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Glucose utilization *in vivo* and insulin-sensitivity of rat brown adipose tissue in various physiological and pathological conditions

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Brown-adipose-tissue glucose utilization rate and its insulin-sensitivity were measured *in vivo* in the anaesthetized rat by a 2-deoxy[1-^{3}H]glucose technique. Glucose utilization can be increased 60-fold by insulin, to reach extremely high rates. Glucose utilization and its insulin-sensitivity are modulated in accordance with physiological or pathological conditions.

INTRODUCTION

Brown adipose tissue is known as a tissue specialized in heat production in response to cold (Himms-Hagen, 1976). More recently it has been shown to be involved as a possible regulator of the energy balance, since thermogenesis could also be elicited in brown adipose tissue by chronic overfeeding (Rothwell & Stock, 1979) and since, in obese rodents, the thermogenic response of this tissue to overfeeding was decreased (Seydoux *et al.*, 1982; Rothwell *et al.*, 1982; Hogan & Himms-Hagen, 1983). This led to numerous studies on brown-adipose-tissue metabolism which have emphasized the role of fatty acids as the potential substrates for cold- or diet-induced thermogenesis (reviewed by Bukowiecki, 1984). However, studies showing a high rate of lipogenesis in brown adipose tissue (reviewed by McCormack, 1982), with glucose as a possible precursor (Sugden *et al.*, 1982; McCormack, 1982), as well as the demonstration of a high capacity for glycolysis in brown adipose tissue (Cooney & Newsholme, 1982), led to the idea that, when glucose is available in the blood, it might also be an important fuel for brown adipose tissue, either directly through glycolysis and oxidation or indirectly after lipid synthesis (McCormack, 1982; Cooney & Newsholme, 1982). Moreover, brown-adipose-tissue glycolytic capacity and lipogenesis are decreased in genetic or acquired obesity (Young *et al.*, 1984; Saito & Shimazu, 1984).

It has been shown *in vivo* that insulin can rapidly modulate the rate of lipogenesis in brown adipose tissue (McCormack & Denton, 1977; Agius & Williamson, 1980; Sugden *et al.*, 1982; Burnol *et al.*, 1983). However, no direct and quantitative evidence has been presented *in vivo* concerning glucose utilization and its insulin-sensitivity in brown adipose tissue. The development of a technique allowing measurement of glucose utilization *in vivo* by a specific tissue of the rat (Ferré *et al.*, 1985) has led us to study basal and insulin-stimulated glucose utilization in rat brown adipose tissue in various physiological (virgin, pregnant, lactating) and pathological (obese Zucker) conditions.

MATERIALS AND METHODS

Animals

Homozygous lean or obese female Zucker rats and female rats of a Wistar strain, bred in the laboratory, were used. They were housed at 23 °C with light from 07:00 to 19:00 h. They had free access to water and chow pellets (carbohydrate 65%, fat 11% and protein 24% of total energy) until 08:00 h. Virgin and 19-day-pregnant rats were 12 weeks old. Lactating rats were 14 weeks old, with ten pups in each litter; age-matched rats whose pups were removed at delivery were used as non-lactating controls. Lean and obese Zucker rats were 12 weeks old and weighed respectively 172 ± 3 g ($n = 5$) and 309 ± 9 g ($n = 5$). All animals were studied at 10:00 h. Rats were anaesthetized with pentobarbital (30 mg/kg body wt. intraperitoneally for Wistar and lean Zucker, 70 mg/kg for obese Zucker); a tracheotomy was performed and a carotid artery catheterized. Injections or perfusions were made through saphenous veins. Body temperature was continuously recorded with a telethermometer (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.) and cautiously maintained at 38 °C with heating lamps and pads.

Euglycaemic–hyperinsulinaemic clamp

This technique was applied as previously described (Leturque *et al.*, 1984). Two rates of insulin infusion were used, allowing attainment of either physiological (250–400 μunits/ml of plasma; clamp A) or maximal (7000 μunits/ml of plasma; clamp B) plasma insulin concentrations.

2-Deoxyglucose injection and blood sampling

2-Deoxy[1-^{3}H]glucose (30 μCi, 20 Ci/mmol; CEA, Saclay, France) was injected in 200 μl of 0.9% NaCl as a bolus through a saphenous vein. In hyperinsulinaemic animals, injection took place once both glycaemia and exogenous glucose infusion were clamped. Blood (50 μl) was sampled via the arterial catheter. At the time of tissue sampling, 2-deoxy[1-^{3}H]glucose concentration was 10%

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of the 1 min value in the different experimental groups. Blood was immediately deproteinized in $Ba(OH)_2/ZnSO_4$ as described by Somogyi (1945), and centrifuged (2 min, 16000 *g*). The supernatant was used for the determination of blood glucose by a glucose oxidase kit (Boehringer, Meylan, France) and of 2-deoxy[1-^{3}H]glucose with a liquid-scintillation counter (Betamatic; Kontron, Vélizy, France). At the end of each experiment, a larger blood sample was taken for plasma insulin determination by a radioimmunoassay previously described (Leturque *et al.*, 1984).

Tissue sampling

After the last blood sample, the rat was killed by cervical dislocation. Interscapular brown adipose tissue was removed and dissected free from adjacent muscles and white adipose tissue. The content of 2-deoxy-[1-^{3}H]glucose 6-phosphate was determined as previously described (Ferré *et al.*, 1985).

Measurement *in vitro* of a correction factor for the discrimination against 2-deoxyglucose (lumped constant)

Rats were killed by cervical dislocation, two pieces (each 100 mg) of interscapular brown adipose tissue were removed and incubated in 2 ml of bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), containing bovine serum albumin (1.5%) (Sigma), 5 mM-glucose, 2 μCi of [5-^{3}H]glucose (CEA) and 1 μCi of 2-deoxy-D-[1-^{14}C]glucose (New England Nuclear, Boston, MA, U.S.A.). Each flask was gassed with O_2/CO_2 (19:1) for 5 min, sealed with a rubber stopper and incubated for 1 h at 37 °C. Three 50 μl samples of the incubation medium were taken and accurately weighed with a Mettler AE 163 (Sofranie, Viroflay, France) balance (accuracy 10 μg) at the beginning and at the end of the incubation. The samples were evaporated to dryness in order to remove the ^3H_2O originating from [5-^{3}H]glucose metabolism, and were analysed for their contents of 2-deoxy[1-^{14}C]glucose and [5-^{3}H]glucose by counting the radioactivity in a liquid-scintillation counter. The correction factor was taken as the ratio 2-deoxy[1-^{14}C]glucose fractional extraction/[5-^{3}H]glucose fractional extraction. Fractional extraction for both glucose and 2-deoxyglucose never exceeded 5%.

Glucose utilization rate per mg of tissue

This was calculated as previously described (Ferré *et al.*, 1985). The integral of arterial-blood [2-deoxy-[1-^{3}H]glucose]/[glucose] was determined from the experimental time points by using a desk calculator.

Statistics

Results are presented as means ± S.E.M. Statistical significance of differences was assessed by Student's *t* test.

RESULTS AND DISCUSSION

The use of the 2-deoxyglucose technique to measure glucose utilization in brown adipose tissue is possible since glucose and 2-deoxyglucose are transported by the same carrier (Czech *et al.*, 1974) and since glucose-6-phosphatase activity is extremely low in this tissue, less than 0.05 μmol/min per g of tissue at 30 °C for warm- or cold-adapted rats (J. Challiss, personal communication). The correction factor for the discrimination against 2-deoxyglucose (lumped constant) was not significantly different for virgin, 19-day-pregnant, non-lactating, lactating Wistar, lean and obese Zucker rats, and all values have been pooled (0.71 ± 0.03, $n = 31$).

In post-absorptive virgin rats, glucose utilization by brown adipose tissue in the basal state (Table 1) is comparable on a wet-weight basis with resting muscle glucose utilization and higher than in white adipose tissue (Ferré *et al.*, 1985). The striking feature is the 60-fold increase in brown-adipose-tissue glucose metabolism in the presence of a physiological increase in plasma insulin. Under these conditions, glucose utilization is much higher than in brain (Ferré *et al.*, 1985). This indicates that brown adipose tissue has a high capacity for glucose utilization, as suggested by enzymic measurement (Cooney & Newsholme, 1982). This also shows that brown adipose tissue is highly insulin-sensitive, since, for a plasma insulin concentration of 350 μunits/ml, for which whole-body glucose utilization is about half of its maximal value (Leturque *et al.*, 1984), glucose utilization is not statistically different from that observed for a maximal plasma insulin value (Table 1). Assuming a weight of about 1–1.5 g for all the brown-adipose-tissue depots, and a whole-body turnover rate of 19.4 μmol/min in virgin rats for an insulin concentration of 350 μunits/ml (Leturque *et al.*, 1984), brown-adipose-tissue glucose utilization would correspond to 8–12% of the whole-body turnover rate, i.e. to a significant contribution.

If brown adipose tissue contributes significantly to the glucose economy of the animal, its metabolism should be modulated by physiological situations in which whole-body metabolism is altered, such as pregnancy and lactation. Indeed, these two situations correspond in the rat to an increased glucose demand (Burnol *et al.*, 1983; Leturque *et al.*, 1984) and are accompanied by hyperphagy.

In 19-day-pregnant rats, in the basal state, glucose utilization by brown adipose tissue is similar to that observed in age-matched virgin rats (Table 1). However, the increase in glucose utilization linked to a physiological or a maximal increment in plasma insulin is significantly decreased in 19-day-pregnant rats (Table 1), despite a nearly similar composition of brown adipose tissue (Agius & Williamson, 1980). Thus brown adipose tissue seems to take part in the overall insulin resistance which characterizes late pregnancy (Leturque *et al.*, 1984).

In lactating rats at peak lactation (12 days), brown-adipose-tissue glucose utilization is decreased as compared with age-matched non-lactating controls in basal conditions. This might be linked to the hypoinsulinaemia and hypoglycaemia which prevail in lactating rats compared with non-lactating controls (Table 1). However, the difference persists in conditions of similar hyperinsulinaemia; it is unlikely then that the 20% lower glycaemia could explain the 55% decrease in glucose metabolism (Table 1). The decreased glucose metabolism of lactating rats is even higher if brown-adipose-tissue glucose utilization is compared with that of virgin animals (Table 1), suggesting that part of the decrease in brown-adipose-tissue glucose metabolism in lactating animals is linked to the preceding gestation. These results are in agreement with studies in lactating rats or mice showing that brown-adipose-tissue thermogenic activity (Trayhurn *et al.*, 1982; Isler *et al.*, 1984) as well as basal or insulin-stimulated lipogenesis (Agius & Williamson, 1980; Agius *et al.*, 1981; Burnol *et al.*, 1983) are decreased compared with virgin animals.

Table 1. Blood glucose, plasma insulin and interscapular-brown-adipose-tissue glucose utilization in the basal state or during euglycaemic–hyperinsulinaemic glucose clamp at two plasma insulin concentrations

Results are means ±S.E.M. for five to six determinations; $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ for significance of differences between virgin and 19-day-pregnant, non-lactating and lactating, lean and obese Zucker; $\dagger P < 0.01$ for significance of difference between clamp A and clamp B.

Experimental group	Treatment	Blood glucose (mM)	Plasma insulin (μunits/ml)	Brown-adipose-tissue glucose utilization (nmol/min per mg)
Virgin	Basal	5.7±0.3	114±16	0.033±0.006
	Clamp A	5.9±0.2	350±50	1.88 ±0.47
	Clamp B	6.3±0.1	6000±400	2.83 ±0.39
19-day-pregnant	Basal	4.5±0.5	142±26	0.022±0.006
	Clamp A	4.2±0.2	450±50	0.36 ±0.12**
	Clamp B	4.4±0.1	7100±600	1.05 ±0.2 ***,†
Non-lactating	Basal	6.1±0.3	106±10	0.056±0.01
	Clamp A	5.5±0.2	367±35	1.00 ±0.16
	Clamp B	6.1±0.2	7800±1100	1.11 ±0.50
12-day lactating	Basal	4.8±0.1	84±10	0.019±0.001**
	Clamp A	4.3±0.1	258±20	0.44 ±0.17*
	Clamp B	4.6±0.1	6100±700	0.58 ±0.10*
Lean Zucker	Basal	5.2±0.2	75±23	0.017±0.003
	Clamp B	5.3±0.2	> 10000	0.65 ±0.11
Obese Zucker	Basal	5.3±0.3	345±73	0.013±0.003
	Clamp B	4.9±0.2	> 10000	0.047±0.006**

Brown-adipose-tissue glucose metabolism in obese animals is of an obvious interest, since defects affecting the thermogenic activity of this tissue have been involved in the development of obesity (see Himms-Hagen, 1984, for a review). We have thus studied brown-adipose-tissue glucose metabolism and its insulin-sensitivity in lean and obese Zucker rats. In the basal state, there was no difference between lean and obese animals in the glucose utilization rate by brown adipose tissue, despite a 4-fold higher plasma insulin concentration in obese rats (Table 1). It can be emphasized that, for this plasma insulin value, glucose utilization by brown adipose tissue is already maximal in virgin Wistar rats (Table 1). This is indicative of a state of insulin resistance in the brown adipose tissue of obese rats. During maximal insulin stimulation, glucose utilization was increased 37-fold in lean rats, but only 4-fold in obese rats, leading to a marked decrease in the maximal rate of glucose metabolism in the latter group. In the Zucker rat, a severe insulin resistance has been described *in vivo* (Terettaz & Jeanrenaud, 1983). The present study shows that a decreased responsiveness to insulin also holds true for brown adipose tissue of obese Zucker rats. Even if the data were computed on a whole-pad-weight basis, which in the 12-week-old obese Zucker rat is 4 times that in its lean littermate (York *et al.*, 1983), it is clear that a large difference would persist. This defect might contribute to the development of the obesity by redirecting glucose towards tissues which have storing but not oxidizing capacity.

In conclusion, we have shown that:

(1) brown-adipose-tissue glucose metabolism is sensitive to insulin;

(2) under physiological insulin stimulation, brown adipose tissue has an extremely high rate of glucose metabolism;

(3) brown-adipose-tissue glucose metabolism can be modulated according to the physiological state; and

(4) in the genetically obese Zucker rat, the response of brown-adipose-tissue glucose metabolism to insulin is markedly impaired.

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