Lactate transport in rat adipocytes: identification of monocarboxylate transporter 1 (MCT1) and its modulation during streptozotocin-induced diabetes

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Abstract We have characterised L-lactate transport in rat adipocytes and determined whether these cells express a carrier belonging to the monocarboxylate transporter family. L-Lactate was taken up by adipocytes in a time-dependent, non-saturable manner and was inhibited (by ~90%) by α-cyano-4-hydroxycinnamate. Lactate transport was stimulated by 3.7-fold upon lowering extracellular pH from 7.5 to 6.5 suggesting the presence of a lactate/proton-cotransporter. Antibodies against mono carboxylate transporter 1 (MCT1) reacted positively with plasma membranes (PM), but not with intracellular membranes, prepared from adipocytes. MCT1 expression was down-regulated in PM of adipocytes from diabetic rats, which also displayed a corresponding loss (~64%) in their capacity to transport lactate. The data support a role for MCT1 in lactate transport and suggest that changes in MCT1 expression are likely to have important implications for adipocyte lactate metabolism.

Key words: Mono carboxylate transporter 1 (MCT1); MCT4; Insulin; Membrane; Diabetes; Gluconeogenesis

1. Introduction

In mammalian cells the production of lactic acid represents one of the key end-points of glucose metabolism which contributes significantly towards acidification of the intracellular compartment. To help maintain intracellular acid–base homeostasis and metabolic control, monocarboxylates such as lactate and pyruvate are transported across the plasma membrane (PM) by a family of monocarboxylate/H^+ co-transporters (MCT) [1]. There are several isoforms of MCT transporters that mediate the transfer of unbranched aliphatic monocarboxylates from C-2 to C-5 (e.g. lactate, C-3) across the PM of mammalian cells. This group of transporters includes the Na^+-dependent monocarboxylate transporters, which are predominantly expressed in intestinal and renal epithelia where they function primarily to aid reabsorption of lactate, pyruvate and ketone bodies [2]. To date, eight members of the MCT family have been characterised, denoted as MCT1–7 and XPCT (MCT8) which are expressed in a tissue-dependent manner possibly reflecting distinct functional roles [1,3,4]. For example, skeletal muscle, a tissue that not only generates large quantities of lactate but one that may also utilise it as a respiratory fuel, expresses MCT1 and MCT4. Expression of both isoforms in this tissue can be rapidly modulated in response to changes in muscular activity suggesting that the turnover of MCT proteins in this tissue may be closely regulated by rates of lactate production, a feature that may be important in helping to regulate intramuscular pH [4].

In addition to its lipogenic and lipolytic functions, adipose tissue is also an important site of lactate production [5]. The overall contribution made by adipose tissue to whole body lactate production can, however, vary considerably depending on the percentage of body fat. In lean individuals, for example, white fat constitutes between 15 and 25% of body mass, whereas in severely obese subjects it may account for as much as 50% of total body mass [6]. Lactate production in adipose tissue is known to be modulated by altered pathology. During insulin lack or insulin resistance, when there is a marked reduction in total glucose metabolism, there is an associated fall in whole body lactate production [7]. In contrast, lactate production is significantly enhanced, accounting for between 45 and 70% of the total glucose metabolised, during obesity when fat cells undergo enlargement and also during fasting [8–10]. Indeed, it is has been suggested that lactate over-production associated with fat cell enlargement may be a factor that contributes to many of the abnormalities that accompany the development of obesity and carbohydrate intolerance [5]. Surprisingly, however, despite the important role played by adipose tissue in the regulation of whole-body lactate dynamics very little is known about the mechanism by which lactate is transported across the fat cell membrane and whether this process is modulated by disease states, such as diabetes. In the present study we have characterised L-lactate transport in rat adipocytes and show, for the first time, that this tissue expresses MCT1, a member of the monocarboxylate transporter family. We also show that this transporter localises specifically to the PM of the adipocyte and that its expression is down-regulated during streptozotocin (STZ)-induced diabetes leading to an associated reduction in lactate transport.

2. Materials and methods

2.1. Reagents and animals

All chemicals were purchased from Sigma unless indicated otherwise in the text. Male Sprague Dawley rats (~250 g, Bantin and Kingman, Hull, UK) were killed by cervical dislocation and epididymal fat-pads removed. Adipocytes were isolated from these by collagenase digestion as described previously [11]. In some experiments, rats were made diabetic by a single subcutaneous injection of 65 mg/kg anhydrous STZ (Sigma) reconstituted in citrate-buffered saline

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(pH 4.5). Control animals were injected with vehicle alone. Urine samples were taken daily to assess diabetic status using Ladstix strips for urine glucose (Bayer Diagnostics) and blood was sampled at the time of killing for analysis of glucose using Glucostix strips (Bayer Diagnostics). Urine of diabetic animals reacted positively for the presence of glucose and blood glucose was typically in excess of 15 mmol/l. Rats were maintained diabetic for a period of 4 days after which period they were killed for adipocyte harvest.

2.2. Lactate uptake in adipocytes

Isolated rat adipocytes were incubated in Krebs buffer (KRP buffer, 120 mM NaCl; 4.8 mM KCl; 1.3 mM $CaCl_2$; 1.2 mM $MgSO_4$; 3.1 mM NaH_2PO_4; 12.5 mM Na_2HPO_4; 200 nM adenosine; 2% bovine serum albumin (w/v, fraction V); pH 7.4) in the absence or presence of inhibitors, if indicated. Uptake was started by addition of L-[^{14}C(U)]lactate (NEN, Boston, MA, USA, specific activity 174.6 mCi/mmol) and [^{3}H]inulin (NEN, specific activity 0.63 mCi/mmol) which was used to measure non-specific cell-associated radioactivity. Uptake was terminated after 10 min by rapid centrifugation through a 100 μl di-isononyl phtalate oil cushion (Fluka, Gillingham, Dorset, UK) for 20 s and samples processed for liquid scintillation counting using a dual channel program.

2.3. Western blot analysis

PMs and low density microsomes (LDMs) from adipose tissue were prepared as described previously [11,12]. Isolated membrane fractions were subjected to SDS–PAGE on 10% polyacrylamide gels as described by Laemmli [13], and electrophoretically transferred onto nitrocellulose membranes. Membranes were incubated with specific polyclonal antibodies against MCT1 or MCT4 (both 1:200, kindly provided by Professor A.P. Halestrap, University of Bristol, UK). Membranes were also immunoblotted with antibodies against GLUT4 (1:500, East Acres, Southbridge, MA, USA), or against rat GLUT5 (1:1000, generously provided by Dr Y. Oka, Japan, [14]). Primary detection was carried out using horseradish peroxidase-conjugated secondary antibody (1:1000, HRP anti-rabbit IgG, SAPU, UK). Immunoreactive bands were detected by the enhanced chemiluminescence method (Amersham, Life Science, Bucks, UK).

2.4. Statistical analysis

Statistical analyses were carried out using a two-tailed Student's *t*-test, data were considered significant at P values less than 0.05.

3. Results and discussion

3.1. L-Lactate uptake in rat adipocytes

We first assessed whether isolated rat adipocytes possessed the capacity to transport L-lactate. Fig. 1A shows that uptake of 50 μM L-lactate was linear for up to 30 min. After this period uptake became non-linear approaching equilibrium by approximately 2 h of incubation. Based on this observation all subsequent experiments were performed over 10 min for convenience. To assess the kinetics of lactate transport in isolated adipocytes, cells were incubated in the presence of increasing concentrations of unlabelled L-lactate (from 50 μM to 50 mM). Lactate uptake was found to be not saturable over the range of concentrations used possibly suggesting that lactate is either transported passively across the PM of adipocytes or that it is taken up by a very low affinity high capacity transporter or channel. This finding is different to that reported for other insulin responsive tissues, such as heart and skeletal muscle, in which uptake of lactate has been shown to be a saturable process [15–17].

3.2. Effect of α-cyano-4-hydroxycinnamate (CHC) on L-lactate uptake

In order to better characterise the process by which lactate was taken up into rat adipocytes, we pre-incubated isolated fat cells for 10 min with 5 mM CHC, a well-established inhibitor of L-lactate uptake [1]. Fig. 2 shows that CHC suppressed L-lactate uptake by ~90% ($P < 0.001$). This inhibition could not be prevented by increasing concentrations of L-lactate suggesting that CHC either acts in a non-competitive fashion or that the highest concentration of lactate used was insufficient to compete out or reverse the inhibition exerted by CHC. If the latter were so, this would imply that the transport process has a much higher affinity for CHC than lactate. This latter possibility cannot be excluded given our inability to saturate L-lactate transport at lactate concentrations as high as 50 mM and reports in the literature indicating that the IC_{50} for CHC inhibition of the monocarboxylate transporter is ~27 μM [2]. CHC is thought to act by binding to the high affinity internal substrate binding site of the monocarboxylate transporter. Thus the substantial inhibition in L-lactate uptake seen in the presence of CHC would support the presence of a membrane bound lactate carrier and suggests that passive movement of lactate across the adipocyte PM is likely to make only a minor contribution towards lactate transport.

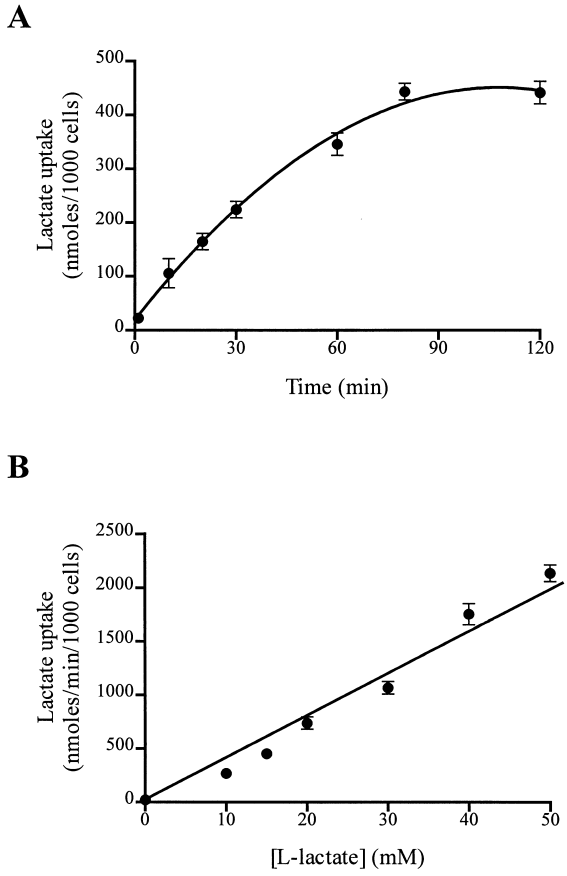

Fig. 1. Time course of L-lactate uptake and the effects of extracellular L-lactate concentration on L-lactate uptake in isolated rat adipocytes. A: The uptake of L-lactate (50 μM) in rat adipocytes was assayed as described in Section 2 for the times indicated. B: Isolated rat adipocytes were incubated in the presence of different concentrations of unlabelled L-lactate and uptake of radiolabelled lactate determined over 10 min as described in Section 2. Values represent the mean ± S.E.M. for up to nine separate experiments, each performed in triplicate.

3.3. Effect of extracellular pH upon L-lactate uptake

Fig. 3 shows that the initial rate of L-lactate uptake was markedly dependent on the extracellular pH. The initial rate

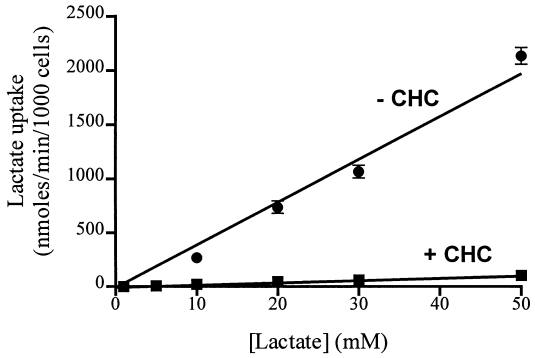

Fig. 2. Effect of CHC on L-lactate uptake in isolated rat adipocytes. Cells were pre-incubated in the absence or presence of 5 mM CHC for 10 min prior to assaying L-lactate uptake at different extracellular concentrations of unlabelled L-lactate as described in the Section 2. Values represent the mean ± S.E.M. for three to nine separate experiments each performed in triplicate.

of lactate uptake at 50 μM was 3.7-fold higher at pH 6.5 compared to that assayed in cells maintained in uptake buffer at pH 7.4. These observations are in line with that reported in other cell types showing that transfer of lactate across the PM involves the cotransport of lactate and a proton [1]. Although it is widely accepted that the Na/H antiporter is the major regulator of intracellular pH [18], the coupled movement of lactate and protons is also likely to make an important contribution towards removal of metabolically produced acid in adipose tissue.

3.4. *Expression and subcellular distribution of MCT1 in rat adipocytes*

The results presented thus far support the presence of a lactate transport protein that is not readily saturated, but which is sensitive to inhibition by CHC and whose activity is enhanced by low extracellular pH. To establish the identity and localisation of the carrier(s), we performed subcellular-fractionation of rat adipocytes to isolate PMs and LDMs (internal membranes). Membrane protein was subjected to SDS–PAGE and immunoblotted with antibodies against two monocarboxylate transporters (MCT1 and MCT4) both of which are expressed in skeletal muscle, another primary insulin target tissue. PM and LDM were also probed with antibodies against the insulin responsive glucose transporter, GLUT4, and the GLUT5 fructose transporter as appropriate marker proteins. In adipocytes that have not been stimulated with insulin, the majority of the GLUT4 is localised intracellularly within specialised storage vesicles that are present in the LDM fraction [19], whereas GLUT5 is expressed only in the PM of rat adipocytes [11]. Consistent with this, expression of GLUT5 and GLUT4 protein was largely restricted to the PM and LDM fractions, respectively (Fig. 4A). When these mem-

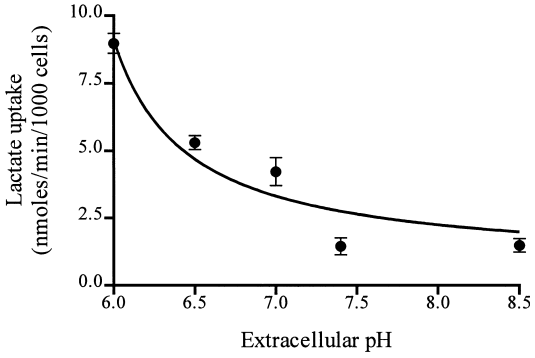

Fig. 3. Effect of extracellular pH on L-lactate uptake in isolated rat adipocytes. Isolated adipocytes were washed twice with KRP buffer (pH of the buffer was adjusted to that required and cell washes lasted no longer than 2 min) prior to assaying uptake of 50 μM L-lactate at the desired pH as described in Section 2. Values are means ± S.E.M. of three separate experiments performed in triplicate.

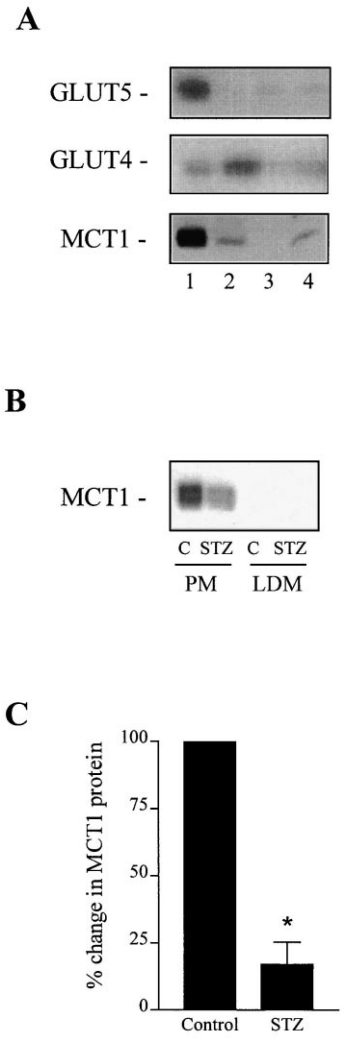

Fig. 4. Representative Western Blots showing the abundance of GLUT5, GLUT4 and MCT1 in rat adipocyte membranes, crude membranes from rat brain and liver and the effects of STZ-diabetes on MCT1 expression in rat adipocytes. A: PMs (1), LDMs (2), brain (3) and liver (4) crude membranes (35 μg of each) were applied to SDS–PAGE gels and immunoblotted using isoform-specific antibodies against GLUT4, GLUT5 and MCT1 as described in Section 2. B: Rats were rendered diabetic with STZ. PMs and low density membranes (LDM) were prepared by subcellular fractionation of isolated adipocytes and subjected to SDS-PAGE and immunoblotting as described in Section 2. Representative immunoblot showing MCT1 expression in control (C) and diabetic (STZ) rats in PM and LDM fractions. C: Densitometric quantification of MCT1 abundance in adipocyte PM from diabetic rats. MCT1 signal densities from control animals were assigned a value of 100%. Values represents means ± S.E.M. ($n = 3$). *Statistically significant change ($P < 0.05$, Student's t-test).

brane fractions were probed with an antibody to MCT1 a single 43 kDa immunoreactive band, which comigrated with that detected in rat liver membranes, was observed in the PM fraction. A much weaker signal was also observed in the LDM and most likely reflected a small degree of contamination of the LDM fraction with PM as, unlike the PM signal, this weaker signal was not consistently observed in different LDM preparations (e.g. see Fig. 4B). When PM and LDM fractions were subsequently probed with an antibody to MCT4 we were unable to detect the presence of this MCT isoform in adipocyte membranes. It is noteworthy, however, that the MCT4 antibody did react positively with a protein of the appropriate molecular size in membranes prepared from rat skeletal muscle (data not shown) indicating that the absence of this carrier in fat cell membranes was not due to problems associated with antibody specificity.

Since lactate is a major product of glucose metabolism and the utilisation of glucose is known to be impaired in insulin responsive tissues during diabetes we investigated whether expression of MCT1 and lactate uptake in adipocytes could be modulated in animals rendered diabetic by STZ treatment. Fig. 4B shows that a 4 day period of STZ-induced diabetes caused a marked reduction in MCT1 expression. We found that MCT1 abundance in the PM fraction of diabetic rat adipocytes fell by approximately 80% (Fig. 4C). In line with the observed fall in MCT1 expression, lactate uptake in adipocytes isolated from diabetic rats also fell by 64% from 1.45 ± 0.31 nmol/min/1000 cells. We currently do not know whether insulin lack itself has a direct effect on MCT1 gene expression or if the fall in MCT1 protein is a consequence of diminished glycolytic metabolism. Previous work has shown that, in absolute terms, adipocytes isolated from lean diabetic rats produce significantly less lactate from glucose when compared with fat cells from lean controls [7]. It is thus conceivable that the amount of lactate produced may act as a signal that regulates the expression of its membrane transport protein. This possibility is supported by studies showing that increased muscular activity (and hence increased lactate production) enhances muscle MCT1 expression [20,21], whereas as muscle inactivity, as a result of muscle denervation, promotes a fall in MCT1 expression [21]. The ability of substrates to regulate the expression of their own carrier proteins is not unprecedented. We, and others, have shown, for example, that increased dietary intake of fructose induces a significant increase in jejunal and renal expression of the GLUT5 fructose transporter in rats [22,23]. The enhanced expression of GLUT5 in both of these tissues is likely to be of physiological importance as it will enable greater intestinal absorption and renal reabsorption of fructose. Whether changes in lactate production also elicit similar effects on MCT expression in adipocytes is currently unknown, but if it does, it raises important questions concerning how cells sense changes in lactate availability and the mechanisms used to signal changes in MCT expression.

In summary, we have shown that the PM of adipocytes express the MCT1 transporter which, we believe, is likely to represent the principal route for lactate transport across the fat cell membrane. Our data indicate that uptake of L-lactate in adipocytes is not saturable, but is acutely stimulated by a fall in extracellular pH and is inhibited substantially by CHC, a classical inhibitor of lactate transport. MCT1 expression is down-regulated in adipose tissue during diabetes possibly reflecting that the reduced conversion of glucose to lactate which prevails during the diabetic state may serve to regulate the 'turnover' of this monocarboxylate transporter. Given the importance of adipose tissue in whole body lactate metabolism it is likely that MCT1 acts as a key intermediary in shuttling lactate to tissues such as liver where it may be used for hepatic gluconeogenesis during fasting and perhaps glycogen synthesis in the post-absorptive state.

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