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METABOLISM OF INTRATUBULAR PROSTAGLANDIN E_2 IN THE RAT KIDNEY

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Urinary concentrations of prostaglandin E_2 are considered an index of intrarenal prostaglandin levels. The extent to which prostaglandins generated in the kidney undergo metabolic transformation prior to excretion is at present unknown.

Prostaglandin E_2 excreted in the urine appears to derive from a pool of prostaglandins produced in the kidney that enters the tubular fluid in the loop of Henle (1,2). Histochemical studies of rat kidney suggest that the prostaglandin metabolizing enzyme 15-hydroxyprostaglandin dehydrogenase is concentrated in the cortical portions of the ascending limb of the loop of Henle and therefore is in a position to intercept prostaglandins that have entered the tubular fluid in medullary portions of the loop before they can reach possible sites of action in more distal portions of the nephron (3). Previous experiments by one of us have shown that, after injection of [^{3}H]-prostaglandin E_2 into the proximal rat renal tubule, emergence of tritium label in the urine was delayed relative to the emergence of simultaneously injected [^{14}C]-inulin (4). In contrast, no difference in the rate of emergence of the two isotopes was seen after injection into the distal tubule. This indicates that prostaglandin E_2 in the tubular fluid is absorbed or adsorbed at some site between the proximal and distal convoluted tubules. If this removal results in exposure of the prostaglandin to metabolizing enzymes, it would be expected that there would be a difference in the chemical nature of products reaching the urine after proximal or distal injection of [^{3}H]-prostaglandin E_2. We have therefore repeated the previous study to determine whether a site of prostaglandin metabolism occurs in the rat nephron, situated between proximal and distal tubules, and capable of altering levels of biologically active prostaglandins that reach late segments of the nephron and are contained in the final urine.

METHODS

Male Wistar rats (8), weighing 250 - 350 g, were anesthetized with an intraperitoneal injection of Inactin and prepared for microinjection studies as previously described (4,5). To permit rapid urine collections, the animals were made diuretic by the infusion of 2.5% NaCl at a rate of 0.1 ml/min through a cannula inserted in the right jugular vein. Animals were placed on a heated animal board, and body temperatures were maintained at 37°. The left kidney was exposed through an abdominal incision (5), and the ureter was catherized with PE-50 polythyelene tubing with an approximate dead space of 50 µl. Tubular transit times were measured following injection of 50-100 µl of a 5% solution of lissamine green into a second jugular cannula and used to distinguish proximal and distal micropuncture sites.

A solution of [^{3}H]-PGE_2 (125 Ci/mmol; New England Nuclear) and [^{14}C]-inulin (2.95 mCi/g) in 0.01 M Tris-HCl, pH 7.4, containing 0.9% NaCl, was prepared with a final concentration

of PGE_2 of 0.3 µg/ml. Aliquots of about 40 nl of this solution were injected into proximal or distal sites and urine was collected for 30 min. Several injections at proximal or distal sites were performed in each rat.

The collected urine was diluted to about 2 ml with water, acidified with 20 µl of formic acid, and extracted three times with 2 ml of ethyl acetate. The combined organic fractions were evaporated under a stream of nitrogen and redissolved either in 50 µl of methanol for analysis by thin layer chromatography or in 100 µl of 32% acetonitrile for high pressure liquid chromatography. High pressure liquid chromatographic analysis was performed with a Waters Radial-Pak C18 column, eluted at 1 ml/min with 32% acetonitrile in water, brought to pH 3.0 with H_3PO_4. After injection of the sample, eluate was collected in 0.5 ml fractions (30 sec) for 25 min, and radioactivity was determined by scintillation counting in 10 ml of Scintiverse (Fisher). The retention time of prostaglandin standards was determined by simultaneous UV-monitoring at 195 nm.

RESULTS AND DISCUSSION

Chromatography of radioactive products recovered from the urine after distal injection of [^{3}H]-prostaglandin E_2 failed to reveal any significant conversion of the prostaglandin to metabolic products during passage through the kidney (Fig. 1). In contrast, a significant fraction of [^{3}H]-prostaglandin E_2 injected into proximal sites was converted to chromatographically distinguishable products before emerging in the urine (Fig. 2). Although there was variability in the extent of conversion, all proximal injections resulted in the formation of products more polar than prostaglandin E_2. It would therefore appear that prostaglandin E_2 that enters the tubular fluid is metabolized at a location between the proximal and distal tubules. This is consistent with the histochemical localization of prostaglandin dehydrogenase activity in the thick ascending limb of the loop of Henle (3). However, the radioactive products emerging in the urine were not the expected products of oxidation of the hydroxyl group on C-15 (and reduction of the double bond between C-13 and C-14), but were more polar than the parent material. These products therefore result either from more extensive metabolism of 15-ketoprostaglandin E_2 or from metabolism by independent pathways.

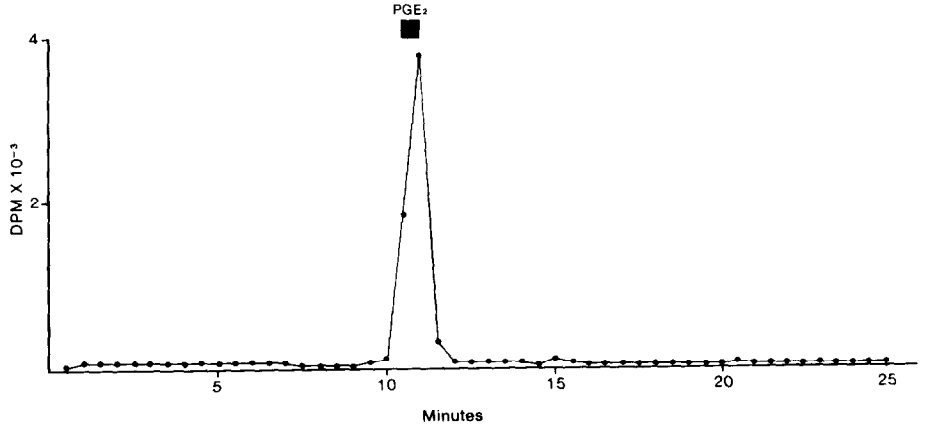

Fig. 1. Representative high pressure liquid chromatographic profile of radioactive products recovered from the urine after microinjection of [^{3}H]-prostaglandin E_2 into the distal tubular fluid. The retention time of prostaglandin E_2, determined by UV-monitoring at 195 nm, is indicated.

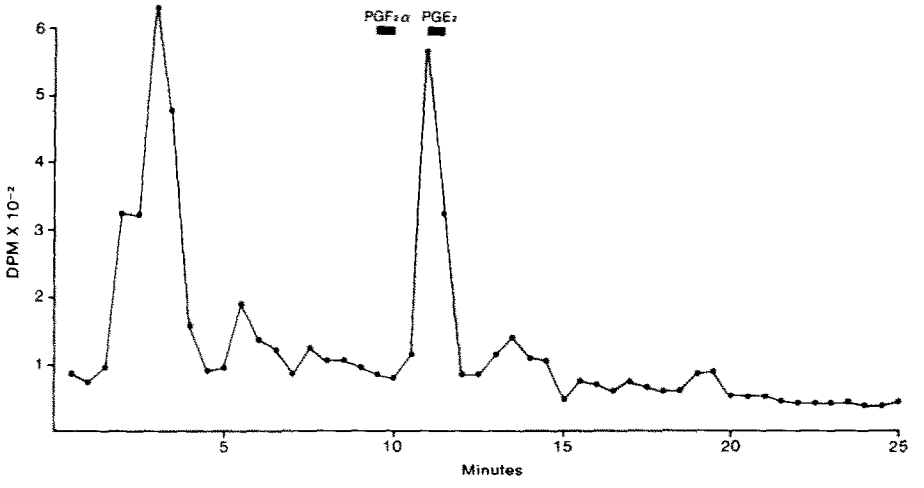

Fig. 2. Representative high pressure liquid chromatographic profile of radioactive products recovered from the urine after microinjection of [^{3}H]-prostaglandin E_2 into the proximal tubular fluid. The retention times of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 standards are indicated. The retention time of 15-ketoprostaglandin E_2 in this chromatographic system is 13-13.5 min.

Green (6) has reported that in the rat, after injection of [^{3}H]-prostaglandin E_2 into a peripheral vein, the bulk of the radioactive material excreted in the urine represents polar products of β- and ω-oxidation, as well as oxidation by prostaglandin dehydrogenase. The sites of these metabolic alterations have not been determined, although the lungs represent a site for oxidation of prostaglandins in the general circulation by prostaglandin dehydrogenase (7). Prostaglandin E_2 injected into the artery of the isolated, Krebs buffer-perfused rabbit kidney is metabolized by β-oxidation as well as by reduction of the 9-keto group and oxidation by prostaglandin dehydrogenase (8,9); similar experiments with isolated rat kidneys showed only metabolism by prostaglandin dehydrogenase (10). The capacity of renal cortical microsomes to catalyze ω-oxidation of prostaglandin E_1 has also been reported (11). Our results suggest that a significant portion of the conversion of prostaglandin E_2 to the β- and ω-oxidation products that appear in the urine may occur during transit through the renal tubule itself.

Two additional points should be noted. First, the extent of metabolism of [^{3}H]-prostaglandin E_2 injected into the tubular fluid may underestimate the extent of metabolism of renally synthesized prostaglandin that enters the tubular fluid in the loop of Henle. The diuretic condition of the rats during the experimental procedure undoubtedly reduces the contact time of intratubular prostaglandin with cells lining the renal tubule. In addition, the concentration of [^{3}H]-prostaglandin E_2 in the solution added to each tubule is probably much higher than that of endogenous prostaglandin E_2 in the tubular fluid, based on a concentration of immunoreactive prostaglandin E_2 in the final urine of about 10 ng/ml (12), and may saturate transport or other rate limiting steps in metabolism.

Second, we observed no significant reduction of intratubular [^{3}H]-prostaglandin E_2 to prostaglandin $F_{2\alpha}$. It might have been anticipated that significant reduction of

prostaglandin E_2 would have been observed, since the rat kidney contains an enzyme, prostaglandin E 9-keto reductase, capable of effecting such reduction *in vitro* (13), and since the ratio of immunoreactive prostaglandin $F_{2\alpha}$ to prostaglandin E_2 in rat urine is several times higher than the ratio of these prostaglandins produced by renal slices (unpublished observation). The absence of reduction of [^{3}H]-prostaglandin E_2 suggests that conversion of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ during passage through the nephron is not responsible, at least in the rat, for the relatively high concentrations of prostaglandin $F_{2\alpha}$ found in urine.

In summary, [^{3}H]-prostaglandin E_2 microinjected into the proximal tubular fluid of the rat kidney was converted to polar metabolites prior to excretion, while that injected into distal sites was not. These results suggest that metabolism of intrarenally generated prostaglandins is a determinant of the levels of biologically active prostaglandin reaching the tubular fluid of late nephron segments. The chemical nature of the prostaglandin metabolites produced by the kidney under these conditions is under current investigation.

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