Biochimica et Biophysica Acta, 598 (1980) 357—365

BBA 78703

EFFECTS OF SCN^- AND NO_3^- ON ORGANIC ANION TRANSPORT IN RABBIT KIDNEY CORTICAL SLICES

JAMES M. GOLDINGER *, BETH DEW. ERASMUS, YONG K. SONG, FRANCIS J. KOSCHIER and SUK KI HONG

Department of Physiology, School of Medicine, State University of New York at Buffalo, Buffalo, NY 14214 (U.S.A.)

(Received August 31st, 1979)

Key words: Anion transport; Thiocyanate; Nitrate; p-Aminohippurate accumulation; (Kidney cortex)

Summary

1. The effects of replacement of Cl^- by either SCN^- or NO_3^- on the accumulation of *p*-aminohippurate, the efflux of pre-accumulated *p*-aminohippurate and kinetics of *p*-aminohippurate uptake were investigated in the rabbit kidney cortical slice.

2. The total replacement of Cl^- in the incubation medium with SCN^- decreased the 60-min slice-to-medium concentration ratio (S/M) of *p*-aminohippurate by 75% and that with NO_3^- by 40%.

3. The decrease in *p*-aminohippurate accumulation by the inorganic ions was found to be specific for organic anion transport since the uptake of the organic cation, tetraethylammonium, was not influenced by inorganic ions.

4. The influence of NO_3^- on *p*-aminohippurate uptake was fully reversible: however, the effect of SCN^- could only be partially reversed.

5. Both SCN^- and NO_3^- significantly increased the K_m value but had no significant effect on the V value of the *p*-aminohippurate uptake process.

6. These findings suggest that both SCN^- and NO_3^- are competitive inhibitors of *p*-aminohippurate transport and, also, that SCN^- appears to bind to a membrane component involved in the transport of *p*-aminohippurate.

Introduction

In studies of renal organic anion transport, inorganic anions, such as NO_3^- and SCN^-, are sometimes used to replace chloride in the supporting medium. The

* To whom correspondence should be addressed.

rationale usually given for this maneuver is that these replacement anions are more permeable than chloride. Little is known about what further alterations in cell function these substitutions may cause: for example, a reduction in *p*-aminohippurate accumulation in the renal slice occurs with alterations in medium inorganic anion composition which can be correlated with the position of the anion in the lyotropic series [1]. The mechanism of this action is not understood. We have recently reported that a disulfonic acid stilbene (4-acetamido-4′-isothiocyano-2,2′-disulfonic stilbene) inhibits *p*-aminohippurate transport in the cortical slice, presumably by virtue of its ability to react electrostatically with amino groups at or near the *p*-aminohippurate site [2]. Since it has been reported that inorganic anions of the lyotropic series are increasingly amino-reactive [3,4], it seemed reasonable to postulate that this property may be responsible for inorganic anion inhibition of *p*-aminohippurate uptake. The aim of this investigation was to systematically examine the effects of substituting NO_3^- and SCN^- for Cl^- on the influx and efflux of *p*-aminohippurate from the rabbit renal cortical slice. Kinetic analysis shows that the effects of SCN^- and NO_3^- on *p*-aminohippurate accumulation are competitive, specific and reversible. Whilst several mechanisms are possible, the data are consistent with the postulate that these inorganic anions act in the same manner as 4-acetamido-4′-isothiocyano-2,2′-disulfonic stilbene.

Methods

Renal slice incubation procedure

The kidneys of New Zealand white rabbits (killed by cervical dislocation) were removed promptly and perfused through the renal artery with ice-cold modified Cross-Taggart medium to remove as much blood as possible. The composition of the medium was as follows (in mM): NaCl, 140; KCl, 10; $CaCl_2$, 1.5; sodium phosphate, 5; at pH 7.4, 25°C.

A Stadie-Riggs microtome was used to make thin (0.4—0.5 mm) renal cortical slices which were stored briefly in cold Cross-Taggart medium. Two slices, each weighing 75—100 mg, were randomly selected from the storage vessel and placed for incubation in flask containing 10 ml of the Cross-Taggart medium. Duplicate flasks were prepared for each experimental point. The incubation was carried out at 25°C in a shaking water bath run at 100 cycles/min. 100% oxygen saturated with water vapor was aspirated through each flask. Unless otherwise stated, the incubation was carried out for 1 h.

Experimental protocol

The first series of experiments was designed to determine the effect of substituting NO_3^- or SCN^- for Cl^- in the incubation medium on *p*-aminohippurate and tetraethylammonium transport by renal cortical slices. All or part of the NaCl and KCl in the medium was replaced by sodium or potassium nitrate or thiocyanate, along with tracer amounts of the ^{14}C-labeled organic ion (New England Nuclear, Boston, MA or Amersham, Arlington Heights, IL). Generally, medium concentrations of *p*-aminohippurate and tetraethylammonium were 75 and 10 μM, respectively (exceptions will be noted). In two experiments, slice uptake of $-S^{14}CN^-$ (Amersham) was measured to determined the steady state distribution of this ion.

The kinetics of *p*-aminohippurate uptake were studied in a second series of experiments. The rate of *p*-aminohippurate uptake was determined during a 10 min incubation at various *p*-aminohippurate concentrations (25—1000 μM) in the presence and absence of 150 mM NO_3^- or 30 mM SCN^-. Preliminary studies showed that *p*-aminohippurate uptake is reasonably linear for at least 40 min under these circumstances [5]. Kinetic parameters (K_m and V for *p*-aminohippurate and K_i for SCN^- and NO_3^-) were derived from conventional Lineweaver-Burk plots.

In the third series, the uptake of *p*-aminohippurate was measured in slices which were preincubated for 30 min in Cross-Taggart medium containing Cl^-, NO_3^- or SCN^- as the principal inorganic anion but without an organic ion. The tissues were subsequently blotted and transferred to a fresh medium containing Cl^-, SCN^-, or NO_3^- and labeled *p*-aminohippurate for a 60 min uptake determination.

In a fourth set of experiments the influence of NO_3^- and SCN^- on the efflux of pre-accumulated *p*-aminohippurate was studied. Renal slices were preincubated for 1 h in a medium containing labeled *p*-aminohippurate (75 μM) and the appropriate inorganic anion. Following a 20 s rinse in *p*-aminohippurate-free medium to remove *p*-aminohippurate adhering to the tissue surface, the slices were transferred at 1-min intervals through a series of 15 chambers containing *p*-aminohippurate-free Cross-Taggart medium at 25°C and aspirated with oxygen. The medium contained Cl^-, NO_3^- or SCN^- as the principal inorganic anion. The rate of efflux was determined over a 15 min period by a method described by Ross et al. [6].

In the last series, the effects of substituting SCN^- for Cl^- on inulin space, tissue water content and intracellular Na^+ and K^+ concentrations were studied. Slices were incubated for 60 min in a medium containing [^{14}C]inulin (0.05 μCi/ml, New England Nuclear) and various concentrations of SCN^- (0—150 mM). Following incubation, each slice was blotted and weighed before and after being dried overnight in an oven (95°C) for the determination of the tissue water content. The dried tissue was extracted in 0.1 N HNO_3 for 48 h and analyzed for [^{14}C]inulin and electrolytes (flame photometry).

The oxygen consumption of renal slices was measured at 25°C using a Clark-type O_2 electrode (YSI model 53) mounted in an air-tight fluid-filled chamber. The initial rate of O_2 consumption (μl O_2/h per mg wet tissue) by a single slice (approx. 100 mg) was computed from measurements of decreasing O_2 tension over the course of a 15 min incubation in 4 ml of Cross-Taggart solution previously equilibrated with 100% oxygen.

Analytical methods

Following incubation, the slices were quickly removed, blotted, weighed and homogenized (100—150 mg slice in 4 ml of 1 N NaOH) with a tissue homogenizer (Polyscience Model RZR-10). Aliquots of the incubation medium and the solubilized tissue were counted for ^{14}C activity in a Beckman LS350 liquid scintillation spectrometer using an external standard channels ratio for quench correction. Quench correction curves were obtained for all solutions used in the experiments. Most uptake data are presented as the slice-to-medium (S/M)

ratio, i.e., the tissue radioactivity (dpm/g wet tissue) divided by that of the medium (dpm/ml medium).

Results

The influence of the progressive substitution of Cl^- with NO_3^- or SCN^- on organic ion uptake in the rabbit cortical slice is shown in Fig. 1. Complete substitution with NO_3^- decreased the S/M of *p*-aminohippurate from 6.80 (control) to 4.10 ($P < 0.005$), whilst that with SCN^- decreased the S/M from 6.12 to 1.48 ($P < 0.005$). In contrast to this inhibitory effect of SCN^- on *p*-aminohippurate uptake, no inhibition of tetraethylammonium uptake was found and, in fact, a slight increase of tetraethylammonium uptake was observed in the presence of SCN^-. These results confirm those of Taggart et al. [1] and, in addition, demonstrate that the effect of these substitute anions is specific for the organic anions (*p*-aminohippurate), having no inhibitory effect on the net accumulation of the organic cation, tetraethylammonium.

The kinetics of *p*-aminohippurate uptake in the presence of SCN^- were examined by means of a Lineweaver-Burk plot of the initial rate of *p*-aminohippurate uptake as a function of *p*-aminohippurate concentration in a medium containing either 150 mM Cl^- or 30 mM SCN^-. Thiocyanate is shown to be a competitive inhibitor of *p*-aminohippurate transport (Fig. 2). The K_m for *p*-aminohippurate was 0.80 mM and the K_i for SCN^- was 53 mM. Similar kinetics were observed when *p*-aminohippurate transport was measured in a medium in which 150 mM Cl^- was replaced by 150 mM NO_3^-. The K_i for NO_3^- inhibition of *p*-aminohippurate uptake was 171 mM.

In experiments to test reversibility of these effects, slices were preincubated in a medium containing Cl^-, SCN^- or NO_3^- (150 mM) for 30 min and then transferred to an incubation medium containing Cl^-, SCN^-, or NO_3^- (150 mM) to determine *p*-aminohippurate uptake for 60 min. The results from these

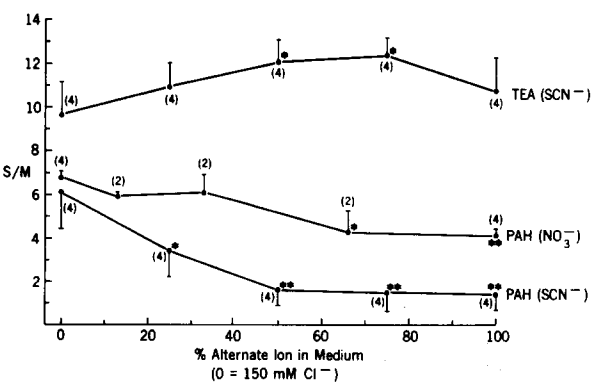

Fig. 1. Slice-to-medium concentration ratio (S/M) of tetraethylammonium (TEA) and *p*-aminohippurate (PAH) in the presence of varying concentrations of NO_3^- and SCN^- as replacements for Cl^- in Cross-Taggart media. Results shown are means ± S.D. for the number of experiments shown in parentheses beside each point. Data were analyzed using the unpaired Student's *t*-test and probabilities shown are for comparison to the 0% substitute values. * $P < 0.05$, ** $P < 0.005$.

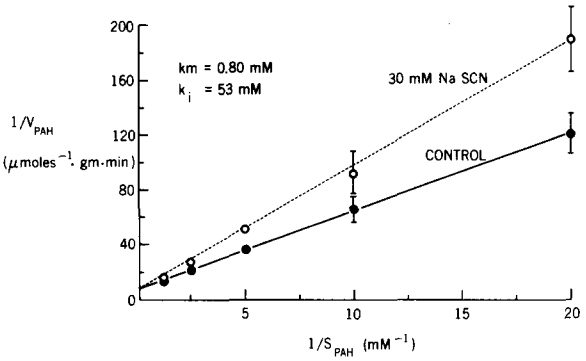

Fig. 2. Lineweaver-Burk analysis of the inhibitory effect of 30 mM SCN^- as a Cl^- replacement on *p*-aminohippurate transport. Initial rate of transport (V) was determined during a 10 min incubation in media containing varying *p*-aminohippurate concentration (S). Values are the mean ± S.E. of three paired experiments.

experiments are shown in Table I. As can be seen in this table (series A), no matter whether the preincubation medium contained NO_3^- or Cl^-, if subsequently the slice was transferred to a Cl^--containing medium, no deviation from the control S/M for *p*-aminohippurate was observed. On the other hand, if the slices were transferred to a NO_3^--containing medium for incubation, the S/M declined significantly regardless of the anion composition of the preincubation medium. Similarly, if the preincubation medium contained Cl^- or SCN^- and the transfer was made into an SCN^--containing medium for incubation (series B), the S/M decreased to 1.64 or 1.33 ($P < 0.005$), respectively. Perhaps the most interesting observation was that, if slices were preincubated in an SCN^--containing medium and then transferred to a medium containing Cl^- for

TABLE 1

UPTAKE OF *p*-AMINOHIPPURATE BY RENAL CORTICAL SLICES PREINCUBATED AND INCUBATED IN MEDIA OF VARYING ANIONIC COMPOSITION

Slice-to-medium concentration (S/M) ratio of *p*-aminohippurate after a 60-min incubation in Cross-Taggart medium containing NO_3^- or SCN^- as a replacement for Cl^-. Prior to this incubation, slices were preincubated for 30 min in a medium containing Cl^-, NO_3^- or SCN^-. The values shown are means ± S.D. Data were analyzed using the unpaired Student's *t*-test and the probabilities shown are compared to the corresponding control in each series, i.e., chloride in both the preincubation and incubation.

Series	Inorganic anion composition		S/M	*n*
	Preincubation	Incubation		
A	Cl^-	Cl^-	6.91 ± 0.28	3
	Cl^-	NO_3^-	4.43 ± 0.36 **	3
	NO_3^-	Cl^-	5.73 ± 1.22	3
	NO_3^-	NO_3^-	3.75 ± 0.40 **	3
B	Cl^-	Cl^-	8.45 ± 2.22	4
	Cl^-	SCN^-	1.64 ± 0.19 **	4
	SCN^-	Cl^-	5.20 ± 1.80 *	4
	SCN^-	SCN^-	1.33 ± 0.34 **	4

* $P < 0.05$.
** $P < 0.005$.

incubation, the S/M decreased to 5.20 from a control value of 8.45 ($P < 0.05$). Assuming complete equilibration with SCN^- during the preincubation, the transfer into the Cl^- medium represents minimally a 100-fold dilution (0.1 g slice per 10 ml of bathing solution) of any SCN^- retained in the slice during transfer. According to our dose vs. response curve (Fig. 1), this concentration of SCN^- (approx. 1.5 mM) would be insufficient to produce the observed effect if SCN^- were acting as an instantaneously reversible inhibitor. The rapidity of SCN^- redistribution was studied in a separate experiment by following the uptake of labeled SCN^-. SCN^- reached a steady-state value ($S/M \approx 0.5$) within 1 min. Therefore, we have assumed that very little SCN^- remains in the slice during incubation.

The efflux of *p*-aminohippurate from slices preincubated and preloaded with ^{14}C *p*-aminohippurate in media containing Cl^-, NO_3^- or SCN^- and subsequently transferred to media containing Cl^-, NO_3^- or SCN^- is reported in Table II. The apparent rate constant (k in min^{-1}) has been shown to be independent of the initial concentration of *p*-aminohippurate in the slice over a wide range [7]. This was reaffirmed in these experiments. For example, when *p*-aminohippurate was preloaded in NO_3^- or SCN^-, much less *p*-aminohippurate accumulated in the slice than when preloaded in a Cl^- medium. However, no effect on k was observed when tissue was transferred to the Cl^- media for efflux measurements. In contrast, if slices were transferred to a medium containing NO_3^- or SCN^- for efflux measurement, an increase in k was observed.

Two sets of experiments were performed to determine what effect, if any, the replacement of Cl^- by SCN^- had on general cell function and viability. Table III demonstrates the influence of progressive replacement of Cl^- in the medium by SCN^- on cell H_2O content (%), extracellular space (S/M inulin) and

TABLE II

EFFLUX OF *p*-AMINOHIPPURATE FROM CORTICAL SLICES PRETREATED AND MEASURED IN MEDIA OF VARYING ANIONIC COMPOSITION

Efflux rate constants of *p*-aminohippurate from slices preincubated and preloaded in Cl^-, NO_3^- or SCN^- (as indicated) for 60 min followed by incubation in Cl^-, NO_3^- or SCN^- during which the efflux was measured. Values shown are means ± S.D. Data were evaluated using the unpaired Student's *t*-test. Probabilities indicated are for comparisons to the corresponding control, i.e., chloride in the preincubation and efflux medium.

Series	Inorganic anion composition		k (min^{-1})	n
	Preincubation and uptake medium	Efflux medium		
A	Cl^-	Cl^-	0.058 ± 0.016	3
	Cl^-	NO_3^-	0.111 ± 0.014 *	3
	NO_3^-	Cl^-	0.059 ± 0.005	3
	NO_3^-	NO_3^-	0.112 ± 0.034 *	3
B	Cl^-	Cl^-	0.040 ± 0.004	6
	Cl^-	SCN^-	0.082 ± 0.003 **	4
	SCN^-	CL^-	0.043 ± 0.009	4
	SCN^-	SCN^-	0.104 ± 0.014 **	6

* $P < 0.05$.
** $P < 0.001$.

TABLE III

Total tissue water content, inulin space and intracellular electrolyte composition (Na^+ and K^+) of slices incubated in media containing varying SCN^- concentrations as a substitute for Cl^-. 100% SCN^- = 150 mM SCN^-. Values are means ±S.E. of three experiments. No value was significantly different from 0% SCN^-.

% SCN	Tissue H_2O %	S/M inulin	$[Na^+]_i$ (μEquiv./g dry wt.)	$[K^+]_i$ (μEquiv./g dry wt.)
0	74.08 ± 1.02	0.339 ± 0.03	74.5 ± 11.9	237.0 ± 36.0
25	76.80 ± 3.45	0.314 ± 0.02	84.6 ± 12.0	249.0 ± 30.3
50	76.00 ± 0.67	0.314 ± 0.01	103.0 ± 10.5	241.3 ± 23.9
75	72.50 ± 3.37	0.349 ± 0.04	94.0 ± 11.8	207.3 ± 56.7
100	76.00 ± 1.88	0.337 ± 0.05	107.9 ± 14.9	243.4 ± 21.7

cellular Na^+ and K^+ concentrations. No significant change in these parameters was noted. Tissue O_2 consumption also was unaffected by replacement of Cl^- with SCN^-. Control O_2 consumption was 2.81 ± 0.29 μl O_2/mg per h compared to 2.91 ± 0.29 μl O_2/mg per h for tissue incubated in a medium with total SCN^- replacement ($n = 4, P > 0.05$).

Discussion

NO_3^- and SCN^- are shown to be competitive inhibitors of *p*-aminohippurate transport and it can be assumed from the kinetic data that at least one site of interaction between *p*-aminohippurate and NO_3^- or SCN^- is at the membrane, since in these 10-min uptake studies little *p*-aminohippurate accumulation in the tissue occurs. Furthermore, the effect of these ions appears to be specific for organic anions, assuming *p*-aminohippurate is a prototype, because no inhibitory effect was observed on the uptake of the organic cation, tetraethylammonium. In fact, a significant increase in tetraethylammonium accumulation in the presence of SCN^- was observed, which may be related to the fact that SCN^- is more permeable than Cl^-. A biphasic dose response was observed (Fig. 1): however, no further experiment was performed to investigate this effect.

It has been suggested that incubation with SCN^- and NO_3^- results in tissue deterioration [8], but our data uncovered no evidence of this. O_2 consumption, cell electrolytes and cell H_2O used as indices of cell integrity were not significantly altered by SCN^- and NO_3^- incubation. There may have been a tendency for cell $[Na^+]$ to increase; however, no statistical significance could be established. Furthermore, in the case of NO_3^- in the uptake and efflux experiments and SCN^- in the efflux experiments, the influence of these ions was completely reversible, i.e., preincubation in either SCN^- or NO_3^- had no effect on efflux if subsequently transferred to a Cl^--containing medium (Tables I and II). However, a residual effect of SCN^- on *p*-aminohippurate uptake was observed after preincubation in SCN^- and transfer to a normal medium (Table I, series B). This was unexpected since the only SCN^- in the medium after transfer would have to come from the slice, and it would be diluted 100-fold by the chloride medium (see above). Furthermore, SCN^- exit from the cell is extremely rapid.

In SCN^- uptake studies a steady-state accumulation of SCN^- (S/M = 0.5) was reached in 1 min. This is in agreement with earlier reports [1]. Nevertheless, a 30% inhibition of *p*-aminohippurate uptake was observed after preincubation with SCN^-. This suggests that SCN^- might be bound to some site necessary for normal *p*-aminohippurate accumulation. Whilst the data suggest that SCN^- and NO_3^- act at the cell membrane to inhibit anion transport, there is no compelling evidence that this is the case, or if this is so, that it is the only site. Examination of our efflux measurements shows that the apparent efflux rate constant was increased in the presence of NO_3^- or SCN^- regardless of the preincubation condition. This enhancement of efflux has been observed with other competitive inhibitors and has been interpreted as an unbinding of *p*-aminohippurate from an intracellular compartment [7]. Intracellular binding of *p*-aminohippurate has been reported to occur to a limited extent and therefore remains a possible site of SCN^- action [9].

The enhanced efflux and decreased influx could possibly be explained by a change in the basolateral transmembrane potential, particularly in view of the high permeability of the basolateral membrane to SCN^- and NO_3^-. Podevin et al. [8], however, saw little effect on the basolateral membrane potential upon incubation in NO_3^- and SCN^- in cortical slices and Kimura and Spring [10] report only a change of a few millivolts with isosmotic replacement of Cl^- with NO_3^- in *Necturus* proximal tubules.

It has been suggested that *p*-aminohippurate may be driven by coupling to another anion [11]. The competitive interaction of SCN^- and NO_3^- with *p*-aminohippurate would be consistent with the idea that SCN^- and NO_3^- share a common carrier. Thus, the observed stimulation of efflux could suggest counterflow. However, attempts to demonstrate counterflow of *p*-aminohippurate have been unsuccessful [12].

An alternative explanation is that these ions are interacting electrostatically with positive sites, probably amino groups, on the basolateral membrane in competition with *p*-aminohippurate. Such a possibility is tenable since Scatchard and Black reported that the binding of inorganic anions to purified albumin solutions occurred in the order $Cl^- < NO_3^- < SCN^-$ [3]. Furthermore, the amino reactivity of inorganic ions has been demonstrated in human erythrocytes [4]. We have recently shown that 4-acetamido-4′-isothiocyano-2,2′-stilbene-disulfonic acid inhibits *p*-aminohippurate transport in a competitive manner [2]. The mechanism of action of these stilbene inhibitors is presumably related to their amino reactivity. At present, the data do not allow us to distinguish between the above hypotheses. Further resolution awaits more information regarding the nature of *p*-aminohippurate accumulation in the cell.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Pamela Cattrall and Marcie Sponholtz. This research was supported in part by Public Health Service Grants AM-18918, AM-05437 and 507-RR-05400-16.

References

1 Taggart, J.V., Silverman, L. and Trayner, E.M. (1953) Am. J. Physiol. 173, 345—350
2 Hong, S.K., Goldinger, J.M., Song, Y.K., Koschier, F.J. and Lee, S.H. (1978) Am. J. Physiol. 234, F302—F308
3 Scatchard, G. and Black, E.S. (1949) J. Phys. Colloid Chem. 53, 88—99
4 Wieth, J.O. (1970) J. Physiol. 207, 581—609
5 Gerencser, G.A., Park, Y.S. and Hong, S.K. (1973) Proc. Soc. Exp. Biol. Med. 44, 440—444
6 Ross, C.R., Pessah, N.I. and Farah, A. (1968) J. Pharmacol. Exp. Ther. 160, 381—386
7 Farah, A., Frazer, M. and Stoffel, M. (1963) J. Pharmacol. Exp. Ther. 139, 120—128
8 Podevin, R.A., Boumendil-Podevin, E.F. and Priol, C. (1978) Am. J. Physiol. 235, F278—F285
9 Welch, L.T. and Bush, M.T. (1970) Am. J. Physiol. 218, 1751—1756
10 Kimura, G. and Spring, K.R. (1979) Am. J. Physiol. 236, F295—F301
11 Berner, W. and Kinne, R. (1976) Pflügers Arch. 361, 269—277
12 Ross, C.R. and Farah, A. (1966) J. Pharmacol. Exp. Ther. 151, 159—167