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Hyperinsulinaemia increases insulin action *in vivo* in white adipose tissue but not in muscles

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The effect of 4 days of stable hyperglycaemia and resulting hyperinsulinaemia on insulin-induced glucose utilization by individual rat tissues was studied *in vivo*. The treatment produced a net increase in the glucose utilization index under both basal and insulin-stimulated (euglycaemic/hyperinsulinaemic clamp) conditions in white adipose tissue. On the contrary, glucose utilization was unchanged in aerobic muscles but was decreased in glycolytic skeletal muscles during the clamp.

INTRODUCTION

In obese humans and in several animal models of obesity hyperinsulinaemia is thought to play a key role in insulin-resistance (Jeanrenaud *et al.*, 1985). However, it has been shown in rats made obese by lesion of the ventromedial hypothalamus (VMH) that at the beginning of the syndrome hyperinsulinaemia was associated with increased insulin action, especially in white adipose tissue (Pénicaud *et al.*, 1986, 1989). Some data suggest that this situation can be reproduced by short-term over-insulinization. On the one hand, hyperinsulinaemia induced by 4 days of glucose infusion resulted in an increase in insulin efficiency (Laury *et al.*, 1989). On the other hand, insulin-stimulated glucose uptake by adipose tissue *in vitro* was increased in tissue from rats rendered hyperinsulinaemic by short-term insulin infusion (Trimble *et al.*, 1984; Wardzala *et al.*, 1985). The purpose of the present study was to determine the effects of short-term endogenous hyperinsulinaemia *in vivo* on subsequent insulin action on glucose utilization in individual tissues. We performed a euglycaemic/hyperinsulinaemic clamp coupled with an injection of 2-deoxy[^{3}H]glucose (Ferré *et al.*, 1985) in animals previously infused for 4 days with either saline or glucose.

MATERIALS AND METHODS

Animals

Female Wistar rats, 3 months old and weighing 200–220 g, were used. They were maintained in animal quarters with a day/night cycle (light on from 6:00 h to 18:00 h) and had free access to water and laboratory chow (UAR 113). The technique for long-term glucose infusion described previously was used (Ktorza *et al.*, 1981). The infusion of hyper-osmotic (30%) glucose at a rate of 30 μl/min, to induce a slight hyperglycaemia (150–200 mg/dl), was started 2 days after surgery and lasted 4 days. Control rats were catheterized and infused with saline. Plasma glucose and insulin concentrations were measured five times daily on blood samples taken from the tail vessels.

Euglycaemic/hyperinsulinaemic clamp

Rats were anaesthetized with pentobarbital (50 mg/kg, intraperitoneal). A catheter was inserted in the left carotid artery for blood sampling. Infusions (insulin, labelled 2-deoxyglucose, unlabelled glucose) were carried out via butterfly needles that were inserted into the saphenous veins. A tracheotomy was performed to avoid respiratory problems during the course of anaesthesia. Body temperature was maintained at 38 °C using a heating lamp.

A 200 μl blood sample was first withdrawn for determination of basal blood glucose and plasma insulin levels. Then euglycaemic/hyperinsulinaemic clamp was performed as described previously (Pénicaud *et al.*, 1989). Briefly, exogenous insulin (pig monocomponent insulin; Actrapid Novo, Copenhagen, Denmark; 0.7 units/h per kg) was infused at a constant rate (20 μl/min). Blood samples (25 μl) were taken every 5 min and blood glucose was determined immediately with a glucose analyser (Yellow Spring Instruments, Yellow Spring, OH, U.S.A.). Measurement of glycaemia allowed adjustment of the rate of unlabelled glucose infusion (10%, w/v). At the end of the clamp, three blood samples were drawn for determination of steady-state plasma insulin.

Glucose utilization in individual tissues

A tracer dose of 2-deoxy[1-^{3}H]glucose (20 Ci/mmol; 20 μCi/rat; CEA, Saclay, France) was injected in 100 μl of 0.9% NaCl as a bolus through a saphenous vein in animals submitted or not to an euglycaemic clamp. The injection took place 50 min after the beginning of the clamp. At 1, 3, 5, 10, 15, 20, 30, 40 and 60 min after the injection, blood samples were taken via the arterial catheter for determination of 2-deoxy[1-^{3}H]glucose radioactivity and blood glucose concentrations. Rats were killed by cervical dislocation 60 min after the 2-deoxy[1-^{3}H]glucose injection and tissues were immediately removed and their 2-deoxy[1-^{3}H]glucose-6-phosphate contents were determined as described previously (Ferré *et al.*, 1985). Glucose utilization was then calculated, except that the value found was not corrected by the discrimination factor (lumped constant) for 2-deoxyglucose in glucose metabolic pathways. Therefore the results are expressed as an index of glucose utilization.

Analytical methods

Blood samples for determination of 2-deoxyglucose specific radioactivity were deproteinized with 250 μl of $ZnSO_4$ and 250 μl of $Ba(OH)_2$ and immediately centrifuged. A sample of the supernatant was used for the determination of glucose concentration with a glucose oxidase kit (Boehringer, Mannheim, Germany). Another sample (100 μl) was counted in a liquid scintillation spectrometer for the determination of blood

Abbreviations used: HG rats, glucose-infused rats; VMH, ventromedial hypothalamus.

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2-deoxy[1-^{3}H]glucose. Blood samples were immediately centrifuged at 4 °C and the plasma was frozen at −20 °C for subsequent determination of insulin concentration. Insulin was quantified by radioimmunoassay with rat or pig insulin as standard.

Results are expressed as means ± S.E.M. Statistical significance was determined by Student's t test for unpaired data.

RESULTS AND DISCUSSION

During the 4 days of glucose infusion, plasma glucose was maintained in the range 152–197 mg/dl (mean ± S.E.M.: 173 ± 5 mg/dl, $n = 7$), whereas it fluctuated from 115 to 122 mg/dl in controls (119 ± 1 mg/dl, $n = 7$). Hyperglycaemia resulted in an increase in the plasma insulin concentration (638 ± 34 μunits/ml; range 540–809 μunits/ml) in comparison with control rats (42 ± 2 μunits/ml; range 35–49 μunits/ml). In order to maintain the hyperglycaemia in the above range, we had to increase the glucose infusion rate throughout the infusion period. Insulinaemia did not change significantly during this period. Taken together, these results corroborate our previous observation demonstrating an increased tissue responsiveness to insulin in glucose-infused (HG) rats (Laury *et al.*, 1989). At the end of this period, plasma glucose and insulin concentrations returned very quickly (around 30 min) to levels similar to those of control rats (results not shown).

The measurement of glucose utilization by individual tissues was performed 3 h after the end of glucose infusion, i.e. when plasma glucose and insulin concentrations were similar in control and HG rats (Table 1). During the clamp study, the levels of glucose and insulin were identical in the two groups of animals (Table 1).

In the basal state, the glucose utilization indexes of muscles were similar in control and HG rats (Table 2). This was true for oxidative (soleus, adductor longus and diaphragm) as well as glycolytic (extensor digitorum longus, tibialis anterior and epitrochlearis) muscles (Ariano *et al.*, 1973; Green *et al.*, 1984). During the euglycaemic/hyperinsulinaemic clamp, glucose utilization was strongly stimulated by insulin in oxidative muscles in both groups of rats (Table 2). In glycolytic muscles, the stimulation of glucose utilization by insulin was far less pronounced in HG than in control rats (Table 2). A differential response to insulin in muscles submitted to a period of over-insulinization depending on the type of muscle considered was suggested by previous works. Thus decreased insulin action in quadriceps (mainly a glycolytic muscle) of rats submitted to long-term over-insulinization (Kruszynska *et al.*, 1987), and in hindlimb perfused for 5 h with glucose plus insulin, has been reported (Richter *et al.*, 1988). On the other hand, in rats submitted to 2 weeks of chronic hyperinsulinaemia (with concomitant hypoglycaemia), insulin-sensitivity of incubated soleus muscle was slightly increased (Wardzala *et al.*, 1985), and incubation of this muscle for 5 h with insulin increased insulin action (Young *et al.*, 1986). Moreover, in rats rendered hyperinsulinaemic by exogenous insulin infusion, insulin-stimulated glucose utilization was unchanged in oxidative muscles but was decreased in glycolytic muscles (Cusin *et al.*, 1990).

In white adipose tissue, the glucose utilization index was, in the basal state, significantly higher in HG than in control rats in subcutaneous (inguinal fat pad) and profound (periovarian fat pad) locations despite the identical plasma insulin concentrations in the two groups of rats (Tables 1 and 2). During the euglycaemic/hyperinsulinaemic clamp, the glucose utilization index was increased by a factor of 2 in both pads and in both

Table 1. Plasma glucose and insulin concentrations in control (C) and HG rats under basal conditions and during a hyperinsulinaemic/euglycaemic clamp

Results are means ± S.E.M. of seven determinations. *Difference is statistically significant when compared with basal conditions; $P < 0.001$.

	Plasma glucose (mg/dl)		Plasma insulin (μunits/ml)	
Rats	Basal	Clamp	Basal	Clamp
C	105 ± 4	99 ± 6	49 ± 8***	342 ± 23
HG	103 ± 5	91 ± 4	65 ± 7***	290 ± 16

Table 2. Glucose utilization index in tissues of control (C) and HG rats either in the basal state or during an euglycaemic/hyperinsulinaemic clamp

Results are means ± S.E.M. of seven determinations. Statistically significant differences when compared with control values under the same situation are indicated by *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Statistically significant differences when compared with basal conditions are indicated by †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$.

	Glucose utilization index (ng/min per mg wet wt.)			
	Basal		Clamp	
	C	HG	C	HG
Oxidative muscles				
Soleus	5.0 ± 1.5	7.6 ± 1.7	12.8 ± 1.2††	14.8 ± 1.0††
Adductor longus	6.0 ± 0.3	7.2 ± 1.2	23.8 ± 3.4†††	24.5 ± 2.4†††
Diaphragm	11.2 ± 3.1	19.0 ± 1.0	63.0 ± 9.0†††	62.9 ± 7.0†††
Glycolytic muscles				
Extensor digitorum longus	3.8 ± 0.4	2.9 ± 0.4	15.9 ± 0.8***†††	5.8 ± 1.0†
Tibialis anterior	6.5 ± 1.7	3.1 ± 0.5	20.6 ± 2.5**††	9.2 ± 1.0†††
Epitrochlearis	1.7 ± 0.1	1.9 ± 0.2	22.7 ± 3.3**†††	8.3 ± 1.3†††
White adipose tissue				
Periovarian	1.8 ± 0.3*	7.3 ± 1.9	3.7 ± 1.0***	14.2 ± 1.7†
Inguinal	1.6 ± 0.2**	12.1 ± 2.6	3.5 ± 0.8***†	18.9 ± 2.9†

groups, so that these indexes were still 4- and 6-fold higher in HG than in control rats (Table 2). This shows an increased insulin action in the white adipose tissue of HG rats. Increased glucose uptake in the white adipose tissue of rats submitted to a short period of exogenous hyperinsulinaemia has been reported *in vitro* (Trimble *et al.*, 1984; Wardzala *et al.*, 1985) and *in vivo* (Cusin *et al.*, 1990). Our results demonstrate that short-term endogenous hyperinsulinaemia increases glucose utilization in white adipose tissue *in vivo*.

Hyperinsulinaemia and insulin-resistance are two main features encountered in animal models of obesity (Jeanrenaud *et al.*, 1985). However, there is a report of an increased insulin action at the beginning of obesity (Pénicaud *et al.*, 1986), and the time course of insulin-resistance varies according to the tissue. In muscles, insulin-resistance appears quite early during the development of obesity (Crettaz *et al.*, 1980; Pénicaud *et al.*, 1987; Doré *et al.*, 1988). Furthermore, in rats made obese by lesion of the VMH nuclei, early insulin-resistance was observed in glycolytic but not in oxidative muscles (Pénicaud *et al.*, 1989). In white adipose tissue, the efficiency of insulin with regard to glucose utilization is increased at the beginning of genetic or experimental obesity (Le Marchand-Brustel *et al.*, 1978; Pénicaud *et al.*, 1987, 1989). The similar results obtained in the present study, in non-obese hyperinsulinaemic rats, and in VMH-lesioned rats with respect to both oxidative and glycolytic muscles and white adipose tissue (Pénicaud *et al.*, 1989) suggest that the short-term hyperinsulinaemia which is present in the obese animals could be responsible for the change observed (Jeanrenaud, 1978).

In conclusion, the present data demonstrate that 4 days of hyperglycaemia and the resulting hyperinsulinaemia induce an increased insulin action on glucose utilization in white adipose tissue and a slight decrease in the glucose utilization of glycolytic muscles. These changes are similar to those observed in animals made hyperinsulinaemic (and hypoglycaemic) by exogenous insulin infusion and in animal models of obesity, particularly in VMH-lesioned rats (Pénicaud *et al.*, 1989). This suggests a crucial role for insulin in the changes described.

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