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Evidence for a lactate transport system in the sarcolemmal membrane of the perfused rabbit heart: kinetics of unidirectional influx, carrier specificity and effects of glucagon

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The kinetics and specificity of L-lactate transport into cardiac muscle were studied during a single transit through the isolated perfused rabbit heart using a rapid (15 s) paired-tracer dilution technique. Kinetic experiments revealed that lactate influx was highly stereospecific and saturable with an apparent $K_t = 19 \pm 6$ mM and a $V_{max} = 8.4 \pm 1.5$ μmol/min per g (mean ± S.E., $n = 14$ hearts). At high perfusate concentrations (10 mM), the inhibitors α-cyano-4-hydroxycinnamate ($K_i = 7.3$ mM), pyruvate ($K_i = 6.5$ mM), acetate ($K_i = 19.4$ mM) and chloroacetate ($K_i = 28$ mM) reduced L-lactate influx, and K_i values were estimated assuming a purely competitive interaction of the inhibitors with the monocarboxylate carrier. The monocarboxylic acids [^{14}C]pyruvate and [^{3}H]acetate were themselves transported, and sarcolemmal uptakes of respectively 38 ± 1% and 70 ± 8% were measured relative to D-mannitol. Perfusion of hearts for 10–30 min with 0.15 or 1.5 μM glucagon increased myocardial lactate production and simultaneously inhibited tracer uptake of lactate, pyruvate and acetate. It is concluded that a stereospecific lactate transporter exhibiting an affinity for other substituted monocarboxylic acids is operative in the sarcolemmal plasma membrane of the rabbit myocardium.

Introduction

It is widely accepted that cardiac muscle is capable of oxidizing a variety of substrates including lactate, glucose and fatty acids for energy production (see review, Ref. 1), and yet there has been some controversy about which is the preferred exogenous fuel [2–5]. Although ketone bodies, pyruvate and acetate may also be oxidized in the heart, their normal plasma concentrations are too low for them to serve as the primary substrates, and L-lactate appears to be the preferred metabolite [3]. Furthermore, the observation that arterial lactate concentration increases to between 5–12 mM during exercise [6] provides an important role for lactate in cardiac metabolism.

In cardiac muscle, only very limited evidence suggests that lactate influx is saturable [7,8], and no information is available concerning the specificity of this putative sarcolemmal carrier system. Recently, Mann and Yudilevich [9] reported preliminary data on carrier-mediated L-lactate transport in the perfused heart. In this paper we report the first detailed characterization of a monocarboxylate transport system in the rabbit myocardium and examine the kinetics of unidirectional L-lactate influx, its isomeric specificity, and

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inhibition by structural analogues such as cyano-4-hydroxycinnamate, pyruvate, chloroacetate and acetate. In view of glucagon's antiarrhythmogenic actions in the heart and its ability to induce myocardial glycogenolysis and lactate production [10], we also investigated its regulatory effect on monocarboxylate transport.

Methods

Animals and perfusion techniques. Adult New Zealand rabbits of either sex and 2–3.5 kg in weight were killed by a rapid blow to the head. As previously described [11,12] the isolated heart was perfused through a cannula in the aorta at constant flow (1 ml/min per g) with a heated (38°C) oxygenated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate solution (pH 7.4) containing 10 g/l bovine serum albumin (Cohn Fraction V, Sigma). The coronary sinus effluent drained from the ventral surface of the horizontally mounted beating heart. Various concentrations of unlabelled inhibitors were added to the control perfusate and the use of a two-way tap permitted rapid switching from one solution to another.

Sarcolemmal influx and efflux measurements. Transport of monocarboxylates across the sarcolemmal membrane of intact cardiac muscle was studied against the background of 5.5 mM D-glucose and a low concentration of free fatty acids (0.04–0.1 mM) associated with the 10 g/l bovine albumin. In vivo uptake of lactate has also been measured from plasma containing at least 0.4–0.5 mM free fatty acids and glucose [1,3], and L-lactate appears to be the preferred substrate [3].

As reviewed previously by Yudilevich and Mann [13], a high resolution paired-tracer dilution technique was used to quantify sarcolemmal transport. In these experiments hearts were pre-equilibrated with a given concentration of unlabelled L-lactate (0.15–20 mM) or an unlabelled inhibitor (10 mM) for 4 min before measuring tracer uptake. Sarcolemmal uptake was assessed during a single transit through the heart by comparing the coronary sinus dilution profiles for labelled D-mannitol (extracellular reference, M_r 180) and a test substrate following a rapid arterial bolus (100 μl in 1–2 s) injection of a mixture containing both tracer molecules. Each tracer injectate was made up to volume with the same solution perfusing the heart. Usually thirty successive 100 μl samples collected in 30–40 s, plus a final 4 min sample, were processed. As an example, the time-course of L-[^{14}C]lactate uptake was obtained by estimating uptake in each of the collected venous samples: uptake = 1 − (L-[^{14}C]lactate/D-[1-^{3}H]mannitol) (Fig. 1). In the presence of a fixed concentration of unlabelled substrate, the maximal fractional tracer uptake serves as an index of unidirectional uptake. Assuming that uptake across the sarcolemmal membrane is proportional to the substrate concentration, an integration along the length of the microvascular exchange site may be used to calculate the initial influx (see review, Ref. 13). Unidirectional lactate influx was measured at different perfusate concentrations (0.15–20 mM) and estimated from the average maximal tracer uptake (U_{max}) using the equation: $v = -F \cdot \ln(1 - U_{max}) \cdot C_a$ where C_a is the unlabelled L-lactate concentration and F the coronary flow in ml/min per g of heart wet weight [11,12]. The logarithmic transformation corrects for the exponential loss of the labelled test substrate.

Tracer efflux was estimated from the integrated venous recoveries of both extracellular and test molecules over the total 4–5 min collection period: efflux (%) = $1 - U_T/U_{max}$, where U_T denotes the overall uptake for the test substrate relative to D-mannitol [14].

Injection solutions and counting of radioactive samples. Each paired-tracer injectate usually contained 2.8 μCi of ^{14}C and 14 μCi of ^{3}H. 2 ml of scintillant (Beckman Ready-Solv HP) were added to all collected samples and appropriate channel and injectate mixture standards. The ^{3}H and ^{14}C activities were determined concurrently using a Beckman LS 7500 liquid scintillation counter. All data were analyzed using a Basic programme written for a microcomputer.

Lactate assay. Myocardial lactate production was estimated by assaying the coronary sinus effluent using a specific assay for lactic acid (Sigma Assay No. 826-UV) and recording the absorbance at 340 nm on a Beckman DU Spectrophotometer. These procedures have previously been described in detail [14].

Chemicals, peptide and radioactive molecules. L-Lactate, D-lactate, pyruvate, acetate, chloroacetate,

α-cyano-4-hydroxycinnamate and crystalline glucagon (extracted from bovine and porcine pancreas) were obtained from Sigma, Poole, U.K. The radioactively labelled molecules L-[U-^{14}C]lactate (153 mCi/mmol), [^{14}C]pyruvate (8.7 mCi/mmol), [^{3}H]acetate (5 mCi/3 mg in 0.5 ml), D-[1-^{14}C]mannitol (45 mCi/mmol) and D-[1-^{3}H]mannitol (17 Ci/mmol) were purchased from New England Nuclear, Dreieich, F.R.G.

Results

Fig. 1A illustrates paired coronary sinus dilution curves obtained for L-[^{14}C]lactate and D-[^{3}H]mannitol (extracellular reference) during perfusion of the isolated rabbit heart with 0.15 mM unlabelled L-lactate. The lower recovery of L-[^{14}C]lactate relative to D-[^{3}H]mannitol indicates sarcolemmal uptake of the monocarboxylate, since the capillary endothelium of the rabbit heart is highly permeable to these small solutes [11,12]. When the time-course of L-[^{14}C]lactate uptake was analyzed (Fig. 1B), it was apparent that uptake reached a maximum (U_{max}) 8 s after the appearance of the tracers in the coronary sinus effluent. The uptake profile then decreased due to tracer efflux from the myocardium. Subsequent perfusion of the same heart with 10 mM unlabelled pyruvate inhibited L-[^{14}C]lactate uptake by 69% (Fig. 1B). In the same experiment 10 mM L-lactate cross-inhibited [^{14}C]pyruvate uptake by 34% (data not shown) and the mean inhibition observed was 34 ± 2% (n = three hearts).

Table I summarizes the tracer uptake and efflux values measured for monocarboxylic acids in hearts perfused with 5.5 mM D-glucose as the only extracellular substrate. Unidirectional uptake of [^{3}H]acetate was significantly higher than the uptake measured for L-[^{14}C]lactate or [^{14}C]pyruvate. Tracer efflux of L-lactate and pyruvate was respectively 1.7- and 1.4-fold higher than that observed for acetate. As chromatographic analyses were not performed, is was not possible to confirm whether efflux of tracer reflected the native substrate or a labelled metabolite.

In another series of experiments, the kinetics of L-lactate influx were investigated in order to establish whether transport was carrier-mediated. When hearts were perfused successively with different unlabelled L-lactate concentrations ranging from 0.15 to 20 mM, L-lactate influx appeared saturable with an apparent $K_t = 19 \pm 6$ mM and $V_{max} = 8.4 \pm 1.5$ μmol/min per g (Fig. 2). The inset in Fig. 2 illustrates the inhibitory effect of elevated L-lactate concentrations on the unidirectional uptake measured for L-[^{14}C]lactate. Tracer

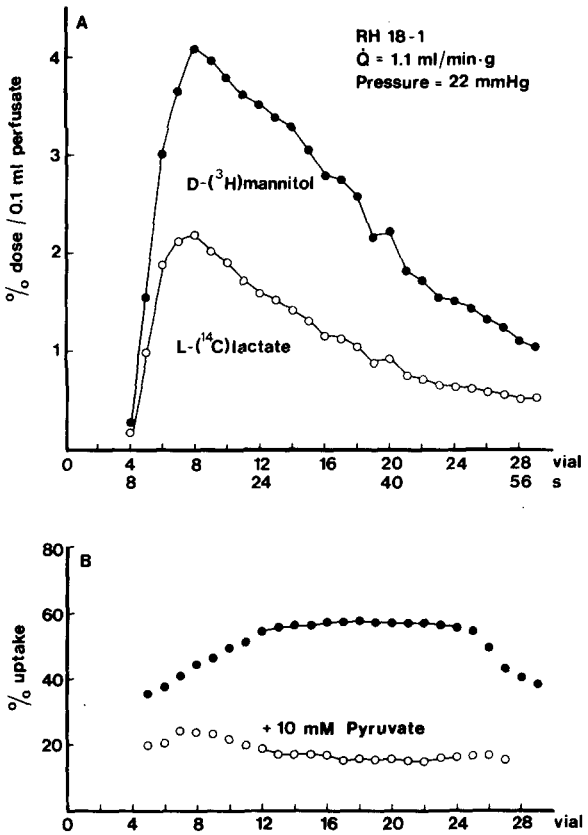

Fig. 1. Sarcolemmal uptake of L-lactate in the rabbit heart. (A) paired venous tracer dilution profiles for D-[1-^{3}H]mannitol (extracellular reference) and L-[^{14}C]lactate following a bolus (100 μl in 1–2 s) injection of this tracer mixture into the perfusate supplying the coronary arteries. Tracer concentrations in successive coronary sinus samples (100 μl) have been normalized with respect to the radioactive doses injected and plotted against the collection time. (B) L-[^{14}C]Lactate uptake from a perfusate containing 0.15 mM L-lactate was estimated from the data shown in panel (A): uptake (%) = [1–([^{14}C]lactate/[^{3}H]mannitol)] × 100. This figure also illustrates the time-course of L-[^{14}C]lactate uptake (dilution data not shown) observed in a subsequent run in the same heart perfused with 10 mM unlabelled pyruvate. In each case the maximal tracer uptake (U_{max}) was calculated from the average of the joined data points. This preparation was perfused at a constant flow rate of 1.1 ml/min per g.

TABLE I

UNIDIRECTIONAL UPTAKE AND EFFLUX OF MONOCARBOXYLIC ACIDS AT THE SARCOLEMMAL MEMBRANE OF THE PERFUSED RABBIT HEART

A time-course of uptake for these substrates was assessed relative to an extracellular marker, D-[^{14}C or ^{3}H]mannitol, and the maximal uptake (U_{max}) was estimated as shown in Fig. 1B. Tracer efflux was quantified simultaneously from the total integrated recoveries of the test substrate and D-mannitol over a 4–5 min collection period: efflux (%) = $[1-(U_T/U_{max})]\times 100$, where U_T denotes the overall uptake of test substrate. The injectate concentrations of the labelled substrates are specified as hearts were perfused with a Krebs solution containing 5.5 mM D-glucose as extracellular substrate. Values are given as mean ± S.E. and n = number of animals.

	n	Injectate (mmol/l)	% Maximal uptake	% Tracer efflux
L-[1-^{14}C]Lactate	16	0.04	43 ± 2	73 ± 6
[^{14}C]Pyruvate	4	0.93	38 ± 1	53 ± 15
[^{3}H]Acetate	4	2.54	70 ± 8	38 ± 1

lactate uptake measured at 0.15 mM (42 ± 2%, $n = 12$) was reduced to 13 ± 1% ($n = 3$) during perfusion of the heart with 20 mM L-lactate. Higher concentrations of L-lactate were not tested in order to avoid titration with hydroxyl ions.

The specificity of L-lactate influx was investigated by perfusing hearts with a number of different inhibitors (10 mM) known to interact with a monocarboxylate transport system. The stereoselectivity of L-lactate transport is shown in Table II, since influx was markedly inhibited by 10 mM L-lactate but unaffected by the D-isomer. Moreover, pyruvate and the aromatic analogue α-cyano-4-hydroxycinnamate were also effective inhibitors of L-lactate influx. Acetate and chloroacetate reduced L-lactate influx to a lesser extent. Based on this inhibition data we also estimated the respective inhibitor constants, K_i, by means of a 'velocity ratio' [15] assuming that the observed inhibitions were competitive. In Table III the tested inhibitors have been arranged in order of increasing K_i and the K_i/K_t ratio is also given for comparison. Pyruvate and α-cyano-4-hydroxycinnamate were more effective inhibitors than L-lactate itself or acetate and chloroacetate.

In view of glucagon's known ability to enhance

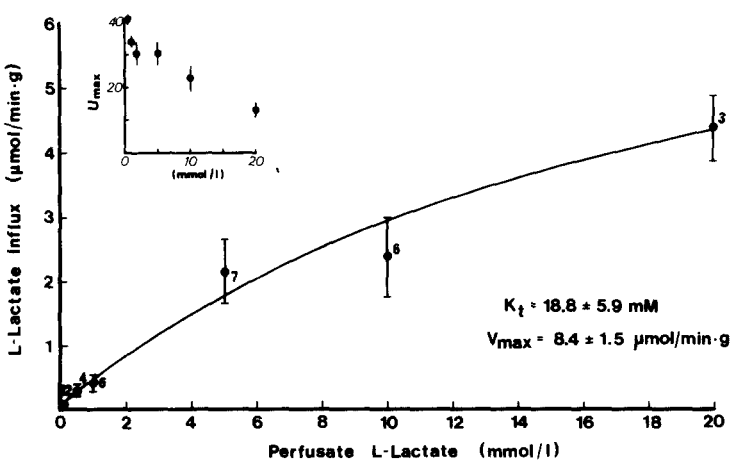

Fig. 2. Kinetics of unidirectional L-lactate influx across the sarcolemmal membrane of the perfused rabbit heart. Influx of lactate was measured during successive perfusion of hearts with two to five different unlabelled L-lactate concentrations (0.15–20 mM). Each paired-racer mixture contained unlabelled L-lactate at a final concentration equal to that of the perfusate under study. Hearts were equilibrated with each perfusate for 4 min before injecting the mixture intraarterially. Michaelis-Menten kinetic constants were estimated by fitting a single rectangular hyperbola to mean influx values weighted for the reciprocal of the standard error at each concentration. The vertical 'lines denote the standard error of the mean and n = number of observations in a total of 14 hearts. The inset illustrates the effect of increasing the perfusate concentration of L-lactate upon the U_{max} for L-[^{14}C]lactate in these 14 experiments.

TABLE II

INHIBITION OF L-LACTATE TRANSPORT AT THE SARCOLEMMAL MEMBRANE OF THE PERFUSED RABBIT HEART

Unidirectional influx of L-lactate was measured at a perfusate concentration of 0.15 mM in the absence (J_c) and then the presence (J_i) of an unlabelled inhibitor at 10 mM. The percentage change in L-lactate influx was calculated from $[1-(J_i/J_c)]\times 100$. Values are given as the mean ± S.E. of n number of observations in at least three hearts.

Inhibitor (10 mM)	% Inhibition in L-lactate influx
L-Lactate	58 ± 9 (5)
D-Lactate	4 ± 5 (4)
α-Cyano-4-hydroxycinnamate	58 ± 3 (3)
Pyruvate	63 ± 8 (3)
Acetate	36 ± 6 (4)
Chloroacetate	27 ± 3 (3)

myocardial lactate production [10], we were interested in studying its effects on sarcolemmal transport of monocarboxylates. In hearts perfused with 5.5 mM D-glucose as substrate, 1.5 μM glucagon inhibited L-[^{14}C]lactate uptake (Fig. 3) and the mean inhibition observed in eight experiments was 41 ± 4% ($P < 0.002$). When we examined the dose response of glucagon's inhibitory effect, we noted

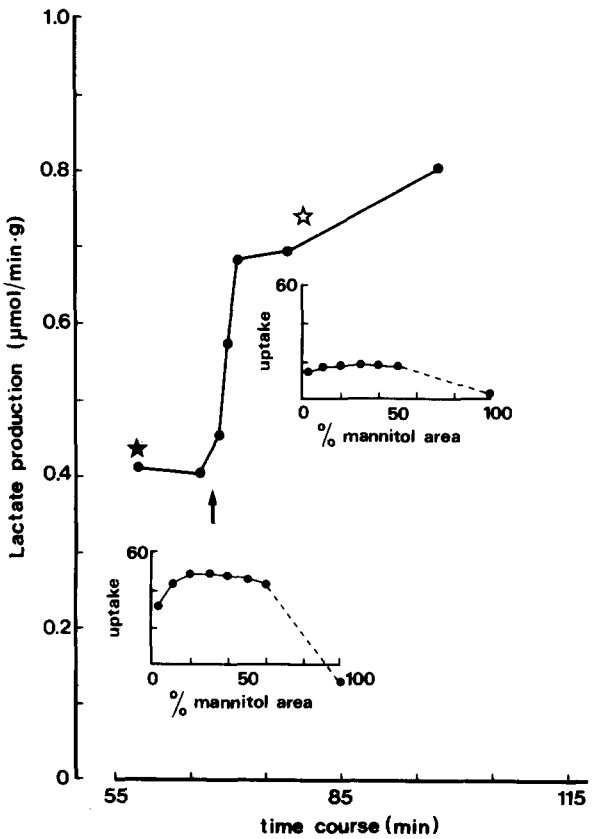

Fig. 3. Effects of glucagon on tracer L-lactate uptake and lactate production by the perfused myocardium. Hearts were perfused with a lactate-free solution in the absence and then the presence of 1.5 μM glucagon. The insets represent the uptake curves obtained for L-[^{14}C]lactate before (★) and 20 min after switching the perfusate to one containing glucagon (☆). The arrow indicates the start of continuous glucagon infusion. The uptake of L-lactate is plotted against the accumulated reference tracer recovery in successive coronary sinus samples, and the dotted lines indicate the relationship between the rapid unidirectional uptake and the uptake measured in a final 4 min venous sample. Lactate production was estimated by assaying the effluent using a specific assay for lactic acid (Sigma Assay No. 826-UV).

TABLE III

KINETIC INHIBITION OF L-LACTATE INFLUX IN THE PERFUSED RABBIT HEART

L-Lactate influx was assessed at a perfusate concentration of 0.15 mM in the absence and then presence of a single inhibitor at 10 mM concentration. The estimate of K_i was based on the K_t value of 18.8 mM measured for L-lactate (see Fig. 2) and was calculated using a velocity ratio [15] in which the uninhibited rate of influx may be related to the inhibited rate provided the inhibition is competitive. When hearts were perfused with D-lactate, no significant inhibition was observed and hence K_i tended to infinity. Values are given as mean ± S.E. and n = number of animals.

	n	K_i (mmol/l)	K_i/K_t
Pyruvate	3	6.5 ± 2.4	0.3 ± 0.1
α-Cyanohydroxycinnamate	3	7.3 ± 0.8	0.4 ± 0.04
L-Lactate	5	12.2 ± 3.5	0.6 ± 0.2
Acetate	4	19.4 ± 4.1	1.0 ± 0.2
Chloroacetate	3	27.6 ± 5.8	1.5 ± 0.3
D-Lactate	4	∞	-

that L-lactate influx was already reduced by 0.075 μM glucagon. Glucagon also reduced [^{14}C]pyruvate and [^{3}H]acetate uptake by respectively 44 ± 2% ($n = 5$, $P < 0.005$) and 39 ± 11% ($n = 3$, $P < 0.01$). During perfusion with 1.5 μM glucagon myocardial production of lactate increased from 0.68 ± 0.06 to 0.94 ± 0.10 μmol/min per g ($P < 0.05$), and perfusion pressures increased from 22 ± 3 to 32 ± 4 mmHg ($P < 0.005$).

Discussion

Saturation kinetics and pH dependence

This study provides the first direct evidence for a carrier-mediated stereospecific L-lactate transport system in the sarcolemmal membrane of the perfused rabbit heart. The existence of specific monocarboxylate carrier has previously been documented in the blood-brain barrier [16–18], erythrocytes [19–23], Ehrlich ascites tumour cells [24], liver [25,26], placenta [27,28], skeletal muscles [29,30], and intestinal [31] and renal [32] brush border and basolateral membrane vesicles. As in our experiments in cardiac muscle (Fig. 2), lactate transport in human erythrocytes [19–23], ascites tumour cells [24], hepatocytes [25], placenta [27,28], skeletal muscle [30] and intestine [31] appears to occur via a low-affinity system, with influx saturating only at concentrations above 10 mM. In many of these studies, including our own, lactate influx may also have been mediated by a non-saturable component. In perfused liver [25] and renal basolateral membrane vesicle [32] uptake was found to virtually increase linearly with substrate concentration. Transport of L-lactate across the blood-brain barrier of adult rats has also been reported to occur by carrier-mediated and non-saturable processes, and unlike the above studies, half-maximal saturation ($K_t = 0.9–3$ mM) of the mediated influx was achieved at low extracellular L-lactate concentrations [16,18].

Alterations in the pH gradient across certain cell membranes have been shown to influence the apparent affinity for L-lactate transport. In ascites tumour cells the K_t for L-lactate was pH-dependent, with values of 4.6 mM and 20 mM at pH 6.2 and 7.2, respectively [24]. The latter K_t value agrees closely with our K_t value of 19 mM determined at pH 7.3–7.4 (Fig. 2). In the perfused liver [25], isolated hepatocytes [26] and erythrocytes [20–23], influx of L-lactate or other monocarboxylates increased progressively with a decrease in extracellular pH. Although lactic acid has a pK'_a of 3.9 and is almost completely ionized at physiological pH, it has been suggested that at low extracellular pH nonionic diffusion could occur via the lipid phases of the membrane [20]. More recent findings in human erythrocytes appear to rule out facilitated diffusion of undissociated lactic acid over a pH range of 5.5–8.7 [23]. Interestingly, superfusion of skeletal [33] and cardiac [34] muscle fibres with external L-lactate was shown to reduce intracellular pH; however, the internal acidification proceeded slowly and only within the order of minutes rather than seconds. It remains to be seen whether pH gradients or anion exchanges influence the rapid (15 s) sarcolemmal uptake of L-lactate in the perfused heart.

Selectivity of the sarcolemmal lactate transporter

In the perfused heart, L-lactate influx was stereoselective (Table II) and similar observations have been reported for erythrocytes [19–21], intestinal basolateral and brush-border membrane vesicles [31], renal basolateral (but not brush-border) membrane vesicles [32] and isolated hepatocytes [26]. Essentially equal rates of transport for D- and L-isomers of lactate have been measured in ascites tumour cells [24], perfused liver [25] and placenta [27,28].

Inhibition of L-lactate transport by various inhibitors was tested at equimolar perfusate concentrations (10 mM) in order to obtain estimates of K_i (see Ref. 15) for direct comparison with published studies. Cyanohydroxycinnamate was chosen on the basis that this aromatic analogue competitively inhibited a specific red cell monocarboxylate carrier [19], although recently the specificity of its inhibition has been questioned (see review, Ref. 35). As illustrated in Table II, perfusion of the heart with 10 mM cyanohydroxycinnamate inhibited L-lactate influx by 58%. The K_i estimated in our study was 7.3 mM (Table III) and considerably higher than values reported by Halestrap [19] for human erythrocytes ($K_i = 50–60$ μM) and by Spencer and Lehninger [24] for ascites tumour cells ($K_i = 0.5$ mM). Moreover, in basolateral membrane vesicles isolated from rat enterocytes 2 mM cyanohydroxycinnamate inhibited L-lactate uptake by only 20%, whereas a 50% inhibition was observed in brush-border membranes [31].

The substituted monocarboxylic acid pyruvate was the most effective inhibitor of L-lactate influx (Table II). Our K_i value of 6.5 mM (Table III) compares favorably with the K_i estimate of 6.3 mM reported for ascites tumour cells [24], although in human erythrocytes the K_i was 1.6 mM

[19]. Pyruvate also exhibits a higher affinity for the monocarboxylate carrier than L-lactate or D-lactate in the perfused liver [25] and blood-brain barrier [16]. Although acetate is taken up avidly during a single circulation through the heart [36], it is a much less efficient inhibitor of L-lactate influx than pyruvate (Table II). The K_i of 19 mM determined for acetate in the heart (Table III) is similar to that measured in Ehrlich ascites cells in which acetate was suggested not to be transported by the lactate carrier [24]. Unlike in Ehrlich ascites cells, chloroacetate was a poor inhibitor ($K_i = 28$ mM) of L-lactate uptake in cardiac muscle (Tables II and III). A large number of monocarboxylates serve as substrates for transport in erythrocytes and ascites cells, and in the blood-brain barrier pyruvate, acetate, L-lactate, propionate and butyrate all exhibit a measurable affinity for the monocarboxylate transport system [16]. By contrast, the sodium-dependent L-lactate transport system in renal brush-border membranes is not inhibitable by pyruvate, acetate, hydroxybutyrate or propionate [32].

Effects of glucagon on monocarboxylate transport

As reviewed by Farah [37], glucagon has virtually no effect on the rate and contractility of the isolated perfused rabbit heart, although it is known that glucagon induces myocardial glycogenolysis [10], lactate production and lipolysis via a lysosomal lipolytic system [38]. When rabbit hearts were perfused with high concentrations of glucagon (0.075–1.5 μM), myocardial lactate production was increased and uptake of tracer L-lactate (Fig. 3), pyruvate and acetate was reduced (data not shown). It is conceivable that uptake of these labelled monocarboxylates may have been inhibited by the elevated interstitial concentrations of endogenously released lactate and/or fatty acids rather than glucagon inducing a modulation of the lactate carrier. It seems unlikely that the small increase in perfusion pressure induced by glucagon could have significantly reduced the surface area available for solute exchange.

It is concluded that a low-affinity, stereospecific lactate transport system is operative in the sarcolemmal membrane of the perfused heart. Moreover, the carrier exhibits a higher affinity for pyruvate and cyanohydroxycinnamate but much lower affinities for acetate and chloroacetate. Our study provides a powerful model for evaluating the influence of circulating hormones, hypoxia, ischemia and diabetes on carrier-mediated transport of monocarboxylates and other metabolites in the heart.

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