Biochimica et Biophysica Acta, 693 (1982) 265–272
Elsevier Biomedical Press

BBA 71446

INHIBITORY EFFECT OF UNCONJUGATED BILIRUBIN ON *p*-AMINOHIPPURATE TRANSPORT IN RAT KIDNEY CORTEX SLICES

MARÍA M. ELÍAS, ELBIO J. COMÍN, MARTA E. GROSMAN, SUSANA A. GALEAZZI and EMILIO A. RODRIGUEZ GARAY *

Instituto de Fisiología Experimental, CONICET-Universidad Nacional de Rosario, Suipacha 570, 2000 Rosario (Argentina)

(Received May 18th, 1982)

Key words: Anion transport; p-Aminohippurate transport; Bilirubin; (Kidney cortex slice)

(1) The effects of unconjugated bilirubin on the accumulation of *p*-aminohippurate, kinetics of *p*-aminohippurate uptake, the efflux of pre-accumulated *p*-aminohippurate and water and electrolyte distribution were investigated in the rat kidney cortical slice. (2) The addition of unconjugated bilirubin to the incubation medium decreased the 60 min slice-to-medium concentration ratio of *p*-aminohippurate. (3) The decrease in *p*-aminohippurate accumulation by unconjugated bilirubin was found to be more pronounced by increasing the concentration of pigment in the medium. (4) The rate of uptake of *p*-aminohippurate as a function of *p*-aminohippurate concentration differed in aerobiosis and anaerobiosis, and unconjugated bilirubin decreased only the uptake of *p*-aminohippurate in aerobic conditions. (5) The efflux of pre-accumulated *p*-aminohippurate decreased when unconjugated bilirubin concentration in the medium was low (10–20 μM) but the efflux increased when the concentration of pigment was much higher (100 μM). (6) The addition of unconjugated bilirubin to the medium (40–100 μM) increased intracellular sodium and total tissue water content, and decreased intracellular potassium and oxygen consumption of tissue. However the slices incubated with low concentration of pigment (20 μM) did not exhibit significative changes in cellular functional parameters. (7) These findings suggest that unconjugated bilirubin impairs *p*-aminohippurate transport by a complex mechanism that might involve binding of pigment to sites necessary for anion transport, although effects related to pigment toxicity or to its oxidative decomposition are not excluded.

Introduction

It is known that *p*-aminohippurate is actively transported into the cells of the renal proximal tubule across the peritubular membrane with a subsequent translocation to the lumen down its concentrations gradient [1,2]. In some species *p*-aminohippurate transport process in renal cortical tissue is influenced by pH changes [3,4], and by some inorganic ions [5,6] and organic substances [7–9]. Unconjugated bilirubin, a degradation or catabolic product of hemoproteins, exhibits toxic effects on several tissues interfering with mitochondrial reactions [10,11] and with the activity of some enzymes [12–14]. Furthermore, unconjugated bilirubin interacts with biological membranes [15,16], and may be responsible for the impairment of transport processes [17–19]. In this connection we reported the interaction of unconjugated bilirubin with peritubular cell membranes of isolated rat kidney interfering with the transport mechanism of *p*-aminohippurate [20,21]. Therefore the aim of this study was to get more insight into the mechanism involved in the effect of unconjugated bilirubin on renal *p*-aminohippurate transport by using the slice technique.

* To whom correspondence should be addressed.

Methods

Animals. Male Wistar rats weighing 300–350 g were used as kidney donors for all studies. Animals were allowed free access to a standard diet and tap water until used.

Slices and medium preparation. The animals were anaesthetized with sodium pentobarbital (50 mg/kg body wt.) and the kidneys, exsanguinated by tying the aorta, were promptly removed and placed in cold saline solution (pH 7.4). Renal cortical slices were obtained freehand (approx. 0.5 mm thickness), and then placed in ice-cold saline solution until they were used for incubation. Unless otherwise stated, all incubations were carried out at 27°C in a Dubnoff metabolic shaker under 95% O_2/5% CO_2 atmosphere. The tissues underwent a 20-min equilibration period in flasks containing 4 ml of a Krebs-Ringer solution before the addition of the test compounds. Each incubation flask contained five pieces of tissue (total 82 ± 2 mg wet wt./flask) randomly selected from the storage vessel. The bathing medium consisted of Krebs-Ringer solution enriched with glucose (10 mM), sodium lactate (10 mM), sodium acetate (10 mM) and sodium pyruvate (10 mM), pH 7.4 [22]. When using unconjugated bilirubin, this was previously dissolved in 0.1 M NaOH and albumin solution being the molar ratio of unconjugated bilirubin to albumin of 20:1. The amount of albumin was suitable to disperse unconjugated bilirubin through non specific adsorption in order to inhibit a rapid aggregation and precipitation of unconjugated bilirubin, although it was mostly in a diffusible form [23]. The final concentration of unconjugated bilirubin in the medium oscillated between 20 and 100 μM.

Effect of unconjugated bilirubin on p-aminohippurate accumulation in kidney slices. After the completion of the equilibration period the slices were transferred to flasks containing 4 ml of fresh Krebs-Ringer solution containing *p*-aminohippurate ($7 \cdot 10^{-5}$ M), and then incubated for 60 min. Then the slices were removed from the incubation medium, blotted dried on filter paper, weighed, placed in 1 ml of distilled water, and throughly homogenized. 1-ml aliquots of the incubation medium were obtained, and from this point homogenates and media were treated identically. 10% trichloroacetic acid (1.5 ml) and distilled water (2.5 ml) were added to each sample, allowed to stand for 30 min, and then centrifuged [24]. *p*-Aminohippurate concentration was determined in the supernatans. Data were expressed as the slice-to-medium concentration ratio S/M, where S equals mg of *p*-aminohippurate per g of wet tissue, and M mg of *p*-aminohippurate per ml of medium. Percent of change in S/M ratio of the slice incubated in the presence of unconjugated bilirubin was calculated for each experiment, as compared with controls incubated without unconjugated bilirubin.

Effect of unconjugated bilirubin on the kinetics of p-aminohippurate uptake. Following the equilibration period, the slices were transferred to flasks containing 4 ml of fresk medium and *p*-aminohippurate. The rate of *p*-aminohippurate uptake by renal cortical slices was determined during a 15 min incubation at various *p*-aminohippurate concentrations (50–2000 μM). In other set of experiments *p*-aminohippurate uptake was analyzed in the presence of unconjugated bilirubin. At the end of the incubation the slices were treated as above described. Duplicate flasks for each experimental point were incubated in a medium containing inulin (4 mg/ml) for estimation of the extracellular water. The concentration of *p*-aminohippurate in the tissue was calculated by simple substraction of extracellular *p*-aminohippurate calculated from the inulin space. The apparent kinetic parameters (K_m and V_{max}) were derived from conventional Eadie-Hofstee plots [25]. Kinetics studies were also carried out under a gas phase of 100% N_2 to determine *p*-aminohippurate uptake and the effect of unconjugated bilirubin in the absence of oxygen.

Effect of unconjugated bilirubin on p-aminohippurate efflux from tissue. The efflux of *p*-aminohippurate from renal cortical tissue was determined by using slices preloaded with *p*-amino[^{14}C]hippurate (spec. act. 40.7 mCi/mmol). The slices were preincubated for 60 min at 25°C in 95% O_2/5% CO_2 atmosphere, in a medium containing *p*-aminohippurate ($7 \cdot 10^{-5}$ M). The slices were removed, rinsed and transferred at 30-s intervals through a series of flasks containing 3 ml of *p*-aminohippurate-free fresh Krebs-Ringer solution, at 25°C in a 95% O_2/5% CO_2 atmosphere. The quantity of compound collected from each runout

flask was plotted against runout time and the efflux rate constant was calculated. To determine the effect of unconjugated bilirubin on the efflux of *p*-aminohippurate, the runout continued through flasks containing unconjugated bilirubin.

Effect of unconjugated bilirubin on cellular functional parameters. In the last series of experiments, the effect of unconjugated bilirubin on inulin space, tissue water content, intracellular Na^+ and K^+ concentration, and tissue respiration were studied. Slices were incubated for 60 min in 4 ml of a medium containing inulin (4 mg/ml) and various concentrations of unconjugated bilirubin (0–100 μM). Following incubation, the slices were blotted and weighed and then homogenized for determination of the inulin space. Duplicate series of slices were dried following incubation during 24 h at 100°C for determination of total water content of tissue. The dried tissue was then extracted in 0.1 M HNO_3 for 48 h and analyzed for electrolytes. Values were corrected for extracellular water estimated for each experiment, and expressed as mequiv./l of intracellular water [26]. The oxygen consumption of slices was measured in a Warburg respirometer (Braun, Melsungen, F.R.G.), prior saturation with oxygen of the incubation medium. The data were given as the initial rate of oxygen consumption (μl O_2/h per mg wet tissue).

Analytical methods. Determination of inulin in homogenates and media was performed by the method of Roe et al. [27]. *p*-Aminohippurate concentration was measured by Brun's technique modified as described [28]. Electrolyte concentration was determined by flame photometry. Radioactivity was measured in low potassium counting vials containing 200 μl of sample, 2 ml of Hyamine 10X in methanol (1 M), and 15 ml of scintillation fluid (2.2 M diphenyloxazole and 0.31 mM 1,4-bis-2-(5-phenyloxazolyl)benzene in dry toluene). Measurement of radioactivity was carried out in a liquid scintillation spectrometer (Packard Instr. Co., U.S.A.) and with [^{14}C]benzoic acid as internal standard. Counting efficiency ranged from 70 to 80%.

Chemicals. All chemicals were of highest grade commercially available. Inulin, *p*-aminohippurate, crystalline unconjugated bilirubin, and Hyamine 10X were purchased from Sigma Chemical Co., U.S.A. [^{14}C]Benzoic acid was from Packard Instr. Co., U.S.A. and *p*-amino[^{14}C]hippurate from New England Nuclear, U.S.A.

Statistics. Values were the mean ± S.E. The results for each experiment represent the average of triplicate determinations. The significance of differences was determined using Student's *t*-test. Regression lines were calculated by the method of least squares.

Results

Effect of unconjugated bilirubin on the accumulation of p-aminohippurate in renal cortical slices

The accumulation of *p*-aminohippurate estimated as *S/M* ratio decreased significantly with the increase of unconjugated bilirubin in the medium as shown in Table I.

Effect of unconjugated bilirubin on the kinetics of p-aminohippurate uptake

The kinetics of *p*-aminohippurate uptake as a

TABLE I

EFFECT OF UNCONJUGATED BILIRUBIN (UB) ON *p*-AMINOHIPPURATE ACCUMULATION BY RENAL CORTICAL SLICES

n, number of experiments. n.s., not significantly different.

Experimental group	*S/M* ratio [a]	% decrease	*n*	*P*
Controls	3.07 ± 0.10	–	25	–
+ UB (20 μM)	2.57 ± 0.27	16.3	5	n.s.
+ UB (40 μM)	2.07 ± 0.07	32.6	3	< 0.01
+ UB (100 μM)	1.81 ± 0.06	41.0	3	< 0.001

[a] Data are mean values ± S.E.

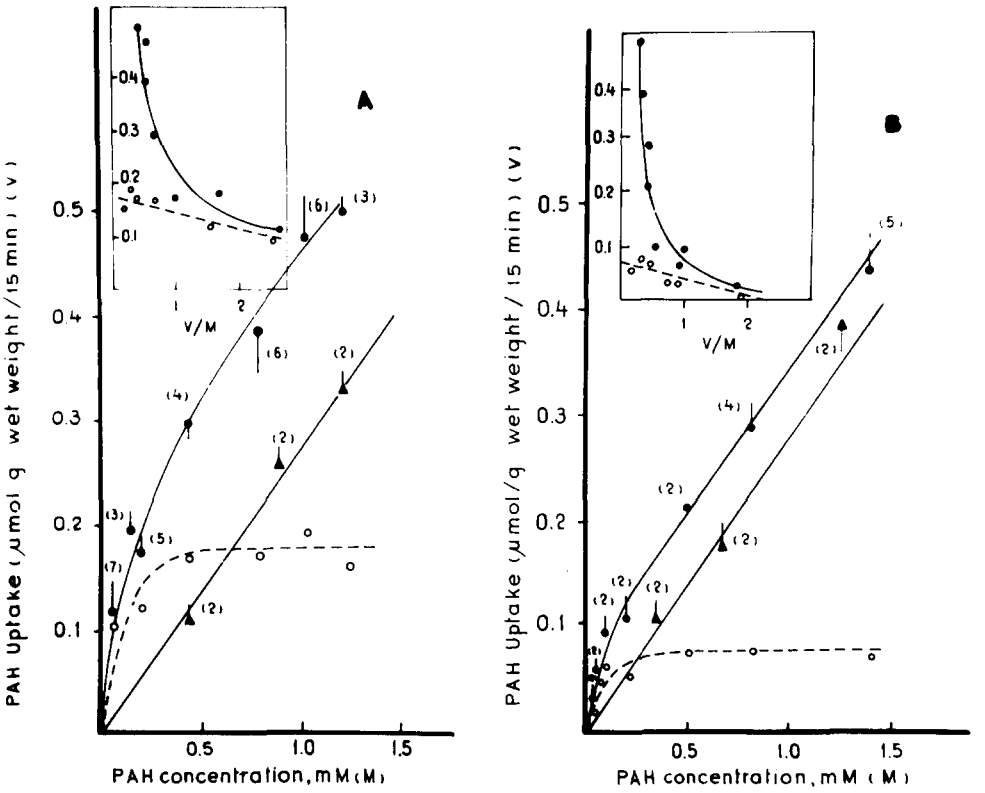

Fig. 1. Rate of uptake of *p*-aminohippurate (PAH) by rat renal cortical slices in the absence (A) and presence (B) of unconjugated bilirubin (100 μM). The results from incubations under 95% O_2/5% CO_2 (●) and under 100% N_2 (▲) were expressed as mean ± S.E. The number of experiments is indicated in the parentheses. Dotted lines represent the points (○) resulting from the substraction between the values corresponding to the nonlinear uptake curve and the interpolated ones on the linear uptake curve (fitted by the least-squares method using a TEXAS, TI59). The insets show Eadie-Hofstee plots of the data. Dotted lines indicate the values resulting from the substraction.

function of *p*-aminohippurate concentration in the absence or presence of unconjugated bilirubin were examined under either 95% O_2/5% CO_2 or 100% N_2. The rate of uptake under aerobic condition was nonlinear and higher than the linear uptake of *p*-aminohippurate in anaerobic condition. As shown in Fig. 1 addition of unconjugated bilirubin to the assay medium inhibited only the uptake of *p*-aminohippurate in aerobic conditions. Fig. 1 also shows the resulting points obtained when the linear component was substracted from the non linear function. The points approached a stable value which was decreased by the presence of unconjugated bilirubin in the medium. Eadie-Hofstee plots of the data represented in the insert of Figs. 1A and 1B indicated an apparent K_m of about 29 μM in the presence of unconjugated bilirubin (100 μM) and 23 μM in the absence of pigment. Conversely the V_{max} was lower in the experiments with the addition of unconjugated bilirubin (0.07 μmol/15 min per g of tissue) as compared with controls without unconjugated bilirubin (0.17 μmol/15 min per g of tissue). A similar effect although less marked was produced by lower un-

TABLE II

EFFECT OF UNCONJUGATED BILIRUBIN (UB) ON THE KINETICS OF *p*-AMINOHIPPURATE EFFLUX

Runout rate constants (k_e) are given as mean ± S.E. *n*, number of experiments.

Experimental group	k_e (s^{-1}) ($\times 10^3$)	*n*	*P*
Controls	2.3 ± 0.1	4	–
+ UB (10–20 μM)	0.8 ± 0.2	2	< 0.01
+ UB (100 μM)	5.1 ± 1.0	3	< 0.01

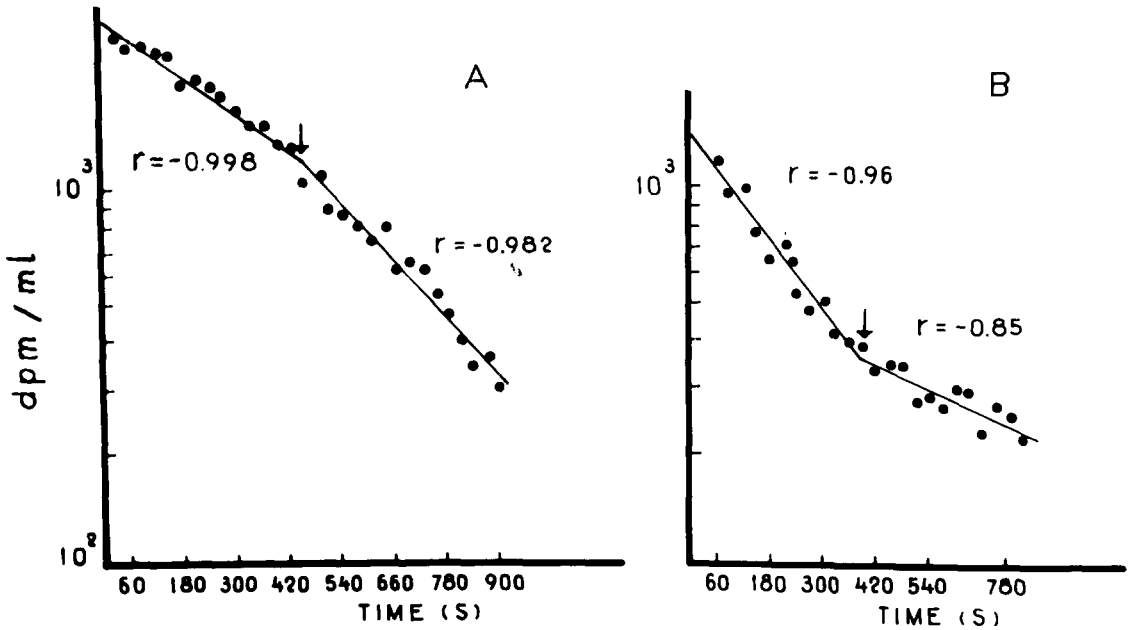

Fig. 2. Typical data of runout of *p*-aminohippurate from rat renal cortical slices in the absence and presence of unconjugated bilirubin (UB). Slices were preloaded with *p*-amino[^{14}C]hippurate for 60 min, rinsed and transferred through a series of flasks containing no *p*-aminohippurate at 30-s intervals. Arrow indicates point where efflux into the medium containing unconjugated bilirubin was begun. (A) unconjugated bilirubin 100 μM. $c_t = 2775$ (dpm/ml) $e^{-0.002\ s^{-1} \cdot t}$, c_t(UB) = 4093 (dpm/ml) $e^{-0.003\ s^{-1} \cdot t}$, $K \neq K$(UB) ($P < 0.05$). (B) Unconjugated bilirubin 10 μM. $c_t = 1364$ (dpm/ml) $e^{-0.003\ s^{-1} \cdot t}$, c_t (UB) = 565 (dpm/ml) $e^{-0.001\ s^{-1} \cdot t}$, $K \neq$ K(UB) ($P < 0.01$).

TABLE III

EFFECT OF UNCONJUGATED BILIRUBIN (UB) ON CELLULAR FUNCTIONAL PARAMETERS

Data are mean value ±S.E., n.s., not significantly different. There were no significant differences in summed concentrations of intracellular Na^+ and K^+ among the four groups when considering the slices in which both cations were determined. $Q\ O_2$, oxygen consumption. The number of experiments is indicated in the parentheses.

Parameters	Controls	+UB (20 μM)	+UB (40 μM)	+UB (100 μM)
Total tissue water	80.1 ±0.9	80.0 ±0.01	82.4 ± 0.6	85.6 ±0.9
(% of wet wt.)	(10)	(3)	(10)	(6)
		n.s.	n.s.	$P < 0.01$
Inulin space	0.30±0.01	0.29±0.02	0.31± 0.07	0.27±0.01
(ml/g wet wt.)	(28)	(5)	(6)	(5)
		n.s.	n.s.	$P < 0.01$
Intracellular Na^+	50.52±2.87	47.38±7.4	102.86±14.5	92.0 ±5.1
(mequiv./l H_2O)	(17)	(8)	(10)	(5)
		n.s.	$P < 0.01$	$P < 0.01$
Intracellular K^+	65.55±1.8	65.02±4.0	44.29± 2.5	33.64±2.06
(mequiv./l H_2O)	(25)	(8)	(10)	(9)
		n.s.	$P < 0.05$	$P < 0.001$
$Q\ O_2$	1.29±0.05	Undetermined	0.98± 0.11	1.02±0.04
(μl/h per mg wet wt.)	(4)		(4)	(4)
			$P < 0.05$	$P < 0.02$

conjugated bilirubin concentrations in the medium with V_{max} values of 0.09 μmol/15 min per g (unconjugated bilirubin concentration 40 μM) and 0.13 μmol/15 min per g (unconjugated bilirubin concentration 20 μM). Thus, the effect of unconjugated bilirubin on *p*-aminohippurate uptake was to decrease the V_{max} of the uptake without significantly affecting the apparent K_m.

Effect of unconjugated bilirubin on the efflux of pre-accumulated p-aminohippurate

The efflux rate constant in the absence of unconjugated bilirubin had a value of 0.0023 s^{-1} as can be seen in Table II. When the slices were transferred to a medium containing unconjugated bilirubin, the efflux rate constant was decreased at low unconjugated bilirubin concentration in the medium but it was increased when unconjugated bilirubin concentration was high. Fig. 2 represents typical data of runout in the absence and presence of unconjugated bilirubin in the medium.

Effect of unconjugated bilirubin on general cell function and viability

Table III shows that the slices incubated in a medium of low unconjugated bilirubin concentration (20 μM) did not exhibit significant change in cell function. When unconjugated bilirubin concentration in the medium increased, cell function and viability were impaired as shown by increased intracellular sodium and total tissue water content and decreased intracellular potassium and oxygen consumption of tissue.

Discussion

The active step in tubular secretion of organic anions in the mammalian kidney is considered to be situated at the peritubular membrane leading to intracellular accumulation and further diffusion into the luminal fluid [1,2]. It has been pointed out that tubular lumina are collapsed in slices of renal cortical tissue [29], and that transport events observed in this preparation reflect preponderantly processes localized at the antiluminal cell phase [30,31]. Therefore accumulation of *p*-aminohippurate by slices reflects the rate of active transport from medium to cell and passive efflux from cell to medium localized at the peritubular cell membrane.

In previous investigations we observed that unconjugated bilirubin which circulated within the peritubular capillaries of an isolated rat kidney preparation interacted with pericapillary membranes and impaired the urinary excretion of *p*-aminohippurate [20,21]. In this study we used renal cortical slices because they seemed appropriate to give insight into the mechanism responsible for the effect of unconjugated bilirubin on *p*-aminohippurate transport at the peritubular site.

The results described in this paper showed that *S/M* ratio for *p*-aminohippurate, indicative of its steady state accumulation, was diminished by unconjugated bilirubin. The decrease of *p*-aminohippurate accumulation might be due to: (1) a decrease in the active transport of *p*-aminohippurate, (2) an increase in the passive efflux of *p*-aminohippurate, (3) a combination of the two. The data also indicated that the change produced by unconjugated bilirubin was different at low ($\leqslant$ 20 μM) and high ($\geqslant$ 40 μM) concentrations of pigment. At low concentrations of pigment the changes produced on *p*-aminohippurate transport were a diminished V_{max} with the apparent K_m remaining constant, and a decrease in the efflux rate constant. The mentioned effects were in accordance with data obtained in the isolated rat kidney [21]. Since no modifications in total water content and ionic distribution could be detected in the presence of low unconjugated bilirubin concentrations, we can assume that cellular metabolism determining *p*-aminohippurate transport was unaffected. However it is possible that unconjugated bilirubin binding to sites necessary for *p*-aminohippurate transport could depress its translocation by noncompetitive inhibition [32]. The effect of high unconjugated bilirubin concentration in the medium might be due to an additional mechanism also involved. In this connection it was seen that *p*-aminohippurate accumulation by renal cortical tissue was inversely related to the magnitude of the membrane potential [33]. As unconjugated bilirubin may behave as an organic anion in biological buffer [34] it seems possible that membrane potential changed by pigment diffusion into cell. Moreover unconjugated bilirubin entering the cell might produce unbinding of *p*-aminohippurate from an intracellular protein [35], thus enhancing its rate of efflux as observed with other inhibitors of *p*-

aminohippurate transport [6].

The Eadie-Hofstee plots of the kinetic data indicated the existence of at least two different components of *p*-aminohippurate transport in renal cortical slices. Although such a phenomenom was not observed by some authors [3,5–7], it was also described by other investigators and explained as due to geometry problems in slices [2,28,31], or to the existence of two different functional components [36–38]. Our data were in agreement with the last point of view since only one component apparently was involved in the uptake of *p*-aminohippurate by the slices incubated in anaerobiosis. Therefore we can assume that the kinetics of *p*-aminohippurate uptake by renal cortical tissue may involve at least two components namely aerobic and anaerobic. The aerobic component followed simple Michaelis-Menten kinetics with a high affinity for *p*-aminohippurate, and may be inhibited by unconjugated bilirubin in a non competitive manner, whereas the anaerobic component seemed not to be affected by unconjugated bilirubin. Nevertheless, the mechanism postulated for unconjugated bilirubin inhibitory effect on *p*-aminohippurate transport, particularly in the presence of high unconjugated bilirubin concentrations, might be well attributed to the extensively studied pigment toxicity [10,12,14]. Impaired functional cell parameters seen in Table III were in agreement with unconjugated bilirubin toxic effects on membrane systems [17,18], and with the view that unconjugated bilirubin may exert effects on the physico-chemical state of membranous phospholipids [14,39].

Whatever would be the explanation for the results described in this paper, the lack of effect of unconjugated bilirubin on *p*-aminohippurate uptake in anaerobic conditions remains intriguing. In this regard it is not possible to discard that unconjugated bilirubin promoted lipid peroxidation of rat renal cortical slices in the presence of oxygen as described for some compounds that produced impairment of tubular cell function and diminished accumulation of *p*-aminohippurate by tissue [40]. In this sense the oxidative decomposition of unconjugated bilirubin in albumin-free solution that may be prevented by exclusion of oxygen [41] might produce degradation products or free radicals [42] responsible of the effect on membrane components. Further resolution awaits more information regarding the nature of the anaerobic component of *p*-aminohippurate transport in rat renal cortical slices, and about the chemical structure of unconjugated bilirubin under aerobic and anaerobic conditions of tissue incubation.

Acknowledgement

This investigation was supported by a grant from Consejo Nacional de Investigaciones Científicas y Tècnicas (CONICET), Argentina. The valuable technical assistance of Miss Elena Ochoa is gratefully acknowledged.

References

1 Tune, B.M., Burg, M.B. and Patlak, C.S. (1969) Am. J. Physiol. 217, 1057–1063
2 Burg, M.B. and Orloff, J. (1969) Am. J. Physiol. 217, 1064–1068
3 Park, Y.S. and Solomon, S. (1977) Am. J. Physiol. 233, F382–F387
4 Blomstedt, J.W. and Aronson, P. (1980) J. Clin. Invest., 931–934
5 Hayashi, H. and Hoshi, T. (1979) Arch. Int. Pharmacodyn. Thér. 240, 103–115
6 Goldinger, J.M., Erasmus, B.D., Song, Y.K., Koschier, F.J. and Hong, S.K. (1980) Biochim. Biophys. Acta 598, 357–365
7 Podevin, R.A. and Boumendil-Podevin, E.F. (1975) Biochim. Biophys. Acta 375, 106–114
8 Dantzler, W.H. and Bentley, S.K. (1980) Am. J. Physiol. 238, F16–F25
9 Hong, S.K., Goldinger, J.M., Song, Y.K., Koschier, F.J. and Lee, S.H. (1978) Am. J. Physiol. 234, F302–F307
10 Mustafa, M.G., Cowger, M.L. and King, T.E. (1969) J. Biol. Chem. 244, 6403–6414
11 Hackney, D.D. (1980) Biochem. Biophys. Res. Commun. 94, 875–880
12 Kashiwamata, S., Shungi, K., Semba, R. and Susuki, F. (1979) J. Biol. Chem. 254, 4577–4584
13 Kashiwamata, S., Asai, M. and Semba, R. (1981) J. Neurochem. 36, 826–829
14 Kawai, K. and Cowger, M.L. (1981) Res. Commun. Chem. Pathol. Pharmacol. 32, 123–135
15 Tipping, E., Ketterer, B. and Cristodoulides, L. (1979) Biochem. J. 180, 327–337
16 Girotti, A. (1978) J. Biol. Chem. 253, 7186–7193
17 Corchs, J.L., Serrani, R. and Palchick, M. (1979) Biochim. Biophys. Acta 555, 512–518
18 Yoshioka, T., Sekiba, K. and Utsumi, K. (1980) Cells Struct. Funct. 5, 209–213
19 Semba, R.K. and Kashiwamata, S. (1980) Biochim. Biophys. Acta 632, 290–297

20 Elías, M., Comín, E.J., Galeazzi, S. and Rodriguez Garay, E.A. (1981) Clin. Sci. 61, 765–771
21 Comín, E.J., Elías, M.M., Grossman, M. and Rodriguez Garay, E.A. (1981) Res. Commun. Chem. Pathol. Pharmacol. 34, 169–172
22 Cross, R.J. and Taggart, J.V. (1950) Am. J. Physiol. 161, 181–190
23 Nelson, T., Jacobsen, J. and Wennberg, R.P. (1974) Pediatr. Res. 8, 963–967
24 Carpenter, H.M. and Mudge, G.H. (1980) J. Pharmacol. Expt. Ther. 213, 350–354
25 Hofstee, B.H. (1952) Science 116, 329–331
26 Kleinzeller, A., Kostyuk, P.G., Kotyk, A. and Levaa, A.A. (1969) in Laboratory Techniques in Membrane Biophysics, (Passow, H. and Stampfli, R., eds.), pp. 69–84, Springer Verlag, New York
27 Roe, H.H., Epstein, J.H. and Goldstein, N.P. (1949) J. Biol. Chem. 178, 839
28 Waugh, W.H. and Beall, P.T. (1974) Kidney Int. 5, 429–436
29 Wedden, P.P. and Vyas, B.T. (1978) Kidney Int. 14, 158–168
30 Foulkes, E.C. (1971) Biochim. Biophys. Acta 241, 815–822
31 Tune, B.M. and Burg, M.B. (1971) Am. J. Physiol. 221, 580–585
32 Koushanpour, E., (1976) in Renal Physiology. Principles and Functions, pp. 121–149, W.B. Saunders Co. Philadelphia
33 Podevin, R.A., Boumendil-Podevin, E.F. and Priol, C. (1978) Am. J. Physiol. 235, F278–F285
34 Gray, C.H., Kulczycka, A. and Nicholson, D.C. (1961) J. Chem. Soc. 442, 2276–2285
35 Kirsch, R., Fleischner, G., Kamisaka, K. and Arias, I.M. (1975) J. Clin. Invest., 1009–1019
36 Maxild, J., Møller, J.V. and Sheikh, M.I. (1980) Biochim. Biophys. Acta 601, 490–499
37 Maxild, J., Møller, J.V. and Sheikh, M.I. (1981) J. Physiol. 310, 273–283
38 Sheikh, M.I., Maxild, J. and Møller, J.V. (1981) Biochem. Pharmacol. 30, 2141–2146
39 Brodersen, R. (1979) J. Biol. Chem. 254, 2364–2369
40 Fujimoto, Y., Yasuoka, F. and Fujita, T. (1981) Jap. J. Pharmacol. 31, 1013–1019
41 Ostrow, J.D. and Branham, R.V. (1970) Gastroenterology 58, 15–25
42 Cohen, A.N. and Ostrow, J.D. (1980) Pediatrics 9, 283–294