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Prostaglandin E_2 Effect from the Luminal Side on Renal Tubular ^{22}Na Efflux: Tracer Microinjection Studies (39653)[1]

MICHAEL L. KAUKER
With technical assistance of Hien Nguyen
The University of Tennessee, Center for the Health Sciences, Department of Pharmacology, Memphis, Tennessee 38163

Renal prostaglandins (PG) have been implicated as possible mediators of the antihypertensive actions of the kidneys, perhaps through blunting vasoconstrictor responses or by increasing the renal elimination of sodium and water. PGE_2, the principal renal PG, is synthesized primarily in the medulla and papilla. However, several of the proposed mechanisms of its effect would require a cortical site of action. Prostaglandins of renal origin have been identified in the urine (1), and a tubular route of medullocortical PG transfer has been proposed. Previous studies in our laboratories (2), using tubular microinjection techniques, showed that about one-third of the PGE_2 reaching the loop of Henle was reabsorbed and did not re-enter the tubule; another third was temporarily delayed, and the remainder traversed it without tubular interaction. PGE_2 efflux beyond early distal puncture sites was very low (<14%). These studies suggested inhibition of sodium and water reabsorption in the distal nephron as a result of the action of PGE_2 at the luminal side rather than at the peritubular side. Alternatively, the PGE_2 that exited in the loop could have altered the function of the ascending tubule or some peritubular structures such as the efferent or afferent arterioles.

The present experiments were designed to examine the effects of PGE_2 from the luminal side on net tubular ^{22}Na efflux beyond the proximal convoluted tubule during 2.5% saline diuresis in rats. Sodium recovery was significantly increased by PGE_2. It is suggested that luminal PGE_2 action may represent an important component of the direct tubular effects of endogenous renal prostaglandins.

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Methods. Male Wistar rats weighing 296 ± 31 g were anesthetized ip with Inactin, 100 mg/kg of body weight. Blood pressure was recorded from the cannulated right carotid artery. The animals were infused iv throughout the experiment with 2.5% saline at a constant rate of 0.05 ml/min to permit rapid serial urine collections. After an equilibration period of 45 min or longer, late proximal surface convolutions were punctured in the left kidney with sharpened volumetric micropipets having an outer tip diameter of 5–8 μm and an average volume of 39 ± 13 nl. Tubules were identified by lissamine green administration and, transit time was measured as described previously (2, 3). Each selected nephron was microinjected at the same site with a control and a PGE_2-containing radioactive solution. The radioactive mixtures were prepared as follows. (i) PGE_2 droplet: Isotonic saline, 0.5 ml, containing 100 μCi of [^{3}H]inulin was mixed with 0.5 ml of saline containing 100 μCi of ^{22}Na, and the solution was colored by adding 50 mg of lissamine green. This radioactive stock solution was mixed with a solution of PGE_2 in isotonic saline (10 μg/ml). The final PGE_2 concentration of the droplet was 5 μg/ml, and the average dose in a single intratubular injection was 200 pg.

(ii) The control droplet was prepared as the PGE_2 droplet except that the radioactive stock was mixed 1:1 with isotonic saline only.

The droplets were divided into small aliquots (0.01 ml each) and stored frozen in sealed glass capillaries. Each aliquot was used only once to prevent contamination or degradation of PGE_2. The injection rate was adjusted to maintain tubular diameter in the vicinity of the micropipet and to prevent retrograde flow. Injection rates averaged 29 ± 9 and 29 ± 8 nl/min for the control and

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PGE_2 injections, respectively. Urine samples were collected separately from the catheterized left and right ureters into vials containing 10 ml of scintillation counting liquid (Triton). Ten 30-sec, three 60-sec, and one 120-sec consecutive samples were collected from the left microinjected kidney, and four 150-sec samples from the right kidney. Total collection time was 10 min from both sides.

Reference standards were prepared by depositing a volume of radioactive mixture (same as the subsequently microinjected volume) directly into counting vials. Radiochemicals were at least 98% pure as shown by the supplier (N.E.N. Boston, Mass.). In all samples, ^3H and ^{22}Na activities were measured simultaneously in a liquid scintillation spectrometer with external standardization as previously described (2–4). The general method of preparation and sample handling and the formulas used to calculate the values presented here have been previously described (2, 5–7). Data are presented as the mean ± one standard deviation (SD). Statistical significance was evaluated by the Student's *t*-test for paired variables.

Results. Forty technically successful microinjections were made in 10 rats. Half of these served as controls and the other half contained PGE_2 in a concentration of 5 pg/nl in addition to [^3H]inulin and $^{22}NaCl$. The data are summarized in Table I. Mean systemic blood pressure and the rate of urine flow from the two kidneys were similar during control and PGE_2 injections. Late-proximal and early-distal tubular transit times were also unaltered, suggesting that renal function was similar during the two injections. Injection time as well as pipet volume and injection rate (see Methods) were similar. Inulin recovery was complete in both cases, indicating that inulin, once deposited in the lumen, remains in the tubule and can be quantitatively recovered in the urine. ^{22}Na recovery from the left microinjected kidney was slightly, but significantly, higher when the injected solution contained PGE_2. The increase was even greater in total ^{22}Na recovery, revealing that PGE_2 significantly reduced the net sodium efflux in nephron segments beyond the late proximal puncture site.

In Fig. 1, the time course of inulin and sodium recoveries during control and PGE_2 injections are illustrated. Inulin recovery usually had a high peak and was complete within 5 min after ending the injection. The recovery pattern was similar for the control and PGE_2 injections. Recovery peaks were lower for ^{22}Na than for inulin in both control and PGE_2 injections. The appearance of sodium was somewhat delayed beyond inulin (30–60 sec), and its recovery terminated sooner (30–40 sec) in most cases. However, a temporal dissociation between ^{22}Na and [^3H]inulin could not be detected during either of the injections. The recovery lines did not cross on the upslope or downslope, nor could a difference be detected in mean tubular transit times of sodium and inulin. Precession of sodium over inulin was not expected or observed since these were luminal rather than close arterial injections.

In Fig. 2, the cumulative inulin and sodium recovery patterns are illustrated. Inulin recovery followed a similar course during control and PGE_2 injections and reached 100%. Sodium recovery, on the other hand,

TABLE I. EFFECT OF LUMINAL PGE_2 ON RENAL FUNCTION.

Function	Control	PGE_2	Δ	*P*
Blood pressure (mmHg)	115 ± 17[a]	114 ± 17	1 ± 1	NS[a]
Urine flow, left kidney (μl/min)	36 ± 8	37 ± 10	1 ± 1	NS
Urine flow, right kidney (μl/min)	37 ± 12	38 ± 11	1 ± 1	NS
Late-proximal transit time (sec)	11 ± 1	11 ± 1	0	NS
Early-distal transit time (sec)	35 ± 5	35 ± 4	0	NS
Injection time (sec)	83 ± 29	81 ± 27	2 ± 4	NS
Inulin recovery (%)	99.5 ± 3.2	100.8 ± 4.1	1.3 ± 1.1	NS
^{22}Na Recovery, left (%)	26.5 ± 12.4	28.9 ± 12.4	2.4 ± 1.4	<0.025
^{22}Na Recovery, total (%)	29.6 ± 13.3	34.1 ± 13.2	4.5 ± 1.7	<0.01

[a] Data are presented as mean ± SD. $N = 20$.
[b] NS, $P > 0.1$.

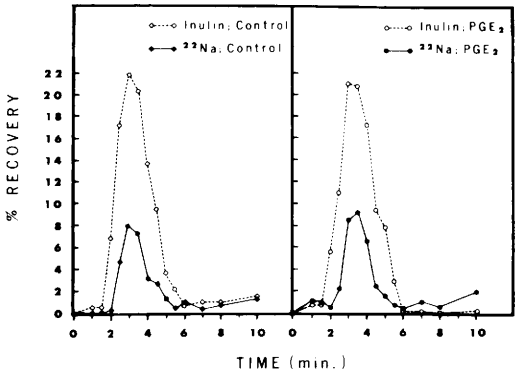

FIG. 1. Patterns of [^{3}H]inulin and ^{22}Na recoveries after microinjection into late-proximal convolutions of the rat nephron with and without PGE$_2$. Diuresis was induced by iv infusion of 2.5% saline.

plateaued around 32% during the control injection and was increased to about 37% recovery when PGE$_2$ was administered simultaneously. This general pattern of reduced ^{22}Na efflux in the presence of PGE$_2$ was observed in 16 of the 20 paired injections. In some cases, the difference was marked, whereas in four injection pairs, a slight reverse effect was found, that is, PGE$_2$ appeared to increase ^{22}Na efflux slightly.

Discussion. The results of the present study reveal that PGE$_2$, applied to the luminal side of renal tubules, increases slightly, but significantly, the urinary recovery of simultaneously administered ^{22}Na. Since renal function and the method and site of injection were similar under the two conditions, the increased ^{22}Na recovery could best be attributed to its reduced net transtubular efflux under PGE$_2$ action. The inhibition of sodium efflux was low, about 3–4% of the amount injected. However, these rats were in intense hypertonic saline diuresis with markedly inhibited baseline sodium reabsorption as indicated by the high urine flow and ^{22}Na recovery of the control. It is difficult to reduce sodium transport further under these conditions. Nevertheless, the net sodium efflux decreased from 70 to 65%, or about 7% of the control value. In anti-diuresis, the same nephron segments may reabsorb more than 97% of the solute and water delivered to them and excrete less than 3%. Therefore, if the PGE$_2$ effect is of similar magnitude in anti-diuresis, luminal action may account for an increase in sodium excretion from less than 3% of distal delivery to more than 8%. This is similar to the usual two- to three-fold increase observed when PG synthesis goes from low to high, or when exogenous PGE$_2$ is infused into the renal artery.

Equal partition of medullary PG between peritubular blood and tubular fluid has been reported (8), and PG of renal origin have been identified in urine (1), indicating accessibility of the renal tubule to PG. Vascular or other indirect effects of this luminal PG should be minimal. It is therefore postulated that luminal PGE$_2$ may modulate extracellular fluid volume in the following manner. A portion of the PGE$_2$ liberated in the medulla in response to a variety of stimuli enters the loop of Henle and is delivered via the tubular route to its site of action, where it reduces the net efflux of solute and water. This luminal PGE$_2$ action may represent a significant component of the direct tubular effects of intrarenal prostaglandins.

The mechanism of PG action on ^{22}Na efflux is not apparent. It is likely that the fraction of luminal PGE$_2$ previously shown to be temporarily delayed in the loop (2) interacts with tubular cells and reduces the activity of a sodium pump or increases the backleak of reabsorbed sodium. It should be emphasized that the effect of PGE$_2$ on ^{22}Na recovery in the present studies was small.

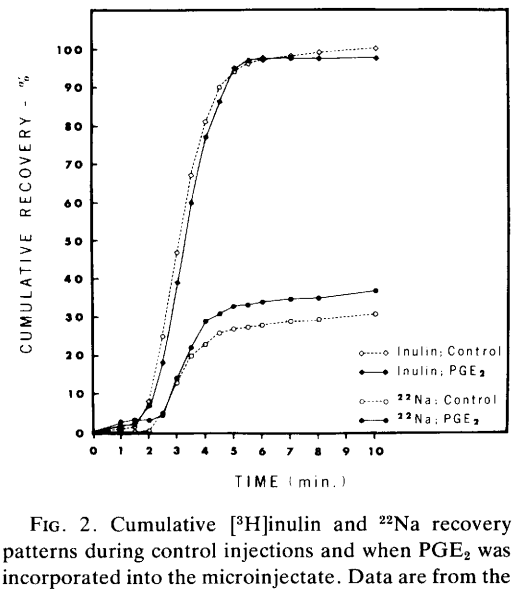

FIG. 2. Cumulative [^{3}H]inulin and ^{22}Na recovery patterns during control injections and when PGE$_2$ was incorporated into the microinjectate. Data are from the same experiment as in Fig. 1.

Alternate mechanisms of action of renal PG on extracellular fluid balance should be considered. Redistribution of RBF, antagonism of the renin–angiotensin aldosterone system, altered efferent and afferent arteriolar tone, and, perhaps, a peritubular cortical site of action of distally absorbed PG may contribute to the overall renal effects.

Summary. Using renal micropuncture techniques, the effect of luminal PGE_2 on the net efflux of simultaneously administered ^{22}Na was examined in rats in hypertonic saline diuresis. [^{3}H]Inulin and $^{22}NaCl$ were microinjected into the lumen of late-proximal convoluted tubules with and without PGE_2, and the urinary recovery of ^3H and ^{22}Na were followed. Sodium recovery was significantly higher in the presence of PGE_2 while renal function and the method and site of injection were similar.

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