Regulation of Semicarbazide-Sensitive Amine Oxidase Expression by Tumor Necrosis Factor-α in Adipocytes: Functional Consequences on Glucose Transport

NATHALIE MERCIER, MARTHE MOLDES, KHADIJA EL HADRI, and BRUNO FÈVE

Unité Mixte de Recherche 7079, CNRS-Paris VI, Centre de Recherches Biomédicales des Cordeliers, Paris, France

Received September 17, 2002; accepted December 4, 2002

ABSTRACT

Membrane-associated semicarbazide-sensitive amine oxidase (SSAO) is mainly present in the media of aorta and in adipose tissue. Recent works have reported that SSAO activation can stimulate glucose transport of fat cells and promote adipose conversion. In this study, the murine 3T3-L1 preadipocyte cell line was used to investigate SSAO regulation by tumor necrosis factor-α (TNF-α), a cytokine that is synthesized in fat cells and known to be involved in obesity-linked insulin resistance. SSAO mRNA and protein levels, and enzyme activity were decreased by TNF-α in a dose- and time-dependent manner, with no change of SSAO affinity for substrates or inhibitors. SSAO inhibition caused by TNF-α was spontaneously reversed along the time after TNF-α removal. The decrease in SSAO expression also occurred in white adipose tissue of C57BL/6 mice treated with mTNF-α. Overall, we demonstrated that reduction in SSAO expression induced by the cytokine had marked repercussions on amine-stimulated glucose transport, in a dose- and time-dependent manner. This effect was more pronounced than the inhibiting effect of TNF-α on insulin-stimulated glucose transport. Moreover, the peroxisome proliferator-activated receptor γ agonists thiazolidinediones did not reverse either TNF-α effect on amine-sensitive glucose transport or the inhibition of SSAO activity, whereas they antagonized TNF-α effects on insulin-sensitive glucose transport. These results demonstrate that TNF-α can strongly down-regulate SSAO expression and activity, and through this mechanism can dramatically reduce amine-stimulated glucose transport. This suggests a potential role of this regulatory process in the pathogenesis of glucose homeostasis dysregulations observed during diseases accompanied by TNF-α overproduction, such as cachexia or obesity.

Copper-containing amine oxidases form a specific family of enzymes that deaminate some aromatic or aliphatic amines to generate ammonia, hydrogen peroxide, and the corresponding aldehydes. An original member of this group is a membrane-bound amine oxidase, highly inhibited by semicarbazide, and often referred to as the “tissue-bound” semicarbazide-sensitive amine oxidase (SSAO) (Lyles, 1996; Jalkanen and Salmi, 2001). This enzyme readily oxidizes exogenous (e.g., benzylamine) or endogenous (e.g., methylamine and aminooacetone) primary amines. SSAO, which is identical to vascular adhesion protein-1, has been cloned in different species (Zhang and McIntire, 1996; Morris et al., 1997; Bono et al., 1998; Smith et al., 1998; Moldes et al., 1999). SSAO activity and transcripts have been detected in a variety of tissues and cell types (Lyles, 1996), but they are prominently expressed in vascular smooth muscle cells (Lyles, 1996), endothelial cells of lymph venules (Jalkanen and Salmi, 2001), and in white and brown adipocytes (Barrand and Callingham, 1982; Raimondi et al., 1991).

The physiological and pathophysiological roles of SSAO are still unclear and depend on the cell type on which the enzyme is expressed (Lyles and Pino, 1998; Jalkanen and Salmi, 2001; El Hadri et al., 2002). In fat cells, it has been recently documented that SSAO expression is strongly induced during preadipocyte differentiation (Moldes et al., 1999; Fontana et al., 2001) and that SSAO chronic activation promotes terminal adipocyte maturation (Fontana et al., 2001; Mercier et al., 2001). Furthermore, SSAO is not exclusively present at the plasma membrane of adipocytes, but is also detectable in vesicles containing the insulin-sensitive glucose transporter GLUT4 (Morris et al., 1997; Enrique-Tarancon et al., 1998). The acute effect of insulin on glucose transport can be mimicked by SSAO substrates (Enrique-Tarancon et al., 1998,
2000; Marti et al., 1998; Fontana et al., 2001; Morin et al., 2001) through the release of hydrogen peroxide (Enrique-Tarancon et al., 1998; Marti et al., 1998). SSAO substrates can also promote IRS-1 and -3 tyrosine phosphorylation, and stimulate phosphatidylinositol 3-kinase activity and GLUT4 translocation to the plasma membrane (Enrique-Tarancon et al., 2000). A recent study (Marti et al., 2001) has underlined the potential therapeutic interest of SSAO in the control of glycemia: an acute or chronic administration of the synthetic SSAO substrate benzylamine in combination with low doses of vanadate enhances glucose tolerance and reduces hyperglycemia in streptozotocin-induced diabetic rats. Interestingly, several studies have reported that SSAO activity is increased in serum from diabetic (Boomsma et al., 1999; Meszaros et al., 1999) or obese (Meszaros et al., 1999) patients. Taken together, these observations suggest that adipocyte SSAO could play a significant role in the control of glucose homeostasis.

TNF-α is a cytokine involved in the clinical and metabolic disturbances observed in obesity-related insulin resistance: TNF-α is overexpressed in adipose tissue of obese rodents or humans, and administration of TNF-α to animals induces insulin resistance, whereas neutralization of TNF-α improves insulin sensitivity (Hotamisligil, 2000; Moller, 2000). Otherwise, results from knockout mice deficient in TNF-α or its receptors indicate that the cytokine can regulate in vivo insulin sensitivity and is involved at least partly in the onset of obesity-associated insulin resistance (Hotamisligil, 2000; Moller, 2000). However, the exact contribution of TNF-α to the pathophysiology of insulin resistance observed in human obesity remains controversial (Moller, 2000).

Multiple cellular and molecular mechanisms could account for the metabolic effects of TNF-α on adipose tissue. Thus, the cytokine potently suppresses the expression of genes encoding proteins involved in fatty acid uptake and lipogenesis. TNF-α also inhibits preadipocyte differentiation (Torti et al., 1985) and provokes apoptosis (Prins et al., 1997). TNF-α stimulates lipolysis and increases free fatty acids through different mechanisms. It is also well known that in adipocytes, TNF-α strongly inhibits insulin-stimulated glucose transport (Stephens and Pekala, 1991; Szalkowski et al., 1995), through different alterations in insulin signaling pathway (Hotamisligil, 2000; Moller, 2000).

Considering the TNF-α-induced alterations in adipocyte hexose transport, and as regards to the promoting effect of SSAO activation on fat cell glucose uptake, the aim of our study was to investigate the impact of TNF-α on the expression and function of this membrane-associated amine oxidase. Using the murine preadipose 3T3-L1 cell line, we demonstrate that TNF-α decreases SSAO expression without altering adipocyte differentiation level. This effect is also observed in white adipose tissue of TNF-α-injected mice. Overall, this TNF-α-induced down-regulation of SSAO expression is involved in a dramatic reduction in amine-stimulated glucose transport.

**Materials and Methods**

**Cell Culture.** Stocks of murine 3T3-L1 preadipocytes were maintained as described previously (Moldes et al., 1999). For experiments, cells were seeded at a density of 10^5/cm² in plastic culture dishes (Falcon, Cowley, UK) and were grown in DMEM supplemented with 10% fetal calf serum (Biomedical, Boussens, France), 100 units/ml penicillin, and 50 μg/ml streptomycin (Invitrogen, Carlsbad, CA) in a 10% CO₂ humidified atmosphere. Adipocyte differentiation was initiated by administration of 0.5 μM of adipogenic agent, vitamin A (100 μM), dexamethasone (0.25 μM), and insulin (1 μg/ml) for 48 h, then cells were refed by DMEM containing 10% fetal calf serum and 1 μg/ml insulin. Using this protocol, more than 95% of the cells acquired an adipocyte morphology at day 8 after confluence. After washing, mature adipocytes were kept for 16 h in a defined medium consisting of DMEM/Ham’s F-12 medium (2:1, v/v) and 0.1% bovine serum albumin. Thereafter, cells were maintained either in the absence or in the presence of mTNF-α (Sigma-Aldrich, St. Louis, MO) at concentrations and for periods of time mentioned under Results.

**Animals.** Four-week-old male C57BL/6 mice received a single intraperitoneal injection of 1 μg of recombinant mTNF-α. Forty-eight hours after TNF-α administration, animals were killed and epididymal fat pads were quickly excised and immediately frozen in liquid nitrogen until preparation of tissue extracts. Experiments were undertaken according to the Guidelines for the Care and the Use of Experimental Animals.

**Cell and Tissue Extracts, Biochemical Determinations, and Enzyme Assays.** 3T3-L1 cells were washed twice in PBS, harvested, and homogenized in 25 mM Tris, pH 7.5, 1 mM EDTA (20 strokes in a Dounce homogenizer, pestle B). A fraction of the homogenate was stored at –80°C. The remaining fraction was centrifuged at 10,000g for 10 min at 4°C, and the supernatant was kept at –80°C until use. The 10,000g pellet was resuspended in the homogenization buffer and stored at –80°C. Aliquots of homogenates, supernatants, and resuspended pellets were used to determine protein content by the method of Lowry, using bovine serum albumin as a standard. Triacylglyceride concentration was determined with the Infinity triglycerides kit (Sigma Diagnostics, St. Louis, MO).

For preparation of tissue extracts, the epidydimal fat depot was homogenized in a buffer consisting of KH2PO4 1 mM, pH 7.8, 250 mM sucrose. After a centrifugation at 600g for 5 min at 4°C, the pellet and the fat cake were discarded, and the supernatant was kept at –80°C until SSAO activity measurement.

SSAO activity was tested by measurement of hydrogen peroxide production by the fluorometric method of Matsumoto et al. (1982). Briefly, 25 μg of cell homogenates was preincubated for 30 min at 37°C, in a final volume of 100 μl containing 40 mM sodium phosphate, pH 7.4, 1 mM homovanillic acid, 1 mM sodium azide, and 1 mM pargyline to inhibit monoamine oxidase A and B. An SSAO inhibitor was present in cell extract for 30 min at 37°C before the addition of the substrate. Incubation was initiated by the addition of the SSAO substrate (benzylamine, methlyamine, tyramine, or β-phenylethylamine) and was carried out in duplicate for 1 h at 37°C. Reaction was stopped with 1 mM semicarbazide, and 1.2 ml of 0.1 N NaOH was added. Fluorescence intensity was measured with excitation at 323 nm and emission at 426 nm. As blank tests, assays were incubated in parallel without substrate addition. Preliminary experiments were performed to ensure that SSAO activity was tested at the initial rate of reaction. Apparent kinetic constants for SSAO substrates were determined within the following concentration ranges: 3 to 300 μM for benzylamine, 0.1 to 10 mM for methlyamine, 0.1 to 10 mM for tyramine, and 30 μM to 3 mM for β-phenylethylamine. To determine IC50 and K values for amine oxidase inhibitors, SSAO activity was determined in the presence of 30 μM benzylamine as a substrate. The concentration ranges of inhibitors used were as follows: 1 μM to 1 mM semicarbazide, 1 to 100 μM aminoguanidine, 0.1 to 100 nM phenelzine, 1 to 100 nM hydrazine, 3 to 100 nM hydralazine, and 30 nM to 3 μM benserazide. Kinetic parameters were determined using the nonlinear regression analysis curve fitting procedure of the ENZFITTER program ( Biosoft-Elsevier, Cambridge, UK).

Monoamine oxidase activity was tested under conditions identical to those for SSAO, except that pargyline was omitted and replaced by...
1 mM semicarbazide, and tyramine or serotonin was used as substrate. Polyamine oxidase activity was also tested with the same procedure, using \(N^2\)-acetyl spermine as a substrate.

G3PDH DH was assessed by reverse transcription of NADH at 340 nm at 25°C (Mercier et al., 2001). The standard mixture contained 50 mM triethanolamine-HCl buffer, pH 7.5, 1 mM EDTA, 0.13 mM \(\beta\)-NADH, 1 mM dihydroxyacetone phosphate, 1 mM 2-mercaptoethanol, and variable amounts of 10,000 g cell supernatants.

Adenyl cyclase activity was measured on 10,000 g pellet fractions as described previously (El Hadri et al., 1997).

All enzyme substrates and inhibitors were from Sigma-Aldrich.

**Western Blot Analysis.** Cells were washed three times with cold PBS, harvested, and homogenized in 1 ml of HES buffer (20 mM Hepes, pH 7.4, 1 mM EDTA, 250 mM sucrose) supplemented with a cocktail of anti-proteases (Complete; Roche Diagnostics, Indianapolis, IN). Homogenates were centrifuged at 200,000 g for 90 min at 4°C. The resulting pellets were resuspended in 30 mM Hepes buffer, pH 7.4, and stored at –80°C.

SDS-polyacrylamide gel electrophoresis was performed with a mini protein III apparatus (Bio-Rad, Hercules, CA). Proteins (30 µg/lane) were boiled for 5 min at 95°C, separated on a 7.5% polyacrylamide-SDS gel, and electroblotted onto 0.45-µm polyvinylidene difluoride membrane (Immobilon-P; Millipore Corporation, Bedford, CA) in 0.1% SDS, 192 mM glycine, and 25 mM Tris, pH 8.3. The membrane was dipped in a methanol bath and then blocked with 5% fish gelatin, 0.1% Tween 20 in TBS buffer (20 mM Tris, 137 mM NaCl, pH 7.6) for 45 min at room temperature. After an initial washing in TBS buffer containing 0.1% Tween 20, the membrane was incubated overnight at 4°C with primary antibodies (rabbit polyclonal antibody against bovine lung SSAO, dilution 1:2000; Lizcano et al., 1998) in TBS buffer containing 2% fish gelatin and 0.05% Tween 20. After four washes with 0.1% Tween 20-TBS, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit IgG (dilution 1:20,000 in TBS buffer containing 0.05% Tween 20 and 2% fish gelatin), for 1 h at room temperature. The membrane was then washed in 0.1% Tween 20-TBS. Detection of immune complex was performed using an enhanced chemiluminescence detection kit for Western blot (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK) on a X-OMAT-AR Kodak film (Eastman Kodak, Rochester, NY).

**RNA Analysis.** Total RNA was extracted from 3T3-L1 adipocytes and from epididymal white adipose tissue with the RNA-PLUS kit procedure (QuiBIO, Ulm, France), followed by 2% agarose gel electrophoresis (M. Tevfik Dorak). Sequences of the sense and antisense oligonucleotides were 5′-CCCCCTGCCCTAT-TACCG-3′ and 5′-AAAAACCCAGCCCTTGAGAGA-3′ for SSAO; and 5′-GGGAGCCTGAGAAACCGC-3′ and 5′-GGTTCGGGAGTAGG-TAATT-3′ for 18S ribosomal RNA.

**Determination of 2-Deoxyglucose Uptake.** For determination of hexose transport, cells were seeded in 24-well plates, and were grown, differentiated, and exposed to TNF-α as mentioned above. When the thiazolidinedione rosiglitazone was tested for its ability to reverse TNF-α effect on glucose transport, it was added into the culture medium together with the cytokine. The uptake of glucose was determined using [1,2-3H]deoxyglucose ([3H]DOG) (ICN Pharmaceuticals, Costa Mesa, CA), a nonmetabolizable analog of glucose. Mature 3T3-L1 adipocytes were rinsed three times with Krebs-Ringer buffer containing 12 mM Hepes, pH 7.4 and then preincubated for 2 h at 37°C in 500 µl of the Krebs-Ringer buffer containing 12 mM Hepes, pH 7.4. When indicated, semicarbazide (1 mM) was added into the preincubation period 90 min before measurement of hexose transport. Sodium orthovanadate (100 µM), insulin (100 nM), or benzylamine (300 µM) was introduced 60 min before [3H]DOG-[3H]DOG uptake measurement. Glucose uptake measurement was initiated by addition of [3H]DOG (0.2 mM, 0.5 µCi/well). After 5 min at 37°C, the experiment was terminated by rapidly aspirating the radioactive incubation buffer and rinsing the cells three times with ice-cold PBS. The cells were solubilized with 1% SDS, and radioactivity was determined by scintillation counting. To determine non-carrier-mediated glucone transport, 10 µM cytochalasin B was used in parallel wells. Non-carrier-mediated glucose uptake accounted for less than 3% of total glucose transport. Preliminary experiments were performed to ensure that [1,2-3H]deoxyglucose uptake was tested at the initial rate of the transport. The results were expressed as picomoles of glucose transported per minute per well.

**Statistical Analyses.** Results are presented as means ± S.E. The statistical comparison of data between groups was assessed with analysis of variance using the STATVIEW software.

**Results**

TNF-α, through multiple effects on lipid and carbohydrate metabolism, has been implicated in the pathogenesis of obesity-linked insulin resistance (Hotamisligil, 2000; Moller, 2000). Because recent studies have shown that SSAO activation can promote hexose uptake and contribute to glucose homeostasis (Enrique-Tarancon et al., 1998, 2000; Marti et al., 1998, 2001), it was of interest to evaluate the possible
action of TNF-α on adipocyte SSAO expression and its consequences on SSAO-mediated glucose transport.

**TNF-α Down-Regulates SSAO Expression in Vitro and in Vivo.** SSAO regulation by TNF-α was first investigated in vitro, using the murine preadipose 3T3-L1 cell line as a model. Although SSAO transcripts and enzyme activity are virtually absent in 3T3-L1 preadipocytes, there is a dramatic increase in SSAO mRNA levels and enzyme activity when cells undergo adipose conversion (Moldes et al., 1999).

In a first set of experiments, 3T3-L1 mature adipocytes were exposed to various TNF-α concentrations for 24 or 48 h. Results shown in Fig. 1A indicate that SSAO mRNA levels were dramatically decreased in the presence of increasing concentrations of TNF-α. Repression of SSAO transcript abundance was statistically detectable at a cytokine concentration of 10 pM and was maximal at 1 nM (8-fold reduction in SSAO mRNA levels compared with control), giving a half-maximal effect between 100 and 300 pM. These TNF-α-induced changes in SSAO mRNA levels were followed by corresponding variations in SSAO protein (Fig. 1B). Western blot analysis of protein extracts from control or cytokine-exposed 3T3-L1 adipocytes show a slight reduction in SSAO protein levels at 100 pM TNF-α (90 ± 4.5% of control), with a maximal effect at 1 nM of the cytokine (37.5 ± 3.5% of control). In agreement with variations in SSAO mRNA and protein levels, SSAO enzyme activity was significantly decreased at 100 pM and was maximally inhibited at 1 nM TNF-α (25 ± 3.7% of control value), giving a half-maximal effect at about 200 pM (Fig. 1C).

The time dependence of SSAO down-regulation by TNF-α was also studied. For that purpose, 3T3-L1 mature adipocytes were exposed for 1 to 72 h to a constant concentration of TNF-α (0.5 nM). Note that whatever the time exposure to TNF-α, all cell extracts were prepared at the same time point, implicating that the first cells treated by the cytokine correspond to the 72-h condition. The same procedure was used for all subsequent time-dependent experiments. SSAO mRNA expression was significantly decreased after a 3-h exposure to TNF-α (60% of control value) (Fig. 2A), and the maximal effect was observed after a 24-h treatment by the cytokine and represented 25% of control levels. SSAO protein expression (Fig. 2B) was moderately decreased (53% of control protein level) after a 12-h exposure to TNF-α. Maximal reduction in protein levels was obtained after 48 h (39% of control value). A significant reduction in SSAO enzyme activity was detectable from a 24-h exposure to TNF-α (63% of control value) and continued to decrease until 72 h (34% of control value) (Fig. 2C). These initial dose- and time-dependent studies show a good correlation between the TNF-α-induced down-regulation of SSAO mRNA and protein levels and enzyme activity, and suggest that the repression in SSAO gene expression by the cytokine is responsible for the decrease in SSAO activity.

To verify that TNF-α effect on SSAO was primarily related to a decrease in $V_{\text{max}}$ enzyme activity and was not a consequence of a modification of SSAO affinity toward its substrates or inhibitors, kinetic parameters of SSAO activity were analyzed with different SSAO substrates (Table 1) and inhibitors (Table 2). 3T3-L1 mature adipocytes were cultured in the absence or in the presence of 0.3 nM TNF-α for 48 h. This submaximal concentration of TNF-α was chosen because it induced a clear but moderate decrease in SSAO maximal activity, thus allowing an exact determination of $K_m$ and $K_i$ values for substrates and inhibitors, respectively. As shown in Table 1, $K_m$ values of SSAO for the four tested substrates, i.e., benzylamine, methylamine, tyramine, and β-phenylethylamine, were not modified by TNF-α treatment.
On the contrary, $V_{\text{max}}$ values obtained with each SSAO substrate were reduced by 45 to 56% in TNF-α-treated cells compared with $V_{\text{max}}$ values of control cells. Likewise, the $K_i$ values of SSAO for several well-characterized inhibitors remained unaffected by TNF-α exposure (Table 2). Thus, TNF-α-induced alterations in SSAO enzyme activity really correspond to a reduction in $V_{\text{max}}$ without change in enzyme affinity for its substrates or inhibitors.

TNF-α is known to inhibit adipocyte differentiation and to induce adipocyte dedifferentiation (Torti et al., 1985). Because SSAO represents a marker of terminal adipocyte differentiation, it was of importance to verify that under our experimental conditions, the TNF-α-induced decrease in SSAO expression was not related to a general reduction in adipocyte maturation level. Thus, G3PDH activity and triglyceride concentration, two classical biochemical markers of adipose conversion (Pairault and Green, 1979; Gaillard et al., 1989), were measured in parallel with SSAO activity in control and TNF-α-treated cells. First, 3T3-L1 differentiated adipocytes were exposed to various concentrations of TNF-α for 48 h (Fig. 3A). G3PDH activity was only slightly inhibited in the presence of the highest TNF-α concentration tested. TNF-α at 0.3 nM induced a 6% decrease in G3PDH activity (Fig. 3A), whereas the corresponding SSAO activity was reduced by 52% (Fig. 1C). The maximal inhibition (27%) in G3PDH activity was observed at 1 nM TNF-α, a concentration at which SSAO activity was decreased by 75%. Even at the highest TNF-α concentrations, cell triglyceride content remained unchanged (Fig. 3A). Second, 3T3-L1 mature cells were exposed to 0.5 nM TNF-α for various periods of time (6–72 h) (Fig. 3B). G3PDH activity was weakly decreased (20–34% inhibition compared with control) from 24 to 72 h of TNF-α exposure, whereas triglyceride content of cytokinetreated cells was not significantly modified. However, SSAO activity was markedly reduced (37–66% inhibition compared with control) during the same interval (Fig. 2C). Thus, there was a striking discrepancy between the very poor TNF-α-induced inhibition in adipocyte maturation level and the strong inhibition of SSAO expression and enzyme activity induced by the cytokine. This observation underlines that the down-regulation of SSAO by TNF-α really corresponds to a specific gene and protein modulation and is not related to a general dedifferentiating effect.

To evaluate the capacity of mature adipocytes to spontaneously reverse the TNF-α-induced inhibition in SSAO activity, 3T3-L1 adipocytes were initially cultured for 48 h in the absence or in the presence of 1 nM TNF-α, a concentration that potently down-regulated SSAO expression. Thereafter, cells were rinsed extensively, and TNF-α was omitted in some dishes previously cultured in the presence of the cytokine. SSAO activity was then tested on cell homogenates at different intervals (12–72 h) after TNF-α removal (Fig. 4). As expected, a 48-h exposure to 1 nM TNF-α caused a dramatic 70% decrease in SSAO activity (time 0 after TNF-α removal). When TNF-α was maintained in the culture medium, SSAO activity continued to be down-regulated, reaching 6% of control SSAO activity after an additional 72-h exposure to the cytokine. When TNF-α was removed after an initial 2-day treatment, there was an initial decrease in SSAO activity that likely reflected the slow turnover of SSAO protein and/or a persistence in TNF-α biological effect. Thereafter, from 48 h after TNF-α removal, there was a marked increase in SSAO activity that reached 60% of the control value at 72 h.

To test whether TNF-α effect on amine oxidases was restricted to SSAO, the activity of two amine oxidases present in fat cells, monoamine oxidase and polyamine oxidase, was also tested in control and TNF-α-treated 3T3-L1 adipocytes. SSAO activity was measured in the presence of 1 mM pargyline to block any residual monoamine oxidase activity. Under these conditions, benzylamine, methylamine, tyramine, and β-phenylethylamine oxidase activities were totally inhibited by a prior incubation with 1 mM semicarbazide, indicating that they corresponded to a SSAO activity (data...
TABLE 1  
Kinetic parameters of SSAO activity in 3T3-L1 adipocytes cultured in the absence or in the presence of TNF-α
Cell homogenates were prepared from mature 3T3-L1 adipocytes exposed or not to 0.3 nM TNF-α for 48 h. Enzyme activity towards benzylamine, methylamine, tyramine, and β-phenylethylamine were performed as described under Materials and Methods, with a prior incubation with 1 mM of the monoamine oxidase-selective inhibitor pargyline. Since in control or TNF-α-treated cells, less than 5% of amine oxidase activity remained when cells extracts were preincubated in the presence of 1 mM semicarbazide, it corresponded to the SSAO activity (not shown). $K_m$ and $V_{max}$ values were calculated by the nonlinear regression analysis curve-fitting procedure of the ENZFITTER program. The percentage of amine oxidase activity observed in TNF-α-treated compared with control cells is also mentioned in the right column. Results represent the mean ± S.E. of four to five separate experiments performed in duplicate.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (nmol/h/mg)</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (nmol/h/mg)</th>
<th>Percentage of Control $V_{max}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>TNF-α</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyllamine</td>
<td>21.6 ± 2.0</td>
<td>31.6 ± 1.0</td>
<td>17.5 ± 1.7</td>
<td>17.2 ± 1.4**</td>
<td>56</td>
</tr>
<tr>
<td>Methylamine</td>
<td>430.6 ± 12.0</td>
<td>24.2 ± 1.6</td>
<td>415.6 ± 6.1</td>
<td>12.9 ± 0.5**</td>
<td>53</td>
</tr>
<tr>
<td>Tyramine</td>
<td>1295.1 ± 288.1</td>
<td>5.1 ± 0.7</td>
<td>1124.0 ± 246.8</td>
<td>2.3 ± 0.2**</td>
<td>45</td>
</tr>
<tr>
<td>β-Phenylethylamine</td>
<td>108.9 ± 7.5</td>
<td>9.0 ± 0.8</td>
<td>133.8 ± 24.9</td>
<td>4.9 ± 0.1*</td>
<td>54</td>
</tr>
</tbody>
</table>

* $p < 0.05$; ** $p < 0.01$; TNF-α-treated versus control cells.

TABLE 2  
Inhibition of SSAO activity by different compounds in control and TNF-α-exposed cells
Cell extracts were prepared from mature 3T3-L1 adipocytes cultured in the absence or presence of 0.3 nM TNF-α for 48 h. 3T3-L1 cell homogenates were preincubated for 30 min at 37°C in the presence of various concentrations of each inhibitor. Pargyline at 1 mM was also added in the assay to inhibit monoamine oxidase activity. Benzyllamine (30 μM) was then added as substrate for 1 h. $IC_{50}$ values were obtained by computer analysis with the ENZFITTER program. Apparent $K_i$ values of SSAO towards the different SSAO inhibitors were then calculated by the equation of Cheng and Prusoff. Results represent the mean ± S.E. of four separate experiments.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_i$ (nM)</th>
<th>Control $V_{max}$</th>
<th>TNF-α $V_{max}$</th>
<th>Percentage of Control $V_{max}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semicarbazide</td>
<td>5720 ± 390</td>
<td>4510 ± 240</td>
<td>5400 ± 140</td>
<td></td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>5200 ± 340</td>
<td>4440 ± 490</td>
<td>5400 ± 140</td>
<td></td>
</tr>
<tr>
<td>Phenelzine</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Hydrazine</td>
<td>2.4 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Hydralazine</td>
<td>58.2 ± 6.6</td>
<td>47.9 ± 8.2</td>
<td>47.9 ± 8.2</td>
<td></td>
</tr>
<tr>
<td>Benserazide</td>
<td>64.4 ± 5.2</td>
<td>49.1 ± 6.2</td>
<td>49.1 ± 6.2</td>
<td></td>
</tr>
</tbody>
</table>

not shown). In contrast, monoamine oxidase activity was measured using serotonin or tyramine as substrates and in the presence of 1 mM semicarbazide to block SSAO activity. Under these conditions, serotonin or tyramine oxidase activities were totally inhibited by a prior incubation with 1 mM pargyline, indicating that they corresponded to a monoamine oxidase activity (data not shown). After a 48-h exposure, to 0.3 nM TNF-α, there was a clear and significant decrease in SSAO activity, regardless of the SSAO substrate used in the assay (Table 3). In contrast, in TNF-α-treated cells, monoamine oxidase activity tested in the presence of serotonin or tyramine was increased by 42 and 22%, respectively. Polyamine oxidase activity was assayed using N1-acetyl spermidine as a substrate and was similar between control and cytokine-exposed 3T3-L1 adipocytes.

We have previously reported that effectors of the cAMP signaling pathway, (-)-isoproterenol and forskolin, are able to decrease SSAO mRNA levels and enzyme activity (Moldes et al., 1999). Thus, we examined whether a chronic exposure to TNF-α could modify cAMP production and participate to the cytokine-induced down-regulation in SSAO activity. Adenyl cyclase activity was measured in membranes from control cells and from 3T3-L1 adipocytes treated with 0.5 nM TNF-α for 48 h. As observed in 3T3-F442A adipocytes (El Hadri et al., 1997), cell exposure to TNF-α led to a moderate but nonsignificant 1.5-fold increase in basal adenyl cyclase activity (0.58 ± 0.04 and 0.88 ± 0.18 pmol of cAMP/min/mg protein in control and TNF-α-treated cells, respectively; $n = 8$; $p = 0.095$). As controls and in agreement with TNF-α effect on 3T3-F442A fat cells (El Hadri et al., 1997), we observed that the cytokine decreased (−)-isoproterenol-stimulated adenyl cyclase activity and did not modify forskolin-stimulated adenyl cyclase activity (data not shown). However, this slight TNF-α-induced increase in basal cAMP production did not seem involved in the down-regulation of SSAO activity by the cytokine. Indeed, the cAMP-dependent protein kinase selective inhibitor H89 did not prevent the reduction in SSAO activity caused by the cytokine (data not shown).

Finally, to more completely characterize TNF-α effect on SSAO expression, we investigated the consequences of an in vivo administration of the cytokine on the expression and activity of the amine oxidase. Four-week-old male C57BL/6 mice received a single intraperitoneal injection of 1 μg of TNF-α, and animals were sacrificed 48 h after injection. SSAO mRNA levels were examined in epididymal white adipose tissue by real time RT-PCR analysis (Fig. 5A). There was a marked 51% decrease of SSAO mRNA levels in TNF-α-injected mice compared with control animals. Likewise, SSAO activity was tested in epididymal fat pad extracts using benzylamine or methylamine as substrates. As shown in Fig. 5B, SSAO activity of TNF-α-injected mice was impaired compared with that of control animals, with a 33 and 34% reduction in benzylamine and methylamine oxidase activities, respectively. Thus, TNF-α is able to suppress SSAO expression both in vitro and in vivo.

**Functional Consequences on Glucose Uptake of TNF-α-Induced SSAO Down-Regulation.** Recent studies have reported that SSAO activation in response to its substrates can stimulate glucose transport in adipocytes (Enrique-Tarancon et al., 1998, 2000; Marti et al., 1998; Fontana et al., 2001; Morin et al., 2001). Otherwise, TNF-α is well known to inhibit insulin-stimulated glucose transport (Stephens and Pekala, 1991) and to be involved in the pathogenesis of obesity-associated insulin resistance (Hotamisligil, 2000; Moller, 2000). It was thus consistent to examine whether TNF-α-induced down-regulation of SSAO could also be implicated in the modulation of amine-stimulated glucose transport.

In this aim, preliminary experiments were performed on mature 3T3-L1 adipocytes treated or not with 0.5 nM TNF-α for 48 h. [3H]DOG incorporation was then estimated after a
TNF-α Modulation of SSAO-Mediated Glucose Uptake

1-h incubation with 100 nM insulin or 300 μM benzylamine, in the absence or in the presence of 100 μM sodium orthovanadate for the same period. In rat or 3T3-L1 adipocytes, low concentrations of sodium orthovanadate have been shown to be required to activate glucose transport in response to SSAO substrates (Enrique-Tarancon et al., 1998; Marti et al., 1998). As shown in Fig. 6A, benzylamine alone did not modify [3H]DOG uptake. When 3T3-L1 cells were preincubated in the presence of sodium orthovanadate, at a concentration (100 μM) that had no significant effect alone, there was a clear potentiation of benzylamine action on glucose transport. Under these conditions, benzylamine caused a 2.35-fold increase in basal [3H]DOG uptake. In contrast, insulin-stimulated glucose transport was not influenced in the presence of sodium orthovanadate. As previously documented in several studies (Stephens and Pekala, 1991; Szalkowski et al., 1995), TNF-α exposure led to a decrease in insulin-stimulated glucose transport. When cells were treated with 0.5 nM TNF-α for 48 h, the insulin-stimulated [3H]DOG uptake over basal transport was reduced by 40%. The presence of sodium orthovanadate did not influence TNF-α effect on insulin-activated hexose uptake. Under the same conditions, amine-stimulated [3H]DOG uptake was more strongly inhibited by TNF-α exposure, because in response to benzylamine hexose transport over basal was reduced by 66% in comparison with control cells. This observa-

**TABLE 3**

Effects of TNF-α on several amine oxidase activities present in mature 3T3-L1 adipocytes

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Substrate</th>
<th>Culture condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSAO</td>
<td>Benzylamine (100 μM)</td>
<td>Control, TNF-α</td>
</tr>
<tr>
<td></td>
<td>Methylamine (3 mM)</td>
<td>25.0 ± 2.3, 15.4 ± 1.6***</td>
</tr>
<tr>
<td></td>
<td>Tyramine (10 mM)</td>
<td>16.5 ± 0.5, 11.5 ± 0.7**</td>
</tr>
<tr>
<td></td>
<td>β-Phenylethylamine (1 mM)</td>
<td>3.6 ± 0.1, 2.1 ± 0.2**</td>
</tr>
<tr>
<td>MAO</td>
<td>Serotonin (100 μM)</td>
<td>7.4 ± 0.6, 4.1 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>Tyramine (1 mM)</td>
<td>4.7 ± 0.3, 6.7 ± 0.4*</td>
</tr>
<tr>
<td>PAO</td>
<td>N⁴-Acetyl-spermidine (1 mM)</td>
<td>56.5 ± 3.9, 68.5 ± 0.5**</td>
</tr>
</tbody>
</table>

\*p < 0.05; \**p < 0.02; \***p < 0.01; TNF-α-treated versus control cells.

Table 3 shows the effects of TNF-α on several amine oxidase activities present in mature 3T3-L1 adipocytes. The data were obtained from experiments performed in duplicate. Control G3PDH activity was 1104.6 ± 56.6 nmol of NADH/min/mg of protein, and control triglyceride concentration was 2.19 ± 0.11 mM. B, cells were exposed for various periods of time to 0.5 nM TNF-α. Thereafter, the cells were washed and refed, and the procedure was pursued in control cells (open diamonds) and in some of TNF-α-treated cells (closed squares), whereas a part of the dishes initially exposed to the cytokine were shifted into a TNF-α-free medium (closed triangles). Cells homogenates were prepared at various intervals (0–72 h) after TNF-α removal, and SSAO enzyme activity was measured with a fluorometric detection of hydrogen peroxide production. Results represent the mean ± S.E. of two independent experiments and are expressed as percentage of control value at each time point.
tion suggests that down-regulation in SSAO expression could play a role in the dramatic TNF-α-induced decrease in amine-stimulated glucose transport.

In subsequent experiments, because sodium orthovanadate was required to observe benzylamine effect on [3H]DOG uptake, it was added in all assays, including those testing basal glucose transport. To ensure that the induction of [3H]DOG uptake by benzylamine was the consequence of SSAO activation, semicarbazide was added 30 min before benzylamine addition (Fig. 6B). Whereas semicarbazide alone had no effect on glucose transport, it completely abolished benzylamine-stimulated [3H]DOG uptake in control cells.

To further characterize the functional consequences of TNF-α-induced repression of SSAO expression on glucose transport, 3T3-L1 cells were grown in 24-well plates until day 8 after confluence. Thereafter, cells were maintained in DMEM/Ham’s F-12 supplemented with 0.1% bovine serum albumin and treated (filled columns) or not (open columns) with 0.5 nM TNF-α for 48 h. Cells were then tested for [3H]DOG (final concentration 0.2 mM, 0.5 µCi/well) incorporation. A. when indicated, 100 µM sodium orthovanadate (NaV), 100 nM insulin, and 300 µM benzylamine (BZM) were added 60 min before [3H]DOG uptake measurement. Results are expressed as percentage of control basal uptake (without any treatment) and represent the mean ± S.E. of six independent experiments performed in triplicate. Basal glucose uptake was 86.2 ± 9.0 pmol of [3H]DOG/min/well and is represented with a dotted line. B. when mentioned, 1 mM semicarbazide (SCZ) was preincubated with 3T3-L1 cells 90 min before [3H]DOG incorporation measurement. Sixty minutes before glucose transport test, 100 µM NaV was added in all wells, whereas 300 µM BZM was added in half wells. Control basal uptake was 74.7 ± 5.8 pmol/min/well and is represented with a dotted line. Results represent the mean ± S.E. of four independent experiments performed in triplicate. *, p < 0.05; **, p < 0.01; BZM- or insulin-stimulated glucose transport versus control basal uptake. †, p < 0.05; ††, p < 0.01; TNF-α-treated versus control cells.
transport, 3T3-L1 mature adipocytes were exposed for 48 h to various concentrations of TNF-α before [3H]DOG uptake measurements (Fig. 7). In control cells, benzylamine induced a 2.3-fold increase in basal glucose transport. Benzylamine-stimulated glucose transport was significantly reduced at 0.1 nM TNF-α and was totally abolished between 0.5 and 1 nM of the cytokine, the half-maximal effect being between 0.25 and 0.3 nM. It was noticeable that in slight contradiction with a previous study (Cornelius et al., 1990), TNF-α had only a weak inducing effect (≤20%) on basal glucose transport. In fact, we observed a more pronounced induction in basal glucose transport after a shorter (12-h) exposure to the cytokine (data not shown). Interestingly, it has been previously reported that basal glucose transport was only slightly increased in 3T3-L1 adipocytes cultured in the absence of insulin (Ranganathan and Davidson, 1996).

We also examined the time dependence of the TNF-α-induced reduction in SSAO-mediated glucose transport. 3T3-L1 mature adipocytes were exposed for 6 to 72 h to a constant concentration of TNF-α (0.5 nM) before testing hexose uptake. In agreement with SSAO activity modulation, the reduction in benzylamine-stimulated [3H]DOG uptake was detectable from 24 h after TNF-α exposure and persisted to decrease after 72 h (Fig. 8).

It has been clearly demonstrated that the inhibiting effects of TNF-α on insulin-stimulated glucose transport can be counteracted in the presence of thiazolidinediones (Szalkowski et al., 1995; Peraldi et al., 1997). Thus, we investigated whether the TNF-α-induced decrease in amine-stimulated glucose uptake was reversible in the presence of the insulin-sensitizing compounds thiazolidinediones. 3T3-L1 adipocytes were cultured for 48 h with or without 0.5 nM TNF-α, and in the absence or in the presence of 1 μM rosiglitazone, a potent peroxisome proliferator-activated receptor γ agonist. Then, basal, insulin- and benzylamine-stimulated glucose uptakes were tested. As expected, almost all of the inhibitory effect of TNF-α on insulin-stimulated glucose transport was antagonized by the concomitant addition of rosiglitazone; 77 ± 6% of the suppressive action of TNF-α on insulin-induced [3H]DOG uptake was reversed in the presence of the thiazolidinedione (data not shown). In contrast, rosiglitazone did not modify the preventing effect of the cytokine on benzylamine-stimulated glucose uptake (Fig. 8A). Rosiglitazone alone did not alter basal or benzylamine-mediated glucose transport. In agreement with the results on glucose uptake, rosiglitazone did not reverse the inhibitory effect of TNF-α on SSAO enzyme activity (Fig. 8B). Rosiglitazone alone did not change SSAO activity.

**Discussion**

In the present work, we observed that in adipocytes, a chronic exposure to TNF-α led to a potent down-regulation in SSAO enzyme activity. Several data strongly suggest that the inhibitory effect on enzyme activity is likely related to a decrease in gene and protein expression. First, the half-maximal efficient TNF-α concentration and the magnitude of maximal repression were similar for mRNA or protein expression and for enzyme activity. Otherwise, the down-regulation in SSAO activity occurred in the same period than the decrease in protein expression and was preceded by the reduction in SSAO mRNA levels. Finally, our in vivo studies indicate a good correlation between the reduction in SSAO mRNA steady-state levels and that of enzyme activity. Identification of the TNF-α receptor subtypes and intracellular signaling pathways as well as the transcriptional or post-transcriptional mechanisms at the basis of the regulation of
we observed an additional 24-h interval before the very progressive restoration in enzyme activity. Although we cannot exclude that our culture conditions in a defined serum-free medium modify protein half-life or that the biological effect of TNF-\(\alpha\) on SSAO expression is particularly persistent, it is likely that SSAO displays a physiological slow turnover. Consequently, variations in SSAO expression could only represent a long-term adaptive mechanism for regulating the activity of this amine oxidase.

It is noteworthy that SSAO modulation by TNF-\(\alpha\) is not restricted to this type of amine oxidase. In contrast to SSAO, monoamine oxidase activity is moderately induced in response to the cytokine. Monoamine oxidase activity is largely expressed in adipocytes (Pizzinat et al., 1999) and is induced during adipose conversion (Fontana et al., 2001). Studies using monoamine oxidase A- and B-selective inhibitors have shown that most of this activity was attributable to monoamine oxidase A expression (Pizzinat et al., 1999). In our study, we did not delineate whether TNF-\(\alpha\)-induced monoamine oxidase activity was related to an increase in monoamine oxidase A, or monoamine oxidase B, or a combination of both. Regardless of the mechanism at the basis of the up-regulation of monoamine oxidase activity by TNF-\(\alpha\), it would be of interest to investigate whether regulation of SSAO and monoamine oxidase correspond to independent biological events, or whether an intracellular cross talk exists between the two types of amine oxidase and regulates the redox equilibrium of adipocytes in a coordinated manner.

A fundamental question was to determine whether the TNF-\(\alpha\)-induced down-regulation of SSAO expression could significantly contribute to the reduction in benzylamine-stimulated glucose transport observed after cytokine exposure. Enrique-Tarancon et al. (2000) have recently shown that SSAO-mediated hexose uptake shares several signaling pathways with the insulin-sensitive glucose transport, such as activation of IRS proteins and phosphatidylinositol 3-kinase and translocation of the glucose transporter GLUT4 to the plasma membrane. Otherwise, numerous studies have reported that TNF-\(\alpha\) can alter the expression and/or function of several key steps in the insulin-sensitive glucose pathway, including alterations in phosphorylation of the insulin receptor or IRS-1, or decreased GLUT4 expression (Hotamisligil, 2000; Moller, 2000). Thus, the targeting by TNF-\(\alpha\) of several signaling molecules common between insulin- and SSAO-mediated effects may be sufficient to explain all the inhibitory effect of the cytokine on benzylamine-stimulated glucose transport. However, several lines of evidence support the view that the down-regulation of SSAO expression caused by TNF-\(\alpha\) has a key role in the decreased hexose transport in response to SSAO substrates. First, after TNF-\(\alpha\) exposure, the decrease of benzylamine-stimulated glucose transport is much more pronounced than that of insulin-sensitive glucose uptake, thus indicating that different and/or additional mechanisms contributed to the alterations in amine-activated glucose uptake. Second, the kinetics and dose-response curve of TNF-\(\alpha\)-induced down-regulation in benzylamine-stimulated glucose uptake are consistent with those of the TNF-\(\alpha\)-provoked reduction in SSAO protein expression and enzyme activity. Both amine-stimulated glucose transport and enzyme activity were decreased from 24 h after cytokine addition and continued to lower at 72 h. Moreover, after a 48-h treatment by TNF-\(\alpha\), both the inhibitory effects on glu-

---

**Fig. 9.** Thiazolidinediones do not reverse TNF-\(\alpha\) effects on benzylamine-stimulated glucose transport and SSAO activity. A, mature 3T3-L1 adipocytes grown in 24-well plates were maintained for 48 h in the absence (open columns) or in the presence (filled columns) of 0.5 nM TNF-\(\alpha\), and in the absence (\(-\)) or in the presence (\(+\)) of rosiglitazone (1 \(\mu\)M). Cells were preincubated with 100 \(\mu\)M NaV, and in the absence (basal transport) or in the presence of 300 \(\mu\)M benzylamine (BZM) 1 h before \(^{14}\)HIDG incorporation measurement. Results are given as percentage of control basal uptake and represent the mean \(\pm\) S.E. of six separate experiments carried out in triplicate. Basal glucose uptake was 71.2 \(\pm\) 10.0 pmol/min/well. B, mature 3T3-L1 cells were treated (filled columns) or not (open columns) with 0.5 nM TNF-\(\alpha\) exposed or not to 1 \(\mu\)M rosiglitazone for 48 h. Cell homogenates were used to evaluate SSAO enzyme activity with a fluorometric detection of hydrogen peroxide production. Results are expressed as percentage of control SSAO activity (cells without any treatment) and represent the mean \(\pm\) S.E. of five independent experiments performed in duplicate. Control SSAO activity was 24.84 \(\pm\) 0.96 nmol of H\(_{2}\)O\(_{2}\)/h/mg of protein. *, \(p < 0.05\); **, \(p < 0.01\); benzylamine-stimulated glucose transport versus basal transport, \(p < 0.05\); ††, \(p < 0.01\); †††, \(p < 0.001\); TNF-\(\alpha\)-treated versus control cells.

SSAO gene expression by the cytokine will require further investigation.

Interestingly, SSAO protein seems to display a slow turnover. There was obviously a time lag between the cytokine-induced reduction in SSAO transcript abundance and the down-regulation in protein expression and enzyme activity. Furthermore, after TNF-\(\alpha\) removal from the culture medium,
cose transport and on SSAO activity were detectable from 100 pM of the cytokine, with a half-maximal concentration being in the 200 to 300 pM range. 3) Finally, experiments performed with the thiazolidinedione rosiglitazone are also very informative. The insulin-sensitizing compounds thiazolidinediones, through peroxisome proliferator-activated receptor γ activation, are known to counteract TNF-α effects on insulin-sensitive glucose transport, adipocyte differentiation, and protein synthesis (Szalkowski et al., 1995; Peraldi et al., 1997). However, in our study, rosiglitazone completely failed to antagonize TNF-α effects on benzylamine-stimulated glucose transport. This was paralleled by the absence of rosiglitazone action to reverse TNF-α-induced suppression of SSAO enzyme activity. This indicates that the down-regulation of SSAO expression by TNF-α does not involve a PPARγ-dependent mechanism. Moreover, this strongly suggests that the main mechanism at the basis of TNF-α-induced decrease in benzylamine-stimulated glucose transport is the inhibition of SSAO gene and protein expression.

Recently, considerable attention has been focused on the possible involvement of the cytokine in the pathogenesis of obesity-associated insulin resistance (Hotamisligil, 2000; Moller, 2000). TNF-α overproduction has been observed in adipose tissue of several rodent models of obesity, as well as in obese humans (Hotamisligil, 2000; Moller, 2000). Exogenous administration of TNF-α to animals can induce insulin resistance, whereas neutralization of endogenous TNF-α can restore insulin sensitivity in genetically obese rats displaying high levels of TNF-α expression in adipose tissue (Hotamisligil, 2000; Moller, 2000). Moreover, genetic ablation of TNF-α or its receptors was reported to improve insulin sensitivity in various models of insulin resistance, including genetically, chemically, or diet-induced obese mice (Hotamisligil, 2000; Moller, 2000). The exact involvement of TNF-α in the pathophysiology of insulin resistance in obese humans and in noninsulin-dependent diabetes mellitus is still a matter of controversy, but it is reasonable to believe that TNF-α, in addition to other cytokines and metabolic factors, could contribute to the balance of tissular insulin sensitivity. Several recent works have reported that SSAO substrates stimulate glucose transport in rodent (Enrique-Tarancón et al., 1998, 2000; Marti et al., 1998; Fontana et al., 2001) or human adipocytes (Morin et al., 2001). This amine-stimulated glucose transport may thus represent an original mechanism by which circulating or locally produced amines can regulate glucose homeostasis. Acute administration of the SSAO substrate benzylamine can thus reduce plasma glucose levels independently to variations in plasma insulin levels (Enrique-Tarancón et al., 2000; Marti et al., 2001). However, it must be kept in mind that modulations of glucose uptake or of plasma glucose levels observed in the presence of SSAO substrates correspond to pharmacological manipulations. So far, there is no definitive evidence that establishes a physiological and fundamental role of SSAO in glucose homeostasis or that demonstrates a pathophysiological involvement of SSAO in disorders of carbohydrate metabolism.

In agreement with our in vitro studies, TNF-α-injected mice displayed a clear reduction in SSAO mRNA levels and enzyme activity. Low levels of SSAO transcripts and activity have also been reported in white adipose tissue of the Zucker fa/fa rat (Moldes et al., 1999), a genetic rodent model of obesity that overexpresses TNF-α in fat cells (Hotamisligil, 2000). Thus, the overproduction of TNF-α observed in adipose tissue of rodent models of obesity or in human obesity could reduce SSAO expression, and in turn may contribute to disturbances in glucose homeostasis. Studies on the effects of SSAO substrates on carbohydrate metabolism in animals overexpressing TNF-α in adipose tissue or in obese mice lacking TNF-α or its receptors would be helpful to ascertain the physiological relevance of our observations.

In the present work, we have only investigated the consequences of TNF-α-induced SSAO down-regulation on amine-stimulated glucose transport. Hoxose transport activation by SSAO substrates can be totally prevented in the presence of catalase and antioxidants (Enrique-Tarancón et al., 1998; Marti et al., 1998; Morin et al., 2001), indicating that hydrogen peroxide generated by SSAO was involved in the stimulation of glucose transport. However, in fat cells, besides its action on glucose transport, exogenously provided hydrogen peroxide has been shown to promote glucose oxidation or incorporation into glycogen, to stimulate lipogenesis or to inhibit lipolysis (Moldes et al., 1999 and references therein). Thus, through hydrogen peroxide production, SSAO may mediate pleiotropic functions in adipocytes. For instance, the antilipolytic effect of SSAO has recently been reported in human fat cells (Morin et al., 2001). As a consequence, the reduction in SSAO expression and activity caused by TNF-α may decrease the antilipolytic properties of the amine oxidase. The resulting increase in free fatty acids may represent an additional mechanism to alter insulin sensitivity (Arner, 2001).

Acknowledgments

We thank Dr. M. Unzeta for the generous gift of the polyclonal antibody against SSAO.

References


