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STIMULATORY AND INHIBITORY EFFECTS OF SULFHYDRYL REAGENT ON *p*-AMINOHIPPURIC ACID TRANSPORT BY ISOLATED RENAL TUBULES

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Summary

Isolated tubules from rabbit kidney cortex were treated with several different sulfhydryl reagents in an attempt to determine whether sulfhydryl groups are involved in organic acid transport. Disulfide reagents such as sodium tetrathionate and 6,6'-dithionicotinic acid were found to exert a biphasic effect on *p*-aminohippuric acid transport, i.e. transient stimulation followed by inhibition. In contrast, treatment of tubules with the mercaptide-forming reagent, *p*-chloromercuribenzoate, caused only inhibition of organic acid transport. Treatment of tubules with reductants such as dithiothreitol or mercaptoethanol blocked the stimulatory effect of tetrathionate without affecting the inhibitory effect of this oxidant. The inhibition caused by *p*-chloromercuribenzoate, however, was largely reversible when tubules were treated with reductants. The results suggest that the renal organic acid transport system contains sulfhydryl groups and that its activity is increased when some of these groups are oxidized.

Introduction

The importance of SH groups in the transport of sugars and amino acids across biological membranes has been well established [1—6]. The use of different sulfhydryl reagents has helped to identify and characterize these groups. With regard to the effects of sulfhydryl reagents on organic acid transport, the only well documented studies were concerned with diuretic and non-diuretic mercurials. In these observations the depression of renal organic acid transport [7—9] was attributed to an effect on tissue metabolism rather than a direct

Abbreviation: PCMB, *p*-chloromercuribenzoate.

action on organic acid transport. In this report we have compared the effects of a mercurial reagent to those of disulfide reagents on the accumulation of *p*-aminohippuric acid by isolated rabbit renal tubules.

The results indicated that, in function of time, the action of the disulfide reagents, sodium tetrathionate and 6,6′-dithionicotinic acid was biphasic i.e. a transient stimulation followed by an inhibition of *p*-aminohippuric acid uptake, whereas with the mercurial, *p*-chloromercuribenzoate (PCMB), only the latter action could be detected. Our experiments also demonstrated that the activation of *p*-aminohippuric acid transport by tetrathionate could be blocked by the reductants dithiothreitol and mercaptoethanol. This report presents these results and other experiments describing the different effects of PCMB and tetrathionate on electrolyte and organic acid transport in isolated renal tubules.

Materials and Methods

Chemicals

Bacterial collagenase was purchased from Worthington, bovine serum albumin and dithiothreitol from Calbiochem, sodium tetrathionate was from Fluka and was recrystallized as described by Gilman [10]; PCMB was from Aldrich. 6,6′-dithionicotinic acid was from Newcell Biochemicals, *p*-amino-[1-^{14}C]hippuric acid, 20—40 Ci/M, and [*Me*-^{3}H]inulin 5 Ci/mM, were from New England Nuclear Corporation. All other chemicals were reagent grade and were obtained from Merck-Darmstadt.

Methods

Suspensions of rabbit renal cortical tubules were prepared according to Burg and Orloff [11]. The acetate Ringer solution used for the preparation and incubation of the isolated tubules contained: 135 mM NaCl; 10 mM KCl, 10 mM sodium acetate, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.5 mM $CaH_4(PO_4)_2$, 20 mM Tris · HCl, (pH 7.4 at 25°C), 5 mM glucose, with 1.5% wt./vol bovine serum albumin.

Treatment of the tubules with the different sulfhydryl reagents (tetrathionate, 6,6′-dithionicotinic acid, PCMB) was performed in conical centrifuge tubes as follows: the investigated chemical was incubated in acetate Ringer solution containing 3% bovine serum albumin for 30 min to allow time for an equilibrium of the reagent with the albumin to be reached. The reaction was started by adding an equal volume (usually 5 ml) of bovine serum albumin-free suspension of tubules. Incubation was carried out for the stated time with a gas phase containing 100% O_2. The reaction was terminated by dilution and centrifugation at 50 × *g* for 2 min. Control tubules were treated as above but omitting the sulfhydryl reagent. In experiments designed to test the reversibility of the effects of sulfhydryl reagents on *p*-aminohippuric acid and electrolyte transport, treated and control tubules were incubated with and without 84 mM mercaptoethanol or 20 mM dithiothreitol for 5 min in an atmosphere of 100% N_2. The reaction was ended as described above. All treatments were carried out at 25°C. The subsequent incubations with *p*-aminohippuric acid were performed in vessels described by Burg and Orloff [11] at 25°C with a gas phase containing 100% O_2. The final suspension contained from 2 to 5% of tissue (wet wt./vol.). The final concentration of *p*-amino[1-^{14}C]hippuric acid in the bathing medium

was 0.15 mM. [*Me*-^{3}H]inulin was also added giving a final radioactivity of about 1 μCi/100 ml. After completion of incubation, the suspension was transferred to a special centrifuge tube maintained at 0°C [12]. Centrifugation was performed for 5 min at 5000 × *g* in a refrigerated centrifuge. The supernate was decanted, the superficial layer of cells removed by suction, and the remaining tissue plugs were weighed before and after dessication at 70°C.

In experiments designed to examine the effects of sulfhydryl reagents on *p*-aminohippuric acid uptake in the absence of active electrolyte transport slices of rabbit renal cortex were used in place of isolated tubules as attempts to prepare (Na^+, K^+)-depleted and ouabain-poisoned separated tubules resulted in fragmentation of most of the cells. Slices of cortex approximately 0.4 mm thick were prepared with a Stadie-Riggs microtome. They were leached for 80 min at 25°C in the following leaching solution: 145 mM choline chloride, 20 mM Tris · HCl (pH 7.3) and 2 mM ouabain, as recently reported [13]. Treatment of these ouabain-poisoned slices with sulfhydryl reagents was performed for the times indicated at 25°C in the leaching solution. The reaction was terminated by transferring the suspension of slices to a beaker covered with surgigal gauze. The slices were washed free of reagents by pouring leaching solution over the pieces of tissue. Control ouabain-poisoned slices were treated as above but omitting the sulfhydryl reagent. Tissues were rapidly blotted and 4—6 slices (30—60 mg wet weight) were transferred for 20 min at 25°C to 5 ml of *p*-amino-[1-^{14}C]hippuric acid (0.14 mM) and [*Me*-^{3}H] inulin containing solution identical in composition to that used for leaching or modified by replacing the choline chloride with an equivalent amount of NaCl. The gas phase was 100% N_2. Preparation of the samples for analysis was similar for the separated tubules and slices [14].

The radioactivities of ^{14}C and ^{3}H of the tissue extracts and of the incubation media were measured simultaneously, each in duplicate, in a Mark 1 Nuclear Chicago liquid scintillation counter, as previously described [15]. The *p*-aminohippuric acid in the incubation media was measured using the method of Smith et al. [16]. Na^+ and K^+ analyses were performed using a flame photometer (Netheler and Hinz, Gmbh, Hamburg, G.F.R.). Tissue contents of *p*-aminohippuric acid, water, and electrolytes were corrected for extracellular contamination by using the inulin space measured for each sample [14].

The accumulation of *p*-aminohippuric acid was usually expressed as the distribution ratio, which is the ratio of organic acid concentration in cellular water to that in the medium.

Results were expressed as means ± S.D. Statistical analyses were performed by the Student's *t* test.

Results

Effect of SH reagent concentration on p-aminohippuric acid and electrolyte transport

The results in Figs. 1 and 2 compare the effects of two sulfhydryl reagents, PCMB and tetrathionate, on *p*-aminohippuric acid and electrolyte transport by isolated renal tubules. With a reagent exposure time of 20 min followed by a 30 min incubation period with *p*-aminohippuric acid alone, both the mercaptide-

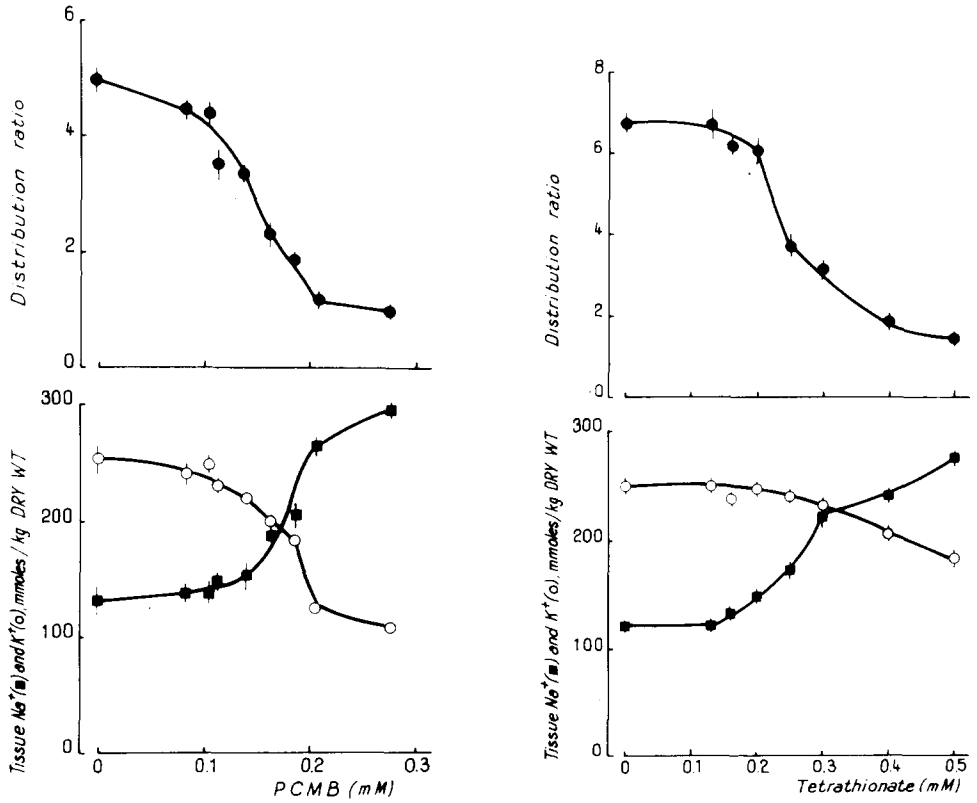

Fig. 1. Inhibition of *p*-aminohippuric acid and electrolyte transport as a function of PCMB concentration. Tubules were treated with the specified concentrations of PCMB for 20 min as indicated under Materials and Methods. Incubation with labeled *p*-aminohippuric acid was for 30 min. Each point is the mean of three experiments. Vertical lines denote ± 1 S.D. Absence of the vertical line indicates that S.D. was negligible.

Fig. 2. Inhibition of *p*-aminohippuric acid and electrolyte transport as a function of tetrathionate concentration. Experimental conditions were identical with those in Fig. 1 except that tetrathionate replaced PCMB.

forming reagent, PCMB, and the oxidizing reagent, tetrathionate, caused inhibition of *p*-aminohippuric acid and electrolyte transport. Figs. 1 and 2 show that whereas the shape of the curve relating organic acid transport to sulfhydryl reagent concentration was comparable whether PCMB or tetrathionate was used, a different pattern emerged for the effects of these two reagents on electrolyte content. As can be seen in Fig. 1, the net loss of K^+ induced by PCMB was almost exactly compensated by the increase in tissue Na^+, whereas tetrathionate caused a net gain of Na^+, 2—5-times higher than the tissue K^+ loss. At least in part, this difference may be accounted for by the enlargement of the intracellular space which was significantly higher with tetrathionate than with PCMB (results not shown).

In these experiments, the sulfhydryl reagents were added to the Ringer solution containing bovine serum albumin 30 min prior to the addition of tubules. Since these reagents react with albumin, the concentration of reagent to which

the tubules were actually exposed was less than the total PCMB or tetrathionate concentration of the medium.

Effect of SH reagent exposure time on p-aminohippuric acid transport

The effects of different exposure times of tubules to various sulfhydryl reagents on steady state *p*-aminohippuric acid uptake were investigated. The most striking feature with the disulfide oxidizing agents (tetrathionate and 6,6′-dithionicotinic acid) was the biphasic action, while with PCMB the inhibitory action only could be detected. As illustrated in Fig. 3, a significant stimulation could be noted after a 2-min exposure to either 0.25 mM ($P < 0.0005$) or 0.50 mM of tetrathionate ($P < 0.005$). The maximum stimulatory effect of tetrathionate was between 3 and 5 min of exposure. Then this transient stimulation disappeared and was followed by inhibition of organic acid transport. With 0.5 mM 6,6′-dithionicotinic acid, the kinetic response was also clearly biphasic. In contrast with PCMB (0.28 mM) inhibition of organic acid uptake occurred even with time exposures as short as 18 s. An 18 or 60 s exposure to a lower level of PCMB (0.08 mM) had no effect on *p*-aminohippuric acid uptake. With this lower concentration of the mercurial, however, increasing the duration of exposure caused slight but statistically significant inhibition of organic acid transport ($P < 0.05$).

The stimulatory effect of tetrathionate on *p*-aminohippuric acid transport was also detected when the organic acid uptake period was reduced. Results in

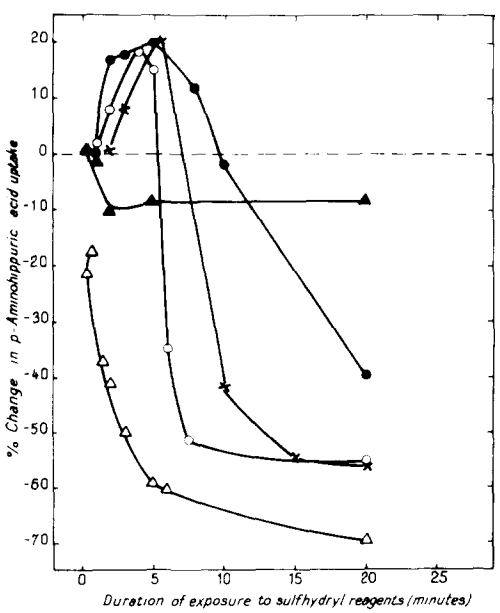

Fig. 3. Stimulation and inhibition of *p*-aminohippuric acid uptake with various sulfhydryl reagents. Tubules were treated with various sulfhydryl reagents with paired controls for the times shown on abscissa. Incubation with labeled *p*-aminohippuric acid was for 30 min. Each point represents the mean per cent change in organic acid uptake for three or four paired suspensions. Concentration of reagent: 0.25 mM tetrathionate, (●); 0.50 mM tetrathionate, (○); 0.50 mM 6,6.-dithionicotinic acid, (X); 0.08 mM PCMB, (▲); 0.28 mM PCMB, (△).

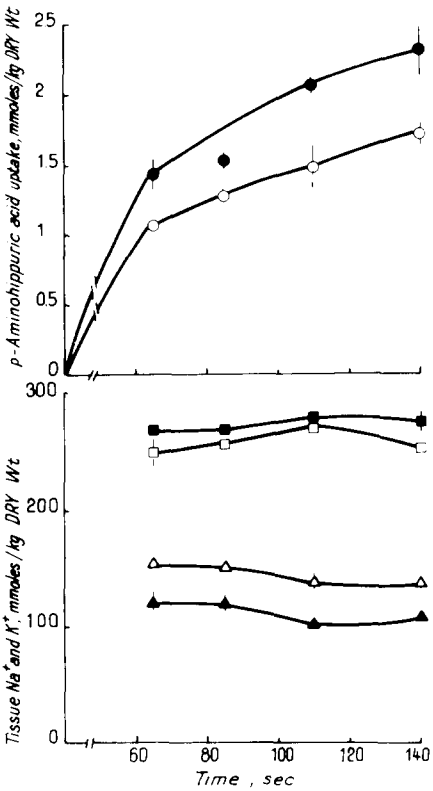

Fig. 4. Stimulation of *p*-aminohippuric acid and Na^+ transport by tetrathionate. Tubules were treated with or without 0.25 mM tetrathionate for 5 min at 25°C. Control and treated tubules were then incubated with labeled *p*-aminohippuric acid for the times shown on abscissa. Organic acid uptake: control, (○); tetrathionate, (●). Tissue K^+: control, (□); tetrathionate, (■). Tissue Na^+: control, (△); tetrathionate, (▲). Each symbol represents the mean of three epxeriments and vertical lines denote ± 1 S.D. When S.D. is not shown it did not extend beyond the symbols.

Fig. 4 demonstrate that tetrathionate caused statistically significant stimulation of *p*-aminohippuric acid accumulation ($P < 0.01$) at the earliest time period that can be reasonably investigated with the method used, i.e., 65 s. Of interest is the observation that the stimulatory effect of tetrathionate on *p*-aminohippuric acid uptake was associated with reduction in tissue Na^+ content. For all the incubation times investigated tetrathionate treated tubules contained significantly less Na^+ ($0.0005 < P < 0.0025$) than paired control tubules. The data of Fig. 4 also show that K^+ tissue content is higher in tetrathionate-treated tubules compared to controls. This increase, however, was not large enough to demonstrate statistically. These effects occurred without significant changes in the cell water content. Mean tissue water content was 2.02 ± 0.067 and 2.04 ± 0.051 kg/kg dry wt. for control and treated tubules, respectively. Each value represents the mean ± S.D. of 12 tubule suspensions.

Reversibility of the effects of SH reagents on p-aminohippuric acid transport

Thiol compounds reversed the stimulation of *p*-aminohippuric acid uptake

TABLE I

REVERSIBILITY OF THE STIMULATORY EFFECT OF TETRATHIONATE ON *p*-AMINOHIPPURIC ACID TRANSPORT BY ISOLATED RENAL TUBULES

Treatments were as indicated under Materials and Methods. Incubations were carried out with 0.15 mM labeled *p*-aminohippuric acid for the specified times. Each value represents the mean ± S.D. of either 6 (Expt. 1) or 3 (Expts. 2 and 3) experiments.

Expt. No.	First treatment (5 min)	Second treatment (5 min)	Incubation time (min)	Distribution ratio	Percent change
1	None	None	2	2.88 ± 0.28	
	None	Dithiothreitol, 20 mM	2	2.71 ± 0.13	−6
	Tetrathionate, 0.25 mM	None	2	3.89 ± 0.18 *	35
	Tetrathionate, 0.25 mM	Dithiothreitol, 20 mM	2	3.13 ± 0.15	9
2	None	None	30	4.82 ± 0.12	
	None	Dithiothreitol, 20 mM	30	4.48 ± 0.16 **	−7
	None	Merceptoethanol, 84 mM	30	4.25 ± 0.21 **	−11.8
	Tetrathionate, 0.5 mM	None	30	6.05 ± 0.16 *	25.5
	Tetrathionate, 0.5 mM	Dithiothreitol, 20 mM	30	4.79 ± 0.09	−0.6
	Tetrathionate, 0.5 mM	Mercaptoethanol, 84 mM	30	4.87 ± 0.07	1
3	None	None	30	4.97 ± 0.34	
	None	Dithiothreitol, 20 mM	30	5.08 ± 0.24	2.3
	None	Mercaptoethanol, 84 mM	30	5.19 ± 0.40	4.4
	Tetrathionate, 0.25 mM	None	30	5.93 ± 0.43 **	19.2
	Tetrathionate, 0.25 mM	Dithiothreitol, 20 mM	30	5.07 ± 0.21	2
	Tetrathionate, 0.25 mM	Mercaptoethanol, 84 mM	30	4.75 ± 0.09	−4.4

* $P < 0.0005$.

** $P < 0.05$ vs. respective control values.

by tetrathionate. Dithiothreitol and mercaptoethanol almost completely abolished the tetrathionate effect (Table I). This reversal could not be attributed on an inhibition caused by the thiol compounds, since these reagents exerted either no statistically significant effect on *p*-aminohippuric acid transport (Expts. 1 and 3) or an inhibitory effect that was small compared to the stimulatory effect of tetrathionate (Expt. 2).

Thiol compounds substantially antagonized the depressive effect of PCMB on organic acid and electrolyte transport. As summarized in Table II, both mercaptoethanol and dithiothreitol reversed by approximately 70% the inhibition of organic acid uptake by PCMB. The reversal of the effect on electrolyte transport was more marked, 93% for K^+ and 86% for Na^+. In contrast, thiol compounds did not antagonize substantially the depression of *p*-aminohippuric acid uptake by tetrathionate (Table II). The only apparent effect of thiol treatment was the fall in tissue Na^+ content.

Effects of SH reagents on p-aminohippuric acid uptake by (Na^+, K^+)-depleted and ouabain-poisoned slices

As recently reported [13], the imposition of an extracellular to intracellular Na^+ gradient in the absence of active electrolyte transport induces a stimulation of *p*-aminohippuric acid uptake. The data in Table III show that the Na^+-dependent anaerobic entrance of *p*-aminohippuric acid is inhibited significantly by PCMB. In Expt. 1 a 20 min exposure to a 0.4 and 2.2 mM level of PCMB

TABLE II

EFFECTS OF THIOL COMPOUNDS ON PCMB OR TETRATHIONATE INHIBITION OF *p*-AMINOHIPPURIC ACID AND ELECTROLYTE TRANSPORT BY ISOLATED RENAL TUBULES

Treatments were as described under Materials and Methods. Incubation with labeled *p*-aminohippuric acid was for 30 min. Each value represents the mean ± S.D. of three experiments.

Exp. No.	First treatment (20 min)	Second treatment (5 min)	Distribution ratio	Tissue K^+ (mmol/kg dry wt.)	Tissue Na^+ (mmol/kg dry wt.)
1	None	None	3.58 ± 0.17	233 ± 4	174 ± 10
	None	Dithiothreitol, 20 mM	3.79 ± 0.10	243 ± 3	175 ± 3
	None	Mercaptoethanol, 84 mM	3.51 ± 0.07	236 ± 3	182 ± 7
	PCMB, 0.19 mM	None	1.10 ± 0.01 *	135 ± 4 *	288 ± 11 *
	PCMB, 0.19 mM	Dithiothreitol, 20 mM	2.58 ± 0.03 *	217 ± 3 **	199 ± 6 ***
	PCMB, 0.19 mM	Mercaptoethanol, 84 mM	2.60 ± 0.09 *	215 ± 4 **	211 ± 9 **
2	None	None	8.54 ± 0.20	304 ± 11	147 ± 3
	Tetrathionate, 0.5 mM	None	2.37 ± 0.09 *	205 ± 4 *	364 ± 20 *
	Tetrathionate, 0.5 mM	Dithiothreitol, 20 mM	2.93 ± 0.14 *	205 ± 1 *	270 ± 8 *
	Tetrathionate, 0.5 mM	Mercaptoethanol, 84 mM	2.70 ± 0.06 *	226 ± 7 *	288 ± 5 *

* $P < 0.0005$.

** $P < 0.005$.

*** $P < 0.05$ vs. respective control values.

TABLE III

EFFECTS OF PCMB AND TETRATHIONATE ON *p*-AMINOHIPPURIC ACID UPTAKE BY (Na^+, K^+)-DEPLETED AND OUABAIN-POISONED SLICES

Sodium- and potassium-depleted and ouabain-poisoned slices were treated for 20 min in the leaching solution alone (control) or for the specified times in the leaching solution containing PCMB (0.4—2.2 mM), or tetrathionate (0.5—2 mM). When treatment with tetrathionate was less than 20 min, slices were maintained for an appropriate time in the leaching solution alone before adding the disulfide reagent so that the overall treatment period was exactly 20 min. Incubation with labeled *p*-aminohippuric acid was for 20 min; the gas phase was 100% N_2. Details are given under Materials and Methods. Each value represents the mean ± S.D. of 4—10 experiments.

Expt. No.	Treatment	Incubation conditions	Distribution ratio
1	None, control	145 mM choline chloride	0.64 ± 0.02
	PCMB		
	0.4 mM, 20 min	145 mM choline chloride	0.54 ± 0.05 *
	2.2 mM, 20 min	145 mM choline chloride	0.48 ± 0.01 *
	None, control	145 mM NaCl	1.79 ± 0.18
	PCMB		
	0.4 mM, 20 min	145 mM NaCl	1.07 ± 0.07 **
	2.2 mM, 20 min	145 mM NaCl	0.55 ± 0.04 **
2	None, control	145 mM choline chloride	0.57 ± 0.04
	Tetrathionate		
	0.5 mM, 3 min	145 mM choline chloride	0.60 ± 0.05
	0.5 mM, 15 min	145 mM choline chloride	0.67 ± 0.07 *
	None, control	145 mM NaCl	1.57 ± 0.13
	Tetrathionate		
	0.5 mM, 3 min	145 mM NaCl	1.41 ± 0.14
	0.5 mM, 5 min	145 mM NaCl	1.46 ± 0.06
	0.5 mM, 15 min	145 mM NaCl	1.48 ± 0.07
3	None, control	145 mM choline chloride	0.52 ± 0.03
	Tetrathionate		
	0.5 mM, 5 min	145 mM choline chloride	0.54 ± 0.04
	0.5 mM, 15 min	145 mM choline chloride	0.57 ± 0.06 ***
	0.5 mM, 20 min	145 mM choline chloride	0.48 ± 0.04 **
4	None, control	145 mM choline chloride	0.54 ± 0.01
	Tetrathionate		
	2 mM, 20 min	145 mM choline chloride	0.48 ± 0.02 *
	None, control	145 mM NaCl	1.32 ± 0.08
	Tetrathionate		
	2 mM, 10 min	145 mM NaCl	1.20 ± 0.05 ***
	2 mM, 20 min	145 mM NaCl	1.15 ± 0.03 **

* $P < 0.02$.
** $P < 0.002$.
*** $P < 0.05$ vs. respective controls.

decreased the uptake by 40 and 70%, respectively in the presence of NaCl, and only by 16 and 25%, respectively in the presence of choline chloride. This table (Expts. 2 and 3) also shows that treatment of ouabain-poisoned slices with 0.5 mM tetrathionate caused significant stimulation of *p*-aminohippuric acid uptake in a choline-medium at 15 min. This stimulation ranged from 10 to 17% and was followed by inhibition when the length of exposure to the agent was increased. The lack of tetrathionate stimulation in the Na^+ medium could be explained by the fact that the Na^+ gradient increased *p*-aminohippuric acid

transport by about 3-fold, thus rendering any further small effect undetectable. With a higher level of tetrathionate (Expt. 4), a modest but significant inhibition of organic acid transport was detected in both the presence or absence of external Na^+.

Discussion

The present work demonstrates that *p*-aminohippuric acid transport in isolated renal tubules can be either stimulated or inhibited by sulfhydryl reagents depending on their nature and on the experimental conditions. With 6,6′-dithionicotinic acid and tetrathionate, which are known to oxidize specifically -SH groups [17—19], we observed a stimulation of organic acid uptake followed by an inhibition whereas with the mercaptide-forming reagent, PCMB, only the latter effect could be detected. The tetrathionate stimulation and the PCMB inhibition could be reversed with dithiothreitol or mercaptoethanol but the inhibitory effect of tetrathionate could not be reversed. Several facts suggest that the stimulatory action of 6,6′-dithionicotinic acid and tetrathionate is due to effects of these reagents at the level of the tubular surface membrane. Firstly, stimulation of uptake occurs for very short time exposures of the tubules to these reagents. Secondly, 6,6′-dithionicotinic acid [20], and probably also tetrathionate [21], does not readily enter cell membranes, and thirdly, this effect is detected at the earliest time period that can be investigated with the method used (65 s) suggesting that this oxidant increases the initial rate of *p*-aminohippuric acid entry.

These results support the view that the *p*-aminohippuric acid transport system or part of it might exist in two states: a normal -SH form and an activated disulfide-sulfhydryl form. This view, which can account for the various observed effects, is presented schematically in Fig. 5. In this scheme we con-

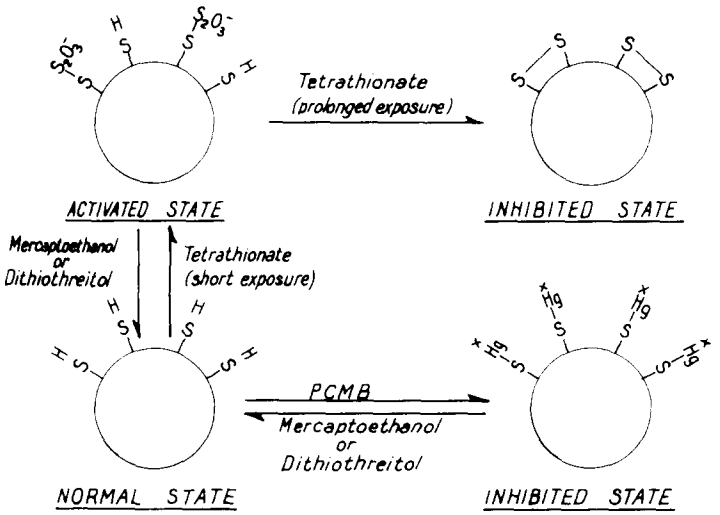

Fig. 5. Hypothetical scheme to explain the effects of various sulfhydryl reagents on transport of *p*-aminohippuric acid by isolated renal tubules. For details, see Discussion.

ceive that the basal state of the organic acid transport system in isolated tubules is in a reduced form since its activity is inhibited by the mercaptide-forming agent PCMB. The finding that the inhibitory process is largely reversed as a result of treatment with reductants suggests that the main action of PCMB is not secondary to irreversible membrane changes or to reactions of this agent with groups other than sulfhydryl. Tetrathionate [18,19] and possibly 6,6′-dithionicotinic acid [17] oxidize protein -SH groups through a two-step reaction. Initially, tetrathionate reacts with superficial -SH groups to form sulfenyl thiosulfates which subsequently react with less accessible -SH groups to form symmetrical disulfides. The first step is a reversible process. The second step, in contrast, has been found to be either hardly reversible [22] or not at all [19]. Thus, the reversible conversion of the basal form of the organic acid transport system to the activated form is most likely to be due to formation of mixed disulfides. Hence, activation could be attributed to limited oxidation of the basal state of the organic acid transport system. The irreversible inhibition of *p*-aminohippuric acid transport caused by prolonged tetrathionate exposure, on the other hand, could be accounted for by permanent membrane structural alterations secondary to extensive formation of disulfide bonds.

Czech et al. [4] reported that oxidants stimulated the glucose transport system in brown fat cells whereas reductants inhibited this transport. In the present study reductants do not affect control *p*-aminohippuric acid transport rates. However, all the reagents used, including the reductants, were not present during the organic acid uptake period in order to avoid possible intereference between these reagents and *p*-aminohippuric acid uptake. The possibility exists, therefore, that -SH groups formed as a consequence of treatment with reductants are reoxidized by O_2 and traces of heavy metal ions [23] during the organic acid uptake period, thus masking a possible inhibitory effect.

The data obtained with reductants, oxidants and PCMB are consistent with the notion that a sulfhydryl-disulfide interchange might play a role in the regulation of organic acid transport comparable to the mechanism first proposed by Czech et al. [4] for hexose transport by brown fat cells. Although the available data are apparently consistent with the above concept they do not exclude other hypotheses. The present experiments show that the effects of the sulfhydryl reagents on *p*-aminohippuric acid uptake are associated with parallel effects on electrolyte tissue content. Since Na^+ and K^+ ions are required for organic acid transport in mammalian kidney cortex [24—29], it is possible that the observed effects on *p*-aminohippuric acid uptake may be indirect and mediated by changes in active electrolyte transport. Against this are the findings of the experiments using ouabain-poisoned slices: PCMB and a high concentration of tetrathionate were found to diminish, and a short exposure to a low concentration of tetrathionate was found to stimulate organic acid uptake (Table III). Nevertheless the magnitude of the responses to the sulfhydryl reagents was diminished in comparison to the results obtained using metabolically active isolated tubules. Hence part of the effects of these agents could be mediated by an alteration in (Na^+, K^+)-ATPase but it is also known that in preparations using slices rather than tubules [11,30,31] the rate of *p*-aminohippuric acid uptake and its sensitivity to various agents is decreased.

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