The Lethal Form of Cushing’s in 7B2 Null Mice Is Caused by Multiple Metabolic and Hormonal Abnormalities

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The neuroendocrine-specific protein 7B2, which serves as a molecular escort for proPC2 in the secretory pathway, promotes the production of enzymatically active PC2 and may have non-PC2 related endocrine roles. Mice null for 7B2 exhibit a lethal phenotype with a complex Cushing's-like pathology, which develops from intermediate lobe ACTH hypersecretion as a consequence of interruption of PC2-mediated peptide processing as well as undefined consequences of the loss of 7B2. In this study we investigated the endocrine and metabolic alterations of 7B2 null mice from pathological and biochemical points of view. Our results show that 7B2 nulls exhibit a multisystem disorder that includes severe pathoanatomical and histopathologic alterations of vital organs, including the heart and spleen but most notably the liver, in which massive steatosis and necrosis are observed. Metabolic derangements in glucose metabolism result in glycogen and fat deposition in liver under conditions of chronic hyperglycemia. Liver failure is also likely to contribute to abnormalities in blood coagulation and blood chemistry, such as lactate acidosis. A hypoglycemic crisis coupled with respiratory distress and intensive internal thrombosis most likely results in rapid deterioration and death of the 7B2 null. (Endocrinology 143: 2324–2332, 2002)

The neuroendocrine protein 7B2 has a critical role in the proteolytic conversion and activation of proPC2, the enzyme responsible for the proteolytic conversion of many peptide hormone precursors (1, 2). The 7B2 protein acts as an intracellular binding protein for proPC2, facilitates its maturation, and is required for its enzymatic activity. Processing of many important peptide precursors does not occur in 7B2 nulls. This interaction of 7B2 with proPC2 is most likely the main function of 7B2; however, other roles of the 7B2 molecule have also been proposed (3–5). In humans the 7B2 gene is located near the Prader-Labhart Willi syndrome region on chromosome 15 Patients with this hereditary syndrome exhibit no PC2 immunoactivity in supraoptical and paraventricular nuclei, and prohormone convertase 1 activity is slightly diminished; although the possible involvement of 7B2 in Prader-Willi syndrome has been postulated (6), it is not likely to represent the primary cause of the disease.

7B2 null mice exhibit a very complex pathology that in many respects resembles Cushing’s disease; this gene deletion represents a lethal phenotype in 129/Sv mice (7). 7B2 nulls exhibit severe pathologies of liver, pancreas, and pituitary and die in the prepubertal or pubertal ages. The Cushing’s-like disease developed by 7B2 null mice takes the form of pituitary-dependent hyperadrenocorticotosteronism, severe hypoglycemia, hyperproinsulinemia, adrenal hyper trophy, pituitary hypothyroidism, and altered islet cell morphology (7). Unlike classical Cushing’s disease, which results from excess ACTH secretion from pituitary adenomas, 7B2 null mice develop this disease from intermediate lobe ACTH hypersecretion. These mice resemble mice deficient in the dopamine receptor 2, which also develop a specific form of Cushing’s-like disease; both kinds of null mice suffer from adrenal hypersecretion of corticosterone because of excess circulating ACTH (8, 9).

The diagnosis of Cushing’s disease seems to be insufficient to account for the sudden death of 7B2 nulls within a specific period of life, the prepubertal to pubertal phase (4–6 wk old). Because they are devoid of PC2 activity, 7B2 null mice exhibit deficiencies in the processing of proglucagon and to a lesser extent proinsulin (7). This lack of PC2-mediated peptide processing is not a symptom of typical Cushing’s disease and most likely accounts for many of the differences of this null model with Cushing’s. However, certain clinical symptoms of 7B2 null mice are consistent with those of Cushing’s, such as abnormal fat disposition, especially on the back and around the neck. Other clinical symptoms that are consistent with severe Cushing’s include changes in skin; compared with wild-type (WT) mice, 7B2 null mice exhibit marked thinning of the skin and dermal atrophy with hyperkeratosis. Livers of 7B2 nulls were previously found to be markedly abnormal, with severe fat vacuolization. However, all of these clinical signs are not sufficient to explain the sudden expiration of 7B2 null mice, in which rapid deterioration and death occur within a 6-h time frame. In this report we have further investigated the pathological changes occurring the 7B2 null, with emphasis on the actual cause of death.

Materials and Methods

Animals

129/Sv 7B2 null and WT mice were derived from crosses of heterozygous 7B2 parents. The genotype of the animals was determined by PCR methodology. All animals had free access to food and water, a constant photoperiod of a 12-h dark/12-h light cycle, constant housing temperature, and constant humidity. All mice were housed and fed identically. Animal care and use was approved by the institutional Division of Animal Care, Louisiana State University Health Sciences

Abbreviations: PC2, Prohormone convertase 2; WT, wild-type.
Center (New Orleans, LA). We investigated the deaths of 19 7B2 null mice and the same number of 7B2 WT animals.

When used, anesthesia using Avertin produced better results in terms of recovery and was better tolerated than ketamine-xylazine anesthesia. Animals were anesthetized ip with Avertin (administered at a 2.5% solution in 0.9% NaCl; 0.015–0.020 ml/g mouse body weight) before venopuncture from the retroorbital plexus or heart. Avertin in the dose mentioned above produced short-term anesthesia without serious side effects (such as convulsions and respiratory failure) and yielded rapid recovery.

**Necropsy**

All animals in these experiments were subjected to necropsy, performed either immediately after death or within a short period after death (2–3 h), maintaining carcasses at 4–8 C. Samples for biochemical investigations were collected and frozen at −20 C, and samples for histopathologic evaluation were placed in tubes containing zinc formaldehyde. Representative sections of organs and grossly identifiable lesions were processed, dehydrated, blocked in formalin, and then stained with hematoxylin and eosin for microscopic analysis.

**Biochemical and clinical chemistry analysis**

**Blood glucose.** Every second day blood glucose was measured using a standard glucometer (Lifescan One Touch, Milpitas, CA). Blood was obtained either by lateral tail venopuncture or cardiac or retroorbital plexus venopuncture. Plasma glucose was also measured using a kit for glucose (Sigma, St. Louis, MO) for comparison with data obtained with a Lifescan glucometer. We did not detect significant differences in glucose levels between the two methods.

**Analysis of tissue free glucose and glycogen**

Free tissue glucose was measured by a quantitative, enzymatic method for glucose determination (10). After homogenization of previously weighed tissue in 1 n acetic acid and centrifugation at 10,000 rpm, the supernatants were deproteinized using 0.3 n barium hydroxide and zinc sulfate. After centrifugation the clear supernatant was used for quantitative enzymatic glucose determination using a kit (Sigma). The reaction is based on the coupled enzymatic reactions of glucose oxidase and peroxidase followed by reaction with the chromogenic oxygen acceptor 3,3′-dianisidine. The intensity of the brown color was measured at 475 nm and is proportional to the original glucose concentration. Plasma for glucose analysis was prepared immediately after venopuncture (subanesthesia using Avertin) from the retroorbital plexus for 7B2 nulls (in light of their very bad health) and via cardiac puncture for 7B2 WT animals.

For glycogen analysis (11), fresh wet liver and muscle tissues were measured and minced in a Petri dish with a small amount of PBS solution and placed in an tube (Eppendorf, Brinkmann Instruments, Westbury, NY). Homogenization with sonication was performed in 500 μl ice-cold 1 n acetic acid, and samples were then boiled in a water bath for 45 min for digestion of glycogen. After boiling, samples were placed on ice and centrifuged at 10,000 rpm 4 C/10 min. Supernatants were collected in tubes containing zinc formaldehyde. After boiling, samples were placed on ice and then stained with hematoxylin and eosin for microscopic analysis.

**Metyrapone and glucagon treatment**

Metyrapone (2-methyl-1, 2-di-3-pyridyl-1 propane, obtained from Aldrich Chemical Co., Inc., Milwaukee, WI) was dissolved in 0.5% methylcellulose (Sigma) and force fed to mice at 1 mg/10 g body weight twice per day (12). Groups treated with metyrapone alone as a control received 0.9% saline solution intramuscularly. Groups treated with metyrapone and glucagon (50 μl of 0.05 mg/ml, Elli Lilly and Co., Indianapolis, IN) received drugs im. Animals were fed twice per day at the same time.

**RIA of corticosterone and ACTH**

Plasma was prepared from blood obtained through venopuncture. Plasma was collected in tubes (Eppendorf) and stored at −70 C until the assay procedure. The ImmuneChem 125I Corticosterone RIA (ICN Biomedicals, Inc., Costa Mesa, CA), specifically designed for use in laboratory mice and rats, was used to measure plasma corticosterone. For measuring plasma ACTH, we used the ACTH RIA kit (human ACTH 1–39 assay kit, Nichols, San Juan Capistrano, CA). This assay is specific for intact ACTH 1–39.

**Lactate and magnesium levels in plasma and tissues**

The level of plasma lactate and magnesium in plasma and tissues was determined using Sigma kits. Lactate was measured using an enzymatic method and the absorbance of the colored product was measured at 540 nm. Magnesium was measured using a quantitative, colorimetric method, and the absorbance of the product, a pink magnesium-calmagite complex, was measured at 520 nm.

**Results**

**7B2 null mice exhibit severe cardiorespiratory failure, convulsions, and hypothermia just before death**

7B2 null animals were closely observed during the 6 h before death. One day before death, the animals exhibited respiratory insufficiency, with a very short and intensive breathing pattern. Occasionally animals separated themselves from others in the cage but continued to eat. Two to three hours before death, 7B2 null mice exhibited severe dyspnea, cardiorespiratory failure, and convulsions; hypothermia then appeared, which was followed by coma. In the moment of death, mice occasionally exhibited low blood glucose levels of around 16 mg/dl (four of six cases measured just at death). Just before death, strong convulsions were followed by periods of short and difficult breathing. During this period, animals constantly assumed a dorsal side position, unusual to rodents. In summary, observation of 7B2 nulls during their terminal period shows that they exhibit rapid deterioration within a 6-h period, followed by sudden death.

**Gross pathology of 7B2 null mice at necropsy**

7B2 nulls exhibited a typical Cushingoid phenotype, with small muscle mass, and typical alterations of the skin with hyperemia and dermal atrophy. The fur was significantly thinner, with regional alopecia and a specific buffalo hump in the dorsal cervical region.

Features of the gross pathology of the 7B2 null mouse are shown in Fig. 1. Significant hepatomegaly was observed (Fig. 1B; compare with control in Fig. 1A), and the liver was yellow, indicating macroscopic signs of fatty change. The total mass of wet liver in 7B2 null mice was 1.1 ± 0.08 g, n = 8, compared with WT mice, which had a weight of 0.41 ± 0.01 g (n = 7; P < 0.001, compared with controls). The hepatomegaly occasionally produced strong tension on the diaphragm. Perforations of the gallbladder (Fig. 1C), hemothorax (Fig. 1C), and/or intraabdominal hematomas were often present. The spleens were decreased in size and retroperitoneal fat deposits were increased. The total mass of spleen in 7B2 null mice was 14.1 ± 0.4 mg wet weight, compared with the mass detected in 7B2 WT mice, 68.1 ± 2.6 mg (P < 0.001). Polyphagia in 7B2 nulls was recognizable by the frequent presence of a greatly distended stomach, always full of food. Significant lymphoid atrophy was present. Cardiomegaly (141 ± 13 mg wet weight in 7B2 nulls, compared with 52 ± 4 mg in WT mice; P < 0.001) was present with
pigmentation on the ventral side of the heart, and significant myocardial calcification was detectable on palpation. Cryptorchidism was often detected in males. We observed hypotrophic alterations in the anterior pituitaries in all null mice. In contrast, the adrenal was hypertrophic, sometimes presenting an intensive red color.

Urine of dying 7B2 nulls collected during necropsy was always ketonegative.

Histopathologic (microscopic) findings in 7B2 null mice at necropsy

The most significant organ microscopic findings were observed in the liver. All mice had microvesicular and macrovesicular steatosis (Fig. 2A) in a perivenular (acinar zone 2 and 3) or panacinar pattern. Hepatic necrosis was also present and ranged from focal to widespread in a midzonal, periportal, or combined pattern (Fig. 2B). For comparison, normal liver histology (WT mouse) is shown in Fig. 2C. In one instance, massive necrosis was observed. Pulmonary edema and congestion were present and most likely represented an agonal event. The gross observations of myocardial calcifications were confirmed by the presence of dystrophic calcification in the myocardium by microscopy.

Serological profile of 7B2 nulls shows no evidence of infection

Because of chronically high levels of circulating corticosterone and significant spleen hypotrophy, 7B2 nulls are probably immunosuppressed. The 7B2 nulls were tested for serological titers of mouse hepatitis virus, Sendai virus, mouse Pneumonia virus, Reovirus type 3, Theiler’s murine encephalomyelitis, Ectromelia, Mycoplasma pulmonis, Parvovirus, epizootic diarrhea of infant mice, and lymphocytic choriomeningitis. However, the basic serology profile in 7B2 nulls suggests that animals are not infected with these pathogens (data not shown).

129/Sv 7B2 null mice are severely hypoglycemic at the time of death

Blood glucose, measured in blood obtained by lateral tail venopuncture, was less than 20 mg/dl several hours before death (Fig. 3A). The normal level of blood glucose in 7B2 nulls is approximately 75 mg/dl (7). One to 2 d before death, the level of glucose significantly decreased; at this time the animals also appeared to become hypothermic.

Liver glycogen levels are increased in 7B2 nulls

The distribution of free glucose in tissues of 7B2 WT and null mice was significantly different between the two genotypes (Fig. 3B). 7B2 nulls exhibited a high level of free tissue glucose in the liver. Because circulating glucose is low, this free glucose is apparently unable to be released into the peripheral circulation. However, 7B2 null brains had a higher level of free tissue glucose, compared with WT animals. Muscle tissues (musculus gastrocnemius and musculus soleus) of 7B2 nulls showed a significantly decreased level of free

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**Fig. 1. Necropsy of 7B2 nulls reveals many severe pathological alterations. A, Necropsy of a 7B2 WT mouse; B, a typical example of hepatomegaly, with significant fat deposition in the retroperitoneal space and back in 7B2 nulls. On the left side near the thorax, a huge hematoma, which produces pressure on the thorax and reduced lung volume, is visible. C, Intraabdominal bleeding with a significant amount of fresh blood, necrosis in gastrointestinal tract, perforation of gallbladder, and alterations in chest architecture. All animals were subjected to necropsy performed either immediately after death or within several hours after death; carcasses were maintained at 4–8 °C.**
FIG. 2. Histopathologic findings in 7B2 nulls. A, Liver sections from a 7B2 null mouse demonstrating microvesicular steatosis in hepatocytes. The black arrow shows hepatocytes with steatosis and (B) extensive midzonal necrosis (necrosis is outlined by arrows). P, Hepatic portal triad; CV, hepatic central vein. C, a liver section from 7B2 WT mouse.

FIG. 3. 7B2 null mice die with severe hypoglycemia as a consequence of significant changes in glucose metabolism. A, Blood glucose measured after lateral tail venopuncture. The levels of blood glucose were followed in six 7B2 null mice from 3 wk of age until death. In four out of six animals, we measured blood glucose immediately before death. B, Levels of plasma glucose in 7B2 WT and null mice and free tissue glucose measured in wet liver, brain, kidney, and muscle. Results of glucose in plasma are in mg/dl plasma; for tissue glucose, results are represented in grams of glucose per gram of wet tissue. Six animals of both genotypes (WT and null) were used in these experiments. ***, Significant difference ($P < 0.0001$). C, Distribution of glycogen in liver and muscle tissue in 7B2 nulls and WT mice. Glycogen measured as glucose residues (g) using an enzymatic method after digestion of glycogen molecules in g wet liver tissue and muscle tissue in 7B2 WT and 7B2 null mice. ***, Significant difference ($P < 0.0001$ in 7B2 WT vs. levels detected in 7B2 WT mice). Each bar represents the mean ± SD of 14 animals for liver in WT mice, 13 animals for liver in nulls, 7 animals for muscle glycogen in WT mice, and 8 animals in muscle tissue of null mice.
tissue glucose, compared with the levels detected in 7B2 WT controls.

7B2 null mice exhibited levels of liver glycogen five times higher than those found in the livers of WT mice (Fig. 3C). Significant differences between the sexes of 7B2 null mice in the concentration of liver glycogen were detected (P < 0.05; data not shown). 7B2 males had less liver glycogen than 7B2 females of both genotypes, both in WT and null (P < 0.05).

**Blood glucose is elevated in the 7B2 null after treatment with metyrapone**

Treatment of 7B2 WT animals with the steroid synthesis inhibitor metyrapone (at a concentration of 1 mg/10 g of body weight twice per day) resulted in a significant increase in the level of blood glucose (Fig. 4A) (P < 0.001 vs. control group treated with vehicle). Metyrapone treatment was carried out for 21 d; no significant change was detected between 5 d and 21 d of treatment.

Different effects were obtained using metyrapone in the same dose and time of treatment in 7B2 null mice. The group treated with metyrapone exhibited no significant changes in the level of blood glucose. However, a group of six 7B2 null mice treated with combination of metyrapone and glucagon (Fig. 4B) exhibited a significant increase in blood glucose. During the first 3 d of treatment with metyrapone, animals in the metyrapone group and the group with the combination of metyrapone and glucagon were tested for reactions to glucagon administered intramuscularly. Both groups showed significant changes in level of blood glucose after intramuscular administrations of glucagon, compared with the untreated group and the group of mice treated with methylcellulose. After the third day, one group was continued on treatment with metyrapone alone, and another group was continued on a combination of metyrapone with glucagon. Only the group treated with the combination of metyrapone and glucagon exhibited a significant increase in blood glucose, compared with untreated animals and animals treated with the metyrapone vehicle, methylcellulose. The level of glucose was maintained at about 120 mg/dl until the end of treatment. Changes in behavior such as increased activity and increased aggressiveness during handling were noticeable during the first 10 d of treatment, particularly in the group treated with the combination of metyrapone and glucagon. However, this change was transient, and treated animals in both groups died in the same critical period, between 4 and 5 wk old.

**Hypercorticosteronism can be rescued by metyrapone treatment in 7B2 null mice**

Figure 5A shows results of the corticosterone RIA in 7B2 null mice, supporting previous results (7) that indicate that 7B2 nulls exhibit much higher levels of corticosterone, compared with WT controls. Treatment of 7B2 WT mice with metyrapone produced a significant decrease of plasma level of corticosterone vs. the group of 7B2 WT animals treated with vehicle. A significant decrease in plasma corticosterone was detected, from 150 ng/ml plasma corticosterone in the control group to less than 50 ng/ml of plasma corticosterone in a group treated with metyrapone (Fig. 5B). 7B2 nulls treated with metyrapone alone or metyrapone in combination of glucagon exhibited a significant decrease (P < 0.0001) of circulating corticosterone (less than 100 ng/ml), compared with animals in the group treated with methylcellulose (circulating corticosterone < 500 ng/ml) (Fig. 5C).

Treatment of 7B2 WT mice with metyrapone induced a small increase in plasma ACTH, compared with both control groups (Fig. 5D). The plasma ACTH of 7B2 nulls treated with metyrapone was not significantly different vs. both control groups (Fig. 5E).
7B2 nulls exhibit severe lactic acidosis and hypomagnesia

The level of lactate in the plasma of 7B2 null mice was five to six times higher than in WT mice (Fig. 6A). No significant differences were observed between the sexes, but significant differences were noted between genotypes (P < 0.0001).

The level of magnesium in the plasma and the heart of 7B2 nulls was significantly reduced (P < 0.0001) vs. the level detected in 7B2 WT animals (Fig. 6B). However, the levels of magnesium in the livers of 7B2 null mice were significantly increased 2-fold, compared with 7B2 WT mice. A similarly increased level of magnesium was also detected in the brains of 7B2 null mice. Muscle tissue showed no significant differences between WT and null genotypes.
The neuroendocrine protein 7B2 is a highly conserved secretory protein present in all prohormone-producing cells; the presence of this protein is required for the proper activation of the convertase PC2. Defects in the expression or activity of PC2 in the neuroendocrine system and brain could be expected to produce complex disturbances of endocrine functions, alterations in main metabolic processes, and changes in behavior. Although many pathological features of the 7B2 null can be ascribed to the presence of chronically increased levels of corticosterone because of excess adrenal stimulation arising from aberrant POMC processing, the pathophysiological progression leading to sudden death between 4 and 6 wk of age has not been totally clarified. Because the 7B2 null may mirror human diseases involving point mutations of PC2 or 7B2, we further investigated the cause of death of this interesting null mouse.

**Discussion**

The neuroendocrine protein 7B2 is a highly conserved secretory protein present in all prohormone-producing cells; the presence of this protein is required for the proper activation of the convertase PC2. Defects in the expression or activity of PC2 in the neuroendocrine system and brain could be expected to produce complex disturbances of endocrine functions, alterations in main metabolic processes, and changes in behavior. Although many pathological features of the 7B2 null can be ascribed to the presence of chronically increased levels of corticosterone because of excess adrenal stimulation arising from aberrant POMC processing, the pathophysiological progression leading to sudden death between 4 and 6 wk of age has not been totally clarified. Because the 7B2 null may mirror human diseases involving point mutations of PC2 or 7B2, we further investigated the cause of death of this interesting null mouse.

**Interpretation of necropsy findings**

During necropsy of 19 7B2 null animals, all animals exhibited certain typical pathological alterations present in Cushing’s disease as well as several pathologies not typical of that disease. Severe fatty and necrotic changes in the livers as well as hepatomegaly and intensive bleeding in the thoracic and abdominal cavities were always observed.

The patterns of steatosis were not specific but resembled those seen in acute fatty liver of pregnancy (acinar zones 2 and 3), Reye’s syndrome (panacinar), and Cushing’s syndrome (13) in humans. The hepatic findings in these mice also indicate acute liver failure and the observations of apparent bleeding diathesis most likely result from a decrease in liver-dependent coagulation factor synthesis, a common, and one of the first, clinical manifestations of liver failure. Another possible contributing factor to the observed hemorrhage could be vascular fragility, a typical manifestation of Cushing’s disease. These processes, in concert, could explain the systemic hemorrhage seen in the 7B2 null mice at necropsy. Intraabdominal bleeding has been frequently noted in 7B2 null newborns (7 and unpublished results).

Glucose and fat metabolism are altered in 7B2 nulls

Severe hypoglycemia is a common metabolic abnormality seen in human and veterinary medicine. A life-threatening condition, hypoglycemia is most often easily diagnosed and rapidly treated with a satisfactory outcome. If not recognized and promptly treated, hypoglycemia may cause irreversible central nervous system injury and, rarely, results in death (14). During their first 3 wk, 7B2 nulls exhibit moderate hypoglycemia with daytime blood glucose levels of 75 mg/dl. This phenomenon is likely to be caused by the lack of circulating glucagon. Approximately 1–2 d before death, blood glucose rapidly decreases to 20–25 mg/dl. This severe hypoglycemic condition can occasionally be more even drastic immediately before death at 16 mg/dl (in four cases). Superimposed on a preexisting chronic hypoglycemic condition complicated by acute liver failure with associated bleeding diathesis and metabolic derangement, severe hypoglycemia most likely represents the precipitating cause of death in 7B2 nulls.

Liver glycogen measurements support the hypothesis that glucose is immobilized in 7B2 nulls. Indeed, the increase in liver glycogen suggests that 7B2 nulls exhibit a unique form of glycogen storage-like disease. Glycogen storage disease type 1a, caused by a deficiency in glucose phosphatase, is characterized by severe hypoglycemia, liver enlargement, growth retardation, hyperlipidemia, and hyperuricemia (15). The 7B2 null mice have a relatively similar clinical picture. It is interesting to note that male double nulls for PC2 and 7B2 do not exhibit a lethal form of Cushing’s (Laurent, V., and I. Lindberg, unpublished data) and also do not exhibit high levels of liver glycogen (0.072 g/g vs. 0.120 g/g for 7B2 nulls). Thus, high levels of liver glycogen are indicative of a metabolic abnormality associated with the disease process.

In the 7B2 null, glucose is apparently not able to diffuse from the tissues into the circulation. Abnormal distribution of free tissue glucose was especially prominent in the liver of 7B2 nulls, in which the free glucose level was four times higher than the level detected in 7B2 WT animals. The liver and brain represent two areas of aberration in glucose metabolism in the 7B2 null. In accordance with our observations...
of severe hypoglycemia, the liver and brains of 7B2 nulls contain a high concentration of free tissue glucose and glucose as glycogen. There are two explanations for this, a defect in the cleavage of glucogen molecules into molecules of glucose or a defect in the release of glucose from the liver into the circulation. 7B2 nulls do not produce substantial levels of mature glucagon, the hormone responsible for release of glucose from the liver into the circulation (7). However, the PC2 null, which also lacks glucagon, exhibits no comparable alterations in glucose metabolism (16). Correct levels of circulating glucagon, balanced with proper levels of insulin, are required to maintain normal blood glucose levels (16). The glycogen storage-like disease present in 7B2 null mice could represent a consequence of the effects of chronically elevated corticosterone on glucose metabolism combined with an increase in circulating proinsulin forms and the absence of mature glucagon in 7B2 nulls.

Pituitaries of PC2 null mice contain high levels of intact ACTH 1–39, but the PC2 null does not exhibit high levels of corticosterone, most likely because of the lack of highly increased circulating ACTH in this null model; the increased ACTH in the pituitary is apparently not subject to release in the PC2 null (16a). Excluding slight hypoglycemia, PC2 nulls do not exhibit Cushingoid pathologies similar to the 7B2 nulls and like 7B2 nulls, PC2 nulls also lack active glucagon and exhibit increased proinsulin forms. A distinguishing feature between these two null models is that the PC2 nulls exhibit normal expression of the 7B2 protein, distributed in organs and in circulation; the fact that the PC2 nulls show no signs of illness points to additional important roles for the 7B2 protein unrelated to PC2-mediated effects.

Corticosterone normally plays an important counterregulatory role during hypoglycemia and augments glucose production, decreases glucose utilization, and accelerates lipolysis (17). Generally glucocorticoids counteract the effects of insulin and stimulate gluconeogenesis (18). Thus, chronically increased circulating corticosterone, in combination with effects on glucagon, would be expected to contribute to many of the pathological alterations in sugar and fat metabolism seen in the 7B2 null.

A major contributing cause of death in 7B2 nulls is likely to be the process of fat replacement in the liver. Hepatic steatosis, observed in our pathohistological analysis, is the end result of abnormal triglyceride synthesis and secretion. Our results are in agreement with other studies of the causes of sudden death resulting from hepatic steatosis with hypoglycemia, hypomagnesemia, and high serum levels of FFA (19).

Treatment of WT mice with metyrapone, a potent inhibitor of corticosterone synthesis, produced a significant increase in blood glucose. However, treatment of 7B2 nulls with metyrapone produced the opposite result. These differences between WT and null mice confirm our hypothesis as to significantly different patterns of glucose utilization in the 7B2 null. Glucagon treatment of 3-wk-old animals was very successful and increased blood glucose to above 100 mg/dl in many animals. However, animals from the metyrapone-treated group and in the group treated with the combination of metyrapone and glucagon died in the same time period, with the same clinical signs as untreated 7B2 nulls.

Hypomagnesemia

A high level of magnesium in the liver can indicate serious alterations in normal liver function. Hypomagnesemia in the plasma and heart tissue of 7B2 null mice suggests that a contributing cause of immediate death in 7B2 nulls might be hypomagnesemia. This pathological alteration is present in chronic liver and renal disease, heart failure, and coma and generates a life-threatening condition. The reason that 7B2 nulls exhibit lower magnesium levels is unclear but might be secondary to alterations in blood chemistry.

Lactic acidosis

Dying 7B2 nulls are in severe lactic acidosis, with levels of plasma lactate in dying 7B2 null mice about 6-fold higher than levels measured in 7B2 WT mice. Excessive concentrations of circulating lactate can produce severe tissue oxygen deprivation, tissue hypoxia, weakness, stupor, fatigue, and circulatory failure with coma (20, 21). Chronic lactic acidosis might be a factor in the process of organ necrosis (confirmed in our histopathologic observations), particularly in the liver. Blood lactate reflects both the production and metabolism of lactate. Normally, the liver is able to remove more lactate than is produced by the body, but the liver in 7B2 nulls is apparently not able to metabolize lactate. This observation supports our conclusion that the liver in 7B2 nulls is highly dysfunctional. Lactic acidosis is a highly critical condition, and, with hypoglycemia, potentially also represents a major cause of immediate death in 7B2 nulls.

In conclusion, 7B2 null mice exhibit a unique form of Cushing’s disease with many atypical symptoms, such as hypoglycemia. Cushing’s and non-Cushing’s symptoms in the 7B2 null join with other metabolic abnormalities to generate a multisystem disorder. The severe hypoglycemia caused by the lack of glucagon, increased proinsulin forms, and chronically elevated corticosterone, coupled with alterations in blood coagulation, all combine to result in multiple organ pathologies including liver failure and precipitate further abnormalities in blood coagulation and blood chemistry, such as lactic acidosis. A hypoglycemic crisis coupled with respiratory distress and intensive internal thrombosis then results in abrupt death.

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