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Hypoxia inhibits semicarbazide-sensitive amine oxidase activity in adipocytes

Xavier Repessé [a,b,*], Marthe Moldes [c,d], Adeline Muscat [a,c,d], Camille Vatier [c,d,e], Gérard Chetrite [a,c,d,e], Thomas Gille [f,g], Carole Planes [f,g], Anna Filip [h], Nathalie Mercier [h,i], Jacques Duranteau [j,k], Bruno Fève [a,c,d,e]

[a] UMR S_1185, INSERM, Université Paris-Sud, Le Kremlin-Bicêtre, France
[b] Service de Réanimation Médico-Chirurgicale, pôle Thorax-Vaisseaux-Abdomen-Métabolisme, Hôpital Ambroise Paré, Assistance Publique-Hôpitaux de Paris, Boulogne-Billancourt, France
[c] Centre de Recherche Saint-Antoine, INSERM, UMR S_938, Sorbonne Universités, Université Paris 6, Paris, France
[d] Institut Hospitalo-Universitaire ICAN, Paris, France
[e] Service d'Endocrinologie, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris, Paris, France
[f] Université Paris 13, Sorbonne Paris Cité, EA2363 Bobigny, France
[g] Service d'Explorations Fonctionnelles, Hôpital Avicenne, Assistance Publique-Hôpitaux de Paris, Bobigny, France
[h] INSERM U1116, Faculté de Médecine, Vandoeuvre-les-Nancy, France
[i] Université de Lorraine, Nancy, France
[j] Service d'Anesthésie-Réanimation, Hôpital Bicêtre, Assistance Publique-Hôpitaux de Paris, Le Kremlin-Bicêtre, France
[k] Microcirculation, Bioénergétique, Inflammation et Insuffisance Circulatoire Aigue, Equipe Universitaire 3509, Paris VII-Paris XI-Paris XIII, Paris, France

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ABSTRACT

Semicarbazide-sensitive amine oxidase (SSAO), an enzyme highly expressed on adipocyte plasma membranes, converts primary amines into aldehydes, ammonium and hydrogen peroxide, and is likely involved in endothelial damage during the course of diabetes and obesity. We investigated whether *in vitro*, adipocyte SSAO was modulated under hypoxic conditions that is present in adipose tissue from obese or intensive care unit. Physical or pharmacological hypoxia decreased SSAO activity in murine adipocytes and human adipose tissue explants, while enzyme expression was preserved. This effect was time-, dose-dependent and reversible. This down-regulation was confirmed *in vivo* in subcutaneous adipose tissue from a rat model of hypoxia. Hypoxia-induced suppression in SSAO activity was independent of the HIF-1-α pathway or of oxidative stress, but was partially antagonized by medium acidification. Hypoxia-induced down-regulation of SSAO activity could represent an adaptive mechanism to lower toxic molecules production, and may thus protect from tissue injury during these harmful conditions.

1. Introduction

Oxidative stress corresponds to an imbalance between reactive oxygen species (ROS) production and their clearance by the anti-oxidative systems, either non-enzymatic or enzymatic. Oxidative stress state is observed in many pathological conditions, in particular in intensive care unit (ICU) patients, who have a major oxidative stress due to inflammation or ischemia-reperfusion during sepsis or acute respiratory distress syndrome (ARDS) (Crimi et al., 2006; Roth et al., 2004). This stress likely exacerbates tissue damage and increases mortality (Gutteridge and Mitchell, 1999). Many trials have attempted to decrease this imbalance by antioxidant supplementation, but results remain controversial (Crimi et al., 2006). Among the molecules involved in oxidative stress, hydrogen peroxide plays a key role in injured tissues, alongside with superoxide and hydroxyl radicals (Marnett et al., 2003). Beyond their deleterious implication in ICU patients, recent findings have suggested that ROS could play a central role in the pathophysiology of obesity- and type 2 diabetes-associated insulin resistance, and could influence the mechanisms that affect adipogenesis and mature adipocyte functions (Bournat and Brown, 2010; Tiganis, 2010).

Abbreviations: ARDS, acute respiratory distress syndrome; $CoCl_2$, cobalt chloride; DFO, deferoxamine; DMOG, dimethyloxalyglycin; DPI, diphenylene iodonium; FCS, fetal calf serum; FFA, free fatty acid; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; G3PDH, glycerol-3-phopshate dehydrogenase; HIF, hypoxia-inducible factor; H_2O_2, hydrogen peroxide; HRP, horseradish peroxidase; ICU, intensive care unit; MTT, 3-(4,5 dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; ROS, reactive oxygen species; SCAT, subcutaneous adipose tissue; SSAO, semicarbazide-sensitive amine oxidase; TNF, tumor necrosis factor; TTFA, thenoyltrifluoroacetone; VAT, visceral adipose tissue.

* Corresponding author. Service de Réanimation Médico-Chirurgicale, pôle Thorax-Vaisseaux-Abdomen-Métabolisme, Hôpital Ambroise Paré, 9 avenue Charles de Gaulle, Assistance Publique-Hôpitaux de Paris, 92104 Boulogne-Billancourt, France. Tel.: +33 149094409; fax: +33 149095892.
E-mail address: xavier.repesse@apr.aphp.fr (X. Repessé).

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Semicarbazide-sensitive amine oxidase (SSAO; EC; 1.4.3.6) is a pleiotropic enzyme converting primary amines in the corresponding aldehydes, ammoniac and hydrogen peroxide (H_2O_2) (Jalkanen and Salmi, 2001). In mammals, SSAO exists both in a membrane-bound and a circulating soluble form (Boomsma et al., 2005). Tissue-bound SSAO is preferentially expressed in adipocytes, vascular smooth muscle cells, and in endothelia of a subset of vessels, and is a transmembrane protein with the catalytic activity located in the extra-cellular compartment. The tissue origin of soluble SSAO is not clearly understood, but it is well established that this form is increased in diabetes mellitus, congestive heart failure or liver cirrhosis (Boomsma et al., 1995, 1999; Garpenstrand et al., 1999; Kurkijarvi et al., 2000; Meszaros et al., 1999). In these chronic diseases, it has been suggested that SSAO was responsible for vascular lesions and was an independent marker of mortality in cardiac failure (Boomsma et al., 2000). At cellular and molecular levels, SSAO-mediated aldehyde generation could play a central role in endothelial or vascular smooth muscle cell dysfunction (Boomsma et al., 2003; Gokturk et al., 2003; Sole et al., 2008; Yu and Zuo, 1993). Alternatively, SSAO-mediated H_2O_2 production could represent another effector of vascular wall injury. Collectively, both experimental and clinical investigations support the view that in several chronic diseases, SSAO could mediate adverse cardiovascular events.

Considering the putative pathophysiological relevance of SSAO, especially in diabetes mellitus, it is also questionable whether the enzyme could also be involved in acute tissue injury observed in ICU. On the opposite, a negative modulation of SSAO expression and/or function in obese, diabetic, or ICU patients could be considered as a protective mechanism that could alleviate the magnitude of tissue damage. This could represent a relevant phenomenon for ICU patients in some tissues such as under-skin, gut, adipose tissue that are often sacrificed during acute injury to preserve heart or brain (Kashyap et al., 1975).

In the present work, we hypothesized that hypoxia, a condition frequently encountered in adipose tissue from obese patients or in ICU, could modulate SSAO activity, and in turn could promote or prevent tissue damage. Indeed, dioxygen is necessary for the amine oxidase enzymatic activity and its consumption has even been used to measure SSAO enzyme activity in purified preparations with the use of Clark electrode (Shen et al., 2012). Considering the very high SSAO expression in fat cells, and the abundance of adipose tissue in subcutaneous tissues and in digestive tract, the adipocyte may thus represent a prototypic target for SSAO regulation under hypoxic conditions. We thus examined whether SSAO expression and function were modulated in adipocytes cultured under hypoxic conditions. We demonstrate that in fat cells, hypoxia induces a strong down-regulation in SSAO activity. This suppression does not involve a hypoxia-inducible factor (HIF)-1-α-dependent mechanism, or a modulation in cell redox status. The modulation of SSAO activity could be mediated by modification of acid–base equilibrium. SSAO down-regulation by hypoxia could thus represent an adaptive mechanism to prevent tissue damage in ischemic tissues.

2. Experimental procedures

2.1. Cell culture

SSAO regulation was studied either in two extensively validated murine models of white preadipocytes, the 3T3-L1 and 3T3-F442A (Green and Kehinde, 1976; Green and Meuth, 1974) cell lines, or in the brown preadipose cell line T37i (Zennaro et al., 1998), or on human adipose tissue explants (Boomsma et al., 2005). Stocks of murine 3T3-L1 and 3T3-F442A were maintained in DMEM containing 25 mM D-glucose and supplemented with 10% donor calf serum. For experiments, cells were grown in DMEM containing 10% fetal calf serum (FCS). Differentiation of 3T3-F442A cells was initiated by the addition of 1 μg/ml insulin at confluence. For 3T3-L1 cells, induction of differentiation was achieved by a treatment with dexamethasone (0.25 μM), insulin (1 μg/ml), and 1-methyl-3-isobutylxanthine (0.1 mM) for the 48-h period following confluence. After removal of induction mixture, 3T3-L1 or 3T3-F442A cells were refed with DMEM supplemented with 10% FCS and 1 μg/ml insulin. At day 7 after confluence, more than 90% of 3T3-L1 or 3T3-F442A cells had the morphology of mature adipocytes.

Stocks of T37i cells were maintained in DMEM/HamF12 (D/H) (1:1; v:v) containing 15 mM D-glucose, and supplemented with 10% FCS. For experiments, T37i cells were grown in the same medium, then induced to differentiate in D/H containing 10% FCS, 2 nM T3, and 20 nM insulin. More than 95% of T37i cells acquired the morphotype at day 7 following confluence.

Human explants were obtained in healthy individuals from subcutaneous abdominal adipose tissue derived from plastic surgery. Informed consent was given for each tissue sample. Pieces of 5–6 g were cut thinly to get fragments, which were maintained during 24 h in D/H without serum.

After washing with PBS, mature murine adipocytes and human explants were placed under hypoxic conditions, either by a physical method with a hypoxic chamber (Oxoïd, Dardilly, France) containing 95% diazote (N_2) and 5% carbon dioxyde (CO_2), either with a pharmacological method with deferoxamine (DFO) or cobalt chloride ($CoCl_2$).

2.2. Animals

Experimental protocols were approved by The Ethics Committee in Animal Experiment Charles Darwin (Ce5/2011/05), done in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU) for animal care, and conducted in accordance with French laws for animal care (authorization number: A-93-086 for NV and A-93-072 for AP).

SSAO regulation was also studied *in vivo* in a rat model of hypoxia. Experiments were conducted in twelve 21-day-old male Sprague–Dawley rats. Four rats (Hypoxia 48h) were exposed to hypoxic conditions during 48 hours in a hypobaric chamber maintained at a pressure of 328 mmHg (≈6500 m) by a vacuum source at flow rates sufficient to prevent CO_2 buildup. Four rats were exposed to the same hypoxic conditions during 24 hours (Hypoxia 24h) and four control rats were maintained in normoxic conditions (Control). Animals were sacrificed with pentobarbitone and they were carefully dissected to obtain two kinds of samples. For each rat were sampled two pieces of visceral-adipose tissue (VAT) and two pieces of subcutaneous adipose tissue (SCAT). All of the procedures were in accordance with institutional guidelines for animal experimentation.

2.3. Enzyme assays

3T3-L1, 3T3-F442A, and T37i cells were washed twice in phosphate-buffered saline, harvested, and homogenized in 0.2 M KH_2PO_4/K_2HPO_4 buffer, pH 7.6, and stored at –80 °C. The VAT and the SCAT of the rats, and the human explants were homogenized by grinding, the fat cake was removed by a brief centrifugation, and the supernatant was kept at –80 °C until use. Aliquots of the homogenates or supernatants were used to determine protein content by the BCA method (Ultima, Interchim, Montluçon, France) using bovine serum albumin as a standard. SSAO enzyme activity was tested by H_2O_2 production on cell homogenates by a fluorimetric method using Amplex Red™ (Invitrogen, Cergy Pontoise, France) as a fluorimetric probe. The assay was performed in a final volume of 200 μl consisting 0.2 M KH_2PO_4/K_2HPO_4 buffer, pH 7.6, 200 μM Amplex Red™, 1 mM pargyline to block monoamine oxidase A and B activities, 1 mg/ml horseradish peroxidase (HRP), and 10 μg cell homogenate. When indicated, 1 mM of the SSAO inhibitor