ages and to broaden the scope of the model. In particular, the ability of this reconstruction technique to size experimental infarcts needs to be tested in animals with acute inferior and nontransmural (subendocardial) myocardial infarcts. The present data, however, suggest that a three-dimensional model may be used for accurate estimation of experimental infarct size in dogs with acute anterior infarcts. This method has potential application for the quantitation of infarct size in humans.

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17 November 1976; revised 17 January 1977

Transport Interaction of Cystine and Dibasic Amino Acids in Renal Brush Border Vesicles

Abstract. *The uptake of cystine by vesicles prepared from rat kidney brush borders occurs by two distinct transport systems. The higher affinity system is inhibited by the dibasic amino acids lysine, arginine, and ornithine. The lower affinity system, unaffected by dibasic amino acids, appears to correspond to that observed by studying uptake of cystine by kidney slices.*

Human cystinuria, an inherited disorder characterized by hyperexcretion in the urine of cystine and the dibasic amino acids, lysine, arginine, and ornithine, has focused attention on the nature of the renal tubule reabsorptive mechanism for these substances. Dent and Rose (*1*) postulated that these four amino acids have a common transport process in the renal tubule cell, which is defective in this disease, a conclusion strengthened by the fact that lysine infusion in both man and dogs produced an increase in cystine excretion (*2*). Experiments with both rat and human kidney cortex slices in which the cellular uptake of radioactive cystine, lysine, and arginine was studied revealed that the dibasic amino acids shared a common transport system, but that cystine uptake was by an independent mechanism; lysine and arginine did not inhibit cystine uptake by the renal cortical slice (*3, 4*). Indeed, this dichotomy was strengthened by the finding that dibasic amino acid uptake by renal cortex slices from human cystinuric patients was defective, but that of cystine was not (*4*). Further support for the separate nature of the renal transport process for cystine and dibasic amino acids came from the description of patients with hyperdibasic aminoaciduria without cystinuria (*5*) and with cystinuria without dibasic aminoaciduria (*6*) as well as from dogs with cystinuria without significant dibasic aminoaciduria (*7*).

However, Greth *et al.* (*8*) have demonstrated in vivo that cystine can enter the renal tubule cell via the basal lateral membrane in rats under conditions where luminal transport does not occur. It thus appeared that in the rat kidney cortical slice significant uptake of cystine might occur through the basal lateral membranes, obscuring transport at the luminal brush border membrane and leaving the interaction of cystine and dibasic amino acids at the luminal membrane as a distinct but undetectable possibility. Silbernagl and Deetjen (*9*) have reported that, in micropuncture studies of rat proximal tubules, arginine inhibits the tubule reabsorption of cystine, thus supporting an interaction of the amino acids in a brush border transport process.

Although the "black box" experimental techniques used previously have provided valuable insights into renal tubule transport mechanisms, the data regarding the nature of events at the luminal brush border membrane have been inferential. The ability to isolate rat renal tubule brush border membranes (*10*) and to study the entry characteristics of amino acids into vesicles prepared from these membranes (*11, 12*) provides the opportunity to examine the interaction of cystine with dibasic amino acids at the membrane locus of transport. The results of such studies form the basis of our report.

Rat brush border membranes were isolated by the method of Booth and Kenny (*13*). Vesicles were prepared by homogenization and centrifugation in hypotonic THM buffer [1 m*M* tris plus Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) plus 100 m*M* mannitol, *p*H 7.4] as previously reported (*14*) and uptake of cystine in the presence of a sodium gradient at *p*H 7.4 determined by methods described by McNamara *et al.* (*12*) in a THM (20 m*M* tris plus 20 m*M* Hepes plus 60 m*M* mannitol). ^{14}C-labeled cystine (291 mc/mmole) was purchased from New England Nuclear Corporation and found to be chromatographically pure. The vesicle preparation showed an alkaline phosphatase enrichment of 12-fold compared to the starting material. Michaelis-Menten constants (K_m) were calculated assuming a two-component system by linear and nonlinear regression analysis as reported (*12*).

Figure 1 shows a Lineweaver-Burk plot of the concentration dependence of the initial rate (0.5 minute) of vesicle uptake of cystine from 0.018 to 0.89 m*M*. The data reveal a two-limbed curve with observed transport parameters of K_{m_1} of 0.031 m*M*, V_{max_1} of 0.322 nmole per milligram of protein per 0.5 minute, and K_{m_2} of 0.481, V_{max_2} of 1.80. By regression analysis of the data, the calculated

Fig. 1. Influence of L-cystine concentration and of 1 m*M* L-lysine on the initial rate of uptake of cystine by brush border vesicles. Uptake was studied in the presence of a sodium gradient. Freshly prepared brush border vesicles were suspended in tris-Hepes-mannitol buffer (20 m*M*, 20 m*M*, 60 m*M*) at 22°C to a protein concentration of 0.3 to 0.4 mg/ml. The standard uptake experiment consisted of 0.5 ml of membrane vesicle which was added at time 0 to a disposable test tube (10 by 75 mm) containing 0.1 μc of L-[^{14}C]cystine, 0.1 μc of 3-*O*-methyl-D-[^{3}H]glucose, and unlabeled amino acid to the desired concentration in 5 μl of 0.5*N* HCl plus 50 μmole of NaCl. The incubation, which had a final *p*H of 7.4, was stirred (Vortex) for 6 seconds and transferred by Pasteur pipette to a Millipore-filter apparatus after 30 seconds. Uptake was stopped by rapid filtration through a Millipore filter (H0.45 nm) and washed once with 5 ml of buffered saline. Filters were air dried and counted in a Packard Tricarb Scintillation Spectrometer using 3-*O*-methyl-D-[^{3}H]glucose as a space measurement as described (*12*). Solid circles represent uptake of L-[^{14}C]cystine and open circles show its uptake in the presence of 1 m*M* L-lysine.

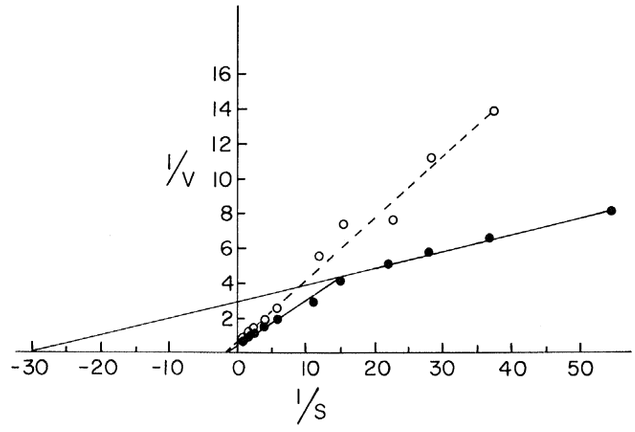

Table 1. Effect of unlabeled amino acids on uptake of ^{14}C-labeled cystine by brush border membrane vesicles. Values given are means ± standard error for 8 to 16 determinations.

Additions	Concentration (m*M*)	Percent of [^{14}C]cystine uptake (0.5 minute) at	
		0.027 m*M*	0.27 m*M*
None		100.0 ± 4.3	100.0 ± 4.4
Arginine	1	45.8 ± 2.8*	72.6 ± 4.6*
	3	33.5 ± 4.8†	
Lysine	1	46.0 ± 2.8*	75.5 ± 3.0*
	3	33.2 ± 4.3†	
Ornithine	1	48.7 ± 3.7*	77.9 ± 3.6*
Glycine	1	88.1 ± 5.3	
Valine	1	102.9 ± 4.5	
α-Aminoisobutyric acid	1	102.2 ± 6.5	
Proline	1	85.6 ± 4.9	
Phenylalanine	1	88.0 ± 5.2	

*$P < .01$ for differences from control. †$P < .05$ for differences between values with 3 m*M* and 1 m*M* of the same added amino acid.

parameters were found to be $K_{m_1} = 0.033$, $V_{max_1} = 0.205$, $K_{m_2} = 0.93$, $V_{max_2} = 1.98$. In previous studies with rat kidney cortex slices, only a single transport component was observed with a K_m of 0.8 m*M* over a substrate range of 0.07 to 1.2 m*M* (*14*). Below this range, uptake by slices did not obey saturation kinetics. The interpretation of the studies of cystine uptake with kidney cortex slices was complicated by the fact that the intracellular form of the amino acid was cysteine (*15*). In our study, however, uptake by membrane vesicles is independent of reduction to cysteine since thin-layer chromatography (*16*) of the vesicle contents after incubations up to 30 minutes demonstrated only the presence of radioactive cystine.

The effect of several amino acids on the initial rate (0.5 minute) of vesicle uptake of cystine in the presence of a sodium gradient was examined (Table 1). At 0.027 m*M* cystine only the dibasic amino acids at 1 m*M* concentration caused a 55 percent inhibition. At 3 m*M* cystine, the inhibition by arginine and lysine was about 66 percent. Kinetic analysis of concentration-dependent uptake of cystine in the presence of 1 m*M* lysine is shown in Fig. 1. The presence of lysine altered the kinetic parameters such that only one transport component was discerned corresponding to the high K_m system. It thus appears that lysine interacted with the low K_m system. The 67 percent inhibition of 0.027 m*M* cystine uptake observed in the presence of 3 m*M* lysine (Table 1) can be correlated with the cessation of that amount of entry expected from the low K_m component (62 percent) of total transport. Table 1 also shows a 25 percent inhibition of 1 m*M* lysine on vesicle uptake of 0.27 m*M* cystine. Again, if one partitions the total entry of cystine at the latter concentration into its separate velocities by regression analysis, the expected inhibition of the low K_m system should reduce entry by 29 percent.

These results deomonstrate the transport interaction of cystine and dibasic amino acids for the first time in a membrane vesicle preparation from rat kidney. Previous studies with rat kidney slices exhibited uptake of cystine by the high K_m system only (*14*), which showed no interaction with dibasic amino acids (*3*). Studies with brush border membrane vesicles reveal a similar lack of interaction of the high K_m system with dibasic amino acids. The low K_m higher affinity transport system, inhibitable by dibasic amino acids, was apparently obscured by the more complex situation in slices, especially by any transport occurring at the basal lateral membrane. In light of our findings the postulate of Dent and Rose (*1*) that cystine and dibasic amino acids have a common transport system which is defective in human cystinuria receives support. The observations that cystine uptake by kidney slices from human patients and cystinuric dogs is not defective should now be reinterpreted.

A defective low K_m transport system may be present in human cystinuric kidney but this may not be the sole abnormality. Still to be explained is the apparent secretion of cystine by humans (*17*) and dogs (*7*) with this disorder as well as the interaction of cysteine and dibasic amino acids in the efflux transport process of renal tubule cells (*18*).

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19. Supported by NIH grant AM 10894, and a grant from the John A. Hartford Foundation.

3 January 1977

Angiotensin: Physiological Role in Water-Deprivation–Induced Thirst of Rats

Abstract. *Cerebroventricular infusion of P-113, the blocking agent of angiotensin II, into rats for 75 minutes prior to their being allowed to drink, significantly attenuated their water intake when they had been deprived of water for 30 hours. However, a similar infusion had no effect on the food intake in rats fasted for 30 hours. The results indicate a physiological role for angiotensin II in the drinking response of rats deprived of water.*

The possible role of the renin-angiotensin system in thirst was first suggested by Fitzsimons (*1*), who showed that drinking was increased in rats subjected to partial aortic constriction and that the response was attenuated by nephrectomy. Later, others demonstrated that both intravenous and intracranial administration of renin or angiotensin II provoke thirst (*2*).

Even though exogenous angiotensin II clearly stimulates thirst, it is not known whether this hormone plays any physiological role in drinking. Some workers have proposed that drinking induced by angiotensin is a pharmacological effect rather than a physiological one (*3*). In previous experiments we found that single intraventricular injections of the blocking agent P-113 (sar^1-ala^8-angiotensin II, where sar is sarcosine and ala is alanine) of angiotensin II attenuated drinking in water-deprived rats (*4*). However, the procedure we used to administer a bolus of P-113 to the animals 1 to 2 minutes prior to the drinking test may have obscured the results. The experiments presented here were designed to test in a more controlled manner the hypothesis that endogenous angiotensin plays a role in thirst.

We used 52 male Sprague-Dawley rats, each weighing 300 to 400 g. They were fed on rat food (Teklad) containing 1 percent NaCl, and water was freely available. The rats were housed individually and exposed to photoperiods of 12 hours of light and 12 hours of darkness. Stereotaxic techniques were used on anesthetized animals to fix a 22-gauge guide tube over the right lateral ventricle, 0.5 to 1 mm anterior to the interventricular foramen (*5*). The end of the guide was 1 to 2 mm above the ventricle. During the 6 to 10 days between surgery and experimentation, the rats were conditioned at least five times to the type of handling to which they would be exposed on the day of the experiment: they were removed from their cages, restrained manually, and weighed; the protective rubber cap above the guide tube was removed and replaced, and then the rats were returned to their cages.

Water intake (from Richter tubes) and weight gain (measured daily for 2 or 3 days prior to experimentation) were used as crude indices of general recovery from surgery and of normal appetitive behavior. No animal was studied which was drinking less than 20 ml per day or was not gaining weight. Animals were grouped so that their water intakes 24 hours prior to the test were similar in the control and experimental groups.

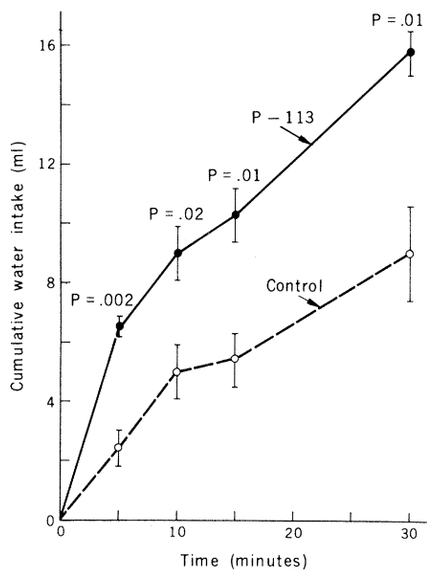

Fig. 1. The results of experiment A, showing the cumulative water intake of rats deprived of water for 30 hours and then given access to water. Infusions into the lateral ventricle (3.3 μl/min) of artificial cerebrospinal fluid with or without P-113 (20 ng/μl) began 30 minutes before the rats were given access to water and continued during the 30-minute test period. Values are means ± standard error (N = 4).

At 0800 hours on the day prior to experiments A and B, water tubes were removed from the cages. The next afternoon each rat was removed from its cage and a 27-gauge needle connected to Teflon tubing was inserted through the guide tube into the right lateral ventricle. The animal was then returned to its cage and the tubing (previously filled with infusate) was connected to a Braun-Melsungen syringe pump set to deliver 3.3 μl per minute. Usually, three control and three experimental animals were infused simultaneously. The infusate was either artificial cerebrospinal fluid (Na^+, 150 mM; Cl^-, 133 mM; K^+, 3.1 mM; Ca^{2+}, 1.2 mM; Mg^{2+}, 1 mM; HCO_3^-, 24.5 mM; phosphate, 0.5 mM; glucose, 50 mg/100 ml) or artificial cerebrospinal fluid containing 20 ng/μl of P-113.

After 30 minutes (experiment A) or 75 minutes (experiment B) of infusion, water tubes were returned to the cage (1400 hours); the associated disturbance aroused the rats. Over the next 30 minutes, during which the ventricular infusions continued, we measured water intake at 5-minute intervals to the nearest 0.5 ml. Thereafter, methylene blue (0.2 percent) in artificial cerebrospinal fluid was infused intraventricularly for 10 to 15 minutes. The animals were then killed and perfused intravascularly with saline followed by formalin-saline. The next morning, the brains were sectioned and examined for ventricular staining. Data from any animal not stained in the right lateral, third, and fourth ventricles were excluded from the results.

In experiment C we evaluated the effect of P-113 on another appetitive behavior, food intake. Twenty-four rats were fasted for 30 hours; thereafter they were infused (exactly as in experiment B). After 75 minutes of infusion, water tubes were removed and food was presented to the animals for a 10-minute test period during which the infusion was continued. Thereafter, each rat was killed and treated as in experiments A and B. In addition, the amount of food eaten was determined by emptying the stomach and upper duodenum and drying and weighing the contents.

Figure 1 shows the results for experiment A. It is evident that P-113 amplified