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NEPHROLOGY FORUM

Prostaglandins and Bartter's syndrome

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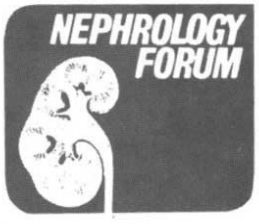

Case presentation

A 46-year-old woman was admitted to the metabolic ward of New England Medical Center Hospital (NEMCH) for evaluation of hypokalemia and metabolic alkalosis. The patient was in good health until four months earlier, when preoperative determination of serum electrolytes disclosed a serum potassium concentration of 3.0 mEq/liter and a bicarbonate concentration of 31 mEq/liter. A hysterectomy was performed without complications, and on discharge 80 mEq of potassium chloride per day was prescribed. One month later she was admitted to another hospital because of supraventricular tachycardia; hypokalemia had persisted. Evaluation revealed no evidence of organic heart disease and the arrhythmia was presumed to be secondary to hypokalemia.

Two weeks later, she was readmitted for further evaluation. Blood pressure was 120/80 mm Hg; cardiac examination revealed normal S_1 and S_2 sounds and a grade I/VI systolic ejection murmur at the lower left sternal border; abdominal examination revealed no abdominal masses; no edema was present. The remainder of the physical examination was normal. Laboratory findings revealed: serum creatinine, 0.7 mg/dl; BUN, 15 mg/dl; serum sodium, 138 mEq/liter; serum potassium, 2.9 mEq/liter; chloride, 96 mEq/liter; and bicarbonate, 34 mEq/liter; hemogram, serum calcium, serum phosphorus, serum albumin, serum bilirubin, blood glucose, serum uric acid, and transaminase levels were normal. Peripheral plasma renin activity on a diet containing 80 mEq of sodium was 3.5 ng/ml/hour; 24-hour urinary aldosterone excretion was 32 ng. An intravenous pyelogram and barium enema were normal. Therapy with 100 mg of spironolactone per day was initiated, and potassium chloride (100 mEq per day) was continued.

Although the serum potassium concentration initially rose to 3.4 mEq/liter, within two weeks it fell again to 2.8 mEq/liter. The dose of potassium chloride was increased to 180 mEq/day and on this regimen, the serum potassium concentration varied between 3.2 and 3.5 mEq/liter. Within several weeks, however, the serum potassium concentration fell to 3.1 mEq/liter. Potassium chloride was increased to 200 mEq/day, spironolactone was discontinued, and 80 mg of triamterene per day was added to the regimen but was soon discontinued because the patient developed headaches.

On admission to NEMCH, physical examination revealed the blood pressure to be 128/84 mm Hg supine and 125/80 mm Hg upright. Laboratory findings were: hemoglobin, 12.5 g/dl; hematocrit, 38%; white blood cell count, 6900/mm³ with 48% polymorphonuclear leukocytes, 47% lymphocytes, and 3 monocytes; serum creatinine, 0.8 mg/dl; BUN, 15 mg/dl; serum sodium, 142 mEq/liter, serum potassium, 2.7 mEq/liter; serum chloride, 98 mEq/liter; and serum bicarbonate, 32 mEq/liter. Serum magnesium was 1.3 mEq/liter. Excretions of 17-hydroxycorticosteroids and 17-ketosteroids were normal. Toxicologic screening for diuretic agents was negative.

Results of the metabolic balance studies in consecutive 5-day periods are given in Table 1. All measurements were made on the fourth to fifth day of the respective dietary regimen.

Following period IV, potassium intake was reduced to 70 mEq/day; sodium intake remained at 10 mEq/day. The patient began to lose weight and after 3 days noted dizziness when standing. On physical examination, her blood pressure fell from 118/70 to 88/55 mm Hg, and her pulse rate increased from 90 to 120 beats per minute on standing; therefore one liter of normal saline was administered intravenously over 6 hours, and oral sodium and potassium intake were increased to 250 to 270 mEq/day, respectively. This change in intake increased her blood pressure to 120/80 mm Hg without orthostatic changes and caused a weight gain of approximately 2 kg. Serum potassium was 2.9 mEq/liter. No data were collected during this interval because the patient did not attain a steady state.

Following period V she was allowed unrestricted sodium chloride intake, and indomethacin (150 mg/day) was continued. After 4 days the serum potassium was 3.6 mEq/liter and she was discharged from the hospital. She continued to take indomethacin but was not given potassium supplementation.

The patient felt well and continued taking indomethacin, but the drug was stopped 18 months later because she developed upper gastrointestinal bleeding. Ibuprofen was substituted for indomethacin at that time and potassium chloride, 60 mEq/day, was added to the regimen. This treatment has been continued for an

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Table 1. Summary of patient data

Period[a]	I	II	III	IV	V
Dietary regimen					
Intake (mEq/day)					
Sodium	100	250	250	10	100
Potassium	70	70	270	270	70
Medications	none	none	none	none	indomethacin (150 mg/day)
Serum potassium (mEq/liter)	2.4	2.6	2.8	2.8	3.5
Serum bicarbonate (mEq/liter)	30	30	28	30	2.7
Plasma renin activity (ng/ml/hour)					
Supine	7.8	3.2	2.1	6.8	1.16
4 hours upright	32.9	17.0	10.3	15.8	10.4
Aldosterone					
Plasma (ng/dl)	3.3	<3.0	3.0	9.7	<3.0
Urine (μg/day)	67.3	60	128	434	19.6
Urinary prostaglandins[b] ng/day)					
PGE_2	371	269	318	521	31
$PGF_{2\alpha}$	513	256	371	653	—
Angiotensin infusion test (quantity of angiotensin II required to raise diastolic BP 20 mm Hg) (ng/kg/min)	90	—	29	—	19

[a] Between periods IV and V the patient developed transient postural hypotension, and detailed observations could not be made (see text).

[b] Analyses carried out in the laboratory of Dr. Michael Dunn at Case-Western Reserve University School of Medicine.

additional 18 months. Aside from a recurrence of supraventricular tachyarrhythmia treated effectively with digitalis and propranolol, the patient has remained well. Serum potassium has ranged from 3.4 to 3.7 mEq/liter.

Discussion

DR. MICHAEL J. DUNN *(Director, Division of Nephrology, University Hospitals of Cleveland, and Hanna Payne Professor of Medicine, Case-Western Reserve University, Cleveland, Ohio):* I plan to develop the case that prostaglandin overproduction is merely a secondary phenomenon in Bartter's syndrome, and that it is not the proximal cause of most of the clinical and physiologic features of this entity. In doing so, I will consider the biochemistry and physiology of prostaglandins, the observations on plasma and urinary prostaglandins in patients with the syndrome, the possible mechanisms of prostaglandin overproduction, and the nature of the disturbance of the renin-angiotensin-aldosterone systems in these patients.

Biochemistry and physiology of prostaglandins. In order to discuss the role of prostaglandins in Bartter's syndrome, we should review the biochemistry and physiology of prostaglandins in the kidney. Figure 1 summarizes the biochemical pathways for prostaglandin synthesis in the kidney [1-

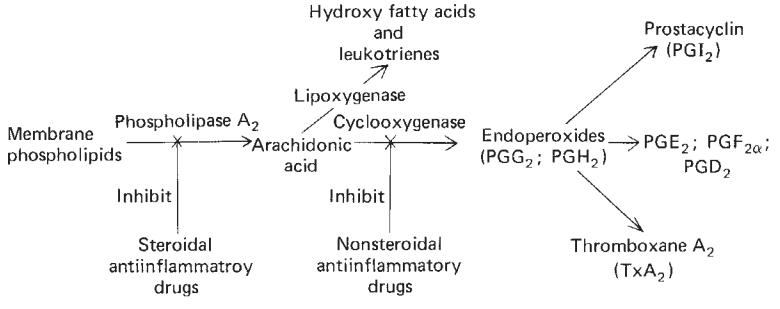

Fig. 1. *Renal conversion of arachidonic acid to prostaglandins, thromboxanes and other products.* The existence of the lipoxygenase products is unproved in the kidney.

3]. The substrate, arachidonic acid, is a 20-carbon fatty acid with 4 double bonds (C20:4). Arachidonic acid is stored within cells as phospholipid, cholesterol, or triglyceride esters. In the kidney, phospholipids, especially phosphatidylcholine, are the largest reservoirs of arachidonic acid. Control of the availability of arachidonic acid is an important regulator of prostaglandin biosynthesis. Virtually all known stimuli of prostaglandin synthesis increase the release of arachidonate from phospholipids rather than directly increase cyclooxygenase activity. Release of arachidonate from phospholipids requires deacylation of an ester linkage, and this deacylation is controlled by phospholipase A_2. Steroidal antiinflammatory drugs, such as prednisone or dexamethasone, partially inhibit phospholipase. After release of arachidonic acid from phospholipids, the arachidonate is either reacylated to the phospholipid or is oxygenated via the cyclooxygenase and lipoxygenase pathways. Fatty acid cyclooxygenase, primarily found in endoplasmic reticulum, dioxygenates arachidonic acid to the labile prostaglandin endoperoxides. Aspirin, indomethacin, meclofenamate, ibuprofen, naproxen, tolmetin, phenylbutazone, fenoprofen, and other nonsteroidal antiinflammatory drugs all inhibit fatty acid cyclooxygenase. Whereas aspirin irreversibly acetylates the cyclooxygenase, the other drugs reversibly inhibit the enzyme. Cyclooxygenase turnover is rapid in the kidney; the inhibitory effects of these drugs therefore is brief, and normal prostaglandin synthesis returns within 12 to 36 hours after administration of the nonsteroidal antiinflammatory drug is discontinued. Although lipoxygenase has been found in platelets and granulocytes, it is uncertain whether lipoxygenase exists in the kidney. Lipoxygenase products include the hydroxy fatty acids and the leukotrienes. The hydroxy fatty acids are chemotactic to leukocytes, and the leukotrienes contract vascular and bronchial smooth muscle (slow-reacting substance of anaphylaxis is a leukotriene) [4]. The prostaglandin (PG) endoperoxides are enzymically converted to PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (prostacyclin), and thromboxane A_2 (TxA_2). The more stable products, PGE_2, $PGF_{2\alpha}$, and PGD_2, have been recognized for many years as normal renal prostaglandins. Only recently have PGI_2 and TxA_2 been demonstrated as products of cyclooxygenase in normal human and animal kidneys [5].

The kidney is anatomically and physiologically a complex organ, and it is not surprising that prostaglandin synthesis varies greatly among different portions of the nephron. Overall, prostaglandin synthesis, expressed per milligram of tissue protein, is greatest in the papilla and medulla and least in the outer cortex. However, one should not conclude that the physiologic importance of cortical prostaglandins is minor; I will return to this topic later. Table 2 summarizes the current knowledge about prostaglandin and thromboxane synthesis in different portions of the kidney. Specific renal prostaglandins probably are synthesized by the same cells in which the prostaglandins exert a physiologic action. In other words, the prostaglandins act directly at their nephron site of synthesis. The measurement of prostaglandins in urine or renal venous blood indicates only total renal synthesis. Obviously it is difficult, if not impossible, to surmise from these measurements the relative contributions of glomeruli, tubules, interstitial cells, etc. to the urinary and renal venous prostaglandin concentrations. We can safely assume, however, that practically all renal venous and urinary prostaglandins originate in the kidney, excluding platelet and granulocyte artifact in blood and contamination of urine with seminal fluid. Therefore, the increased urinary levels of prostaglandins in patients with Bartter's syndrome indicates renal overproduction of the measured prostaglandin. Increased prostaglandin synthesis in Bartter's syndrome certainly occurs in the medullary interstitial cells and probably in glomeruli and cortical vessels as well.

Table 2. Renal sites of prostaglandin synthesis

Tissue[a]	Relative amounts synthesized[b]
Medullary interstitial cells [6]	$PGE_2 \gg PGF_{2\alpha}$. No PGI_2 or TxA_2
Collecting tubules (papillary) [7]	$PGE_2 > PGI_2 > PGF_{2\alpha} > PGD_2$
Cortical tubules (proximal and distal) [8]	$PGE_2 > PGF_{2\alpha} > TxA_2$. ? PGI_2
Cortical arterioles [9]	$PGI_2 > PGF_{2\alpha} > PGE_2$
Glomeruli [8]	$PGE_2 \geq PGF_\alpha > TxA_2 > PGI_2 = PGD_2$

[a] In vitro studies of isolated portions of the kidney or of cells in culture.

[b] These measurements are approximate, especially when made by radiometric thin-layer chromatography. The in vivo synthesis of these products can differ substantially because of cofactors, substrate concentration, etc.

Prostaglandins and thromboxanes exert multiple physiologic and pharmacologic actions in the kidney. Table 3 summarizes the best-established renal actions of these compounds [3]. Some of these actions probably are unimportant in normal physiologic processes. For example, inhibition of prostaglandin synthesis has no effect on renal blood flow or glomerular filtration in normal conscious animals

Table 3. Renal actions of prostaglandins

1.	Renal blood flow
	a. Vasodilation: PGE_2, PGI_2
	b. Vasoconstriction: TxA_2; $PGF_{2\alpha}$ (weak)
2.	Glomerular filtration rate[a]
	a. Increase: PGE_2, PGI_2
	b. Decrease: TxA_2
3.	Renin secretion
	a. Increased: PGI_2, PGE_2, PGD_2
	b. Decreased: ? TxA_2
4.	Natriuretic: PGE_2, PGI_2, PGD_2?
5.	Water diuretic: PGE_2

[a] Increments of GFR only occur if vasoconstriction (e.g., resulting from angiotensin II) precedes administration of PGE_2 or PGI_2.

or humans, but the same drugs can reduce significantly renal blood flow and filtration rate if renal vasoconstriction is present [10]. Therefore angiotensin II, catecholamines, and alpha-adrenergic neural input apparently elicit a compensatory increment of renal prostaglandin production that partially counteracts the renal vasoconstriction. Indomethacin therapy in patients with Bartter's syndrome generally reduces the glomerular filtration rate and presumably lowers renal blood flow. The renin-stimulatory effects of PGI_2 and PGE_2 might account partially for the renin overproduction in Bartter's syndrome. The importance of prostaglandins as regulators of sodium and water excretion is unknown. Although PGE_2 has unequivocal natriuretic and diuretic potency, it seems that other endocrine as well as physical factors exert a more potent and controlling influence over sodium and water excretion. Inhibition of renal prostaglandin synthesis does not completely eliminate the renal losses of chloride or potassium in patients with Bartter's syndrome.

Some characteristics of Bartter's syndrome. Before discussing some of the important features of this patient's illness, I would like to summarize the usual findings in Bartter's syndrome [11-13]. These patients have hypokalemia and hypochloremic alkalosis, both of which are refractory to potassium replacement therapy. Plasma renin activity is increased, and angiotensin II and aldosterone levels are elevated. Hyperuricemia and hypomagnesemia sometimes are present. Growth is retarded in children. The renal juxtaglomerular cells become hyperplastic, as can the renal medullary interstitial cells. The pressor response to angiotensin II is blunted although the patient is normotensive. Urinary prostaglandins are generally, but not invariably, increased, whereas plasma PGE_2 and $PGF_{2\alpha}$ levels are normal. Renal chloride wasting, presumably due to a chloride-reabsorption defect in the thick ascending limb of Henle's loop, has been documented. Most of the signs and symptoms of Bartter's syndrome are secondary to severe, chronic potassium depletion and include weakness, malaise, muscle cramping, polyuria, and nocturia. Treatment, although helpful, is not curative. Therapeutic measures include potassium supplements, spironolactone, triamterene, propranolol, indomethacin, and ibuprofen [11-13].

Several unusual aspects of this case make suspect the diagnosis of Bartter's syndrome in this patient. In most patients with Bartter's syndrome, the disease is symptomatic and has been diagnosed by the time the patient is 25 years old [11]. Patients have been reported, however, in whom the diagnosis was not established until age 35, 41, or 52 years. The patient under discussion would be the second-oldest, at the time of diagnosis, in the literature. Another unusual feature was her lack of symptoms. Very few cases are discovered incidentally, without the patient complaining of weakness, cramps, and polyuria [12]. It is also atypical for a patient with Bartter's syndrome to develop severe sodium depletion with postural hypotension after sodium restriction, as occurred between periods IV and V (see Table 1). These factors—namely age at onset, lack of symptoms, and postural hypotension—make it imperative that we rule out surreptitious vomiting or diuretic abuse as an explanation for this woman's clinical disorder.

Observations about prostaglandins. In 1976 and early 1977, four papers appeared that stimulated interest in the possible pathophysiologic importance of prostaglandins in Bartter's syndrome. Fichman and coworkers reported that indomethacin reduced plasma renin activity and aldosterone levels, decreased renal losses of sodium and potassium, reversed the pressor resistance to angiotensin II, and increased serum potassium [14]. Although they measured an increased blood concentration of PGA_2 in their patient who had Bartter's syndrome, this value represented radioimmunoassay artifact, because PGA_2 is not measurable in human plasma [15]. Verberckmoes et al also successfully treated a patient with indomethacin and improved the hyperreninemia, hyperaldosteronism, and the hypokalemia [16]. They observed histologic hyperplasia of the renal medullary interstitial cells, cells that have been found to produce large quantities of prostaglandins, especially PGE_2 [6]. Verberckmoes et al hypothesized that renal prostaglandins, synthesized by the hyperplastic medullary interstitial cells, ac-

counted for most of the changes in endocrine and electrolyte status in their patient [16]. Donker and coworkers reached similar conclusions after treating 3 patients with indomethacin [17]. Gill and colleagues, using radioimmunoassay and gas chromatography-mass spectroscopy, verified that renal excretion of prostaglandins is enhanced in patients with Bartter's syndrome [18]. Urinary PGE_2 was increased in 4 patients, whereas a metabolite of PGE_2, attributable to systemic (i.e., nonrenal) production and degradation of PGE_2, was not increased. Indomethacin or ibuprofen decreased urinary excretion of PGE_2 by 66% to 96% and partially reversed the biochemical abnormalities of the syndrome [18]. Subsequently, many investigators have confirmed both the presence of increased excretory rates for prostaglandins and the improvement after therapy with nonsteroidal inhibitors of prostaglandin synthesis [19–27]. Twenty-four children and adults (20 females and 4 males) in 5 separate reports had moderate (twofold) to marked (tenfold) increments of urinary PGE_2 [20–24]. Also, PGD_2 and 6-keto-$PGF_{1\alpha}$, the stable degradation product of PGI_2, were increased in the urine of 5 women with Bartter's syndrome [28]. Urinary 6-keto-$PGF_{1\alpha}$ probably provides a reasonable measure of renal PGI_2 synthesis [29, 30]. Cortical and medullary microsomes obtained from human kidneys synthesize large quantities of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ but lesser amounts of PGE_2 and TxA_2 [5]. In patients with Bartter's syndrome, therefore, overproduction of PGI_2 as measured by increased excretion probably occurs in the renal medulla as well as in the cortex. These findings gain significance in light of the recently described changes in platelet aggregation and the potent renin-stimulatory actions of PGI_2. Although most investigators have documented raised levels of urinary PGE_2, this increase is not an invariable finding. Dray observed that urinary excretion of PGE_2 and $PGF_{2\alpha}$ was normal in 7 adults with the syndrome, whereas 8 children had three- to fourfold elevations of urinary PGE_2 and $PGF_{2\alpha}$ [31]. Peripheral venous plasma concentrations of PGE_2 and $PGF_{2\alpha}$ were normal in the children and the adults [31]. This latter observation reinforces an earlier report by Gill et al, who found normal excretion of a PGE metabolite to be an index of circulating PGE_2 rather than of renal PGE_2 [18]. Stoff and coworkers [21] and O'Reagan et al [32] observed defects in platelet aggregation in patients and parents of patients with the syndrome. Platelet aggregation, measured in vitro with platelet-rich plasma, was abnormal in response to ADP, collagen, and epinephrine. Therapy with indomethacin for 3 to 5 days substantially improved the aggregation responses and reduced the abnormally high levels of cyclic AMP in the platelets [21]. Normal platelets acquired the aggregation defect after they were incubated in plasma from patients with Bartter's syndrome, but the stability of the unknown plasma factor ruled out PGI_2 as the explanation. In fact, researchers disagree as to whether PGI_2 is a circulating "hormone" at all [33, 34]. Both PGD_2 and 6-keto-PGE_1 are stable prostaglandins with potent actions on platelet adenylate cyclase and aggregation, and these characteristics could explain the in vitro results [35, 36].

In the patient under discussion, excretion of PGE_2 and $PGF_{2\alpha}$ was normal or slightly increased. We measured these prostaglandins using radioimmunoassay after acidification, solvent extraction, and silicic acid-column chromatography [37]. Normal 24-hour excretion of PGE_2 and $PGF_{2\alpha}$ ranges from 150–300 and 400–700 ng/day, respectively, using this method. Therefore, the values for PGE_2 excretion were clearly increased only in period IV, during sodium restriction. Plasma levels of PGE_2 and $PGF_{2\alpha}$ were normal or undetectable on most of our determinations in this patient.

Overproduction of prostaglandins: Cause or effect? Is overproduction of prostaglandins, especially renal prostaglandins, a primary or a secondary abnormality in Bartter's syndrome? In my opinion, increased prostaglandin synthesis is a secondary phenomenon (Table 4). If it were a primary abnormality, one would expect all patients to have enhanced synthesis, excretion, or both, of prostaglandins. However, some patients have no detectable abnormalities of prostaglandin excretion. Treatment with indomethacin or ibuprofen reduces prostaglandin excretion to subnormal levels, yet the clinical syndrome is only partially reversed [19]. If prostaglandins played a primary and central role, then the efficacy of cyclooxygenase-inhibitor drugs would be greater. Gill and Bartter concluded that a reabsorptive defect for chloride transport in the ascending limb of Henle's loop is the basic abnormality in patients with the syndrome [38]. Theoretically, prostaglandins could account for this reabsorptive defect, since (*1*) PGE_2 inhibits chloride reabsorption in microperfused tubular segments of the thick ascending limb of Henle's loop in the rabbit, and (*2*) inhibitors of prostaglandin synthesis augment tubular chloride reabsorption [39, 40]. However, the chloride reabsorptive defect persists after indomethacin or ibuprofen treatment despite a reduction in renal prostaglandin synthesis [20, 38].

Table 4. Renal overproduction of prostaglandins: A secondary event in Bartter's syndrome

1. Increased prostaglandin excretion is a variable finding
2. Reduction of prostaglandin synthesis, after indomethacin treatment, is not curative
3. Tubular reabsorptive defect for chloride is not improved by indomethacin
4. Vomiting and diuretic abuse mimic Bartter's syndrome, with increased excretion of PGE_2 present in all three conditions

Patients with Bartter's syndrome can be differentiated from patients who vomit covertly: urine chloride levels are quite high in the former [38] and low in the latter group [41]. The chloride determination in today's patient favors a diagnosis of Bartter's syndrome or diuretic abuse, as the urine chloride was approximately equal to the chloride intake. Diuretic abuse seems unlikely because the urine was screened for diuretics and was negative. Finally, it should be noted that patients with psychogenic vomiting or diuretic abuse (so-called "pseudo-Bartter's" syndrome) have increased excretory rates of PGE_2 [23, 41-43]. Since these patients share most of the clinical and laboratory alterations seen in true cases of the syndrome, it seems likely that increased renal prostaglandin synthesis is a secondary rather than a primary abnormality.

Possible mechanisms of prostaglandin excess. Table 5 lists some of the possible explanations for secondary overproduction of prostaglandins in patients with Bartter's syndrome. Plasma angiotensin II is increased in the syndrome, and angiotensin II is a potent stimulus of prostaglandin synthesis in the kidney [37, 44, 45] and vasculature [46]. Since, as I will discuss later, PGE_2 and PGI_2 are potent stimuli of renin secretion and thereby increase plasma angiotensin II, it is impossible to know which alteration is primary in these patients, i.e., increased renal prostaglandin synthesis or increased angiotensin II formation. Although methodologic difficulties can obscure the absolute values, it appears that plasma bradykinin is elevated in Bartter's syndrome [22]. Bradykinin stimulates prostaglandin synthesis in most cells, including whole kidney [37, 45, 47] and renal medullary interstitial cells [48]. Although urinary kallikrein also is increased in Bartter's syndrome [20, 22], urinary kinins are decreased [22]. Treatment with indomethacin suppresses plasma bradykinin and urinary kallikrein; this suggests that prostaglandins also might stimulate the kallikrein-kinin-kininogen system [20, 22]. It is unknown whether plasma vasopressin is elevated in untreated Bartter's syndrome, although chronic mild decrements of effective circulatory volume could increase plasma vasopressin. Studies in normal rats and in rats with diabetes insipidus confirmed the stimulatory effect of vasopressin on renal prostaglandin synthesis [49-51].

Controversy surrounds the possible importance of potassium depletion as an explanation for enhanced renal prostaglandin synthesis [52]; Table 6 summarizes this issue. Galvez et al reported that potassium depletion in dogs augmented PGE_2 excretion, caused polyuria and hyperreninemia, and induced vascular resistance to angiotensin's pressor action [53]. Additionally, in vitro studies with rabbit renomedullary interstitial cells [54] and papillary slices from rabbits and humans [55] showed augmentation of PGE_2 synthesis after reductions in extracellular potassium. Using rats, Hood and Dunn [56] as well as Berl et al [57] were unable to detect a change in PGE_2 excretion [56] or renal medullary prostaglandin content [57] despite having induced significant potassium depletion with accompanying hypokalemia and polyuria. Furthermore, neither indomethacin nor meclofenamate improved the maximum urine osmolality in these experiments. Acute potassium depletion (219 ± 21 mEq negative balance) induced by treatment with furosemide for three days did not increase PGE_2 excretion in 5 healthy female volunteers [58]. Chronic potassium depletion, accompanying primary aldosteronism [59] or DOCA administration in humans [60], does not increase renal excretion of PGE_2. Hypokalemic patients who have primary mineralocorticoid excess also have suppressed plasma angiotensin II levels unlike patients with Bartter's syndrome, those who self-induce vomiting, or those who abuse

Table 5. Possible causes of prostaglandin overproduction in Bartter's syndrome

1. Increased plasma angiotensin II
2. Increased plasma bradykinin
3. Increased plasma vasopressin
4. Potassium depletion
5. Polyuria

Table 6. Potassium depletion and urinary PGE_2

1. Potassium depletion in dogs leads to increased urinary PGE_2
2. Potassium depletion in rats leads to no changes in urinary prostaglandins
3. Potassium depletion in humans due either to primary aldosteronism or to DOCA administration leads to no changes of urinary PGE_2
4. In Bartter's syndrome, treatment with potassium salts can increase urinary PGE_2

diuretics. Nasjletti et al came to a different conclusion regarding the effects of aldosterone and DOCA on urinary PGE in the rat [61]; after 10 to 14 days of aldosterone or DOCA administration, they found significant elevations of prostaglandin and urinary kallikrein excretion [61]. Additionally, potassium supplements (240 mEq per day) increased rather than decreased PGE_2 excretion in 2 patients with Bartter's syndrome who also had increased urine volumes [25]. Finally, there is little evidence to suggest that potassium depletion directly stimulates renal prostaglandin synthesis in vitro.

Polyuria, induced either by water loading or by potassium or magnesium therapy, increases PGE_2 excretion in humans [25] and dogs [62]; however, polyuria in normal rats given 5% dextrose as drinking water did not increase urinary prostaglandin excretion (Hood and Dunn, unpublished data). Most patients with Bartter's syndrome have polyuria; it is possible therefore that polyuria augments renal excretion of PGE_2. These studies were performed in patients who had polyuria of brief duration, however, and the relevance of these findings to a chronic situation is unknown.

In summary, although it is likely that renal overproduction of prostaglandins in Bartter's syndrome is a secondary event, we do not know what specifically triggers the increased prostaglandin synthesis. Angiotensin II and bradykinin are plausible explanations, but their primary role must be questioned because inhibition of prostaglandin synthesis reduces plasma angiotensin II (i.e., plasma renin activity) and bradykinin.

Renin, angiotensin, and aldosterone. Renin release by the kidney undoubtedly is partially controlled by renal prostaglandin synthesis. Evidence supporting this statement has come from experiments in which renin secretion has been stimulated by prostaglandins and prostaglandin precursors, and also from experiments using inhibitors of prostaglandin synthesis. Arachidonic acid, infused into the renal artery, stimulates renin secretion; however, arachidonic acid must be converted to prostaglandin since blockade of fatty acid cyclooxygenase with indomethacin nullifies the renin stimulation by arachidonic acid [63, 64]. Some disagreement exists as to which prostaglandin is the critical stimulus to renin release. It is possible, and indeed likely, that several prostaglandins stimulate renin production by the kidney. Evidence has been presented in experimental animals that PGI_2, PGE_2, and PGD_2 can increase renin secretory rates (Fig. 2). Yun and his coworkers have shown that intrarenal infusions of PGE_2 and PGE_1 increase renin secretion in anesthetized dogs [65, 66]. Inhibition of endogenous renal prostaglandin synthesis by pretreatment of the dogs with indomethacin might explain the clear-cut stimulation of renin release by PGE_2, although prior studies reported equivocal results. In the nonfiltering kidney, PGE_2 retained its ability to stimulate renin secretion [67, 68]; this finding suggested that prostaglandins act directly on juxtaglomerular cells regardless of changes in delivery of glomerular filtrate to the macula densa. Intrarenal infusion of $PGF_{2\alpha}$ did not affect renin secretion. Hockel and Cowley infused PGE_2, 2 μg/min, into the renal ar-

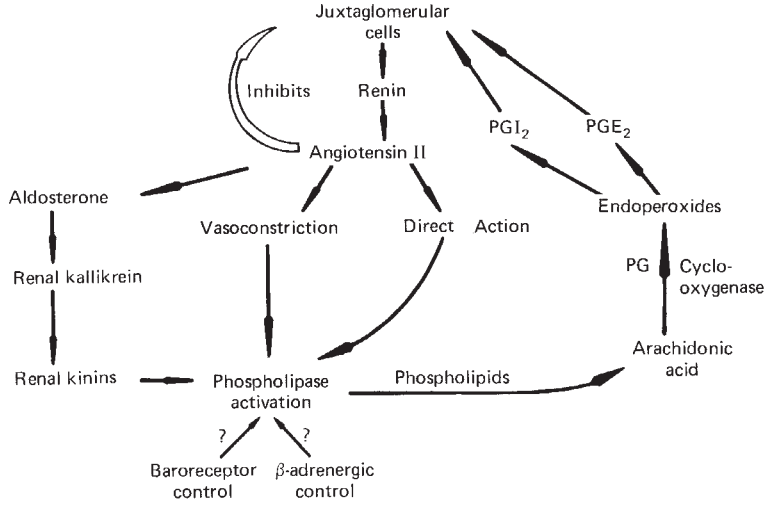

Fig. 2. *Interrelationship of prostaglandin, renin-angiotensin, and kallikrein-kinin systems.* Indomethacin and similar drugs inhibit the cyclooxygenase enzyme that converts arachidonic acid to endoperoxides (Data from Ref. 10).

teries of uninephrectomized dogs over a 7-day period [69]. Plasma renin activity increased rapidly, remained elevated throughout the infusion, and was strongly correlated with accompanying elevations of mean arterial pressure. If prostaglandin overproduction were a primary abnormality in Bartter's syndrome, one might expect similar responses, namely hypertension with hyperreninemia. Gerber et al also studied the role of prostaglandins as stimuli of renal renin release [67, 68, 70, 71]. To reduce the effect of other mechanisms that control renin secretion, they studied dogs that had undergone renal denervation, had had pretreatment with propranolol and indomethacin, and had a nonfiltering kidney. Under these circumstances, PGE_2 and PGI_2 increased renin secretion; PGE_2 and PGI_2 were either equipotent renin stimuli [68] or PGI_2 was more potent [71]. Seymour et al confirmed that PGI_2 and PGD_2 stimulate renin release from the kidney, regardless of tubular flow to the macula densa [72, 73]. Since 13,14-dihydro-PGE_2, a slowly metabolized product of PGE_2, was the most potent renin stimulus, Gerber et al suggested that different rates of prostaglandin degradation by the kidney might account for the different potencies of PGE_2 (rapidly degraded) and PGI_2 (slowly degraded) [71]. Two groups were unable to show that rabbit cortical slices incubated with PGE_2 stimulate renin release [74, 75], whereas PGI_2 is stimulatory over concentrations of 10^{-5} to 10^{-7}M [75]. Inhibitors of prostaglandin synthesis generally reduce but rarely obliterate renal renin secretion. Reduction of renin release by indomethacin and other inhibitors of fatty acid cyclooxygenase has been shown in normal animals and humans *(1)* under basal conditions; *(2)* after baroreceptor stimulation of renal juxtaglomerular cells; *(3)* after beta-adrenergic stimulation; and *(4)* after administration of furosemide [76–83]. Reduction of plasma renin activity after indomethacin administration can be variable, especially in subjects with very low sodium intakes, e.g., 10 mEq/day [77, 80]. There is uniform agreement that indomethacin, ibuprofen, and aspirin significantly reduce plasma renin activity in patients with Bartter's syndrome [14, 16, 17–26] as well as in patients with surreptitious vomiting or diuretic abuse [23, 41–43]. Plasma renin activity decreased in the patient under discussion after she received indomethacin therapy (see Table 1; compare periods I and V).

Most patients with Bartter's syndrome, as well as those with covert vomiting, laxative abuse, or overuse of diuretics, have secondary hyperaldosteronism. The extent of the increased aldosterone secretion is blunted by the concomitant suppressive effect of hypokalemia. Nonsteroidal inhibitors of prostaglandin synthesis reduce or partially normalize the excretory rates for aldosterone [22]. Glasson et al also observed inhibitory effects of indomethacin on aldosterone excretory rates in normal individuals treated with spironolactone or given 10 to 15 mEq of sodium [82]. Although much of the suppression of aldosterone secretion is secondary to reduction of plasma renin activity, recent work suggests that indomethacin directly inhibits aldosterone synthesis in the adrenal gland. Campbell and coworkers reported suppression by indomethacin of angiotensin II-stimulated aldosterone release in vivo and in vitro with rat adrenal cells [83]. Miller, Douglas, and Dunn have confirmed that indomethacin reduced aldosterone release by approximately 50% from purified preparations of rat adrenal zona glomerulosa cells that had been stimulated by angiotensin II, ACTH, or potassium [84] (Fig. 3). Meclofenamate produces similar results [84]. Although these data suggest a direct role for prostaglandins in aldosterone synthesis or release, Miller et al found no increments in PGE synthesis by the adrenal cells after stimulation of aldosterone production, and arachidonic acid had no stimulatory action on aldosterone release despite a three- to fivefold increment of PGE_2 [84].

Patients with Bartter's syndrome and normal pregnant women share many similarities (Table 7) [85]. Pregnant women have increases in plasma renin activity, angiotensin II, and aldosterone [86]. The vasculature is vasodilated in pregnancy, and Bay and Ferris reported increased peripheral plasma and urine concentrations of PGE_2 [87]. Urinary kallikrein also increases during pregnancy [88]. Gant et al demonstrated resistance to the pressor actions of angiotensin II during normal pregnancy [89]. It is unknown whether increased prostaglandin synthesis explains the resistance to angiotensin II in pregnancy. Patients with Bartter's and pseudo-Bartter's syndromes have a similar resistance to the pressor actions of angiotensin II and norepinephrine [23]. Indomethacin given to pregnant women [90], patients with Bartter's syndrome [20, 23, 91], or patients with surrepititious vomiting [23] increases the pressor response to angiotensin II. However, this finding does not provide direct evidence of increased vascular synthesis of prostaglandins in these conditions because indomethacin potentiates the pressor response to angiotensin II in normal individuals [92, 93]. Figure 4 shows the effect of 150 mg of indomethacin on the angiotensin

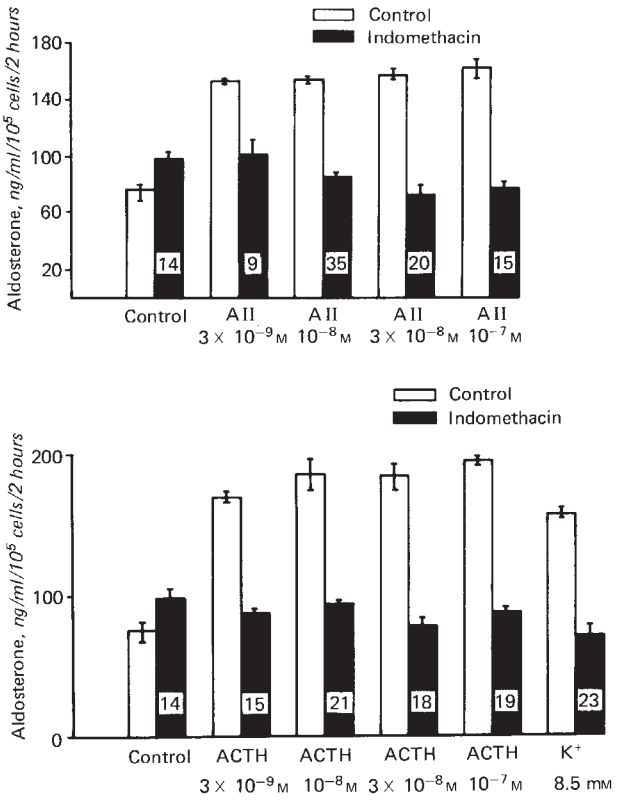

Fig. 3. *Effects of indomethacin on aldosterone and PGE_2 synthesis in adrenal zona glomerulosa cells.* Indomethacin, 3×10^{-6} M, was incubated with rat adrenal glomerulosa cells with angiotensin II, ACTH, or potassium. The percentage of PGE_2 synthesis remaining after indomethacin is indicated within the histogram bars (Data from Ref. 84).

II-induced increment in mean blood pressure in 10 normal men [92]. Indomethacin enhanced significantly the vasoconstrictor action of angiotensin II in these normal men. This response in healthy subjects makes interpretation of similar tests in patients with Bartter's syndrome difficult. In other words, one should not assume that the diminished pressor responsiveness to angiotensin II in patients with Bartter's syndrome is secondary to overproduction of prostaglandins simply because indomethacin reverses the "resistance."

As anticipated, the woman we are discussing had resistance to angiotensin as demonstrated by the infusion test. The initial infusion rate of 90 ng/kg/min required to raise the blood pressure by 20 mm Hg is extremely high. When the test was repeated during period II, a rate of 29 ng/kg/min was required, which is also high. Indomethacin improved angiotensin sensitivity, but the value of 19 ng/kg/min was twice the normal value of 8 to 12 ng/kg/min. Radfar et al reported 8 patients with Bartter's syndrome

Table 7. Similarities between Bartter's syndrome and normal pregnancy

1. Increased plasma renin activity and angiotensin II
2. Increased aldosterone
3. Generalized vasodilation
4. "Resistance" to angiotensin II
5. Indomethacin potentiates pressor response to angiotensin II
6. Increased urinary PGE_2
7. Increased urinary kallikrein

who required an angiotensin II infusion rate of 25 ± 5 ng/kg/min to increase blood pressure by 20 mm Hg before indomethacin treatment, and 10 ± 2 ng/kg/min after indomethacin [23]. Four patients with psychogenic vomiting required 102 ng/kg/min to raise their blood pressures 20 mm Hg; administration of indomethacin returned sensitivity to normal (i.e., 12 ± 2 ng/kg/min) [23].

In summary, abundant evidence exists that renal synthesis of PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ is increased in patients with Bartter's syndrome. There are no data to support nonrenal or systemic overproduction of PGE_2 or $PGF_{2\alpha}$. Because a platelet aggregation defect, reversible after indomethacin administration, has been established in these pa-

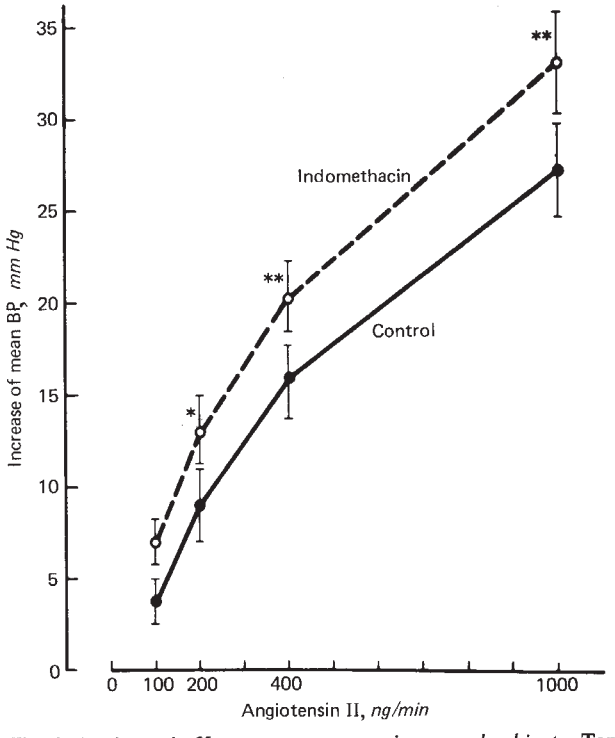

Fig. 4. *Angiotensin II pressor responses in normal subjects.* Ten normal men (mean weight of 69.8 kg) received angiotensin II before and after indomethacin (50 mg every 6 hours for 3 doses). Each dose of angiotensin was given intravenously for 20 minutes (*$p < 0.025$; **$p < 0.01$) (Data from Ref. 92).

tients, some researchers have surmised that other prostaglandins (e.g., PGI_2, PGD_2, 6-keto-PGE_1) are increased in plasma. The cause of the increased renal synthesis of prostaglandins, although unknown, seems clearly a secondary event in patients with Bartter's syndrome. Drugs that inhibit fatty acid cyclooxygenase and thereby reduce prostaglandin synthesis have a salutary effect in these patients. Indomethacin and other nonsteroidal inhibitors of prostaglandin synthesis reduce plasma renin activity and aldosterone excretory rates, reverse the vascular insensitivity to angiotensin II, and increase the plasma potassium.

Questions and answers

DR. JEROME P. KASSIRER: We now know a great deal about derangements in the renin-angiotensin-aldosterone system and the kallikrein-prostaglandin system in patients with Bartter's syndrome, but our understanding of the physiology fails to provide a coherent, integrated picture of relations between these disturbances. In your opinion, is this an accurate reflection of the current situation?

DR. DUNN: I think it is. We do know enough to say that prostaglandin overproduction is secondary, but we don't know how much of the renin elevation is due to prostaglandin overproduction or how much of the prostaglandin overproduction is due to renin elevation. We can best integrate this information by saying that the primary abnormality is tubular: either a chloride reabsorptive defect in the thick ascending limb or a potassium reabsorption defect in the distal tubule. How would those events trigger prostaglandin overproduction? You are absolutely right that we understand many physiologic and pathophysiologic interrelations but that we do not understand the temporal and sequential mechanisms.

DR. KASSIRER: You implied that potassium depletion could be excluded as the cause of prostaglandin overproduction, but I am not convinced that the models of potassium depletion studied approximated closely the physiologic state of patients with Bartter's syndrome. In animals treated with mineralocorticoids and in patients with primary aldosteronism, for example, volume tends to be expanded, whereas in patients with Bartter's syndrome, volume is probably normal or low.

DR. DUNN: That's right. Those animals are imperfect models of potassium depletion because they are volume expanded, have aldosterone or DOCA excess, and have suppressed renin and angiotensin levels, whereas the patient with Bartter's syndrome has either normal or slightly low plasma volume and very high levels of renin and angiotensin. Researchers at several laboratories agree that dietary potassium depletion in rats does not increase renal prostaglandin overproduction, but these studies might be inapplicable to humans.

DR. KASSIRER: Was potassium deficiency induced by administration of DOCA in these experiments?

DR. DUNN: Potassium depletion was induced via dietary deprivation; although potassium deprivation generally increases plasma renin activity, we did not measure plasma renin in these experiments.

DR. JORDAN J. COHEN: As you noted, Gill and Bartter hypothesized that the fundamental defect in Bartter's syndrome involves the chloride transport mechanism in the ascending limb [38]. Do we know how prostaglandins affect chloride transport in the kidney? Also, do prostaglandin synthetase inhibitors alter the chloride transport defect in Bartter's syndrome?

DR. DUNN: I don't know whether all the answers to that question are in. If the chloride reabsorptive defect is a primary abnormality, and researchers disagree about that, you could theorize that the defect results from prostaglandins, because PGE_2 does inhibit chloride reabsorption in the microperfused thick ascending limb in the rabbit [39]. Higashihara and coworkers have shown that acute reduction of prostaglandin synthesis augments chloride reabsorption in the rat [40]. These data suggest that prostaglandins inhibit the movement of chloride from the luminal fluid out of the tubule in the thick ascending limb and perhaps more distal segments. Gill and Bartter studied chloride reabsorption in Bartter's syndrome before and after indomethacin administration. Because indomethacin did not improve the chloride reabsorptive defect, they concluded that excessive prostaglandins did not explain the chloride transport abnormality [38]. I neglected to emphasize the importance of measuring urinary chloride to differentiate patients with Bartter's syndrome, whose urinary chloride levels should approximate the dietary intake, from patients with covert or surreptitious vomiting, in whom urinary chloride is very low. In most other respects, the patient with self-induced vomiting can mimic the patient with Bartter's syndrome and even can manifest a beneficial response to indomethacin. Patients who are diuretic abusers have high urinary chloride levels and therefore mimic even more closely patients with Bartter's syndrome. You must

rule out diuretic abuse by assaying the urine for diuretics because patients are not always forthright about the medications they take.

DR. JEFFREY S. STOFF *(Renal Unit, Beth Israel Hospital, Boston, Mass.):* I would like to comment on several aspects of Dr. Dunn's superb presentation. One of the issues he raised concerns the question of whether the increase in urinary prostaglandin excretion in Bartter's syndrome is a primary or secondary event. The recent observation by Drs. Gill and Bartter suggests that the chloride reabsorption defect is independent of prostaglandins since it was not reversed by treatment with indomethacin [38]. However, indomethacin treatment generally fails to fully suppress urinary prostaglandin excretion to normal in these patients. The failure to reverse the defect in chloride transport by indomethacin thus still might be the result of increased amounts of prostaglandins acting on tubular transport. In fact, several reports have indicated that PGE_2 inhibits chloride transport at the thick ascending limb of Henle's loop [39, 40].

Secondly, the effect of potassium on regulation of prostaglandin metabolism remains controversial, with conflicting data derived from several different models. The studies by Drs. Dunn and Berl utilizing the potassium-depleted rat failed to demonstrate an increase in PGE_2 excretion [56, 57], whereas Ferris and colleagues reported an increase in PGE_2 excretion in the potassium-depleted dog [94]. These latter studies agree with the findings by Zusman and Keiser on the effect of potassium on renal medullary interstitial cells grown in tissue culture [95]. We have explored this question in humans by inducing potassium depletion using a potassium-restricted diet and a cation-binding resin (Kayexalate®) [96]. We successfully induced a reversible defect in urinary concentrating ability in these acutely potassium-depleted subjects. However, PGE_2 excretion did not increase with, nor was the defect corrected by, indomethacin treatment. These results thus failed to provide evidence for potassium depletion as a cause of increased PGE_2 excretion in humans.

DR. KASSIRER: What was the extent of the potassium deficiency in these experimental subjects?

DR. STOFF: Serum potassium decreased an average of 1 mEq/liter, and total-body potassium counting indicated a 10% to 12% decline, but the latter result was quite variable. The concentrating defect was about 30% to 40% of maximal concentrating ability.

DR. DUNN: Your findings are interesting, Dr. Stoff, because we tried unsuccessfully to reverse the concentrating defect with meclofenamate in potassium-depleted rats. Berl, Aisenbery, and Linas reported a similar lack of effect of indomethacin on urine concentration in potassium-depleted rats [57].

DR. KASSIRER: I presume, therefore, that in your potassium-depleted subjects the serum potassium concentration was approximately 3 mEq/liter. Of course, because patients with Bartter's syndrome frequently have lower serum potassium concentrations and, by inference, greater potassium deficits than did the subjects you studied, it is still possible that a greater degree of potassium deficiency could be responsible for the prostaglandin abnormalities. I am not an advocate of the notion that potassium deficiency is the proximate cause of prostaglandin overproduction in Bartter's syndrome, but I am not willing to dismiss this hypothesis without more convincing evidence.

DR. RANDALL ZUSMAN *(Cardiac Unit, Massachusetts General Hospital, Boston, Mass.)*: When Bartter's syndrome was first known to be associated with elevated PGE_2 excretion, it was assumed that prostaglandin overproduction was a proximal event in the pathogenesis of the disorder. Further investigation disclosed, however, that prostaglandin overproduction was not causative. The data from Ferris' laboratory provided evidence in the dog that hypokalemia itself was the stimulant to PGE_2 overproduction [53], but data from your lab indicated that hypokalemia had no effect on urinary PGE_2 excretion in the rat [56]. We have shown that rabbit renomedullary interstitial cells incubated in potassium-free buffered solution synthesize larger amounts of PGE_2 than do cells incubated in potassium-containing buffers [48, 95]. These data suggest that acute potassium depletion stimulates PGE_2 excretion in vitro. Subsequent work by Dusing, who used rabbit renomedullary slices, also demonstrated that potassium depletion stimulates PGE_2 synthesis in vitro [55]. Can you reconcile these apparent differences or add any more information about the rat interstitial cell in vitro? Does it respond like the rabbit interstitial cell? How can we integrate the in vivo and in vitro data?

DR. DUNN: We have not done experiments altering extracellular potassium in the rat medullary interstitial cell, so I really don't know. How much did you change the extracellular potassium in order to effect a change in PGE_2 production by the interstitial cell? Was it in the range of 5 to 10 mmol?

DR. ZUSMAN: If PGE_2 synthesis is measured in the rabbit renomedullary interstitial cell in tissue culture, and the potassium concentration in the medium is varied from 2 to 7 mEq/liter, PGE_2 synthesis is inhibited approximately 7% for each 1 mEq/liter

increase in potassium concentration [98]. At potassium concentrations below 2 mEq/liter, there is a striking increase in prostaglandin synthesis. These experiments test the effects of acute potassium depletion, but we do not know the intracellular potassium concentration under these conditions. As Dr. Kassirer implied before, the chronic potassium depletion that characterizes patients with Bartter's syndrome and those who vomit surreptitiously might result in a profound intracellular potassium deficit and in increased PGE_2 excretion.

DR. DUNN: I cannot reconcile the differences between the in vitro results you quote and the difficulty with in vivo results; only one laboratory has shown in the dog—and nobody else has been able to show in humans or rats—that negative potassium balance stimulates prostaglandin synthesis. I agree that these negative results might be accounted for by the modest potassium depletion induced in the experiments I alluded to.

DR. ZUSMAN: I doubt that species differences account for these disparate results. To postulate that the rat is more resistant to potassium depletion than is the rabbit is not a satisfactory explanation.

DR. ROBERT S. BROWN (*Chief, Renal Unit, Beth Israel Hospital, Boston, Mass.*): I would like to add some evidence from our laboratory that tends to exclude hypokalemia as the primary stimulus for the increase in prostaglandins in Bartter's syndrome. We have used platelet aggregometry to identify a prostaglandin-like substance in the plasma of patients with Bartter's syndrome. This substance can induce an aggregation defect in platelets from healthy individuals as well as in those who have Bartter's syndrome. We have studied small numbers of patients who abuse diuretics and those with surreptitious vomiting; both groups had comparable degrees of hypokalemia but in neither were we able to demonstrate a defect. The platelet defect is not present in patients with primary hyperaldosteronism or in potassium-depleted normal individuals; this prostaglandin-like factor therefore seems to be unique to Bartter's syndrome rather than secondary to potassium depletion.

Dr. Dunn questioned the effect of indomethacin. Indomethacin, a complex drug, has a variable inhibitory action on different cyclooxygenases. It is known that some cyclooxygenases are more resistant to indomethacin, and we should not assume that if urinary PGE_2 is decreased by 80% that other prostaglandins also will be reduced by 80%; other prostaglandins might be reduced by much less than 80%. For instance, the platelet aggregation defect in patients with Bartter's syndrome who are on low-sodium diets is alleviated only slightly by indomethacin, whereas urinary PGE_2 is greatly reduced to normal levels. The administration of indomethacin decreases urinary prostaglandins in these patients but fails to correct the platelet defect caused by a prostacyclin-like substance in the plasma [21].

DR. DUNN: Have you observed a platelet aggregation defect in relatives of patients with Bartter's syndrome?

DR. BROWN: We haven't looked at normal relatives, but the familial occurrence of Bartter's syndrome is well known. It is a good suggestion to study the family members who do not clinically appear to have Bartter's syndrome.

DR. DUNN: Has the platelet defect been a consistent finding?

DR. BROWN: Only in patients who have Bartter's syndrome.

DR. STOFF: I would like to comment on our studies with the circulating plasma factor in Bartter's syndrome [21]. We have neutralized the antiaggregatory effect of the plasma factor with antisera directed against PGI_1 [96]. These sera crossreact with prostacyclin and 6-keto PGE_1 and to a lesser extent with 6-keto $F_{1\alpha}$. We are now generating an antiserum to 6-keto PGE_1 to test whether this circulating plasma factor is in fact 6-keto PGE_1. We think that these studies support the idea that prostacyclin metabolites are present in high concentration in the plasma of patients with Bartter's syndrome and that these substances contribute to the high renin state as well as to the defect in platelet function.

DR. DUNN: Would you agree with my interpretation of your published data that the plasma factor that induced the platelet aggregation defect in normal platelets cannot be prostacyclin because, unlike prostacyclin, it was stable for more than one hour during the incubation?

DR. STOFF: You are right. There is little doubt that the circulating factor is not prostacyclin, as the plasma factor is much more stable.

DR. AARON SPITAL (*Renal Unit, Rhode Island Hospital, Providence, Rhode Island*): Is it possible that the vascular insensitivity in patients with Bartter's syndrome is related to the high levels of circulating angiotensin? Also, does indomethacin reduce these high levels of angiotensin and thereby free up receptors that previously might have been saturated?

DR. DUNN: That is a good question. We know that the higher the endogenous renin level, the less sensitive is the vasculature to infused angiotensin II, as the receptor occupancy from endogenous angiotensin II is high. Patients with Bartter's syn-

drome have similar vascular insensitivity to norepinephrine; I think that vascular insensitivity to angiotensin II in patients with Bartter's syndrome cannot be explained solely by angiotensin II receptor occupancy.

DR. JOHN T. HARRINGTON: What happens to the platelet defect in a patient with Bartter's syndrome in whom sodium intake is restricted to 7 to 10 mEq/day? Is either the platelet dysfunction or the increase in prostaglandin synthesis negated?

DR. BROWN: The platelet defect persists. In fact, sodium restriction worsens the platelet defect even though the prostaglandins (PGE_2) decrease in the urine.

DR. NICOLAOS MADIAS (*Renal Service, NEMCH*): Has anyone studied patients with Bartter's syndrome following either acute or chronic interruption of the renin-angiotensin system with, for example, a converting enzyme inhibitor? What happens to their blood pressure and their aldosterone levels?

DR. DUNN: Rudin et al reported that blood pressure is very dependent on the angiotensin II levels and that these patients are quite sensitive to saralasin. Indomethacin reversed the vasodepressor effects of saralasin in 2 patients [26].

DR. MADIAS: What happens to the prostaglandin level?

DR. DUNN: To my knowledge, no one has measured PGE_2 before and after saralasin administration in patients with the syndrome.

DR. ANDREW LEVEY (*Renal Fellow, NEMCH*): Is urinary prostaglandin excretion elevated in other hyperreninemic states such as cardiac failure, cirrhosis, nephrotic syndrome, and volume depletion? Are these clinical disorders characterized by hyperreninemia but not with an increase in urinary PGE excretion?

DR. DUNN: There have been no reports of measurements of prostaglandins in congestive heart failure, but one study did report that patients with congestive failure who are given indomethacin can have precipititous decreases in renal function [98]. Boyer, Zia, and Reynolds [99] and Zipser and his collaborators [100] reported that urinary prostaglandins were increased in patients with cirrhosis and ascites and that after indomethacin or ibuprofen administration, the patients had decreases in renal blood flow and GFR. Zambraski and I are studying the effects of chronic bile duct ligation in dogs on renal susceptibility to indomethacin. Within 60 to 90 days of biliary ligation, a single, intravenous dose of indomethacin, 2 mg/kg, causes reductions of renal blood flow and GFR (unpublished results). Conditions associated with decreases in effective circulating volume, such as sodium depletion, nephrosis, heart failure, and cirrhosis with ascites, are associated with high plasma angiotensin II and increased alpha-adrenergic activity as compensatory responses. A greater need exists, therefore, for renal prostaglandins to modulate these constrictor influences. If you interrupt prostaglandin synthesis with indomethacin, renal function is compromised. Patients with Bartter's syndrome, similarly, have high angiotensin II levels; most series that have measured GFR before and after indomethacin treatment have shown a 10% to 25% decrement in GFR, sometimes transient and sometimes fixed.

DR. LEVEY: Should we interpret elevated urinary PGE as a sign of hyperrenemia?

DR. DUNN: In general, plasma renin activity and urinary prostaglandins are positively correlated, especially in disease states. But some researchers have found that salt depletion in normal humans has no effect on urinary prostaglandins. Dr. Zusman, did you find increments of urinary PGE_2 after sodium depletion of normal subjects?

DR. ZUSMAN: No, we did not find an effect of dietary sodium content on urinary PGE_2 excretion [22]. I had some reservations, however, about the validity of the assay for urinary PGE_2, and therefore I think that the effect of sodium intake on urinary prostaglandin excretion has to remain an open question for now.

DR. COHEN: Why did salt depletion in this patient cause urinary prostaglandins to increase?

DR. DUNN: Urinary prostaglandins certainly did increase with salt depletion (period IV), probably because the plasma angiotensin II increased and possibly because there were increments in plasma bradykinin as a result of the aldosterone stimulation.

DR. SPITAL: What is the evidence that vasopressin stimulates prostaglandin production?

DR. DUNN: The evidence for that can be found in several systems. Dr. Zusman showed that rabbit renomedullary interstitial cells in vitro increase PGE_2 synthesis in response to arginine vasopressin [48]. We and a group from Vanderbilt have published work on rats with diabetes insipidus (Brattleboro rat) showing that these animals, which lack vasopressin, have much lower urinary excretion of PGE_2 and $PGF_{1\alpha}$ [49-51]. We treated the rats with pitressin tannate in oil or with dD-AVP, a vasopressin analogue without pressor activity. We found that vasopressin treatment for as little as one or two

days in the Brattleboro rat increases urinary prostaglandin excretion about threefold [49]. We recently extended those studies in collaboration with Drs. Kinter and Beeuwkes at Harvard Medical School. There was a linear dose-response relationship between dD-AVP and urinary PGE_2; the evidence is quite good, at least in the rat model, that renal prostaglandins are stimulated by vasopressin [50]. No similar evidence is available in humans.

DR. MADIAS: Has anyone observed pregnant patients with Bartter's syndrome? The similarities between the syndrome and pregnancy prompt me to ask whether such patients have any hormonal or hemodynamic changes during pregnancy as compared to baseline.

DR. DUNN: I know of no studies of pregnant patients who have Bartter's syndrome. I am puzzled by the difference between pregnancy and Bartter's syndrome, because hormonally these women are similar. Perhaps the antialdosterone effect of progesterone is important.

DR. KASSIRER: Another difference is the lack of a renal chloride wasting defect in the normal pregnant woman.

DR. COHEN: I understand why you think the changes in prostaglandins are secondary in Bartter's syndrome. I also understand that indomethacin has effects other than those occurring via the prostaglandin system. But I am still puzzled by the improvement that most, if not all, patients with Bartter's syndrome experience when given indomethacin. If prostaglandins are not intimately involved in the pathogenesis of the electrolyte disturbance, how can you explain the improvement that occurs?

DR. DUNN: I didn't say that the elevation of prostaglandins was unimportant in Bartter's syndrome; I only said it was secondary. If the prostaglandins, renin, and aldosterone are reduced, the potassium balance becomes positive. These people feel poorly largely because they are potassium depleted. If one raises the potassium levels in these patients by reducing renin via reductions of prostaglandins, they feel better.

DR. COHEN: But aren't you also suggesting that positive feedback drives prostaglandin production in this syndrome?

DR. DUNN: Yes. Prostaglandins stimulate renin and angiotensin, and renin stimulates prostaglandin production.

DR. COHEN: What stops it?

DR. DUNN: Probably the negative feedback of angiotensin II on renin production by the juxtaglomerular cells. Ultimately, a new steady state is established in which a high angiotensin II level inhibits renin production despite continued production of prostaglandin.

Acknowledgments

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