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# Specificity of the $Na^+$-Dependent Monocarboxylic Acid Transport Pathway in Rabbit Renal Brush Border Membranes

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**Summary.** The substrate specificity of a $Na^+$-dependent transport pathway for L-lactate was studied in rabbit renal brush border membrane vesicles. $J_{max}$ for L-lactate transport was unaffected by the presence of a fixed concentration of two different short-chain monocarboxylic acids, while the apparent $K_t(K_a)$ for L-lactate increased, and this is compatible with competitive inhibition. The inhibitor constants ("$K_i$"'s) for the transport pathway for the two solutes examined closely corresponded to the respective "$K_i$"'s derived from a Dixon plot. A broad range of compounds were then tested as potential inhibitors of L-lactate transport, and the "$K_i$"'s thereby derived yielded specific information regarding optimal substrate recognition by the carrier. A single carboxyl group is an absolute requirement for recognition, and preference is given to 3 to 6 C chain molecules. Addition of ketone, hydroxyl and, particularly, amine groups at any carbon position, diminishes substrate-carrier interaction. Intramolecular forces, notably the inductive effects of halogens, may play a role in enhancing substrate-carrier interaction; however, no correlation was found between $pK_a$ and "$K_i$" for the substrates examined. We conclude that a separate monocarboxylic acid transport pathway, discrete from either the D-glucose, $\alpha$ or $\beta$ neutral amino-acid, or dicarboxylic acid carriers, exists in the renal brush border, and this handles a broad range of monocarboxylates.

**Key Words** monocarboxylate carrier · lactate · renal brush border membranes · $Na^+$-cotransport

## Introduction

Recent studies indicate the existence of a $Na^+$-dependent monocarboxylic acid transport pathway in the luminal membrane of rabbit [4, 8, 9] and rat [1, 3] renal cortex. This carrier is distinct from those for sugars, amino acids and dicarboxylic acids, but there is evidence to suggest that pyruvate and ketone bodies share this pathway. The purpose of the present study was to characterize the structural requirements for optimal substrate recognition by the monocarboxylate carrier. The results indicate that the carrier interacts with a wide range of monocarboxylic acids, and preference for recognition is determined by carbon chain length, and the nature and position of substituted moieties.

## Materials and Methods

Purified rabbit renal cortical brush border membrane vesicles were prepared by a previously described $Ca^{++}$-aggregation method [14]. The specific activity in the final pellet of the luminal membrane marker, trehalase, was consistently enriched 10-fold over that of the initial tissue homogenate, and the preparation was essentially free of the basolateral marker $Na^+$, $K^+$-ATPase, and the mitochondrial marker succinate dehydrogenase. The membranes were suspended in a medium consisting of 410 mM sorbitol, 50 mM KCl, and 1 mM HEPES[1]-Tris[2] (H–T) buffered to pH 7.5, and then stored in 350 µl aliquots at −190° C for up to 5 days [12]. Protein concentration (19 to 25 mg/ml) was determined by the BioRad protein assay (BioRad Laboratories).

One second uptakes of $^{14}C$-labeled L-lactate were measured at 22 °C by a previously reported technique [8]. The final composition of the incubation buffer was 100 mM NaCl (or 100 mM ChCl), 50 mM KCl, $^{14}C$ L-lactate (20 µM), 40 mM Tris buffered to pH 7.5 with HEPES, and sufficient sorbitol to produce a final osmolarity of 511 mOsm (iso-osmotic with the intravesicular buffer). The electrical potential difference (PD) and pH gradient across the brush border membrane were eliminated by the addition of the potassium ionophore, valinomycin (25 µg/ml), and the proton ionophore, FCCP[3] (75 µM) to both the incubation media and vesicle suspension [15].

$^{14}C$-labeled L-lactate was obtained from New England Nuclear (Boston, Mass.). Valinomycin was supplied by Sigma Chemical Company (St. Louis, Missouri) and FCCP by Pierce Chemical Company (Rockford, Illinois). All other chemicals used were of the highest commercial grade available.

## Results

### *Kinetics of* L-*Lactate Transport*

Previous studies by this laboratory have demonstrated that L-lactate transport across the renal brush border is predominantly $Na^+$-dependent [4, 8]. Preliminary experiments showed that over the concentration range of L-lactate used (0.02

[1] HEPES: N-2 hydroxyethylpiperazine-N′-2-ethanesulfonic acid.
[2] Tris: Tris(hydroxymethyl) aminomethane.
[3] FCCP: *p*-trifluormethoxyphenylhydrazone.

**Table 1.** Kinetics of L-lactate transport in the absence and presence of propionate and 3-bromopropionate

| Inhibitor (mM) | L-lactate | | | Inhibitor |
|---|---|---|---|---|
| | $J_{max}$ (nmol/mg min) | $K_t$ (mM) | "$K_a$" (mM) | "$K_i$" (mM) |
| None | 32.0±3.0 | 0.42±0.08 | – | – |
| propionate (0.25) | 36.0±3.0 | – | 1.0±0.1 | 0.18 |
| 3-bromopropionate (1.0) | 26.0±4.4 | – | 2.0±0.4 | 0.27 |

$^{14}$C L-lactate uptake (20 μM) was measured at 1-s time points in the absence and presence of propionate (0.25 mM) and 3-bromopropionate (1.0 mM). For calculation of "$K_a$" and "$K_i$" values *see* text. Intra- and extravesicular media as described in Fig. 1. Kinetic parameters (±SD) were derived from a single representative experiment, using a computer and an iterative nonlinear regression program.

to 10.0 mM) the $Na^+$-independent component of transport accounted for less than 13% of total uptake. Kinetic parameters determined for total solute flux were: maximal influx ($J_{max}$) 50±1 nmol/mg min, and Michaelis constant ($K_t$) 0.48±0.03 mM [8]. Since the $Na^+$-independent component of L-lactate uptake contributes little to total solute flux, L-lactate uptake by the brush border may be considered to be essentially via a single, $Na^+$-dependent pathway[4].

*Kinetics of Inhibition*

The kinetics of L-lactate transport were also determined in the presence of a fixed concentration of either propionate or 3-bromopropionate. The results from such an experiment are shown in Table 1. 0.25 mM propionate increased the apparent Michaelis constant for L-lactate transport from 0.42±0.08 mM ($K_t$) to 1.0±0.1 mM ($K_a$). Under these conditions the $J_{max}$ was unchanged: 32.0±3.0 nmol/mg min in the absence of propionate vs. 36.0±3.0 nmol/mg min in the presence of 0.25 mM propionate. In a parallel experiment with 1.0 mM 3-bromopropionate, $J_{max}$ was 26.0±4.4 nmol/mg min and the $K_a$ was 2.0±0.4 mM. The simplest explanation for these observations is competitive inhibition between propionate, or 3-bromopropionate, and L-lactate, for a common transport pathway.

The inhibitor constants ("$K_i$"'s) for propionate and 3-bromopropionate as inhibitors of L-lactate transport were calculated from the $K_t$ for L-lactate and the respective $K_a$'s for L-lactate in the presence of propionate or 3-bromopropionate of concentrations ($i$), according to [7]:

$$K_i = \frac{K_t(i)}{K_a - K_t}.$$

In the experiments described, the "$K_i$" for propionate was 0.18 mM and "$K_i$" for 3-bromopropionate, 0.27 mM (Table 1).

An alternate method for deriving inhibitory constants employs the use of a fixed low (20 μM) concentration of substrate, while the concentration of potential inhibitor is varied. Figure 1 *A* presents the results of an experiment showing the inhibitory effects of increasing concentrations of two monocarboxylic acids and L-lactate on the uptake of 20 μM $^{14}$C L-lactate. A reciprocal transformation of the Michaelis-Menten equation (Dixon plot) was used to estimate the concentration of inhibitor producing 50% inhibition of L-lactate transport (Fig. 1 *B*). Since 20 μM L-lactate is much lower than the $K_t$ for L-lactate transport, this concentration of inhibitor is taken as an estimate of the "$K_i$" for that inhibitor [7]. "$K_i$"'s for the two monocarboxylates presented in Fig. 1 were: 3-bromopropionate 0.26 mM and 4-bromobutyrate 22.5 mM; the value for L-lactate was 0.58 mM. Recall that the calculated "$K_i$" value for 3-bromopropionate was 0.27 mM (Table 1). The results of these two experimental protocols both suggest a single site of interaction between these monocarboxylic acids.

## Structural Specificity of the Transport System

*Length of the Carbon Chain.* The structural specificity of the transport pathway was examined using several groups of compounds as inhibitors, and "$K_i$"'s were derived from the Dixon plot according to the experimental protocol outlined above. Each group of compounds provided specific information regarding required elements for recognition by the carrier's receptor site. The "$K_i$" of each compound for the transport system was derived from a single experiment. Four to eight different compounds were examined in each of 12 experiments, and, in addition, L-lactate was included in each experiment as an internal control. Two to three experiments were performed on successive days using vesicles which had been prepared from the same group of animals and then stored at −190 °C. The mean "$K_i$" for L-lactate for 12 experiments was 0.60±0.04 mM; "$K_i$"'s determined

[4] This $Na^+$-dependent monocarboxylate carrier is quite distinct from the $Na^+$-independent, pH-driven anion transporter described for dog brush border membranes (S.E. Guggino, G.J. Martin, and P.S. Aronson, 1982. *Kidney Int.* **21**:274).

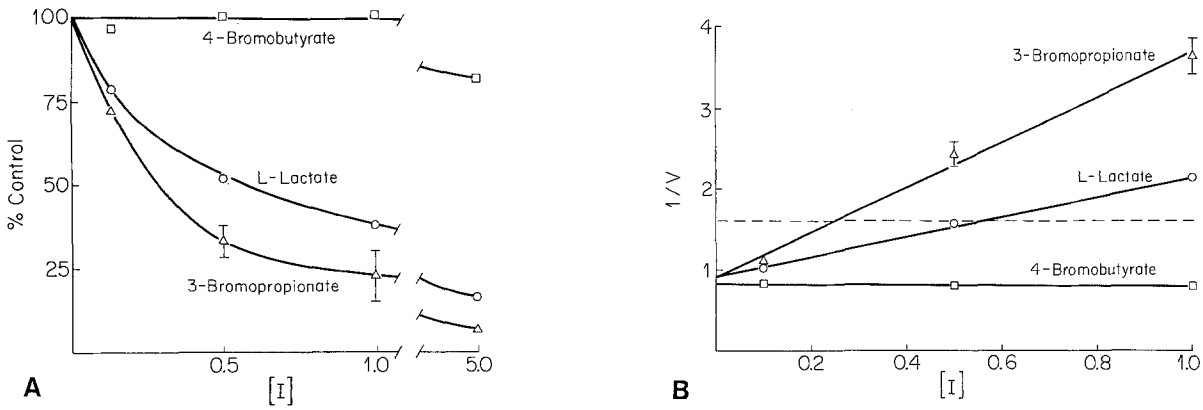


**Fig. 1.** *A.* L-lactate uptake measured as a function of inhibitor concentration. Brush border membrane vesicles were pre-equilibrated with 410 mM sorbitol, 50 mM KCl, 1 mM H-T buffer at pH 7.5 (osmolarity 511 mOsm), valinomycin 25 μg/ml, and 75 μM FCCP. Incubation buffer contained 100 mM NaCl, 50 mM KCl, 40 mM Tris, sufficient HEPES to attain pH 7.5, 20 μM $^{14}$C L-lactate, 0.1 to 5.0 mM of inhibitor or L-lactate where indicated, valinomycin 25 μg/ml, and 75 μM FCCP. Sorbitol was added to attain a final osmolarity of 511 mOsm. The reaction was stopped after 1 s incubation with ice-cold sorbitol (510 mOsm) at pH 7.5 (1 mM H-T buffer). Units of the ordinate are percent of control; the abscissa mM. Control flux = 1.23 ± 0.04 nmol/mg min. *B.* Dixon plot ($1/V$ vs. $I$) of data shown in *A*. Lines were fitted to all data points using a least-squares analysis, but the points above 1 mM were omitted from the graph. The "$K_i$" for L-lactate was 0.58 mM and the regression coefficient ($r$) was 0.99, the "$K_i$" for 3-bromopropionate was 0.27 mM, $r = 0.99$, and the "$K_i$" for 4-bromobutyrate was 22.5 mM, $r = 0.96$

**Table 2.** Inhibitor constants for a homologous series of monocarboxylic acid

| Number of carbon atoms | Compound | Mol wt | Structure | $pK_a$[a] | "$K_i$"[b] (mM) | $r$ |
|---|---|---|---|---|---|---|
| 1 | formic acid | 46.0 | $HCOO^-$ | 3.75 | >60 | 0.78 |
| 2 | acetic acid | 60.1 | $CH_3COO^-$ | 4.76 | 1.0 | 0.99 |
| 3 | *n*-propionic acid | 74.1 | $CH_3CH_2COO^-$ | 4.88 | 0.29 | 0.99 |
| 4 | *n*-butyric acid | 88.1 | $CH_3(CH_2)_2COO^-$ | 4.82 | 0.16 | 0.99 |
| 4 | isobutyric acid | 88.1 | $CH_3$ $CH_3$ CH $COO^-$ | 4.86 | 0.33 | 0.99 |
| 5 | *n*-valeric acid | 102.1 | $CH_3(CH_2)_3COO^-$ | 4.86 | 0.21 | 0.99 |
| 6 | *n*-caproic acid | 116.2 | $CH_3(CH_2)_4COO^-$ | 4.88 | 0.37 | 0.99 |
| 7 | *n*-heptanoic acid | 130.2 | $CH_3(CH_2)_5COO^-$ | 4.89 | 0.97 | 0.99 |

[a] At 25 °C [5, 10].

[b] Inhibitor constants ("$K_i$"'s) in this and all subsequent Tables were obtained from Dixon plots (Fig. 1 *B*) with inhibitor concentrations of 0.1 to 10.0 mM.

on day 1 or day 5 of storage were similar. This "$K_i$" for L-lactate transport is similar to the $K_t$ for L-lactate (0.48 ± 0.03 mM) previously reported [8].

Table 2 presents inhibitor constants of a homologous series of straight-chain monocarboxylic acids. (Compounds with eight or more carbon atoms were not examined as they are poorly soluble in water over the concentration range used.) The initial substrate in this series, formic acid, (1 C compound) had a calculated "$K_i$" of >60 mM. Increasing the carbon chain length from 1 to 2 atoms produced a marked decrease in "$K_i$" from >60 mM to 1.0 mM. The "$K_i$"'s reached a minimum value of 0.16 to 0.37 mM with 3 to 6 C compounds, and appeared to increase again (0.97 mM) as carbon chain length increased to 7. From this homologous series of compounds it thus appears that 3 to 6 C chain substrates are optimal for interaction with the carrier site.

Isobutyric acid, the branched-chain derivative of the 4 C compound butyric acid, was examined. The "$K_i$" for the former analog was 0.33 mM vs. "$K_i$" 0.16 mM for butyric acid. Thus when compared to its parent compound the presence of a branched carbon chain approximately doubles the "$K_i$" of the substrate for the transport system.

*Requirement for a Carboxyl Group.* Absolute requirement for a carboxyl group is evident from the "$K_i$"'s of the 3 carbon compounds examined and presented in Table 3. Replacement of the carboxyl group by either a hydroxyl group (propanol) an amino group (1-amino-2 propanol), an amide

**Table 3.** Inhibitor constants for 3-carbon compounds where the carboxyl group has been substituted

| Compound | Mol wt | Structure | "$K_i$" (mM) | $r$ |
|---|---|---|---|---|
| *n*-propionic acid | 74.1 | $CH_3CH_2COO^-$ | 0.29 | 0.99 |
| propanol | 60.1 | $CH_3(CH_2)_2OH$ | 49 | 0.53 |
| 1,2 propanediol | 76.1 | $CH_3CH(OH)CH_2OH$ | >60 | 0.99 |
| 1,3 propanediol | 76.1 | $HOCH_2(CH_2)_2OH$ | >60 | 0.54 |
| 3-amino-1-propanol | 75.1 | $H_2NCH_2(CH_2)_2OH$ | 31 | 0.84 |
| 1-amino-2-propanol | 75.1 | $CH_3CH(OH)CH_2NH_2$ | >60 | 0.99 |
| propionamide | 73.1 | $CH_3CH_2CONH_2$ | 23 | 0.96 |
| methyl acetate | 74.1 | $CH_3CO_2CH_3$ | >60 | 0.87 |
| ethanesulfonic acid | 110.1 | $CH_3CH_2SOOO^-$ | >60 | 0.99 |

group (propionamide), or an ester (methyl acetate), increased the "$K_i$" by at least two orders of magnitude. The presence of a hydroxyl group at the $\beta$- or $\alpha$-carbon position, or an amino group at the $\alpha$-carbon position of the propanol molecule, did not alter the poor inhibitory effect of the compound ("$K_i$" 31 to >60). Furthermore, interaction with the carrier site demands the presence of a $COO^-$ group, since substitution by a $SOOO^-$ group (ethanesulfonic acid) results in an increase in "$K_i$" from 0.29 mM to >60 mM.

*Effect of Amino Group Substitution.* As indicated in Table 4 the presence of a $NH_2$ moiety at all positions tested, i.e. $\alpha$-, $\beta$-, $\gamma$- or $\delta$-carbon, markedly increased the "$K_i$" for the substrate examined, when compared to its respective parent compound; e.g. "$K_i$" for L-alanine was >60 mM vs. 0.29 for propionate.

**Table 4.** Effect of amino group ($NH_2$) substitution on inhibition of L-lactate transport

| Number of carbon atoms | $NH_2$ at C | Compound | Mol wt | Structure | $pK_a$ | "$K_i$" (mM) | $r$ |
|---|---|---|---|---|---|---|---|
| 2 | * | acetic acid | 60.1 | $CH_3COO^-$ | 4.76 | 1.4 | 0.99 |
| | $\alpha$ | L-glycine | 75.1 | $H_2NCH_2COO^-$ | 2.35 | >60 | 0.43 |
| 3 | * | *n*-propionic acid | 74.1 | $CH_3CH_2COO^-$ | 4.88 | 0.29 | 0.99 |
| | $\alpha$ | L-alanine | 89.1 | $CH_3CH(NH_2)COO^-$ | 2.34 | >60 | 0.99 |
| | $\beta$ | $\beta$-alanine | 89.1 | $H_2NCH_2CH_2COO^-$ | 3.55 | 58 | 0.46 |
| 4 | * | *n*-butyric acid | 88.1 | $CH_3(CH_2)_2COO^-$ | 4.82 | 0.16 | 0.99 |
| | $\alpha$ | DL-$\alpha$-aminobutyric acid | 103.1 | $CH_3CH_2CH(NH_2)COO^-$ | 2.29 | 27 | 0.79 |
| | $\beta$ | DL-$\beta$-aminobutyric acid | 103.1 | $CH_3CH(NH_2)CH_2COO^-$ | –[a] | >60 | 0.58 |
| | $\gamma$ | $\gamma$-aminobutyric acid | 103.1 | $H_2NCH_2(CH_2)_2COO^-$ | 4.03 | >60 | 0.73 |
| 5 | * | *n*-valeric acid | 102.1 | $CH_3(CH_2)_3COO^-$ | 4.86 | 0.21 | 0.99 |
| | $\alpha$ | DL-norvaline | 117.2 | $CH_3(CH_2)_2CH(NH_2)COO^-$ | 2.32 | 22 | 0.87 |
| | $\delta$ | $\delta$-aminovaleric acid | 117.2 | $H_2NCH_2(CH_2)_3COO^-$ | 4.27 | >60 | 0.51 |

* Parent compound. [a] Value unavailable.

**Table 5.** Effect of ketone group (–CO) substitution on inhibition of L-lactate transport

| Number of carbon atoms | –CO at C | Compound | Mol wt | Structure | $pK_a$ | "$K_i$" (mM) | $r$ |
|---|---|---|---|---|---|---|---|
| 2 | * | acetic acid | 60.1 | $CH_3COO^-$ | 4.76 | 1.4 | 0.99 |
| | $\alpha$ | glyoxylic acid | 74.0 | $HCOCOO^-$ | –[a] | 47.2 | 0.86 |
| 3 | * | *n*-propionic acid | 74.1 | $CH_3CH_2COO^-$ | 4.88 | 0.29 | 0.99 |
| | $\alpha$ | pyruvic acid | 88.1 | $CH_3COCOO^-$ | 2.49 | 0.65 | 0.99 |
| 4 | * | *n*-butyric acid | 88.1 | $CH_3(CH_2)_2COO^-$ | 4.82 | 0.16 | 0.99 |
| | $\alpha$ | $\alpha$-ketobutyric acid | 102.1 | $CH_3CH_2COCOO^-$ | –[a] | 0.61 | 0.99 |
| | $\beta$ | acetoacetic acid | 108.0 | $CH_3COCH_2COO^-$ | 3.59[b] | 1.0 | 0.99 |
| 5 | * | *n*-valeric acid | 102.1 | $CH_3(CH_2)_3COO^-$ | 4.86 | 0.21 | 0.99 |
| | $\alpha$ | $\alpha$-ketovaleric acid | 131.8 | $CH_3(CH_2)_2COCOO^-$ | –[a] | 0.19 | 0.99 |
| | $\gamma$ | levulinic acid | 116.1 | $CH_3CO(CH_2)_2COO^-$ | 4.64[b] | 1.5 | 0.90 |

* Parent compound. [a] Value unavailable. [b] At 18 °C.

*Effect of Ketone or Hydroxyl Group Substitution.* In contrast to amino groups, ketone substitution (Table 5) at the α-C position does not interfere markedly with substrate-carrier interaction; e.g. "$K_i$" for pyruvate 0.65 mM vs. "$K_i$" for propionate 0.29 mM. Substitution of a ketone group at the β- or γ-C position increased the "$K_i$" for transport of that compound when compared to α-C ketone substitution; e.g. 4 C: "$K_i$" for acetoacetic acid 1.0 mM vs. "$K_i$" for α-ketobutyric acid 0.61 mM.

α-C hydroxyl substitution (Table 6) results in "$K_i$" values very similar to those derived for ketones: "$K_i$" for L-lactate (−OH) 0.60 mM vs. "$K_i$" for pyruvate (−CO) 0.65 mM. Comparable with the observed effects of ketone group substitution, β- or γ-C hydroxylation increased the "$K_i$" for that compound by at least eightfold; e.g. "$K_i$" for L-lactate 0.60 mM vs. "$K_i$" for β-OH propionate 11.5 mM. Taken together these results demonstrate that: a) 3 to 5 C analog with either a ketone or a hydroxyl group at the α-C position interact in an almost identical manner with the carrier site, b) ketone or hydroxyl group substitution at subsequent carbon positions decrease substrate-carrier interaction, and c) a ketone or hydroxyl moiety at the α-C position increases the "$K_i$" for that substrate for the transport pathway by two- to threefold (e.g. "$K_i$" for propionate 0.29 mM vs. "$K_i$" for L-lactate 0.60 mM or "$K_i$" for pyruvate 0.65 mM).

Three additional observations regarding the transport of ketone and hydroxyl analogs should be noted. First, the presence of both a ketone and hydroxyl moiety at adjacent carbons of the same compound markedly increases the "$K_i$" for transport for that substrate: "$K_i$" for pyruvate ($CH_3COCOO^-$) 0.65 mM vs. "$K_i$" for β-hydroxy pyruvate ($CH_2OHCOCOO^-$) 29 mM. Second, 2 C compounds, i.e., glyoxylic acid (−CO) and glycolic acid (−OH) are both poorly recognized by the carrier, their respective "$K_i$'s" being 47 and 8.4 mM. Third, in comparing the L- and D-isomers of lactic, the "$K_i$" values are little different ("$K_i$" for L-lactate 0.60 mM vs. $K_i$ for D-lactate 0.96 mM), suggesting that stereospecificity at the α-C position is not an important determinant of substrate-carrier interaction.

*Effect of Halogens.* Substrates incorporating a bromide (Br) chloride (Cl) or iodide (I) moiety at the various C positions were tested, and the results are presented in Table 7. Compounds containing Br appear to be given preference for binding over either Cl or I; e.g. 2-bromopropionate, 2-chloropropionate and 2-iodopropionate had "$K_i$"'s of 0.15, 0.42 and 0.76 mM, respectively. There appears to be little difference in terms of substrate recognition whether the halogen is at the α- or β-C position; e.g. Br: 2-bromopropionic acid "$K_i$" 0.15 mM vs. 3-bromopropionic acid "$K_i$" 0.26 mM, or Cl: 2-chloropropionic acid "$K_i$" 0.42 mM vs. 3-chloropropionic acid "$K_i$" 0.33 mM. γ- or δ-C halogen substitution appears to have a destabilizing effect on substrate-carrier interaction; e.g. 4 C compounds: 2-bromobutyric acid "$K_i$" 0.78 mM vs. 4-bromobutyric acid "$K_i$" 22.5 mM.

In considering the 2C compounds, the unsubstituted acid (acetic acid) as well as its ketone (glyoxylic acid) and hydroxyl (glycolic acid) deriv-

**Table 6.** Effect of hydroxyl group (−OH) substitution on inhibition of L-lactate transport

| Number of carbon atoms | −OH at C | Compound | Mol wt | Structure | $pK_a$ | "$K_i$" (mM) | $r$ |
|---|---|---|---|---|---|---|---|
| 2 | * | acetic acid | 60.1 | $CH_3COO^-$ | 4.76 | 1.4 | 0.99 |
| | α | glycolic acid | 76.1 | $HOCH_2COO^-$ | 3.83 | 8.4 | 0.97 |
| 3 | * | *n*-propionic acid | 74.1 | $CH_3CH_2COO^-$ | 4.88 | 0.29 | 0.99 |
| | α | L-lactic acid | 90.1 | $CH_3CH(OH)COO^-$ | 3.86 | 0.60 | 0.92–0.99 |
| | α | D-lactic acid | 90.1 | $CH_3CH(OH)COO^-$ | | 0.96 | 0.99 |
| | β | β-hydroxypropionic acid | 90.1 | $HOCH_2CH_2COO^-$ | 4.51 | 11.5 | 0.99 |
| | β | β-hydroxypyruvic acid[b] | 103.1 | $HOCH_2COCOO^-$ | –[a] | 29 | 0.99 |
| 4 | * | *n*-butyric acid | 88.1 | $CH_3(CH_2)_2COO^-$ | 4.82 | 0.16 | 0.99 |
| | α | DL-α-hydroxybutyric acid | 104.1 | $CH_3CH_2CH(OH)COO^-$ | –[a] | 0.61 | 0.99 |
| | β | β-hydroxybutyric acid | 104.1 | $CH_3CH(OH)CH_2COO^-$ | 4.70 | 4.5 | 0.99 |
| | γ | γ-hydroxybutyric acid | 104.1 | $HOCH_2(CH_2)_2COO^-$ | 4.72 | 19 | 0.99 |
| 5 | * | *n*-valeric acid | 102.1 | $CH_3(CH_2)_3COO^-$ | 4.86 | 0.21 | 0.99 |
| | α | DL-α-hydroxyvaleric acid | 158.1 | $CH_3(CH_2)_2CH(OH)COO^-$ | –[a] | 0.98 | 0.99 |

* Parent compound. [a] Value unavailable. [b] In addition to an −OH at the β-C there is a −CO at the α−C.

**Table 7.** "$K_i$"'s of halogenated compounds on L-lactate transport

| Number of carbon atoms | Halogen at C | Compound | Mol wt | Structure | $pK_a$ | "$K_i$" (mM) | $r$ |
|---|---|---|---|---|---|---|---|
| 2 | * | acetic acid | 60.1 | $CH_3COO^-$ | 4.76 | 1.4 | 0.99 |
| | α | 2-bromoacetic acid | 139.0 | $BrCH_2COO^-$ | 2.83[b] | 0.25 | 0.99 |
| | α | 2-chloroacetic acid | 94.5 | $ClCH_2COO^-$ | 2.89[b] | 0.54 | 0.99 |
| | α | 2-iodoacetic acid | 186.0 | $ICH_2COO^-$ | 3.15[b] | 0.29 | 0.99 |
| 3 | * | *n*-propionic acid | 74.1 | $CH_3CH_2COO^-$ | 4.88 | 0.29 | 0.99 |
| | α | 2-bromopropionic acid | 153.0 | $CH_3CH(Br)COO^-$ | 2.97[b] | 0.15 | 0.99 |
| | β | 3-bromopropionic acid | 153.0 | $BrCH_2CH_2COO^-$ | 3.99[b] | 0.26 | 0.99 |
| | α | 2-chloropropionic acid | 108.5 | $CH_3CH(Cl)COO^-$ | 2.88[b] | 0.42 | 0.99 |
| | β | 3-chloropropionic acid | 108.5 | $ClCH_2CH_2COO^-$ | 4.10[b] | 0.33 | 0.99 |
| | β | 3-iodopropionic acid | 200.0 | $ICH_2CH_2COO^-$ | 4.07[b] | 0.76 | 0.99 |
| 4 | * | *n*-butyric acid | 88.1 | $CH_3(CH_2)_2COO^-$ | 4.82 | 0.16 | 0.99 |
| | α | 2-bromobutyric acid | 167.0 | $CH_3CH_2CH(Br)COO^-$ | —[a] | 0.78 | 0.99 |
| | γ | 4-bromobutyric acid | 167.0 | $BrCH_2(CH_2)_2COO^-$ | —[a] | 22 | 0.96 |
| | γ | 4-chlorobutyric acid | 122.6 | $ClCH_2(CH_2)_2COO^-$ | 4.52[c] | 14 | 0.79 |
| 5 | * | *n*-valeric acid | 102.1 | $CH_3(CH_2)_3COO^-$ | 4.86 | 0.21 | 0.99 |
| | α | 2-bromovaleric acid | 181.0 | $CH_3(CH_2)_2CH(Br)COO^-$ | —[a] | 0.91 | 0.99 |
| | δ | 5-bromovaleric acid | 181.0 | $BrCH_2(CH_2)_3COO^-$ | —[a] | 18 | 0.99 |
| | δ | 5-chlorovaleric acid | 136.6 | $ClCH_2(CH_2)_3COO^-$ | —[a] | 0.65 | 0.99 |

* Parent compound. [a] Value unavailable. [b] At 18 °C. [c] At room temperature.

**Table 8.** Inhibition of L-lactate transport by other "model" $Na^+$-cotransported substrates

| Compound | Mol wt | Structure | $pK_a$ | "$K_i$" (mM) | $r$ (mM) |
|---|---|---|---|---|---|
| Succinic acid | 118.1 | $H_2C-COO-$<br>$-OOC-CH_2$ | 4.21<br>5.64 | 20.5 | 0.66 |
| D-glucose | 180.2 | CHO<br>HCOH<br>HOCH<br>HCOH<br>HCOH<br>$CH_2OH$ | — | >60 | 0.95 |
| β-alanine | 89.1 | $H_2NCH_2CH_2COO^-$ | 3.55 | 58 | 0.46 |
| L-alanine | 89.1 | $CH_3CH(NH)_2COO^-$ | 2.34 | >60 | 0.99 |

atives, all interact poorly with the carrier site as demonstrated by relatively high "$K_i$"'s of 1.4, 47 and 8.4 mM, respectively (*see* Tables 2, 5 and 6). In contrast the Br, Cl and I compound all have "$K_i$" values even lower than that of the parent compound (2-bromoacetic acid "$K_i$" 0.25 mM, 2-chloroacetic acid "$K_i$" 0.54, and 2-iodoacetic acid 0.29 mM vs. acetic acid "$K_i$" 1.4 mM).

*Interaction with Other "Model" $Na^+$-Cotransported Substrates.* Succinate [14], glucose [11], L-alanine and β-alanine [6], all extensively studied "model" $Na^+$-cotransported substrates, were tested as potential inhibitors of L-lactate transport. "$K_i$"'s for these compounds are summarized in Table 8, and range from 20.5 to >60 mM.

## Discussion

The results presented demonstrate the existence in rabbit renal cortical microvillus membranes of a pathway that interacts with a broad range of monocarboxylic acids. Using two independent experimental protocols, similar "$K_i$"'s were derived for 3-bromopropionate inhibition of L-lactate transport (Table 1, Fig. 1). Although this observation is strong evidence that the two substrates examined interact with a common transport receptor, it does not necessarily demonstrate that the inhibitor is itself transported. However, the observation that among the monocarboxylates tested, pyruvate, β-OH butyrate [8, 9], and D-lactate transport (*unpublished observations*) are predominantly $Na^+$-dependent, is consistent with such a hypothesis.

Interaction of solutes with the L-lactate transporter requires the presence of a single, negative-charged carboxyl group. Replacement of the carboxyl group with either an amino, amide, hydroxyl group or methyl ester, precludes recognition by the transport pathway (Table 2). Interestingly, not even the sulfonyl group is recognized. Succinate, a dicarboxylic acid, interacts poorly with the carrier site (Table 8) supporting the observation that

a single carboxyl group is required for recognition by the transport pathway. The latter result is compatible with previous observations by this laboratory, namely, the demonstration of separate pathways for mono- and polycarboxylic acids [8], and poor interaction between a series of monocarboxylic acids and the dicarboxylic acid receptor site [14]. Furthermore, the structural requirements for optimal substrate-carrier interaction have been defined for polycarboxylic acids [14], sugars [11] and neutral amino acids [6] so that poor interaction between the above three compounds, and the transport pathway studied herein, supports the hypothesis of an independent monocarboxylic acid carrier site.

Molecular size appears to be an additional important determinant for recognition by the carrier site. Thus, *n*-propionate (3C), *n*-butyrate (4C) *n*-valerate (5C) and *n*-caproate (6C) appear to have the highest affinity (Table 2). It is feasible that longer or shorter molecules cannot interact optimally with the receptor site simply as a result of steric considerations. Our present observations closely correspond to those of Ullrich and Rumrich [13] who studied monocarboxylic acid transport in the microperfused proximal tubule of the rat. These authors found that all small fatty acids from acetate to octonoate are accepted by the system, with propionate (3C) and butyrate (4C) showing the greatest interaction. In contrast to our observations and to those of Ullrich's group [13], Barac-Nieto, Murer and Kinne [1] were unable to demonstrate any inhibition of L-lactate transport by either propionate or acetate in brush border membrane vesicles isolated from rat kidney. The source of this discrepancy is at present unclear.

The nature and position of the substituted polar group, appears to be a third determinant of substrate-carrier interaction. When considering $-OH$ or $-CO$ derivatives it is apparent that the more distant the substituted moiety is from the carboxyl group, the poorer the inhibitory capacity of that analog. A common denominator appears to be propensity for the inhibitor to form hydrogen bonds. Hence, "optimal" substrate-carrier interaction occurs with unsubstituted compounds, since a methylene group ($-CH_2-$) has the least capacitance for hydrogen bond formation. An $-OH$ group can form approximately two hydrogen bonds with water, thereby rendering the compound more hydrophilic and, therefore, less lipid soluble, which impedes its inhibitory capacity. Since many ketones ($-CO$) on being dissolved in water may undergo rapid transformation to a hydroxyl compound, the very similar "$K_i$" values for ketone and hydroxyl groups, at least at the $\alpha$-C position, is readily explained. Amine groups can form approximately three hydrogen bonds with water; thus, one would predict that ketone, hydroxyl, or amine substitution would have a similar effect in terms of recognition by the receptor site. Clearly, the aminated analogs are poor inhibitors of L-lactate transport when compared to their respective ketone or hydroxyl analogs. The simplest explanation for this observation is that at pH 7.5, the condition under which the experiment was performed, the amine radical is positively charged ($NH_3^+$) since the $pK_{a2}$ of all the tested aminated compounds was $>9.0$. Consequently, the positive charge at the $\alpha$-carbon position appears to hinder substrate-carrier interaction by electrostatic effects.

Further evidence that the monocarboxylic acid receptor site gives preference to substrates that possess a hydrophobic tail is obtained by two additional independent observations. First, the presence of both hydroxyl and keto groups at adjacent carbon atoms of a single compound (Table 6), markedly decreases the inhibitory capacity of that analog when compared to either the parent compound or substrates containing only a single substituted moiety. Second, is the observation that substitution of a $NH_3^+$ group at any carbon position destabilizes substrate-carrier binding.

In addition to the requirement for a single carboxyl group and a hydrocarbon tail, there is evidence to suggest that intramolecular forces have an effect on substrate-carrier interaction. This is best displayed by the halogenated 2C compounds. While ketone, hydroxyl and amine group substitution at the $\alpha$-C position markedly impede the inhibitory capacity of that substrate when compared to the parent compound (acetic acid), the halogenated derivatives have the opposite effect, i.e., they enhance substrate-carrier interaction. This property of halogens to increase substrate permeability for a lipid membrane is best explained by "inductive effects" [2]. Briefly, the electronegative halogen withdraws electrons from the adjacent parts of the same molecule thereby reducing the strength of the hydrogen bond formed when the latter atom accepts a proton from water. Proton dissociation is thereby promoted which in turn lowers the $pK_a$. While low $pK_a$ values correspond with low "$K_i$" values with regard to L-lactate inhibition by 2 C compounds, our present results failed to show any correlation between $pK_a$ and the inhibitory capacity for the other compounds examined. Presumably, in molecules with longer carbon chains, other intramolecular forces override the "inductive effects" of halogens.

The role of the monocarboxylic acid pathway in the reclamation of important metabolites fil-

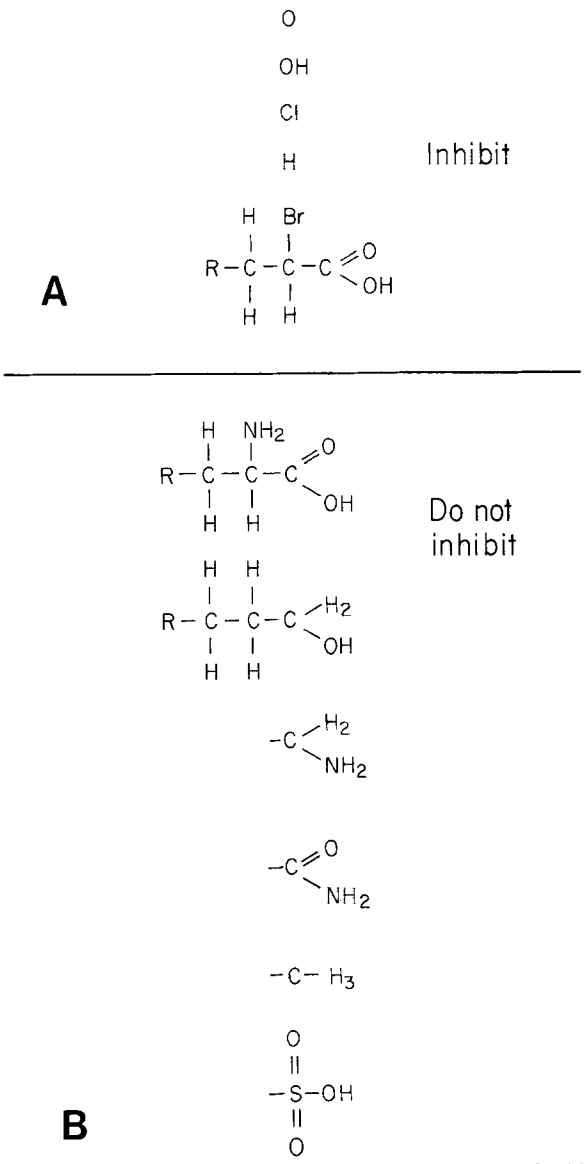


**Fig. 2.** Model of the basic structural elements required for binding of substrate to the monocarboxylic acid receptor site, after Ullrich and Rumrich [13]. (*A*): Compounds that interact with the transporter with the substituted moieties at the α-carbon position listed in order of preference. (*B*): Structural elements poorly recognized by the receptor site

tered by the glomerulus is obvious. Compounds of particular note include acetate, pyruvate, L-lactate and the ketone bodies aceto-acetate and β-OH butyrate. The present results are consistent with those of Garcia, Benuvides and Valdivieso [3] who have shown that pyruvate and ketone bodies share a common transport pathway in brush border vesicles isolated from rat kidney.

## Conclusions

The specificity of a transport pathway, defined by optimal substrate recognition, was investigated by testing the inhibitory capacity of a series of monocarboxylic acids and other compounds on L-lactate transport. Depicted in Fig. 2 is the schematic molecular structure of compounds that interact (*A*) or are poorly recognized (*B*) by the receptor site. The following are our conclusions:

1. The monocarboxylic acid receptor site interacts with a broad range of short-chain monocarboxylic acids.

2. A single negative-charged carboxyl group is an essential prerequisite for recognition; absence of a carboxyl group, or the presence of more than one carboxyl group, precludes recognition.

3. Optimal carbon chain length is 3 to 6 C atoms; any decrease or increase in carbon atoms impedes the transport process.

4. Hydroxyl or ketone substitution at the α-C position diminishes substrate-carrier interaction when compared to the unsubstituted parent compound. Hydrogen bonding appears to be a common denominator since the unsubstituted compound has the least capacitance for this form of bond formation and optimal interaction with the receptor site.

5. A positively charged amino group ($NH_3^+$) at the α-carbon diminishes substrate interaction with the carrier, and this is, presumably, due to electrostatic repulsion at the receptor.

6. Preference is given to compounds that possess a hydrophobic tail, i.e., $R=CH_2$ through $R=(CH_2)_4$ (*see* Fig. 2). The presence of an $-OH$, $-CO$, or $-NH_3^+$ group at the β or subsequent carbon positions renders the molecule increasingly more hydrophilic thereby hindering substrate-carrier interaction.

7. The inductive effect of halogens enhances substrate-carrier interaction for 2C compounds; however, no consistent pattern equating $pK_a$ to "$K_i$" could be elicited for other compounds.

8. Neither succinate, D-glucose, L-alanine nor β-alanine interacts with the receptor, suggesting that an independent monocarboxylic acid transporter exists in rabbit renal microvillus membranes.

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