Stimulatory Effect of Cold Adaptation on Glucose Utilization by Brown Adipose Tissue

RELATIONSHIP WITH CHANGES IN THE GLUCOSE TRANSPORTER SYSTEM*

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The effect of cold adaptation (4 °C) on the in vivo glucose utilization and on the number of properties of the glucose transporters has been studied in brown adipose tissue of normal rats. Glucose utilization was assessed in vivo by the 2-deoxyglucose method. Glucose transporters in plasma and microsomal membranes were quantified by the [3H]cytochalasin B-binding assay. After cold adaptation the in vivo glucose utilization by brown adipose tissue increased 21-fold compared to controls (22 °C). The number of glucose transporters in plasma membranes of brown adipose tissue increased from 75 to 436 pmol/g tissue and that of total glucose transporters (plasma + microsomal membranes) from 438 to 754 pmol/g tissue. In addition, cold adaptation increased the Hill coefficient of the plasma membrane transporter for cytochalasin B from 0.90 to 2.03 and decreased the \( K_d \) from 100 to 54 nm. This study shows that cold adaptation promotes: (a) a translocation of glucose transporters from an intracellular pool to plasma membranes; (b) an increased number of plasma membrane glucose transporters unaccounted for by the translocation process (e.g., de novo synthesis); (c) an increase in the Hill coefficient for cytochalasin B that could also represent changes in the properties of the transporters vis-à-vis glucose, (e.g., positive cooperativity); and (d) a decrease in the \( K_d \) value for cytochalasin B.

In rodents adapted to a cold environment, brown adipose tissue (BAT) is a major site of nonshivering thermogenesis (1). To fulfill this increased energy expenditure, the substrate requirement of BAT is augmented during cold exposure. Together with fatty acids, glucose may be an important fuel for this tissue (2, 3), either as a source of carbon for fatty acid synthesis or as a direct source of energy through its oxidation. Indeed, it has been observed that brown adipose tissue from cold-adapted rats utilizes more glucose when incubated in vitro than that of controls kept at normal temperature (4). In BAT, as in white adipose tissue, glucose transport appears to be the rate-limiting step for glucose utilization (5). Preliminary data have shown that cold adaptation produces an increase in the total number of glucose transporters in BAT plasma membranes of mice (24), although the underlying mechanism(s) of this effect was not investigated. The aim of the present study was therefore to measure the in vivo glucose utilization by BAT of control rats kept at 22 °C and of cold-adapted (4 °C) ones and to attempt relating changes in glucose utilization to changes in the number or in the state of the glucose transporters of plasma and microsomal membranes. The data show that, in brown adipose tissue of cold-adapted rats, glucose utilization is markedly increased.

This cold-induced metabolic effect is accompanied by a translocation of glucose transporters from an intracellular pool to the plasma membranes, by a further increase in the number of plasma membrane glucose transporters (suggestive of an increased "de novo" synthesis), by an increase in the Hill coefficient of plasma membrane glucose transporter for cytochalasin B, possibly reflecting the occurrence of a positive cooperativity between plasma membrane transporters, and by a decrease in \( K_d \) for cytochalasin B.

**EXPERIMENTAL PROCEDURES**

Normal male rats of the Zucker (FA/FA) strain (253 ± 5 g) were used throughout the experiments. They were initially all maintained at constant temperature (22 °C) with a fixed (12 h) light cycle. The animals were fed ad libitum with a standard laboratory chow (UAR, Epinay/Orge, France). For cold adaptation the rats were transferred for 12 days to a room refrigerated at 4 °C. At the time of the experiments the animals were 12-weeks-old.

BAT glucose utilization was studied in vivo in conscious rats, using the 2-deoxyglucose technique described by Ferré et al. (6). This technique was as follows: at the age of 10 weeks, the rats were implanted, under anesthesia (Nembutal, 70 mg/kg body weight) with a cardiac catheter placed via the jugular vein and fixed on the skull with acrylic cement as described elsewhere (7, 8). Three to 4 days after the surgery the animals had regained their normal body weight and were placed at 4 °C or kept at room temperature. The catheters were rinsed every third day. On the day of the experiment, 40 pCi of 2-deoxy-\( ^3 \)H]glucose were injected through the catheter that was then rinsed with a 0.2-mI isotonic saline solution and used for blood sampling at 1, 3, 5, 10, 20, 40, and 60 min. Blood was collected in heparinized tubes and plasma used for measurements of immunoreactive insulin (9). Following plasma deproteinization in Ba(OH)\( _2/ \)ZnSO\( _4 \) supernatants were used for the determination of blood glucose levels (glucose oxidase kit) and for those of 2-deoxy-\( ^3 \)H]glucose (6). After the last blood sample, the rats were killed by the intravenous injection of 0.8 ml of Nembutal. Interscapular brown adipose tissue was removed and dissected from adjacent tissues. Its content in 2-deoxy-\( ^3 \)H]glucose 6-phosphate was determined as in (6). The rate of glucose utilization derived from the amount of 2-deoxy-\( ^3 \)H]glucose 6-phosphate was calculated using a mathematical formula previously justified (6). It implied the determination of a correction factor (referred to as lumped constant) used for the possible discrimination of 2-deoxyglucose against glucose. Such a correction factor...
Cold Adaptation and Brown Adipose Tissue Glucose Transporters

Glucose utilization by interscapular BAT in rats kept at normal temperature or cold-adapted at 4°C

12-week-old normal male rats of the Zucker (FA/FA) strain. Cold adaptation was of 12-day duration at 4°C. Glucose utilization was assessed 60 min after 2-deoxy-D-[3H]glucose administration via a chronic cardiac catheter to conscious rats (see "Experimental Procedures"). Results are the mean ± S.E. of three individual determinations.

Table I

| Control rats (22°C) | Cold-adapted rats (4°C) |
|---------------------|-------------------------|
| BAT glucose utilization (mg/min × mg of tissue) | 10.2 ± 0.4 | 210.5 ± 16.4 |
| Plasma insulin (microunits/ml) | 32.5 ± 2.5 | 37.5 ± 7.8 |
| Plasma glucose (mm) | 7.4 ± 0.3 | 7.8 ± 0.3 |

*2p < 0.001 compared to control at 22°C.
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Plasma and microsomal membranes were obtained as described under "Experimental Procedures." Results are the mean ± S.E. of six individual preparations for each group of rats.

| TABLE II |
|-----------------|-----------------|-----------------|
|                 | 5' Nucleotidase  | NADPH-cytochrome-c reductase | Protein |
|                 | Specific activity | Recovery | Specific activity | Recovery | Specific activity | Recovery |
|                 | microunits/ mg protein | % | milliunits/mg protein | % | mg/g BAT | % |
| Control (22 °C) |                    |       |                  |       |               |       |
| Homogenate     | 1.65 ± 0.13       | 32.5 | 41.3 ± 5.7      | 6.1 | 184.4 ± 18.0 | 2.5 |
| Plasma membranes | 22.05 ± 3.00      | 4.8  | 99.1 ± 5.5      | 12.2 | 4.5 ± 0.3    | 2.5 |
| Microsomes     | 4.35 ± 0.52       | 5.5  | 271.0 ± 15.1    | 0.5 | 3.3 ± 0.2    | 1.8 |
| Cold-adapted (4 °C) |               |       |                  |       |               |       |
| Homogenate     | 0.98 ± 0.10*     |       | 46.3 ± 3.5      | 7.3 | 349.6 ± 26.2* | 2.4 |
| Plasma membranes | 20.58 ± 0.85     | 4.9  | 152.0 ± 10.1*   | 0.7 | 7.7 ± 0.7*   | 2.0 |
| Microsomes     | 3.78 ± 0.32*     |       | 416.2 ± 33.0*   | 0.7 | 6.4 ± 0.6*   | 2.0 |
|                 | p < 0.005 compared to control. |
|                 | p < 0.05. |

| TABLE III |
|-----------------|-----------------|-----------------|
|                 | Hill coefficient, Kd, and number of D-glucose inhibitable cytochalasin B-binding sites in plasma and microsomal membranes of brown adipose tissue from control and cold-adapted normal rats |
| Values obtained after correction for cross-contamination. Plasma and microsomal membranes were obtained as described under "Experimental Procedures." Results are the mean ± S.E. of four (control) and three (cold-adapted) independent preparations. * = specific cytochalasin B-binding activities have been adjusted to those which would have been observed had the membrane fractions been free of cross-contamination. Adjustments were based on the enzyme marker specific activities and on the assumption that only 5'-nucleotidase activity is localized specifically to the plasma membrane and that NADPH-cytochrome-c reductase is a specific marker for microsomal membranes. |
|                 | Control rats (22 °C) | Cold-adapted rats (4 °C) |
|                 | Plasma membranes | 0.90 ± 0.03 | 2.93 ± 0.19* |
|                 | Microsomes       | 1.14 ± 0.04 | 1.34 ± 0.05* |
|                 | Kd (nM)          | Plasma membranes | 99 ± 4     | 54 ± 3* |
|                 |                 | Microsomes       | 91 ± 5     | 61 ± 6* |
|                 | Cytochalasin B-binding sites* (pmol/mg of membrane protein) | Plasma membranes | 5.51 ± 0.62 | 27.07 ± 2.20* |
|                 |                 | Microsomes       | 13.43 ± 1.09 | 7.96 ± 0.63* |
|                 | Cytochalasin B-binding sites* (pmol/g of tissue) | Plasma membranes | 75 ± 8 | 436 ± 35* |
|                 |                 | Microsomes       | 363 ± 30 | 318 ± 25 |
|                 | Total           | 438 ± 37 | 754 ± 55* |
|                 |                 | p < 0.005. |
|                 |                 | p < 0.05. |
|                 |                 | p < 0.001. |

Further shown by Figs. 2 and 3 (panels B and D). As can be seen, binding curves obtained in the BAT membranes fit a rectangular hyperbola in control (22 °C) rats (Fig. 2, panels B and D), whereas a sigmoidal shape was observed in BAT membranes of cold-adapted rats (Fig. 3, panels B and D). For this reason, the data had to be analyzed according to the Hill equation (16). Kd and Hill coefficient were determined in individual experiments as described under "Experimental Procedures," and are presented on Table III. In BAT plasma membranes of control rats, the Hill coefficient was close to 1 and Kd was 99 nM. Cold adaptation resulted in a doubling of the Hill coefficient and a significant decrease in the Kd of the plasma membranes. The properties of the glucose transporter in the microsomal membranes of control animals were very similar to those of the plasma membranes. Cold adaptation promoted slight changes in the Hill coefficient of microsomal membranes and a decrease in the Kd toward cytochalasin B.
Fig. 2. [3H]Cytochalasin B-binding sites in brown adipose tissue plasma and microsomal membranes from control rats (22 °C). [3H]Cytochalasin B binding to plasma and microsomal membranes from control rats has been measured at six standard concentrations in the absence (○) or presence (■) of 500 mM d-glucose (panels A and C), as described under "Experimental Procedures." The differences of the values obtained in the presence of d-glucose from their respective values obtained in the absence of d-glucose were analyzed according to the Hill equation (16) in order to obtain the number of cytochalasin B-binding sites (Rn, in picomoles per milligram of membrane protein), the Kd (dissociation constant, in nanomolar), and the Hill coefficient (Hc). Using the values of Rn, Kd, and Hill coefficient thus found, the binding curve was plotted (panels B and D). Kd and Hill coefficient were calculated from the averaged curves shown by the figure. Results are the means ± S.E. of four independent preparations in duplicate determinations.

Table III also summarizes the results of the cytochalasin B-binding sites in the plasma and microsomal membranes from control (22 °C) and cold- (4 °C) adapted rats. The measured specific cytochalasin B-binding activities have been adjusted to those which would have been observed, had the membrane fractions been free of cross-contamination. Adjustments were based on the enzyme marker specific activities and on the assumptions that 5′-nucleotidase activity is localized specifically to the plasma membrane and NADPH-cytochrome-c reductase is a specific marker for microsomal membranes. These corrections did not modify the Hill coefficient or the Kd. As can be seen by Table III, cold adaptation produced a significant increase (by 5–6-fold) in the number of glucose transporters associated to the plasma membranes and a decrease (by 40%) in those present in the microsomal fraction, when expressed per milligram of protein. Furthermore, the total number of glucose transporters (plasma + microsome membranes) was increased by 2-fold by cold adaptation.

**DISCUSSION**

The present data show for the first time that, when the experiments are performed at 4 °C, glucose utilization by BAT is markedly increased (21-fold) by cold adaptation (Table I). This is in keeping with other data that suggested indirectly an increased glucose utilization by this tissue, used in particular for heat dissipation and fatty acid synthesis during cold adaptation (2–4). This study also shows the basic characteristics of the glucose transporters of brown adipose tissue of rats and underscores the major changes which are occurring in the glucose transporters after cold adaptation. As depicted in Table III, exposure to cold results in a 5–6-fold increase (on a per milligram of membrane protein or on a per gram of tissue basis) in the number of the glucose transporters in the plasma membranes, together with a 40% decrease in the number of the glucose transporters in microsomal membranes (when expressed on a per milligram of protein basis but not per gram of tissue).

In addition, cold adaptation brings about other changes in the BAT glucose transporters. Thus, when data are analyzed according to the Hill equation (16) to obtain a precise evaluation of the properties of glucose transporters, as well as the Hill coefficient, it is observed that this latter value is close to 1 in both plasma and microsomal membranes of control rats maintained at 22 °C. In marked contrast, cold adaptation increases the Hill coefficient of plasma membrane transporters (to a value of 2.0) and, to a lesser extent (1.3), that of the microsomal ones. This suggests the existence of a positive cooperativity amongst the glucose transporters that would be induced by cold adaptation. Functionally, this could mean that the binding of each molecule of cytochalasin B increases the affinity for the next one and that cold adaptation would thus change the properties of the glucose transporters by making them more efficient. Cold adaptation also increases the affinity of the glucose transporter for cytochalasin B by decreasing the Kd. Hill coefficient and Kd of the glucose transporters are measured toward cytochalasin B. They could
reflect changes in the affinity and cooperativity of the transporters toward glucose and thereby contribute, in addition to the well-characterized translocation process, to the increased glucose uptake. Indeed, additional mechanisms involving "activation" of transporters have been postulated (20, 21) to explain the stimulatory effect of insulin on glucose transport in white adipocytes or the inhibitory effect of hormones counterregulating insulin-stimulated glucose transport (22, 23).

It can be speculated that the marked increase in glucose utilization produced by cold adaptation could be due to an increased glucose uptake. This view is based on the finding that cold adaptation produced a 5–6-fold increase of the plasma membrane glucose transporter number when expressed per gram of tissue, together with a doubling of the plasma and microsomal membrane Hill coefficients toward cytochalasin B indicating the appearance of positive cooperativity. A marked increase in the Hill coefficient and a decrease in $K_d$ toward cytochalasin B are also observed in BAT plasma membranes after insulin, a condition known to markedly increase glucose utilization (3).

These data together are compatible with the view that cold adaptation produces a dual effect on brown adipose tissue: (a) a marked increase in the total number of the glucose transporters (plasma + microsomal membranes), possibly due to a stimulation of their de novo synthesis (b) a stimulation of the translocation of these transporters from the intracellular pool to the plasma membrane in a way that may be similar to that observed after the addition of insulin to isolated white adipose tissue (15, 17) or to isolated diaphragm (18, 19); and (c) changes in the properties of the transporter vis-à-vis cytochalasin B that may possibly reflect changes in the properties of the system for glucose as proposed by others (20, 21). All these modifications may participate in the marked increase in glucose utilization measured in brown adipose tissue after cold adaptation.

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