Influence of NSAID-Induced Inhibition of Renal Prostaglandin Synthesis on Inorganic Sulfate Clearance in Rats (43374)

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Abstract. **The objective of the present investigation was to examine the influence of inhibition of renal prostaglandin synthesis on the renal clearance of inorganic sulfate, an electrolyte involved in the biotransformation of both exogenous and endogenous substrates. Homeostasis of inorganic sulfate is maintained predominantly by renal reabsorption in the proximal tubule. Using a crossover study design, the renal clearance of sulfate was assessed in conscious female Lewis rats during control periods and following the infusion of two structurally dissimilar nonsteroidal anti-inflammatory drugs, ibuprofen (IBU) and indomethacin (INDO). Animals were infused with IBU or INDO to achieve steady state concentrations of 59 ± 8 μg/ml (mean ± SD) of IBU and 22 ± 3 μg/ml of INDO. At these serum concentrations, IBU and INDO produced >80% decrease in the urinary excretion of prostaglandin (PG) E_2. Treatment with either IBU or INDO significantly increased the renal clearance of sulfate, but did not alter the glomerular filtration rate as assessed by creatinine clearance. The role of prostaglandins in the effects of IBU and INDO on sulfate homeostasis was investigated by examining the influence of concomitant intraarterial PGE_2 administration (infusion of 0.1 μg/min) on nonsteroidal anti-inflammatory drug-induced alterations in sulfate renal clearance. Although PGE_2 alone did not significantly alter the renal clearance of inorganic sulfate or that of creatinine, the PGE_2 infusion abolished the effects of IBU on sulfate renal clearance. Concomitant PGE_2 administration also significantly increased the sulfate reabsorption rate in INDO-treated animals; other parameters were not significantly changed, although the fractional reabsorption of sulfate tended to increase ($P = 0.17$). The reason for the less pronounced effect on PGE_2 on the INDO-sulfate interaction is as yet unknown, but may be partly due to additional mechanisms involved in the INDO-induced alterations in sulfate clearance. The results of these studies suggest that prostaglandin inhibition represents one mechanism whereby IBU can alter the renal clearance of inorganic sulfate.** [P.S.E.B.M. 1992, Vol 199]

Inorganic sulfate is a physiologic anion that is involved in the conjugative biotransformation of both exogenous and endogenous compounds, reactions that serve detoxification, pharmacologic, and biosynthetic functions (1). Inorganic sulfate is formed in the body from the oxidation of sulfhydryl-containing amino acids (2) and is excreted predominantly in unchanged form in the urine (3, 4). The renal clearance of inorganic sulfate in humans and animals is about 10–35% of the glomerular filtration rate (GFR) under normal physiologic conditions and increases to a rate approximately equal to GFR when serum sulfate concentrations are increased (5–7), suggesting saturable reabsorption and little, if any, tubular secretion. Sulfate reabsorption occurs predominantly in the proximal tubule, and its luminal transporter has been demonstrated to be sodium dependent and electroneutral and to exhibit high specificity (8). Sulfate exit across the contraluminal membrane of the epithelial cells is mediated by an anion exchanger (9). The renal tubular reabsorption of inorganic sulfate represents the major mechanism for sulfate homeostasis in humans and animals.

Prostaglandins are synthesized in the kidneys and

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have a well-established role in the physiologic regulation of renal blood flow, GFR, and the urinary excretion of electrolytes and water (10, 11). These actions are evident under conditions in which renal perfusion is impaired and may represent direct actions of the prostaglandins (PG) or actions secondary to the modulation of other hormones. PGE_2 is quantitatively the most important prostaglandin synthesized along the rat nephron (12, 13), and PGE_2 itself has been shown to increase sodium and phosphate excretion (11, 14, 15). The potential role of prostaglandins in the regulation of sulfate renal transport in the proximal tubule has not been examined previously. Therefore, the objectives of the present investigation were: (i) to examine the influence of two nonsteroidal anti-inflammatory drugs (NSAID), namely ibuprofen (IBU) and indomethacin (INDO), on the renal clearance of inorganic sulfate in rats; and (ii) to examine the potential reversal of NSAID-induced alterations in sulfate disposition by the concomitant infusion of prostaglandin E_2.

Materials and Methods

Chemicals. IBU, INDO, mefenamic acid, and PGE_2 were purchased from Sigma Chemical Co. (St. Louis, MO), potassium iodide was from J. T. Baker Chemical Co. (Phillipsburg, NJ), and potassium hydrogen phthalate was from Fisher Scientific (Fair Lawn, NJ). INDO sodium trihydrate was obtained as a gift from Merck, Sharp, and Dohme Research Lab (Rahway, NJ). All solvents were high-performance liquid chromatography grade.

Preparation of Solutions. IBU was dissolved in N/2 NaOH and diluted to volume with normal saline (4 mg/ml), and the pH of the solution was adjusted to 7.4 with HCl. The volume of N/2 NaOH did not exceed 5% of the final volume. INDO sodium trihydrate was dissolved in normal saline. Infusion solutions for IBU and INDO were obtained by diluting the bolus injection solutions with normal saline. All control solutions were prepared exactly the same way except that drug was omitted. PGE_2 was dissolved in 95% ethanol to give a 1-mg/ml solution that was diluted with saline to 0.01 mg/ml just prior to use.

Preliminary Pharmacokinetic Studies. Preliminary studies were conducted to determine the clearance (CL) and the apparent volume of distribution at steady state (V_{ss}) for INDO in rats. Briefly, INDO was given as an intravenous bolus injection of 10 mg/kg. CL and V_{ss} were calculated by noncompartmental methods (16). IBU pharmacokinetic parameters in the rat have been published previously (17) and these values, along with our experimental values for INDO, were used to calculate the bolus doses and infusion rates for these drugs. Dosage regimens were designed to achieve steady state concentrations of 70 μg/ml for IBU and 23 μg/ml for INDO. The concentration of IBU chosen is similar to the peak concentration seen clinically after administration of a single 800-mg oral dose (18, 19). The concentration of INDO represents the peak concentration that would be attained after the administration of a 5-mg/kg dose, the standard dose used to inhibit prostaglandin synthesis in rats (20, 21). A dose of 2.5 mg/kg is insufficient to decrease the urinary excretion of PGE_2 in healthy rats (21). We also examined a lower INDO concentration (2.4 ± 0.3 μg/ml) in rats, since this concentration is more similar to that observed clinically after therapeutic doses of INDO (22). No significant alterations in the urinary excretion of PGE_2 were found at this low INDO serum concentration.

Study Design. *A comparison of the effects of IBU and INDO on renal prostaglandin inhibition and sulfate homeostasis.* IBU and INDO were studied on separate occasions. Seven female Lewis rats (Charles River, Wilmington, MA) weighing 200–215 g in the IBU study and 190–219 g in the INDO study were utilized. Each rat received one NSAID (treated day) plus its vehicle (control day) in a randomized crossover study, with the two study days separated by 72 hr. INDO-treated rats had right jugular and bladder cannulas implanted under ether anesthesia 2 days prior to the first study day. IBU-treated rats had right carotid cannulas implanted in addition to the other two cannulas. IBU and INDO were given as an intravenous bolus injection of 27 mg/kg and 5 mg/kg followed by a constant infusion of 33 μg/min and 1.5 μg/min (0.0206 ml/min), respectively, for 4 or 6 hr. For IBU, blood samples were obtained at 2, 3, and 4 hr after the start of the infusion and urine was collected between 2 and 4 hr. For INDO, blood samples were obtained at 4, 5, and 6 hr and urine was collected between 4 and 6 hr after initiation of the infusion. Bladders were flushed with 3 ml of distilled water over 3 min just prior to and at the end of the urine collection period. The effects of INDO and its vehicle were studied between 4 and 6 hr, instead of between 2 and 4 hr, since in preliminary studies more pronounced effects on inorganic sulfate disposition were noted during this later time interval.

The effect of exogenous prostaglandin E_2 administration during NSAID treatment. IBU ($n = 6$) and INDO ($n = 18$) were studied separately in crossover studies (NSAID-treated and NSAID-PGE_2-treated). Female Lewis rats weighing 158–178 g (IBU study) and 176–207 g (INDO study) had right jugular, right carotid, and bladder cannulas implanted under light ether anesthesia 2 days prior to the first study day. A randomized crossover study design was employed, with 72 hr between study days. Animals were given an intravenous bolus injection of IBU or INDO, followed by a constant infusion for 4 or 6 hr, respectively. Additionally, animals received either PGE_2 or the PGE_2 vehicle via the carotid artery at a rate of 0.1 μg/min starting 30 min before beginning the urine collection on the first study

day. On the second study day, animals were crossed over to the alternate treatment (PGE_2 or PGE_2 vehicle). Two groups of control animals received only the PGE_2 infusion or only the PGE_2 vehicle infusion, along with either the IBU or INDO vehicles. Serum samples (1 ml) were obtained via the jugular vein at the midpoint of the urine collection period. Urine was collected between 2 and 4 hr in the IBU study and between 4 and 6 hr during the INDO study. Preliminary studies had demonstrated that this dose of PGE_2 can increase the urinary excretion of PGE_2, after IBU treatment, to values similar or greater than control values.

Analytical Methods. IBU and INDO were analyzed by modifications of a high performance liquid chromatography method (23). IBU serum concentrations were quantitated using a C_{18} column (Partisphere 5 μm; Whatman, Clifton, NJ), a mobile phase of acetonitrile, water, methanol, and 85% phosphoric acid (30:37:5:0.05), and UV detection was at 196 nm. Serum protein was precipitated by adding one part serum to two parts acetonitrile containing mefenamic acid (40 μg/ml), the internal standard. At IBU serum concentrations of 10 and 87 μg/ml, the intraday coefficients of variation were 2.7% and 1.4%, respectively, while the interday coefficients of variation at these concentrations were 4.1% and 2.3%, respectively. This same assay was employed for INDO, except that the mobile phase was changed to acetonitrile, water, methanol, and 85% phosphoric acid (46:47:6.4:0.6), and UV detection was at 254 nm.

Inorganic sulfate was determined by single-column anion chromatography (24) using a conductivity detector (model 213A; Wescan Instruments, Inc., Santa Clara, CA), an anion exchange column (Wescan), and a mobile phase of 4 m*M* potassium hydrogen phthalate (pH 4.5). In a preliminary study, it was established that IBU and INDO did not interfere with the analysis of sulfate when added *in vitro*.

Serum and urine concentrations of potassium, calcium, and magnesium were measured by atomic absorption spectroscopy (model 613; Perkin Elmer, Norwalk, CT). Phosphorus and creatinine concentrations were determined using commercially available kits (Sigma). Urinary PGE_2 was measured by radioimmunoassay (New England Nuclear, E.I. Du Pont de Nemours & Co., Boston, MA).

Data Analysis. The renal clearance of creatinine and electrolytes was calculated as the urinary excretion rate divided by the midpoint serum concentration. Clearance ratios were determined by dividing the renal clearance of inorganic sulfate by the renal clearance of creatinine. Since sulfate in serum is not bound to plasma proteins (6) and, therefore, is completely ultrafilterable, the renal filtration rate of inorganic sulfate was calculated as the product of serum sulfate concentration and creatinine renal clearance. The renal tubular reabsorption rate of sulfate was estimated as the difference between the renal filtration and urinary excretion rates, assuming negligible renal tubular secretion of sulfate (5, 6). The fraction of the filtered sulfate that was reabsorbed was calculated by dividing the reabsorption rate by the filtration rate.

Statistical Analysis. Statistical analysis was by paired *t*-test for the crossover studies, with $P < 0.05$ defined as statistically significant. All results are expressed as mean ± 1 SD.

Results

Mean serum steady state concentrations of 59 ± 8 μg/ml and 22 ± 3 μg/ml (mean ± SD) were obtained for IBU and INDO, respectively, after a bolus dose and infusion of these compounds. The dosing regimen used for INDO was based on pharmacokinetic parameters (CL of 20.7 $ml \cdot hr^{-1} \cdot kg^{-1}$; V_{ss} of 0.22 liters/kg) determined after a 10 mg/kg iv dose. At the serum concentrations of IBU and INDO obtained in this investigation, greater than 80% inhibition of the urinary excretion rate of PGE_2 was achieved.

IBU caused a statistically significant increase in the urinary excretion rate and renal clearance of sulfate, and in the clearance ratio of sulfate to creatinine (Fig. 1). This was accompanied by a significant decrease in the fraction of sulfate reabsorbed in the kidneys and a decrease in the serum sulfate concentrations ($P = 0.06$). INDO treatment produced a significant decrease in serum sulfate concentrations and a small, but statistically significant, increase in sulfate renal clearance and

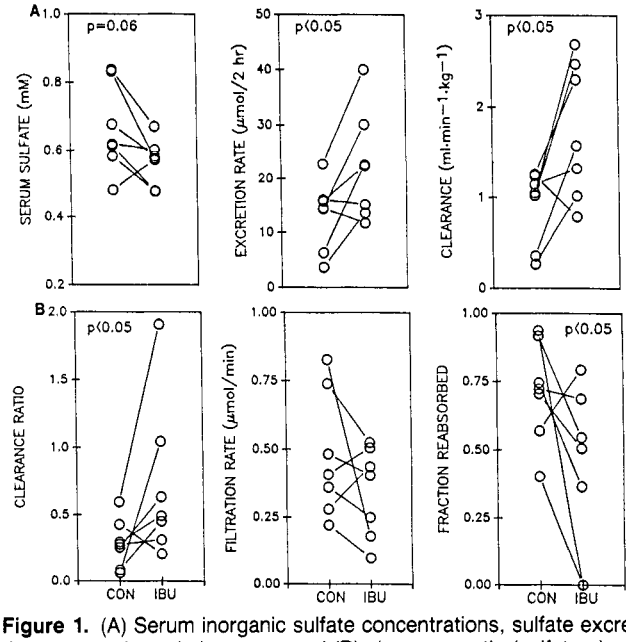

Figure 1. (A) Serum inorganic sulfate concentrations, sulfate excretion rate, and renal clearance, and (B) clearance ratio (sulfate clearance divided by creatinine clearance), sulfate filtration rate, and the fraction of filtered sulfate that is reabsorbed for rats during control (CON) and ibuprofen-treated (IBU) periods. Paired data for individual rats are connected by solid lines, $n = 7$.

clearance ratio, with no change in the sulfate excretion rate (Fig. 2). There was also a significant decrease in the fraction of sulfate reabsorbed by the kidney. The alteration in sulfate clearance after INDO treatment was smaller in magnitude than that observed after IBU treatment. The GFR, as assessed by creatinine clearance values, was unchanged following NSAID therapy (3.05 ± 1.63 ml·min^{-1}·kg^{-1} in IBU-treated rats vs 3.56 ± 1.18 ml·min^{-1}·kg^{-1} in controls; 4.58 ± 1.71 ml·min^{-1}·kg^{-1} in INDO-treated rats vs 4.18 ± 1.88 ml·min^{-1}·kg^{-1} in controls). Although creatinine clearance is not necessarily an accurate estimate of GFR in rats (25, 26), a lack of change with drug treatment suggests no drug-induced alteration in renal function. IBU treatment did not significantly alter the renal clearances of potassium, calcium, magnesium, or phosphorus (data not presented). There was a small, but statistically significant, decrease in serum calcium concentrations (4.60 ± 0.27 vs 4.83 ± 0.16 mEq/liter in controls).

The administration of PGE_2 alone, at a rate of 0.1 μg/min, did not alter the renal clearances of creatinine or sulfate in control rats. However, there was a significant decrease in serum sulfate concentrations after PGE_2 administration (0.76 ± 0.12 vs 0.85 ± 0.12 mM in controls, $n = 15$, $P < 0.05$). In this same group of rats, there were no significant alterations in the serum concentrations or renal clearances of potassium, calcium, or magnesium (data not presented). The renal clearance of phosphorus was significantly increased in the PGE_2-treated group (0.90 ± 0.38 vs 0.51 ± 0.39 ml·min^{-1}·kg^{-1} in controls, $n = 15$, $P < 0.05$), with no change in the serum concentration of phosphorus. Sodium clearance was not assessed after IBU or PGE_2 administration since sodium was present in the IBU and INDO vehicles that were administered in these studies.

In the investigations designed to examine the effect of concomitant PGE_2 treatment in NSAID-treated rats, the urinary excretion of PGE_2 increased from 20.1 ± 16.6 and 13.8 ± 5.42 pg/min in IBU- and INDO-treated rats, respectively, to 224 ± 180 and 189 ± 107 pg/min after concomitant PGE_2-IBU and PGE_2-INDO treatment. There was no difference in creatinine clearance between the NSAID-treated and NSAID-PGE_2-treated animals. Animals that received concomitant PGE_2 and IBU treatment exhibited significantly lower values for the urinary excretion rate and renal clearance of inorganic sulfate and higher values for the fraction of filtered sulfate reabsorbed, compared with those values obtained when the animals received IBU alone (Fig. 3). Infusing INDO-treated rats with PGE_2 resulted in a significant increase in the reabsorption rate ($P < 0.05$); the fractional reabsorption of sulfate tended to increase as well ($P = 0.17$) (Fig 4). Sulfate renal clearance and serum concentrations were not significantly altered with concomitant PGE_2 treatment. This lack of effect of PGE_2 administration does not appear to be a time-dependent phenomenon, since we also observed, in an additional study, no statistically significant reversal of INDO-induced sulfate alterations when sulfate dispo-

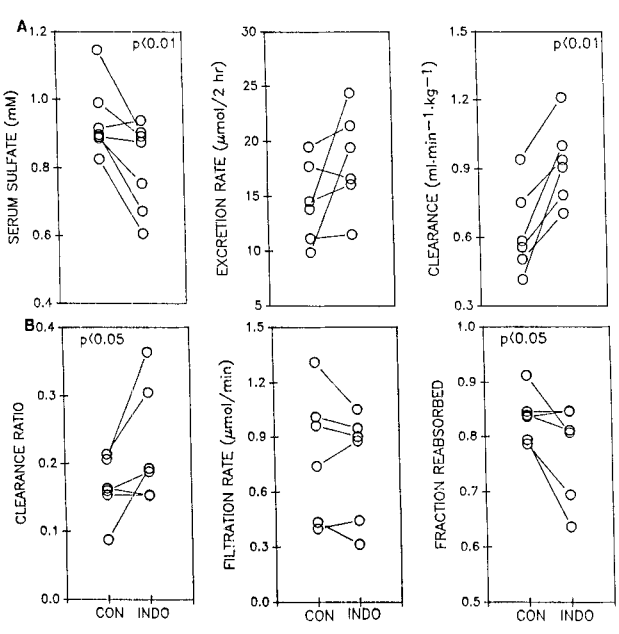

Figure 2. (A) Serum inorganic sulfate concentrations, sulfate excretion rate, and renal clearance, and (B) clearance ratio (sulfate clearance divided by creatinine clearance), sulfate filtration rate, and the fraction of filtered sulfate that is reabsorbed for rats during control (CON) and indomethacin-treated (INDO) periods. Paired data for individual rats are connected by solid lines, $n = 6$ or 7.

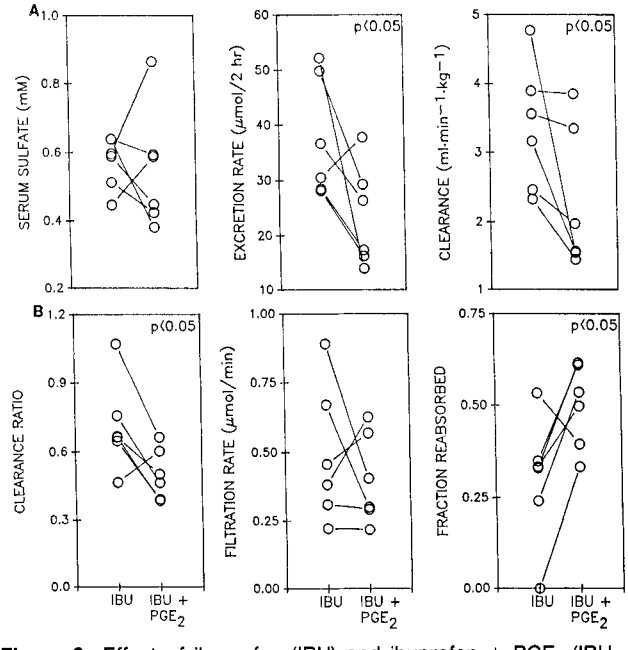

Figure 3. Effect of ibuprofen (IBU) and ibuprofen + PGE_2 (IBU + PGE_2) on (A) inorganic sulfate serum concentration, sulfate excretion rate, and renal clearance, and (B) clearance ratio (sulfate clearance divided by creatinine clearance), sulfate filtration rate, and the fraction of filtered sulfate that is reabsorbed. Paired data for individual rats are connected by solid lines, $n = 6$.

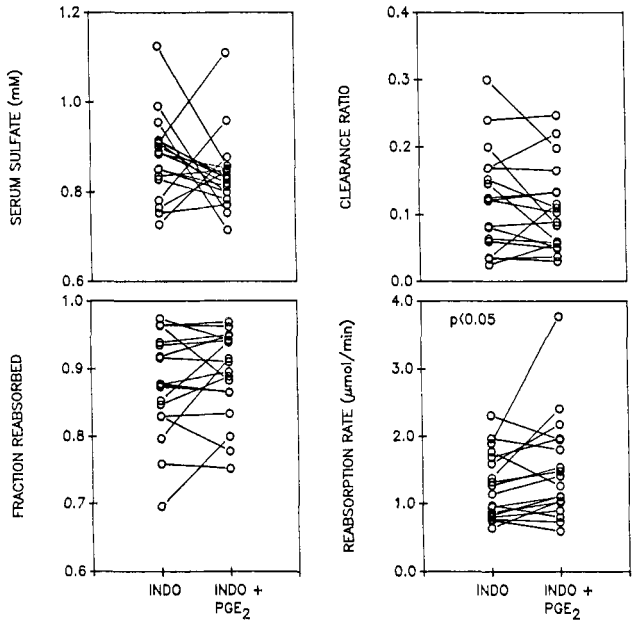

Figure 4. Effect of indomethacin (INDO) and indomethacin + PGE_2 (INDO + PGE_2) on inorganic sulfate serum concentration, clearance ratio, fraction reabsorbed, and reabsorption rate. Paired data for individual rats are connected by solid lines, $n = 18$.

sition was examined between 2 and 4 hr (the same time period used in the IBU study).

Discussion

Inorganic sulfate is eliminated from the body predominantly in unchanged form by urinary excretion (3, 4), the extent of which is controlled by the capacity-limited reabsorption of sulfate in the proximal tubule (5–7). In the present investigation, both IBU and INDO treatment increased the renal clearance of inorganic sulfate by decreasing its fractional renal reabsorption. Alterations in the reabsorption of sulfate will initially increase the urinary excretion rate and renal clearance of sulfate and consequently decrease serum sulfate concentrations. However, as steady state conditions are approached, the urinary excretion rate of sulfate will return to its initial level, as long as the rate of formation of sulfate is unaltered, while renal clearance will remain increased. With IBU, the urinary excretion rate, determined between 2 and 4 hr after initiating an infusion of IBU, was increased and there was only a small alteration in serum sulfate concentrations, suggesting that steady state conditions have not been achieved. In the INDO study, when clearance was determined between 4 and 6 hr, the urinary excretion of sulfate tended to be higher than that of controls, but this was not statistically significant, suggesting that steady state conditions for sulfate disposition were approached. If the primary effect of these NSAID was to decrease the sulfate serum concentrations, by decreasing the formation of sulfate or through its utilization in sulfate conjugation reactions, one would expect a decreased renal clearance and increased fractional reabsorption of sulfate, as has been reported following acetaminophen treatment in both rats (7) and humans (27). This is opposite to what was observed, suggesting that the NSAID-sulfate interaction is renal in nature. However, it is possible that nonrenal mechanisms, in addition to the renal mechanism, may contribute to the experimentally observed interaction.

The results of the present investigation indicate that prostaglandins may be involved, at least in part, in the IBU-induced alterations in the renal clearance of inorganic sulfate. Plasma concentrations of NSAID achieved in the IBU and INDO studies produced >80% inhibition of urinary PGE_2 excretion, a measure of renal prostaglandin synthesis (28). The administration of PGE_2 concomitantly with IBU resulted in a reversal of the alterations in sulfate renal clearance and fractional sulfate reabsorption observed following IBU treatment, but only a trend toward reversal was seen in the INDO-treated rats, possibly due, at least in part, to the difficulty in detecting significant changes due to the smaller magnitude of the INDO-induced effects. The fact that two structurally dissimilar NSAID both alter sulfate homeostasis and that concomitant PGE_2 administration can reverse the IBU-induced alterations and produces a trend toward reversal in the INDO-treated animals suggests that prostaglandins may play a role in the modulation of sulfate renal reabsorption. However, the differences in the magnitude of the observed effects of IBU and INDO on sulfate disposition, along with the inability of exogenous PGE_2 administration to reverse the INDO-induced changes in sulfate disposition, might indicate that other mechanisms may also be involved in the renal interaction between INDO and sulfate. Pharmacologic effects of INDO, other than inhibition of prostaglandin synthesis, may either be responsible for the observed changes in sulfate homeostasis or, alternatively, may oppose the effects of PGE_2 administration. The numerous prostaglandin-unrelated actions of INDO, including inhibition of cAMP degradation and inhibition of the cellular efflux of cAMP, have been reviewed by Dunn and Zambraski (10). Additionally, INDO has been demonstrated to inhibit the active secretion of exogenously administered PGE_2, which may have limited the renal availability of PGE_2 (29); however, the urinary excretion of PGE_2 after concomitant INDO and PGE_2 treatment increased to similar levels observed after concomitant IBU and PGE_2 treatment, suggesting no difference in the availability of exogenous PGE_2 in IBU- and INDO-treated rats. An alternative hypothesis is that IBU, INDO, or their metabolites may directly inhibit the renal transport of inorganic sulfate. Previous studies have demonstrated that salicylate can increase the renal clearance of inorganic sulfate (30) at concentrations at which

there are no significant alterations in the urinary excretion of PGE_2 (31), and that this interaction is due, at least in part, to the direct inhibition of the renal transport of inorganic sulfate (32).

It is of interest that the effect of inhibition of renal prostaglandin synthesis on inorganic sulfate excretion differs from that seen with other electrolytes: inhibition of prostaglandin synthesis generally results in a decreased, not an increased, fractional excretion of electrolytes (10, 33). However, these changes are usually observed in anesthetized animals or in animals under conditions in which renal perfusion is impaired. Little or no alteration in renal blood flow, GFR, and electrolyte excretion is generally observed in conscious, healthy animals or humans (10, 33). In the present investigation, there were no significant alterations in GFR or in potassium, calcium, magnesium, or phosphorus excretion following IBU treatment. As well, PGE_2 treatment by itself, at the dose used in this investigation, decreased the serum concentrations of inorganic sulfate and increased the renal clearance of phosphorus, but did not alter the renal clearances of potassium, magnesium, or calcium, and did not alter GFR. Previous studies in healthy animals have also demonstrated that the administration of similar doses of PGE_2 did not significantly influence renal function (34, 35).

The results of the present investigations suggest that renal prostaglandin inhibition may alter the renal transport of inorganic sulfate in rats. IBU treatment results in a decreased renal reabsorption of sulfate in conscious rats, an effect that can be reversed by the administration of PGE_2. INDO treatment also increases the renal clearance of inorganic sulfate in rats, but its effects are of a smaller magnitude and concomitant PGE_2 administration produces only a trend toward reversal, a finding that may be explained by considering the many other renal pharmacologic actions of INDO besides inhibition of prostaglandin synthesis.

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