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Endogenous epinephrine protects against obesity induced insulin resistance

Michael G. Ziegler^a, Milos Milic^a, Ping Sun^a, Chih-Min Tang^a, Hamzeh Elayan^b, Xuping Bao^a, Wai Wilson Cheung^a, and Daniel T. O'Connor^a

^aDepartment of Medicine, UCSD, 9500 Gilman Drive, La Jolla, CA 92093-0838

^bDepartment of Pharmacology, Faculty of Medicine the University of Jordan, Amman, Jordan

Abstract

Epinephrine (E) is a hormone released from the adrenal medulla in response to low blood sugar and other stresses. E and related β 2-adrenergic agonists are used to treat asthma, but a side effect is high blood sugar. C57BL/6 mice prone to overfeeding induced type II diabetes had the PNMT gene knocked out to prevent E synthesis. These E deficient mice were very similar to control animals on a 14% fat diet. On a 40.6% fat diet they gained 20 to 33% more weight than control animals and increased their blood glucose response to a glucose tolerance test because they became resistant to insulin.

Although the short term effect of β 2-agonists such as E is to raise blood glucose, some long acting β 2-agonists improve muscle glucose uptake. Endogenous E protects against overfeeding induced diabetes. Since adrenal E release can be impaired with aging and diabetes, endogenous E may help prevent adult onset diabetes.

Keywords

Phenylethanolamine N-methyltransferase; PNMT; mouse; glucose; diabetes mellitus

Introduction

Epinephrine (E), also called adrenaline, is the quintessential stress hormone. When E is administered it increases blood glucose by inhibition of insulin release, stimulation of glucagon release, hepatic glycogenolysis and hepatic and renal gluconeogenesis (Cryer, 1993). Paradoxically, while the β -adrenergic agonist E acutely increases glucose and decreases insulin sensitivity, the β -adrenergic antagonists are also associated with increased blood glucose and decreased insulin sensitivity (Jacob et al., 1996). Epinephrine is synthesized from norepinephrine (NE) by the enzyme phenylethanolamine N-methyltransferase (PNMT) and is a more potent β 2-adrenergic agonist than NE. In addition to stimulating the release of metabolic substrate, E stimulates metabolic rate. Both E and NE

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Corresponding author. Michael G. Ziegler; UCSD, 200 W Arbor St. San Diego, CA92103-8341, USA; Tel.: 1 619 543 6180, fax: 1 619 543 7716; mziegler@ucsd.edu .

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stimulate β_3 receptors to promote fat metabolism, although this is predominantly a response to neuronal NE release. However, physiologic E levels also stimulate a thermogenic response in humans (Cannon et al., 2004). Conversely, β -blocking drugs are associated with weight gain (Sharma et al., 2001).

Weight gain and stress are characteristics of industrialized societies, so the stress hormone E might play a role in the current epidemic of type II diabetes. By far the highest concentrations of E and PNMT are found in the adrenal medulla, but about half of all PNMT is outside the adrenal (Kennedy et al., 1995), so removing the adrenal medulla might leave important stores of E while altering release of glucocorticoids from the adrenal cortex. We have created a PNMT knockout mouse and backcrossed it into a mouse that develops type II diabetes in response to overfeeding. This animal demonstrates a role for endogenous E in type II diabetes.

Materials and Methods

Animals

Phenylethanolamine N-methyltransferase PNMT^{-/-} cells were injected into C57BL/6 blastocysts and the chimeras were back crossed into C57BL/6 mice as reported previously (Bao et al., 2007). Mice were housed at a constant temperature and on a 12-h light, 12-h dark cycle. All experimental procedures for the studies were approved by the Institutional Animal Care and Use Committee of the University of California San Diego. The animal care facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Experiment 1: effects of high fat diet on weight gain, insulin and glucose tolerance

15 PNMT^{+/+} and 13 PNMT^{-/-} male mice were fed a normal diet (14% kcal fat; Harlan Teklad 8604, Madison, WI). 10 PNMT^{+/+} and 10 PNMT^{-/-} mice received a high-fat diet (40.6% kcal fat; Harlan Teklad TD.96132 Adjusted Fat Diet) for 10 weeks ad libitum. Animals were weighed bi-weekly and, at the end of 10-weeks, the intraperitoneal glucose tolerance test (ipGTT) and intraperitoneal insulin tolerance test (ipITT) were performed. Prior to each test, mice were fasted for 6 h. Then, a baseline blood sample was taken from their tail and each mouse received either ip glucose, 1g/kg body weight or ip insulin 0.85 U/kg body weight (Novolin R by Novo Nordisk Pharmaceuticals, Princeton, NJ). Tail blood samples were drawn at 15, 30, 45, 60, 90, and 120 min after the injection and were analyzed immediately for glucose content using HemoCue B-Glucose Analyzer (HemoCue, Lake Forest, CA). Catecholamine levels were determined by the radioenzymatic method of Kennedy and Ziegler (Kennedy et al., 1990).

Experiment 2: glucose differences found in study 1 and sex-specific effects

To investigate the etiology of glucose differences found in experiment 1 and sex-specific effects, mice of both genders were subjected to further testing. PNMT^{+/+} (9 males and 10 females) and 10 PNMT^{-/-} (6 males and 4 females) mice were fed a high fat diet for 13 weeks. An additional 13 PNMT^{+/+} (7 males and 6 females) mice were given the β -adrenergic blocking drug propranolol 79 100-150 mg/kg (Sigma Chemical Co., St. Louis, MO) in their drinking water along with the high fat diet to provide a pharmacologic block of some E stimulation. Mice were weighed weekly and, at the end of 13-week high fat diet, underwent dual energy X-ray absorptiometry (DEXA) scan to determine their body composition. After mice were fasted overnight (with water supply) to minimize the effect of ingested food and then euthanized, we used a PIXImus mouse densitometer (MEC Lunar Corp., Minster, OH) to determine their lean, and fat masses.

Statistical Analysis

Statistical software package SAS version 9.0 was used for statistical analysis. All values are expressed as mean $\pm$ SEM. Comparison of means of weight gain between PNMT $^{-/-}$ and PNMT $^{+/+}$ mice was performed with Student's *t* test. Comparison of means of weight gain among PNMT $^{-/-}$, PNMT $^{+/+}$ and PNMT $^{+/+}$ with β -blocker mice was performed with one-way ANOVA plus post hoc analysis with the Least Significant Difference method. Glucose tolerance and insulin tolerance were analyzed with the General Linear Model. Values of $P < 0.05$ were considered statistically significant.

Results

In the first experiment, 9 PNMT $^{-/-}$ mice and 9 PNMT $^{+/+}$ were fed a diet with 40.6% calories from fat (Table 1). In the second experiment, mice were fed the same diet for 13 weeks (Table 2). Knockout of the PNMT gene effectively blocked production of epinephrine (Fig. 1). Overall, mice unable to make the β -adrenergic agonist E tended to gain more weight and mice receiving a β -blocker gained more weight on a high fat diet.

When the PNMT $^{-/-}$ mice unable to synthesize E ate a normal diet, they had blood glucose levels slightly, but not significantly lower than PNMT $^{+/+}$ mice. However, those on a high fat diet reversed this relationship (Fig. 2) leading to a significant diet by genotype interaction. The high fat diet also prolonged the duration of elevated glucose levels following a glucose challenge.

In order to determine whether the higher blood glucose response to a glucose challenge was due to insulin resistance, we measured blood glucose levels following an injection of insulin. The insulin sensitivity of PNMT $^{+/+}$ and PNMT $^{-/-}$ animals on a 14% fat diet did not differ. The mice that ate a high fat diet were relatively insulin resistant and the PNMT $^{-/-}$ animals were the most insulin resistant (Fig. 2).

Discussion

The PNMT $^{-/-}$ mice weighed the same as PNMT $^{+/+}$ animals when raised on a customary 14% fat diet and their glucose tolerance and insulin sensitivity did not differ. All of the mice used in this experiment were C57B/6 animals, which develop obesity and type II diabetes on a high fat diet (Petro et al., 2004). The PNMT $^{-/-}$ mice gained 20% to 33% more weight on a high fat diet, but their final body fat, muscle mass and total body weight did not significantly differ from control animals. Nevertheless, removing E synthesis significantly worsened their response to glucose because they became more insulin resistant than control animals placed on a high fat diet.

The short term effect of pharmacologic doses of E is to increase blood glucose and diminish insulin sensitivity (Westfall et al., 2010). Surprisingly, normal E production had the opposite effect, protecting against diet induced hyperglycemia and insulin resistance. There is reason to believe this is due to E stimulation of β_2 receptors. PNMT $^{-/-}$ mice still had normal amounts of NE. E stimulates β_2 receptors better than NE, but has similar potency at α , β_1 and β_3 receptors (Westfall et al., 2010). Skeletal muscle is the most important site for glucose disposal and responds to β_2 stimulation. A chronic E infusion enhanced rat muscle insulin sensitivity (Jensen et al., 2005). Some β_2 -agonists in pharmacologic doses lead to muscle hypertrophy in rats cattle, pigs, poultry and sheep (Petrou et al., 1995) by decreasing breakdown of muscle protein (Navegantes et al., 2002). E facilitates insulin binding to exercising muscle (Jensen et al., 2005) and increases muscle blood flow.

The short term effect of β 2-agonist drugs is to increase insulin release. Six hours after administration of β 2-stimulating drugs insulin sensitivity is diminished, but that effect is short lived (Sternbauer et al., 1998). While short acting β 2-agonists worsen glucose tolerance, chronic administration of long acting β 2-agonists improves insulin sensitivity (Castle et al., 2001). On the other hand, chronic use of β -blockers increases glycohemoglobin (Kveiborg et al., 2006), decreases insulin sensitivity and causes weight gain (Sharma et al., 2001) in humans. Thus, the long term effect of chronic administration of β 2-agonists is to increase muscle growth, blood flow, insulin binding and insulin sensitivity. Our study indicates that the long term effect of endogenous E is protection against the insulin insensitivity that accompanies a high fat diet. We have previously shown that endogenous E also protects against exercise induced blood pressure elevation and cardiac remodeling in mice on a normal fat diet (Bao et al., 2007). The effect of endogenous E on the hypertension that frequently accompanies a high fat diet is an important future research topic.

Human overfeeding has become a worldwide phenomenon, as has type II diabetes (Davis et al., 2009). Adrenal E release and the E response to stress decreases with age (Seals et al., 2000) and deficient E responses are more common in diabetics (Cryer et al., 2003). Although E is generally thought to raise blood glucose, this is an acute effect of pharmacologic doses. This study shows that endogenous E actually protects against the insulin resistance and high blood glucose that accompanies dietary excess.

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Fig. 1.
Adrenal catecholamines in mice on high fat diet. Values are presented as mean $\pm$ S.E.M.

Fig. 2.

Glucose tolerance (A) and insulin tolerance (B) of PNMT^{-/-} and PNMT^{+/+} mice on a high fat diet and normal diet. (A) Blood glucose of PNMT^{-/-} and PNMT^{+/+} mice after a normal or high fat diet were measured after a glucose injection. A repeated measures general linear model analysis showed an effect of the high fat diet to raise glucose levels ($P < 0.001$) and an interaction between genotype and diet ($P = 0.0002$). In addition there was a time by genotype by diet effect ($P < 0.001$). (B) Mice fasted for 6 hours received an insulin injection and had blood glucose measured for two hours. For clarity of display, standard error bars are omitted and responses to insulin are normalized to the baseline value. There was a significant between subject effect of diet ($P < 0.001$) and genotype ($P < 0.05$).

Table 1

Final weight and weight gain of mice in experiment 1.

	PNMT ^{+/+}	PNMT ^{-/-}
Final Weight (g)	46±1	48±1
Weight Gain (g)	12±1	16±1 ^a

^aP < 0.05, by Student's *t* test.

Table 2

Body mass characteristics of mice in experiment 2.

	PNMT ^{+/+}	PNMT ^{+/-}	PNMT ^{-/-}	β -blocker PNMT ^{+/+}
Final Weight (g)	38 $\pm$ 3	40 $\pm$ 2	41 $\pm$ 1	42 $\pm$ 1
Weight Gain (g)	8 $\pm$ 1	9 $\pm$ 1	10 $\pm$ 1	12 $\pm$ 1 ^a
Muscle Mass (g)	18 $\pm$ 0.4	N/A	17 $\pm$ 1	18 $\pm$ 1
Fat Mass (g)	18 $\pm$ 1	N/A	16 $\pm$ 1.5	18 $\pm$ 1

^aP < 0.05 vs. PNMT^{+/+} (ANOVA).