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DO THIOSULFATE AND SULFATE UTILIZE THE SAME RENAL TRANSPORT PROCESS?

W. O. BERNDT

Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, N.H. 03755 (U.S.A.)

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SUMMARY

Several workers have discussed the possibility that SO_4^{2-} and $S_2O_3^{2-}$ utilize the same renal transport mechanism. For example in *Lophius americanus* BERGLUND AND FORSTER[1] concluded that there existed a single secretory system for the divalent anions, SO_4^{2-} and $S_2O_3^{2-}$. BERGLUND *et al.*[2] have proposed a similar interaction for the reabsorptive process. The present work was undertaken to examine whether or not the correspondence suggested by the *in vivo* studies was substantiated by *in vitro* studies. Several parameters were examined for both SO_4^{2-} and $S_2O_3^{2-}$ accumulation by rat renal slices and in most circumstances good agreement was not noted. For example, although the differences noted between rat and rabbit tissue were the same for both anions, and the estimated initial rates of uptake for SO_4^{2-} and $S_2O_3^{2-}$ were approximately the same, other similarities were less obvious. The apparent K_m values for the two uptake processes were different. The patterns of response to phlorizin in the presence of and absence of glucose were different for $S_2O_3^{2-}$ than those reported in the literature for SO_4^{2-}. Differences were also noted in the $S_2O_3^{2-}$ uptake at different temperatures compared to literature reports for SO_4^{2-}. Also $S_2O_3^{2-}$ uptake was greater in the absence of Na^+ than was SO_4^{2-} uptake. Finally, no effects of amino acids were noted on $S_2O_3^{2-}$ accumulation, while certain amino acids reduced SO_4^{2-} uptake.

INTRODUCTION

The complex nature of $S_2O_3^{2-}$–SO_4^{2-} interaction in renal tissue has been commented on by several workers[1-3]. BERGLUND AND FORSTER[1] described the occupation of a common transport site by both anions in aglomerular fish, *Lophius americanus*. In the dog a more complicated situation was reported by BERGLUND *et al.*[2]. These workers interpreted their data to indicate that SO_4^{2-} and $S_2O_3^{2-}$ were reabsorbed by a common carrier mechanism, although the affinities of the two ions for the carrier were quite different. Furthermore, while $S_2O_3^{2-}$ secretion was noted, no such activity was reported for SO_4^{2-} although SO_4^{2-} could interfere with $S_2O_3^{2-}$ secretion. Finally, MUDGE *et al.*[3] while recognizing both proximal tubular secretion

and reabsorption of $S_2O_3^{2-}$, found SO_4^{2-} interaction difficult to characterize in terms of usual secretory or reabsorptive phenomena.

Other characteristics of renal SO_4^{2-} transport by dog kidney have been reported, however. COHEN *et al.*[4] found glucose to lower the SO_4^{2-} T_m and that this effect was reversed by phlorizin. DEYRUP[5] obtained data in rat renal cortex slices similar to the intact dog data. She found that glucose depressed SO_4^{2-} accumulation and that this effect was reversed by phlorizin. Further, BERGLUND AND LOTSPEICH[6] noted that several amino acids reduced the sulfate T_m and again DEYRUP[7] has found a correlation between these *in vivo* data and those obtained with isolated tissues. Again utilizing rat renal cortex slices she found that certain amino acids, *e.g.* arginine and alanine, depressed SO_4^{2-} accumulation. The present study was intended to examine the relationship of SO_4^{2-} to $S_2O_3^{2-}$ transport in the rat renal cortex system. This was attempted by examining the effects on $S_2O_3^{2-}$ movement of factors known to affect SO_4^{2-} uptake. Also certain rate, temperature, and species comparisons were made.

METHODS

The technics utilized in this study were essentially the same as reported earlier[8]. For each experiment the kidneys from three to six rats were pooled and the cortical slices prepared free-hand. The incubations were performed in modified Krebs–Ringer phosphate solutions. In those instances where the Na^+ concentration was reduced significantly osmolality was maintained by the addition of sucrose. For example in the solution containing 40 mM K^+ and 15 mM Na^+ ("low Na^+"), the osmolality was maintained with sucrose. In the solution with 40 mM K^+, but not designated as low Na^+, the Na^+ concentration was 95 mM and no sucrose was present. In all incubations the final $S_2O_3^{2-}$ concentration was 10 μM and that of SO_4^{2-} 8 μM. In each case the ^{35}S-labeled material was used to monitor uptake by the slices. Unless otherwise stated the incubations were performed in a Dubnoff shaker or New Brunswick Metabolyte (80–90 oscillations per min) for durations of 2 h at 25° in the presence of 100 % O_2.

After the experimental period the tissues were blotted, weighed, homogenized in distilled water, and the proteins precipitated with trichloracetic acid. Medium samples were also treated with trichloroacetic acid and aliquots of both tissue and medium extracts were counted for radioactivity in a liquid scintillation spectrometer equipped with an external standard for quench correction. The data are expressed as the distribution ratio, *i.e.* the ratio of cell water radioactivity to that in the bathing solution. When appropriate statistical calculations were performed using Student's *t* test.

RESULTS

Effects of Na^+ and K^+ on SO_4^{2-} and $S_2O_3^{2-}$ uptake

In Table I are presented the steady-state distribution ratios for both divalent anions, as measured in the same experiments. The uptake of these ions was essentially the same in the presence of 5 mM K^+. With both anions the accumulation was more than doubled by elevation of the K^+ concentration to 40 mM. In the presence

TABLE I

COMPARISON OF UPTAKE OF $S_2O_3^{2-}$ AND SO_4^{2-} IN THE PRESENCE OF DIFFERENT Na^+ AND K^+ CONCENTRATIONS

Incubations were for 2 h at 25°. When the Na^+ concentration was 15 mM and the K^+ 40 mM, sucrose was added to maintain osmolality.

Bathing solution (mequiv/l)		*N*	*Distribution ratio ± S.E.*	
Na^+	*K^+*		*SO_4^{2-}*	*$S_2O_3^{2-}$*
140	5	4	3.48 ± 0.13	3.48 ± 0.25
100	40	4	8.09 ± 1.64	8.30 ± 0.35
15	40	4	7.18 ± 1.35	16.25 ± 1.55

of 40 mM K^+ and low Na^+, however, no similarity in the response of SO_4^{2-} and $S_2O_3^{2-}$ was noted. SO_4^{2-} accumulation was not affected by reduction of the bathing solution Na^+ concentration, whereas the $S_2O_3^{2-}$ uptake was again doubled.

Effects of various amino acids

Several amino acids were tested for their effects on $S_2O_3^{2-}$ accumulation (Fig. 1). For these experiments the tissues were incubated in a high K^+–low Na^+ medium in order to enhance accumulation. The expectation was that in the presence of an elevated $S_2O_3^{2-}$ distribution ratio a depressant effect of the amino acids would be detected more readily. Amino acids were tested in this system because several workers with both *in vivo* and *in vitro* procedures (*e.g.* DEYRUP[7], BERGLUND AND LOTSPEICH[6]) showed that some amino acids could interfere with SO_4^{2-} transport. In the present system no effect was noted on the $S_2O_3^{2-}$ uptake. This did not represent a peculiarity of this particular experimental system, however, because, as seen in the same figure, it was possible to block SO_4^{2-} uptake with L-arginine, D-alanine and taurine.

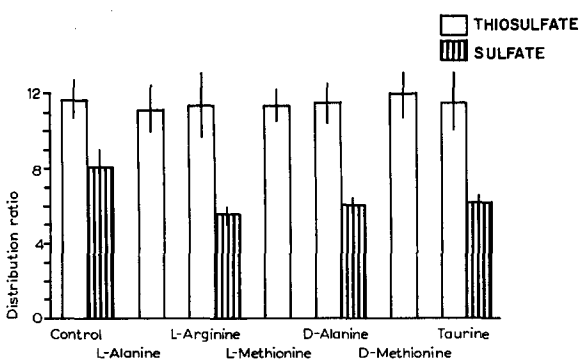
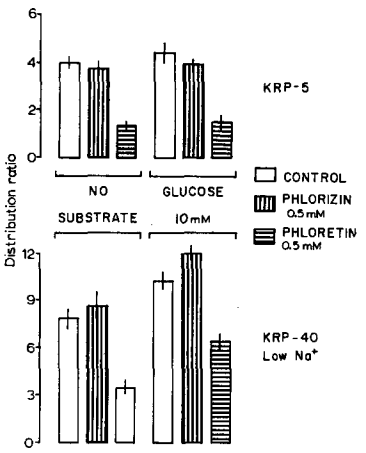


Fig. 1. The effect of several amino acids on the $^{35}S_2O_3^{2-}$ and $^{35}SO_4^{2-}$ distribution ratios. Each bar represents the mean and the vertical line the standard error for four experiments. The amino acids were employed in concentrations of 0.5 mM.

Interaction of glucose, phlorizin and phloretin

The effects of glucose, phlorizin (0.5 mM) and phloretin (0.5 mM) on $S_2O_3^{2-}$ accumulation in two bathing media are presented in Fig. 2. In the presence of a balanced salt solution, only phloretin altered the $S_2O_3^{2-}$ distribution ratio. The presence of glucose did not alter $S_2O_3^{2-}$ uptake nor did its presence affect the actions of either phlorizin or phloretin. The substrate and inhibitor effects were also the same in the presence of 40 mM K^+ and 15 mM Na^+.



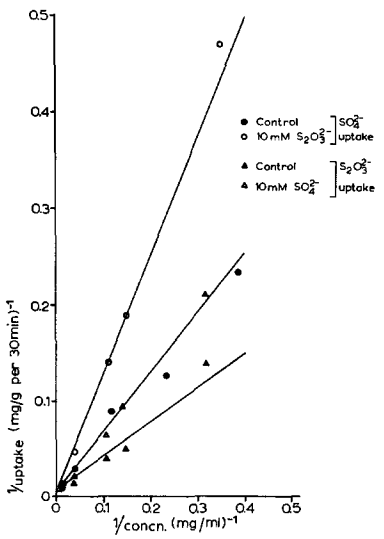


Fig. 2. The interactions of phlorizin, phloretin and glucose on $S_2O_3^{2-}$ uptake. The bars and vertical lines represent the means and standard errors for four experiments. See text for details.

Fig. 3. Doubly reciprocal plots for $^{35}S_2O_3^{2-}$ uptake in the presence and absence of SO_4^{2-} and for $^{35}SO_4^{2-}$ uptake in the presence and absence of $S_2O_3^{2-}$. Each point is the mean of triplicate experiments.

SO_4^{2-}–$S_2O_3^{2-}$ interaction

Doubly reciprocal plots for the uptakes of SO_4^{2-} and $S_2O_3^{2-}$ in the presence and absence of these ions as inhibitors are presented in Fig. 3. The straight-line relationships indicate the existence of Michaelis–Menten kinetics. The two reactions show different apparent K_m values, however, *i.e.* the slopes of the two control uptakes are different. The inhibitor studies show that each ion can competitively block the accumulation of the other.

No attempt was made to calculate specific K_m values or inhibitor constants for these reactions. These studies were performed in slices and the velocities were 30-min uptakes not initial uptake rates. Therefore under these experimental conditions it is not clear what these calculated quantitative values would mean. In any event the specific values are not needed to show that different K_m's exist and that competitive interactions are possible.

Other effects

The optimal temperature for SO_4^{2-} accumulation appears to be 37° (refs. 5, 7 and 9). In Table II are presented the steady-state distribution ratios for $S_2O_3^{2-}$ at

TABLE II

EFFECT OF TEMPERATURE ON STEADY-STATE ACCUMULATION OF $S_2O_3^{2-}$ BY RAT RENAL CORTEX SLICES

Incubations were performed in presence of 5 mM K^+.

Temp.	*Distribution ratio ± S.E.*	*N*
0°	2.43 ± 0.12	4
25°	3.47 ± 0.23	6
37°	0.84 ± 0.020	4

TABLE III

COMPARISON OF ESTIMATED INITIAL RATES OF UPTAKE OF SO_4^{2-} AND $S_2O_3^{2-}$ IN PRESENCE OF DIFFERENT CONCENTRATIONS OF K^+

The uptakes were performed at 25° and the rates were estimated from samples collected during the first 20 min of incubation. Each value is the mean of three experiments.

K^+ concn. (mM)	*Uptake (μg/g wet tissue per min)*	
	SO_4^{2-}	*$S_2O_3^{2-}$*
5	0.067	0.070
40	0.173	0.125

TABLE IV

COMPARISON OF $S_2O_3^{2-}$ AND SO_4^{2-} ACCUMULATION BY ISOLATED RABBIT AND RAT RENAL CORTEX SLICES

The steady-state uptakes were performed in the presence of 5 mM K^+ at 25°.

	Distribution ratio ± S.E.		*N*
	SO_4^{2-}	*$S_2O_3^{2-}$*	
Rat	2.10 ± 0.13	2.56 ± 0.29	4
Rabbit	1.02 ± 0.01	1.52 ± 0.07	4

three temperatures. Maximal uptake was found at 25° with somewhat less uptake at 0°. At 37°, however, the accumulation was inhibited markedly. The distribution ratio at this temperature was slightly less than one, *i.e.* net uptake of $S_2O_3^{2-}$ was not seen. Furthermore, these data stand in constrast to the earlier report by DEYRUP[7] where $S_2O_3^{2-}$ uptake was examined briefly at both 37° and 25° and no difference was reported. The nature of this discrepancy is not clear, but may related to differences in protocol, *e.g.* the concentration of $S_2O_3^{2-}$ employed in the two studies was different.

In Table III are presented data which summarize the estimated initial rates of uptake for both anions in the presence of two K^+ concentrations. In both cases the rate of ion accumulation by the tissue was enhanced by elevation of the bathing

solution K^+, although the effect on $S_2O_3^{2-}$ appeared to be somewhat less than that on SO_4^{2-}.

DEYRUP[5] gave a detailed description of species differences associated with SO_4^{2-} uptake by rat renal tissue. In the present study only rabbit and rat slices have been compared with respect to SO_4^{2-} and $S_2O_3^{2-}$ uptake and the data are presented in Table IV. The SO_4^{2-} data agreed well with those reported earlier[5] in that rat renal slices accumulated SO_4^{2-}, but rabbit slices did not. Thiosulfate was also accumulated by rat tissue, and in this case the rabbit also demonstrated the ability to take up the anion. The extent of $S_2O_3^{2-}$ accumulation by rabbit tissue was less than that noted for rat, but was significantly greater than the SO_4^{2-} uptake.

DISCUSSION

Evidence has been presented[7,8] to indicate that $S_2O_3^{2-}$ can reduce SO_4^{2-} accumulation and that SO_4^{2-} can block $S_2O_3^{2-}$ uptake. In each case a 100–1000-fold excess of one ion was required to block the uptake of the other. The mechanism of these inhibitory effects is not clear. Most of the data presented here, however, support the position that SO_4^{2-} and $S_2O_3^{2-}$ do not utilize the same transport mechanism. In any event there are more experimental situations reported here in which the two ions behave differently than in which their behavior is the same.

Although, none of the effects of the amino acids on SO_4^{2-} uptake observed in this laboratory or as reported by others[7] were very dramatic, it is clear from the present experiments that there were no amino acid effects on $S_2O_3^{2-}$ uptake. Similar discrepancies exist with respect to the effects of glucose and phlorizin. DEYRUP[5] found an unequivocal, albeit modest, reduction of SO_4^{2-} accumulation by glucose which was reversed by phlorizin. It was proposed that the glucose effect was somehow mediated through glucose accumulation by the tissue and phlorizin acted by inhibiting glucose uptake. Whatever the mechanisms involved in the case of SO_4^{2-}, it is clear that with $S_2O_3^{2-}$ these cannot be invoked. Glucose was never found to retard the accumulation of $S_2O_3^{2-}$. In the presence of either a low or a high concentration of K^+ no glucose effect was noted (ref. 8 and Fig. 2). Phlorizin was found to be without effect in any of the experimental situations tested. The effect of phloretin on $S_2O_3^{2-}$ uptake, however, agrees with what was reported for SO_4^{2-} (ref. 7), *i.e.* in both cases the accumulation of label was reduced. These data taken together are a powerful argument for significant differences in the SO_4^{2-} and $S_2O_3^{2-}$ transport processes. It is not possible, however, to determine whether the processes differ at the level of substrate–carrier interaction or with respect to some other aspect of the transport system, *e.g.* the energy supply.

Another difference in the transport characteristics was revealed by examining the effects of temperatures. Earlier work by DEYRUP[5,7,9] indicated that SO_4^{2-} uptake by rat renal cortex slices was optimal at about 37°. On the basis of data presented here it is clear that this is not the case for $S_2O_3^{2-}$. Of the three temperatures examined in this study only 37° resulted in a marked depression of $S_2O_3^{2-}$ accumulation although the uptake at 0° was significantly lower than that at 25°. The nature of the relatively high steady-state $S_2O_3^{2-}$ distribution ratio at 0° is not clear from these data. It is possible that this accumulation represents an adsorption process at this

lower temperature. Passive adsorption as a possible mechanism of uptake at the higher temperatures has been eleminated, however.

Furthermore, the uptake of these ions at different Na^+ and K^+ concentrations also demonstrates the dissimilarity of the transport systems. The steady-state distribution ratios for $S_2O_3^{2-}$ and SO_4^{2-} were increased by about the same amount in response to a 35 mequiv/l reduction in the bathing solution Na^+ concentration and a similar elevation in the K^+ concentration. When the Na^+ concentration was reduced further, however, the two transport systems responded differently, *i.e.* the $S_2O_3^{2-}$ uptake was doubled while the SO_4^{2-} uptake was unaffected. Although these data indicate a fundamental difference in the two systems, no mechanism for the effects of Na^+ and K^+ can be offered.

No attempt was made to do a detailed study of the species differences noted with $S_2O_3^{2-}$ uptake. However, in the two species examined it does appear that a significant difference exists between the uptake of SO_4^{2-} and $S_2O_3^{2-}$. With respect to SO_4^{2-}, these data do agree with those reported by DERYUP[5]. That is the rat slices accumulated SO_4^{2-} while the rabbit slices did not. The rat slices also accumulated $S_2O_3^{2-}$ with the steady-state distribution ratio somewhat greater than for SO_4^{2-}. In the rabbit slices, however, $S_2O_3^{2-}$ was accumulated to a significantly greater extent than was SO_4^{2-}. Again, therefore, a difference appears to exist between the two anion transport systems.

In summary, it appears that despite the fact that SO_4^{2-} and $S_2O_3^{2-}$ can interfere each other's accumulation, these two ions are taken up by rat (possibly rabbit) slices by different mechanisms. These uptake processes respond differently to certain amino acids, glucose, phlorizin, temperatures, and inorganic electrolytes. The specific mechanisms involved, however, can not be defined from these data.

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