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Substrate and inhibitor specificity of monocarboxylate transport into heart cells and erythrocytes

Further evidence for the existence of two distinct carriers

Robert C. POOLE,*‡ Susan L. CRANMER,* Andrew P. HALESTRAP* and Allan J. LEVI†
Departments of *Biochemistry and †Physiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, U.K.

A range of short-chain aliphatic monocarboxylates, both unsubstituted and substituted with hydroxy, chloro and keto groups, were shown to inhibit transport of L-lactate and pyruvate into both guinea-pig cardiac myocytes and rat erythrocytes. The carrier of heart cells exhibited a higher affinity (approx. 10-fold) for most of the monocarboxylates than did the erythrocyte carrier. A notable exception was L-lactate, whose K_m for both carriers was similar. The K_i values of the two carriers for inhibitors such as phenylpyruvate and α-cyanocinnamate derivatives were also different. The high affinity of the heart cell carrier for ketone bodies and acetate may be physiologically important, since these substrates are used as fuels by the heart.

INTRODUCTION

L-Lactate and the ketone bodies, β-hydroxybutyrate and acetoacetate, are important fuels for the heart [1,2]. Transport of these compounds across the cardiac sarcolemmal membrane is therefore of considerable physiological importance. We have shown recently that the transport of lactate and pyruvate into ventricular myocytes is largely carrier-mediated at physiological substrate concentrations [3]. This monocarboxylate carrier, like that of erythrocytes [4,5], hepatocytes [6,7] and a number of other cell types [8], is inhibited by α-cyano-4-hydroxycinnamate. However, the transporter in ventricular myocytes has a 10-fold higher affinity for pyruvate (approx. 70 μM) and much less pronounced stereoselectivity for L- over D-lactate than the carrier in erythrocytes and hepatocytes [3].

The experiments described in the present paper provide a more extensive characterization of the substrate specificity of the cardiac myocyte monocarboxylate carrier. For many carriers this can be done by using alternative substrates to *trans*-accelerate transport. Although such an approach has been used for the erythrocyte monocarboxylate carrier [4,5], it is not possible with isolated cardiac myocytes, since many of the preloaded substrates would be rapidly metabolized by the cells. Therefore we have used alternative substrates as competitive inhibitors of the uptake of ^{14}C-labelled pyruvate or L-lactate into the cells. Previous data for the erythrocyte and cardiac myocyte carriers have shown that K_i values for L-lactate, D-lactate and pyruvate as competitive inhibitors of the transport of each other are equivalent to the K_m values for their own transport [3,4]. Such observations indicate that the K_m value of the carrier for a monocarboxylate represents the dissociation constant for the substrate–transporter complex [3]. If it is assumed that this conclusion applies to other short-chain monocarboxylates, estimates of K_m values for a range of substrates may be derived from inhibition experiments. In this paper we report such experiments, which demonstrate further differences between monocarboxylate carriers of erythrocytes and cardiac myocytes.

EXPERIMENTAL

Materials

Monocarboxylates, with the exceptions given below, were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Glycollic acid, α-cyanocinnamate, α-cyano-4-hydroxycinnamate and α-fluorocinnamate were obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K. All other chemicals were from the sources given previously [3,9]. Monocarboxylates were added to incubations from unbuffered stock solutions in water (neutralized with NaOH if required). Radiochemicals were from Amersham International, Amersham, Bucks., U.K.

Preparation of erythrocytes and cardiac myocytes for measurement of transport

Fresh rat erythrocytes in citrate buffer were washed once with Krebs–Henseleit medium [10], and then a further twice with citrate buffer (84 mM-sodium citrate/1 mM-EGTA, neutralized to pH 7.4 with NaH_2PO_4) before resuspension at 10% haematocrit in citrate buffer. 4,4′-Di-isothiocyanostilbene-2,2′-disulphonate ('DIDS') and acetazolamide were added to final concentrations of 10 μM and 100 μM respectively. These inhibitors prevent the operation of the inorganic-anion exchanger, which might otherwise contribute to the transport of monocarboxylates [4,5,11].

Ventricular myocytes were isolated from guinea-pig hearts and prepared for transport assays as described previously [3]. The final incubations were in citrate buffer (above), but without the inclusion of EGTA. As described previously, the use of citrate buffer in these experiments results in a trans-membrane pH gradient (alkaline-inside) and so increases monocarboxylate uptake on the proton-linked carrier [3,4]; this allows more accurate measurement of transport.

Assay of initial rates of monocarboxylate uptake

Pyruvate uptake into guinea-pig cardiac myocytes was measured at 0 °C over a 15 s period by a silicone-oil filtration technique as recently described [3]. Competing substrate or inhibitor was added with the [^{14}C]pyruvate and ^3H_2O used to initiate transport. Uptake of 0.5 mM-L-lactate and pyruvate into rat erythrocytes was measured over a 15 s period by a similar centrifuge-stop technique, but at 6 °C without filtration through oil [4,12]. All measurements of initial rates of transport were performed in duplicate. In all cases, uptake of substrate was corrected for non-carrier-mediated transport, determined as the

‡ To whom reprint requests should be addressed.

uptake occurring in the presence of 2 mM-α-cyano-4-hydroxycinnamate. This concentration of inhibitor was shown previously to inhibit transport on both carriers by > 95% [3,4]. Carrier-mediated transport of pyruvate and lactate into the cells in the presence of competing monocarboxylates is expressed as a percentage of the control rate, ±S.E.M. for the given number of cell preparations. To estimate K_i values for competing substrates, the equation for competitive inhibition was used, with the relevant values for substrate and inhibitor concentrations. K_m values of the carriers for L-lactate (3.0 mM for rat erythrocytes; 2.3 mM for guinea-pig cardiac myocytes) and pyruvate (0.96 mM for rat erythrocytes; 0.066 mM for guinea-pig cardiac myocytes) are taken from refs. [3] and [7]. For the erythrocyte carrier, the K_m value for D-lactate is known to be greater than 25 mM ([5]; R. C. Poole, unpublished work), whereas that of the cardiac myocyte transporter has been estimated at 4.2 mM [3]. It should be emphasized that competitive inhibition has been assumed in the calculations of K_i, and that the values determined can only be approximate, since they are obtained from rates of transport measured at a single substrate and inhibitor concentration.

RESULTS AND DISCUSSION

Substrate specificity

Table 1 shows the effects of a range of monocarboxylates (2.5 mM) on the uptake of 0.5 mM-L-lactate or -pyruvate into rat erythrocytes, or of 40 μM-pyruvate into cardiac myocytes. These concentrations of substrates are below their K_m values for transport (see above), which is desirable when studying competitive inhibition. Such conditions also allowed substantial uptake of radioactivity at 15 s. Although pyruvate was used as the substrate with cardiac myocytes, and L-lactate with erythrocytes, estimates of K_i values for rat erythrocytes were similar regardless of the substrate used (see Table 1 and its legend). We have shown previously that, when both K_m and K_i values for a substrate are measured directly, they are very similar [3,7]. The data of Table 1 also confirm the similarity of the K_i values of pyruvate, L-lactate and D-lactate to their K_m values determined previously (see the Experimental section).

Inspection of Table 1 reveals a number of similarities in the substrate specificities of the two carriers. Firstly, unsubstituted acids, and those substituted with hydroxy, chloro, or keto groups are substrates; for both systems the affinity is in the order keto-acids > chloro-acids > unsubstituted > hydroxy-acids. Aliphatic monocarboxylates that branch at C-2, such as 2-hydroxy-2-methylpropionate, do not interact with either carrier. This was shown previously for the human erythrocyte monocarboxylate carrier [5]. There are also a number of significant differences between the two carriers. There appears to be no stereoselectivity for D- or L-2-chloropropionate on either carrier, whereas for lactate there is almost complete stereoselectivity for the L- over the D-isomer with erythrocytes, but only partial stereoselectivity (approx. 2-fold) with the heart cell carrier. The absence of stereoselectivity of either carrier for the 2-chloropropionates indicates that the hydrogen of the hydroxy group on lactate may be involved in determining stereoselective interactions. Affinities for substituted monocarboxylates vary with the position of substitution in the order C-2 > C-3 ≈ C-4 for both transporters. However, the difference between substitutions at C-2 and C-3 is greater for the erythrocyte than for the cardiac myocyte carrier (compare 2-, 3- and 4-hydroxybutyrate). Glyoxylate is not a substrate for the erythrocyte carrier (the present work; reference [5]), and a poor substrate for the cardiac myocyte carrier. The present data also show oxamate to interact, albeit weakly, with the rat erythrocyte transporter, whereas the myocyte carrier has an affinity some 15-fold higher for this substrate. Glyoxylate and oxamate are analogues of pyruvate which are incapable of keto–enol tautomerization. Although it has been suggested that it is the enol forms of keto acids which bind to the carrier [8], the present data on the heart cell carrier do not support this conclusion.

The most significant difference between the monocarboxylate carriers of erythrocytes and cardiac myocytes is the much higher affinity of the heart cell transporter for a wide variety of

Table 1. Inhibition of L-lactate and pyruvate transport in rat erythrocytes and guinea-pig cardiac myocytes by aliphatic monocarboxylates

Initial rates of transport of 0.5 mM-L-lactate into rat erythrocytes and of 40 μM-pyruvate into cardiac myocytes were measured as described in the Experimental section, and are given as percentages of the control values, ±S.E.M. for experiments with the numbers of cell preparations shown in parentheses. K_i values for monocarboxylates were estimated as described in the text; some additional values derived from inhibition of pyruvate transport in rat erythrocytes (mean values for two experiments) are shown in square brackets for comparison with those obtained with L-lactate as substrate.

		Erythrocytes		Cardiac myocytes		K_i (erythrocyte)
Competing substrate (2.5 mM)		Rate (% of control)	K_i (mM)	Rate (% of control)	K_i (mM)	K_i (cardiac myocyte)
C_2	Acetate	62±2.4 (5)	3.5	17.6±4.4 (5)	0.33	10.6
	Glyoxylate	97±4 (5)	69	87±0.8 (6)	10.4	6.6
	Oxamate	89±4.8 (5)	17	43±5 (6)	1.18	14.4
	Glycollate	76±3.2 (5)	6.6	49±3.6 (6)	1.5	4.4
C_3	Propionate	41±3.6 (6)	1.49 [1.34]	17±5 (5)	0.32	4.6
	Pyruvate	23±2 (6)	0.64	0.1±3 (8)	< 0.1	
	L-Lactate	51±2.5 (6)	2.23 [2.01]	52±5 (5)	1.69	1.3
	D-Lactate	96±1.6 (6)	51	70±4 (5)	3.6	14
	L-2-Chloropropionate	30±2.8 (6)	0.92 [1.01]	12±4 (6)	0.2	14.4
	D-2-Chloropropionate	31.5±3.6 (6)	0.98 [0.81]	8.5±5 (6)	0.14	7
C_4	2-Oxobutyrate	8±1.2 (5)	0.19 [0.29]	1.6±5 (5)	< 0.1	
	3-Oxobutyrate	62±2.2 (5)	3.49	15±4.5 (5)	0.27	12.9
	D/L-2-Hydroxybutyrate	37±1.6 (4)	1.26	20±2.5 (6)	0.38	3.3
	D/L-3-Hydroxybutyrate	79±2.7 (5)	8.1	35±4 (5)	0.84	9.6
	4-Hydroxybutyrate	77±2.4 (5)	7.2	33±4 (5)	0.77	9.4
	2-Hydroxy-2-methylpropionate	98±3.6 (5)	> 100	100±6.5 (5)	> 100	

Table 2. Inhibition of L-lactate and pyruvate transport in erythrocytes and cardiac myocytes by aromatic monocarboxylates

Initial rates of uptake of 0.5 mM-L-lactate into rat erythrocytes and of 0.04 mM-pyruvate into guinea-pig cardiac myocytes were as described in the Experimental section. Inhibitors were present only during flux measurement. Results are given as means ± S.E.M. for the numbers of cell preparations shown in parentheses.

Inhibitor	Concn. (mM)	Rate (% of control)	
		Erythrocytes	Cardiac myocytes
Phenylpyruvate	2.5	26 ± 1.2 (5)	4.6 ± 3.3 (6)
α-Cyanocinnamate	0.1	83 ± 3.8 (7)	42 ± 3.9 (5)
α-Cyano-4-hydroxycinnamate	0.1	25 ± 2.5 (6)	45 ± 2.2 (5)
α-Fluorocinnamate	0.1	89 ± 3.7 (7)	66 ± 4.6 (6)
trans-Cinnamate	0.1	93 ± 3.8 (7)	82 ± 5.0 (4)

monocarboxylates; L-lactate is a notable exception. These differences cannot be ascribed to the different species used, since the properties of rat and guinea-pig erythrocyte monocarboxylate carriers are similar ([3,8]; R. C. Poole, unpublished work). For a range of substrates, the ratio of the estimated K_i values for the erythrocyte carrier to those of the cardiac myocyte carrier ranges between approx. 3 (2-hydroxybutyrate) and 13 (3-oxobutyrate). For the physiological substrates 3-oxobutyrate (acetoacetate), 3-hydroxybutyrate (β-hydroxybutyrate) and acetate, values for this ratio are 13, 9.6 and 10.6 respectively. The affinities of the myocyte carrier for these substrates are within their physiological concentration range [13,14].

Inhibitor specificity

Aromatic monocarboxylates, most notably the α-cyanocinnamate derivatives, are inhibitors of lactate and pyruvate transport across cell membranes [4,15]. In Table 2 we compare the effectiveness of such compounds on the plasma-membrane monocarboxylate carriers of cardiac myocytes and erythrocytes. The data show phenylpyruvate to be a more potent inhibitor of the cardiac myocyte carrier than of the rat erythrocyte transporter. For cinnamic acid derivatives there were also differences between the two transporters. The order of effectiveness for the erythrocyte transporter was α-cyano-4-hydroxycinnamate $\gg$ α-cyanocinnamate $\geqslant$ α-fluorocinnamate $\geqslant$ *trans*-cinnamate, whereas that for the myocyte transporter was α-cyano-4-hydroxycinnamate $\approx$ α-cyanocinnamate $>$ α-fluorocinnamate $>$ *trans*-cinnamate.

General conclusion

The present work shows further significant differences between the monocarboxylate carriers of cardiac myocytes and erythrocytes. Our data differ from those of Trosper & Philipson [16], who failed to observe a high affinity for pyruvate and a number of other monocarboxylates. These workers used a preparation of sarcolemmal membrane vesicles from dog heart. Such preparations could be contaminated with other membrane fractions, and are likely to be heterogenous with respect to vesicle size and orientation. In addition, they did not attempt to correct for the component of transport which is due to diffusion; this can be quite significant under many conditions [3,7]. In physiological terms, the most important finding in the present work is the high affinity of the heart cell carrier for both acetate (K_i = 0.33 mM) and the ketone bodies acetoacetate (K_i = 0.27 mM) and β-hydroxybutyrate (K_i = 0.84 mM). Since ketone bodies and acetate are readily oxidized by the heart [2], these observations suggest an important metabolic role for the monocarboxylate carrier in this tissue.

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