Volume 235, number 1,2, 224-228 FEB 06136 August 1988

Carrier-mediated uptake of L-(+)-lactate in plasma membrane vesicles from rat liver

Irene Quintana, Antonio Felipe, Xavier Remesar and Marçal Pastor-Anglada

Unitat de Bioquimica i Biologia Molecular B, Departament de Bioquimica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Received 30 May 1988; revised version received 10 June 1988

Plasma membrane vesicles from rat liver transported L-lactate into the inner vesicular space. Kinetic analysis of L-lactate uptake gave a K_m value of approx. 2.9 mM. Selective inhibition was found in a similar pattern to that described for the hepatic lactate carrier. L-Lactate transport was enhanced when a pH gradient was created across the plasma membrane. Vesicles obtained from fasted rats showed a higher uptake of L-lactate than those from fed rats, when incubated with physiological concentrations of L-lactate.

L-Lactate; Sugar transport; Plasma membrane vesicle; (Rat liver)

1. INTRODUCTION

Lactate is a major gluconeogenic substrate in liver. It has been shown that in those situations where glucose homeostasis is lost, e.g. during short periods of fasting, pregnancy or lactation, lactate uptake by liver is enhanced [1,2]. As deduced from observations made on isolated hepatocytes [3,4], lactate transport across the plasma membrane is carrier-mediated, especially when lactate concentrations in the medium are in the physiological range. However, L-lactate uptake in these experiments was estimated by using non-specific inhibitors of lactate metabolism. An alternative method has been used by Edlund and Halestrap [5] who incubated isolated hepatocytes with lactate at 0°C and calculated the carrier capacity at 37°C from the activation energy of the lactate carrier of human erythrocytes. Because plasma membrane vesicles from rat liver have proven to be a useful tool for the study of amino acid uptake [6,7], we decided to carry out partial purification of plasma membranes from rat liver with a lactate carrier activity, as a first step to further monitoring of lactate uptake under those situations where the liver is actively taking up this substrate in vivo.

Correspondence address: M. Pastor-Anglada, Unitat de Bioquímica i Biologia Molecular B, Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

2. MATERIALS AND METHODS

Livers from fed and 24 h-starved rats of the Wistar strain (over 200 g body wt) were excised after decapitation and used for partial purification of plasma membrane vesicles, according to the method of Van Amelsvoort et al. [6] as adapted by Pastor-Anglada et al. [8]. Membrane fractions were characterized according to their contamination by other subcellular membranes and by their morphological and functional integrities. The level of contamination was assessed by measuring the activities of some enzyme markers: glucose-6-phosphatase (EC 3.1.3.9) for endoplasmic reticulum [9], *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30) for lysosomes [10] and cytochrome-*c* oxidase (EC 1.9.3.1) for mitochondria [11]. Protein was determined according to Peterson [12]. 5'-Nucleotidase activity (EC 3.1.3.5) was measured as described by Aronson and Touster [13] and used as a marker for plasma membrane. The morphological integrity of the purified vesicles was assessed either by electron microscopy or from evidence of an osmotic sensitive space, as indicated by Sips et al. [14]. In order to investigate the functional integrity of our membrane preparations, their capability to take up concentratively L-[2,3-^{3}H]alanine (Amersham, England) in front of an electrogenic gradient of Na^+ was determined by filtration as in [8]. Incubations with L-[U-^{14}C]lactate (Amersham) were performed in the same medium as that used for L-alanine studies, which

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

basically consisted of 0.25 M sucrose, 0.20 mM $CaCl_2$, 10 mM Hepes, 10 mM $MgCl_2$, 100 mM NaSCN and, where stated, inhibitors at the desired concentrations (pH 7.5). Calculations of substrate uptake rates were made by considering their specific activities in the medium (from 16 to 66 μCi/μmol for [2,3-^{3}H]alanine and from 5 to 11 μCi/μmol for L-[U-^{14}C]lactate), and by correcting either by μg membrane protein or by units of 5'-nucleotidase activity, as described [7]. Statistical comparisons, when necessary, were performed by using Student's *t*-test.

3. RESULTS

3.1. *Enzyme markers*

The partial purification of plasma membranes yielded 8–12-fold enrichment of 5'-nucleotidase activity and a recovery of about 10–12% of the original activity in the homogenate. Relative specific activities between membrane and homogenate fractions for *N*-acetyl-β-D-glucosaminidase and cytochrome oxidase were lower than 0.4 and recoveries were less than 0.5%. A 1.7-fold purification was achieved for glucose-6-phosphatase with recovery amounting to 2–2.5% of the original activity. All these parameters were found to be similar between preparations from either fed or fasted rats and within the range of previously reported data [6,8,14]. L-lactate dehydrogenase activity was absent in our plasma membrane preparations.

3.2. *Structural and functional integrity of membrane vesicles*

Samples of pooled plasma membranes from either fed or fasted rats were incubated for 30 min in the presence of 0.25 mM L-alanine and increasing amounts of sucrose in order to give a set of osmolarities ranging from 0.4 to 1.5 osmol. Alanine vesicular content decreased with increasing osmolarity (correlation coefficients 0.89 and 0.96 for fed and fasted rats, respectively). The intravesicular space and sensitivity to osmotic pressure were similar in the two types of membrane preparations.

The time course of alanine transport into pooled plasma membrane vesicles from either fed or fasted rats, incubated in a medium with 100 mM sodium sulfocyanate, was monitored. The uptake was highly concentrative, since it reached an overshoot 6–7-fold higher than the values determined at equilibrium (not shown).

Further evidence of the functional integrity of our membrane preparations was obtained from the fact that Na^+-dependent transport of L-alanine could be partially inhibited by near-saturating amounts of methyl-aminoisobutyric acid, thus suggesting the presence of A and ASC carriers in these vesicles (not shown).

3.3. *Uptake of L-lactate*

The time course of the content of L-lactate retained by membrane preparations is shown in fig.1. Equilibrium was reached at 30 min and remained unaltered until 180 min after the beginning of the experiment. It was also observed that substrate retention was linear over the first 15–20 min of incubation.

In order to ascertain whether lactate retention by membrane vesicles was due to actual transport into the vesicular space, we performed two further experiments. In the first, a light disruptive treatment of membrane vesicles with 0.5% Triton X-100 was performed after incubation of 1 mM L-lactate for 15 min. The amounts of substrate retained were almost negligible (0.015 ± 0.004 pmol/μg protein) when compared with untreated samples

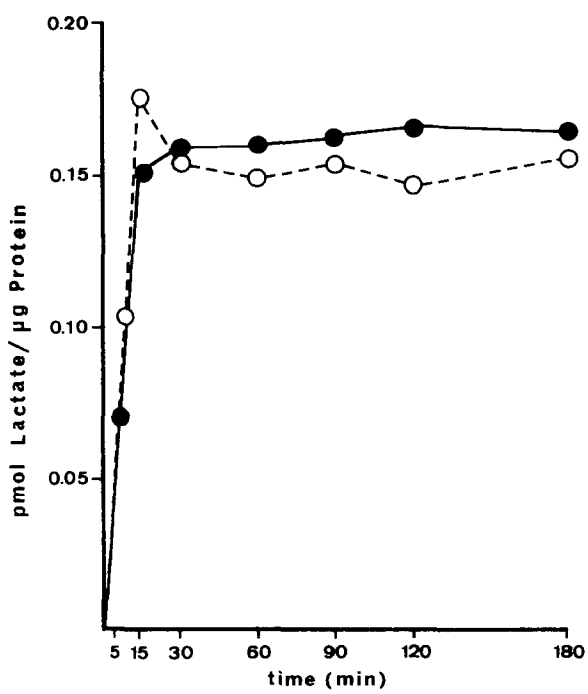

Fig.1. Time course of L-lactate retention by plasma membrane vesicles from either fed (●) or fasted (○) rats.

(0.148 ± 0.006 pmol/μg protein, means ± SE for three experiments), therefore suggesting that L-lactate had been transported into the vesicular space. In the second, membrane preparations were incubated with either L-lactate or L-alanine for 90 min. The apparent vesicular volume was calculated from the amounts of substrate retained and its concentration in the medium. With either L-lactate or L-alanine, the apparent volume was very similar (table 1), further suggesting the internalization of L-lactate rather than its binding to the plasma membrane.

Because L-lactate uptake could be due to simple diffusion instead of being carrier-mediated, we decided to demonstrate that its transport could be selectively inhibited (table 2). When 1 mM L-lactate was incubated with several potential inhibitors, we found that the most effective was α-cyano-3-hydroxycinnamate, a known inhibitor of L-lactate transport into either erythrocytes or liver cells [3,15]. D-Lactate was able to inhibit L-lactate uptake to a significant extent, as well as pyruvate and β-hydroxybutyrate. L-Alanine failed to affect L-lactate transport at the assayed concentrations.

In order to characterize further L-lactate uptake as well as its inhibition by α-cyano-3-hydroxycinnamate, we incubated membrane preparations for 15 min with increasing concentrations of L-lactate, in either the presence or absence of 5 mM inhibitor. Lineweaver-Burk plots of the kinetic data (fig.2) showed that α-cyano-3-hydroxycinnamate induced a sharp decrease in V_{max} (0.58 vs 0.09 pmol/15 min per μg protein) and to a lesser extent, in K_m (2.9 vs 1.1 mM).

Table 1

Apparent volumes of plasma membrane vesicles from livers of fed and fasted rats as measured from either L-alanine or L-lactate content equilibrium

	L-Lactate	L-Alanine
Fed (nl/mg protein)	160 ± 4	205 ± 24
(nl/U 5′-nucleotidase)	885 ± 29	1059 ± 124
Fasted (nl/mg protein)	138 ± 7	154 ± 39
(nl/U 5′-nucleotidase)	915 ± 45	891 ± 119

Pooled vesicles from either fed or fasted rats were incubated in triplicate, as described in section 2, in the presence of either 1 mM L-lactate or 1 mM L-alanine until equilibrium was reached. Then, apparent volumes were calculated from the substrate vesicular content and the substrate concentration in the medium. Results are means ± SE of three experiments. Results are expressed either per mg protein or per unit of 5′-nucleotidase activity (1 unit = 1 pmol P_i formed per s)

Table 2

Inhibition of L-lactate transport into plasma membrane vesicles from fed rats

Inhibitor	Transport (pmol/μg protein)	% inhibition
None	0.171 ± 0.014	-
α-Cyano-3-hydroxy-cinnamate (5 mM)	0.062 ± 0.000	63
Pyruvate (5 mM)	0.183 ± 0.017	-
(15 mM)	0.124 ± 0.012	27
β-Hydroxybutyrate (10 mM)	0.190 ± 0.018	-
(100 nM)	0.051 ± 0.002	70
L-Alanine (10 mM)	0.174 ± 0.021	-
D-Lactate (10 mM)	0.065 ± 0.006	62

Plasma membrane vesicles were incubated with 1 mM L-lactate for 15 min, as described in section 2, in the presence of several inhibitors at the concentrations indicated above. Results are means ± SE of 3–5 different incubations

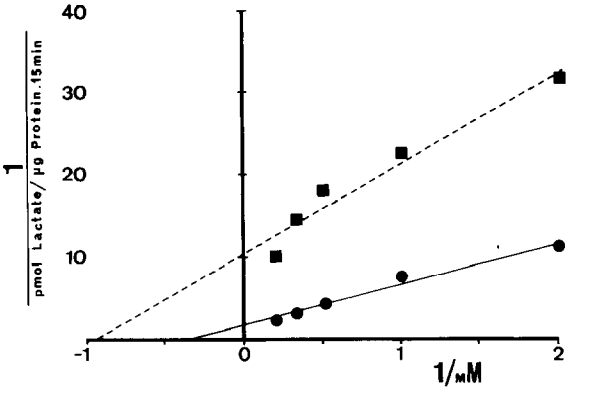

Fig.2. Lineweaver-Burk plots of L-lactate uptake by plasma membrane vesicles from rat liver, in either the presence (■) or absence (●) of 5 mM α-cyano-3-hydroxycinnamate.

Because a pH gradient across the plasma membrane enhances L-lactate uptake in isolated hepatocytes [4], we decided to reproduce this finding in our plasma membrane preparations. Hence, incubation media were prepared in order to give final pH values after mixing with membranes of over 7.2, 6.2 and 5.6. The pH inside the vesicular space was 7.5 under all of the above conditions. L-Lactate uptake measured at pH 7.2 was compared with transport rates at either pH 6.2 or 5.6, by means of the paired *t*-test. A significant

Table 3

L-Lactate uptake by plasma membrane vesicles from either fed or fasted rats

	[L-Lactate]	
	1 mM	2 mM
Fed (pmol/μg protein)	0.152 ± 0.004	0.395 ± 0.032
(pmol/U 5'-nucleotidase)	0.787 ± 0.021	2.046 ± 0.160
Fasted (pmol/μg protein)	0.223 ± 0.010[a]	0.452 ± 0.016
(pmol/U 5'-nucleotidase)	1.494 ± 0.070[b]	3.028 ± 0.110[a]

Pooled plasma membrane vesicles from either fed or fasted rats were incubated for 15 min as indicated in section 2. Results are means ± SE of three different incubations. Statistical significance vs 'fed' values was established by using the Student's t-test: [a] $p<0.01$, [b] $p<0.001$

enhancement of L-lactate transport was observed (15% at pH 6.2, 29% at pH 5.6; $p<0.05$).

In an attempt to ascertain whether plasma membrane vesicles could be useful as a tool for the study of hepatic metabolic adaptations that occur in altered physiological states, we decided to monitor L-lactate transport into vesicles from either fed or fasted rats. It was observed (table 3) that L-lactate uptake was enhanced in preparations obtained from starved animals.

4. DISCUSSION

To our knowledge, the only attempt at the partial purification of plasma membrane vesicles with a lactate carrier activity is that of Welch et al. [16]. However, these authors failed to demonstrate transport into the inner vesicular space, but rather they reported an unusual binding of L-lactate to the membrane preparations. This binding was specific but irreversible, did not reach equilibrium and even increased exponentially when incubation was prolonged for several hours. Because they obtained vesicles from hepatocytes previously isolated by collagenase treatment, it is likely that the different yields in our respective studies may be explained by partial denaturation of the carrier protein due to collagenase perfusion. In fact, our first experiment performed to determine whether L-lactate retained by vesicles had been internalized, was based on the study of Welch et al. [16]. These authors treated their membrane vesicles with 0.5% Triton X-100 and failed to remove L-lactate from preparations. We repeated it under the same conditions and observed that L-lactate retention was almost abolished. Furthermore, the finding that the apparent vesicular volumes were similar when calculated from either L-lactate or L-alanine content at equilibrium makes it rather likely that L-lactate is actually taken up and not bound to the surface of the plasma membrane.

The pattern of inhibition we report here is in agreement with previous literature. The great effectivity of α-cyano-3-hydroxycinnamate agrees with the results of Welch et al. [16], who showed inhibition of L-lactate binding to plasma membrane vesicles and with recent data obtained by Edlund and Halestrap [5] using isolated hepatocytes. Although high stereoselectivity of the liver L-lactate carrier has been recently claimed by Fafournoux et al. [4], these authors reported that the percent inhibition of L-lactate transport into isolated hepatocytes by either L- or D-lactate was very similar. Furthermore, Schwabb et al. [17] showed that D-lactate uptake in perfused rat liver was carrier-mediated and inhibited by either α-cyano-3-hydroxycinnamate, L-lactate or pyruvate. We also observed significant inhibition of L-lactate transport by pyruvate, although it was probably less effective than that reported by Fafournoux et al. [4] but more than that found by Welch et al. [16] when studying inhibition of L-lactate binding by pyruvate. In any case, the possibility that both substrates were transport by the same agent across the plasma membrane is challenged by the great differences in L-lactate and pyruvate concentrations in the portal vein [2]. The inhibition of L-lactate uptake by β-hydroxybutyrate is in good agreement with a recent report by Metcalfe et al. [18], who showed that in either isolated hepatocytes or perfused rat liver, ketone bodies inhibited lactate removal. The lack of inhibition of L-lactate uptake by L-alanine is in agreement with the known characteristics and specificity of amino acid transport into liver cells.

The K_m value for L-lactate uptake into plasma membrane vesicles from rat liver is of the same magnitude as that reported by others for isolated rat hepatocytes [4]. Moreover, it is in the physiological range of L-lactate concentrations in the portal vein, thus suggesting that substrate availability might be one of the factors eventually modulating the unidirectional liver uptake of L-

lactate. The enhancement of L-lactate transport by inducing a pH gradient across the plasma membrane is in good agreement with what has been previously reported to occur in isolated rat hepatocytes [4].

All of these data taken together show that L-lactate transport into our membrane vesicles is not mediated by simple diffusion, but rather by a carrier which is probably the same as that reported previously by others in this and other cell types. Thus, the main finding of this work is that a lactate carrier is functional in plasma membrane vesicles from rat liver. Furthermore, our results suggest that the carrier may respond to physiological stimuli, such as depletion of substrate availability during fasting, by increasing its capacity to take up L-lactate, at least when transport is measured at physiological concentrations of substrate. This is in good agreement with the enhanced liver fractional extraction rates for L-lactate reported in fasted rats when measured in vivo [1].

REFERENCES

[1] Rémésy, C. and Demigné, C. (1983) Ann. Nutr. Metab. 27, 57–70.
[2] Casado, J., Remesar, X. and Pastor-Anglada, M. (1987) Biosci. Rep. 7, 587–592.
[3] Monson, J.P., Smith, J.A., Cohen, R.D. and Iles, R.A. (1982) Clin. Sci. 41, 159–170.
[4] Fafournoux, P., Demigné, C. and Rémésy, C. (1985) J. Biol. Chem. 260, 292–299.
[5] Edlund, G.L. and Halestrap, A.P. (1986) Biochem. Soc. Trans. 14, 1190–1191.
[6] Van Amelsvoort, J.M., Sips, H.J. and Van Dam, K. (1978) Biochem. J. 174, 1083–1086.
[7] Bourdel, G. and Forestier, M. (1982) FEBS Lett. 143, 81–85.
[8] Pastor-Anglada, M., Remesar, X. and Bourdel, G. (1987) Am. J. Physiol. 252, E408–E413.
[9] De Duve, C., Pressman, B.C., Gianetto, R. and Appelmans, F. (1955) Biochem. J. 60, 604–617.
[10] Carroll, M. (1978) Biochem. J. 60, 604–617.
[11] Rafael, J. (1983) in: Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) pp.266–273, Verlag Chemie, Weinheim.
[12] Peterson, G.L. (1979) Anal. Biochem. 100, 201–220.
[13] Aronson, N.N. jr and Touster, O. (1974) Methods Enzymol. 31, 90–102.
[14] Sips, H.J., Van Amelsvoort, J.M.M. and Van Dam, K. (1980) Eur. J. Biochem. 105, 217–224.
[15] Halestrap, A.P. and Denton, R.M. (1974) Biochem. J. 138, 313–316.
[16] Welch, S.G., Metcalfe, H.K., Monson, J.P., Cohen, R.D., Henderson, R.M. and Iles, R.A. (1984) J. Biol. Chem. 259, 15264–15271.
[17] Schwabb, A.J., Bracht, A. and Scholz, R. (1979) Eur. J. Biochem. 102, 537–547.
[18] Metcalfe, H.K., Monson, J.P., Welch, S.G. and Cohen, R.D. (1986) J. Clin. Invest. 78, 743–747.