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EFFECTS OF INHIBITORS OF GLUCONEOGENESIS ON WEAK ORGANIC ACID UPTAKE IN RAT RENAL TUBULES

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Abstract—Using inhibitors of gluconeogenesis (phenylpyruvate, α-cyano-4-hydroxycinnamate, quinolinate, D-malate, aminooxyacetate), we analysed mechanisms by which the gluconeogenic substrates, lactate and pyruvate, as well as a short-chain fatty acid, acetate, stimulate the uptake of a weak organic acid, fluorescein, in the rat kidney. We have shown that these inhibitors modified both the rate of glucose production from lactate and pyruvate in the renal cortex fragment suspension and the stimulatory effects of the metabolic substrates on fluorescein uptake in superficial proximal tubules in the renal cortex slices. The peculiarities of the effects of lactate and pyruvate on the uptake were correlated with the partial divergence of the pathways of gluconeogenesis from these precursors. The linkage of the weak organic acid uptake with gluconeogenesis is interpreted in terms of the hypothesis that the uptake is controlled by the cytoplasmic pyridine nucleotide redox potential, which is maintained with the participation of certain processes involved in glucose synthesis.

Secretion of weak organic acids [e.g. *p*-aminohippurate (PAH†)] in renal proximal tubules (RPTs) is considered to depend on aerobic oxidative metabolism [1, 2]. Metabolic substrates such as pyruvate, lactate, fatty acids and intermediates of the tricarboxylic acid (TCA) cycle are known to stimulate the uptake of weak organic acids in RPTs [3–6]. However, attempts to reveal a close correlation between the effects of these substrates on PAH uptake, oxygen consumption and intracellular ATP content in the RPT cells was not successful [3, 4, 7]. Thus, it should be admitted that the stimulatory effects of metabolic substrates on the transport system under consideration cannot be accounted for by activation of ATP production only [8].

Recently, it has been postulated [9, 10] that PAH transport into RPTs through the basolateral membrane occurs by means of its exchange with intracellular dicarboxylates (e.g. α-oxoglutarate) with an outward concentration gradient of the dicarboxylates being maintained at the expense of their Na-dependent uptake. Results confirming this model were obtained using basolateral membrane vesicles. Experiments with renal cortex slices supported the possibility of the PAH–dicarboxylate exchange, but the overall PAH uptake could not be accounted for by such an exchange only [11].

Most metabolic substrates stimulating the uptake in RPTs of weak organic acids cannot directly exchange with PAH. However, many substrates undergo metabolic transformation in the mammalian RPTs [reviews: 8, 12, 13], and so it could well be assumed that some metabolic substrate might stimulate the uptake of weak organic acids due to their exchange for certain product(s) of its transformation in the RPT cells [6]. The most important metabolic process participating in the substrate interconversion in the RPTs is gluconeogenesis (GNG). The mechanisms of GNG and its regulation have been studied in some detail in the RPTs of the rat kidney [reviews: 13, 14]. In the present work we studied effects of some substrates and inhibitors of GNG on the uptake of weak organic acids in the rat RPTs. The results suggest that the mechanisms regulating weak organic acid uptake and GNG in the RPTs overlap to a certain extent.

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† Abbreviations: PAH, *p*-aminohippurate; GNG, gluconeogenesis; RPT, renal proximal tubule; TCA, tricarboxylic acid; LDH, lactate dehydrogenase; CHC, α-cyano-4-hydroxycinnamate; PMS, phenazine methosulfate; OAA, oxaloacetate; AOA, aminooxyacetate; T/M, tubule/medium.

MATERIALS AND METHODS

Chemicals. Fluorescein was obtained from Koch-Light Laboratories Ltd (Colnbrook, U.K.); quinolinate, aminooxyacetate (AOA), α-cyano-4-hydroxycinnamate (CHC) and D(+)-malate from the Sigma Chemical Co. (St Louis, MO, U.S.A.); and ouabain, EDTA, Tris, pyruvate, L-lactate, phenylpyruvate, monofluoroacetate and L-cycloserine from Serva (Heidelberg, F.R.G.). Other reagents were of commercial grade.

Determination of fluorescein uptake in renal cortex slices. Kidneys of fed male rats (Wistar, 180–250 g) from a local source were used. The animals were quickly decapitated and the kidneys removed and decapsulated. The outermost slices (0.5–0.8 mm) of the cortex were prepared by hand with the aid of a razor blade, the surface of the kidney being left intact. The slices were preincubated at 20–22° for 60 min in an aerated, substrate-free physiological solution (modified Krebs–Ringer phosphate buffer) containing (mmol/L): NaCl 104.7; KCl 15.3; $CaCl_2$

1.5; $MgSO_4$ 2.5; $NaHCO_3$ 3.6; NaH_2PO_4 3.3; Na_2HPO_4 4.8; pH 7.1–7.3 (20°).

After preincubation the slices weighing 50–80 mg were incubated in 10 mL of fresh, aerated medium containing fluorescein (disodium salt, uranine, C.I. 45350) with additions as indicated. The dye is known to share a common transport system with PAH in rat RPTs [2, 15]. The amount of fluorescein accumulated in superficial renal tubules was measured with the aid of a laboratory-built microfluorimeter with a contact objective lens [16]. The measurements of the fluorescein luminescence were performed in the tubules located at the constant depth (approx. 40 μm) from the intact surface of the kidney. These tubules have been characterized previously with the aid of a polarization epi-microscope [15]. It was concluded that they should be regarded as the proximal convoluted tubules (presumably, S2 segments). The lumen of these tubules was opened and the oxygen supply of the tubule cells was not limited since the pyridine nucleotides in their mitochondria were reduced by little more than 50–60% in comparison with their reduction in the presence of amytal. Fluorescein uptake in the rat RPTs during incubation in a substrate-free medium was not limited by the content of endogenous substrates (for incubation up to 30 min), as followed from the data showing the energy-dependent stimulation of fluorescein uptake by cadmium ions [17]. In the present work, effects of substrates and inhibitors on the fluorescein uptake were examined at 20 min, i.e. on the rising phase of the uptake curve.

The primary and secondary filters of the microfluorimeter had wide enough bands of transmission to avoid a significant influence of intracellular pH changes on the intensity of the fluorescein luminescence. To rule out an overestimation of the fluorescein uptake in the RPTs due to probable intracellular binding, the results were expressed in the form of a normalized concentration ratio, tubule/medium (T/M), the value of this ratio for equilibrium fluorescein accumulation during incubation at 2–4° being considered as unity.

On the surface of each slice the luminescence intensity in 40 different convoluted RPTs was measured; the intensity of background luminescence was subtracted. The measurements were repeated on slices from three to four animals, so that each point represents the mean of 120–160 individual records. The uptake data are presented as means ± 2 SE.

Preparation of renal cortex fragment suspension. To obtain metabolic correlates for the uptake data, we needed an adequate experimental model for determination of metabolite levels and oxygen consumption. The cortical slices were rejected because of inherent diffusion limitations resulting in anoxic zones inside the slices. The preparation of choice was the cortex fragment suspension obtained without treating the tissue with proteases to avoid undesirable alterations in the cellular metabolism. The tubule fragment isolation technique was adopted from Guder *et al.* [18].

After preincubation at 20–22° for 60 min, the outermost cortical slices were blotted slightly, weighed, chilled and squeezed by a press through a stainless steel sieve (average diameter of pores about 580 μm) into the ice-cold medium (1:5 w/v). The tissue was suspended with a glass rod and the suspension was centrifuged at 50 *g* for 5 min. The supernatant was removed by suction and the sediment was diluted with fresh medium up to the original volume, stirred and centrifuged at 50 *g* for 5 min. The procedure was repeated and the final loose, whitish pellet was resuspended in the original volume. The resulting suspension was kept on ice. Typical yields averaged 90–120 mg protein/1 g wet weight of cortex tissue.

This fragment suspension consisted mostly of tubules and isolated cells and stained poorly with Trypan blue. Most tubules accumulated fluorescein; some tubules had the lumen kept open. Cell viability in the suspension was estimated by measuring lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27; LDH) release, which averaged 2.6 ± 0.3%, 3.2 ± 0.3%, 3.4 ± 0.4% and 4.7 ± 0.5% after 0, 20, 40 and 60 min of incubation, respectively.

The RPT cells in the preparation retained their functional activity to a certain extent, as followed from the fact that the oxygen consumption in the suspension was inhibited by ouabain, and the rate of glucose production from lactate or pyruvate was constant at least during 60 min of incubation.

Measurement of metabolite levels. The cortex fragment suspension (final content 4–8 mg protein/mL of the incubation medium) was usually incubated at 20–22° for 30 min in flasks containing 1 mL of the standard physiological solution (see above for composition) containing 0.5% bovine serum albumin (fraction V, defatted, Sigma Chemical Co.) in a shaker at 70 cycles/min. At the end of incubation period, 0.1 mL of ice-cold 30% $HClO_4$ was added to the vessels. All the following procedures were carried out at 2–4°. After 15 min the vessel contents were neutralized to a final pH of 7.5–7.7 by adding 0.4 mL Tris (1.5 M)–EDTA (5 mM) (pH 9.7–10.2) and immediately centrifuged at 10,000 *g* for 10 min. The supernatants (clear extracts) were collected for determination of glucose, lactate, pyruvate and ATP, while the pellets were used for measurements of protein content according to a modified Lowry method [19].

It should be noted that the measured metabolite levels represent the sum of the concentrations in the medium *plus* the tubules. The content of glucose in the incubation vessels at zero time was subtracted.

Glucose was determined by the glucose oxidase technique; for assays of lactate, pyruvate and ATP standard enzymatic methods were used [20]. Enzymes for metabolite determinations were obtained from Boehringer (Mannheim, F.R.G.).

LDH activity was measured spectrophotometrically [20] in 0.02 mL aliquots of the incubation medium after 0, 20, 40 and 60 min of incubation and in the homogenates. Percentage LDH release was calculated as percentage of total LDH activity present in the incubation vessels.

Registration of oxygen consumption. Oxygen consumption was measured polarographically at 20–22° (magnetically stirred chamber with the Clarke-

Table 1. Comparison of the effects of lactate, pyruvate and acetate on fluorescein uptake in cortex slices and the rates of gluconeogenesis and oxygen consumption in the cortex fragment suspension

Substrate (mM)	Fluorescein uptake (% of stimulation)	Oxygen consumption* (% of stimulation)	GNG rate (nmol/mg protein/30 min)
None	—	—	1.89 ± 0.14 (17)
Acetate (1)	+24 ± 2% (12)	+27 ± 6% (7)	1.82 ± 0.14 (6)
Lactate (5)	+44 ± 3% (18)	+30 ± 3% (22)	7.39 ± 0.35 (13)
Pyruvate (5)	+20 ± 3% (7)	+27 ± 4% (17)	7.92 ± 0.52 (13)

Cumulative data concerning relative effects of the substrates on fluorescein uptake and oxygen consumption (as compared to appropriate controls), as well as the rates of glucose synthesis in the presence of these substrates, are presented. The slices and the cortex fragment suspension were incubated at 20–22° for 20 and 30 min, respectively, as described in Materials and Methods.

In parenthesis: for fluorescein uptake, number of separate series; for oxygen consumption and GNG rates, number of separate experiments.

The data are presented as means ± SE.

* Basal oxygen consumption rate averaged 165 ± 8 nmol O_2/mL/60 min (24 recordings).

type electrode and universal polarograph PU-1 (Gomel, Belorussia). Aliquots of the cortex fragment suspension (final content of the tissue 1–2 mg protein/mL) were introduced into the chamber equilibrated with air. Once a stable recording was achieved, various substrates and inhibitors were added to the chamber as concentrated aqueous stock solutions. The oxygen tension in the chamber was monitored as a function of time. The measurements lasted usually 7–10 min. Data are expressed as the percentage change in oxygen consumption rate (slope during the experimental period/slope during control conditions).

Data concerning the oxygen consumption and metabolite levels are presented as means ± SE for the numbers of separate preparations indicated in parentheses.

RESULTS

Exogenous substrates

Lactate, pyruvate (both at 5 mM) and acetate (1 mM) were used in the present study as metabolic substrates. Lactate and pyruvate which penetrate freely across the basolateral membrane into the rat RPT cells [21] are known to be the glucose precursors in the rat renal cortex *in vitro* [review: 13]. Acetate entering the rat RPT cells by active transport and/or passive diffusion [22] is not a gluconeogenic substrate.

Data revealing the effects of these substrates on glucose production, oxygen consumption and fluorescein uptake are presented in Table 1. In particular, it followed from these data that the rates of glucose production, as well as those of oxygen consumption, in the presence of lactate and pyruvate, were similar under the conditions employed in the present study. In contrast, fluorescein uptake in the RPTs was stimulated by lactate to a much greater extent than by pyruvate. Pyruvate, at concentrations below 5 mM, did not increase the fluorescein uptake, whereas the stimulatory effect of lactate was the same at concentrations from 0.5 to 5 mM (data not shown).

To investigate whether an involvement of the TCA cycle was necessary for the substrates tested to stimulate fluorescein uptake, we used an inhibitor of aconitase [citrate (isocitrate) hydrolase, EC 4.2.1.3], fluoroacetate [23]. As seen in Fig. 1, fluoroacetate (1 mM) neither affected directly fluorescein uptake nor modified the effect of lactate on its. However, the effects of pyruvate and acetate on the fluorescein uptake were augmented by fluoroacetate.

It should be noted that the fluoroacetate concentration (1 mM) used in these experiments can hardly be too low to inhibit aconitase, since fluoroacetate at this concentration inhibited markedly both GNG from lactate (Table 2) and the basal oxygen consumption (data not shown) in the cortex fragment suspension. Besides, 1 mM fluoroacetate completely blocked the stimulatory effects of lactate, pyruvate and acetate on oxygen consumption in the suspension (data not shown).

The above results suggested that mechanisms of the stimulatory action of lactate, pyruvate and acetate on fluorescein uptake should be associated with metabolic pathway(s) other than the TCA cycle. Thus, we investigated further the fluorescein uptake, as affected by some inhibitors of GNG.

First, it was tested how these inhibitors affected the rate of glucose production in the RPTs under the conditions employed in the present work. Data peculiar to GNG in the rat kidney were obtained (Table 2). In particular, the rate of glucose production in the presence of lactate or pyruvate was markedly diminished by CHC, an inhibitor of mitochondrial pyruvate transport [24], and by phenylpyruvate, an inhibitor of pyruvate carboxylase [pyruvate: CO_2 ligase (ADP-forming), EC 6.4.1.1] [25]. An inhibitor of phospho*enol*pyruvate carboxykinase [orthophosphate: oxaloacetate carboxylase (phosphorylating); EC 4.1.1.31], quinolinate [26], inhibited GNG from lactate more

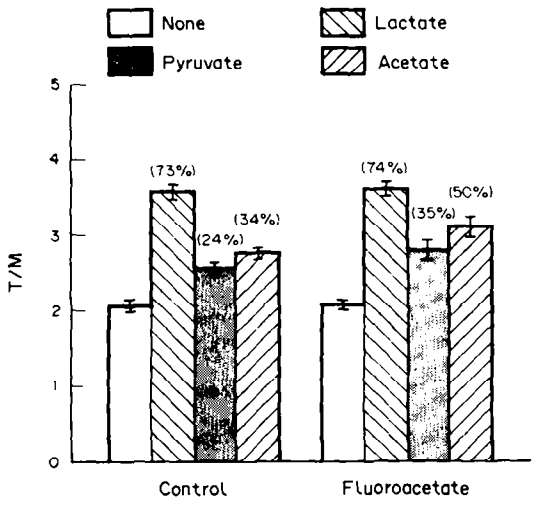

Fig. 1. Influence of fluoroacetate on the metabolic substrate effects on fluorescein uptake. Slices were preincubated in aerated, substrate-free medium at 20° for 60 min and then incubated with fluorescein (0.05 mM) at 20° for 20 min. Concentrations: fluoroacetate and acetate, both at 1 mM; lactate and pyruvate, both at 5 mM. Each bar represents a mean of 120–160 individual records on slices from three to four rats. In parenthesis: the extent of fluorescein uptake stimulation. The vertical lines show the 95% confidence limits.

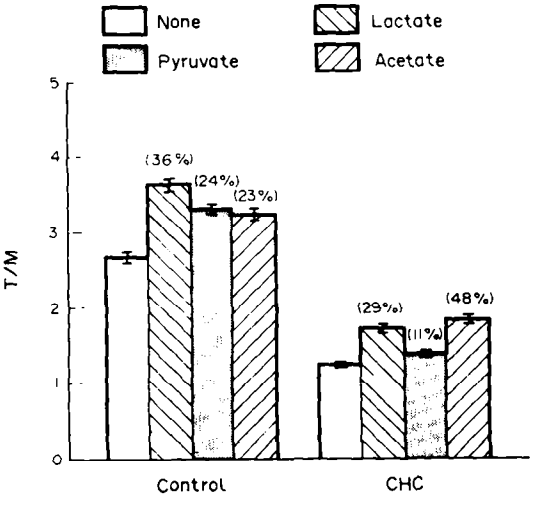

Fig. 2. Influence of CHC (0.1 mM) on the metabolic substrate effects on fluorescein uptake. Conditions and abbreviations as in the legend to Fig. 1.

efficiently than from pyruvate; D-malate, an inhibitor of cytosolic malate dehydrogenase (L-malate: NAD^+ oxidoreductase; EC 1.1.1.37) [27], decreased GNG from pyruvate but augmented that from lactate whereas an inhibitor of aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase; EC 2.6.1.1), aminooxyacetate [27], inhibited GNG from lactate but did not affect that from pyruvate.

The results showing an influence of these inhibitors on the stimulatory effects of the metabolic substrates on fluorescein uptake are presented in Figs 2–4. As follows from these data, CHC (Fig. 2) and phenylpyruvate (Fig. 3) lowered the stimulatory effects of lactate and pyruvate, whereas the effect of acetate was significantly increased by these agents. The effect of lactate was decreased by quinolinate but augmented by D-malate (Fig. 4). [Note that GNG from lactate was affected by these inhibitors in similar ways (Table 2)]. D-Malate completely blocked the stimulatory effects of pyruvate and acetate. Quinolinate abolished the effect of pyruvate, while the effect of acetate on fluorescein uptake was not altered by this inhibitor.

To reveal a possible involvement of the cytoplasmic

Table 2. Effects of the inhibitors tested on the rates of glucose production in the cortex fragment suspension

Inhibitor (mM)	GNG rate in the presence of Lactate (5 mM) (nmol/mg protein/30 min)	Effect* (%)	Pyruvate (5 mM) (nmol/mg protein/30 min)	Effect* (%)
Expt 1				
None	6.58 ± 0.12	—	6.56 ± 1.06	—
D-Malate (2)	8.68 ± 0.81	+32	4.13 ± 0.53	−30
CHC (0.1)	3.69 ± 0.28	−44	2.09 ± 0.46	−68
AOA (1)	2.62 ± 0.44	−60	6.26 ± 0.55	−5
Expt 2				
None	8.60 ± 0.37	—	7.53 ± 0.24	—
Fluoroacetate (1)	4.83 ± 0.12	−44	6.53 ± 0.46	−13
Quinolinate (2)	5.05 ± 0.14	−41	5.57 ± 0.10	−26
Phenylpyruvate (1)	2.43 ± 0.18	−72	2.45 ± 0.25	−67

The cortex fragment suspension was incubated at 20–22° for 30 min.

The data are presented as means ± SE; each value is an average of 3–4 determinations from separate experiments.

* Stimulation (+) or inhibition (−) as compared to an appropriate control.

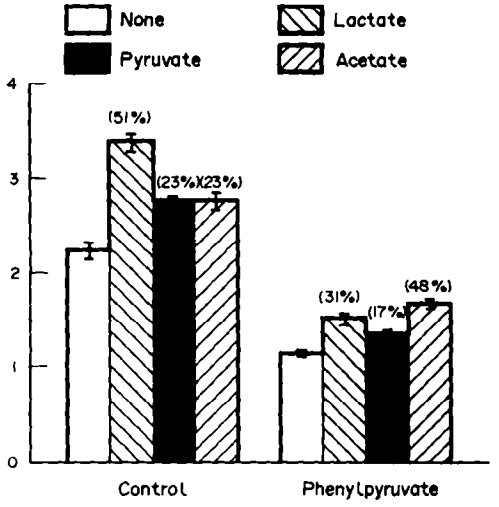

Fig. 3. Influence of phenylpyruvate (1 mM) on the metabolic substrate effects on fluorescein uptake. Conditions and abbreviations as in the legend to Fig. 1.

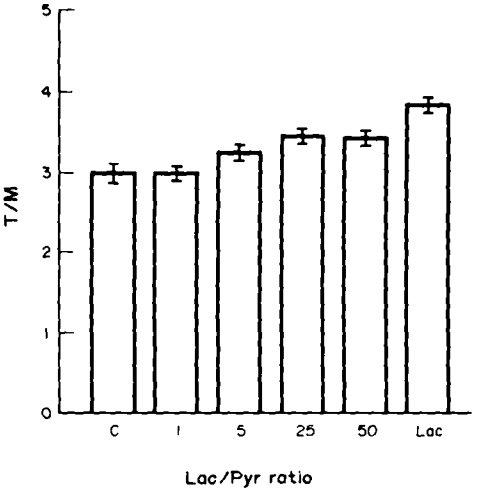

Fig. 5. Dependence of the fluorescein uptake on the lactate/pyruvate (Lac/Pyr) concentration ratio in the incubation medium. Lac/Pyr ratio was changed by varying the concentration of pyruvate at constant concentration of lactate (5 mM). Initial values of the ratio are depicted. C, the substrate-free medium; Lac, lactate (5 mM) only in the incubation medium. Other conditions and abbreviations as in the legend to Fig. 1.

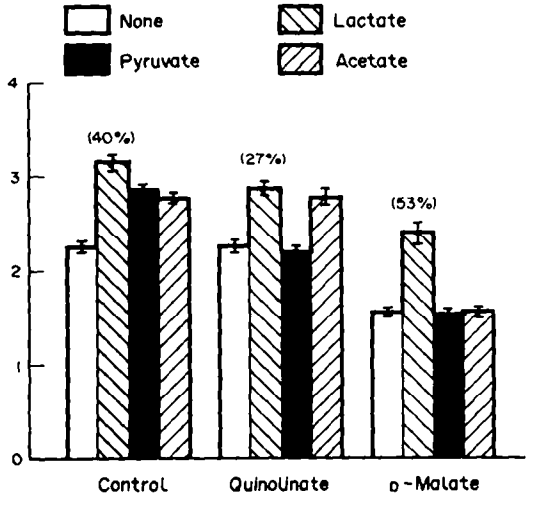

Fig. 4. Influence of quinolinate (2 mM) and D-malate (2 mM) on the metabolic substrate effects on fluorescein uptake. Conditions and abbreviations as in the legend to Fig. 1.

redox potential in the regulation of weak organic acid uptake in the RPTs, we investigated the dependence of fluorescein uptake on the ratio of lactate and pyruvate concentrations in the incubation medium. As seen in Fig. 5, when raising this ratio, the uptake increased. [It should be emphasized that similar data were obtained when the incubation lasted 2 min (data not shown).]

An electron donor, phenazine methosulfate (PMS) (20 μM), added to the incubation medium significantly diminished the fluorescein uptake (see below), but the relative stimulatory effect of lactate in the presence of this oxidizing agent was 2.5 times higher than in controls (data not shown). Preincubation of the slices with PMS for 60 min also resulted in both the inhibition of fluorescein uptake and marked augmentation of the effects of pyruvate, acetate and, especially, lactate (data not shown).

Endogenous substrates

As follows from Figs 2–4, CHC, phenylpyruvate and D-malate significantly inhibited fluorescein uptake in the absence of any exogenous substrates. Since the transport system for weak organic acids in RPTs plays a part in the secretion of a great variety of xenobiotics [reviews: 2, 28] the possibility cannot be excluded that the tested agents competed with fluorescein for a transport carrier. Thus, we investigated the kinetics of inhibition of fluorescein uptake by these substances. As seen in Fig. 6, the inhibitory effects of D-malate, phenylpyruvate and CHC on the dye uptake did not decrease significantly when the concentration of the dye in the incubation medium was raised, which suggested that the inhibition was not the result of competition.

Regarding the possible influence of the GNG inhibitors on cellular energy metabolism in the rat RPTs, some conclusions may be drawn from the data presented in Table 3. It follows from these data that the inhibitors being tested lowered neither the glucose production from endogenous substrates nor the ATP level in the renal cortex fragment suspension. Besides, we did not observe any correlation between their effects on fluorescein uptake in the RPTs and oxygen consumption in the suspension.

It was noteworthy that some inhibitors of GNG

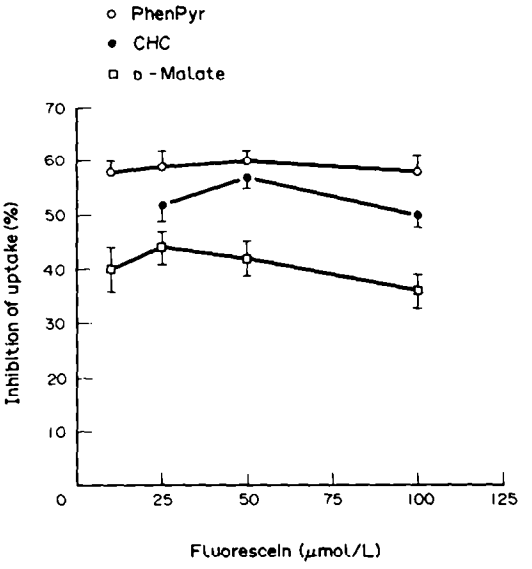

Fig. 6. Dependence of the inhibitory effects of phenylpyruvate (PhenPyr; 1 mM), CHC (0.1 mM) and D-malate (2 mM) on the fluorescein concentration in the incubation medium. Ordinate: percentage of inhibition of fluorescein uptake. The data are presented as means ± SE (N = 3). Other conditions as in the legend to Fig. 1.

did not inhibit the fluorescein uptake (Table 3): AOA, stimulated it, while quinolinate had no effect under these conditions. [Note that L-cycloserine (1 mM) stimulated the fluorescein uptake in the rat RPTs to the same extent as AOA.] The effects of the inhibitors tested on fluorescein uptake in the absence of exogenous substrates correlated with alterations in the cytoplasmic pyridine nucleotide redox potential, estimated as the ratio of lactate/pyruvate concentration in the tissue. In this context it was of interest that PMS prevented both the inhibitory effect of D-malate and the stimulatory effect of AOA on fluorescein uptake in the substrate-free medium (Fig. 7) The AOA effect was abolished and the effect of D-malate diminished in the presence of phenylpyruvate. In turn, D-malate completely blocked the stimulation of fluorescein uptake by AOA.

DISCUSSION

As follows from Results, there are certain differences in the effects of lactate and pyruvate on fluorescein uptake in rat RPTs. These differences can be associated with peculiarities in the metabolic pathways of these substrates. The transformations of lactate and pyruvate in rat RPT cells start in the mitochondria, and in both cases the substrate entering into the mitochondria is pyruvate. In the mitochondria pyruvate can be: (1) decarboxylated to acetyl-CoA and further oxidized through the TCA cycle, and/or (2) carboxylated by pyruvate carboxylase to oxaloacetate (OAA), which in turn enters into either the TCA cycle or GNG [reviews:

Table 3. Comparison of effects of the inhibitors tested on fluorescein uptake, oxygen consumption, glucose synthesis and metabolite levels

Inhibitor (mM)	Changes in		GNG rate	Content of			
	Fluorescein uptake	O_2 consumption (% control)	(nmol/mg protein/30 min)	ATP	Lactate (nmol/mg protein)	Pyruvate	Lac/Pyr
None	—	—	1.9 ± 0.1 (17)	6.4 ± 0.7 (19)	7.8 ± 0.1 (22)	0.31 ± 0.03 (23)	25
D-Malate (2)	−34 ± 2 (16)	−3 ± 4 (18)	2.1 ± 0.2 (5)	4.9 ± 1.0 (6)	6.6 ± 0.1 (9)	0.46 ± 0.07 (8)	14
CHC (0.1)	−56 ± 2 (5)	−20 ± 4 (8)	2.3 ± 0.3 (6)	8.4 ± 0.7 (9)	7.7 ± 0.1 (15)	0.64 ± 0.09 (13)	12
Phenylpyruvate (1)	−52 ± 2 (10)	−4 ± 3 (6)	2.3 ± 0.6 (3)	10.9 ± 1.5 (6)	10.1 ± 0.1 (15)	1.04 ± 0.10 (13)	10
Quinolinate (2)	+1 ± 3 (6)	−14 ± 4 (11)	1.9 ± 0.8 (3)	6.0 ± 1.8 (6)	9.1 ± 0.1 (10)	0.41 ± 0.09 (6)	22
AOA (1)	+22 ± 2 (12)	−18 ± 4 (9)	1.6 ± 0.5 (3)	ND	ND	ND	—
L-Cycloserine (1)	+22 (2)	ND	ND	6.1 ± 1.0 (6)	7.7 ± 0.1 (6)	0.29 ± 0.05 (6)	27

Cumulative data concerning the effects of inhibitors tested on fluorescein uptake in the rat renal cortex slices, and oxygen consumption, glucose synthesis and lactate, pyruvate and ATP levels in the cortex fragment suspension in the absence of exogenous substrates are presented.
In parenthesis: for fluorescein uptake, number of separate series; for oxygen consumption, GNG rate and metabolite levels, number of separate experiments.
ND, not determined.
The data are presented as means ± SE.

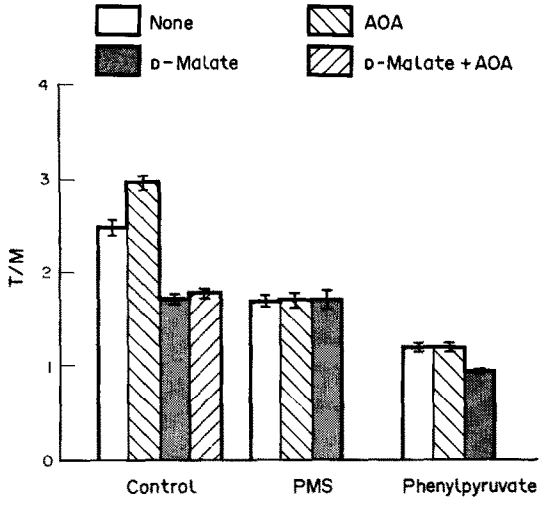

Fig. 7. Influence of PMS (0.02 mM) and phenylpyruvate (1 mM) on the effects of AOA (1 mM) and D-malate (2 mM) on fluorescein uptake in the absence of exogenous substrates. Conditions and abbreviations as in the legend to Fig. 1.

12, 13]. As to the oxidation, it should be emphasized that lactate and pyruvate stimulated oxygen consumption in the cortex fragment suspension to the same extent. Besides, the failure of fluoroacetate to suppress the effects of lactate and pyruvate suggested that the initial steps of the TCA cycle did not limit the energization of fluorescein uptake under the conditions employed in the present study. On the other hand, some acknowledged inhibitors of GNG affected the fluorescein uptake, which could not be explained by either competition with the dye for a transport mechanism or an influence on the cellular energy metabolism. These inhibitors modified to some extent the stimulatory effects of lactate and pyruvate on the uptake, but none of them (except for D-malate) diminished the effects of the non-gluconeogenic substrate, acetate.

The principal difference between lactate and pyruvate, as regards GNG from these precursors in rat RPTs, is in the means by which the cytosol is supplied with reducing equivalents: during GNG from lactate and pyruvate the reducing equivalents are generated by the LDH and malate dehydrogenase reactions, respectively [26, 29]. The consideration that the cytoplasmic redox potential is regulated by a couple of these two near-equilibrium reactions, as introduced for rat hepatocytes [30], seems to be fruitful for interpreting the results obtained in the present work. In such a case, the addition of lactate to the medium would result in a reductive shift in the cytoplasmic pyridine nucleotide redox potential [31]. The enhanced (as compared to pyruvate) effect of exogenous lactate on fluorescein uptake would follow from the reduction of cytoplasmic pyridine nucleotides. The data showing that fluorescein uptake increases when the lactate/pyruvate ratio in the incubation medium is raised support this assumption, as do data concerning the influence of PMS on the stimulatory effect of lactate on the uptake. Thus, it may be proposed that the uptake of weak organic acids in the rat RPTs is regulated by the cytoplasmic pyridine nucleotide redox potential. This hypothesis, in our opinion, can explain how inhibitors of GNG affect fluorescein uptake. The rate of GNG is dependent on both the cytoplasmic redox potential and the flow of C_4 acids from mitochondria, with the former having a biphasic effect on the rate of glucose production from lactate and pyruvate [32]. This may be an explanation for some quantitative discrepancies in the effects of the inhibitors tested on fluorescein uptake and GNG rates.

It is of great interest that the inhibitors of GNG affect fluorescein uptake in the absence as well as the presence of exogenous substrates. Fatty acids, the principal endogenous substrates of oxidation in the RPTs [8, 33], cannot serve as glucose precursors in vertebrates [34]. The process which could be sensitive to some GNG inhibitors is glycolysis, but the glycogen content of the rat RPT cells is too low to energize cellular functions *in vitro* for a prolonged period [reviews: 12, 13]. Besides, glucose synthesis *de novo* is initiated by endogenous precursors, which shows that the cytoplasmic redox potential in the rat RPT cells does not favor the glyceraldehyde-3-phosphate dehydrogenase [D-glyceraldehyde 3-phosphate: NAD^+ oxidoreductase (phosphorylating); EC 1.2.1.12] reaction to flow in the "glycolytic" direction even though exogenous substrates are omitted.

The inhibition of fluorescein uptake by PMS, as well as the correlation of its inhibition by the GNG inhibitors with the intracellular lactate/pyruvate ratio, suggests that in the absence of exogenous substrates the uptake is also regulated by the cytoplasmic redox potential. If so, the inhibitory action of D-malate on the uptake proves that reducing equivalents in the cytosol are generated under these conditions by the malate dehydrogenase reaction.

None of the GNG inhibitors tested influenced the rate of glucose production from endogenous substrates. At the same time, both the inhibitor of mitochondrial pyruvate transporter, CHC, and that of pyruvate carboxylase, phenylpyruvate, markedly inhibited fluorescein uptake in the absence of exogenous lactate or pyruvate. The effects of the GNG inhibitors tested on fluorescein uptake in the substrate-free incubation medium were to some extent similar to their effects on the rate of glucose production from pyruvate. Thus, it may be concluded that endogenous pyruvate is needed for reducing equivalents to be generated in the RPT cells in the absence of exogenous substrates. This implies that the maintenance of the cytoplasmic redox potential in the rat RPTs, just as in the rat hepatocytes [25], is not tightly coupled to the GNG rate, but seems to be related to certain "basal" processes exporting reducing equivalents from mitochondria.

The rate of malate exchange between the mitochondrial matrix and the cytosol is far higher than that of glucose production in the rat RPTs [35], which suggests the operation of a "futile" cycle dealing with the transfer of reducing equivalents. The existence of at least three "futile" cycles in rat

RPTs with re-circulation of pyruvate and malate through the mitochondrial membrane is postulated. They are: (1) pyruvate–OAA–malate–OAA–phospho*enol*pyruvate–pyruvate with the participation of pyruvate kinase (ATP: pyruvate 2-*O*-phosphotransferase; EC 2.7.1.40) [36]; (2) pyruvate–OAA–malate–pyruvate with the participation of "malic" enzyme [D-malate; $NADP^+$ oxidoreductase (OAA-decarboxylating); EC 1.1.1.40] [37] and (3) pyruvate–OAA–malate–OAA–pyruvate with the participation of OAA decarboxylase (EC 4.1.1.3) [38]. These cycles very probably operate regardless of the presence of exogenous substrates. It may well be that with changing circumstances different "futile" cycles come into operation. In the absence of exogenous substrates the cycle with the participation of OAA decarboxylase is more probable, since under these conditions fluorescein uptake is inhibited by D-malate, but is not affected by quinolinate. The stimulatory effect of acetate on the uptake is somehow related to the operation of such a cycle, as revealed by the fact that it is prevented by D-malate. In the presence of quinolinate, the effect of pyruvate on fluorescein uptake is completely blocked, and hence the cycle with the participation of phospho*enol*pyruvate carboxykinase may be involved here.

The data obtained so far cannot be interpreted with regard to the "exchange model" described in the Introduction. However, it is evident that the mechanisms regulating weak organic acid uptake in the RPTs are rather complicated, and the problem of coupling the uptake to cellular metabolism demands much further research.

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