Overexpression of β₂-adrenergic receptors in mouse liver alters the expression of gluconeogenic and glycolytic enzymes

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Submitted 9 March 2004; accepted in final form 24 November 2004

Erraji-Benckekroun, Loubna, Dominique Couton, Catherine Postic, Isabelle Borde, Jesintha Gaston, Jean-Gérard Guillet, and Claudine André. Overexpression of β₂-adrenergic receptors in mouse liver alters the expression of gluconeogenic and glycolytic enzymes. Am J Physiol Endocrinol Metab 288: E715–E722, 2005; doi:10.1152/ajpendo.00113.2004.—In the livers of humans and many other mammalian species, β₂-adrenergic receptors (β₂-ARs) play an important role in the modulation of glucose production by glycolysis and gluconeogenesis. In male mice and rats, however, the expression and physiological role of hepatic β₂-ARs are rapidly lost with development under normal physiological conditions. We previously described a line of transgenic mice, F28 (André C, Erraji L, Gaston I, Grimmer G, Briand P, and Guillet JG. Eur J Biochem 241: 417–424, 1996), which carry the human β₂-AR gene under the control of its own promoter. In these mice, hepatic β₂-AR levels are shown to increase rapidly after birth and, as in humans, be maintained at an elevated level in adulthood. F28 mice display strongly enhanced adenylyl cyclase responses to β-AR agonists in their livers and, compared with normal mice, have increased basal hepatic adenylyl cyclase activity. In this report we demonstrate that, under normal physiological conditions, this increased β₂-AR activity affects the expression of the gluconeogenic and glycolytic key enzymes phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and α1-pyruvate kinase and considerably decreases hepatic glycogen levels. Furthermore, we show that the effects of β₂-adrenergic ligands on liver glycogen observed in humans are reproduced in these mice: liver glycogen levels are strongly decreased by the β₂-AR agonist clenbuterol and increased by the β-AR antagonist propranolol. These transgenic mice open new perspectives for studying in vivo the hepatic β₂-AR system physiopathology and for testing the effects of β-AR ligands on liver metabolism.

β₂-adrenergic receptor; glycogen; glucose; phosphoenolpyruvate carboxykinase; t-pyruvate kinase

THE INTERACTION OF CIRCULATING CATECHOLAMINES with adrenergic receptors plays an important role in the regulation of lipid, protein, and carbohydrate metabolism. Epinephrine enhances lipolysis in adipose tissue, glycogenolysis in muscle, and glucose production in liver that result from both direct stimulation of glycogenolysis and indirect stimulation of gluconeogenesis (3, 21, 26).

Both α₁- and β₂-adrenergic receptors (α₁-ARs and β₂-ARs) are expressed in the liver and are involved in the direct effect of catecholamines. Whereas α₁-ARs are linked to the phosphoinositide turnover/calcium mobilization, β₂-ARs mediate their effects via the adenylyl cyclase/cAMP-signaling pathway. The relative contribution of the two adrenergic receptor subtypes to catecholamine-induced glycogenolysis depends on animal species, sex, and physiological state (16, 19).

In the livers of most species, including humans, the number of both α₁- and β₂-ARs is high, and catecholamine-stimulated glycogenolysis occurs primarily via β₂-ARs in normal physiological conditions (3, 17, 21, 26, 28, 34). In male rats and mice, however, there is a reversal in predominance from β₂-ARs to α₁-ARs during development. The β₂-AR density, β₂-AR-induced cAMP responses, and β₂-AR-dependent glycogenolysis become nearly irrelevant in the livers of these animals at adulthood (10, 16, 23, 27, 33). Although the physiological role of the hepatic β₂-ARs has been most extensively analyzed in rats, the human hepatic β₂-AR system has been far less studied and its physiopathological importance not well characterized.

Our laboratory produced a line of transgenic mice (F28), which carry the human β₂-AR gene under the control of its own promoter. In these animals, the transgene is expressed in muscle, heart, brain, lung, and liver, as revealed by the presence of transgenic mRNA and an increased β₂-AR binding activity (2). The most striking difference between β₂-AR expression in F28 mice and wild-type controls (Co) is undoubtedly in the liver. We report that, in F28 males, hepatic β₂-AR expression increases rapidly after birth to levels comparable to those reported in humans (8, 29) and much higher than those of normal mice. As in humans, the F28 mice maintain an elevated level of β₂-AR expression in adulthood and display strongly enhanced adenylyl cyclase responses to β-AR agonists in their livers. Basal hepatic adenylyl cyclase activity in F28 mice is approximately double that of Co mice (1, 2).

Our earlier reported observations (1) suggest that the strongly increased expression of β₂-ARs in the liver of F28 mice generates physiological changes. Indeed, young adult F28 mice are naturally protected against fulminating liver apoptosis induced by anti-Fas antibodies, most likely due to the β₂-AR-linked increase of hepatic basal adenylyl cyclase activity. Important physiological alterations are also suggested by the fact that F28 mice have a significantly lower body mass and body mass index than Co mice (data given in MATERIALS AND METHODS, Animals and tissues; Ref. 2).

In this study, we investigated the metabolic and physiological consequences of the overexpression of β₂-ARs in F28
mice. Because cAMP plays a key role in the regulation of glycogen turnover and gene transcription of gluconeogenic and lipolytic enzymes and determines the phosphorylation state of key regulatory enzymes (14, 31, 32), we focused on glycogen storage, expression of the gluconeogenic key enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase), and expression of the key glycolytic enzymes glucokinase (GK) and 1-phosphatase (1-PK).

MATERIALS AND METHODS

Animals and tissues. Unless indicated otherwise, 8-wk-old F28 male mice (2) were used for this study. As hepatic β-AR expression is the same in C57BL/6 males as in nontransgenic littermate males, C57BL/6 mice (Iffa Credo, Lyon, France) were used as controls (Co) for comparison. The body masses (body mass index) of the F28 and the Co mice were 15.5 ± 0.6 (0.20 ± 0.01) and 25 ± 1 (0.24 ± 0.01), respectively (n = 15). All mice were housed in colony cages with a 12:12-h light-dark cycle. Except for the fasting experiments, mice had free access to water and food (granulated diet; in terms of energy: 65% carbohydrate, 11% fat, 24% protein; UAR, Epinay sur Orge, France). Mice were killed by means of an intraperitoneal pentobarbital sodium injection (70 mg/kg body wt) followed by cervical dislocation. The livers were dissected out without 30 s, immediately frozen, powdered in liquid nitrogen, and kept at −80°C until further analysis. In some experiments, hindlimb muscles were also rapidly removed, frozen, powdered in liquid nitrogen, and kept at −80°C until glycogen assays were performed.

For the fasting and refeeding studies, mice from each species (F28, C57BL/6) were divided into two groups. The first group (fasted group) was fasted for 24 h, and the second one (refed group) was fasted for 24 h and then refed for 18 h with a high-carbohydrate diet (in terms of energy: 72.2% carbohydrate, 1% fat, 26.8% protein). The animals were killed and the livers sampled as described above.

All animal procedures were conducted in accordance with French government regulations (Services Vétérinaires de la Santé et de la Production Animale, Ministère de l’Agriculture).

Plasma membranes. Plasma membranes were prepared as described previously (2). Briefly, tissues from 1-, 7-, 14-, 21-, 30-, 60-, 90-, and 180-day-old mice were homogenized in 50 mM Tris, 5 mM EDTA, and 250 mM sucrose supplemented with a protease inhibitor cocktail (Roche, Mannheim, Germany). The particulate fraction (membrane preparation) was isolated by centrifugation at 1,500 g for 10 min and resuspended in 75 mM Tris, 5 mM EDTA, and 12.5 mM MgCl2 supplemented with the protease inhibitor cocktail and 10% glycerol. All procedures were performed at 4°C. The membrane preparations were stored at −80°C until use. For binding studies, the membranes were washed twice with PBS plus 10% glycerol and resuspended in PBS immediately before the assay; for adenylyl cyclase studies, they were washed once with and resuspended in 25 mM Tris and 1 mM EDTA. Protein concentrations were determined with a bicinchoninic acid protein assay kit (Pierce, Rockford, IL) using BSA as standard.

Binding experiments. We proceeded as described previously (2). Saturation binding experiments were performed on 10–20 μg of plasma membrane proteins with the β-AR antagonist [125I]iodocyanopindolol ([125I]ICYP, 2000 Ci/mmol; Amersham, Arlington Heights, IL). For competition binding experiments, membranes were incubated with 75 pM [125I]ICYP plus 50 nM of the β2-AR-selective antagonist ICI-118551 or 500 nM of the β2-AR-selective antagonist CGP-20712A (both ligands from Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK). Non-specific radioligand binding was evaluated by incubating in the presence of 1 μM (−)propranolol (Sigma Chemical, St. Louis, MO).

Adenylyl cyclase assay. The assays for evaluation of the responsiveness of adenylyl cyclase to isoproterenol, Gpp(NH)p, and forskolin were performed as described elsewhere (2). To determine the maximal agonist-induced adenylyl cyclase response, incubations were done in presence of 10 μM (−)-isoproterenol. For determination of the maximal G protein-induced and the maximal non-receptor-mediated stimulation of adenylyl cyclase, incubations were done in the presence of 100 μM Gpp(NH)p and 1 mM forskolin, respectively.

Administration of antagonists and agonists. Extreme care was taken to avoid stressful experimental conditions for the animals, since these can alter the catecholamine release and thus possibly confound the β2-adrenergic agonist or antagonist actions. The agonist clenbuterol (Sigma Chemical) and the antagonist propranolol (Avlocardyl; Zeneca-Pharma, Cergy, France) were therefore administered via ingested beverage instead of injection (drug concentration: 2.6 and 7 mg/l water, respectively). The treatments were initiated at 5:00 PM and lasted until the next day for clenbuterol and 2 days later for propranolol.

Food consumption. Commercial rodent pellets were powdered and given to the animals in feeding trays for powdered food (UAR), allowing the quantification of the amount of ingested food. Two individual series of daily measurements were made for 1 wk each on three individual animals from each group (F28, C57BL/6). The quantity of daily ingested food was expressed as a percentage of the animal’s body mass.

Blood glucose determination. A Glucomatic Esprit apparatus (Bayer) was used for determination of the blood glucose concentration. Glucose content was measured, as indicated by the manufacturer, in a drop of blood taken from the tail vein.

Liver and muscle glycogen determination. Glycogen content was determined as described elsewhere (18). Briefly, 200 mg of powdered tissue stored at −80°C were homogenized with an Ultra-Turax in 1 ml of HClO4 at 4°C for extraction and deproteinization. Two hundred microliters of the homogenate were first treated for 2 h at 40°C with α-amyloglucosidase in acid buffer for glycogen hydrolysis and then centrifuged at 14,000 rpm for 10 min after acidification with HClO4. The remaining 800 μl of the homogenate were immediately neutralized with solid KHCO3 and centrifuged at 14,000 rpm for 10 min to determine the tissue glucose amount that did not result from glycogen hydrolysis. The glucose content was assayed in both supernatants, using a UV method for the determination of β-glucose (Boehringer Mannheim kit) according to the manufacturer’s instructions. Tissue glycogen was expressed as millimoles of glucose resulting from glycogen hydrolysis per gram of tissue.

Histological revelation of glycogen. Ten-micrometer-thin cryostat sections were made from livers of untreated, clenbuterol-treated, and propranolol-treated 8-wk-old F28 and Co mice killed either at 8:00 AM or at 5:00 PM as indicated. Glycogen was visualized with periodic acid-Schiff staining (9).

Northern blot analysis. Total RNA was extracted from 200 mg of liver tissue by use of the guanidine thiocyanate method (4). Northern blot analysis was performed as described (24). mRNA (20 μg) was run on 1% agarose gels and transferred onto Hybond N+ membranes (Amersham). Northern blot analyses were performed using a 569-bp fragment spanning exons 1–4 of the mouse PEPCK cDNA (35), a 1.25-kb EcoRI-HindIII fragment from the rat G-6-Pase cDNA (11), a 700-bp PvuII fragment of the rat GK cDNA, and a 1.8-kb pair PstI fragment from the rat cDNA PK G4 as specific probes for the PEPCK, G-6-Pase, GK, and i-PK mRNA, respectively (24). To normalize the signals, blots were hybridized with a 700-bp HindIII-EcoRI fragment cyclophilin-specific probe derived from a rat cDNA plasmid (24). Band intensity was determined by optical density (NIH Image, version 1.62).

Real-time quantitative RT-PCR. Real-time quantitative RT-PCR analysis was done as described (6). Total RNA (500 ng) was reverse transcribed for 1 h at 42°C in a 20-μl final volume reaction containing 50 mM Tris·HCl, 75 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, 250 mM random hexamers (Promega), 250 ng of oligo(dT) (Promega), 2 mM each of dNTPs, and 100 units of Superscript II reverse transcriptase. After each PCR cycle, the melt curve analysis was performed to ensure that a single PCR product was generated.
transcriptase (Invitrogen). Real-time quantitative RT-PCR analysis was performed starting with 6.25 ng of reverse-transcribed total RNA in a final volume of 10-μl PCR reaction, with 0.5 μM of the earlier described (6) cyclophilin-, β-PK-, and GK-specific primers (Invitrogen) and 2 mM MgCl₂, using 1 × light cycler DNA Master SYBR Green I mix in a light cycler instrument (Roche Applied Science).

Samples were incubated in the light cycler apparatus for an initial denaturation at 95°C for 10 min followed by 40 cycles. Each cycle consisted of 95°C for 15 s, 58°C for 7 s, and 72°C for 15 s. SYBR Green I fluorescence emission was determined after each cycle. The relative amounts of the different mRNAs were quantified by using the second derivative maximum method of the light cycler software. Cyclophilin was used as an invariant control, and the relative quantification for a given gene was corrected to the cyclophilin mRNA values. Amplification of specific transcripts was confirmed by melting curve profiles generated at the end of each run. PCR specificity and product length were further checked by agarose gel electrophoresis and ethidium bromide staining.

Statistical analysis. All results are presented as means ± SE. The significance of differences between means of two groups was assessed by using Student’s t-tests for unpaired variables and between more than two groups by using ANOVA, which was followed, when significant, by post hoc Bonferroni-corrected t-tests. Significance of difference between F28 and control mice was indicated in the figures by ns (not significant) for P ≥ 0.05, * for P < 0.05, and ** for P < 0.02. Differences were considered statistically significant at P < 0.05.

RESULTS

Hepatic β₂-AR system expression in F28 and Co mice. [125I]CYP was used to specifically label β-ARs in liver membrane preparations of F28 and Co mice taken at different ages between 1 and 180 postnatal days (PN). The radioligand bound to a single class of sites with similar Kd values to all extracts. The geometric means of all values (and range limits) were 3.9 (2.8–5.6) and 3.2 (2.2–4.7) pM, respectively, for F28 and control mice. The maximal binding capacity (Bmax) values produced the developmental patterns of β-AR accumulation shown in Fig. 1A.

In Co animals, the hepatic β-AR density was highest at birth and decreased with development, dropping by nearly 50% in the 1st wk after birth and then declining progressively to ≈15% of the initial value at 3 mo of age. The hepatic β-AR expression in F28 mice was similar to that of Co mice in the immediate postweaning period but increased thereafter and, at PN14, reached a maximal level that was maintained for ≥6 mo. Competition binding experiments with the β₁- and β₂-AR selective antagonists CGP-20712A and ICI-118551, respectively, indicated that β₁-AR numbers remained at a constant low level for all chosen ages in both Co and F28 mice (14 ± 3 and 23 ± 3 fmol/mg, respectively). Therefore, the developmental patterns of receptor expression in Fig. 1A mainly reflect β₂-AR density changes.

We further investigated the relationship between β-AR expression and G protein-adenyl cyclase signal transduction system. We measured the adenylyl cyclase response to forskolin (1 mM), which stimulates the enzyme directly (Fig. 1B); to Gpp(NH)p (100 μM), which activates all G proteins (Fig. 1C); and to the β-AR agonist isoproterenol (10 μM) (Fig. 1D). The developmental patterns of the forskolin-induced adenylyl cyclase response in F28 and Co mice were similar (Fig. 1B).

**Fig. 1.** Ontogenetic pattern of the accumulation of β-adrenergic receptors (β-ARs) (A) and the adenylyl cyclase (AC) response induced by 1 mM forskolin (FK; B), 100 μM 5’-guanylylimidodiphosphate (Gpp(NH)p; C), or 10 μM (-)-isoproterenol (isop; D). Bmax, maximal binding capacity. Measurements were done on particulate fractions of livers from 1-, 7-, 14-, 21-, 30-, 60-, 90-, and 180-day-old control (Co, ◦) and F28 (●) mouse livers as indicated in MATERIALS AND METHODS. Data shown are means ± SD values obtained from 3–6 individual triplicate experiments, each performed on a different membrane preparation. Statistical differences between F28 and Co mice are indicated by * and ** as described in MATERIALS AND METHODS.
However, the ontogenetic patterns of total G protein and β-AR agonist-induced responses were different (Fig. 1, C and D, respectively). Gpp[NH]p-induced responses were slightly, but significantly, higher for F28 than for Co mice (P < 0.05 at PN1, 14, 30, 60, and 90). A large difference was observed in the isoproterenol-induced response. In F28 mice, the response showed a marked increase over Co mice, reaching maximum level within 2 wk after birth, whereas in Co mice the response decreased to levels close to basal activity (4.2 ± 0.8 pmol·min⁻¹·mg protein⁻¹). Both types of mice maintained their respective characteristics across the 6-mo age range studied.

Liver glycogen and glycemia in F28 and Co mice under normal physiological conditions. The basal adenyl cyclase activity in the F28 mouse liver is about twofold higher than in Co mice (1). We investigated whether this affects the glycogen accumulation/degradation and/or blood glucose concentration under normal physiological conditions. Normally, hepatic glycogen stores are degraded during the postabsorptive phase and refilled during the absorptive phase. Analyses were therefore performed at the beginning (8:00 AM) and the end (5:00 PM) of the postabsorptive phase. We visualized hepatic glycogen stores histochemically in liver cryostat slices (see Fig. 3) and assayed the overall liver glycogen content in tissue homogenates (Fig. 2A).

For untreated F28 mice (F28nt), the overall tissue glycogen yield at 8:00 AM was approximately one-half of that in Co mice (Fig. 2A1), yet glycogen was demonstrable in most hepatocytes of both Co and F28nt mice (Fig. 3, A and B, respectively). At 5:00 PM, the overall glycogen yield decreased by 60–70% in both Co and F28nt mice (Fig. 2A1). Glycogen remained detectable in most Co mouse hepatocytes (Fig. 3G), but glycogen-depleted areas were observed in F28nt mouse livers (Fig. 3H).

The lower glycogen levels in F28nt mouse livers did not appear to be due to a deficit in food absorption: consumed food mass vs. body mass was similar for both groups of animals (Fig. 2C). Administration of the β-AR antagonist propranolol (Coprop, F28prop), however, considerably reduced the difference in glycogen levels (Fig. 2A1) and glycogen distribution between the two animals at 8:00 AM and 5:00 PM (Fig. 3, E–F and K–L, respectively), supporting the idea that the decreased glycogen levels in F28nt may be due to the overexpression of hepatic β2-ARs.

The hepatic β2-adrenergic system plays an important role in the maintenance of blood glucose homeostasis via glycogenolysis and gluconeogenesis. Because glycogen storage is modified in F28nt compared with Co mice, we checked whether blood glucose levels were changed. Levels of plasma glucose in F28nt mice were slightly but significantly lower than in Co mice at 8:00 AM and 5:00 PM, and this decrease in F28 mice was inhibited by propranolol (Fig. 2B).

The muscles of F28 mice have higher levels of β2-ARs than those of Co mice (2). The differences in glycogen content between the animals could be a result of lower blood glucose caused by increased glucose uptake in the muscle. To examine the validity of this hypothesis, we assayed glycogen in hindlimb muscles of F28nt and Co mice (Fig. 2A2). Muscle glycogen levels in F28nt animals did not differ statistically from those in Co mice. Moreover, glycogen levels in the

![Fig. 2. Effect of β2-AR overexpression on liver glycogen (A1), muscle glycogen (A2), blood glucose (B), and food consumption (C). A1–2: glycogen yield from livers (A1) and hindlimb muscles (A2) of 8-wk-old Co and F28 mice, untreated (nt) or treated with clenbuterol (clenb) or propranolol (prop), was measured at the beginning [8:00 AM: nt (n = 7), clenb (n = 8)] and the end [5:00 PM: nt (n = 4), prop (n = 5)] of the postabsorptive phase. Glycogen was quantified in tissue homogenates as indicated in MATERIALS AND METHODS. Tissue glycogen is given as millimoles glucose produced by hydrolysis of glycogen/g of tissue. Data are given as means ± SE of 2–3 individual determinations performed per animal. B: blood glucose was measured by Glucomatic Esprit apparatus (Bayer). Data correspond to means ± SE of measurements done respectively on 25–40 untreated, 10 clenbuterol-treated, and 10 propranolol-treated 2- to 6-mo-old animals of both species. C: food consumption was monitored as described in MATERIALS AND METHODS. Quantity of daily ingested food was expressed as % of the animal’s body mass. Data shown are means ± SE of measurements done on 6 animals per group. Statistical differences between F28 and control mice are indicated by * (not significant), *, and **, as described in MATERIALS AND METHODS.](/UserImages/343/9540449/1422002/4042002.png)
muscles did not change significantly upon treatment of the animals with propranolol.

Effect of clenbuterol on liver glycogen and glycemia in F28 and Co mice. In vitro experiments on membrane preparations (Fig. 1D) or on intact liver lobes (1) showed that β-adrenergic receptor agonists can induce strong adenylyl cyclase responses in F28nt livers and small adenylyl cyclase responses in Cont livers, despite the low β-AR density. We examined the extent to which oral administration of the β2-AR agonist clenbuterol affected glycogen storage and blood glucose in Co and F28 mice (Coclenb, F28clenb).

At 8:00 AM, glycogen deposition was observed in most Coclenb hepatocytes, whereas F28clenb livers were severely glycogen depleted (Fig. 3, C and D). Compared with Cont, total glycogen levels were decreased by 40% in Coclenb and 90% in F28clenb (81% vs. F28nt; Fig. 2A). Glycogen levels measured at 8:00 AM in Coclenb resembled those measured in F28nt mice (Fig. 2A). Also, at 5:00 PM, liver glycogen in Coclenb and F28clenb differed considerably: depletion of glycogen stores in the liver was only partial for Coclenb (Fig. 3I) but nearly total for F28clenb (Fig. 3J).

Mice consumed less food when treated with clenbuterol, yet differences of the clenbuterol effects in F28clenb compared with Coclenb mice were most likely not due to differences in food consumption since amounts of ingested food vs. body mass remained similar for both animal groups (Fig. 2C).

Clenbuterol had no effect on blood glucose levels. At 8:00 AM, no statistical difference was observed in the level of blood glucose concentrations in F28Clenb and Coclenb compared with F28nt and Cont, respectively (Fig. 2B).

Expression of GK, β-PK, G-6-Pase, and PEPCK. After demonstrating that hepatic β2-AR overexpression considerably perturbs the hepatic glycogen storage/degradation, we examined whether this affects the expression of the GK, β-PK, G-6-Pase, and PEPCK genes (Fig. 4, A–D). Levels of β-PK, G-6-Pase, and PEPCK mRNA in Coent mice were the lowest at the beginning of the postprandial period (8:00 AM) and increased significantly (P < 0.02) during this period (Fig. 4, B–D). Levels of GK mRNA did not change significantly during the postprandial period (P > 0.05; Fig. 4A). At 8:00 AM, there was no statistical difference between GK mRNA levels in Cont and F28nt mice (Fig. 4A), whereas β-PK and especially G-6-Pase and PEPCK mRNA levels were higher in F28nt than in Cont (Fig. 4, C and D). At 5:00 PM, however, β-PK and G-6-Pase mRNA levels (Fig. 4, B and C) were lower in F28nt than in Cont, whereas GK and PEPCK mRNA levels (Fig. 4, A and D) were similar in both animals.

The modulation of β-PK and GK gene transcription is an immediate index of the modulation of the glycolytic rate. The expression of both genes can be acutely regulated by 24-h fasting and refeeding with high-carbohydrate diet (6). To further examine the extent to which hepatic β2-AR overexpression affects the expression of both enzymes, we monitored β-PK and GK gene expression and blood glucose levels in Co and F28 mice in both fasting and refeeding conditions. In both groups of mice, GK and β-PK mRNA levels were low after a 24-h fast and markedly increased upon refeeding with a high-carbohydrate diet (Fig. 5, A and B). However, GK mRNA levels were significantly higher, and β-PK mRNA levels significantly lower, in refed F28 mice compared with Co. Blood levels of E719
glucose levels decreased to similar levels in F28 and Co mice upon fasting (Fig. 5C). Upon refeeding, blood glucose levels increased over Co and F28nt levels (Fig. 2B) but were significantly lower in F28 than in Co mice (Fig. 5C).

**DISCUSSION**

We previously described a transgenic mouse line, F28, that carries the human β2-AR gene under the control of its natural promoter (2). Unlike wild-type Co mice, hepatic β2-AR levels in these mice increase after birth. At young adulthood, these mice have 10-fold more functional β2-ARs in their livers than wild-type Co mice. With this high β2-AR expression, F28 mice have β2-AR levels comparable to those reported in the human liver (8, 29). As in humans, they maintain this elevated level of β2-AR expression through adulthood and display strongly enhanced hepatic adenylyl cyclase responses to β2-AR agonists (Fig. 1). Their basal hepatic adenylyl cyclase activity is also higher than in Co mice, probably due to an increased number of constitutively active β2-ARs (1).

A substantial body of data (reviewed in Ref. 20) indicates that the liver plays a central role in the control of food intake, metabolism, and body weight. Under normal physiological conditions in human and many animal species, the hepatic β2-AR/adenylyl cyclase system is a major modulator of many metabolic processes (20, 23, 26, 28, 33). In an earlier study (1), we demonstrated the potential of the F28 mouse model for studying the physiopathological importance of the hepatic β2-AR system and found that F28 mice were naturally protected against lethal and fulminant Fas-dependent liver apoptosis, probably due to increased basal hepatic β2-AR activity. In the present study, we focused on the metabolic changes induced by increased β2-AR expression in the F28 mouse liver and examined the effects of β-adrenergic ligands on the liver’s metabolism.

In the human liver, glycogenolysis occurs primarily via a β2-AR/cAMP-dependent mechanism (26, 28), whereas, in the adult rat liver, hepatic glycogenolysis is mediated by a cAMP-independent mechanism involving α1-ARs (16, 28). Our study demonstrates that, in F28 mice, the hepatic β2-AR system is also a major modulator of liver glycogen accumulation and/or degradation. Under normal physiological conditions, we found that the F28 mouse liver contains about one-half the glycogen of the normal mouse liver (Figs. 2A and 3). These deficiencies did not appear to be due to a different nutritional behavior, as food consumption was similar for both mouse lines (Fig. 2C). Differences appear to

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**Fig. 4.** Expression of glucokinase (GK), l-pyruvate kinase (l-PK), glucose-6-phosphatase (G-6-Pase), and phosphoenolpyruvate carboxykinase (PEPCK) in livers of untreated Co and F28 mice. Total mRNA extracts were prepared from livers of 8-wk-old Co and F28 mice taken at 8:00 AM and 5:00 PM. The mRNA specific for glycolytic enzymes GK (A) and l-PK (B) and gluconeogenic enzymes G6Pase (C) and PEPCK (D) were quantified by Northern blot analysis, as described in MATERIALS AND METHODS. Data given are means ± SE of results obtained from 6 animals per group. Significance of difference between F28 and Co mice is indicated by ns, *, or **, as described in MATERIALS AND METHODS.

**Fig. 5.** A and B: GK and l-PK gene expression in livers of fasted and refed Co and F28 mice. Co and F28 mice were divided into 2 groups. 1 group (fasted; n = 4) was fasted for 24 h, the other group (refed; n = 4) was fasted for 24 h and then refed for 18 h with a high-carbohydrate diet. Livers were thereafter dissected out, and total RNA was extracted and analyzed for GK (A) and l-PK (B) gene expression by real-time quantitative RT-PCR, as described in MATERIALS AND METHODS. Results were normalized to cyclophilin mRNA values. C: blood glucose levels in fasted and refed Co and F28 mice. Measurements were done by Glucomatic Esprit apparatus (Bayer). Results are presented as means ± SE; n = 4/group. Significance of difference between the values obtained for F28 and Co mice is indicated by ns, *, or **, as described in MATERIALS AND METHODS.
be due neither to the higher β2-AR levels nor to an increased glucose uptake in the muscles of F28 mice (2); indeed, glycogen levels in the muscles of control mice did not differ significantly from those in F28 mice and were not altered upon treatment of the animals with propranolol. It can, however, reasonably be hypothesized that decreased glycogen accumulation and/or increased glycogen degradation is due to the approximately twofold increased hepatic basal β2-AR activity in F28 mouse liver (1). This is supported by the fact that differences in liver glycogen levels were almost totally abolished upon pretreatment with the β-AR antagonist propranolol. Perhaps one of the most interesting features of the F28 hepatic β2-AR system is that the effect of the β2-AR agonist clenbuterol on liver glycogen is strongly increased: compared with untreated animals, liver glycogen is moderately decreased in C0−/− mice but severely depleted in F28+/− mice (Figs. 2 and 3).

Regulation of cAMP levels by glucagon, insulin, and catecholamines accounts in large part for minute-to-minute hormonal control of pathway flux in fed animals and during the transition from fed to starved. cAMP plays a key role in the regulation of gene transcription of gluconeogenic and lipolytic enzymes: it enhances the transcription of genes encoding hepatic enzymes, such as PEPCK, which in turn induces regulation of gene transcription of gluconeogenic and lipolytic genes. These regulations should lead to increased blood glucose levels, which were not observed in the F28 mice.

These apparently contradictory regulations might, however, reflect a series of compensatory adaptations that might take place in the F28 mice to maintain euglycemia. The interrelationship between the different mechanisms that are involved in vivo in glycogenolysis, gluconeogenesis, and blood glucose homeostasis is extremely complex, and the precise modulatory role of the F28 hepatic β2-AR system remains to be clarified.

In conclusion, F28 mice maintain, as do humans, an elevated level of β2-AR expression in their livers in adulthood and display strongly enhanced adenylyl cyclase responses to β-AR agonists. This study showed that, under normal physiological conditions, the increased β2-AR activity alters the expression of the gluconeogenic and glycolytic key enzymes phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and triokinase and decreases hepatic glycogen levels and blood glucose levels. Moreover, the effects of β-adrenergic ligands on liver glycogen in humans were reproduced in these mice: liver glycogen levels were strongly decreased by the β2-AR agonist clenbuterol and increased by the β-AR antagonist propranolol. Because of these physiological characteristics, these transgenic mice offer new possibilities for studying in vivo the hepatic β2-AR system physiopathology and for testing the effects of β-AR ligands on liver metabolism.

ACKNOWLEDGMENTS

We thank Drs. Pascale Briand and Tarik Issad (Cochin Institute, Paris) for helpful discussions, Drs. Diane Currier and Mark D. Underwood (Columbia University, New York State Psychiatric Institute, New York) for revising the manuscript, and Fadila Benhamed for performing the real-time RT-PCR analysis.

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GRANTS

This work was supported by grants from the Association de Recherche contre le Cancer and the Ligue Nationale contre le Cancer.

REFERENCES


3. Arinze IJ and Kawai Y. Adrenergic regulation of glycogenolysis in isolated guinea-pig hepatocytes: evidence that beta2-receptors mediate increases in the expression of hepatic GK lead to a decreased blood glucose level (24). In this context, it is worth noting that the blood glucose levels measured in the F28 mice closely resemble those observed in transgenic mice that overexpress GK (24).

The decreased L-PK (Fig. 5) gene transcription in the liver of the F28 mice compared with Co mice might be caused by the increased hepatic basal adenylyl cyclase activity. cAMP is known to negatively regulate the L-PK gene transcription (5, 14). The decreased L-PK gene transcription is consistent with a decreased glycolytic flux and fits the strongly increased expression of the gluconeogenic enzyme PEPCK (Fig. 4). These regulations should lead to increased blood glucose levels, which were not observed in the F28 mice.

In conclusion, F28 mice maintain, as do humans, an elevated level of β2-AR expression in their livers in adulthood and display strongly enhanced adenylyl cyclase responses to β-AR agonists. This study showed that, under normal physiological conditions, the increased β2-AR activity alters the expression of the gluconeogenic and glycolytic key enzymes phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and triokinase and decreases hepatic glycogen levels and blood glucose levels. Moreover, the effects of β-adrenergic ligands on liver glycogen in humans were reproduced in these mice: liver glycogen levels were strongly decreased by the β2-AR agonist clenbuterol and increased by the β-AR antagonist propranolol. Because of these physiological characteristics, these transgenic mice offer new possibilities for studying in vivo the hepatic β2-AR system physiopathology and for testing the effects of β-AR ligands on liver metabolism.


