Impaired Glucose Homeostasis in Mice Lacking the $\alpha_{1b}$-Adrenergic Receptor Subtype*

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To assess the role of the $\alpha_{1b}$-adrenergic receptor (AR) in glucose homeostasis, we investigated glucose metabolism in knockout mice deficient of this receptor subtype ($\alpha_{1b}$-AR$^{-/-}$). Mutant mice had normal blood glucose and insulin levels, but elevated leptin concentrations in the fed state. During the transition to fasting, glucose and insulin blood concentrations remained markedly elevated for at least 6 h and returned to control levels after 24 h whereas leptin levels remained high at all times. Hyperinsulinemia in the post-absorptive phase was normalized by atropine or methylatropine indicating an elevated parasympathetic activity on the pancreatic $\beta$ cells, which was associated with increased levels of hypothalamic NPY mRNA. Euglycemic clamps at both low and high insulin infusion rates revealed whole body insulin resistance with reduced muscle glycogen synthesis and impaired suppression of endogenous glucose production at the low insulin infusion rate. The liver glycogen stores were 2-fold higher in the fed state in the $\alpha_{1b}$-AR$^{-/-}$ compared with control mice, but were mobilized at the same rate during the fed to fast transition or following glucagon injections. Finally, high fat feeding for one month increased glucose intolerance and body weight in the $\alpha_{1b}$-AR$^{-/-}$, but not in control mice. Altogether, our results indicate that in the absence of the $\alpha_{1b}$-AR the expression of hypothalamic NPY and the parasympathetic nervous activity are both increased resulting in hyperinsulinemia and insulin resistance as well as favoring obesity and glucose intolerance development during high fat feeding.

The catecholamines noradrenaline and adrenaline bind to different adrenergic receptors (AR)† subtypes, which belong to the superfamily of the G protein-coupled receptors. Three $\beta$ ($\beta_1$, $\beta_2$, $\beta_3$), three $\alpha$ ($\alpha_1A$, $\alpha_2A$, $\alpha_2C$), and three $\alpha_1$ ($\alpha_1A$, $\alpha_1D$, $\alpha_1E$) AR subtypes have been characterized at the molecular level. The AR subtypes are widely expressed in different brain regions and peripheral organs (1, 2) where they contribute to mediate some important effects of catecholamines on cardiovascular and metabolic functions (3–8).

With regard to glucose metabolism, ARs are involved in the regulation of numerous functions such as insulin and glucagon secretion as well as hepatic glucose production and storage (6, 9). An injection of adrenaline mobilizes glucose from the liver by increasing glycogenolysis and glucoseogenesis (7). This effect is mediated by both the $\beta_2$-AR and $\alpha_1$-AR subtypes via the CAMP and calcium-dependent pathways, respectively, thus leading to hyperglycemia (10). In addition, catecholamines decrease insulin secretion by binding to the $\alpha_1$-AR on $\beta$ cells (11, 12) and increase glucagon secretion by binding to $\beta_2$-AR present in pancreatic $\alpha$ cells (13). The combined effects of catecholamines on both liver and endocrine pancreas are thus an important component of the counter-regulatory response to hypoglycemia.

The effect of catecholamines on glucose metabolism could either be direct at the postsynaptic ARs on target cells, as for regulation of insulin and glucagon secretion, or indirect, acting on ARs expressed on regulatory neurons in various brain areas. The $\alpha_{1b}$-AR has been mapped to different brain regions, including several hypothalamic nuclei, in particular the arcuate nucleus, the paraventricular and lateral hypothalamic nuclei as well as in the brain stem (in the nucleus of the tractus solitarius) (2). These brain structures control several important homeostatic functions such as insulin and glucagon secretion, glucose utilization or production, energy expenditure, and food intake. In addition to their sensitivity to catecholamines (14), these central regions, in particular the arcuate nucleus and the nucleus of the tractus solitarius, are also regulated by peripheral signals such as insulin, leptin, and glucose levels (15–18). Arcuate nucleus neurons secrete the orexigenic peptides neuropeptide Y (NPY) and agouti gene-related proteins (AgRP) as well as the anorexigenic peptides $\alpha$-MSH, derived from proopiomelanocortin (POMC), and cocaine and amphetamine-regulated transcript (CART). Whereas the orexigenic peptide production is decreased by leptin, insulin, and glucose, these factors increase expression of the anorexigenic peptides (19, 20). The physiological role of NPY has been particularly well studied. Its intracerebroventricular administration stimulates feeding, increases insulin secretion and fat metabolism and induces insulin resistance (21).

Identifying the specific receptor subtype that mediates the various effects of catecholamines is of considerable therapeutic interest, but has proved difficult because of a lack of subtype specific drugs. In this study we investigated glucose metabolism in knockout mice deficient in the $\alpha_{1b}$-AR subtype (19). Our results provide the evidence that in the absence of the $\alpha_{1b}$-AR, the parasympathetic activity is increased leading to increased insulin secretion, higher hepatic glycogen stores and impaired insulin action. This is associated with increased hypothalamic
NPY and AgRP mRNA levels in the presence of hyperleptinemia indicating that leptin is inefficient in controlling the expression of these orexigenic peptides in the absence of the α1b-AR. Furthermore, the mice lacking the α1b-AR displayed glucose intolerance and increased body weight under high fat feeding.

MATERIALS AND METHODS

Animals—12–16-week-old male mice from our colony were housed individually with inverted dark light cycle 8:00 a.m. to 8:00 p.m. During the feeding period, blood was collected between 1 and 2 p.m. In fasting experiments the mice were housed at 8 a.m. in clean new cages without food for 24 h (Sigma). Muscles from the total hind limb have been grounded and pulverized in a dry ice-cold state and stored at −70 °C until use.

Analytical Determinations—Blood parameters: plasma glucose concentrations were determined by a glucose oxidase method (Trinder kit, Sigma Diagnostic). Plasma insulin concentration was determined by ELISA (Merodia, Uppsala, Sweden), from 10 μl of plasma sampled from the tail vein during the intraperitoneal glucose tolerance tests or radioimmunoassays in all other instances (Linco, St Charles, MO).

Plasma free fatty acids were determined by an enzymatic colorimetric method (NEFA C, Wako, Neuss, Germany). In all other instances, plasma free fatty acids were determined by gas chromatography using M13 as the elongation primer.

For body composition, the mice were killed, and the whole carcasses were dried until constant weight. Total body fat content was determined by the soxhlet extraction method using petroleum benzine.

A sample of the supernatant was incubated in the presence of amyloglucosidase (Sigma) for 2 h at 37 °C in a shaking incubator. After centrifugation the glucose present in the supernatant was assayed by a glucose oxidase method (Sigma).

RT-PCR Analysis—The liver glycogen content was determined from a slice of the main lobe of the liver. The muscle glycogen content was determined after muscles from the total hind limb have been ground and pulverized in dry ice-cold state and stored at −70 °C until use. Briefly, 50–100 mg of the tissues were dissolved in NaOH, 1 μl at 55 °C for 1 h, neutralized with HCl (1 μl) and spun down. A sample of the supernatant was incubated in the presence of amyloglucosidase (Sigma) for 2 h at 37 °C in a shaking incubator. After centrifugation the glucose present in the supernatant was assayed by a glucose oxidase method (Sigma).

RT-PCR Analysis—For the α1b-AR and cyclinphilin mRNA determinations, pancreatic islets were isolated and total RNA extracted as previously described (24, 25). Three micrograms of total RNA were reverse-transcribed into cDNA using 200 units of Superscript II reverse transcriptase (RT) (Invitrogen) for 60 min at 42 °C in a total volume of 20 μl containing 0.7 μM oligo(dT), 1 μM dNTP, 1× first-strand buffer (Invitrogen), 10 μM dithiothreitol. Three microliters of the RT reaction were amplified by PCR using 5 units of Taq polymerase (Amersham Biosciences) in a 50-μl reaction containing 0.25 μM both forward and reverse primers, 1× PCR buffer (Amersham Biosciences), and 125 μM dNTP. The primer sequences designed using the Genetics Computer Group program suite were as follows: 5′-CAATGGACGACCAAGATTGTGG-3′ and 5′-TGGAGAAGCTGAGAAAG-3′ for the α1b-AR; 5′-GGCTCGAGACTGAGAGAAGA-3′ and 5′-TCAGCTTTGCGAGAGAAGA-3′ for cyclinphilin. PCR was performed as follows: 45 s at 94 °C, 50 s at 62 °C and 45 s at 72 °C. Twenty cycles were performed with a 0.5 °C decrease in the annealing temperature. Then 20 cycles were performed using an annealing temperature of 52 °C followed by 10 min at 72 °C. PCR products were separated on a 1% agarose gel, revealed with ethidium bromide and transferred on nylon membranes (Hybond, Amersham). Detection was enhanced by hybridization with a random-primed [32P] labeled probe for the specific genes. The probes were cloned into the pCR2.1 vector (Invitrogen) using the TOPO TA cloning kit (Invitrogen) and sequenced (Macrogen, The Netherlands) using M13 as the elongation primer.

Statistical Analysis—The significance of the results was calculated using the unpaired Student’s t test. Mean values were considered different when p < 0.05.

RESULTS

Blood Parameters—Fig. 1, A–D, shows the blood glucose and plasma insulin, glucagon and leptin levels in the fed state and during an initial 6 h period of fasting. In the fed state, blood glucose levels were similar in control and α1b-AR−/− mice (Fig. 1A), they however remained significantly higher in the α1b-AR−/− mice during the first six hours of fasting but eventually returned to the level of the control mice after 24 h of fasting (3.6 ± 0.2 versus 3.7 ± 0.2 mEq/l in mutant and control mice, respectively).
The plasma insulin concentration in the α1b-AR–/– mice returned to the value of the control mice after 24 h of fasting (Fig. 1C). After 24 h of fasting, these levels were still 6.5 ± 2.0 versus 1.9 ± 1.9 ng/ml in mutant and control mice, respectively. The high leptin levels in mutant mice could in part be explained by an elevated total body fat content, which was increased by 60% (5.33 ± 0.76 g versus 3.34 ± 0.40 g in mutant and control mice, respectively), even though body weights were similar (34.5 ± 1.0 g versus 34.0 ± 1.3 g for mutant and control mice, respectively). The increased postabsorptive insulinaemia may also contribute to the elevated leptin levels.

Free fatty acid levels were identical in control and mutant mice in the fed state (0.648 ± 0.060 versus 0.650 ± 0.054 mmol/L, respectively), but were lower in the mutant as compared with control mice after 6 h of fasting (0.838 ± 0.083 versus 1.159 ± 0.116 mmol/L, p < 0.05, respectively). This suggests that the increased body fat mass observed in the mutant mice results, at least in part, from a decreased lipolysis linked to the lack of the α1b-AR, which is known to mobilize free fatty acid from the adipose tissue (8).

Increased Parasympathetic Activity in Pancreatic β Cells—In several species, including the mouse, insulin secretion is stimulated by the parasympathetic system via cholinergic muscarinic receptors (27, 28). To investigate whether the parasympathetic nervous system plays a role in the postabsorptive hyperinsulinaemia of the α1b-AR–/– mice, the insulin plasma levels were measured 30 min after the injection of atropine or methyl-atropine in 6-h fasted mice. Injection of these cholinergic blockers reduced the plasma insulin levels of the α1b-AR–/– mice to the values of the control mice (Fig. 2). The blood glucose concentration was not affected by the injection of the anticholinergic drugs thus excluding that the reduction in insulin concentration resulted from changes in glycemia and that the modest elevation in blood glucose levels in fed mice was the cause of the hyperinsulinaemia (5.9 ± 0.2 versus 5.4 ± 0.2 mmol/L in mutant and control mice, respectively). Similar saline or methyl-atropine injections in 6 h fasted control and mutant mice did not reveal any decrease in leptin levels induced by muscarinic blockade. Leptin levels were (ng/ml ± S.E.): control mice, saline: 2.72 ± 0.074 (n = 7), control mice, m-atropine: 2.79 ± 0.042 (n = 8), α1b-AR–/– mice, saline: 5.81 ± 0.84 (n = 8), α1b-AR–/– mice, m-atropine: 6.61 ± 0.12 (n = 8).

To investigate whether the α1b-AR could play a direct role in the control of normal β cell function, we compared its mRNA expression in liver and pancreatic islets isolated from control mice. Fig. 3 shows, by RT-PCR and Southern blot analysis, that the α1b-AR mRNA was expressed at high level in the liver, as expected, whereas it was absent from pancreatic islets of control mice. Thus, this receptor does not play any direct role in regulating insulin secretion by pancreatic β cells and dysregulation of these cells secretory activity mutant islets is best explained by a modification of the parasympathetic nervous activity.

Measurements of Hypothalamic Neuropeptide mRNAs—Increase in parasympathetic nervous activity in pancreatic β cells could be due to higher expression of hypothalamic NPY (29). We therefore measured the mRNA of NPY and AgRP normalized to the GAPDH mRNA content, was significantly increased in the α1b-AR–/– mice (Fig. 4). Thus, the absence of the α1b-AR leads to elevated levels of NPY and AgRP even in the presence of elevated plasma leptin levels. This may cause the observed increase in parasympathetic nervous activity.

Intraperitoneal Glucose Tolerance Tests—Intraperitoneal glucose tolerance tests performed in 6 h fasted control and α1b-AR–/– mice revealed normal tolerance in both groups (Fig. 5A). However, the plasma insulin concentrations were 3-fold
Glucose Turnover—Normal glucose tolerance in the presence of exaggerated insulin secretion and hyperinsulinemia in the fasted state suggests the presence of insulin resistance in the \( \alpha_{1b}\)-AR \(^{-/-} \) mice. To directly evaluate this possibility, whole body glucose turnovers were determined in 6-h fasted control and \( \alpha_{1b}\)-AR \(^{-/-} \) mice. When expressed as increase over fasting control mice (Fig. 5A), the glucose turnover rate was associated with a reduced whole body glycogen synthesis rate in the \( \alpha_{1b}\)-AR \(^{-/-} \) mice in all conditions studied (Fig. 6D). However, the glycolytic rate was normal (Fig. 6C). Hepatic glucose production was lower in the basal state, but was poorly suppressed by physiological hyperinsulinemia in \( \alpha_{1b}\)-AR \(^{-/-} \) as compared with control mice (Fig. 6B). High insulin levels however completely suppressed endogenous glucose production. Altogether these data indicate a state of hepatic insulin resistance in the \( \alpha_{1b}\)-AR \(^{-/-} \) mice.

Glycogen Content—Activation of the hepatic \( \alpha_{1b}\)-AR is known to stimulate glycogenolysis (7, 10). To determine whether the absence of this receptor from the liver would modify glycogen metabolism we compared the hepatic glycogen stores during the fed to fast transition in mutant and control mice. In the fed state, the glycogen stores were 2-fold higher in the \( \alpha_{1b}\)-AR \(^{-/-} \) than in control mice (Fig. 7, A and B). In control mice the hepatic glycogen content progressively decreased by more than 80% within the first three hours of fasting. In the \( \alpha_{1b}\)-AR \(^{-/-} \) mice after 6 h of fasting the glycogen stores still represented ~60% of the initial content and was reduced by 80% after 24 h of fasting. However, the initial rates of glycogen degradation, expressed as the amount (\( \mu g \)) of glycogen degraded per minute, during the first 3 h of fasting were similar in control and mutant mice (~2.2 \( \mu g/g.min \)) and ~1.7 \( \mu g/g.min \) for control and mutant mice, respectively). Furthermore, intraperitoneal injection of glucagon at 1 mg/kg, lead to a 70% reduction in glycogen stores within 1 h in control mice whereas a similar reduction required ~3 h in the mutant mice (Fig. 8, A and B). Again, the rate (\( \mu g/min \)) of glycogen mobilization following glucagon injection was similar in both mouse strains (Fig. 8C). Thus, even though the glycogen stores were increased in \( \alpha_{1b}\)-AR \(^{-/-} \) mice, their rate of mobilization during fasting or a glucagon test was normal (Fig. 8C).

In contrast, muscle glycogen content was reduced in the mutant mice when compared with control in both fed (352 ± 80 and 612 ± 127 \( \mu g/mg \), respectively) and after six hours of fast (146 ± 71 and 292 ± 65 \( \mu g/mg \), respectively).
Glucose Tolerance and Body Weight Following High Fat Diet Feeding—To explore whether the lack of the α1b-AR might result in more pronounced metabolic changes upon a nutritional stress, mice were fed for one month a high fat diet. A glucose tolerance test was then performed in 6-h fasted mice before and after high fat feeding. The results of these experiments are shown in Fig. 5. Normal glucose tolerance but increased glucose-stimulated insulin secretion in α1b-AR<sup>−/−</sup> mice. A, blood glucose was measured 20 min before and 0, 30, 60 and 90 min after the injection of 1 g/kg glucose into the peritoneal cavity of mice fasted for 6 h. B, plasma insulin (microunits/ml) concentration was measured 30 min after the glucose injection. C, the insulin concentration over fasting has been calculated by subtracting the insulin concentration in the fasted state from the concentration measured 30 min after the glucose injection. D, the insulin/glucose ratio has been calculated by dividing the plasma insulin levels (microunits/ml) 30 min after the glucose injection by the corresponding glycemia (μm/ml). The results are the mean ± S.E. of 6 mice. *, p < 0.05 compared with control.

Fig. 6. Insulin resistance and reduced glycogen synthesis rate in α1b-AR<sup>−/−</sup> mice. A, glucose turnover; B, endogenous glucose production; C, glycolysis; and D, glycogen synthesis rates (mg/kg/min) have been measured after 6 h of fasting (Basal) and during an hyperinsulinemic euglycemic clamp using low (4 milliunits/kg/min) and high (18 milliunits/kg/min) rates of insulin infusion. The results are the mean ± S.E. of 6 mice per group. *, p < 0.05 compared with control.

Fig. 7. Hepatic glycogen content is increased in α1b-AR<sup>−/−</sup> mice. The hepatic glycogen content was measured in control (+/+) and α1b-AR<sup>−/−</sup> (−/−) mice in the fed state and during fasting. The glycogen content is in μg of glucose/mg of protein (A) and in percent of the fed value (B). The results are the mean ± S.E. of 6 mice. *, p < 0.05 compared with control.

Glucose Tolerance and Body Weight Following High Fat Diet Feeding—To explore whether the lack of the α1b-AR might result in more pronounced metabolic changes upon a nutritional stress, mice were fed for one month a high fat diet. A glucose tolerance test was then performed in 6-h fasted mice before and after high fat feeding. The results of these experi-
A; Glycogen (µg/mg)  B; Glycogen (% of fed value)  C; Glycogen degradation (µg/mg.min)

Hours after glucagon injection

Fig. 8. Glucagon-induced glycogenolysis in control and α₁b-AR−/− mice. The hepatic glycogen content was measured in control (+/+) and α₁b−−− (−/−) mice at the indicated times following the injection of glucagon. The glycogen content is expressed as µg of glucose/mg of protein (A) and percent of the fed value (B). The glycogen degradation rate is expressed as µg of glycogen/mg of protein × min (C). The results are the mean ± S.E. of six mice. *p < 0.05 compared with control.

Discussion

This study provides evidence that the α₁b-AR subtype plays an important regulatory role in the control of whole body glucose homeostasis. This role is associated to both increased expression of hypothalamic NPY and increased activity of the parasympathetic nervous system. The absence of the α₁b-AR results in hyperinsulinemia, high plasma leptin levels, increased hepatic glycogen content, reduced muscle glycogen content, insulin resistance as well as susceptibility to development of glucose intolerance and obesity during short term high fat feeding.

A striking feature of the α₁b-AR−/− mice is their prolonged hyperinsulinemia during the postabsorptive state, which however returns to the value of the control mice after 24 h of fasting. The elevated insulin plasma levels can be rapidly normalized by administration of the muscarinic receptor antagonists atropine or methyl-atropine. As the latter compound cannot cross the blood brain barrier, it must act on peripheral muscarinic receptors very likely at the level of the pancreatic β cells. These findings strongly suggest that in the α₁b-AR−/− mice the hyperinsulinemia is due to an increased parasympathetic nervous activity at the level of the pancreatic β cells.

The normalization of insulinemia in the 24-h fasted animals could be caused by the progressive decrease in glyceremia. Indeed, the insulinotropic action of acetylcholine is glucose-dependent and normalization of the glyceremic levels is expected to suppress insulin secretion even in the presence of a constant parasympathetic tone.

The activity of the autonomous nervous system is controlled by different hypothalamic neuronal circuits which respond to the body energy status. Circulating leptin, insulin and glucose levels are directly sensed by neurons in the arcuate nucleus, which synthesize NPY and AgRP. Intracerebroventricular administration of NPY leads to increased insulinemia, higher adipocyte glucose utilization and insulin resistance in muscle (30, 31). These effects are suppressed by vagotomy, indicating that NPY effects are transmitted through the parasympathetic nervous system (29). The intracerebroventricular infusion of AgRP is associated with a reduced sympathetic tone leading to a reduced energy expenditure (32). Therefore, the increase in the parasympathetic nervous activity in the α₁b-AR−/− mice may result from the observed increased hypothalamic expression of both NPY and AgRP.

The α₁b-AR−/− mice are hyperleptinemic, but this is not surprising since insulin is a positive regulator of leptin secretion (33). In addition, it has been shown that brain injection of NPY increased leptin expression in the adipose tissue (34). Consequently, the combined action of insulin and NPY could explain the hyperleptinemia of the α₁b-AR−/−. In adult mutant mice, administration of methyl-atropine in 6-h fasted mice, in conditions that normalizes insulinemia, does not reduce the leptin level. This absence of acute effect of muscarinic blockade on leptin levels suggests that the influence of the parasympathetic nervous system on leptinemia may be at two different levels: through a regulation of leptin expression by adipocytes, probably acutely controlled by insulin, and through a regulation of the fat mass, which is increased in the mutant mice.

It is however surprising that the hyperleptinemia does not result in down-regulation of the NPY and AgRP expression thus suggesting a leptin resistance state in the α₁b-AR−/− mice. This apparent lack of leptin effect on NPY and AgRP expression may, however, be explained by the observation that the α₁b-AR is expressed in the arcuate nucleus and that NPY neurons are also regulated by afferent projections from catecholaminergic neurons located in the brain stem (in particular in the nucleus of the tractus solitarius) (2). Destruction of these neurons by immunotoxins leads to increased NPY and AgRP levels (35). Thus, the elevated expression of these orexigenic peptides in the α₁b-AR−/− mice strongly suggests that leptin cooperates with the α₁b-AR to control the level of expression of NPY and AgRP in the hypothalamus.

Euglycemic clamps revealed whole body insulin resistance, as revealed by a 15–20% decrease in glucose turnover in the basal state and during low and high insulin infusion rates. This state of insulin resistance was associated with a marked defect in suppression of endogenous glucose production at physiological, but not high insulin levels. A similar degree of insulin resistance has been reported before for the eNOS−/− or AMP kinase−/− (36, 37) mice, which are also normoglycemic. This mild insulin resistance may explain the slower decrease in glyceremia in the presence of relatively elevated insulin levels in the mutant as compared with the control mice during the fed to fasting transition. It may also be responsible for development of the glucose intolerance upon high fat feeding (Fig. 9).

The mechanism inducing insulin resistance in the α₁b-AR−/− mice may be secondary to the hyperinsulinemia and a down-regulation of the insulin receptors in peripheral tissues (38, 39). A direct effect of the α₁b-AR on muscles is unlikely since the number of α₁-ARs in this tissue is extremely low (22). In addition, insulin resistance cannot be attributed to elevated free fatty acid levels since these were lower in null mice than in controls (see “Results”). Furthermore, we and others (40, 41), have previously shown that muscle glucose transport can be activated by an intracerebroventricular infusion of leptin which increases the sympathetic nervous system activity and that leptin increases the peripheral action of insulin (2). Thus, in the α₁b-AR−/− mice the apparent leptin and insulin resistance may both contribute to perturb the sympathetic nervous transmission in the muscle and reduce glucose clearance as well as insulin sensitivity.

The α₁b-AR is expressed at high level in the mouse liver where it mediates, in the fasting state, the catecholamine-induced activation of glycogenolysis (42). Therefore we sought to assess whether the absence of this receptor would modify hepatic glycogen metabolism. In the fed state, there was a
significant 2-fold increase in glycogen content in the α₁b-AR−/− mice (Fig. 8). This could be due to several factors including the absence of the α₁b-AR-mediated glycogenolysis, the increased insulinemia in the postabsorptive state in face of normal glucagon levels, the increased parasympathetic nervous activity, which stimulates glycogen synthesis (7, 43–46) or a combination of them. Strikingly, the rate of glycogen degradation was similar in control and mutant mice during the initial fed to fasted transition or following an intraperitoneal glucagon injection (Fig. 8). Therefore, despite the fact that the α₁b-AR stimulates glucose mobilization in the liver, its absence did not result in hypoglycemia in the fasted state.

Altogether, our findings indicate that the absence of the α₁b-AR is associated to an increased parasympathetic nervous activity. This may, at least in part, be due to an increase in NPY expression, which is known to activate the parasympathetic nervous system via mechanisms, which are not fully elucidated (29). The increased NPY expression together with the increased parasympathetic activity may be responsible of the increased insulin secretion, insulin resistance in muscle and increased leptin production by adipocytes in the α₁b-AR−/− mice (34).

In support of this hypothesis, it is interesting to highlight that some of the metabolic changes observed in the α₁b-AR−/− mice are the opposite of those displayed by knockout mice lacking the M3 muscarinic cholinergic receptors, which displayed very low insulin and leptin levels as well as decreased body weight (47).

Whereas, usually, increased leptin down-regulates NPY expression and activates the sympathetic nervous system, in the α₁b-AR−/− mice this negative feedback regulation is no longer functional indicating a condition of leptin resistance. This leads us to suggest the hypothesis that the α₁b-AR plays a permissive role on leptin action in neurons of the arcuate nucleus. Therefore, in the absence of the α₁b-AR, leptin no longer inhibits NPY, which is increased thus leading to further increase in leptin production. The α₁b-AR could also control leptin action in other brain areas like the ventro-medial hypothalamus, which is involved in the stimulation of muscle glucose utilization, as previously described (48). Whereas the lack of the α₁b-AR in the central nervous system could result in leptin resistance, the markedly increased levels of leptin observed in the α₁b-AR−/− mice might result, at least in part, from their increased body fat mass which might be linked to decreased lipolysis in the null mice (see “Results”).

In conclusion, our findings indicate that the α₁b-AR plays an important role in the control of glucose homeostasis acting at various levels both in the central nervous system and in peripheral tissues. However, the mechanisms proposed to explain the complex metabolic changes observed in the α₁b-AR−/− mice are far from being conclusive. Future studies will attempt to directly demonstrate, using intracerebral manipulations, the interaction between the α₁b-AR, NPY, and leptin in distinct brain areas and their implications on glucose metabolism.

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