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TRANSPORT INTERACTION OF SUGARS AND AMINO ACIDS IN MAMMALIAN KIDNEY

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SUMMARY

Mutual inhibition of transport between the non-metabolized amino acid, α-aminoisobutyric acid, as well as other amino acids, and the non-metabolized hexose, α-methyl-D-glucoside, was observed in rat kidney cortex slices. The inhibition occurs at the influx stage of the transport process, since the effect could be demonstrated after short incubation periods and was not accompanied by accelerated efflux. Analysis of the interaction showed a non-competitive inhibitory pattern that did not appear completely linear in respect to inhibitor concentration. Accelerated efflux of the sugar could not be induced by external glycine at a concentration (20 mM) which markedly diminishes the steady-state distribution ratio. In respect to mechanism, these findings can be interpreted as competition for a common energy supply or as allosteric inhibition by related, yet separate "carrier" systems.

INTRODUCTION

Hexose inhibition of amino acid transport in rat kidney cortex was reported from this laboratory several years ago[1,2]. The inhibition by the natural hexoses, D-galactose and D-fructose, was non-competitive in nature and then ascribed to production of toxic metabolites. Subsequently, mutual inhibition of hexoses and amino acids has been extensively investigated in mammalian intestine. In addition to the toxic metabolite hypothesis, initially proposed in kidney and extended to intestine[3], other mechanisms have been suggested to explain the phenomenon in intestine but without agreement as to a unifying concept. The controversy has been well summarized both by ALVARADO[4] and by SCHULTZ AND CURRAN[5] within their comprehensive review. The theories proposed have included (1) competition for a common energy source, first suggested by NEWEY AND SMYTH[6]; (2) allosteric competition for a common polyfunctional "carrier"[7]; and (3) stimulation of efflux from the epithelial cell[8]. In order to clarify further the phenomenon in mammalian kidney, we have studied the interaction of amino acids and α-methyl-D-glucoside, a non-metabolized monosaccharide[9] which shares the glucose–galactose transport system[10].

METHODS

The technique for the study of amino acid and α-methyl-D-glucoside transport in kidney cortex slices of 150–180 g Sprague–Dawley rats has been published previously in detail[9,11,12]. Three cortex slices, prepared with a Stadie–Riggs microtome and weighing between 15–30 mg each, were placed in 25 ml flasks containing 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) with the appropriate concentration of the test amino acid or α-methyl-D-glucoside and with tracer quantities of radioactive label (0.25 μC/ml). Flasks were gassed for 30 sec with O_2–CO_2 (95:5,v/v), sealed and incubated for varying lengths of time at 37° in a Dubnoff metabolic shaker. After extraction of the tissue radioactivity into boiling water, the radioactivity of the tissue extract and the media was assayed in a liquid scintillation spectrometer with an 80 % efficiency of counting for ^{14}C. Extracellular fluid space was assessed using [^{14}C]inulin in separate flasks which did not contain radioactive amino acids or α-methyl-D-glucoside. Determination of total tissue water then permits calculation of the distribution ratio (D.R.), *i.e.* the ratio of intracellular to extracellular substrate, as follows:

$$(1)\ \text{Counts/min per ml intracellular fluid} = \frac{\text{counts/min per ml tissue water} - (\text{volume inulin space} \times \text{counts/min per ml media})}{\text{total tissue water} - \text{inulin space}}$$

and

$$(2)\ \text{D.R.} = \frac{\text{counts/min per ml intracellular fluid}}{\text{counts/min per ml extracellular fluid}}$$

Kinetic analysis was derived from curves of concentration dependence conducted over a wide substrate range. For studies involving the interaction of α-aminoisobutyric acid and α-methyl-D-glucoside, slices were first preincubated with either 10 mM α-aminoisobutyric acid or 10 mM α-methyl-D-glucoside for 30 min, followed by a 20-min incubation at varying levels of the other substance. The preincubation was found desirable in order to accentuate the inhibition found, although results similar to those reported below were found without preincubation. Preincubation was not required to dramatically depict the interaction of α-methyl-D-glucoside with glycine. Therefore, slices were incubated for 30 min with varying concentrations of either hexose or glycine as the transported substance in the presence of 5 and 10 mM glycine (*vs.* α-methyl-D-glucoside) or 5 and 25 mM α-methyl-D-glucoside (*vs.* glycine). Parallel incubations in buffer without the appropriate inhibitor were performed with slices from the same group of animals to serve as controls for each experiment. Results were corrected for a diffusion component for Lineweaver–Burk kinetic analysis[12]. We have previously demonstrated that the radioactivity extracted from the tissue fluid is virtually all in the form added to the media as α-aminoisobutyric acid[13], natural amino acids[11,12] or α-methyl-D-glucoside[9]. Efflux of α-methyl-D-glucoside was performed as previously described[9,14].

Uniformly ^{14}C-labeled α-methyl-D-glucoside, specific activity 73.4 mC/mmole, was purchased from Calbiochem and found to be pure by thin-layer chromatography[9]. Unlabeled α-methyl-D-glucoside was obtained from the Pfansteil Co., Waukegan, Ill. and found to be pure by gas–liquid chromatography. [2-^{14}C]Glycine, specific

activity 5.13 mC/mmole and α-amino [1-^{14}C]isobutyric acid, specific activity 8.7 mC/mmole, were purchased from New England Nuclear. Unlabeled amino acids were obtained from Mann Research. Amino acids were found to be pure by thin-layer or paper chromatography. [^{14}C]Inulin, specific activity 2.5 mC/g, was obtained from New England Nuclear.

RESULTS

Mutual transport inhibition by sugar and amino acid

A number of amino acids were noted to inhibit the transport of α-methyl-D-glucoside by renal cortex slices (Table I). The most striking inhibition was produced by glycine, L-methionine and L-histidine. Of particular interest was the inhibition seen with two non-metabolized neutral amino acids, α-aminoisobutyric acid and cycloleucine. Inhibition was seen using lysine, a basic amino acid, but not with proline, an imino acid. Lysine transport is mediated by a separate mechanism shared with other dibasic amino acids[12,15], whereas proline is transported partially via an imino acid system and partially in interaction with the glycine mechanism[16]. Where inhibition was found, it was consistent over a wide range of α-methyl-D-glucoside concentration. The duration of incubation did not appreciably influence the results once a steady-state equilibrium was reached (usually 30 min).

The time course of α-methyl-D-glucoside transport from a 2 mM solution was studied in the presence of the non-metabolized amino acid, α-aminoisobutyric acid at a 10 mM concentration. As can be seen in Fig. 1, inhibition of α-methyl-D-glucoside transport was noted at the earliest time point examined, 15 min, and continued until a steady state level was reached when a 25 % inhibition of transport was observed.

TABLE I

AMINO ACID INHIBITION OF α-METHYL-D-GLUCOSIDE TRANSPORT IN RAT KIDNEY CORTEX SLICES

Cortex slices were incubated for 60 min at 37° with 0.5 μC α-[^{14}C]methyl-D-glucoside *plus* unlabeled hexose to give a final concentration of 0.18 or 1.065 mM in Krebs–Ringer bicarbonate buffer alone (control) or in the presence of the appropriate amino acid (5 mM). Values represent mean distribution ratio (intracellular/extracellular α-[^{14}C]methyl-D-glucoside) of triplicate determinations from kidney cortex slices of at least 9 rats and compared for significance using Student's *t* test.

Inhibitor / *Substrate concn.*:	*Distribution ratio* *0.18 mM*	*1.065 mM*
Control	3.37 ± 0.20	3.38 ± 0.12
α-Aminoisobutyric acid	2.66 ± 0.16 *	2.64 ± 0.11 *
Glycine	2.33 ± 0.09 *	2.45 ± 0.16 *
L-Valine	2.71 ± 0.11 *	2.86 ± 0.11 *
L-Proline	3.18 ± 0.14	3.09 ± 0.16
Cycloleucine	2.94 ± 0.02 **	2.67 ± 0.11 *
L-Lysine	2.87 ± 0.07 **	2.88 ± 0.07 **
L-Methionine	2.47 ± 0.19 *	2.32 ± 0.13 *
L-Histidine	—	2.30 ± 0.18 *
L-Tryptophan	—	3.12 ± 0.42

* Significantly different than control $P < 0.01$.
** Significantly different than control $P < 0.05$.

Similar results were noted when glycine or L-histidine, but not L-proline, replaced α-aminoisobutyric acid as the inhibitor.

Not only was the hexose transport inhibited by amino acids but, reciprocally, α-methyl-D-glucoside inhibited amino acid transport in kidney cortex slices (Table II). As with the amino acid inhibition of α-methyl-D-glucoside transport, the phenomenon appeared at the earliest time point studied. This inhibition did not appear linearly related to hexose concentration, with 5 mM α-methyl-D-glucoside equally as effective as 20 mM α-methyl-D-glucoside.

Kinetic analysis of the inhibition

Lineweaver–Burk analysis of α-methyl-D-glucoside transport over a substrate range of 2.565–10.065 mM indicated first order kinetics with an apparent K_m of 6 mM and apparent v_{max} of 16 μmoles/ml intracellular fluid per 20 min (Fig. 2A). The inhibition by α-aminoisobutyric acid was non-competitive in nature, with a 33 %

TABLE II

INHIBITION OF AMINO ACID TRANSPORT BY α-METHYL-D-GLUCOSIDE

Rat kidney cortex slices were incubated with 0.5 μC of [^{14}C]amino acids, final concentration 65 μM, in buffer alone (control) or in the presence of the appropriate concentration of α-methyl-D-glucoside for 60 min at 37°. Values represent mean distribution ratio ± S.E. of triplicate determinations from slices of 9 rats and are compared for significance using Student's *t* test. All values significantly differ from their respective controls ($P<0.01$).

α-Methyl-D-glucoside concn. (mM)	*Distribution ratio*		
	α-Amino-isobutyric acid	*Glycine*	*L-Valine*
Control	5.38 ± 0.14	10.41 ± 0.19	3.33 ± 0.14
5	3.95 ± 0.11	6.86 ± 0.24	2.44 ± 0.19
20	3.58 ± 0.26	6.13 ± 0.20	2.50 ± 0.10

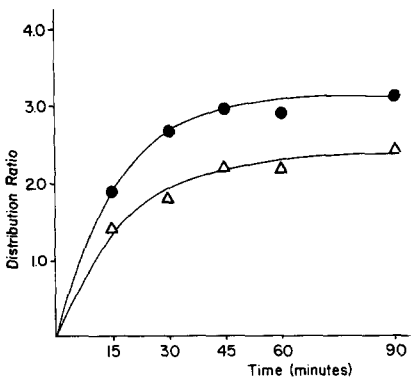

Fig. 1. Effect of 10 mM α-aminoisobutyric acid on the intracellular accumulation of 2 mM α-methyl-D-glucoside by rat kidney cortex. Slices were incubated at 37° for 15–90 min with 0.5 μC α-[^{14}C]methyl-D-glucoside in 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) after gassing with O_2–CO_2 (95:5, v/v). Ordinate, ratio of intracellular/extracellular α-methyl-D-glucoside concentration. Abscissa, time of incubation. ●, control; △, with 10 mM α-aminoisobutyric acid. Each point represents the average of triplicate determinations from 9 rats.

decrease in the v_{max} to a value of 11 μmoles/ml intracellular fluid per 20 min. The hexose inhibited α-aminoisobutyric acid uptake in an identical manner (Fig. 2B) over a substrate range of 0.67–4.065 mM with no effect on the apparent K_m of 3.6 mM. A 44 % decrease was observed in the apparent v_{max}, from 10 to 5.6 μmoles/ml intracellular fluid per 20 min. It should be noted that at the highest concentration of α-methyl D-glucoside studied (10 mM) inhibition of uptake was found with an equal concentration of α-aminoisobutyric acid.

Glycine was noted to be among the most potent inhibitors of α-methyl-D-glucoside transport. As with α-aminoisobutyric acid, 5 mM glycine produced a similar non-competitive inhibition of hexose transport (Fig. 3). A 40 % decrease was observed in the apparent v_{max}, from 26 to 15.5 μmoles /ml intracellular fluid per 30 min with no changes in the apparent K_m of 7.8 mM. A doubling of the glycine concentration to 10 mM provided only a moderate accentuation of inhibition with reduction of the apparent v_{max} to 11 μmoles/ml, a further decrease of only 20 %. Analysis of the inhibition by the method of CLELAND[17], plotting both the intercepts and slopes against the inhibitor concentration, suggested linear non-competitive inhibition in respect to intercepts, but hyperbolic in respect to the slopes, compatible with an allosteric phenomenon.

The renal tubule cell has the ability for concentrative uptake of amino acids over a wide range of substrate concentration. Kinetic analysis of the resultant curves of concentration dependent uptake velocity indicates that two slopes are obtained

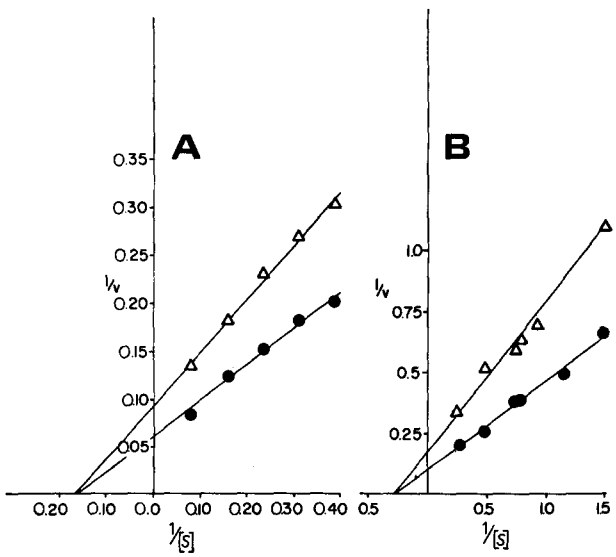

Fig. 2. Lineweaver–Burk plots of reciprocal of velocity ($1/v$) *vs.* reciprocal of substrate concentration ($1/[S]$) illustrating the mutual interaction of α-aminoisobutyric acid and α-methyl-D-glucoside transport. Slices were incubated for 20 min at 37° in 2 ml of Krebs–Ringer bicarbonate buffer containing 0.5 μC ^{14}C tracer and varying concentrations of substrate. A. α-Methyl-D-glucoside transport: ●, buffer alone; △, in the presence of 10 mM α-aminoisobutyric acid. B. α-Aminoisobutyric acid transport: ●, control; △, in the presence of 10 mM α-methyl-D-glucoside. Slices were preincubated for 30 min in either buffer or with the appropriate inhibitor (10 mM) prior to the experiment. Each point represents the mean of triplicate analysis from slices of 9 rats and has been corrected for a diffusion component[12]. v is in μmoles/ml intracellular fluid per 20 min and $[S]$ is in mM.

from which two apparent K_m values for the transport process may be calculated[18,19]. The exact nature of the transport mechanism leading to these observations is unknown, but it has been speculated that there may be two transport systems, one operative at low substrate levels and the other at high levels. Further, the "high K_m" system for glycine appears to be a more general mechanism in that it is shared with

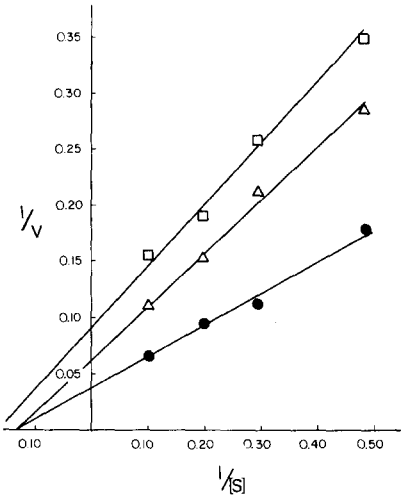

Fig. 3. Reciprocal plot demonstrating inhibition of α-methyl-D-glucoside transport by glycine. Slices were incubated for 30 min at 37° in 2 ml of Krebs–Ringer bicarbonate buffer containing 2–10 mM α-methyl-D-glucoside with 0.5 μC radioactive tracer in buffer alone (control) or in the presence of 5 and 10 mM glycine. Each point represents the mean of triplicate analysis from slices of 9 rats and has been corrected for diffusion[12]. ●, Control; △, 5 mM glycine; □, 10 mM glycine.

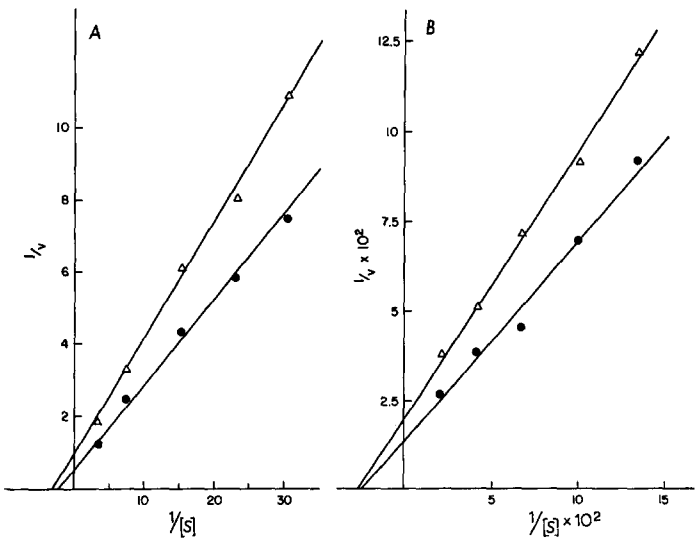

Fig. 4. Lineweaver–Burk plots depicting α-methyl-D-glucoside inhibition of glycine transport over both low (A) and high (B) substrate dependent transport systems. Rat kidney cortex slices were incubated for 30 min at 37° in buffer containing 0.5 μC [^{14}C]glycine *plus* appropriate concentrations of unlabeled amino acid. A. ●, "low" glycine control (0.03–0.3 mM); △, glycine + 5 mM α-methyl-D-glucoside. B. ●, "high" glycine control (5–50 mM); △, glycine + 25 mM α-methyl-D-glucoside. Each point is the mean of triplicate analysis from slices of 9 rats.

the imino acids and other neutral amino acids while at the low, more physiologic concentrations, the transport mechanism is specific for glycine[16,20]. α-Methyl-D-glucoside reciprocally inhibited the transport of glycine in a non-competitive manner. As seen in Fig. 4, similar results were obtained when 5 mM α-methyl-D-glucoside inhibited the low substrate dependent system (A) or when 25 mM α-methyl-D-glucoside inhibited the high system (B). The transport of 50 mM glycine was inhibited by half that concentration of α-methyl-D-glucoside.

Accelerated efflux experiments

In order to test the hypothesis that the amino acid–hexose interaction involves a shared carrier mechanism[4,7], accelerated efflux experiments were performed after preloading slices for 60 min with labeled α-methyl-D-glucoside to give an intracellular concentration of approximately 0.2 mM. Slices were removed, quickly rinsed with saline, blotted and transferred to flasks containing 3 ml of buffer alone or with either unlabeled 20 mM α-methyl-D-glucoside or 20 mM glycine. At intervals of 3 min, the flasks were opened and the appearance of radioactivity into the media determined. After 18 min, the radioactivity in the tissue and media was assayed and the percent remaining in the tissue at each time period was calculated and plotted. Whereas α-methyl-D-glucoside readily promoted accelerated efflux of labeled material (Fig. 5,

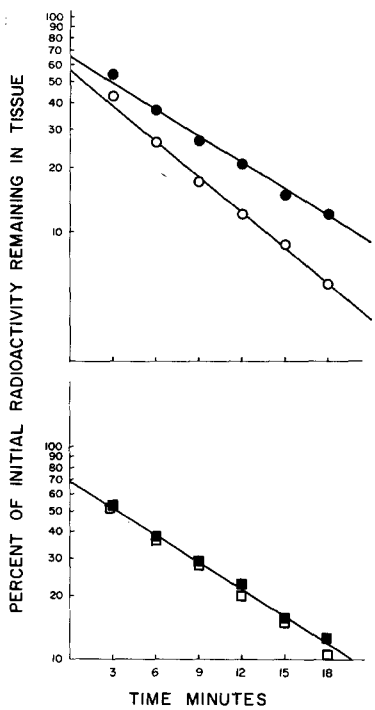

Fig. 5. Accelerated efflux of preloaded α-[^{14}C]methyl-D-glucoside into media containing 20 mM α-methyl-D-glucoside (top) or 20 mM glycine (bottom). Results represent the average of duplicate determinations with tissue from three animals and are plotted on a semilog graph as the percent of initial radioactivity remaining in the tissue with time. Top: ●, media = buffer alone; ○, buffer + 20 mM α-methyl-D-glucoside. Bottom: ■, media = buffer alone; □, buffer + 20 mM glycine.

top) glycine was without effect (bottom). Glycine in this concentration markedly diminishes the steady state distribution ratio of the sugar. Thus, the observed inhibition cannot be explained by promotion of efflux, already suggested by the early inhibition observed during the timed uptake studies. The results also mitigate against the concept of a mutual transport process, since the extracellular hexose readily exchanged for intracellular α-methyl-D-glucoside under identical conditions.

DISCUSSION

Our finding of mutual non-competitive inhibition of transport by two non-metabolized, actively transported substances, α-aminoisobutyric acid, a neutral amino acid, and α-methyl-D-glucoside, a hexose sharing the glucose–galactose transport mechanism, clearly establishes that the amino acid sugar interaction does not solely depend upon metabolism of either substance or formation of toxic metabolites in rat kidney cortex. It is noteworthy that we previously failed to demonstrate inhibition of amino acid transport in renal cortex by a number of other sugars, among these 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, D-mannose, D-xylose and D-ribose[2]. KLEINZELLER[21] has found 2-deoxy-D-glucose transport to be Na^+ independent and not shared with D-glucose or D-galactose. The remaining sugars are either not actively transported or are transported poorly[21]. Thus, at least in kidney cortex slices, the inhibition of amino acid transport appears to be dependent on these hexoses that are Na^+ dependent and actively share the glucose–galactose transport system. The inhibition noted with D-fructose in earlier experiments appears an exception since it is not generally considered to share the glucose–galactose transport mechanism, but it is quite possible that metabolism of fructose is necessary for its inhibition of amino acid transport in renal cortex.

The present experiments provide the first observation in kidney cortex of the reverse inhibition, *i.e.* amino acid inhibition of hexose uptake. Since α-methyl-D-glucoside is not metabolized[9], the inhibition clearly affects transport and is not secondary to an alteration in hexose disposal. Similar inhibition of sugar transport by all actively absorbed amino acids tested has been observed by HINDMARSH *et al.*[22] in hamster intestine. In dogs, renal glucose reabsorption is depressed by infusions of L-lysine, glycine and L-alanine, but not by L-leucine or L-aspartic acid[23].

Some comment should be made regarding the mechanism of the mutual inhibition. The extensive investigation of the phenomenon in intestine has not clarified the interaction; rather controversy has erupted[4,24]. Several mechanisms have been proposed; however, their relevance to the interaction in renal cortex is open to question in view of the known, albeit subtle, difference in these tissues. We could find no promotion of either α-methyl-D-glucoside or glycine efflux from preloaded cortex slices into a media containing high concentrations of the other. Thus, the theory of CHEZ *et al.*[8], postulating accelerated efflux as found in ileal strips exposed to hexoses and alanine, does not explain the phenomenon in kidney cortex.

Two other theories have been put forth to explain the interaction in intestine. ALVARADO[7] initially proposed a polyfunctional "carrier" with multiple reactive sites, but has recently modified this concept to imply allosteric inhibition by closely related, but separate "carrier" mechanisms[4]. NEWEY AND SMYTH[6], however, suggested that actively transported amino acids and sugars compete for a common energy supply,

mediated through ATP. The latter hypothesis has received the most support[25,26] and has recently been promulgated in a unified concept by KIMMICH[27]. Since specific carrier sites have yet to be demonstrated and since the source of energy utilized in active transport has not been delineated, neither of these concepts is subject to experimental proof. Indeed, as ALVARADO[4] has recently suggested, the two hypotheses may not be mutually exclusive.

Our studies with α-methyl-D-glucoside do not allow definitive support for either mechanism in kidney cortex. The kinetic analysis shows a non-competitive mechanism with α-methyl-D-glucoside interacting either with α-aminoisobutyric acid or with glycine. Similar kinetics were observed whether α-methyl-D-glucoside served as the substrate or as the inhibitor and over both high and low substrate dependent systems for glycine transport. If the multiple transport systems for glycine are independent and operate simultaneously, as has been suggested[20], the interaction would appear a generalized rather ubiquitous phenomenon affecting multiple carrier mechanisms. On the other hand, the mutual inhibition with α-methyl-D-glucoside is not observed with proline, an imino acid. This is of particular interest since proline does share part of the glycine transport mechanism[16]. Moreover, inhibition did not appear linear with increasing inhibitor concentrations and a finite concentration beyond which no further inhibition occurred was found. A similar non-linear inhibition has been observed in intestine[22,25]. This would be compatible with an allosteric effect, and is reflected by our kinetic analysis implying a mixed linear–hyperbolic non-competitive inhibition. Alternately, one can equally envision the degree of competition for a common energy supply as dependent on the relative affinity of each substance and changing in relation to the "inhibitor's" use of multiple carrier mechanisms.

There are several lines of indirect evidence that lead us to reject ALVARADO's hypothesis as an entirely suitable explanation for the mutual inhibition observed in rat kidney cortex. (1) Phlorizin, a competitive inhibitor of active hexose transport, paradoxically stimulates amino acid transport[28]. (2) We have recently demonstrated that active hexose transport in kidney cortex develops at a later time than that of amino acids[29]. (3) In the present study, we could not elicit accelerated efflux of α-methyl-D-glucoside by glycine, a potent inhibitor of hexose accumulation under conditions where α-methyl-D-glucoside readily promoted autoexchange.

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