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Semicarbazide-sensitive Amine Oxidase Activity in White Adipose Tissue of the Insulin-deficient Rat

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Abstract

We have investigated whether the effects in white adipose tissue due to insulin deficiency might also be related to an alteration of histamine levels which are regulated by semicarbazide-sensitive amine oxidase.

The lack of circulating insulin induced by streptozotocin produced, in rat white adipose tissue, a loss of affinity of the semicarbazide-sensitive amine oxidase for histamine oxidation. In parallel, a decrease of cell transformation, measured by glycerol-3-phosphate dehydrogenase activity and an augmented sensitivity to histamine lipolysis were observed.

These findings could contribute to the understanding of histamine metabolism and function in diabetic rats and to the knowledge concerning amine oxidases in this animal pathology.

White adipose tissue represents a good source of a tissue-bound benzylamine oxidase conventionally named semicarbazide-sensitive amine oxidase (E.C.1.4.3.6., SSAO). This enzyme is concentrated in adipocytes and it is the only one responsible for the oxidative deamination of histamine in rat white adipose tissue (Raimondi et al 1991). This protein is a typical product of fat cell maturation, as is shown by its occurrence in differentiating pre-adipocytes in culture (Raimondi et al 1990). In these precursor cells, where the transformation is induced by the addition of an adipogenic mixture which contains insulin, SSAO follows the same time-course appearance of the glycerol-3-phosphate dehydrogenase activity (GDH), a marker of the specific lipid acquisition of committed cells (Wise & Green 1978). We could then hypothesize that SSAO participates in cell maturation by oxidative deamination of amines active as growth and differentiating factors. In this respect, the histaminase feature of white adipose tissue SSAO might be considered important for the role of histamine as a tissue- and cell-growth regulator (Brandes et al 1987, 1990). According to those authors, histamine levels in rat white adipose tissue, regulated by SSAO, might be important in controlling adipocyte maturation and metabolism.

Because, so far, all our observations were carried out in-vitro and on mature adipocytes or differentiating pre-adipocytes, we were interested in following SSAO histaminase activity in mature white adipose tissue of diabetic animals. This condition was reached by treating mature rats with streptozotocin (experimental diabetes) which is known to induce a severe insulin deficiency which compromises white adipose tissue functions and cellularity, inducing adipocyte de-differentiation (Geloen et al 1989).

In this model we would follow the fate of SSAO in cells which have already expressed the enzyme, and are then devoid of insulin, the main stimulus which sustains and promotes differentiation of adipose cells.

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Others describing the SSAO of diabetic rat tissues have found an increase of this enzymatic activity in kidney and plasma (Hayes & Clarke 1990). This finding has been further extended by Yu & Zuo (1993) to the possible toxic role of formaldehyde, a product of the oxidative deamination of methylamine produced by plasma SSAO, on endothelial cells. Both results have induced the authors to speculate a role for SSAO in diabetes-induced cardiovascular and renal damage.

On the other hand, some changes in histamine tissue concentrations and in diamine oxidase have also been reported in several diabetic tissues (Fogel et al 1990; Gill et al 1990). In this respect, no information is available, so far, on the SSAO histaminase activity in white adipose tissue of diabetic rats.

Our aim was then to follow SSAO histaminase activity and histamine lipolysis in white adipose tissue of insulin-deficient rats. A change in SSAO activity might represent a further confirmation that SSAO follows and perhaps sustains adipocyte maturation, and might contribute to the knowledge of the amine oxidases in this animal pathology.

Adipocyte homeostasis is mainly controlled by insulin, and the lack of the hormone induces a decreased recruitment of undifferentiated cells from the stromal fraction and changes in adipocyte lipolysis and lipogenesis (Zumstein et al 1980). Because SSAO seems to be linked to cell maturation but also participates in triglyceride breakdown, we hypothesize that diabetes also compromises white adipose tissue SSAO functions. We could suppose that some of the effects in white adipose tissue due to insulin deficiency might also be related to an alteration of histamine levels which are regulated by SSAO.

Materials and Methods

Materials

Male Wistar rats were from the Morini breeding colony, S. Polo D'Elsa, Reggio Emilia, Italy.

Streptozotocin, dihydroxyacetonephosphate dimethylketal, horseradish peroxidase, a glycerol UV determination

kit and a D-glucose determination kit were obtained from Boehringer Mannheim, Germany.

Histamine hydrochloride, bovine serum albumin fraction V and pargyline hydrochloride were purchased from the Sigma Chemical Co., St Louis, MO, USA. Homovanillic acid was obtained from Merck, Darmstadt, Germany. All other reagents were of analytical grade.

Methods

Rats, 200–230 g, were injected in the tail vein with 65 mg kg^{-1} streptozotocin in sterilized citrate buffer pH 4·5. Control animals were treated with citrate buffer alone. After the injection animals had free access to standard chow and were housed under constant temperature (23°C) and lighting conditions (0800–1700 h).

After 1, 3 and 6 weeks, animals were killed by cervical dislocation. The blood was collected in heparinized tubes and the epididymal portions of white adipose tissue were removed. The tissue was rinsed in saline, blotted, dried and weighed. A portion (300 mg) was used for SSAO determination. GDH activity was determined in a small portion of the tissue homogenized in 0·1 M triethanolamine-hydrochloride buffer, pH 7·4, and the remaining tissue was used for lipolysis measurement.

SSAO activity

Cleaned white adipose tissue (300 mg) was homogenized in 3 mL Krebs-bicarbonate buffer, pH 7·4, of the following composition (mM): NaCl 118, $MgSO_4$ 1·5, KCl 4·7, KH_2PO_4 1·5, glucose 11, $NaHCO_3$ 24·6. Homogenate was centrifuged at 900 *g* for 10 min to eliminate the fat cake and cellular debris. The clear supernatant was used for SSAO determination.

SSAO assay was carried out according to Matsumoto et al (1982). Samples (100 μL) were pre-incubated in 1 mM potassium phosphate buffer, pH 7·8, in the presence of 1 mM pargyline, to inhibit monoamine oxidase activity, at 37°C for 30 min and then substrates, benzylamine or histamine, were added and incubated for a further 30 min in the same conditions of temperature. Benzylamine concentrations ranged from 2·5 to 50 μM, whereas the histamine range was from 0·125 to 4 mM. Reactions were stopped by the addition of 2 mL 0·1 M NaOH and fluorescence measured in a Shimadzu fluorimeter.

GDH activity

The enzymatic activity was measured according to Wise & Green (1978). Tissue homogenized in triethanolamine-HCl buffer was centrifuged at 10 000 *g* for 10 min. The clear supernatant (100 μL) was incubated in triethanolamine buffer pH 7·4, containing 25 mM EDTA, NADH (0·1 mg mL^{-1}) at 37°C for 10 min. Dihydroxyacetonephosphate (3 mg mL^{-1}), prepared according to the manufacturer's instructions, was then added and the decrease in optical density was followed spectrophotometrically. Results were expressed as μmol NADH oxidized min^{-1} (mg protein)$^{-1}$.

White adipose tissue lipolysis

White adipose tissue pieces (10–15 mg) from control or diabetic rats were pre-incubated in Krebs-bicarbonate buffer containing 3% bovine serum albumin and pre-incubated for 30 min at 37°C in an atmosphere of 5% CO_2 and 95% O_2. After that time, histamine was added to the medium and incubated for a further 90 min in the same conditions of temperature and oxygenation. Tissue pieces were then removed, blotted, dried and weighed. Glycerol concentration in the medium was measured by an enzymatic method (Eggestein & Kuhlman 1974). Results were expressed as μmol glycerol released in 90 min per g of tissue.

Protein determination

Protein levels of samples were measured by the method of Lowry et al (1951).

Glucose in plasma

Glucose levels were measured by an enzymatic method, using a Boehringer D-glucose assay kit. Blood from control and streptozotocin-treated rats was collected at 1, 3 and 6 weeks after the injection, in heparinized tubes, and plasma was prepared by centrifuging the blood at 1000 *g* for 20 min at room temperature.

Results

Glucose in plasma

Glucose concentration in control rats remained unchanged during the period of the experiment, while in streptozotocin-treated rats glucose levels rose significantly one week after treatment (Table 1).

Body and white adipose tissue weight

At the beginning of the experiment rat weight was 213 ± 6 g. One week after streptozotocin injection, the body and white adipose tissue weight showed a similar increase in control and treated animals (Table 1).

At three and six weeks, treated rats did not gain weight, in contrast to control animals. Moreover, white adipose tissue weight began to decrease progressively from one up to six weeks in diabetic rats, reaching, at six weeks, the lowest ratio with respect to body weight (0·3%).

GDH

The enzymatic activity was measured in the 10 000 *g* supernatant obtained from white adipose tissue homogenate. GDH reached higher levels in control than in diabetic rats (Fig. 1). The enzymatic activity changed with the age of the

Table 1. Body, white adipose tissue weight and glucose plasma in control and streptozotocin-treated rats.

	Glucose (g L^{-1})	Body weight (g)	Tissue weight (g)
Control			
1 week	1·15 ± 0·06	273 ± 11	1·47 ± 0·15
3 weeks	1·42 ± 0·26	378 ± 10	2·74 ± 0·12
6 weeks	1·88 ± 0·30	474 ± 27	5·05 ± 0·46
Treated			
1 week	4·16 ± 0·31*	267 ± 5	1·16 ± 0·12
3 weeks	5·33 ± 0·53*	287 ± 13*	0·70 ± 0·13*
6 weeks	6·80 ± 0·54*	302 ± 20*	0·89 ± 0·26*

Results are means ± s.e. *$P < 0·01$ compared with corresponding controls (n = 10).

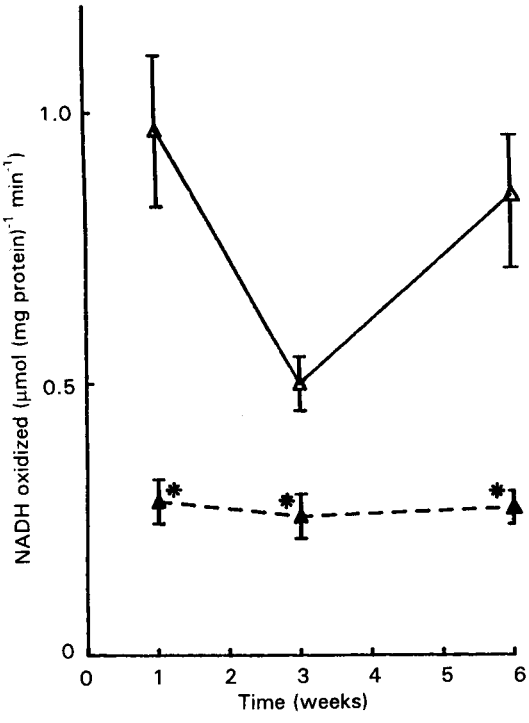

FIG. 1. GDH activity in control (△) and streptozotocin-treated (▲) rats. Results are means ± s.e. of 7–10 enzyme preparations. Enzymatic activity was measured spectrophotometrically. Statistical evaluation of results was performed by Student's t-test for grouped data (*$P < 0.05$).

rats; it was very high after one week, decreased at three weeks and returned to the initial value at six weeks. During that period the weight of control animals almost doubled. A different picture appeared in the streptozotocin-treated rats: the enzyme levels were lower after one week with respect to the control value, and remained almost unchanged during the rest of the experiment.

SSAO

SSAO activity was measured in tissue homogenates, using benzylamine or histamine as substrate under conditions of monoamine oxidase inhibition by pargyline. Benzylamine was used at 50, 20, 12, 10, 5 or 2·5 μM, while histamine concentrations were 4, 2, 1, 0·5, 0·25 or 0·125 mM. Reactions were stopped 30 min after the addition of substrates and results were expressed as nmol of substrate oxidized (mg protein)$^{-1}$ min^{-1}. Apparent K_m and V_{max} values for the oxidation of both substrates were calculated by the method of Wilkinson (1961) and are reported in Table 2.

Control rats. The apparent K_m and V_{max} for benzylamine or histamine oxidation did not change with the age of the animals (Table 2).

Streptozotocin-treated rats. The apparent K_m and V_{max} for benzylamine oxidation at one week did not change from the value measured in the age-matched control animals. At three weeks a slight increase of K_m was observed. This increase was more pronounced at six weeks (Table 2). V_{max} remained unchanged.

Table 2. The apparent kinetic constants for benzylamine and histamine oxidation.

	K_m (μM)		V_{max} (nmol (mg protein)$^{-1}$ min^{-1})	
	Benzylamine	Histamine	Benzylamine	Histamine
Control				
1 week	6·8 ± 1·5	157 ± 20	2·2 ± 0·5	2·0 ± 0·3
3 weeks	2·8 ± 0·5	130 ± 30	1·8 ± 0·5	1·9 ± 0·6
6 weeks	6·8 ± 1·3	174 ± 21	3·0 ± 0·6	1·8 ± 0·4
Diabetes				
1 week	3·9 ± 1·3	130 ± 10	2·0 ± 0·1	1·5 ± 0·1
3 weeks	8·2 ± 2·7	368 ± 40*	1·2 ± 0·3	1·3 ± 0·2
6 weeks	54 ± 10*	523 ± 34*	3·1 ± 1·2	2·2 ± 0·5

Results are means ± s.e. of 7–10 enzyme preparations. SSAO activity was measured fluorimetrically using benzylamine (50, 20, 12, 10, 5, 2·5 μM) and histamine (4, 2, 1, 0·5, 0·25, 0·125 mM) as substrates. *$P < 0.05$ compared with corresponding controls.

The K_m for histamine oxidation was indistinguishable from control rats at one week while it increased at three and maintained this high value up to six weeks; however, V_{max} for histamine oxidation did not change at one, three or six weeks (Table 2).

Histamine lipolysis

The effect of exogenously added histamine (from 10 nM to 100 μM), was measured as glycerol release in white adipose tissue pieces. Basal glycerol release was obtained by incubating tissue for 90 min in the absence of lipolytic stimuli (Table 3).

Control rats. Histamine induced a dose-dependent stimulation of glycerol release in white adipose tissue. It reached a maximum stimulation at 1 μM and its effect did not change during the time course of the experiment. The low efficacy of histamine in stimulating glycerol release was confirmed.

Diabetic rats. Basal glycerol release was enhanced after three and six weeks of streptozotocin treatment compared with control animals. Histamine lipolysis did not change at one

Table 3. Histamine lipolysis in control and streptozotocin-treated rats.

Histamine (μM)	Glycerol released (μmol (g tissue)$^{-1}$/90 min)		
	Controls	Diabetes	
	1, 3, 6 weeks (n = 10)	3 weeks (n = 6)	6 weeks (n = 8)
100	5·9 ± 0·8	6·7 ± 1·8	6·9 ± 1·3
10	6·1 ± 1·1	6·8 ± 1·4	8·8 ± 1·5
1	6·2 ± 0·9*	6·9 ± 1·8	9·8 ± 1·5
0·1	6·0 ± 0·8	7·1 ± 2·2*	13 ± 2·5*
0·01	5·0 ± 1·1	6·3 ± 1·9	8·8 ± 1·1
Basal	4·3 ± 0·8	5·8 ± 1·8	5·3 ± 0·8

The lipolytic activity of increasing concentrations of histamine (from 0·01 to 100 μM) was determined in white adipose tissue pieces from control and streptozotocin-treated rats. Glycerol release in the medium was measured enzymatically and used as lipolytic index. *$P < 0.05$ compared with corresponding controls.

week, whereas an increase of histamine potency was observed after three weeks of streptozotocin treatment. At six weeks, a significant increase of glycerol release was obtained by histamine. At that time the maximum stimulation was obtained at 0·1 μM histamine (Table 3).

Discussion

Benzylamine oxidase activity, which belongs to the same class of diamine oxidase, has been described in rat white adipose tissue and in isolated adipocytes. Among physiological amines screened up to now, adipocyte SSAO is able to oxidize methylamine (Conforti et al 1993) and histamine. This latter feature, shared by other SSAOs (Ignesti et al 1992), might be important in controlling adipocyte maturation and the lipolytic activity of histamine in rat white adipose tissue (Raimondi et al 1993).

Because adipocyte homeostasis is mainly sustained by insulin, we wondered about the effect of the lack of this hormone on white adipose tissue SSAO and lipolysis induced by histamine. We already knew that this enzyme occurred in cells committed to become adipocytes, but we did not know yet what happened to this protein when a regression of adipose phenotype was in progress. Hence we experimentally forced adipocytes to de-differentiate, reproducing in animals the diabetes mellitus of human subjects. We believed it was important to investigate, in this condition, the properties of SSAO when mature white adipose tissue is devoid of the main stimulus which maintains cell transformation.

Insulin deprivation was obtained by treating adult rats (200 g) with 65 mg kg^{-1} streptozotocin, a concentration which should guarantee a mild-to-severe form of diabetes, allowing animals to survive without insulin supplementation. The duration of the experiment was chosen as a compromise between animal survival and the collection of enough tissue for measurements.

Under these conditions, glucose plasma levels rose significantly in the first week and then remained at high levels. During the first week, control rats gained weight as much as the treated ones. After that time, the effect of insulin deficiency on white adipose tissue mass became evident: treated animals lose body and tissue weight. A decrease of GDH activity in treated rats was also observed, suggesting a variation of lipid turnover measurable only in the first week of treatment when the tissue mass had not yet changed. In parallel with GDH, SSAO changes were also evident; SSAO lost affinity for the two substrates studied. The kinetic constants for benzylamine oxidation did not change from one to three weeks in either control or treated rats, whereas an increase of K_m at six weeks was evident.

The kinetic constants for histamine oxidation however changed earlier; the decrease of affinity for histamine was found at three weeks and then remained constant up to 6 weeks. For both substrates we only observed a loss of affinity for amine oxidation with no changes in V_{max} values. The variation of histamine affinity at three weeks of diabetes, which did not yet involve any benzylamine oxidation parameters, might represent a useful model to study the chemical requirements for enzyme-substrate interaction with molecules structurally related to histamine and benzylamine. The general loss of affinity of the enzyme might be compared with a competitive type of inhibition. The presence of such inhibitors in white adipose tissue of diabetic animals was therefore excluded by the linear relationship between protein concentration and enzymatic activity obtained in control and treated rats. Instead, to explain our results, we assume that white adipose tissue is subjected to a severe impairment of its cellularity which could compromise SSAO function, as happens in other diabetic tissues. By the membrane-bound nature of white adipose tissue SSAO, it is therefore also possible that damage of the cell membrane might lead to a different assembly of the protein with an alteration of its function as already described for other proteins (Tomlinson et al 1992).

Our findings are in contrast with the increase in SSAO V_{max} reported by Hayes & Clarke (1990) in plasma and kidney of diabetic rats. Those authors described an augmented SSAO activity without any change in the K_m values for some substrates. Although we are unable, so far, to explain these discrepancies, we hypothesize that decreased SSAO affinities are the result of tissue-specific modifications due to the insulin disappearance.

Considering SSAO histaminase activity, it might also be possible that high histamine levels are necessary, or in part responsible, for white adipose tissue lipid deprivation. Histamine has been reported to exert lipolytic activity in dog (Grund et al 1973), whereas its effect on rat white adipose tissue is uncertain (Nakano & Oliver 1970). We recently reviewed the histamine lipolytic activity in rat white adipose tissue with respect to SSAO enzymatic activity (Raimondi et al 1993). While some evidence of the presence of H_2 receptors on rat adipocytes were reported by us, the nature of histamine lipolysis in tissue pieces was not completely elucidated and we cannot exclude the participation of other lipolytic factors, released by histamine, in the lipolysis induced by histamine. Our results now confirm the weak lipolytic activity of histamine in white adipose tissue pieces; in these experiments the maximum glycerol release was obtained at 1 μM histamine in control rats. In these animals, the dose-response curve of histamine did not change with the age of the animals. No further differences were then measured, with respect to control values, at one week of treatment (data not shown). Later at three and six weeks the maximum response was obtained at 0·1 μM histamine with an increase of the basal glycerol release. At six weeks the maximum effect was obtained at a higher net value of glycerol mobilization with respect to controls. In addition, a progressive decrease of glycerol mobilization was observed in diabetic tissue at high histamine concentrations (1–100 μM). In fact, the maximum percentage of mobilization for 100 μM histamine shifted from 95% in controls to 66 and 14% respectively in three- and six-week diabetic tissue. These latter findings could indicate a more pronounced inhibitory shape of the histamine dose–response curve.

This result seems to suggest a different susceptibility of histamine oxidative deamination in diabetes, and of the receptor-mediated histamine effects. In streptozotocin-treated rats the oxidative deamination (K_m) of histamine might assume less importance while its lipolytic effect seems

augmented. The different behaviour of histamine in these rats with respect to the controls might indicate the participation of this amine in the lipid deprivation of the tissue due to the pathology. This finding might confirm the importance of the histaminergic system in controlling white adipose tissue function in normal and pathological conditions.

In conclusion, in insulin-deprived animals, the participation of histamine in white adipose tissue homeostasis might be sustained by a modification of the cellular systems implied in its physiological role. In fact, white adipose tissue is important in controlling energy homeostasis and this role is altered by a number of diseases including diabetes (Geloen et al 1989) and tumour malignancy (Porat 1989), which seriously affect its anatomy and functions. In diabetic tissue, where cell turn-over seems blocked, SSAO levels remain unchanged (V_{max}), but its oxidative capacity is altered. This finding could be the result of a new cellular homeostasis which leads to an augmented effect of an amine which is usually deaminated by the enzyme. In this respect, histamine could represent an investigative tool, but other physiological substrates might be involved in lipid deprivation and de-differentiation of white adipose tissue. In addition, the disappearance of GDH confirms the inability of diabetic cells to sustain the adipose phenotype, with the block of recruitment of newly transformed cells from the stromal fraction.

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