ELSEVIER

Pharmacological Research 56 (2007) 70–79

Pharmacological research

www.elsevier.com/locate/yphrs

Prolonged treatment with aminoguanidine strongly inhibits adipocyte semicarbazide-sensitive amine oxidase and slightly reduces fat deposition in obese Zucker rats

D. Prévot [a], Z. Soltesz [a], V. Abello [a], E. Wanecq [a], P. Valet [a], M. Unzeta [b], C. Carpéné [a,*]

[a] *INSERM (Institut National de la Santé et de la Recherche Médicale), U858 équipe 3, Université Paul Sabatier, IFR31, Centre Hospitalier Universitaire de Rangueil, Toulouse, France*

[b] *Facultat de Medicina, Departament de Bioquimica, Institut de Neurociències, Universitat autonoma de Barcelona, Bellaterra, Barcelona, Spain*

Accepted 10 April 2007

Abstract

Beneficial effects of aminoguanidine (AG) on diabetic vascular complications result from prevention of protein glycation, inhibition of inductible NO synthase, and inhibition of vascular semicarbazide-sensitive amine oxidase (SSAO). However, influence of AG on adipose tissue deposition has been poorly investigated in obesity. Considering that SSAO is highly expressed in fat cells, and that a SSAO blocker has been recently reported to reduce body weight gain in obese mice, this work aimed to investigate the influence of AG on adipose tissue functions. First, AG was shown to directly inhibit SSAO activity in cultured adipocytes. Although AG did not directly alter lipolytic activity in human adipocytes, it inhibited benzylamine-induced antilipolysis via SSAO (but not NO synthase) inhibition. When AG was i.p. administered to obese Zucker rats (270 μmol kg^{-1} day^{-1} for 3 weeks), treated rats lost their capacity to oxidize benzylamine in a SSAO-dependent manner in adipose tissues and in cerebral vessels. Monoamine oxidase activity was unmodified in liver, skeletal muscles or adipose tissues and tended to increase in brain vessels. AG-treatment did not change body weight gain or hyperinsulinemic state of obese rats but slightly reduced subcutaneous fat deposition. AG did not modify insulin responsiveness in adipocytes but impaired the effects of SSAO substrates, such as glucose transport activation and lipolysis inhibition by methylamine or benzylamine plus vanadate. These results show that complete impairment of SSAO activity produced by AG-treatment in obese rats was likely responsible for a weak limitation of fat deposition. Previously proposed for prophylaxis in diabetes, AG may be useful for treating obesity via its SSAO blocking properties.

Keywords: Obesity; Adipose tissue; Amine oxidase; Semicarbazide; Insulin; Benzylamine; Lipolysis

1. Introduction

Aminoguanidine (AG), a nucleophilic hydrazine derivative of small molecular size (CH_6N_4; mol weight = 74.09), can interact with carbonyl groups of many biological constituents. Chemical interaction of AG with oxoaldehydes prevents the formation of highly reactive advanced glycation end products (AGEs) [1] and is thought to be responsible for most AG pharmacological actions [2]. AG has been proposed for delaying the pathogenesis of vascular complications that are secondary to diabetes since interaction of AGEs with collagen within arterial wall is a major event responsible for the development of diabetic vascular injury. Accordingly, AG has been repeatedly used as an experimental anti-AGEs agent [1], and until very recently the prevention of arterial stiffening and cardiac hypertrophy by AG in rats with type 1 [3] or type 2 diabetes [4] was attributed to inhibition of AGE formation. However, AG is not only scavenging dicarbonyls and limiting AGEs formation. In fact, at micromolar concentrations, AG also inhibits inducible nitric oxide synthase (iNOS) [1,2,5,6], semicarbazide-sensitive amine oxidase (SSAO) [7] and diamine oxidase [2]. These enzymes are expressed in vessels and catalyze the generation of nitric oxide, or the degradation of biogenic and exogenous amines such as methylamine, benzylamine, and histamine. Recent studies have shown that AG modulates age-related changes occurring in arteries not only by acting on AGEs-induced cross-linking [8] but also via vascular NOS activity [9]. Moreover, it has been reported that

* Corresponding author at: INSERM U858 eq 3, IFR 31, Bat. L3, CHU Rangueil, BP 84225, 31432 Toulouse Cedex 4, France.
Tel.: +33 5 62 17 29 55; fax: +33 5 61 33 17 21.
E-mail address: carpene@toulouse.inserm.fr (C. Carpéné).

1043-6618/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phrs.2007.04.002

aorta stiffness can also be modulated by SSAO activity invalidation [10]. Therefore, inhibition of iNOS and SSAO could also be responsible, at least in part, of the beneficial effects of AG on vasculature.

Thus, AG can be currently considered as a relatively selective inducible NOS inhibitor [11], while another L-arginine analogue, namely L-NAME (NG-nitro-L-arginine methyl ester) is well-known as a non-selective NOS inhibitor, also able to interact with endothelial and neuronal NOS. Noteworthy, the AG concentrations required for irreversible SSAO inactivation are lower than those needed for its other pharmacological actions [1,7] while, at very high doses, AG is considered a complex agent [1], able to act as an antioxidant by quenching hydroxyl radical and lipid peroxidation [12], or to inhibit catalase and generate hydrogen peroxide [13,14].

Despite its current limitation for clinical use in diabetes (for review, see [1]), and although chronic AG treatment of diabetic models has often limited the vascular pathogenesis without being able to lower hyperglycemia [12,15,16], several recent findings prompted us to test AG in obesity. Firstly, a highly potent SSAO inhibitor, (*E*)-2-(4-fluorophenethyl)-3-fluoroallylamine (known as FPFA) was recently described to reduce weight gain in obese KKAy mice fed an atherogenic diet [17]. Secondly, SSAO, known to be expressed in endothelial cells, especially in inflammatory conditions [18], is also constitutively elevated in adipocytes [19]. Thirdly, activation of SSAO by its substrates favours glucose uptake and inhibits lipolysis in adipocytes from various species, including man [20]. Lastly, several studies using prolonged AG treatment have already reported a weight gain reduction in aged [8] and diabetic rats [21]. We hypothesized that if endogenous or alimentary amines could reach adipose depots, they could be oxidized by SSAO and generate lipogenic and antilipolytic effects favouring fat deposition. Therefore, SSAO blockade by AG could limit lipid deposition by reducing this anabolic process.

The aim of this study was therefore to investigate *in vitro* and *in vivo* whether AG could inactivate SSAO activity and influence adipose cell lipolytic and lipogenic functions. It was first tested whether AG could directly inhibit SSAO activity in fat cells. The murine 3T3 F442A cultured adipocytes were used for this *in vitro* approach since SSAO expression largely increases during adipogenesis [22] and since SSAO subtrates have been reported to exhibit insulin-like actions in this model [23,24]. Then, short-term effects of AG, L-NAME and semicarbazide were compared on human fat cell lipolysis, in the absence and in the presence of the reference SSAO substrate benzylamine. Lastly, we investigated whether aminoguanidine-treated obese rats (AG-treated) could exhibit SSAO inhibition in diverse tissues, decreased fat deposition, or altered adrenergic and insulin responsiveness. Young Zucker obese rats were chosen to study the influence of AG treatment on obesity on its own, without diabetic complications since they exhibit elevated circulating insulin but remain normoglycemic and since their adipocytes are far from being deeply insulin-resistant [25,26]. Moreover, it has been previously reported that adipose tissue of young obese rats expresses SSAO [22] and does not present the signs of oxidative stress [26] found in obese and diabetic mice [27]. The following results show that, *in vitro*, AG inhibits adipocyte SSAO and impairs the antilipolytic or lipogenic effects of SSAO substrates, while, *in vivo*, it faintly reduces fat deposition in obese rats.

2. Materials and methods

2.1. Drugs and reagents

[^{14}C]-benzylamine (54 mCi mmol^{-1}) was purchased from Amersham Biosciences (Buckinghamshire, UK). [^{14}C]-serotonin (42 mCi mmol^{-1}) and 2-[1,2-^{3}H]-deoxyglucose (2-DG, 40 Ci mmol^{-1}) were purchased from Perkin-Elmer Life and Analytical Sciences (Boston, MA). [^{14}C]-tyramine (7.5 mCi mmol^{-1}), aminoguanidine, benzylamine, methylamine, tyramine, semicarbazide (all hydrochloride salts), sodium orthovanadate, cytochalasin B, fatty acid-free bovine serum albumin, antiproteases cocktail, and other chemicals were from Sigma–Aldrich (St. Louis, MO), unless otherwise specified. Collagenase, enzymes and cofactors for the determination of glycerol were from Roche Diagnostics (Mannheim, Germany).

2.2. Animals and tissue sampling

Female obese Zucker rats of 7 weeks (Crl:ZUC(Orl)-*Lepr^{fa}* from Charles River Laboratories, L'Arbresle, F) were individually housed and treated from the age of 9 weeks for 3 weeks by daily ip injection of saline (control), or by aminoguanidine hydrochloride at 30 mg kg^{-1} (270 μmol kg^{-1}; AG-treated). Lean littermates of both sexes were used for adipocyte preparation. All the animals had free access to water and chow in accordance with the European Communities Council Directives for experimental animal care. Body weight and food intake were measured every 3 days. At the end of treatment, tissues were removed from euthanized animals and immediately frozen at −80 °C until preparation of homogenates for amine oxidase activity assays. Fat depots were carefully dissected and weighed. Samples (perigonadal and perirenal fat pads) from intra-abdominal white adipose tissues (INWAT) were minced in Krebs-Ringer containing 15 mM sodium bicarbonate, 10 mM HEPES, 2 mM pyruvate and bovine serum albumin (35 mg ml^{-1}) (KRBH buffer, pH 7.4) for collagenase digestion and adipocyte isolation. The meninges, the pial membrane and its vessels were removed from the dissected rat brains and microvessels were prepared using the method of Dallaire et al. [28]. Insulin and glucose plasma levels were determined using kits from RIA Pasteur and Biotrol Diagnostics, respectively.

2.3. Human adipocyte preparations

Human adipose cells were prepared by collagenase digestion of subcutaneous adipose tissue pieces obtained from patients undergoing abdominal lipectomy at the plastic surgery department of Rangueil Hospital (Toulouse, France) under the agreement of the local ethics committee. The preparations used for the present study were obtained from 15 women with a mean age of 40 years and a mean body mass index of 25.7 ± 1.2 kg/m^2.