Inhibition of Sodium Transport by Prostaglandin E_2 across the Isolated, Perfused Rabbit Collecting Tubule

JOHN B. STOKES and JUHA P. KOKKO

From the Department of Internal Medicine, The University of Texas Health Science Center, Southwestern Medical School, Dallas, Texas 75235

ABSTRACT This study was designed to examine whether prostaglandin E_2 can directly affect sodium transport across isolated perfused rabbit renal collecting tubules. Changes in transepithelial potential and isotopic sodium fluxes in response to peritubular prostaglandin E_2 were measured. In addition, changes in transepithelial potential of the outer medullary collecting tubule in response to prostaglandin E_2 were also measured. With few exceptions, all rabbits received 5 mg/day desoxycorticosterone acetate for 4–11 days before experimentation. The results of the experiments show that: (*a*) prostaglandin E_2 inhibits the negative transepithelial potential in the cortical collecting tubule as well as the outer medullary collecting tubule; (*b*) prostaglandin E_2 inhibits net sodium transport out of the lumen by inhibiting efflux while backflux is unaffected; (*c*) prostaglandin E_2 produces this inhibition within 15 min, and the effects are dose dependent and reversible. These results suggest that prostaglandin E_2 may modulate sodium transport in vivo and may contribute to the final regulation of sodium excretion.

INTRODUCTION

The collecting duct participates in the final regulation of sodium excretion (1). Recent studies have demonstrated that the cortical collecting tubule (CCT)[1] is the segment sensitive to aldosterone (2, 3) and in this way is capable of regulating sodium reabsorption. Rapid natriuresis in response to a variety of stimuli such as acute volume expansion cannot be explained fully by inhibition of aldosterone because of its long duration of action. These studies were conducted to examine the possibility that a naturally occurring intrarenal substance could modulate sodium transport by the collecting tubule.

The renal medulla is a rich source of prostaglandins (4) and they have been implicated in sodium regulation by many investigators. No evidence currently exists to implicate prostaglandins in renal epithelial transport of salt though such evidence does exist for the jejunum (5), frog corneal epithelium (6), and toad bladder (7). It is difficult to interpret the effects of prostaglandins on renal epithelial transport with clearance techniques because of their well-demonstrated effects on renal blood flow (8, 9) and on vasopressin-induced osmotic water flow (10). The present studies were deisgned, therefore, to examine directly the effect of prostaglandin E_2 (PGE_2), the renal prostaglandin occurring in greatest quantity, on sodium transport across the in vitro-perfused rabbit collecting tubule.

METHODS

Segments of collecting tubule were dissected and perfused in vitro with the same techniques described previously (11). Briefly, female New Zealand white rabbits weighing 1.5–2.5 kg were used in all experiments. All rabbits were fed standard laboratory diet containing approximately 140 meq/kg sodium and 390 meq/kg potassium.

With the exception of a few rabbits so noted, all rabbits received 5 mg/day desoxycorticosterone acetate (DOCA) intramuscularly for 5–8 days before experimentation. This meneuver was done to insure a transepithelial potential difference (PD) lumen negative (2). All animals had free access to food and water before guillotine decapitation.

The kidney was quickly removed, a 1-mm slice was placed in chilled solution identical to the bath, and the appropriate segment of collecting tubule was dissected free.

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[1] *Abbreviations used in this paper*: CCT, cortical collecting tubule; DOCA, desoxycorticosterone acetate; $J_{Na,bl}$, bath-to-lumen sodium flux; $J_{Na,lb}$, lumen-to-bath sodium flux; J_v, net water flux; MCT, outer medullary collecting tubule; PD, transepithelial potential difference; PGE_2, prostaglandin E_2.

The solution was maintained at pH 7.4 by continuous bubbling with 95% O_2 and 5% CO_2. Cortical collecting tubules (CCT) were 1.4–3.0 mm long and outer medullary collecting tubules (MCT) were 0.6–1.2 mm long. All studies were conducted at 37°C. Tubules were perfused with an artificial solution containing NaCl 105 mM, KCl 5 mM, $NaHCO_3$ 25 mM, Na_2HPO_4 2.3 mM, Na acetate 10 mM, $MgSO_4$ 1 mM, $CaCl_2$ 1.8 mM, glucose 8.3 mM, and alanine 5 mM. The bath and dissection solutions were identical to the perfusion solution except for the addition of 5% fetal calf serum. PGE_2 was stored at −20°C in ethanol 10 mg/ml, and the bath solution containing PGE_2 was prepared immediately before use. PGE_2 prepared in this way is stable (12).

During all experiments, except those measuring bidirectional flux, the bath solution was continuously exchanged with a Delta micro-metering pump at 0.5 ml/min (Lab-line Instruments, Inc., Melrose Park, Ill.) while the bath fluid level was maintained by continuous suction at the opposite end. In this system >95% of the bath is exchanged in 5 min, and >99% is exchanged in 10 min. The bath solution can thus be changed continuously without inducing changes in turbulance or temperature. In addition, alterations in bath composition can be effected simply by changing the reservoir. Previous experiments have shown that solute concentration changes less than 2% in 2 h.

The PD was measured by techniques previously reported (13). Lumen-to-bath sodium flux ($J_{Na,lb}$) was calculated from measurements of the disappearance of ^{22}Na (10–20 μCi/ml) from the perfusate. Bath-to-lumen sodium flux ($J_{Na,bl}$) was determined by adding ^{24}Na (75–100 μCi/ml) to the bath (without bath perfusion) and measuring its appearance in the collected fluid. ^{22}Na and ^{24}Na (New England Nuclear, Boston, Mass.) were discriminated by using appropriate channels on a Packard model 3365 gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) and the sample was recounted 14 days later when ^{24}Na activity could not be detected. ^{24}Na activity was corrected to the time at which the counting began. ^{125}I-Iothalamate (Glofil 125, Abbott Laboratories, N. Chicago, Ill.) was added to the perfusate (15 μCi/ml) and collections where the net water flux (J_v) was more negative than −0.1 nl/mm per min were discarded (5–10% of measurements) since this deviation reflects a leak in the seal at the collecting pipette. J_v was calculated according to the expression:

$$J_v = \frac{V_o}{L}\left[\frac{[^{125}I]_o}{[^{125}I]_i} - 1\right],$$

where V_o is the collection rate, L is the length of the tubule, and $[^{125}I]_i$ and $[^{125}I]_o$ are the concentrations of ^{125}I in the perfused and collected fluid, respectively. $J_{Na,lb}$ was calculated according to the expression:

$$J_{Na,lb} = [Na]_i \frac{V_i}{L} \ln \frac{[^{22}Na]_i}{[^{22}Na]_o},$$

where $[Na]_i$ and $[^{22}Na]_i$ are the chemical and isotopic concentrations of Na in the perfusate, $[^{22}Na]_o$ is the isotopic concentration of sodium in the collected fluid, and V_i is the perfusion rate. $J_{Na,bl}$ was calculated according to the following expression which accounts for a significant efflux of sodium (modified from [14]):

$$J_{Na,bl} = \frac{[Na]_b[^{24}Na]_o}{[Na]_i[^{24}Na]_b}\left[\frac{J_{Na,lb}}{1 - \exp - (L \cdot J_{Na,lb}/V_i[Na]_i)}\right],$$

where $[Na]_b$ and $[^{24}Na]_b$ are the respective chemical and isotopic concentrations in the bath and $[^{24}Na]_o$ is the isotopic concentration in the collected fluid.

Tubular perfusion rate was maintained between 3 and 6 nl/min by hydrostatic pressure. An equilibration period of 90–120 min was necessary to allow the control PD to stabilize. Three periods were recorded: control, PGE_2 addition to the bath, and recovery where PGE_2 was removed from the bath. Once the control PD was stable for 15 min, the bath was changed to the solution containing PGE_2.[2] The effect of PGE_2 became apparent within 2 min, and the maximum effect usually began to stabilize within 15 min. When the PD was stable for 10 min the bath was changed to the control bath. The return of a stable potential took 40–50 min.

To insure an unbiased interpretation of the results changes in PD were measured in nine "blind" experiments with solutions prepared by a person not connected with these investigations. The solution contained either 1.0 μM PGE_2 or an equivalent amount of carrier (ethanol 0.004% vol/vol).

Sodium flux measurements were made for two to four collection periods in each of the three periods. Collections were initiated when the PD became stable, and the collected isotopes were counted. Bath perfusion during unidirectional flux experiments was constant, and distilled water was added to the bath during bidirectional flux experiments to replace evaporative losses. Before the beginning of the second period, an appropriate amount of PGE_2 was added to the bath to make the concentration 1.0 μM. During the 40–60 min necessary for recovery of the PD, the bath exchange was continuous. It was again discontinued before the measurement of recovery bidirectional fluxes.

All analyses were done with the Student's t test for paired values, and mean values are reported±standard error.

RESULTS

Effect of PGE_2 on the trasepithelial potential of the cortical collecting tubule and outer medullary collecting tubule. The addition of PGE_2 to the bath caused the magnitude of the negative PD of the CCT and MCT to decrease. No effect on PD was seen when comparable doses of PGE_2 were added to the perfusate ($n = 5$). The peritubular effect was rapid, requiring only minutes to become apparent with a maximal response beginning to stabilize within 15 min. Though on rare occasion the PD became transiently more negative, the majority of responses simply demonstrated an inhibition. The onset of recovery required at least 15 min after complete elimination of PGE_2 from the bath and stable recovery generally required 40–60 min. Fig. 1 displays the change in PD of CCT as a function of PGE_2 concentration of the bathing solution.

Doses of 10 nM resulted in a barely detectable response while doses of 0.1–10 μM effected an increasing response such that at 10 μM the mean inhibition was greater than 80%. The PD of CCT from normal rabbits is difficult to predict and is dependent, at least in part, on the mineralocorticoid activity

[2] Kindly provided by Dr. John Pike, The Upjohn Co., Kalamazoo, Mich.

(2). The administration of DOCA insures a negative PD which is invariably responsive to PGE_2 in doses of 0.1 μM or greater. The pretreatment of the rabbit with DOCA appeared to have no effect on the PD response when compared to tubules of equivalent base-line PD not so treated.

Nine experiments were conducted in a blinded fashion where the experimentor was unaware whether the solution contained PGE_2 (1.0 μM, $n = 6$) or the carrier ($n = 3$). PGE_2 in these experiments caused a reduction in the magnitude of the PD by >26% while the carrier had no effect ($P < 0.001$).

Since PGE_2 is found in high concentrations in the medulla (4), the response of the PD in the MCT was also examined. This segment is morphologically indistinguishable from the CCT at ×400 during the experiment. Fig. 2 demonstrates the response of the negative PD to various doses of PGE_2 in the bath. The response to 1 and 10 μM PGE_2 was similar to that found in the CCT. However, the tubule failed to recover from 0.1 mM in 1 h, the time at which

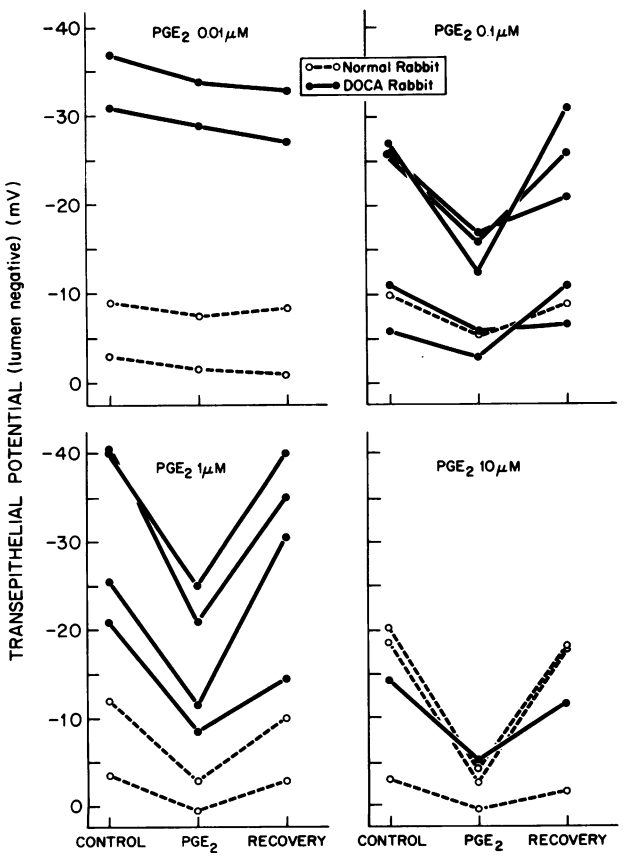

FIGURE 1 Effect of PGE_2 on rabbit CCT PD. PGE_2 was added to the bathing solution in the concentrations shown. In all experiments a stable PD was obtained after 90–120 min. The stable PD resulting from PGE_2 addition is recorded. Recovery was complete within 60 min.

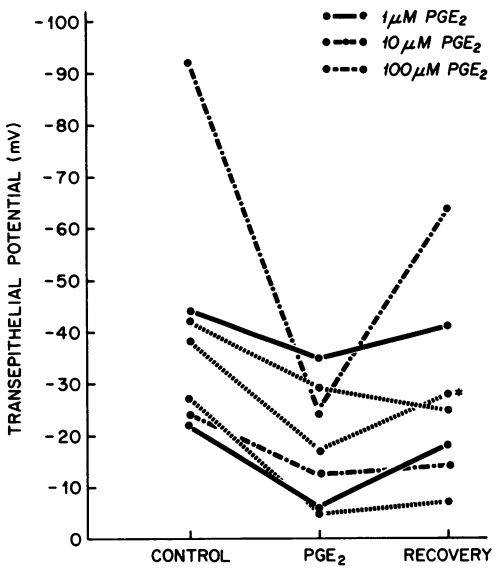

FIGURE 2 Effect of PGE_2 on outer medullary collecting tubule PD. Technique was identical to that used in Fig. 1. All rabbits received DOCA. *Indicates one experiment where recovery from 0.1 mM PGE_2 was allowed to continue for 180 min rather than the usual 60 min. At 0.1 mM no tubule recovered within 60 min.

recovery was virtually complete when smaller doses were used. The one tubule demonstrating recovery after 0.1 mM did so only after 3 h. The finding that PGE_2 inhibits the negative PD in the MCT in a fashion similar to CCT provides stronger evidence that PGE_2 may act similarly in vivo.

Effect of PGE_2 (1.0 μM) on sodium flux. Table I depicts the alterations in simultaneous bidirectional sodium fluxes across the CCT induced by the addition of 1.0 μM PGE_2 to the bath. There was no significant change in backflux ($J_{Na,bl}$) while efflux was significantly inhibited. In an additional six experiments unidirectional lumen-to-bath fluxes were measured under conditions identical to the PD experiments (i.e., with the bath pump on). PGE_2 inhibited efflux in a similar fashion: 25.5±4.0 (control), 15.1±3.5 (PGE_2), and 21.3±4.1 (recovery). The differences are highly significant ($P < 0.001$).

The resulting alteration in sodium transport can be expressed as a change in flux ratio (Table I) or in a net sense. If one assumes a perfusion rate of 2 nl/min of isotonic fluid and a net efflux of 21.2 peq. cm^{-1} per s ($J_{Na,lb} - J_{Na,bl}$), in 2 mm of tubular length, 45% of the sodium load would be reabsorbed. PGE_2 (1.0 μM) would decrease this reabsorption by an average of 40–50%.

There is a greater fall in $J_{Na,lb}$ in these bidirectional flux experiments than in the unidirectional flux experiments, and the recovery in the bidirectional experiments is less complete. These findings are probably

TABLE I

Effect of 1.0 μM PGE_2 Applied to Peritubular Surface of CCT on Transepithelial Potential and Bidirectional Sodium Flux

Experiment	Potential			$J_{Na,lb}$			$J_{Na,bl}$			$J_{Na,lb}/J_{Na,bl}$		
	Control	Exp.	Recovery	Control	Exp.	Recovery	Control	Exp.	Recovery	Control	Exp.	Recovery
	mV			peq·cm^{-1}·s^{-1}			peq·cm^{-1}·s^{-1}			peq·cm^{-1}·s^{-1}		
1	−38	−19	−38	21.6	8.7	13.3	4.8	3.6	4.4	4.5	2.4	3.0
2	−49	−16	−46	30.7	7.0	14.9	4.7	3.9	3.6	6.5	1.8	4.1
3	−35	−14	−66	33.2	8.2	16.6	4.0	4.2	4.1	8.3	1.9	4.0
4	−38	−12	−39	18.9	6.7	7.0	6.2	6.5	4.6	3.0	1.0	1.5
Mean	−40	−15	−47	26.1	7.6	12.9	4.9	4.5	4.2	5.6	1.8	3.2
SEM	±3.1	±1.5	±6.5	±3.4	±0.5	±2.1	±0.5	±0.7	±0.2	±1.2	±0.3	±0.6
P	<0.0025		<0.0125	<0.01		<0.05	NS		NS	<0.025		<0.05

Each flux value is the mean of two to four collections. Exp., experiment.

due to the fact that bidirectional experiments take longer to complete than do unidirectional ones. A longer exposure to PGE_2 and tubule fatigue most likely account for the differences. That this fatigue does not affect membrane permeability is evidenced by the sodium backflux which tended to fall insignificantly with time.

DISCUSSION

The present studies provide the first evidence that PGE_2 can affect renal tubular sodium transport directly. In the setting of stimulated sodium transport across the cortical collecting tubule and outer medullary collecting tubule PGE_2 in doses ranging from 0.1 μM to 0.1 mM when applied to the peritubular surface causes an inhibition of the negative PD (Figs. 1 and 2) and a decrease in the outward sodium flux without a significant change in the influx of sodium (Table I). The effect is rapid, reversible, and dose dependent. The net efflux of sodium can be inhibited by as much as 85% by 1.0 μM PGE_2.

Since the discovery of large quantities of prostaglandins in the renal medulla (4), many attempts have been made to elucidate its mechanisms of action. The general approaches that have been utilized to assess the effect of prostaglandins on renal function in the intact kidney include: (*a*) infusion of prostaglandins into the renal artery (8, 9, 15, 16), (*b*) infusion of sodium arachidonate into the renal artery to stimulate endogenous prostaglandins (17, 18), and (*c*) reduction of endogenous intrarenal prostaglandins by drugs or dietary deficiency (19, 20). Because of concomitant changes in renal blood flow and water excretion, the effects of prostaglandins on renal tubular transport are difficult, if not impossible, to interpret. Because of these difficulties and the controversies surrounding the role of prostaglandins in sodium excretion, we have chosen to study their effects in the in vitro, isolated, perfused tubule. The advantage of the technique is that one can control completely the epithelial environment and thus eliminate such conditions as intrarenal hydrostatic pressure, regional blood flow, and circulating factors which might cause uniterpretable changes.

The sodium fluxes reported in these CCT experiments are two to three times higher than those reported by other investigators (21–24) when they have examined the CCT from rabbits not receiving a mineralocorticoid hormone. These flux data support the electrophysiologic data implying that the collecting tubule is the site where aldosterone exerts its action (2, 3). The capacity of the CCT taken from these DOCA-stimulated rabbits to transport sodium is second only to the proximal convoluted tubule which can transport sodium at a rate of 37 peq·cm^{-1} per s (25). No other segment when evaluated under similar conditions has been found to transport more than 13 peq·cm^{-1} per s (26). The range of fluxes exhibited by the collecting tubule (ranges presumably due to the effect of mineralocorticoid hormones) and the magnitude of its capacity are entirely reasonable considering the teleological importance of maintaining extracellular volume.

The inhibition of sodium transport in the rabbit collecting tubule by PGE_2 may appear to be at variance with the stimulation of sodium transport in the toad bladder by PGE_1, since the toad bladder is sometimes compared with the mammalian collecting tubule. However Besley et al. (27) may have resolved this issue since they have demonstrated an inhibition of sodium transport by PGE_2 while confirming the stimulatory effect of PGE_1 in the theophylline-treated toad bladder.

The evidence that this action of PGE_2 is physiologically significant is circumstantial but supported by four studies. (*a*) Recent determinations of renal PGE_2 by gas chromatography-mass spectrometry (28) demon-

strate levels of $0.19 \pm 0.04\ \mu g \cdot g^{-1}$ in the cortex and $4.36 \pm 1.04\ \mu g \cdot g^{-1}$ in the medulla, concentrations which fall well within the effective dose range utilized in our experiments. (*b*) Histochemical studies have demonstrated prostaglandin dehydrogenase (the enzyme responsible for the degradation of PGE_2) activity in the thick ascending limb of Henle, the distal convoluted tubule, and the inner medullary collecting tubule, a distribution which skips the segments of the collecting tubule examined in this study (29). (c) Inhibition of prostaglandin synthesis is associated with a striking increase in medullary NaCl concentrations (30), a finding which is compatible with the thesis that prostaglandins normally inhibit sodium reabsorption in the collecting tubule. (*d*) Bartter's syndrome, a pathological state identified by hypokalemic alkalosis; high circulating renin and aldosterone; a tendency to renal salt wasting; and a normal blood pressure which is resistant to angiotensin II infusions is also accompanied by high urinary prostaglandins (31–33). Several patients have been reported whose metabolic and pathologic syndrome has been reversed with indomethacin, an inhibitor of prostaglandin synthesis (31, 32). Taken in the aggregate, these studies strongly suggest that PGE_2 may inhibit the reabsorption of sodium by the collecting tubule in vivo.

In summary, we have shown that PGE_2 can inhibit sodium efflux from the lumen of cortical collecting tubules taken from rabbits stimulated by DOCA. The inhibition is rapid, reversible, and dose dependent. In addition, PGE_2 exerts a similar effect on the outer medullary collecting tubule, a fact which greatly enhances the probability that PGE_2 acts in vivo to inhibit directly sodium reabsorption by the collecting tubule.

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