Involvement of SSAO-mediated deamination in adipose glucose transport and weight gain in obese diabetic KKAY mice

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Yu, Peter H., Michael Wang, Hui Fan, Yulin Deng, and Diana Gubisne-Haberle. Involvement of SSAO-mediated deamination in adipose glucose transport and weight gain in obese diabetic KKAY mice. Am J Physiol Endocrinol Metab 286: E634–E641, 2004.—Semicarbazide-sensitive amine oxidase (SSAO) is located on outer surfaces of adipocytes and endothelial and vascular smooth muscle cells. This enzyme catalyzes deamination of methylamine and aminoaetocetone. SSAO can prevent weight gain in obese KKAY mice fed an atherogenic diet. The enzyme contains copper and 6-hydroxydopa quinone as cofactors. Methylamine and aminoaetocetone have been found to be endogenous substrates for SSAO. SSAO-mediated deamination induces adipocyte glucose uptake and lipogenesis. Highly selective SSAO inhibitors can effectively induce glucose uptake. The results suggest that increased SSAO-mediated deamination is related to chronic inflammation and vascular disorders related to diabetic complications. The major health risks associated with obesity are development of cardiovascular diseases, stroke, type 2 diabetes, osteoarthritis, and certain types of cancer. The cause of obesity is multifactorial in nature. Obesity occurs as a result of genetic makeup, acquired changes from feeding control, thermogenesis, and adipogenesis. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The KKAy mice at 9 wk of age confirmed to exhibit hyperglycemia were randomly divided into two groups (n = 16 in each group) as control and SSAO inhibitor-treated groups. For chronic investigation, mice were fed a high-cholesterol atherogenic diet (ICN Pharmaceutical, Aurora, OH). The diet contained 1.25% cholesterol, 7.5% cocoa butter, 5% sodium cholate, and other sugars and minerals.

**SSAO assay.** SSAO activity was determined by a radioenzymatic procedure using 14C-labeled benzylamine as a substrate, following our previously described procedure (44). The SSAO enzyme preparations were preincubated with clorgyline (1 × 10⁻⁶ M) and i-deprenyl (1 × 10⁻⁶ M) at room temperature for 20 min to ensure that any monoamine oxidase activity, if present, was completely inactivated. The enzyme was then incubated in the presence of benzylamine (5 × 10⁻⁵ M, 0.1 μCi) in a final volume of 200 μl at 37°C for 30 min. The enzyme reaction was terminated by adding 200 μl of 2 M citric acid. The oxidized products were extracted into 1 ml of toluene-ethyl acetate (1:1, vol/vol), of which 600 μl were then transferred to a counting vial containing 10 ml of Omnifluor cocktail (New England Nuclear, Boston, MA). Radioactivity was assessed by liquid scintillation counting (Beckman LS-7500, Fullerton, CA). One unit of enzyme activity is defined as one nanomole of product formed per minute per milligram of protein.

**Serum glucose.** The animals were fasted overnight before collection of venous blood. Serum glucose concentration was determined by a coupled enzymatic procedure using hexokinase and glucose-6-phosphate dehydrogenase in the presence of NAD. NADH, the product of the enzymatic reaction, was measured spectrophotometrically. A Sigma glucose diagnostic kit was employed (Sigma, St. Louis, MO).

**2-Deoxyglucose uptake.** Adipocytes were isolated from epididymal fat pads of male Wistar rats and from male CD-1 Swiss White mice. The fat tissues were sliced using a McIwain tissue chopper (Michle Laboratory Engineer, Surry, UK) and digested in Krebs-Ringer buffer containing 25 mM NaHCO₃, 1.16 mM NaCl, 5 mM KCl, 1 mM KH₂PO₄, 1.2 mM MgSO₄, 4% bovine serum albumin, and 1.5 mg/mg collagenase. After incubation for 45 min at 37°C under gentle shaking, the adipocytes were filtered through a 400-mesh sieve and washed with the same buffer without collagenase. The cells were then resuspended in buffer and centrifuged at 3,000 x g for 10 min. The viability of isolated adipocytes was checked by Trypan blue stain microscopically.

2-Deoxyglucose (2-DG) uptake was used for assessment of glucose transport (11). The adipocytes in Krebs-Ringer solution were preincubated with insulin (1 × 10⁻⁷ M), SSAO substrate (1 × 10⁻⁴ M) plus orthovanadate (1 × 10⁻⁴ M), and/or SSAO inhibitor FPFA (1 × 10⁻⁶ M) in a total of 200 μl for 60 min at 37°C. Fifty microliters of 2-DG were then added to make to 1 × 10⁻⁴ M containing 1.5 μCi of tritium-labeled tracer 2-DG. The cells were further incubated for 10 min at 37°C, and uptake was terminated by addition of 100 μl of cytochalasin (100 μM). The cells were centrifuged in microtubes containing 100 μl of silicone oil. The cells (upper part of the tubes) were collected and solubilized with 20 μl of SOLVABLE (New England Nuclear) overnight. The extracts were then transferred to counting vials containing 10 ml of ACS counting fluid (New England Nuclear). Radioactivity was assessed with a liquid scintillation counter (Beckman LS-7500).

**Statistics.** The results were assessed using analysis of variance (ANOVA) followed by Newman-Keuls multiple comparisons. In general, the null hypothesis used for all analyses was that the factor has no influence on the measured variable, and significance was accepted at the >95% confidence level.

**RESULTS**

**Inhibition of weight gain in obese KKAy mice by selective SSAO inhibitor.** FPFA, a potent selective SSAO inhibitor, significantly reduced weight gain in obese KKAy mice fed high-cholesterol diets (Fig. 1). This selective inhibitor at the applied low dose did not exhibit any apparent toxicity to these mice. The effect of the SSAO inhibitor on daily consumption of food and water was monitored for 5 days during week 8 following the treatment. This compound did not affect food and water intake (results not shown). The reduction of body weight is primarily due to reduction of masses of abdominal fat tissues.

**Adipocyte SSAO and its inhibition.** As can be seen in Fig. 2A, FPFA is quite potent at inhibiting mouse adipose SSAO. This inhibitor effectively blocks SSAO in vivo at very low dosages. The ED₅₀ is <0.5 mg/kg intraperitoneally. As can be seen in Fig. 2C, a single dose of FPFA can effectively block SSAO activity. In the present study, a dosage of 2 mg/kg intraperitoneally every 2nd day almost completely blocked the adipose SSAO activity.

Although FPFA is quite potent and selective at inhibiting SSAO activity, it also inhibits type B monoamine oxidase, a mitochondrial flavine enzyme (46). 2-Bromoethylamine (2-BrEA) was therefore employed. 2-BrEA is known to be less potent in inhibiting vascular SSAO activity (42), but it does not inhibit monoamine oxidase activity in vitro (Fig. 2B) and in vivo (Fig. 2D). Both FPFA and 2-BrEA were used in the subsequent studies.

SSAO-mediated deamination enhances glucose uptake in adipocytes in vitro and ex vivo. Enhanced SSAO-mediated deamination has been found to stimulate 2-DG uptake in adipocytes (11). When adipocytes, isolated from both rat and...
human adipose tissues and from cultured adipocytes, were incubated with SSAO benzylamine, glucose uptake was increased. Inhibition of SSAO by semicarbazide can completely block induction of 2-DG uptake by benzylamine. Semicarbazide possesses a hydrazine moiety, which interacts with a large variety of biological constituents, particularly at the high concentrations used in these studies. It may inhibit many other pyridoxal or quinone enzyme activities and thus produces a nonspecific effect. In the present study, highly selective and potent SSAO inhibitors (FPFA and 2-BrEA) were employed. Induction of 2-DG uptake by benzylamine in isolated adipocytes from both rats and mice was confirmed. Figure 3A shows that FPFA at 1 μM, like semicarbazide (at 10^{-3} M), completely blocks benzylamine-induced 2-DG uptake in rat adipose tissue. The effect of SSAO substrates on 2-DG uptake is tissue selective. Benzylamine does not induce 2-DG uptake in isolated skeletal muscle cells under similar experimental conditions (Fig. 3B). Although GLUT4 is present in both adipose and skeletal muscles, SSAO in skeletal muscles is quite low and probably limited to blood vessels in these tissues.

Induction of 2-DG uptake correlates with SSAO activity. As can be seen in Fig. 3, C and D, effects of FPFA and 2-BrEA on 2-DG uptake are dose dependent. SSAO inhibitors do not affect baseline 2-DG uptake in adipocytes.

Although benzylamine proves to be effective at stimulating adipose 2-DG uptake, it is not present endogenously. Methylamine and aminoaetone are known endogenous substrates for SSAO. Both amines have been shown to be capable of inducing adipocyte 2-DG uptake. Also, FPFA blocks such an amine-induced increase in 2-DG uptake (Fig. 4).

The effect of SSAO inhibitors on adipose 2-DG uptake was also demonstrated in an ex vivo experiment. Adipocytes were obtained from mice 2 h after treatment with different doses of FPFA and 2-BrEA. Levels of 2-DG uptake in the presence of methylamine and vanadate were compared with basal 2-DG uptake levels. As seen in Fig. 5, SSAO inhibitors blocked methylamine- and aminoaetone-induced 2-DG uptake. Although SSAO inhibitors block amine-induced 2-DG uptake in adipocytes, they do not seem to affect insulin-elicited 2-DG uptake (results not shown).

Effect of SSAO substrates and inhibitors on blood glucose levels. Blood glucose levels were altered after treatment of either SSAO inhibitors or substrates in both rats and mice. As can be seen in Fig. 6A, 2-BrEA induced a moderate but significant and persistent increase in blood glucose. Interestingly, methylamine caused a reduction in serum glucose level at 2 h after treatment (Fig. 6A). However, the hypoglycemic effect of methylamine is transient and can be completely
blocked by 2-BrEA. Reduction of serum glucose by methylamine was no longer detected 24 h after treatment. Clearly, methylamine is subject to metabolism and disposition, whereas inhibition of SSAO by 2-BrEA is irreversible and long lasting.

A single dose of FPFA (5 mg/kg ip) also caused an increase in blood glucose (Fig. 6B). Hyperglycemia persisted for ≥3 days and was gradually reduced to baseline levels. Alteration in glycemia negatively correlated to SSAO activities. The half-life of SSAO recovery took ~3–4 days (results not shown). It appears that ≥60% of inhibition of SSAO activity is needed to affect blood glucose levels. This is consistent with the in vitro experiment shown in Fig. 3.

Effect of SSAO inhibitor on glucose tolerance. Figure 7 shows the effect of FPFA on glucose and insulin tolerance. The inhibitor was administered 60 min before the challenge of glucose or insulin. A mild hyperglycemia was detected 60 min after SSAO inhibitor treatment. After injection of glucose, blood glucose levels increased for 15–20 min before returning to normal levels. FPFA caused an increase in blood glucose compared with controls (Fig. 7B). This is primarily due to an increase in the level of glucose baseline caused by FPFA. Insulin was able to bring glucose slowly back to basal levels (Fig. 6B). An increase in glucose levels was detected in the SSAO inhibitor-treated group over the control group.

DISCUSSION

Recently, SSAO-mediated deamination has been shown to induce glucose uptake in isolated rat adipocytes (11) as well as in 3T3-F422A and 3T3-L1 adipocyte cell lines (13). Intriguingly, the effect of SSAO substrates is quite comparable to the action of insulin. Induction of glucose uptake can be blocked by SSAO inhibitor semicarbazide. In the present investigation, the highly selective mechanism-based SSAO inhibitors FPFA and 2-BrEA have been applied. Both SSAO inhibitors at low selective concentration were equally as effective as semicarbazide in blocking benzylamine-induced increase in 2-DG uptake in isolated mouse adipocytes. Adipocytes obtained from mice pretreated with SSAO inhibitors also showed a substantial reduction in 2-DG uptake. It was suggested that H2O2 derived from SSAO-mediated deamination might be responsible in induction of glucose transport, since H2O2 is known to...
enhance glucose uptake (12, 20, 31). H$_2$O$_2$ stimulates tyrosine phosphorylation of insulin receptor substrate proteins and GLUT4 translocation (10). H$_2$O$_2$ is produced via SSAO-catalyzed deamination in the adipose compartment. SSAO substrates have also been shown to mimic insulin action on adipocyte differentiation in 3T3 cell lines (13, 24).

In the present study, we also found that the endogenous SSAO substrates methylamine and aminoacetone were capable of inducing adipose 2-DG uptake. In addition, selective SSAO inhibitors blocked such an effect. Our result, that SSAO substrates induce a transient reduction of blood glucose, is consistent with the finding that administration of benzylamine or vanadate reduced serum glucose in streptozotocin-induced diabetic rats (23). Interestingly, selective SSAO inhibitors cause a moderate and persistent increase in blood glucose. These observations suggest that SSAO-mediated deamination may play a role in regulation of glucose uptake in vivo. The increase in blood glucose following blockade of SSAO activity seems to be primarily due to the effect on adipose glucose uptake. Skeletal muscles exhibit negligible amounts of SSAO activity. We have shown that 2-DG uptake in skeletal muscle cells was not affected by SSAO substrates. The fate of increased blood glucose after SSAO inhibitor treatment is uncertain. Increased glycolysis was not detected, as rectal temperature was unchanged by the inhibitor. It is conceivable that glucose may be excreted, but a significant increase in urinary glucose was not detected.

It is interesting to note that the antihypertensive drug hydralazine was previously reported to induce marked hyperglycemia in rats (34) and dogs (25). The mechanism for such hyperglycemia was unclear at the time. Hydralazine has also been shown to be a potent SSAO inhibitor (22). The hyperglycemic effect is probably due to inhibition of SSAO activity and blockade of glucose uptake into adipocytes by hydralazine.

GLUT4, which is located primarily in skeletal muscle cells and adipocytes, is sensitive to insulin. It is considered to be the rate-limiting step for glucose transport into adipose tissue. GLUT4-mediated glucose transport is essential for maintenance of normal glucose homeostasis (50). A marked alteration in GLUT4 gene expression in adipocytes, but not in skeletal muscle, has been shown in human and animal obesity models (7, 15). Regulation of GLUT4 in adipocytes has been shown to be involved in the development of obesity or in adaptation to the obese state. Transgenic mice selectively overexpressing GLUT4 (using fat-specific aP2 promoter/enhancer) in adipocytes become obese (36, 39). Heterozygous knockout of GLUT4 causes hyperglycemia and diabetic histopathology (38), whereas homozygous knockout of GLUT4 induces insulin resistance, glucose intolerance, growth retardation, cardiac hypertrophy, and a shortened life span (50). All forms of obesity are associated with hypertrophy of adipose cells, and more severe forms also show hyperplasia (37). Interestingly, transgenic obese mice selectively overexpressing adipose GLUT4 exhibit dramatic increases in hyperplasia (cell num-
bers increase >2-fold) but not in cell size of adipocytes (36).

KKAy mouse SSAO-mediated deamination mimics the action of insulin in vivo and, at least in part, regulates adipose GLUT4 function. Increased SSAO in KKAy mice (46) may account for the weight gain.

Obesity is associated with type 2 diabetes, and the basis for this link is the ability of obesity to evoke insulin resistance (17, 18). Insulin resistance is linked to the etiology of pathological sequelae such as hyperlipidemia, atherosclerosis, etc. Insulin not only promotes glucose uptake in adipocytes but also enhances adipocyte triglyceride storage and adipocyte differentiation and maturation. SSAO-mediated deamination exerts very similar effects on adipocytes (13, 24, 27). Our observation that selective inhibition of SSAO activity prevents weight gain in KKAy mice is probably a result of a reduction of adipose glucose uptake and subsequent effect on adipose differentiation. Serum SSAO activity is significantly higher in obese people (28, 40) and is positively correlated to body mass index (28). This supports the notion that increased SSAO may be involved in adipose differentiation related to weight gain. An increase in vascular SSAO activity could also concomitantly increase production of toxic aldehydes and oxidative stress, possibly causing vascular damage, protein cross-linkage, plaque formation, and atherosclerosis (41). Increased serum SSAO activity was found in diabetes (4, 14, 26, 29, 49) and in heart patients (3, 5) and diabetic animals (9, 16). SSAO inhibitor has been shown to reduce vascular damage in animal models (43, 44, 47). SSAO-mediated deamination involved in pathogenesis related to obesity, as well as vascular complications associated with type 2 diabetes, warrants further attention and investigation.

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Fig. 6. Effect of methylamine and SSAO inhibitors on serum glucose levels. 

A: effects of a single dose of methylamine (100 mg/kg ip) and/or 2-BrEA (or Br, 25 mg/kg ip) on blood glucose levels assessed at different times. B: time course of effect of a single dose of FPFA (5 mg/kg ip) on mouse blood glucose levels. Data represent means ± SE of ≥5 animals. aP < 0.01 compared with corresponding saline control group.

Fig. 7. Effect of SSAO inhibitor on glucose tolerance in mice. 

A: glucose challenge experiment. FPFA (5 mg/kg ip) was administered to the animals 1 h before injection of glucose (1 g/kg ip). Blood glucose levels were assessed over a period of 90 min. Data represent means ± SE of 9 animals. B: effect of FPFA on insulin-induced (0.75 U/kg ip) reduction of blood glucose. Data represent means ± SE of 5 animals. aP < 0.01 compared with corresponding saline control group.
REFERENCES


