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Renal Fanconi syndrome: developmental basis for a new animal model with relevance to human disease

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Using succinylacetone (SA), a metabolite of tyrosine excreted in excess by infants and children with hereditary tyrosinemia and the renal Fanconi syndrome (FS), we have investigated developmentally-related membrane transport events leading to emergence of the generalized renal tubular dysfunction seen in human FS. SA was found to impair sugar and amino acid uptake by both newborn renal tubules and 7-day renal brush-border membrane vesicles (BBMV). This impairment by SA was due in part to a slowing of substrate cotransport rate of $^{22}Na^+$-entry into BBMV. Concentration-dependent uptake studies indicated SA inhibited the newborn high-affinity transport systems for sugars and amino acids. SA also caused an increase in membrane fluidity and a shift in the thermotropic transition temperature. The demonstrated dual nature of SA's effect on membrane fluidity and O_2 consumption, together with the relative contribution of each component to SA-induced transport impairment helps to provide a basis for an understanding of the age-related increases in glucosuria, aminoaciduria and natriuria seen in infants with FS.

Introduction

Age-related changes in rat renal tubular membrane transport have been widely investigated in order to gain a better understanding of developmental changes which occur in the human nephron. With respect to morphologic and biochemical criteria, the newborn rat approximates the maturational state of the human neonate. Previous investigations have provided a great deal of information about developmental changes in renal tubular transport of iminoglycine [1–3], β-amino acids [4,5], cystine and dibasic amino acids [6,7] and sugars [8,9].

We suggested the possibility that interruption of developmental events leading to maturation of renal tubular function might constitute the basis for the transport abnormalities comprising the human renal Fanconi syndrome [10]. Support for this hypothesis was derived from studies using maleic acid, which induces the renal Fanconi syndrome when administered to adult rats but had no effect on sugar and amino acid uptake by newborn rat renal tubules; indeed, partial maturation of the respective membrane transport system was required for maleate to exert a demonstrable effect on uptake [10]. This led us to a critical reexamination of the utility of the maleic acid adic adult rat kidney model for study of human renal transport dysfunction seen in Fanconi syndrome. Since the Fanconi syndrome is seen predominantly in human infants, these observations caused us to question the validity of the use of maleic acid as a model for the study of this human transport disorder. In addition, the physiological significance of the maleic acid model is questioned because maleic acid is not a metabolic intermediate product of rats or humans.

We have recently described the ability of the compound succinylacetone (SA) to generate glucosuria, proteinuria and aminoaciduria in adult rats [11]. The significance of these studies lies in the unique finding of urinary excretion of SA in infants with hereditary tyrosinemia and the associated renal Fanconi syndrome [12]. In vitro studies using adult rat renal tubules revealed inhibition of sugar and amino acid uptake by SA [11,13]. In adult rat renal brush-border membrane vesicles, SA inhibited sugar and amino acid uptake in part, by a direct membrane interaction and not via

Abbreviations: AIB, α-aminoisobutyric acid; MAG, methyl α-D-glucoside; SA, succinylacetone.

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alteration of cellular metabolism [14]. These observations suggest that SA may be used to generate a physiologically relevant animal model for study of the human renal Fanconi syndrome. To further our understanding of the human renal Fanconi syndrome we have investigated the effects of SA on the development of rat renal tubular sugar and amino acid transport.

Methods and Materials

Animals and tissue preparations

Gravid Sprague-Dawley rats were purchased from Charles River Breeding Labs (Wilmington, MA) at 14 days gestation. After parturition mothers were housed separately and provided Purina Chow and water ad libitum. Renal tubule fragments from newborn rats were prepared from kidneys of pups killed by decapitation within 36 h of age, according to methods previously described [8,9]. Renal tubule fragments were also prepared from seven and 14-day-old animals by the same method. Isolated renal tubule fragments from 150–175 g adult males were prepared and used for comparison with tubules from younger rats. Brush-border membrane vesicles were made from seven-day-old animals starting with whole kidney and utilizing the method of Booth and Kenny [15], modified as we have previously described [16,17]. The final membrane preparation was enriched in alkaline phosphatase 6–7-fold over the starting homogenate, comparable to that previously reported [18,19].

Time-dependent substrate uptake by tubules

Uptake by 2 mM methyl α-D-glucoside (MAG) and 0.065 mM α-aminoisobutyric acid (AIB) by isolated renal tubule fragments was examined in the absence and presence of 4 mM SA. This concentration was selected for several reasons: 4 mM SA has previously been reported to provide optimal levels of transport inhibition for in vitro study [11]; in the affected human, the renal tubule cell contains an undetermined concentration of endogenously-produced SA, as well as being exposed to circulating levels of SA produced in liver; and, reported urinary levels are in this range. Blood levels of SA in tyrosinemic individuals have not been reported. Tubules were suspended in Krebs-Ringer bicarbonate buffer (KRB), pH 7.4 and gassed continuously with 95% O_2/5% CO_2 at 37°C, according to previously described techniques [2,8]. Tubule suspensions contained 10–12 mg tissue/ml. The immediate inhibitory effect on SA on uptake by tubule suspensions has been reported [11,13,14], eliminating a need for preincubation. Substrate uptake studies were initiated by addition of 0.1 μCi of ^{14}C-label per ml of suspension plus sufficient unlabeled material to achieve the desired final concentration, and aliquots removed at specified times. Distribution ratios (DR), defined as the ratio of cpm/μl intracellular fluid to cpm/μl extracellular fluid, were calculated as previously described [2,8]. Data were analyzed for significance by Student's *t*-test and plotted as the mean (±S.E.) distribution ratios versus time of sampling (minutes).

Effect of SA on concentration-dependent uptake by newborn tubules

Concentration-dependent uptake of α-aminoisobutyric acid (0.026–4.0 mM) and methyl α-D-glucoside (0.02–5.0 mM) was examined using newborn tubules in the absence and presence of 4 mM SA. Tubules were incubated as above with 0.1 μCi of ^{14}C-labeled substrate plus the appropriate amount of unlabeled substrate to yield the desired final concentration. Distribution ratios were determined after a 15 min incubation with AIB and a 5 min incubation with MAG, times which we have previously shown inform on the initial uptake of these solutes [2,9]. Distribution ratios and velocities of uptake were calculated and the data examined for significance as above. Results were analyzed as Hofstee or Lineweaver-Burk transformations and apparent transport parameters determined by linear regression analysis for each system.

Substrate uptake by brush-border vesicles from 7-day-old rats

Renal brush-border membrane vesicles were prepared from 7-day-old animals as described above, and the final membrane pellet was resuspended in THM buffer, pH 7.4 (2 mM Tris-Hepes + 100 mM mannitol) to a protein concentration of 0.7–0.85 mg protein/ml. Protein concentration was determined by the Bio-Rad method (Bio-Rad, Richmond, CA). Sodium-gradient stimulated uptake of 0.06 mM glycine and 0.06 mM glucose was examined with and without 4 mM SA, and compared to substrate uptake under Na^+-equilibrated conditions in the absence of SA. Sodium-gradient studies were carried out by addition of 480 μl of membrane suspension to vials containing a mixture of substrate (0.1 μCi ^{14}C-label + unlabeled material), NaCl and SA or THM to yield a final concentration of 0.06 mM substrate in 100 mM NaCl with or without 4 mM SA in total volume of 500 μl. Sodium-equilibrated studies were carried out by addition of membrane suspension preincubated in 100 mM NaCl to tubes containing only substrate as above. Incubations were carried out for 30 s, 1, 3, 5, 20 and 60 min, and stopped by rapid filtration as previously described [17]. Filters were air-dried overnight and radioactivity determined using a liquid scintillation counter. Uptake was calculated as nmol substrate per mg protein, and the data analyzed for significance as above.

Effect of SA on ^{22}Na uptake by brush-border membrane vesicles from 7-day-old rats

For these studies, membranes were prepared from whole kidneys of 7-day-old animals, as described previously [17]. The final membrane preparation contained approx. 0.7 mg protein/ml. Vesicles were suspended in 100 mM NaCl + THM buffer at pH 7.4 for 60 min at 25°C. To initiate incubations, 480 μl of vesicle suspension (approx. 0.35 mg protein) were added to a mixture containing 10 μl of 200 mM SA or 10 μl THM buffer, 5 μl of 6 mM glycine and 5 μl (0.5 μCi) of $^{22}NaCl$ to bring the total volume to 0.5 ml, with final concentration of 0.06 mM glycine and 100 mM NaCl with or without 4 mM SA. The entry of sodium was measured at different times from 15 s to 20 min, after which the incubation was stopped by rapid filtration of the mixture followed by washing the filter with 5 ml of ice-cold buffer containing 154 mM choline chloride, 100 mM mannitol and 2 mM Tris-Hepes, pH 7.4. Filters were air-dried overnight and assayed for radioactivity in a Beckman Gamma 4000 counter. Study of the effects of medium osmolarity on the entry of $^{22}NaCl$ into vesicles was carried out as previously described [14].

Fluorescence polarization studies

For these experiments, the lipid soluble fluorescence probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and DL-12-(9-anthroyl)stearic acid (12-AS) were employed and prepared according to established techniques [14,20]. All studies used either freshly prepared membranes, or membranes stored at −20°C for less than 2 weeks. Estimates of relative membrane fluidity were calculated following fluorescence polarization measurements using a Shimadzu RF-540 spectrofluorophotometer fitted with a thermoregulated sample chamber and automatic polarizers. In these experiments, the term 'fluidity' is used to describe the motional freedom of lipid soluble molecular probes (DPH, 12-AS), within a membrane bilayer. Determination of absolute fluidity is limited in an anisotropic membrane or liposome suspension (as opposed to a homogeneous, isotropic medium) because of the inability to accurately reproduce the three-dimensional structure of the hydrophobic bilayer. Therefore the steady-state fluorescence anisotropy r (the reciprocal of fluidity) is employed to estimate relative degrees of fluidity, following probe incorporation into the bilayer. Values for r were calculated (Ref. 20) from fluorescence polarization measurements using the equation

$$r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$$

where $I_{\parallel}$ and $I_{\perp}$ equal fluorescence intensities parallel and perpendicular, respectively, to excitation plane (excitation wavelength = 360 nm for DPH, 365 nm for 12-AS; emission wavelength = 430 nm for both probes). Scattered light plus ambient medium fluorescence contributed less than 5% to the total fluorescence intensity throughout the temperature range utilized in all studies. Fluorescence anisotropy in cell membranes of liposomes may be further resolved according to the Perrin relationship [21], a modified form of which may be written

$$r = r_{\infty} + (r_0 - r_{\infty})[t_c/(t_c + t_f)]$$

where r_0 represents the maximal limiting anisotropy in the frozen state (0.365 for DPH, 0.285 for 12-AS); t_c, the correlation time; and t_f, the mean lifetime of the excited state. The term r represents the static component of fluidity, or limiting hindered anisotropy, which is related to both bilayer molecular order and the degree of hindrance to probe rotation by packing of bilayer lipids (factors which cannot be distinguished under the present experimental conditions). Here, r_{∞} was calculated according to the equation proposed by Van Blitterswijk et al. [22], where $r_{\infty} = (4r/3) - 0.1$.

To determine the effects of known renal transport inhibitors on brush-border membrane lipid physicochemical properties, anisotropy measurements were performed following membrane incubation (5 min at 37°C) in either SA (4 mM final concentration) or maleic acid (6 mM); and, these values were compared to data obtained in the absence of transport inhibitors (phosphate-buffered saline, alone). This concentration of maleate was selected for study in order to compare its action on membrane fluidity with our earlier studies of age-related, maleate-induced membrane transport inhibition in the rat kidney [10]. Data were analyzed by one-way analysis of variance (ANOVA); and, if a level of significance of $p < 0.05$ was determined, results were finally compared utilizing Newman-Keul's multiple range testing [23].

Results

Effect of SA on sugar and amino acid uptake

(A) Tubules. Newborn tubules actively transport both MAG and AIB, evidenced by their ability to achieve a DR greater than 1 [10]. These capabilities develop at a separate age-dependent rate for each substrate [2,9,10]. Tubules from 7-day- and 14-day-old rats preincubated with 4 mM SA exhibited impaired concentrative uptake of both substrates within 15 min of incubation (Fig. 1). MAG and AIB uptake by newborn, 7- and 14-day tubules was significantly inhibited by SA, as shown in Table I. A comparison with the adult data indicated a more rapid rate of maturation of MAG than of AIB uptake, with SA exerting a greater absolute degree of impairment of MAG than of AIB uptake at all ages.

(B) Brush-border membrane vesicles. Since preparation of vesicles from newborn rat kidney requires free-flow electrophoresis necessitating exposure of the mem-

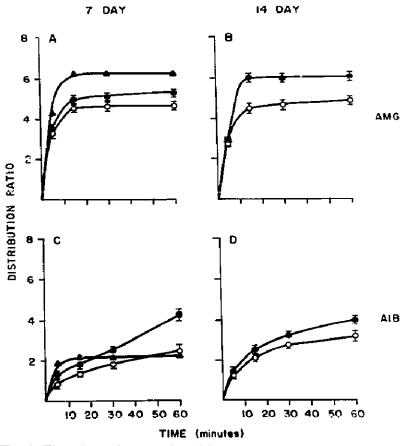

Fig. 1. Time-dependent uptake of 2 mM MAG (A and B) and 0.065 mM AIB (C and D) by 7- and 14-day-old rat renal tubules. Tubules were prepared and incubated as described in Methods. Each ml of suspension contained 0.1 μCi of ^{14}C-labeled substrate plus sufficient unlabeled material to give the desired final concentration. Data are the mean (±S.E.) of the distribution ratios ((cpm/ml intracellular fluid)/(cpm/ml medium)) versus the time of incubation (min). Each point represents at least nine separate determinations, incubated without (●) and with (○) 4 mM SA. Adult renal tubular uptake (▲) is shown for comparison.

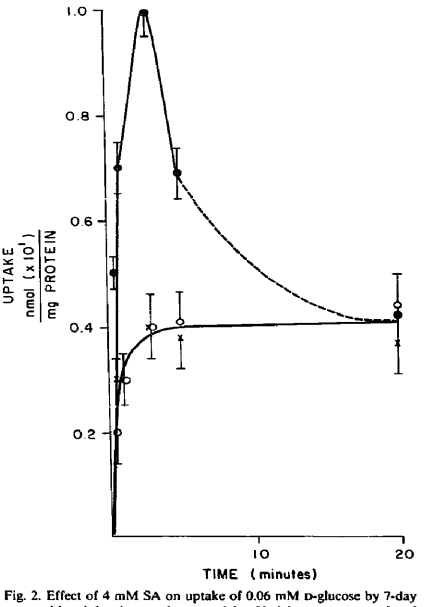

Fig. 2. Effect of 4 mM SA on uptake of 0.06 mM D-glucose by 7-day rat renal brush-border membrane vesicles. Vesicles were prepared and incubated as described in Methods. Incubations were performed at 25°C under 100 mM NaCl gradient conditions with (○) or without (●) 4 mM SA; in addition, uptake was studied under 100 mM NaCl-equilibrated conditions (×). Data shown represent the means (±S.E.) of at least 15 separate determinations, and are plotted as nmol uptake (×10¹) per mg protein versus incubation time in minutes.

branes to conditions differing from those used in preparation of adult membranes [24], we confined our observations to the 7-day-old animal. Uptake of 0.06 mM glucose by 7-day brush-border vesicles was found

TABLE I

Age-related effects of 4 mM SA on concentrative uptake of MAG and AIB by renal tubules

Tubules were made from animals of various ages and incubated under identical conditions described under Methods. Results were calculated as the distribution ratios ((cpm/ml intracellular fluid)/(cpm/ml medium)) and analyzed for significance by Student's *t*-test. Data for adult controls are presented for comparison.

Substrate	15-min Distribution ratio (±S.E.)			
	newborn $n=18$	7-day- $n=18$	14-day $n=30$	adult
MAG (2 mM)				
Control	1.49 (±0.055)	4.82 (±0.10)	5.98 (±0.06)	6.30 (±0.09)
+4 mM SA	1.20 (±0.021) **	4.59 (±0.05) *	4.60 (±0.03) **	
AIB (0.065 mM)				
Control	1.96 (±0.08)	1.82 (±0.25)	2.59 (±0.07)	2.31 (±0.11)
+4 mM SA	1.55 (±0.05) **	1.36 (±0.07) *	2.36 (±0.05) **	

* $P < 0.05$.
** $P < 0.01$.

to be sodium gradient-dependent and exhibited a typical 'overshoot' which was maximal at three minutes of incubation. Addition of 4 mM SA eliminated the overshoot and additionally reduced gradient-driven glucose uptake by the vesicles to a rate which was similar to that observed using Na^+-equilibrated vesicles (Fig. 2). Control, SA-treated and Na^+-equilibrated vesicles achieved the same equilibrium uptake value at 20 min of incubation ($p > 0.6$), indicating no significant difference of the intravesicular volume for solute distribution among the three groups.

In contrast, uptake of 0.06 mM glycine by 7-day membrane vesicles exhibited a different profile of solute uptake. Na^+-gradient stimulated glycine uptake did not result in an overshoot, although by 5 min of incubation, uptake in the presence of a Na^+ gradient significantly ($p < 0.001$) exceeded sodium-equilibrated vesicular solute entry. In the presence of 4 mM SA, initial Na^+-gradient uptake of glycine was the same as control, but plateaued after 1 min. Thus, later uptake by SA-treated vesicles remained substantially lower than that seen under both Na^+-gradient and Na^+-equilibrated conditions (Fig. 3).

Effect of SA on $^{22}NaCl$ entry in 7-day membrane vesicles

In order to examine the effect of SA on entry of $^{22}Na^+$ into renal brush-border membrane vesicles from 7-day-old rats, vesicles were preincubated in 100 mM NaCl to eliminate non-specific sodium-binding to the membrane as previously described [14]. Inclusion of unlabelled glycine during the incubations maximized cotransport-related sodium entry. Osmotic reactivity studies indicated a linear, inverse relationship between osmolarity and $^{22}Na^+$ entry, with $^{22}Na^+$ binding responsible for approximately 15% of total uptake in both control and SA exposed vesicles. The data, shown

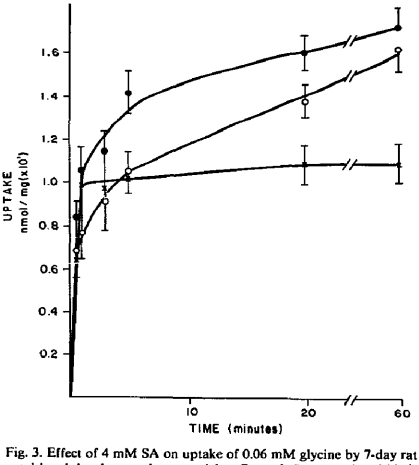

Fig. 3. Effect of 4 mM SA on uptake of 0.06 mM glycine by 7-day rat renal brush-border membrane vesicles. Control, SA-treated and Na^+-equilibrated data points are represented by the symbols ●, × and ○, respectively.

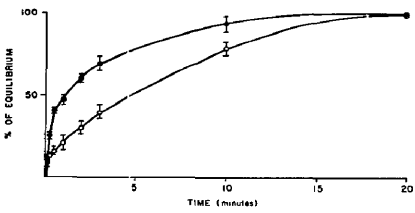

Fig. 4. Effect of 4 mM SA on $^{22}Na^+$-entry into 7-day brush-border membrane vesicles. Vesicles were prepared as described in Methods and preincubated in unlabeled 100 mM NaCl at 25°C for 60 min. Vesicles were then incubated with 0.5 μCi $^{22}NaCl$ with (○) and without (●) 4 mM SA. Data are shown as the mean percentage (±S.E.) of 20-min uptake versus time in minutes and represent at least ten separate determinations. 20-min uptake values did not differ significantly for control and SA-treated vesicles (2.43±0.122 vs. 2.37 ±0.201 pg/mg protein, respectively).

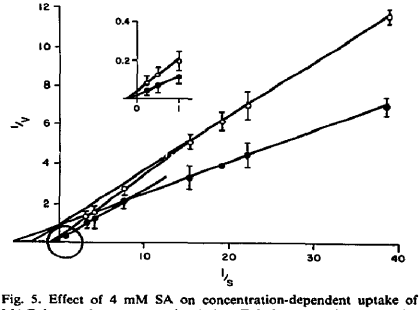

Fig. 5. Effect of 4 mM SA on concentration-dependent uptake of MAG by newborn rat renal tubules. Tubule suspensions were incubated for 5 min as described in Methods. Each ml of suspension contained 0.1 μCi ^{14}C-label, plus sufficient unlabeled material to give the desired final concentrations with (○) or without (●) 4 mM SA. Points shown represent reciprocals of the mean velocities of uptake (V = mmol/l intracellular fluid per 5 min), plotted versus the reciprocal of the various substrate concentrations (S, in mM). Data are from at least 12 separate determinations. Points shown in the inset represent low-affinity MAG uptake by control (●) and SA-treated (○) tubules.

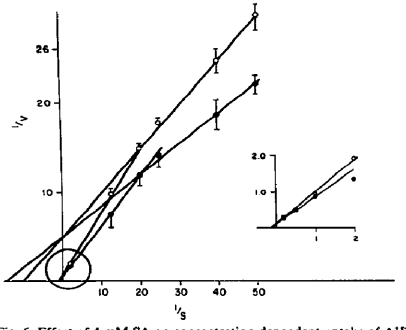

Fig. 6. Effect of 4 mM SA on concentration-dependent uptake of AIB by newborn rat renal tubules. Legend as for Fig. 5 (Note: V = nmol/l per 15 min).

in Fig. 4, indicate that vesicles reached 50% of equilibrium entry within the first 1.5 min of incubation in the absence of SA. In contrast, 4 mM SA-treated vesicles required almost 5 min to achieve 50% of steady-state uptake level. In addition, the rate of entry was significantly reduced ($p < 0.001$) compared to control vesicles by 15 s of incubation.

Effects of SA on concentration-dependent substrate uptake by newborn tubules

Concentration-dependent uptake of MAG by newborn tubules was observed to occur by means of two separate transport systems, as previously described [9]. Addition of 4 mM SA caused a significant reduction of uptake ($p < 0.02$) on the high-affinity system due to competitive inhibition (Fig. 5). Uptake on the low-affinity system, while not significantly reduced, was impaired due to non-competitive inhibition (inset, Fig. 5). AIB uptake was also shown to occur by two separate concentration-dependent systems in the newborn kidney. As with MAG, 4 mM SA significantly inhibited transport on the high-affinity system in a competitive manner ($p < 0.05$) and exerted a minor non-competitive inhibition of solute uptake by the low-affinity system

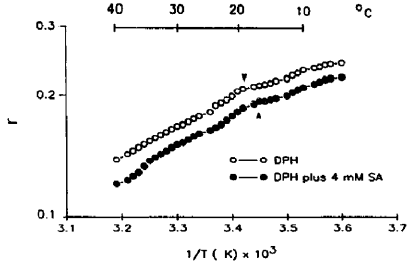

Fig. 7. Representative Arrhenius plot of the fluorescence anisotropy, r, for DPH of renal brush-border membrane vesicles from 7-day-old rats, incubated without (○) and with (●) 4 mM succinylacetone, according to techniques in Materials and Methods. Arrows indicate the thermotropic transition temperatures.

TABLE II

Effect of 4 mM SA on apparent transport parameters for MAG and AIB in newborn tubules

Tubules were incubated for 5 min (MAG), or for 15 min (AIB) at various substrate concentrations under identical conditions, as described in Methods. Velocities of uptake were calculated by linear regression analysis and the data represented as double-reciprocal plots. Each data point shown represents the mean (±S.E.) of the apparent transport parameters calculated from at least three separate experiments, each with four replicate determinations. Significance was determined by Student's t-test. n.s., not significant.

Substrate	K_{m1} (mM)	K_{m2} (mM)	(mmol/l per 5 min)	
			V_{max1}	V_{max2}
MAG				
Control	0.07 (±0.007)	2.0	0.20	5.56
+4 mM SA	0.13 (±0.011)	2.0	0.20	5.46
	$p < 0.02$	n.s.	n.s.	n.s.
	K_{m1} (mM)	K_{m2} (mM)	(mmol/l per 15 min)	
			V_{max1}	V_{max2}
AIB				
Control	0.20 (±0.032)	2.0	1.11	2.25
+4 mM SA	0.48 (±0.023)	2.0	1.11	1.7
	$p < 0.05$	n.s.	n.s.	n.s.

TABLE III

Fluorescence anisotropies for DPH (r_{20}) and 12-AS (r), of renal brush-border membranes incubated in phosphate-buffered saline (PBS) alone (Control) and in PBS containing either SA (4 mM) or maleic acid (6 mM)

Results expressed as mean±S.E. from at least five membrane preparations.

Temp. (°C)	Control	Maleic acid	SA
DPH (r_{20})			
15	0.218+0.011	0.200+0.012	0.159+0.015 **
25	0.179+0.011	0.163+0.010	0.136+0.010 **
37	0.136+0.006	0.128+0.006	0.108+0.012 *
12-AS (r)			
15	0.145+0.002	0.146+0.001	0.136+0.001 *
25	0.126+0.002	0.124+0.001	0.114+0.001 *
37	0.102+0.002	0.099+0.001	0.087+0.001 *

* $p < 0.05$, compared with Control and Maleic acid.

** $p < 0.02$, compared with Control and Maleic acid.

(Fig. 6). Apparent transport parameters for each substrate were calculated and are shown in Table II.

Fluorescence polarization studies

Table III lists values for both the DPH determined static (r_∞) and 12-AS dynamic (r) components of fluorescence anisotropy at 15, 25 and 37°C. Incubation of brush-border membranes with 4 mM SA resulted in a significantly increased membrane relative fluidity (i.e., decreased anisotropy) over all temperatures studied, when compared with membranes preincubated in either 6 mM maleic acid or buffer alone. Arrhenius plots of fluorescence polarization data, shown in Fig. 7, demonstrate the expected downward shift of the membrane lipid thermotropic transition temperature (21.4 ± 0.4 vs. 18.6 ± 0.5°C, control vs. SA; $p < 0.05$, $n = 5$) consequent to SA-induced membrane fluidization.

Discussion

Several earlier studies from our laboratory have documented developmental patterns of rat renal tubular uptake of methyl α-D-glucoside [9], glycine [2], α-aminoisobutyric acid [10] and proline [19,24]. These studies demonstrate: (1) separate age-related differentiation of transport systems for sugars and amino acids occurring at independent rates; (2) multiple uptake systems for both sugars and amino acids which are present in the newborn and change with age; and, (3) a marked difference in response of these systems in newborn and adult tubules to a wide variety of transport inhibitors. As a logical outgrowth of our recent interest in the actions of the inhibitor SA on adult rat renal tubular transport [11,13,14], we have now examined the developmental effects of this metabolite.

The significant impairment by SA of both MAG and AIB uptake in newborn renal tubules is somewhat unique, in view of the relative resistance of the newborn renal tubule to perturbations such as O_2 deprivation and maleic acid inhibition of membrane transport, compared to the adult [10]. These observations suggest that maleate and SA inhibit renal tubular transport through different mechanisms.

The present data describing glucose and glycine uptake by brush-border membrane vesicles prepared from 7-day-old animals correlate well with earlier observations in isolated tubules [2,9,10], as glucose uptake in these vesicles was found to be closer to the mature state than was glycine uptake. The uptake of each substrate was at least partially sodium gradient-dependent. The finding that SA dramatically depressed the rate of substrate-related Na^+ entry into these vesicles could explain in part the inhibitory effects of SA on both substrate transport systems: both substrate and sodium uptake could have been altered by SA-related membrane conformational changes at a site(s) other than the carriers, or SA may exert a direct effect on the membrane transport protein itself. Resolution of this question awaits studies of the effect of SA on Na^+–H^+ exchange and alternate sodium entry pathways. In either case, our present demonstration of substrate transport inhibition by SA in both newborn renal tubules and 7-day brush-border membrane vesicles stands in stark contrast to previous experience with maleic acid [10,26].

Multiple uptake systems exist for MAG and glycine in the newborn renal tubule [2,9]. Immature tubules evidence a low-affinity MAG uptake system, also exhibited by the adult, which results in increasing solute transport with age. The newborn tubule also has a unique high-affinity system which disappears as the nephron matures. Furthermore, the responses of the two newborn MAG uptake systems to metabolic inhibitors were different under identical experimental conditions, one exhibiting a change in K_m, the other in V_{max}. Thus, our observation that SA inhibited uptake only on the high-affinity system is consistent with our earlier findings in adult tubules [2,8,9]. While SA did not significantly impair MAG uptake on the shared, low-affinity system, the data suggest a non-competitive inhibitory effect as reported earlier in adult renal tubules [11] and brush-border membrane vesicles [14]. Similar results were obtained for the effects of SA on the two separate glycine uptake systems in the newborn. Competitive inhibition of glycine uptake was observed on the high-affinity system, while discernible but insignificant inhibition occurred on the low-affinity system, as seen for AIB in adult tubules [13] and for glycine in the adult brush-border membrane vesicle [14].

To determine whether the functional transport alterations induced by SA were related to biophysical changes of the renal brush-border membrane, fluorescence polarization studies were performed. Incubation of membranes with SA resulted in significantly enhanced relative fluidity, indicated by reductions of both r(12-AS) and r_∞ (DPH). Conversely, while SA exerted a marked fluidizing effect on brush-border membranes, maleic acid, another known renal transport inhibitor, did not modulate either anisotropy parameter. These results are particularly intriguing in view of recent studies which suggest direct interrelationships between membrane fluidity and the function of lipid-associated, intrinsic membrane proteins. For example, SA-mediated fluidization of renal proximal tubule brush-border membranes from adult rats was coincident with inhibition of both sugar and amino acid transport [14]. In another animal model, reduction of rabbit ileal basolateral membrane fluidity induced by estrogen was related to inhibition of Na^+/K^+-ATPase specific activity [20]. Furthermore, enzyme activity was restored to control levels following in vitro membrane fluidization. SA influences on rat renal cell membrane function, which include both competitive and non-competitive inhibi-

tion of solute transport in the 7-day animal and competitive inhibition of glycine and non-competitive inhibition of methyl α-D-glucoside uptake in the adult [14], suggest direct interactions between SA and these membranes. It is possible that SA interacts with membrane transport proteins to compete for association sites with solute (competitive inhibition). Alternatively, SA-mediated fluidity changes may influence the availability of solute binding sites or alter the mobility of carrier-solute complex through the membrane (non-competitive inhibition) [27,28].

The lack of effect of maleic acid on the fluidity of renal membranes from 7-day rats is not unexpected, as previous investigations have shown that this compound has no effect on solute transport by rat renal membrane vesicles [26]. Maleic acid inhibition of solute uptake by isolated renal tubules must be due to alteration of cellular metabolism and/or an effect on the transduction of energy for transport in this metabolically active system [29]. While SA also alters renal proximal tubule cell metabolism, these and previous studies [11,13,14] demonstrate a direct effect on physical properties of isolated brush-border membranes as well. Thus, the differences which we have consistently observed in the transport-related actions of SA and maleate on substrate uptake in tubules and brush-border membrane vesicles appear to be primarily related to the dual nature of SA's effects on renal tubular epithelium.

There is a direct, membrane-fluidizing effect of SA on the adult membrane which coincides with diminished Na^+ entry and substrate uptake [14]. This component necessarily contributes less to the overall picture in immature tissue, where membrane fluidity is known to be maximal [17]. On the other hand there is a metabolic action of SA resulting in reversible diminution of O_2 consumption by the adult tubule [11]. In newborn tubules, because of the pre-existing membrane fluidity in such tissue, the metabolic action of SA would be predictably greater in relative proportion to any membrane-fluidizing effect. This hypothesis is supported by the greater degree of SA-inhibition of substrate uptake in the adult tubule, where the membrane and metabolic actions are additive, than in newborn tubule where the latter is predominant. Studies of the effect of SA on O_2 consumption by newborn tubules are currently in progress.

We have proposed the use of succinylacetone to produce a physiologic animal model for study of the human renal Fanconi syndrome [14]. Our present observations strengthen the case for such a proposal for several reasons. The human Fanconi syndrome is a disorder usually associated with infancy and early childhood coinciding with development of tubular function [12]; an ideal model should logically provide the ability to perturb systems under study during their age-related transitional stages, as has been demonstrated here for SA. Such a model should also utilize physiologic substances and/or conditions for its production; our extensive investigations here and elsewhere [11,13,14] substantiate such a claim for SA. Data gathered from such a model should be consistent with clinical observations of the human disorder and help to directly explain the latter. Most especially, the inciting substance should affect both experimental model and human similarly; initial observations indicate that SA inhibits amino acid and sugar uptake by human renal cortical slices (unpublished data). Thus, our present results help provide a basis for understanding the age-related increases in glucosuria, aminoaciduria and natriuria seen in infants with the Fanconi syndrome.

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References

1 Segal, S., Rea, C. and Smith, I. (1971) Proc. Natl. Acad. Sci. USA 68, 327–376.
2 Roth, K.S., Hwang, S.M., London, J.W. and Segal, S. (1977) Am. J. Physiol. 233, F241–F246.
3 Reynolds, R., Roth, K.S., Hwang, S.M. and Segal, S. (1978) Biochim. Biophys. Acta 511, 274–284.
4 Chesney, R.W. and Jax, D.K. (1979) Pediatr. Res. 13, 854–860.
5 Chesney, R.W. and Jax, D.K. (1979) Pediatr. Res. 13, 861–867.
6 Segal, S. and Smith, I. (1969) Proc. Natl. Acad. Sci. USA 63, 926–933.
7 Segal, S. and Smith, I. (1969) Biochem. Biophys. Res. Commun. 35, 771–777.
8 Roth, K.S., Hwang, S.M., Yudkoff, M. and Segal, S. (1976) Life Sci. 18, 1125–1129.
9 Roth, K.S., Hwang, S.M., Yudkoff, M. and Segal, S. (1978) Pediatr. Res. 12, 1127–131.
10 Roth, K.S., Goldmann, D.R. and Segal, S. (1978) Pediatr. Res. 12, 1121–126.
11 Roth, K.S., Spencer, P.D., Higgins, E.S. and Spencer, R.F. (1985) Biochim. Biophys. Acta 820, 140–146.
12 Roth, K.S., Buckalew, V.M. and Chan, J.C.M. (1985) Current Nephrology, pp. 87–137, Year Book Med. Pub., Chicago.
13 Spencer, P.D. and Roth, K.S. (1987) Biochem. Med. Metab. Biol. 37, 101–109.
14 Spencer, P.D., Medow, M.S., Moses, L.C. and Roth, K.S. (1988) Kidney Int. 34, 671–677.
15 Booth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581.
16 Roth, K.S., Medow, M.S., Serabian, M.A. and London, J.W. (1982) Pediatr. Res. 16, 644–648.
17 Medow, M.S. and Segal, S. (1987) Biochem. Biophys. Res. Commun. 142, 849–856.
18 Goldmann, D.R., Schlesinger, H. and Segal, S. (1976) Biochim. Biophys. Acta 419, 251–260.
19 Medow, M.S., Roth, K.S., Goldmann, D.R. Ginkinger, K., Hsu, B.Y.-L. and Segal, S. (1986) Proc. Natl. Acad. Sci. USA 83, 7561–7564.
20 Schwarz, S.M., Bostwick, H.C. and Medow, M.S. (1988) Am. J. Physiol. 254, G687–694.

21 Van der Meer, B.W., Van Hoeven, R.P. and Van Blitterswijk, W.J. (1986) Biochim. Biophys. Acta 854, 38–44.
22 Van Blitterswijk, W.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332.
23 Zar, J.H. (1974) Biostatistical Analysis, pp. 151–163, Prentice Hall Inc., Englewood Cliffs, NJ.
24 Medow, M.S., Roth, L.S., Ginkinger, K. and Segal, S. (1983) Biochem. J. 214, 209–214.
25 Hwang, S.M., Serabian, M.A., Roth, K.S. and Segal, S. (1983) Pediatr. Res. 17, 42–46.
26 Reynolds, R., McNamara, P.D. and Segal, S. (1977) Life Sci. 22, 39–43.
27 Brasitus, T.A., Dudeja, P.K., Worman, H.G. and Foster, E.S. (1986) Biochem. Biophys. Acta 855, 16–24.
28 Shinitzky, M. and Inbar, M. (1976) Biochim. Biophys. Acta 433, 133–149.
29 Roth, K.S., Hwang, S.M. and Segal, S. (1976) Biochim. Biophys. Acta 426, 675–687.