Biochimica et Biophysica Acta 812 (1985) 91–97
Elsevier

BBA 72403

Prostaglandin E_2 transport in rabbit renal basolateral membrane vesicles

E.F. Boumendil-Podevin and R.A. Podevin *

Laboratoire de Physiologie, Faculté de Médecine Xavier-Bichat (Université Paris 7) and INSERM U.251, 16 rue Henri Huchard, 75018 Paris (France)

(Received July 19th, 1984)

Key words: Proximal tubule; H^+ cotransport; Transport inhibitor; Prostaglandin transport; Membrane vesicle; (Rabbit kidney)

We examined the mechanism of prostaglandin E_2 transport in rabbit renal basolateral membrane vesicles which were predominantly oriented right-side-out. In the presence of an inwardly directed H^+ gradient, the initial rate of uptake was markedly accelerated and the influx of prostaglandin E_2 resulted in a transient accumulation (overshoot) above the equilibrium value. Both H^+-independent and H^+-stimulated prostaglandin E_2 uptake were shown to be insensitive to valinomycin-induced K^+ diffusion potentials. Intravesicular probenecid inhibited the pH gradient-stimulated uptake of prostaglandin E_2 but did not affect the pH-stimulated uptake of thiocyanate and acetate which enter membranes via ionic and nonionic diffusion, respectively. Finally, the existence of a Na^+ cotransport or of a K^+ antiport pathway for prostaglandin E_2 could not be demonstrated. Thus, these data demonstrate the presence of an electrically neutral H^+-prostaglandin E_2 cotransport or OH^--prostaglandin E_2 antiport mechanism in the basolateral membrane of the rabbit proximal tubule.

Introduction

Previous studies using various intact cell systems have shown that exogeneously administered prostaglandins enter the proximal tubule by active secretion, presumably via the organic anion secretory pathway [1–3]. It was also found that the transtubular flux of prostaglandin E_2 in microperfused rabbit tubules occurs principally in the S_2 segment, and that the uphill step in active transepithelial secretion is localized to the basolateral membrane [4]. These findings, although they contribute to an understanding of the overall renal handling of prostaglandin E_2, do not define the mechanisms that underlie active prostaglandin E_2 transport across the tubular cell membrane.

In this paper, a purified preparation of basolateral membrane vesicles isolated from the rabbit kidney cortex was used to examine the mechanisms of prostaglandin E_2 uptake. We report the existence of a pH gradient-dependent, carrier-mediated, electroneutral transport mechanism for prostaglandin E_2 uptake. In addition, our results also suggest that there is no Na^+ gradient or K^+ gradient-dependent prostaglandin E_2 transport system in renal basolateral membranes.

Material and Methods

Basolateral membrane vesicles were isolated from the rabbit renal cortex by a recently developed Percoll gradient technique [5]. The quality of the membrane preparations, evaluated by measuring the enrichment of enzyme markers, was the same as recently reported [5]. Studies to ascertain

* To whom reprint requests should be addressed.

Abbreviations: Prostaglandin E_2, [5*Z*,11α,13*E*,15*S*]-11,15-dihydroxy-9-oxoprosta-5,13-dien-1-oic acid; SITS, 4-acetamido-4′-isothiocyano-2,2′-disulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

membrane sidedness indicated that 85–90% of the vesicles were oriented right-side-out [5] Protein concentrations were determined by the method of Lowry et al. [6].

The [^{3}H]prostaglandin E_2 uptake was assayed at 25°C by a rapid filtration technique. Incubations were started by the addition of 10 μl of a suspension of membranes (50–100 μg protein) to 150 μl incubation medium preequilibrated at 25°C, and were terminated by the addition of 1 ml of an ice-cold solution containing 150 mM NaCl, 10 mM Tris-Hepes (pH 7.4) and 1 mM Tris-probenecid. The suspension was immediately collected on a prewetted Sartorius filter (SM 113, pore size 0.6 μM) and the vesicles on the filter were washed with 3 vol., each of 3 ml cold wash buffer.

The uptakes of [^{3}H]acetate and [^{14}C]SCN were measured, using the above-mentioned procedures, to monitor relative changes in the transmembrane pH and potential differences, respectively [7].

All incubation solutions were filtered through 0.45 μm Millipore filters prior to use. The incubation conditions for each experiment are given in the legends to figures and tables. Values for the nonspecific retention of radioactivity on filters were subtracted from the values of incubated samples. All incubations were carried out at least in triplicate. Each experiment was repeated three times with different membrane vesicles.

To determine the identity of the accumulated compound, basolateral membrane vesicles were incubated with 5 nM [^{3}H]prostaglandin E_2 for 30 s or 90 min in the presence of an inward proton gradient. The membranes were then collected by filtration and the accumulated radioactivity was extracted in 200 μl 50% ethanol. Aliquots of the ethanol solutions were spotted on silica-gel plates with wedged-tip divisions [8]. The tritiated prostaglandin E_2 from New England Nuclear served as a marker and was spotted on the same plates. The following solvent systems were used: (1) chloroform/methanol/acetic acid (18:1:1); R_f prostaglandin E_2 0.85; and (2) toluene/dioxane/acetic acid (20:10:1); R_f prostaglandin E_2 0.50. After separation, the distribution of radioactivity was analyzed by scintillation counting of 1 cm^2 chromatogram sections.

The [^{3}H]prostaglandin E_2 (165 Ci/mmol) and [^{3}H]acetic acid (90 mCi/mmol) were obtained from New England Nuclear and [^{14}C]SCN (5–15 mCi/mmol) from ICN Pharmaceutical. Probenecid, SITS and valinomycin were supplied by Sigma. As valinomycin was added to 95% ethanol, control suspensions received equivalent volumes of ethanol. The final ethanol concentration was 1.5%.

Results

As illustrated in Fig. 1, imposition of an inwardly directed proton gradient (pH_{out} 6.0; pH_{in} 7.4) markedly increased the initial rate of prostaglandin E_2 uptake relative to the uptakes in the absence of a transmembrane pH gradient (7.4 in; 7.4 out and 6.0 in; 6.0 out). In the presence of inward H^+ gradient, prostaglandin E_2 uptake reached a maximum at 30 s. Thereafter, the amount of prostaglandin E_2 accumulated slightly decreased, indicating an efflux. The final level of prostaglandin E_2 uptake was significantly higher

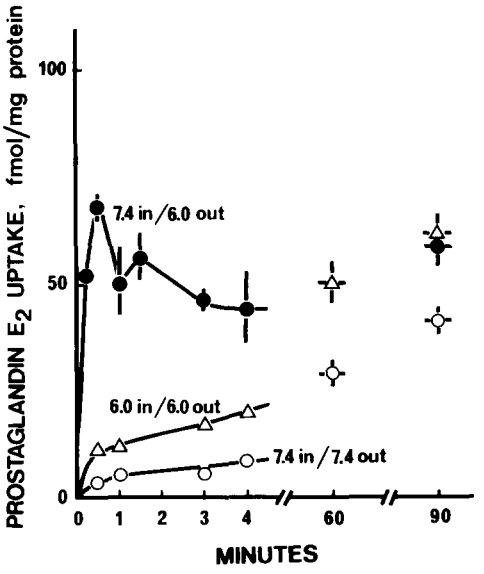

Fig. 1. Effect on an inwardly directed proton gradient on prostaglandin E_2 uptake. Basolateral membrane vesicles were preincubated for 90 min at 20°C in 20 mM K_2SO_4, 150 mM sucrose and 50 mM Tris-Hepes (pH 7.4). Then, 10 μl vesicles were added to 150 μl of a medium containing 5 nM [^{3}H]prostaglandin E_2, 20 mM K_2SO_4, 150 mM sucrose and either 40 mM Tris-Hepes (pH 7.4) (○) or 40 mM Tris-Mes (pH 6.0) (●). In the series denoted by (△; 6.0 in/6.0 out) vesicles were preloaded in 20 mM K_2SO_4, 150 mM sucrose, 50 mM Tris-Mes (pH 6.0) and the uptake of 5 nm [^{3}H]prostaglandin E_2 was then assayed in the same medium. Means ± S.D., $n = 4$.

at pH 6.0 than at pH 7.4, regardless of the initial pH values of the intravesicular fluids.

In the presence of an inward proton gradient, the radioactivity taken up by the vesicles incubated for 30 s or 90 min with [^{3}H]prostaglandin E_2 cochromatographed for 91 ± 3.1 and 86.5 ± 5.6%, respectively, with authentic standard [^{3}H]prostaglandin E_2 (means ± S.D. from three experiments). These results indicate, therefore, that metabolism did not play a significant role in the uptake of prostaglandin E_2 by basolateral membrane vesicles.

From the amount of prostaglandin E_2 taken up at equilibrium (90 min) by the basolateral membrane vesicles, it could be calculated that the intravesicular space averaged 11.5 and 8.0 μl/mg of membrane protein at pH 6.0 and 7.4, respectively. These values were approx. 20-times higher than those previously found for the intravesicular accumulation of L-glutamate [9], urate and L-glucose by the vesicles at equilibrium (unpublished data), suggesting binding of prostaglandin E_2 to the membrane. Other evidence consistent with binding of prostaglandin E_2 to the membrane was obtained from experiments in which the amount of prostaglandin E_2 remaining bound to the membranes after hypotonic lysis was evaluated. As shown in Table I, binding of prostaglandin E_2 at 30 s accounted for 10 and 13% of total uptake in the presence and absence of an initial pH gradient, respectively. In contrast, uptake at equilibrium mostly represented binding, 56 and 82% at pH 7.4 and 6.0, respectively. In the next series of studies, the hypotonic lysis method was used to distinguish between transport and binding of prostaglandin E_2, as a function of time, in the presence of an inward H^+ gradient. As illustrated in Fig. 2, if at each time interval the prostaglandin E_2 uptake was corrected for binding, an overshoot of prostaglandin E_2 influx could be demonstrated. At the peak of the overshoot, the accumulation of pros-

TABLE I

TRANSPORT AND BINDING OF [^{3}H]PROSTAGLANDIN E_2

Basolateral membrane vesicles were preincubated for 90 min at 20°C in 20 mM K_2SO_4, 150 mM sucrose and 40 mM Tris-Hepes (pH 7.4). Then, 10 μl vesicles were added to 150 μl of a medium containing 5 nM [^{3}H]prostaglandin E_2, 20 mM K_2SO_4, 150 mM sucrose and either 40 mM Tris-Hepes (pH 7.4) or 40 mM Tris-Mes (pH 6.0). Experiments were terminated either in the standard way or after lysis of the vesicles as described in the legend to Fig. 2. Means ± S.D., $n = 4$.

External pH	Incubation time (min)	Prostaglandin E_2 uptake (fmol/mg protein)	
		total uptake	binding
7.4	0.5	5.2 ± 0.6	0.7 ± 0.3
6.0	0.5	55.0 ± 7.4	5.3 ± 0.5
7.4	90	31.7 ± 2.2	17.5 ± 0.7
6.0	90	39.5 ± 1.9	32.6 ± 2.9

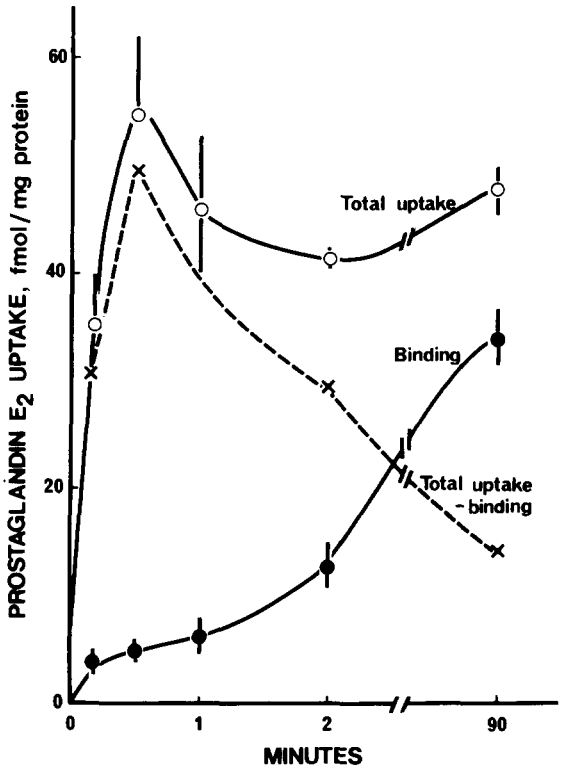

Fig. 2. Transport and binding of prostaglandin E_2 in the presence of an inward proton gradient. Basolateral membrane vesicles were preincubated for 90 min at 20°C in 20 mM K_2SO_4, 150 mM sucrose and 50 mM Tris-Hepes (pH 7.4). Then, 10 μl vesicles were added to 150 μl of a medium containing 5 nM [^{3}H]prostaglandin E_2, 20 mM K_2SO_4, 150 mM sucrose and 40 mM Tris-Mes (pH 6.0). Experiments were terminated either in the standard way, so that total uptake (○) was measured, or by adding 3 ml ice-cold deionized water to vesicles prior to their collection by filtration in order to estimate binding (●). The total uptake − binding values (×) were obtained by subtracting the binding from the total uptake obtained at each time interval. Means ± S.D., $n = 4$.

taglandin E_2 reached about 4-times the final equilibrium value of uptake into an osmotically reactive intravesicular space.

By osmotic methods, we confirmed that the degree of binding of prostaglandin E_2 to the membranes was related to the duration of incubation. From the experiments illustrated in Fig. 3, it can be estimated from extrapolation of the regression lines to infinite medium osmolarity, that under standard conditions (300 mosM), approx. 9% of the uptake at 15 s could be attributed to binding. In contrast, binding of prostaglandin E_2 at 90 min accounts for nearly 70% of total uptake.

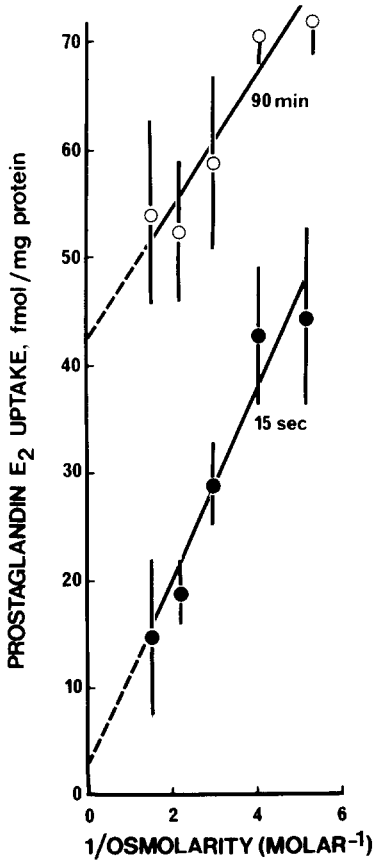

Fig. 3. Effect of medium osmolarity on the prostaglandin E_2 uptake by basolateral membrane vesicles. Membrane vesicles were preequilibrated for 90 min in 10 mM K_2SO_4, 20 mM Tris-Hepes (pH 7.4) and the indicated concentrations of sucrose. Uptake was measured after 15 s (●) or 90 min (○) in a medium containing 5 nM [^{3}H]prostaglandin, 10 mM K_2SO_4, 20 mM Tris-Mes (pH 6.0) and various concentrations of sucrose to give the indicated osmolarity. Means ± S.D., $n = 4$.

To determine the effects of membrane potential and probenecid on pH gradient-stimulated prostaglandin E_2 uptake, initial uptake rates were measured. As illustrated in Fig. 4, pH-stimulated prostaglandin E_2 uptake was linear for 20 s; thus, 10-s uptakes were used to estimate the initial rate of unidirectional transport.

We next tested the possible influence of electrical potential on both pH-stimulated ($H^+_{out} > H^+_{in}$) and pH-independent ($H^+_{out} = H^+_{in}$) uptake of prostaglandin E_2 (Table II)., In the first series of experiments, a K^+ gradient was established, $K^+_{out} > K^+_{in}$. In the absence of a transmembrane pH gradient ($pH_{out} = pH_{in} = 7.4$), addition of valinomycin generated an electropositive interior, as evidenced by a nearly 4-fold increase in SCN^- uptake. Valinomycin, however, had no measurable effect on prostaglandin E_2 uptake, proving the absence of a conductive pathway for this anion. Imposition of an inwardly directed H^+ gradient, in the absence of valinomycin, resulted in a 5-fold increase in SCN^- uptake. A 16-fold stimulation of prostaglandin E_2 uptake measured under identical conditions was observed. Since the transmembrane potentials produced by these two means were com-

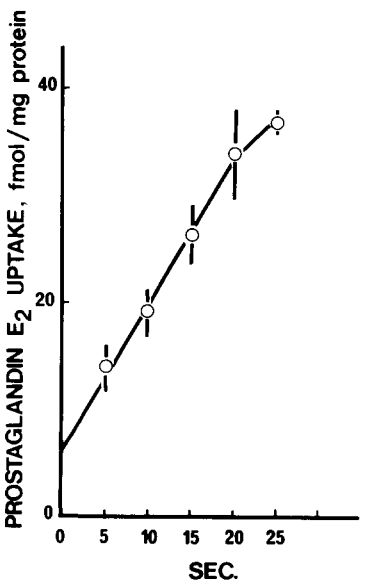

Fig. 4. Time-course of prostaglandin E_2 uptake in the presence of an inward H^+ gradient. Uptake of prostaglandin E_2 was determined under pH gradient conditions as described in the legend to Fig. 2. Means ± S.D., $n = 4$.

TABLE II

INFLUENCE OF TRANSMEMBRANE POTENTIAL ON PROSTAGLANDIN E_2 UPTAKE

In the $K^+_{out} > K^+_{in}$ experiments, basolateral membrane vesicles were preincubated for 90 min at 20°C in (mM) 150 sucrose, 25 Na_2SO_4, 50 Tris-Hepes (pH 7.4) with or without 0.3 mg/ml valinomycin. Samples (10 μl) of vesicles were added to 150 μl of medium containing (in mM) 150 sucrose, 25 K_2SO_4, either 40 Tris-Hepes (pH 7.4) or 40 Tris-Mes (pH 6.0) and either 5 nM [^{3}H]prostaglandin E_2, 14 μM [^{14}C]KSCN or 16 μM [^{3}H]acetate. In the $K^+_{in} > K^+_{out}$ experiment, the experimental conditions and ionic composition of vesicles were the same except that 25 mM Na_2SO_4 was used instead of K_2SO_4 and vice versa. The incubation time was 10 s. Means ± S.D., $n = 4$.

Conditions	Prostaglandin E_2 uptake (fmol/mg protein)		SCN^- uptake (fmol/mg protein)	
	− valinomycin	+ valinomycin	− valinomycin	+ valinomycin
$K^+_{out} > K^+_{in}$				
$H^+_{out} = H^+_{in}$	0.98 ± 0.57	1.37 ± 0.76	1.50 ± 0.35	5.70 ± 1.10 [a]
$H^+_{out} > H^+_{in}$	15.64 ± 0.58	20.13 ± 2.92 [a]	7.60 ± 0.78	15.80 ± 1.18 [a]
$K^+_{in} > K^+_{out}$				
$H^+_{out} = H^+_{in}$	1.88 ± 0.43	2.48 ± 0.65	2.00 ± 0.35	1.40 ± 0.17 [a]
$H^+_{out} > H^+_{in}$	31.76 ± 4.15	28.68 ± 5.84	6.90 ± 1.08	2.25 ± 0.17 [a]

[a] $P < 0.05$ vs. respective control values measured without valinomycin.

parable in magnitude, it is likely that the pH-stimulated prostaglandin E_2 uptake cannot be attributed to the electrical potential. In the presence of a pH gradient, the addition of valinomycin resulted in a 2-fold increase in SCN^- uptake, indicating that the stimulation of SCN^- uptake by the ionophore was additive to the proton gradient effect. By contrast, prostaglandin E_2 uptake was increased by only 30% in the presence of valinomycin. These results confirm the hypothesis that prostaglandin E_2 uptake is poorly potential-sensitive. Moreover, stimulation of prostaglandin E_2 uptake by the ionophore cannot be entirely attributed to the generation of a positive interior. Indeed, we found that, under these conditions, the proton gradient was maintained better in the presence of valinomycin (not shown). In the second series of experiments, an outward K^+ gradient was established. Valinomycin was employed to create an inside-negative K^+ diffusion potential in order to overcome the inside-positive H^+ diffusion potential. The efficacy of this maneouver was evidenced by the finding that the pH-stimulated uptake of SCN^- was completely suppressed by the ionophore. In contrast, valinomycin had no significant effect on pH-stimulated prostaglandin E_2 uptake. Taken together, these results clearly demonstrate that the pH gradient-dependent transport system for prostaglandin E_2 in the basolateral membrane was an electroneutral process.

It is interesting to determine whether probenecid, a classical inhibitor of organic anion transport, may specifically inhibit the pH gradient-stimulated transport of prostaglandin E_2. As summarized in Table III, addition of probenecid to the external medium inhibited the pH-stimulated uptake of prostaglandin E_2 and also the pH-stimulated uptake of acetate and SCN^- which are accumulated via nonionic and ionic diffusion, respectively. Under these conditions, we observed a comparable inhibitory effect of SITS which is considered to be a specific anion-exchange inhibitor. The nonspecific effect of probenecid could be due to its intravesicular accumulation by nonionic diffusion [10] leading to intravesicular acidification. Accordingly, in the next series of studies, the vesicles were loaded with various concentrations of probenecid and diluted 16-fold in probenecid-free media to reduce alterations in the transmembrane electrical potential difference and pH gradients. Under these conditions, probenecid inhibited the pH gradient-stimulated uptake of prostaglandin E_2 in a dose-dependent manner, whereas the pH gradient-stimulated uptake of acetate and SCN^- were unaffected by this agent.

Finally, we examined the possible existence of Na^+ or K^+ cotransport pathways for prostaglandin E_2. As shown in Table IV, imposition of an

TABLE III

EFFECT OF PROBENECID ON pH GRADIENT-STIMULATED UPTAKE OF PROSTAGLANDIN E_2, SCN^- AND ACETATE

Basolateral membrane vesicles were preincubated for 90 min at 20°C in 20 mM K_2SO_4, 150 mM sucrose, 50 mM Tris-Hepes (pH 7.4) with or without probenecid at the specified concentrations. The 10-s uptake of either 5 nM [^{3}H]prostaglandin E_2, 14 μM [^{14}C]KSCN or 16 μM [^{3}H]acetate by controls and probenecid-treated vesicles was assayed with the indicated concentrations of probenecid in the presence or absence of a pH gradient as described in the legend to Fig. 1. Results are expressed as means ± S.D. of three different membrane preparations.

Probenecid (μM)		Uptake (% of control)		
intravesicular	extravesicular	prostaglandin E_2	SCN^-	acetate
0	200	62.8 ± 14.6 [a]	65.4 ± 15.7 [a]	60.0 ± 1.4 [a]
400	25	74.4 ± 12.8 [a]	102.2 ± 20.3	106.0 ± 3.6
800	50	64.9 ± 11.4 [a]	89.2 ± 21.9	104.0 ± 20.9
1200	75	49.0 ± 8.8 [a]	95.8 ± 6.9	91.1 ± 10.4

[a] $P < 0.05$ vs. respective controls.

inwardly directed Na^+ gradient across the vesicle in the presence or absence of an outwardly directed K^+ gradient did not enhance the rate of prostaglandin E_2 uptake. The uptake of prostaglandin E_2 at equilibrium was not affected by the nature of the salt gradient, suggesting an absence of changes in vesicle size or degree of binding. These studies indicate that gradients for Na^+ and K^+ existing across the basolateral membrane in vivo cannot drive prostaglandin E_2 uptake.

TABLE IV

EFFECTS OF EXTERNAL Na^+ AN INTERNAL K^+ ON PROSTAGLANDIN E_2 UPTAKE

Basolateral membrane vesicles were preloaded for 90 min at 20°C in 100 mM sucrose, 100 mM KCl and 40 mM Tris-Hepes (pH 7.4). Uptakes were initiated by diluting the membranes 16-fold into a medium containing 5.2 nM [^{3}H]prostaglandin E_2, 100 mM sucrose, 100 mM KCl, 40 mM Tris-Hepes (pH 7.4) (cation gradients absent) or the same medium in which sucrose was replaced by 100 mM NaCl ($na^+_{out} > Na^+_{in}$) or KCl replaced by 100 mM sucrose ($Na^+_{out} > Na^+_{in}$ and $K^+_{in} > K^+_{out}$. Means ± S.D., $n = 4$.

Conditions	Prostaglandin E_2 uptake (fmol/mg protein)	
	0.5 min	60 min
Without cation gradients	3.81 ± 0.37	33.06 ± 4.87
Na^+ gradient ($Na^+_{out} > Na^+_{in}$)	4.19 ± 0.72	34.16 ± 3.27
Na^+ gradient + K^+ gradient ($K^+_{in} > K^+_{out}$)	4.84 ± 0.48	29.97 ± 2.29

Discussion

Recent studies by Bito [1,3], Rennick [2] and Irish [4] suggest that prostaglandins and their metabolites are secreted by the proximal tubule, essentially via the organic acid secretory pathway. In this report, we present strong evidence for the existence of a carrier-mediated prostaglandin E_2 transport system in the basolateral membrane of the rabbit proximal tubule. The pH gradient ($pH_{out} < pH_{in}$) provided the driving force for the uphill accumulation of prostaglandin E_2.

There is a general agreement that cellular pH in the proximal tubule is high. Bichara et al. [13] and Kleinman et al. [14] have shown that, under normal conditions, cellular pH (7.50 ± 0.008) is more alkaline than medium pH (7.4 ± 0.007). Consequently, a gradient for passive H^+ flux into the cell (across the basolateral cell membrane) and OH^- flux (or HCO_3^-) out of the renal cell exists [11,12,15]. Recent studies have suggested that the passive OH^- efflux from the cell into the interstitium (or the passive H^+ flux from the interstitium into the cell) may be coupled in a countertransport (OH^-/anion) or in a cotransport system (H^+/anion) to account for anion uptake across the basolateral membrane [15–17].

Thus we believe there exists a favorable H^+ gradient across the basolateral membrane as the in vivo driving force for transport of prostaglandin E_2 in the kidney.

The present study provides evidence that the H^+ gradient-dependent uptake of prostaglandin E_2 is the result of the operation of a specific transport system rather than the activation of passive transport processes such as ionic or nonionic diffusion. This was shown by the finding that intravesicular probenecid inhibited the pH gradient-stimulated transport of prostaglandin E_2 but did not decrease the pH-stimulated uptake of SCN^- and acetate which enter membranes via ionic and nonionic diffusion, respectively.

The fact that, in the absence of a preset transmembrane pH gradient, the imposition of a valinomycin-mediated K^+ diffusion potential (inside positive) did not stimulate prostaglandin E_2 uptake indicates the absence of a conductive pathway for the uptake of this anion into vesicles. Accordingly, pH gradient-stimulated prostaglandin E_2 uptake cannot be explained by the generation of an interior H^+ diffusion potential due to the inward H^+ gradient. The present studies show that the pH gradient-stimulated transport of prostaglandin E_2 by the basolateral membrane vesicles is mediated by an electroneutral mechanism. This was particularly clearly demonstrated by the observation that the effect of an inward proton gradient on prostaglandin E_2 transport remained unchanged when the H^+ diffusion potential was negated by valinomycin in the presence of an outward K^+ gradient. These results imply that the movements of either H^+ or OH^- and of the anionic form of prostaglandin E_2 are one for one, i.e., a process in which prostaglandin E_2 is cotransported with H^+ or exchanged for OH^-. A distinction between a cotransport with H^+ and an antiport with OH^- is not yet possible.

To summarise, our study provides evidence consistent with an electrically neutral H^+-prostaglandin E_2 cotransport or OH^--prostaglandin E_2 antiport mechanism in the basolateral membrane of the rabbit proximal tubule. The contribution of this pathway to transepithelial prostaglandin E_2 transport remains to be determined.

Acknowledgements

The technical assistance of Isabelle Caillaud and the secretarial assistance of Françoise Carlier are gratefully acknowledged.

References

1 Bito, L.Z. (1976) Prostaglandins 12, 639–646
2 Rennick, B.R. (1977) Am. J. Physiol.: Renal Fluid Electrolyt. Physiol. 233, F133–F137
3 Bito, L.Z. and Baroody, R.A. (1978) Am. J. Physiol.: Renal Fluid Electrolyt. Physiol. 234, F80–F88
4 Irish; J.M., III (1979) Am. J. Physiol.: Renal Fluid Electrolyt. Physiol. 237, F268–F273
5 Boumendil-Podevin, E.F. and Podevin, R.A. (1983) Biochim. Biophys. Acta 735, 86–94
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
7 Rottenberg, H. (1979) Methods Enzymol. 55, 547–569
8 Stahl, E. (1969) in Thin-layer Chromatography (Stahl, E., ed.), 2nd Edn., pp. 86–105, Springer-Verlag, New York
9 Sacktor, B., Rosenbloom, I.L., Liang, T.C. and Cheng, L. (1981) J. Membrane Biol. 60, 63–71
10 Weiner, I.M. and Mudge, G.H. (1964) Am. J. Med. 36, 743–762
11 Aronson, P.S. (1983) Am. J. Physiol.: Renal Fluid Electrolyt. Physiol. 245, F647–659
12 Warnock, D.G. and Rector, F.C. (1981) in The Kidney, 2nd Edn. (Brenner, B.M. and Rector, F.C., eds.), pp. 440–494, Saunders, Philadelphia
13 Bichara, M., Paillard, M., Leviel, F. and Gardin, J.P. (1980) Am. J. Physiol.: Renal Fluid Electrolyt. Physiol. 238, F445–F451
14 Kleinman, J.G., Brown, W.W., Ware, R.A. and Schwartz, J.H. (1980) Am. J. Physiol.: Renal Fluid Electrolyt. Physiol. 239, F440–F444
15 Ullrich, K.J. (1976) Kidney Int. 9, 134–148
16 Pritchard, J.B. and Renfro, J.L. (1983) Proc. Natl. Acad. Sci. USA 80, 2603–2607
17 Low, I., Friedrich, T. and Burckhard, T. (1984) Am. J. Physiol.: Renal Fluid Electrolyt. Physiol. 246, F334–F342