells

from control mice with those of mice expressing Q227L

Gαs (Fig. 5). Total PDE activity increased ~15% in the

fat cells from the Q227L Gαs mice. Most of the increase

was observed in the PDE activity that was sensitive to

inhibition by the PDE inhibitor Ro-20-1724. Although

PDE activity was increased in the fat cells expressing

Q227L Gαs, the increase in Ro-20-1724-sensitive PDE

was modest and provided only a partial answer.

The expression of a constitutively activated Gαs

would be expected to increase the activation of the

cAMP-dependent protein kinase (PKA). Basal and to-
total PKA activities were measured in adipocytes iso-

lated acutely from mice expressing the Q227L Gαs, as

well as from wild-type, nontransgenic littermates (Fig.

6A). Total PKA activities for adipocytes from Q227L

Gαs mice were not significantly different from those of

adipocytes from wild-type controls. Remarkably, in

contrast to the expected increased PKA activity, we

observed a >50% decline in the amount of basal PKA

activity in the Q227L Gαs mouse adipocytes. These

unexpected results were explored by immunoblotting

with anti-PKA antibodies to ascertain the relative

amounts of the PKA catalytic subunit (PKA cat) in

adipocytes from wild-type and Q227L Gαs mice (Fig.

Fig. 4. Expression of Q227L Gαs, in adipocytes of TG mice increases

basal intracellular cAMP levels. Tissue was excised from 4-mo-old

mice. Fat pads of control WT and TG mice were digested with

collagenase, and cAMP accumulation was measured in the acutely

prepared adipocytes. Isolated adipocytes were incubated in the ab-

sence or presence of isoproterenol (Iso; 0.01–10 μM) for 15 min at

37°C in buffer containing phosphodiesterase (PDE) inhibitor Ro-20-

1724 (100 μM). For measurement of basal cAMP, cells were incu-
bated in the absence (total) or presence (Ro insensitive) of PDE

activity in the absence (total) or presence (Ro insensitive) of PDE

inhibitor Ro-20-1724 (0.1 mM). Results are mean ± SE values from

9 separate experiments. Nine WT and eight Q227L animals were

assayed, each in duplicate. *P < 0.05 (paired t-test).

Fig. 5. Expression of Q227L Gαs, in vivo leads to increased cAMP-
specific PDE activity in adipose tissue. Fat pads excised from 4-mo-

old Q227L mice and WT littermates were homogenized with a glass/
glass homogenizer. The supernatant was collected by 2,000

centrifugation for 10 min at 4°C and immediately assayed for PDE

activity in the absence (total) or presence (Ro insensitive) of PDE

activity in the absence (total) or presence (Ro insensitive) of PDE

activity in the absence (total) or presence (Ro insensitive) of PDE

activity in the absence (total) or presence (Ro insensitive) of PDE

activity in the absence (total) or presence (Ro insensitive) of PDE
The amount of PKA cat, unlike the total PKA activity (Fig. 6A), was found to be equivalent for adipocytes from the wild-type and transgenic mice. In parallel, the relative amounts of the regulatory subunits of PKA were determined in adipocytes from both groups. The expression of RIIα, RIIα, and RIIβ subunits was studied via immunoblotting. Blots were prepared from whole cell extracts, subjected to SDS-PAGE, and then stained with subunit-specific antisera. Significant increases in the expression of all of the PKA regulatory subunits were observed (Fig. 6B). The increases for RIIα and RIIβ were the greatest, increasing in the adipocyte cultures.
cytes from transgenic mice by 70–80%. The expression of the highly abundant RIIβ subunit was increased by >20% in fat tissue of the Q227L Goαs mice. Analysis of the subcellular distribution of PKA catalytic and regulatory subunits revealed quantitative recovery in the postnuclear (2,000 g, 5 min) supernatants rather than in nuclear fractions obtained from whole homogenates of adipose tissue from wild-type as well as Q227L mice (Fig. 6C). Increased expression of R1α, R1α, and RIIβ was also detected in the liver of the Q227L Goαs mice (data not shown).

In fat and liver, analysis of PKA subunit by SDS-PAGE and immunoblotting did not reveal any gross alterations in the stability of RIIβ subunits, R1α, and RIIα (not shown) prepared in the usual cocktail of protease inhibitors (Fig. 6D). The addition of 0.2 mM benzamidine to this cocktail had no dramatic effect on RIIβ subunit recovery, although recoveries seemed slightly lower than greater in the presence of this protease inhibitor (Fig. 6D). Even though the immunoblots were intentionally overexposed, there was little evidence of altered proteolytic processing of the RIIβ subunit of PKA in tissues prepared from transgenic compared with wild-type mice. Tissues excised from a set of older Q227L Goαs transgenic mice (18 mo old) displayed the same expression pattern as observed in the 4-mo-old mice. The quantitative aspects of the immunoblotting of PKA subunits was tested and shown to be essentially linear within the range of protein loading (5–20 μg) used in these studies (Fig. 6E). The increased expression of PKA regulatory subunits, but a normal level of catalytic subunit, provides a likely explanation for the reduction in PKA activity in fat and suggests that chronic elevation of Goαs activity and/or of intracellular cAMP may provoke several adaptive responses that act to dampen the cAMP signaling pathway.

**DISCUSSION**

To achieve the creation of a chimeric mouse model, we employed a transgene in which the expression of Q227L Goαs was regulated by the promoter for the PEPCK gene. The PEPCK promoter offers a number of advantages for this line of investigation (12). The PEPCK promoter is silent in utero, which ensured that the transgene would be carried by a viable pup. Although this design precludes expression until after birth, it seemed a valuable compromise. Germline alterations in the expression of Goαs (i.e., −/− knockouts) to date have not yield viable pups (26). Secondly, the PEPCK promoter is not leaky, is relatively strong, and maintains a level of expression sustained throughout adulthood. We demonstrated that the expression of Q227L Goαs was inducible in vitro in rat hepatoma cells and that the transgene was expressed in vivo in targeted organs. The tissue-selective expression of the PEPCK promoter was most desirable, i.e., we achieved the creation of a “chimeric” mouse in which Q227L Goαs expression was confined to adipose tissue, liver, and skeletal muscle. As deduced from immunoblotting experiments, the expression of Q227L Goαs achieved ~40% of that observed for the endogenous Goαs.

Phenotypically, the Q227L Goαs transgenic mice were quite normal on a gross level. The transgenic mice were fertile, they procreated, and they displayed gross body mass, organ weights, and growth curves that were indistinguishable from those of their nontransgenic littermates or mice of the same age and sex. Although expression of Q227L Goαs was observed in liver, a tissue targeted by the PEPCK gene promoter, we observed no hepatomegaly. Hepatomegaly has been observed in some patients with MAS, who show expression of the constitutively active Goαs in liver among other targeted tissues (18). We did observe, however, a delayed rectification of blood glucose after the administration of a
bolus of glucose. The delay was pronounced and required an additional 2–3 h for the transgenic mice to rectify glucose levels to those of their littermates. Both Goαs (25, 26) and Goa12 (13) have been shown to influence insulin action and glucose metabolism in vivo. Loss-of-function mutants lacking Goα2 display frank insulin resistance (13), whereas gain-of-function mutants of Goα12, such as the Q205L Goα2, yield an insulinomimetic state (1). The Goαs−/− knockouts have proven lethal in mice, whereas the heterozygotes demonstrate increased insulin sensitivity (25). In the current studies the gain-of-function Q227L Goα in mice display the opposite phenotype of the loss-of-function Goαs−/− heterozygotes, showing impaired glucose tolerance.

The modest increase in intracellular cAMP levels that accompanied expression of the gain-of-function, constitutively active Q227L Goα in vivo was unexpected. However, earlier studies provide some precedent for these paradoxical observations. Overexpression of wild-type Goαs targeted to heart created mice with enhanced chronotropic and inotropic responses to sympathetic stimulation, cardiomyopathy with age, and increased apoptosis (2, 3). Although expression of wild-type Goαs in the heart was increased 2.8-fold, basal levels of adenylyl cyclase activity were unaffected (2). These observations support the premise that addition to the possible molar excess of Goαs may have limited consequences for cAMP levels (16). The studies reported herein, however, involved targeted overexpression of a constitutively active mutant of Goαs, not the wild-type form. The expression of a constitutively active form of Goαs in adipocytes did result in elevated basal cAMP levels, but the increase was modest, apparently reflecting some adaptive response(s) provoked by the elevation of intracellular cAMP. The increases in Goα2 expression and cAMP-specific PDE observed in the Q227L Goα mice may partially explain the adaptive response observed in the adipocytes. These adaptive responses may not be universal. In rat vascular smooth muscle cells in culture treated with an adenovirus-directed vector harboring Q227L Goαs, resting cAMP levels were found to increase 10-fold, although the expression of Q227L Goαs was clearly more robust than that obtained with the PEPCK gene promoter used here (8). Our laboratory showed earlier (5) that increasing intracellular cAMP levels in various cells in culture provoked an increase in Goα2 mRNA and a threefold increase in Goα12. Here, we demonstrate a likely correlate in vivo that chronic increases in cAMP levels may increase Goα2 expression in both fat and liver. We examined a second Goα readout, activation of pp60Src nonreceptor tyrosine kinase (11), but found no Q227L Goαs-induced change in the amount of Src or its phosphorylation (data not shown). Thus the cellular context and adaptive changes in PDE and in Goα12 may be important in defining the extent to which expression of Q227L Goα translates into comparable increases in resting cAMP, a primary readout for Goαs.

The most exciting adaptive response observed involves the expression of PKA subunits. Studies in human neoplastic B-(Reh) cells in culture have reported increased expression of both the catalytic subunit and the RI regulatory subunit mRNA in response to elevated cAMP levels, although immunoreactive RIα and Cα levels were observed to decline in response to an eightfold elevation of cAMP by forskolin stimulation (22). In Sertoli MSC-1 cells in culture, elevation of cAMP levels leads to an increase in the expression of RIβ only, not RIα, RIβα, Cα, or Cβ (7, 9). Here we made use of targeted expression of Q227L Goα in mice to address this issue in vivo. In adipocytes from Q227L Goα mice, expression of the catalytic subunit of PKA was unaffected. Expressions of RIα, RIβα, and RIβ, in contrast, were all increased in both fat and liver from Q227L Goα mice. This increased abundance of the regulatory subunits of PKA occurred with no apparent change in the amount of PKA cat, moderating PKA activity and constituting an important part of a possible adaptive response to increased cAMP levels. Taking into consideration the modest changes in cAMP that were noted in response to the expression of the Q227L Goα in vivo, we only can speculate that such adaptive changes, although related to expression of the transgene, are directly related to the change in intracellular cAMP. Indeed, if elevated cAMP levels were driving some of these adaptive changes, the adaptive changes may have already succeeded in attenuating elevated cAMP levels in this interesting mouse model. Levels of expression of PKA regulatory and catalytic subunits as well as PDE have not been analyzed in tissue samples from patients with MAS, but they may well provide a basis for some adaptive correction to the presence of a constitutively active Goαs.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-30111.

REFERENCES