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L-Lactate uptake by rat liver

Effect of food deprivation and substrate availability

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We have studied the role of substrate availability on net L-lactate uptake by liver of anaesthetized fed and 24 h-fasted rats. L-Lactate was infused through a mesenteric vein at infusion rates equivalent to 0, 0.125, 0.25 and 0.5 times the basal turnover rate (R_t). By these means we were able to increase L-lactate portal concentrations up to 5.5 mM, without significant changes in portal pH. In the basal state (0 R_t), a net L-lactate uptake by liver was found in 24 h-fasted animals. No net balance was observed in fed rats. Infusion of L-lactate in fed animals failed to induce a net hepatic uptake, except when L-lactate levels in portal vein were raised above 5 mM. In fasted animals, net L-lactate uptake by liver increased linearly ($r = 0.99$) as a function of L-lactate concentration in the portal vein, even beyond the saturation of its specific carrier. It is concluded that, first, the L-lactate carrier does not limit net L-lactate uptake, and second, that substrate availability is an important factor modulating net L-lactate uptake by liver.

INTRODUCTION

Lactate is the major gluconeogenic substrate taken up by the liver (Casado *et al.*, 1987*b*). Transport across the plasma membrane of the hepatocyte is carrier-mediated and might be, under certain conditions, the limiting step of hepatic removal (Fafournoux *et al.*, 1985; Metcalfe *et al.*, 1986). L-Lactate transport is selectively inhibited by other metabolites, such as pyruvate and ketone bodies, as shown in perfused rat liver (Metcalfe *et al.*, 1986), freshly isolated rat hepatocytes (Metcalfe *et al.*, 1986, 1988) and plasma-membrane vesicles enriched with a L-lactate-carrier activity (Quintana *et al.*, 1988). The carrier might indeed respond to physiological stimuli such as starvation by increasing the capacity to take up L-lactate (Metcalfe *et al.*, 1988); this enhancement is due to a stable change at the plasma-membrane level (Quintana *et al.*, 1988). Thus many interacting factors are likely to modulate the carrier activity *in vivo*, and indeed, when hepatic balances of L-lactate are measured in the anaesthetized intact rat, it is found that the net uptake rates are extremely sensitive to the physiological and nutritional conditions of the animals. For instance, fed rats in a near-post-absortive state show a nil hepatic balance for lactate, but after fasting, when substrate availability is decreased, a net uptake is found (Rémésy & Demigné, 1982; Davis *et al.*, 1987). Thus it seems rather unlikely that substrate availability might be modulating hepatic lactate removal. However, as shown by studies using partial hepatectomy, the hepatic capacity for lactate disposal might exceed its rate of provision to the liver (Schofield & Sugden, 1986). Furthermore, the lactate-uptake rate by liver of 48 h-fasted rats perfused *in situ* is a linear function of L-lactate concentration in the perfusion buffer, at least in the range 0.4–1.5 mM (Sestoft & Marshall, 1986). Thus it is not clear to what extent substrate availability might be modulating lactate disposal by liver *in vivo*.

The goal of the present work was to determine the role of substrate availability in L-lactate net uptake by liver in both fed and fasted rats. Nevertheless a more physiological approach than those of previous studies was used, because portal substrate concentrations were modified by infusing L-lactate into a mesenteric vein of anaesthetized animals.

EXPERIMENTAL

Animals

Some 53 female Wistar rats (200 g body wt.) were purchased from the Laboratory Animal Service of the University of Barcelona. Animals were kept under controlled conditions of temperature (22±2 °C), humidity (40–60%) and light (12 h on/12 h off). All the experiments with fed rats were performed 3 h after the beginning of the light cycle (near-post-absorptive conditions). The fasting animals were used after being kept for 24 h without any food available, but having had access to water *ad libitum*.

Surgery and L-lactate infusions

Rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt. intraperitoneally). After laparatomy, an indwelling catheter was placed into a mesenteric vein and an L-lactate solution buffered with (5.4 mM-KCl/0.44 mM-KH_2PO_4/0.98 mM-$MgCl_2$,$6H_2O$/0.81 mM-$MgSO_4$,$7H_2O$/136.8 mM-NaCl/1.33 mM-Na_2HPO_4/5 mM-$CaCl_2$,$2H_2O$/20 mM-Hepes, pH 7.4) was immediately infused at a rate equivalent to 0.125, 0.25 and 0.5 times the basal lactate turnover rate (19 mg/min per kg body wt., as previously reported in the literature; Valcarce *et al.*, 1985). Control rats were equally infused with the buffered solution without L-lactate. Infusion rate was about 90 μl/min, and no significant changes in volaemia should be expected to occur. After a 5 min infusion, blood was sampled from the afferent and efferent vessels of the liver as previously described (Casado *et al.*, 1987*a*). Direct sampling of the hepatic vein was carefully performed by using a bent needle, which was inserted close to the liver lobe, before the hepatic vein joins the inferior vena cava. The pH of the portal blood was immediately measured by means of a BMS3 MK2 Blood Micro System (Radiometer, Copenhagen, Denmark). Blood samples were deproteinized by adding $HClO_4$ (final concn. 5%, w/v). Supernatants were used for L-lactate and pyruvate determinations by standard fluorimetric methods (Gutmann & Wahlefeld, 1974; Passoneau & Lowry, 1974).

Calculations

To calculate the hepatic balances, we used liver blood flows

Abbreviation used: R_t, basal turnover rate.

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previously measured in our laboratory and reported elsewhere (Casado *et al.*, 1987*c*). These measurements were done by using an indicator-dilution method adapted for small mammals, as described by Rémésy & Demigné (1983). The values we used were 3.8 and 2.4 ml/min per g of tissue for the total liver blood flow and 2.1 and 1.8 ml/min per g of liver for the portal blood flow of fed and fasted rats respectively (Casado *et al.*, 1987*c*). Results on hepatic balances are given as μmol/min. Fractional extraction values were used to reflect the capacity of the liver to adapt its uptake independently of substrate availabilities and were calculated as previously reported (Casado *et al.*, 1987*c*). Statistical differences between either the fed and the fasted state or L-lactate-infused and control rats were tested by Student's *t* test.

RESULTS

L-Lactate and pyruvate concentrations in the aorta and the hepatic and portal veins are shown in Table 1. Basal lactate levels in the three blood vessels were lower in starved than in fed rats. In both nutritional conditions all the infusion rates made lactate concentrations increase in afferent and efferent vessels. In particular, L-lactate levels in porta were raised as high as 5.5 mM when the highest L-lactate infusion rate was used. pH was unaffected in both experimental groups for all the infusion rates tested (pH 7.351 ± 0.02, $n = 53$). After L-lactate infusion, lactate concentrations in the hepatic vein remained lower in the fasted than in the fed state. Similar concentrations were found in the other two vessels for both experimental conditions. Pyruvate concentrations remained unchanged at any L-lactate infusion rate tested. The only significant difference was found when pyruvate levels in the hepatic vein were compared under both nutritional conditions. Fasting induced a marked decrease in pyruvate concentrations in this vessel, reaching values 2–3-fold lower than those of fed rats.

L-Lactate net hepatic balance is shown in Fig. 1(*a*). No net hepatic balance was found in fed rats. Similar results have been described previously (Casado *et al.*, 1987*b*). Only when the L-lactate portal concentration was increased over 5 mM were the values for the fed animals clearly positive, showing a net L-lactate uptake (−0.23 ± 2.09 and 27.0 ± 5.20 μmol/min for 0 and 0.5 R_t (R_t, basal turnover rate) respectively; $P < 0.01$). In fasted animals a basal net uptake by liver was observed (8.05 ± 1.25 μmol/min; $P < 0.01$ versus fed rats, 0 R_t). The net L-lactate uptake by liver in those animals increased linearly as a function of substrate availability ($r = 0.99$).

The results on L-lactate fractional extraction rates are shown in Fig. 1(*b*). Values of fed animals were not different from zero for all portal concentrations up to 3.6 mM. When L-lactate in the portal vein was raised above 5 mM, a significant increase in the fractional extraction rate was observed ($P < 0.01$ versus 0 R_t). Conversely to what was found in the fed state, the fractional extraction rates in fasted animals were always positive and significantly different from those of fed rats ($P < 0.001$). Infusions of L-lactate did not result in any change of fractional extraction in food-deprived animals.

Table 1. L-Lactate and pyruvate concentrations in the hepatic afferent and efferent vessels

The pH value was measured immediately after sampling in the portal vein as described in the Experimental section. Values are expressed as means ± S.E.M. for 6–8 animals. Statistically significant differences were: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus basal (0 R_t); †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ versus fed animals at each L-lactate dose infused (Student's *t* test).

Rats	L-Lactate infused (R_t)...	Lactate (mM) 0	0.125	0.25	0.5	Pyruvate (μM) 0	0.125	0.25	0.5
Fed	Porta	1.66 ± 0.02	2.74 ± 0.16***	3.60 ± 0.11***	5.60 ± 0.20***	112.7 ± 5.1	125.2 ± 10.2	123.5 ± 20.4	169.6 ± 21.4
	Aorta	1.56 ± 0.08	2.31 ± 0.19**	2.42 ± 0.22**	3.51 ± 0.36***	111.8 ± 14.1	115.7 ± 15.3	114.0 ± 15.3	146.0 ± 26.2
	Hepatic	1.64 ± 0.07	2.73 ± 0.19***	2.84 ± 0.23***	3.73 ± 0.20***	188.1 ± 20.3	260.3 ± 33.3	193.8 ± 41.4	214.7 ± 38.3
	pH	7.36 ± 0.01	7.36 ± 0.02	7.33 ± 0.01	7.33 ± 0.02	–	–	–	–
Fasted	Porta	1.16 ± 0.11††	2.61 ± 0.09***	3.32 ± 0.14***	5.44 ± 0.39***	104.0 ± 10.5	126.3 ± 14.5	109.4 ± 10.9	172.9 ± 40.7
	Aorta	0.85 ± 0.13††	1.52 ± 0.26*	1.73 ± 0.35*	2.25 ± 0.25***	86.9 ± 10.9	97.2 ± 11.6	97.2 ± 10.1	114.7 ± 26.3
	Hepatic	0.72 ± 0.16†††	1.36 ± 0.21*††	1.36 ± 0.19*†††	1.89 ± 0.21***†††	80.2 ± 19.6†††	86.8 ± 16.2†††	67.1 ± 10.7†	114.6 ± 22.5†
	pH	7.35 ± 0.02	7.38 ± 0.02	7.38 ± 0.02	7.38 ± 0.02	–	–	–	–

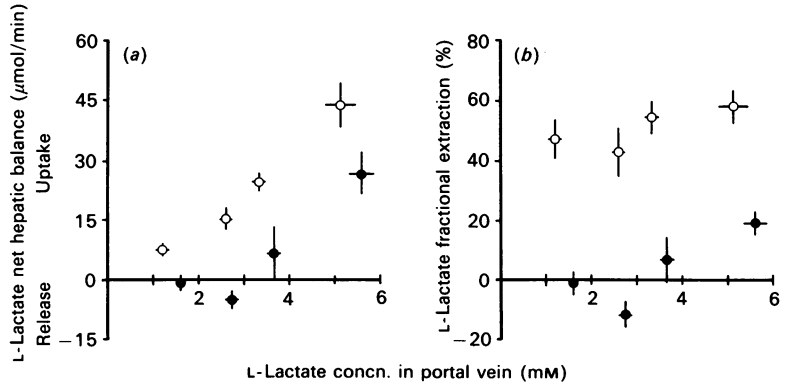

Fig. 1. L-Lactate net hepatic balance (*a*) and fractional extraction (*b*) as a function of L-lactate concentrations in portal vein

Measurements were done in either fed (●) or 24 h-fasted (○) rats. Each point represents the mean ± S.E.M. for 6–8 animals. For details see the Experimental section.

Table 2. Net balance and fractional extraction of pyruvate by livers from fed and 24 h-fasted rats

Values are expressed as means ± S.E.M. for 6–8 animals. Statistically significant differences were found by Student's *t* test: $*P < 0.01$, $**P < 0.001$ versus fed rats at each L-lactate dose infused. For details see the Experimental section.

Rats	Dose	Balance (μmol/min)	Fractional extraction (%)
Fed	0 R_t	−2.17±0.49	−68.7±16.8
	0.125 R_t	−4.02±0.85	−87.9±14.4
	0.25 R_t	−2.15±0.71	−57.6±20.4
	0.5 R_t	−2.17±0.51	−45.8±10.8
Fasted	0 R_t	0.93±0.28**	41.4±7.9**
	0.125 R_t	1.01±0.36**	32.7±8.8**
	0.25 R_t	0.94±0.18*	37.4±7.1*
	0.5 R_t	1.05±0.67*	19.4±10.0**

Pyruvate balance across the liver is shown in Table 2. No effects of L-lactate infusion on net pyruvate uptake or release by liver were observed. Nevertheless, fed animals clearly showed net hepatic production of pyruvate, whereas fasted animals showed a net uptake. The pyruvate fractional extraction rates are also shown in Table 2. Fed animals showed negative values and fasted rats showed positive fractional extraction rates. The infusion of L-lactate did not modify this parameter at any concentration tested.

The evolution of L-lactate/pyruvate ratios in aorta and portal and hepatic veins after L-lactate infusion in fed and 24 h-fasted animals is shown in Figs. 2(*a*) and 2(*b*) respectively. As expected, the L-lactate/pyruvate ratio in portal vein increased with L-lactate infusion rates. However, only a few significant changes ($P < 0.05$) from basal values (0 R_t) were found in hepatic vein at 0.25 and 0.5 R_t. On the other hand, no changes in this parameter were observed in aorta of fed animals. In the same way, the L-lactate/pyruvate ratio in the portal vein of 24 h-fasted animals increased as a function of the L-lactate infused. In hepatic vein an enhancement of this ratio at 0.25 and 0.5 R_t was observed. Furthermore, in aorta this parameter increased significantly in a similar way to that reported for the hepatic vein.

DISCUSSION

In the present work we have been successful in artificially increasing L-lactate concentrations in the portal vein without inducing any significant change in blood pH. This is an important feature of this work, because it has been widely described, not only in liver (Sestoft *et al.*, 1982; Sestoft & Marshall, 1986; Edlund & Halestrap, 1988), but also in other cell systems (Trosper & Philipson, 1987; Balkovetz *et al.*, 1988; Tiruppathi *et al.*, 1988), that the L-lactate carrier is sensitive to a pH gradient across the plasma membrane. It has been suggested, indeed, that this might be the driving force for L-lactate active transport into cells. Thus, any change observed in our experimental model should be attributable not to modifications of the pH gradient, but rather to intrinsic differences whether in L-lactate carrier activity or substrate availability.

The role of the L-lactate carrier in mediating net L-lactate uptake by liver in food-deprived rats has been studied in previous reports (Metcalfe *et al.*, 1988; Quintana *et al.*, 1988), which showed that physiological situations leading to an increased gluconeogenic flux, such as starvation or diabetes, are associated with an enhancement of L-lactate-transport-system activity in liver. To what extent this modification is a true limiting step of L-lactate metabolism in liver, or merely a logical change, but lacking regulatory properties, remained to be established. L-Lactate uptake into liver parenchymal cells through its specific carrier is known to be inhibited by pyruvate and ketone bodies (Metcalfe *et al.*, 1986; Edlund & Halestrap, 1988; Quintana *et al.*, 1988). None of these inhibitors is likely to mediate to a significant extent any of these changes that we report in the present work, because, first, L-lactate/pyruvate ratios were similar between both fed and fasted L-lactate-infused animals and, second, blood ketone-body concentrations needed to exert significant inhibition on L-lactate uptake are much higher than those found in 24 h-starved rats (Sugden *et al.*, 1982). Intrinsic changes in the carrier activity might partially explain the differences found in the present work, but we do not think they have a decisive role in mediating L-lactate net uptake by liver, because no saturability is observed even when L-lactate concentrations in the portal vein reached 5.5 mM. When a similar study to ours was performed by infusing L-alanine, saturation was reached at substrate concentrations in the range of the K_m for Na^+-dependent L-alanine transport in liver parenchymal cells (Fafournoux *et al.*, 1983). This finding agrees with the suggested

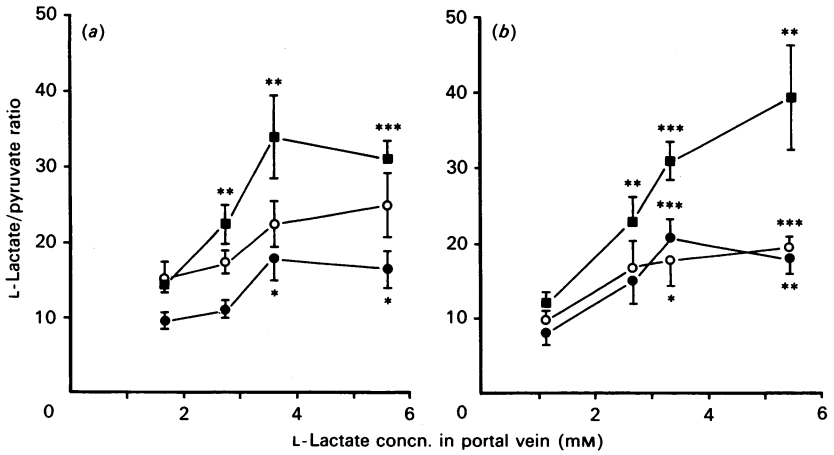

Fig. 2. L-Lactate/pyruvate ratios as a function of L-lactate concentration in portal vein

(*a*) Fed animals; (*b*) 24 h-fasted animals. Symbols: ●, hepatic vein; ■, portal vein; ○, aorta. Each point is the mean ± S.E.M. for 6–8 animals: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus 0 R_t at every L-lactate infusion dose in both fed and 24 h-fasted rats.

role of alanine transport as a limiting step of its own metabolism (Sips *et al.*, 1980; Fafournoux *et al.*, 1983). In our case, it is rather likely that, as suggested by others (Monson *et al.*, 1982; Fafournoux *et al.*, 1985; Edlund & Halestrap, 1988), for L-lactate concentrations greater than the reported K_m of its carrier, undissociated forms of L-lactate might cross the plasma membrane by simple diffusion. Irrespective of this possibility, Edlund & Halestrap (1988) reported for the L-lactate hepatic carrier a much greater $V_{max.}$ than the maximal recorded rate for hepatic gluconeogenesis. This finding is not in agreement with previous data by Fafournoux *et al.* (1985), showing a $V_{max.}$ value 60–70 times lower than that reported by Edlund & Halestrap (1988), but, if the data by these latter authors are correct, lactate transport should never have been considered as a factor that limits lactate utilization by liver parenchymal cells.

The net uptake of 1–3 mM-lactate by the liver of fasted rats, but not of fed rats, suggests that net uptake is determined by the metabolic disposal of lactate during fasting, mainly as gluconeogenic substrate. The metabolic adaptations and their regulatory basis leading to enhanced gluconeogenesis in the liver have been reviewed elsewhere (Pilkis *et al.*, 1988).

The possibility that anaesthesia might modify lactate disposal by liver should not be discarded. It is known that anaesthesia induces a 30% decrease in glucose production by liver in rats in a post-absorptive state (Pénicaud *et al.*, 1987), but it is not known to what extent substrate uptake might be affected. So far, chronic catheters in the hepatic vein of small mammals have not been available, and other approaches trying to study this specific point have been done by indirect means and not in unrestrained conditions (Zorzano & Herrera, 1984). Whether or not pentobarbital anaesthesia might affect substrate uptake is not so important, in view of the lack of saturability for L-lactate net uptake, which is one of the main findings of this work.

Finally, another interesting feature of the present findings is that, despite exogenous L-lactate infusion, L-lactate/pyruvate ratios in artery and hepatic veins are quite well preserved, and they increase to a much lesser extent than the portal-vein ratios, thus suggesting a decisive role of the liver in controlling systemic L-lactate/pyruvate ratios. This effect is being mediated by different mechanisms, depending on the nutritional status of the animal. Thus in fed rats a net pyruvate production by liver is observed, whereas in fasted animals a net hepatic uptake of pyruvate was found.

From all the results discussed above, we can conclude (i) that the L-lactate carrier does not limit hepatic L-lactate uptake (assuming that the measured net uptake approximates to net influx, i.e. that efflux was negligible), and (ii) that the substrate availability is an important factor modulating the uptake of lactate by liver.

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