

The Kallikrein-Kinin System in Bartter's Syndrome and Its Response to Prostaglandin Synthetase Inhibition

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Research Article

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The Kallikrein-Kinin System in Bartter's Syndrome and Its Response to Prostaglandin Synthetase Inhibition

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ABSTRACT The kallikrein-kinin system was characterized in seven patients with Bartter's syndrome on constant metabolic regimens before, during, and after treatment with prostaglandin synthetase inhibitors. Patients with Bartter's syndrome had high values for plasma bradykinin, plasma renin activity (PRA), urinary kallikrein, urinary immunoreactive prostaglandin E excretion, and urinary aldosterone; urinary kinins were subnormal and plasma prekallikrein was normal. Treatment with indomethacin or ibuprofen which decreased urinary immunoreactive prostaglandin E excretion by 67%, decreased mean PRA (patients recumbent) from 17.3 ± 5.3 (S.E.M.) ng/ml per h to 3.3 ± 1.1 ng/ml per h, mean plasma bradykinin (patients recumbent) from 15.4 ± 4.4 ng/ml to 3.9 ± 0.9 ng/ml, mean urinary kallikrein excretion from 24.8 ± 3.2 tosyl-arginine-methyl ester units (TU)/day to 12.4 ± 2.0 TU/day, but increased mean urinary kinin excretion from 3.8 ± 1.3 μ g/day to 8.5 ± 2.5 μ g/day. Plasma prekallikrein remained unchanged at 1.4 TU/ml. Thus, with prostaglandin synthetase inhibition, values for urinary kallikrein and kinin and plasma bradykinin returned to normal *pari passu* with changes in PRA, in aldosterone, and in prostaglandin E. The

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INTRODUCTION

Bartter's syndrome is characterized by hypokalemic alkalosis, hyperreninemia, hyperaldosteronism, and juxtaglomerular cell hyperplasia (1). Patients with this syndrome have normal blood pressure, despite their hyperreninemia, and aldosteronism; their pressor response to intravenously administered angiotensin II or norepinephrine is subnormal (1, 2). Volume expansion with either saline or albumin does not change their blood pressure and does not suppress plasma renin activity (PRA)¹ and aldosterone to normal values (3). When their dietary sodium is restricted, they conserve sodium but continue to lose potassium (1).

In recent studies in patients with Bartter's syndrome, Verberckmoes et al. (4) have found hyperplasia of renomedullary cells and Gill and associates (5) have shown that urinary prostaglandin E is increased, presumably as a result of increased synthetic activity of the renomedullary cells. This group and others (6, 7) have shown that treatment with prostaglandin synthetase inhibitors corrects many of the metabolic and hormonal derangements in the syndrome.

Studies from several laboratories have indicated that the vasodilator kallikrein-kinin system has several

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¹Abbreviations used in this paper: PGE₂, prostaglandin E₂; PRA, plasma renin activity; TAME, α -N-tosyl-L-arginine-[³H]methyl ester; TU, TAME units; UipGEV, urinary immunoreactive prostaglandin E excretion.

interrelationships with the renin-angiotensin-aldosterone system and with prostaglandins. Bradykinin stimulates prostaglandin synthesis in blood vessels and in the kidney, probably by activating enzymes which make substrate available for prostaglandin synthetase (8–11). The response of plasma bradykinin to postural change, sodium depletion, and saline infusion parallels temporally the response of PRA and of angiotensin II (12). Urinary kallikrein, which is derived from the kidney (13), is increased in conditions in which sodium-retaining steroids are present in excess (14–17), but neither plasma nor urinary kinins are affected by sodium-retaining steroids (17).

To study the interrelationships between these systems, we characterized the kallikrein-kinin system, renin-angiotensin-aldosterone system, and prostaglandins in seven patients with Bartter's syndrome before, during, and after prostaglandin synthetase inhibition.

METHODS

Clinical methods. Seven Caucasian women with Bartter's syndrome, ranging in age from 9 to 46 yr (30 ± 16 , mean \pm SD), were studied. All patients were hypokalemic, hyperreninemic, and had juxtaglomerular cell hyperplasia on renal biopsy, vascular hyposensitivity to infusions of angiotensin II, and conserved sodium normally in response to a low sodium intake. All patients but one have always been normotensive. The one exception has occasionally had elevated systolic blood pressures in the range of 150–170 mm Hg, although the averages of her systolic and diastolic pressures are 137/76 mm Hg. Six patients were fed constant diets containing 9 meq Na and 42–90 meq K daily. The diets were supplemented with NaCl and KCl to yield a total intake of 109 meq Na and 250 to 270 meq K daily. One patient, aged 9 yr, received a total intake of 59 meq Na and 165 meq K daily. Fluid intake was constant and approximately 2,500 ml/day throughout the study. After 4 days of control observations, a prostaglandin synthetase inhibitor was given. Indomethacin was administered to six patients for 7–9 days, followed by 4–7 days of posttreatment observations. The dose of indomethacin was 150 mg/day except in two patients, one of whom was given only 112 mg/day because of marked sodium retention; the second was given only 75 mg/day because she was a 9-yr-old girl who weighed only 17 kg. One patient received ibuprofen, 1,600 mg/day.

25 normal Caucasian women, ranging in age from 19 to 58 yr (26 ± 12 is mean \pm SD) were fed constant diets for 1 wk, containing 9 meq Na and 70 meq K daily. The diets were supplemented with 100 meq NaCl and 30 meq KCl daily. Control observations were made on the 5th, 6th, and 7th days. 18 of these normal subjects were studied for 1 additional wk after withdrawal of the supplemental NaCl; six of these normal subjects were repleted and then given a 9-meq sodium diet with indomethacin, 150 mg/day, given on days 5–7 of the diet. Comparisons were made between observations on days 5–7 of sodium depletion alone and sodium depletion plus indomethacin.

All subjects were hospitalized at the Clinical Center, National Institutes of Health, during the study. The protocols for these studies were approved by the Clinical Research Committee of the National Institutes of Health and all subjects gave informed consent in writing.

Biochemical methods. Urine was collected daily in 24-h periods and each voiding was equally divided into two polypropylene containers. One container had 20 ml 6 N HCl and 2 ml of a 0.1-mg/ml solution of pepstatin,² in methanol, while the other container had no additives. Urine was refrigerated during collection and immediately divided into aliquots for measurement of sodium, potassium, creatinine, aldosterone, prostaglandin E, kallikrein, and kinins.

Sodium, potassium, and creatinine in blood and urine, and chloride and CO₂ in blood, were determined by standard techniques as previously described (18). Venous blood for the estimation of PRA by radioimmunoassay (19), plasma bradykinin, and plasma prekallikrein, was taken between 8:00 a.m. and 9:00 a.m. when the patient had been recumbent overnight. Urinary aldosterone was determined by radioimmunoassay (20). PRA was assayed by Hazelton Laboratories, Inc., Vienna, Va., under a special contract. Urinary immunoreactive prostaglandin E-like material was determined by a radioimmunoassay as recently reported (5). This assay gives values that are 0.6- to 3.4-fold higher than those assayed by gas chromatography-mass spectroscopy in the basal state and from 1.25- to 13.7-fold higher with prostaglandin inhibition (5). The reason for this discrepancy is unknown but is probably due to the presence of a prostaglandin or its metabolite as the levels of immunoreactive prostaglandin E are reduced to a similar extent in either assay by treatment of these patients with prostaglandin synthesis inhibitors (5).

Urinary kallikrein. Aliquots of urine were stored under toluene at 4°C. At the completion of each individual study, samples were assayed in duplicate by a modification (21) of the radiochemical esterolytic method of Beaven et al. (22). The esterolytic activity of urine filtered through Sephadex G-25 (fine) (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) was determined with purified α -N-tosyl-L-arginine-[³H]-methyl-ester (TAME) as the substrate. The major TAME esterase in human urine at the pH of this assay is kallikrein (14); the specificity of this assay has been confirmed by bioassay (14). In this study, esterase activity was identified as kallikrein in four patients with Bartter's syndrome and in four normal subjects; antibodies to either human urinary kallikrein or human kininogen were covalently bound to agarose.³ When 0.40 ml of urine was applied to 25 μ l of agarose, covalently bound to kallikrein antibody, only $12.0 \pm 2.5\%$ (mean \pm SEM) of the esterase activity in the urine of patients with Bartter's syndrome was eluted. Similarly, $10.0 \pm 1.5\%$ of the esterase activity of urine from the normal subjects was eluted from this column. In contrast, all esterase activity ($99.0 \pm 2.5\%$) was eluted from the column containing antibody to kininogen. Thus, as previously demonstrated by bioassay, the major TAME esterase in human urine is kallikrein. Standard curves of esterase activity were constructed for each assay with a functionally pure human urinary kallikrein standard (22).³ Four concentrations of standard were used in triplicate and a standard curve was generated by computer by the method of least squares. Values were computed for this curve and expressed as TAME units (TU) excreted per 24 h. 1 esterase unit is defined as that amount of kallikrein which hydrolyzes 1 micromole of TAME per minute at pH 8.0 and 30°C in a titrametric assay (23). A standard

² Kindly supplied by Dr. Walter Troll, State University of New York at Stony Brook, Stony Brook, N. Y.

³ Kindly supplied by Dr. Jack V. Pierce, Laboratory of Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md.

urine was run with each assay to serve as a quality control. In 28 assays it gave a kallikrein value of 1.18 ± 0.15 TU/ml (mean \pm SD). An assay was repeated if this value was exceeded by 2 SD, or if duplicate samples varied by more than 10%.

Prekallikrein. Whole blood (1.7 ml) was drawn into a plastic syringe, emptied into a plastic tube containing 0.3 ml of an acid-citrate-dextrose solution, and mixed immediately at room temperature (21). The tube was centrifuged at room temperature and 200- μ l aliquots of plasma were quickly frozen and stored in plastic tubes at -20°C . Prekallikrein was determined in duplicate by a radiochemical esterolytic assay (21) against the same kallikrein standard used in the assay for urinary kallikrein. Values are expressed as TU per milliliter plasma.

Plasma bradykinin collection and processing. Whole blood was collected and processed by a modification of the method of Talamo et al. (24) and Wintroub et al. (25). 9 ml of blood was collected through a no. 19 gauge siliconized butterfly needle (Abbott Diagnostics, S. Pasadena, Calif.) into a polypropylene syringe containing 0.15 ml of solution that was 0.15 M NaCl with 3.6 mg disodium EDTA, 9 mg polybrene (Aldrich Chemical Co., Milwaukee, Wis.), and 45 nCi bradykinin triacetate [$2\text{-prolyl-3,4 } ^3\text{H(N)}$] (sp act, 50 Ci/mmol). (The latter was purified weekly over CM-Sephadex C-25 with 0.5 M ammonium acetate at pH 6.7 and stored at -70°C .) Blood was mixed well with this solution as it was drawn and was then emptied immediately into a 12-ml polypropylene tube equilibrated at 0°C . Plasma was separated at 12,000 g for 10 min at 4°C with 90–100% recovery of [^3H]bradykinin. Duplicate 2-ml aliquots were placed into two polypropylene tubes and precipitated with TCA (24) with no additional loss of [^3H]bradykinin. The supernate was diluted 10-fold with 3.5 mM phosphate buffer, pH 8.0, and placed over a $1.5 \times 6.5\text{-cm}$ plastic column of QAE-Sephadex A-25 (Pharmacia Fine Chemicals, Inc.) equilibrated with 3.5 mM phosphate buffer (25). The sample was then adjusted to pH 4.0 and placed over a $0.7 \times 1.5\text{-cm}$ IRC-50 (100–200 mesh) column equilibrated in 0.1 N acetic acid (23). The initial effluent was discarded as well as the subsequent 3-ml wash with 0.1 N acetic acid. Bradykinin was then eluted with 5 ml 9 N acetic acid into a siliconized glass tube and flash evaporated at 40°C . The dried sample was reconstituted in 0.5 ml buffer used in the radioimmunoassay (0.2 M Tris-acetate buffer, pH 6.4, with 0.1 M EDTA and 1 mg/ml lysozyme). A 0.1-ml aliquot was used to determine the recovery of [^3H]bradykinin after processing. Mean recovery for 49 samples was $59 \pm 9\%$ (mean \pm SD). Processed samples were stored in 1.5-ml microtest tubes (Brinkmann Instruments, Inc., Westbury, N. Y.) at -20°C before assay and were stable for at least 1 mo. Samples were assayed at the completion of each individual study.

Urine collection and processing for urinary kinins. Urine was collected in 6 N HCl to destroy kininases and in pepstatin to inhibit uropepsin, a urinary enzyme that has been shown to generate methionyl-lysyl-bradykinin in urine at pH 1 to 2 in vitro; urine, collected in pepstatin, does not contain methionyl-lysyl-bradykinin (26). Samples were stored at -20°C and were measured at the completion of each individual study. Urinary kinins, lysyl-bradykinin and bradykinin (27), are stable for up to 1 yr under these conditions. Before assay, urine was thawed adjusted to pH 6.4 and centrifuged at 12,000 g for 10 min.

Bradykinin radioimmunoassay. Urinary kinins and plasma bradykinin were determined by the same radioimmunoassay. The antibody was kindly supplied by Dr. J. V. Pierce who prepared it by immunization of sheep with highly purified human plasma low molecular weight

kininogen I (27) in which the kinin moiety is at the carboxy terminus. This antibody, which was directed primarily to the C-terminal bradykinin moiety, had the following features. (a) It had essentially the same reactivity with bradykinin as with lysyl-bradykinin (the two kinins found in vivo in human urine) and methionyl-lysyl-bradykinin over a 0.04 to 2.0 pmol range; thus, this antibody gives a measure of total urinary kinins. (b) Kinin fragments consisting of amino acid residues 1–4, 1–6, 1–7, 4–7, 3–7, 2–7 were undetectable at the 100-pmol level, but 100 pmol of fragment 1–8 gave the same displacement as 0.25 pmol bradykinin. (c) Its binding by the bradykinin moiety in low molecular weight kininogen I was only 5% of that by the bradykinin released by a similar amount of low molecular weight kininogen I; it did not significantly bind to other forms of kininogen.

The weak binding of kininogen I, which comprises about 20% of the total plasma pool, is inconsequential, as samples are processed to contain virtually no kininogen. Also, the mean value for kinin in 10 urine samples assayed by bioassay with the guinea pig ileum ($19.7 \pm 2.7 \mu\text{g/day}$) was similar to that obtained by our radioimmunoassay ($20.9 \pm 2.4 \mu\text{g/day}$). Agreement between bioassay and radioimmunoassay was very good and showed a correlation coefficient (r) of 0.86 ($P < 0.001$) for these few samples.

Tyrosine-8-bradykinin (New England Nuclear, Boston, Mass.) was iodinated monthly with ^{125}I by the chloramine-T method⁴ and stored at -70°C in Tris buffer, pH 6.4, with 1 mg/ml lysozyme. Bradykinin standard was kindly supplied by Sandoz Ltd., Basel, Switzerland. Its purity and concentration were confirmed by amino acid analysis and bioassay in this laboratory.

In the radioimmunoassay each tube contained a total volume of 0.7 ml which consisted of 0.2 ml ^{125}I -tyrosine-8-bradykinin (13,000 dpm), 0.30 ml antibody (1:10,000 dilution), 0.05 or 0.1 ml sample, and 0.10 or 0.15 ml 0.2 M Tris-acetate buffer, pH 6.4, with 0.01 M EDTA and 1 mg/ml lysozyme. After incubation overnight, free and bound bradykinin were separated with 0.45- μm Millipore filters (Millipore Corp., Bedford, Mass.) and counted in scintillation fluid. A standard curve with values from 125 to 2,000 pg was run with each assay and analyzed by computer program (28) along with duplicate unknown samples. In 20 assays, the antibody gave $35 \pm 5.4\%$ (mean \pm SD) binding of ^{125}I -tyrosine-8-bradykinin at a 1:10,000 dilution of antibody. The slope was -0.99 ± 0.09 in a log-logit plot of dose of bradykinin vs. the fraction of ^{125}I -tyrosine-8-bradykinin bound; 50% inhibition of binding for bradykinin occurred with 300 ± 36 pg and the computed minimal detectable dose (i.e., the smallest amount of bradykinin the assay can determine to be different from zero) was 12 ± 8 pg/sample volume (120 ± 8 pg/ml sample). In those 20 assays, as with all assays, three control urine samples were run, showing interassay variability of 7.6, 9.2, and 10%. Any duplicate samples whose values differed by more than 15% were repeated, and any assay in which there was more than a 2-SD difference in the three quality control samples was repeated. Values for plasma bradykinin were derived by computer from the mean of samples processed in duplicate. Each processed sample was assayed in duplicate, and the values were corrected for recovery of 45 nCi bradykinin triacetate which was added to each syringe before venipuncture.

Statistical methods. Values are reported as mean \pm SEM unless stated otherwise. Student's t test for paired samples was used to determine statistical significance and $P = 0.05$ was considered the upper limit of significance.

⁴ Thomas, Richard J. Personal communication.

RESULTS

Metabolic effects of prostaglandin synthetase inhibition

The plan of the study and the effects of prostaglandin synthetase inhibition on these patients are presented in Fig. 1. In the control period, mean serum potassium was 2.4 ± 0.2 meq/liter, (mean \pm SEM), mean PRA (patients recumbent) was 17.3 ± 5.3 ng/ml per h (16 normal recumbent subjects on 109 meq Na intake had a value of 1.4 ± 0.2 ng/ml per h) and mean urinary aldosterone was 12.0 ± 5.1 μ g/day (19 normal subjects on 109 meq Na intake excreted 4.5 ± 0.5 μ g/day.) Blood pressure with patients supine was $107/70 \pm 6/3$ mm Hg. In addition, as recently reported (5) mean urinary immunoreactive prostaglandin E excretion (UiPGEV) was elevated to 70 ± 11 ng/h as compared to that of normal subjects, who excreted 29 ± 5 ng/h when fed a diet with 109 meq Na/day.

During prostaglandin synthetase inhibition there was an abrupt reduction of UiPGEV to 48% of control ($P < 0.005$) on the first day, and UiPGEV remained at 33 to 38% of control throughout the treatment period. There was significant retention of 51 meq sodium on the 1st ($P < 0.005$) and 45 meq sodium on the 2nd ($P < 0.02$) days of therapy. There was an increase in weight of 0.9 ± 0.2 kg on the 1st day ($P < 0.005$) and of 1.8 ± 0.4 kg by the 5th day. Simultaneous with this early sodium retention, there was significant retention of 87 meq potassium on the 1st ($P < 0.02$) and 45 meq on the 2nd ($P < 0.05$) day. Mean serum potassium rose from 2.4 ± 0.2 meq/liter to 3.2 ± 0.3 meq/liter on the first day ($P < 0.02$) and remained elevated throughout treatment at a level of about 3.0 ± 0.1 meq/liter. Serum CO_2 (not shown) fell, serum chloride rose, and serum sodium was unchanged during treatment. Mean creatinine clearance was significantly reduced ($P < 0.05$) only on the 1st 3 days of treatment by 23, 15, and 10%, respectively. In addition to these effects of prostaglandin synthetase inhibition, there was an early reduction of mean PRA (patients recumbent) from a control value of 17.3 ± 5.3 ng/ml per h to 8.1 ± 3.9 ng/ml per h ($P < 0.05$) (mean of the 1st and 2nd days of treatment), and to 3.3 ± 1.1 ng/ml per h ($P < 0.025$) on the 6th and 7th days. The reduction in PRA was followed by a significant reduction in aldosterone excretion on the 4th through 7th days of treatment. Blood pressure was not significantly changed.

When the prostaglandin synthetase inhibitor was stopped, there was a brisk return of prostaglandin E excretion to $77 \pm 9\%$ and $108 \pm 11\%$ of control on the 1st and 2nd days, respectively, and urinary sodium excretion on these 2 days was over twice that on the last day

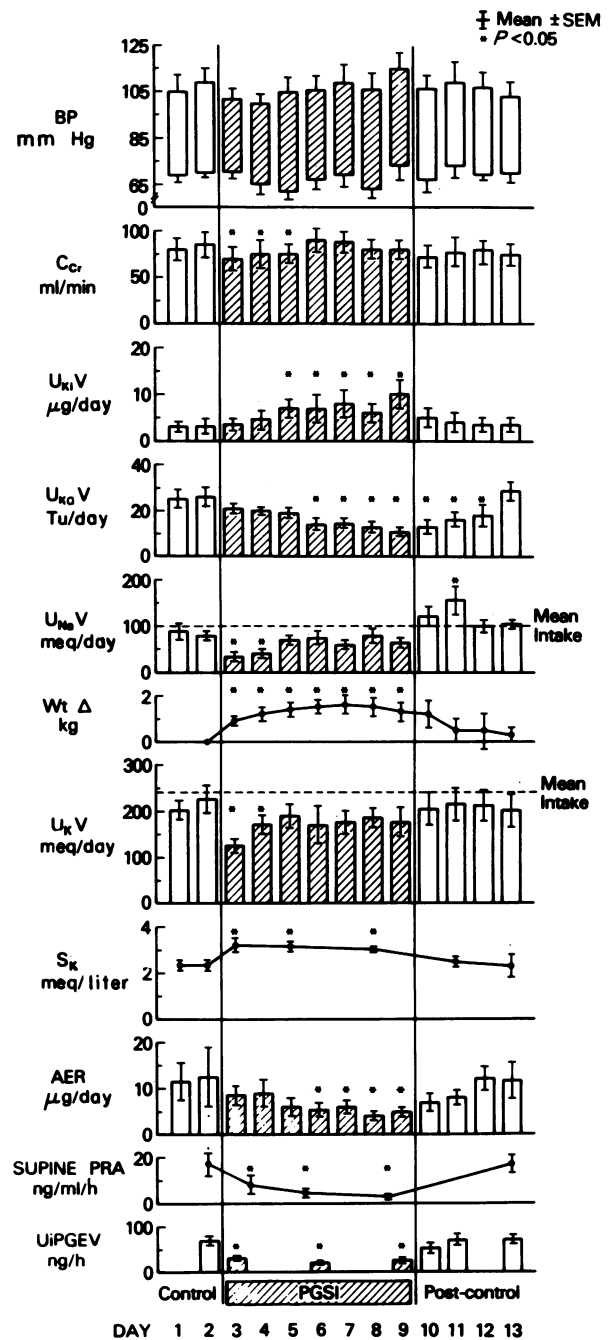


FIGURE 1 Effects of prostaglandin synthetase inhibition (PGSI) in seven patients with Bartter's syndrome on blood pressure (BP), creatinine clearance (C_{cr}), kinin excretion ($U_{KI}V$), kallikrein excretion ($U_{Ka}V$), urinary sodium excretion ($U_{Na}V$), weight change ($Wt \Delta$), urinary potassium excretion (U_KV), serum potassium (S_K), aldosterone excretion rate (AER), plasma renin activity (PRA), and urinary immunoreactive prostaglandin E excretion (UiPGEV). * is $P < 0.05$. When calculated as percentage change, AER is significantly different from control on days 6-9.

of treatment ($P < 0.05$). With this natriuresis, weight, urinary potassium, serum potassium, aldosterone excretion, and PRA returned to control values. In Table I, the mean values in six normal subjects on days 5–7 of 9-meq/day sodium intake are compared to the mean values during indomethacin on days 5–7 of sodium depletion. During indomethacin, UiPGEV, PRA, and aldosterone excretion rate were significantly lower than during sodium depletion alone. Unlike patients with Bartter's syndrome, however, these subjects demonstrated no significant change in creatinine clearance during indomethacin. Although potassium excretion was significantly less ($P < 0.05$), as was aldosterone excretion, this was minor and had no effect on serum potassium.

Kallikrein-kinin system, basal values

Urinary kallikrein (Fig. 2A). Mean urinary kallikrein of 25 normal subjects with daily dietary intakes of 109 meq Na and 100 meq K was 8.2 ± 7.4 TU/day (mean ± 2 SD). These values are similar to those re-

ported previously by Margolius et al. (14) from this laboratory.

All patients with Bartter's syndrome excreted amounts above this range: their mean excretion was 24.8 ± 3.2 TU/day, a value that is significantly different from that of normal subjects ($P < 0.001$). Although 18 normal subjects who were depleted of sodium (9 meq Na diet for at least 5 days) increased their mean kallikrein excretion more than twofold, the patients with Bartter's syndrome still excreted significantly more kallikrein ($P < 0.05$).

Urinary kinin excretion (Fig. 2B). Mean urinary kinins of 25 normal subjects with daily dietary intakes of 109 meq Na and 100 meq K were 13.3 ± 8.7 μ g/day (mean ± 2 SD). These values are similar to those reported by Hial et al. (26) which were obtained by bioassay. Patients with Bartter's syndrome excreted significantly ($P < 0.001$) less kinins (4.3 ± 1.2 μ g/day), than the normal subjects on a 109-meq sodium intake or those who were sodium depleted. The four patients with Bartter's syndrome with the lowest kinin excretion had higher PRA (25.9 ± 6.4 vs. 5.9 ± 0.4 ng/ml per h) higher urinary aldosterone excretion (21.7 ± 9.4 vs. 7.9 ± 3.5 μ g/day), higher kallikrein excretion (28.7 ± 9.4 vs. 19.6 ± 1.3 TU/day), and lower creatinine clearances (63 ± 15 vs. 113 ± 13 cm³/min) than the three patients with the highest kinin excretion.

Plasma bradykinin (Fig. 2C). Mean plasma bradykinin for the 19 normal subjects on an intake of 109 meq Na and 100 meq K was 2.95 ± 1.75 ng/ml (± 2 SD). These values are similar to those reported by Talamo et al. (24). All patients but one with the syndrome had values above this range with a mean of 13.2 ± 4.2 ng/ml ($P < 0.001$). Although 14 sodium-depleted normal subjects had a mean plasma bradykinin (6.0 ± 0.9 ng/ml), which was significantly ($P < 0.05$) higher than that in their sodium-replete state, it was still lower ($P < 0.05$) than the values in the patients with Bartter's syndrome.

Plasma prekallikrein. In 12 normal subjects plasma prekallikrein averaged 1.3 ± 0.4 TU/ml (mean ± 2 SD) on daily Na intakes of 109 meq Na and 100 meq K/day and 1.4 ± 0.1 on daily Na intakes of 9 meq/day. These values are similar to those reported by Imanari et al. (21) Basal plasma prekallikrein in four patients with Bartter's syndrome was 1.4 ± 0.1 TU/ml, and was not abnormal.

Kallikrein-kinin system, prostaglandin synthetase inhibition

The effects of prostaglandin synthetase inhibition on the urinary and plasma kallikrein-kinin system of each patient with Bartter's syndrome are plotted in Fig. 3

TABLE I

Effect of Indomethacin on Sodium-Depleted Normals*

	Control	Indomethacin	P value
	mean \pm SE	mean \pm SE	
C _{Cr} , ml/min	105 \pm 7	110 \pm 9	NS
U _{Ki} V, μ g/day	10.7 \pm 1.9	11.1 \pm 1.0	NS
U _{Ka} V, TU/day	25.4 \pm 5.9	15.8 \pm 4.0	<0.02
U _{Na} V, meq/day	4.3 \pm 1.1	3.6 \pm 1.1	NS
Wt Δ , kg	-1.7 \pm 0.16	-1.24 \pm 0.24	NS
U _K V, meq/day	88 \pm 6	77 \pm 7	<0.05
S _K , meq/liter	3.9 \pm 0.1	4.0 \pm 0.1	NS
AER, μ g/day	82.0 \pm 11.6	60.5 \pm 5.1	<0.05
PRAR, ng/ml/h	4.3 \pm 0.5	3.0 \pm 0.6	NS
PRAU, ng/ml/h	9.9 \pm 1.4	7.2 \pm 1.3	<0.04
PBKR, ng/ml	2.9 \pm 0.5	2.7 \pm 0.6	NS
PBKU, ng/ml	4.2 \pm 0.5	3.6 \pm 0.7	NS
UiPGEV, ng/h	22.3 \pm 4.7	13.4 \pm 2.8	<0.01

Mean effects of indomethacin in sodium-depleted normal subjects. Six normal subjects were fed diets containing 9 meq/day sodium during two periods. Mean control observations were made on days 5–7 during the first period and again on days 5–7 in the second period when indomethacin, 150 mg/day, was given.

*Abbreviations used in this table: C_{Cr}, creatinine clearance; U_{Ki}V, urinary kinin excretion; U_{Ka}V, urinary kallikrein excretion; U_{Na}V, urinary sodium excretion; Wt Δ , weight change; U_KV, urinary potassium excretion; S_K, serum potassium; AER, aldosterone excretion rate; PRA, plasma renin activity, subject recumbent (R) and upright (U); PBK, plasma bradykinin, subject recumbent (R) and upright (U); UiPGEV, urinary immunoreactive prostaglandin E excretion.

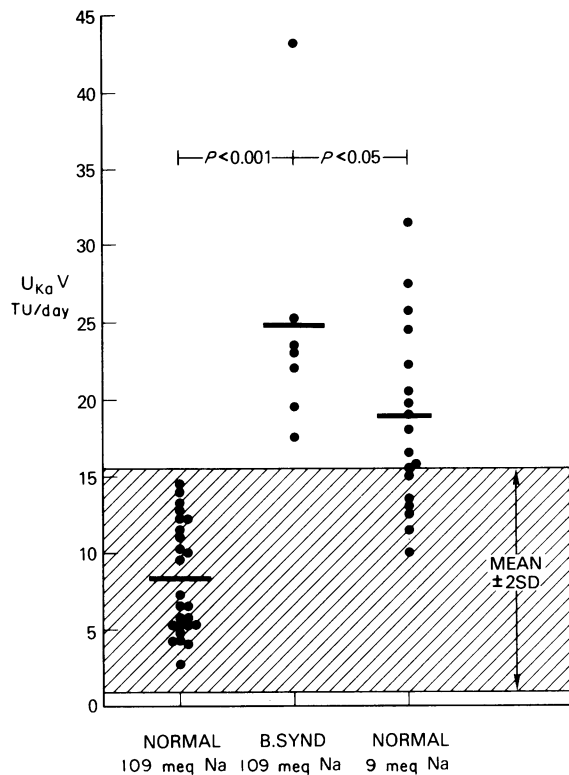


FIGURE 2A Urinary kallikrein ($U_{Ka}V$) in seven patients with Bartter's syndrome (B. Synd) in the basal state compared to that of normal subjects fed 109-meq and 9-meq/day sodium diets. The shaded area is the mean ± 2 SD for normal subjects fed 109 meq sodium/day. Horizontal bars represent the mean for each group.

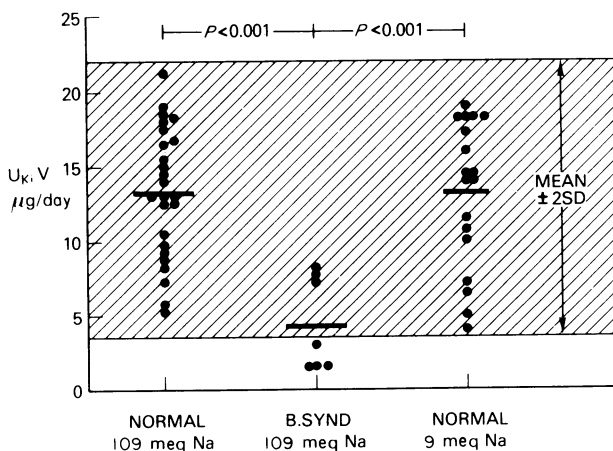


FIGURE 2B Urinary kinin excretion ($U_{Ki}V$) in seven patients with Bartter's syndrome (B. Synd) in the basal state as compared to that of normal subjects fed 109-meq and 9-meq/day sodium diets. The shaded area is the mean ± 2 SD for normal subjects fed 109 meq sodium/day. Horizontal bars represent the mean for each group.

and the effects on daily mean kallikrein and kinin excretion are shown in Fig. 1. The effects of indomethacin on the kallikrein-kinin system of six sodium-depleted normal subjects are presented in Table I.

Urinary kallikrein. Mean urinary kallikrein was significantly decreased by the 4th day of prostaglandin synthetase inhibition in all seven patients with Bartter's syndrome, (Fig. 1) to a normal value of 12.4 ± 2.0 TU/day on the 7th day of treatment from a mean control value of 24.8 ± 3.2 TU/day (Fig. 3). When drug was withdrawn, urinary kallikrein remained significantly decreased after 3 days. In those patients who were followed for longer than 4 days, urinary kallikrein had returned to $88 \pm 3\%$ of control excretion by the 7th day. The daily changes in urinary kallikrein excretion closely paralleled the changes in aldosterone excretion rate (Fig. 1). Mean urinary kallikrein in normal subjects (Table I) was significantly lower during sodium depletion plus indomethacin than during sodium depletion alone.

Urinary kinin. Mean urinary kinins were significantly increased by prostaglandin synthetase inhibi-

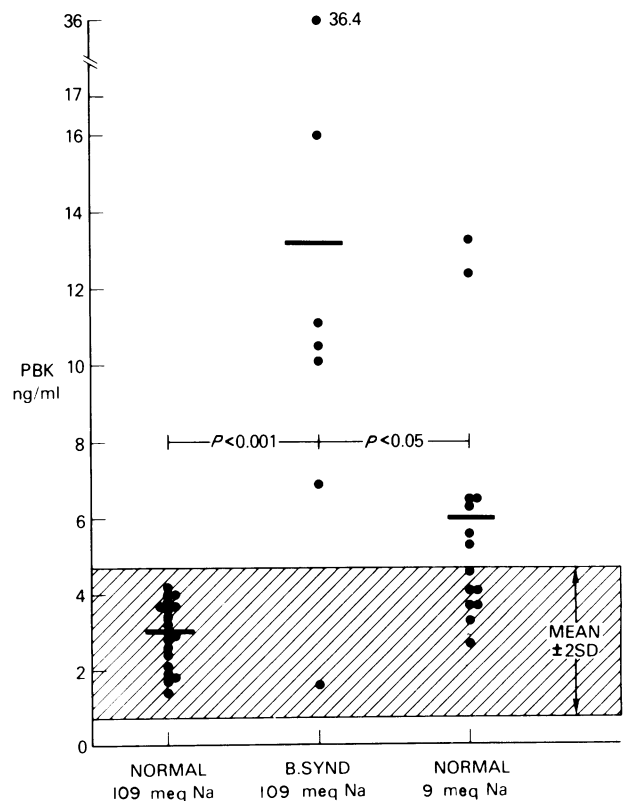


FIGURE 2C Plasma bradykinin (PBK) (patients recumbent) in seven patients with Bartter's syndrome (B. Synd) in the basal state as compared to that of normal subjects fed 109-meq and 9-meq/day sodium diets. The shaded area is the mean ± 2 SD for normal subjects fed 109 meq sodium/day. Horizontal bars represent the mean for each group.

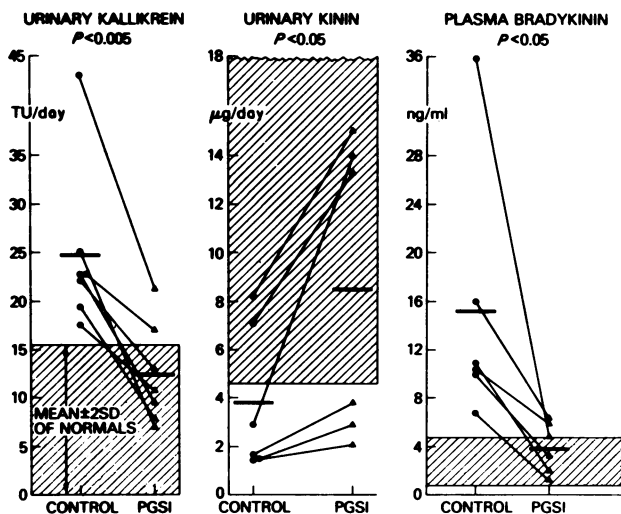


FIGURE 3 Effects of prostaglandin synthetase inhibition (PGSI) on urinary kallikrein excretion (U_{kaV}) on the 7th day of treatment in seven patients, on urinary kinin excretion (U_{kiV}) on the 7th day of treatment in six patients, and on mean plasma bradykinin (PBK) on the 4th to 7th days of treatment in six patients. The shaded area is the mean ± 2 SD for normal subjects fed 109 meq/day sodium. Horizontal bars represent the mean for each group.

tion in all six of the patients in whom they were measured, from control values of 3.8 ± 1.3 to 8.5 ± 2.5 $\mu\text{g/day}$ (Fig. 3). The increase in urinary kinin excretion (Fig. 1) was rapid and maximal ($P < 0.05$) by the 3rd day. (It was elevated by $63 \pm 27\%$ and $72 \pm 33\%$ above control on the 1st and 2nd days, respectively, but the values were not statistically significant.) In response to prostaglandin inhibitors, those patients with the lower kinin excretion showed a greater decrease in U_{iPGEV} ($66 \pm 5\%$ vs. $45 \pm 6\%$), greater sodium retention on the 1st day (62 ± 15 meq compared to 37 ± 15 meq), a greater weight gain by day 7 (2.2 ± 0.5 kg compared to 0.8 ± 0.2 kg), greater potassium retention (98 ± 46 meq vs. 73 ± 11 meq), and a greater rise in serum potassium (1.1 meq/liter vs. 0.6 meq/liter). Thus, not only did the patients who excreted the least kinins have the greater basal hormonal derangements, but they also showed a greater response to prostaglandin inhibitors. When treatment was stopped, urinary kinin excretion fell rapidly to control values by the 1st day, and remained unchanged for the remainder of the period. In the normal subjects indomethacin (Table I) did not significantly affect mean kinin excretion.

Plasma bradykinin (Fig. 3). Mean plasma bradykinin during the latter part of prostaglandin synthetase inhibition (days 4–7) was markedly reduced from a control value of 15.2 ± 4.4 ng/ml to a normal value of 3.9 ± 0.9 ng/ml ($P < 0.05$) in the six patients with Bartter's syndrome in whom it was measured. Mean

plasma bradykinin in recumbent or upright normal subjects was not significantly affected by treatment with indomethacin.

Plasma prekallikrein. Mean plasma prekallikrein was 1.4 ± 0.2 TU/ml in the latter half of treatment and thus not different from the control value of 1.4 ± 0.1 TU/ml.

DISCUSSION

In this study, PRA and bradykinin and urinary kallikrein, aldosterone, and immunoreactive prostaglandin E were elevated in patients with Bartter's syndrome. Despite increased excretion of kallikrein, urinary kinin was subnormal. Inhibition of prostaglandin synthesis restored to normal PRA, plasma bradykinin, urinary kallikrein, kinin, aldosterone, and U_{iPGEV} . The rise in kinin excretion occurred despite a decrease in kallikrein excretion. Prostaglandin inhibition also decreased creatinine clearance, produced sodium and potassium retention and weight gain, and partially corrected the hypokalemic alkalosis that characterizes the syndrome. Prostaglandin inhibition in sodium-depleted normal man decreased U_{iPGEV} , PRA, aldosterone, and urinary kallikrein but did not affect plasma or urinary kinins, sodium excretion, creatinine clearances, or serum potassium.

Bradykinin, a potent vasodilator peptide, is generated by a limited proteolytic system analogous to the renin-angiotensin system (29). The proteolytic enzyme, kallikrein, generates bradykinin from precursor proteins, kininogens; it thus resembles renin, which generates angiotensin I from angiotensinogen. Whereas the potent vasodilatory effects of kinins are partly mediated by prostaglandins (30–32) (bradykinin stimulates synthesis of prostaglandins and bradykinin-induced vasodilatation is attenuated by prostaglandin synthetase inhibition) the vasoconstrictor effects of angiotensin II are attenuated by prostaglandins (32, 33). Current concepts regarding the interplay of these vasoactive systems suggest that the interaction of bradykinin and the prostaglandins may modulate the vasoconstrictor effects of angiotensin II. That the kallikrein-kinin system exists in glands (e.g., exocrine glands and kidneys), and plasma as separate systems, is supported by the following evidence. (a) Glandular kallikrein is not normally found in plasma (29). (b) Enzyme kinetic studies of human plasma prekallikrein show that high molecular weight kininogen (mol wt = 200,000) is hydrolyzed much more rapidly than low molecular weight kininogen (mol wt = 50,000), whereas both substrates are hydrolyzed equally well by human urinary kallikrein (34). (c) Plasma kallikrein generates bradykinin directly, but urinary and glandular kallikreins generate lysyl-bradykinin, which is then converted to bradykinin by an amino-

peptidase (35). (d) Urinary kallikrein is synthesized by the kidney (13), is located on the luminal surface of distal tubular cells (36, 37), and is released into urine (13). Bradykinin infused into a renal artery is hydrolyzed by proximal tubule cells and is poorly recovered in urine (38), and this suggests that urinary kinins probably originate in the distal tubule fluid and not from filtered plasma bradykinin (38, 39). If urinary kinins do originate in the distal tubule as the above evidence suggests, this would argue against degradation by kininases or net reabsorption of kinins in distal tubular fluid (39). Unlike that in blood, kininase activity in human urine is low; only 50% of [³H]bradykinin incubated in human urine at 37°C is destroyed in 8 h (unpublished observation). Thus, it is likely that when samples are properly collected and assayed, the measurement of urinary kinins represents the net excretion of mostly unmetabolized bradykinin and lysyl-bradykinin that are generated in the distal tubule. Although kallikrein has been localized in the renal tubule, the mechanisms which make substrate available to kallikrein in the distal tubule and those factors which may alter the fate of kinins generated in the distal tubule are unknown.

The high levels of plasma bradykinin observed in Bartter's syndrome may partly account for the resistance to the pressor action of angiotensin II and the persistence of normal blood pressure in these severely hyperreninemic patients. When the patients with Bartter's syndrome were treated with prostaglandin synthetase inhibitors, their renin activity was markedly suppressed but their blood pressure did not change. This paradox may be explained by the decrease of plasma renin and plasma bradykinin *pari passu* during prostaglandin inhibition, so that pressor and depressor effects were diminished simultaneously.

The mechanism by which basal plasma bradykinin is elevated and then altered by prostaglandin inhibitors in Bartter's syndrome is not known. Although other studies have indicated a parallelism between changes of plasma bradykinin and those of plasma angiotensin II or renin activity in response to changes of posture, salt depletion, or saline infusion in normal subjects (12, 17), in the present study, treatment of normal subjects with indomethacin decreased PRA but did not affect plasma bradykinin (Table I). This difference in the response of plasma bradykinin in normal subjects and in patients with Bartter's syndrome may be attributed to the much higher basal values of plasma bradykinin and PRA in the patients with Bartter's syndrome (Fig. 1, Table I). Alternatively, this difference may be attributed to the marked antinatriuresis, antikaliuresis, and expansion of the extracellular fluid volume caused by indomethacin in patients with the syndrome but not in the

normals. Nevertheless, available data suggests that the basal levels of plasma bradykinin in patients with Bartter's syndrome were not elevated by increased kinin generation from plasma kininogen by plasma kallikrein as plasma prekallikrein was normal and in two of the patients, so was plasma kininogen. Alternatively, bradykinin may be high because of decreased destruction. Angiotensin converting enzyme, present in the lung and other tissues, inactivates bradykinin (40), and this action is inhibited *in vitro* by several peptides, including angiotensin I, angiotensin II, and the dipeptide product of angiotensin I hydrolysis, histidyl-leucine (41). Although it is tempting to postulate that total bradykinin hydrolysis may be significantly inhibited *in vivo* by the high levels of angiotensin I and II found in this syndrome, there is no evidence that this occurs. In fact, this hypothesis ignores the contribution made by other kininases (42) which are more important in the destruction of plasma bradykinin (43). Indeed, in 10 hypertensive patients with unequivocal biochemical evidence of converting enzyme inhibition with SQ20881, plasma bradykinin was not changed (44). Thus, it appears unlikely that the high levels of bradykinin result from inhibition of converting enzyme by elevated endogenous levels of angiotensin I. Whatever the mechanism for the increased bradykinin it is likely that it modulates the pressor activity of angiotensin II in this syndrome.

Urinary kallikrein excretion is increased in conditions in which sodium-retaining steroids are present in excess (14–17). Consistent with this observation are the report by Lechi et al. (45) and our present findings that patients with Bartter's syndrome excrete more kallikrein than normal subjects. Indeed, patients with Bartter's syndrome excreted three times as much kallikrein as sodium-replete normal subjects, but only one-third more than sodium-depleted normal subjects. When renal prostaglandin synthesis was inhibited in patients with this syndrome, urinary kallikrein decreased slowly and simultaneously with the fall in aldosterone excretion (Fig. 1). When prostaglandin synthetase inhibitor was stopped, urinary kallikrein was slower to return to control than aldosterone. In those subjects followed for longer periods, kallikrein excretion returned to control values. (It has been our experience that kallikrein often lags behind changes in aldosterone by as much as 2–3 days.) In normal subjects, both kallikrein and aldosterone excretion were lower during indomethacin. These data further support the observation, as yet unexplained, that production of urinary kallikrein depends, in part, upon the action of aldosterone.

To our knowledge, an abnormality in urinary kinins has not been reported. In patients with Bartter's syndrome, urinary kinins are not only abnormal, but also

appear to reflect the severity of hormonal derangements and the response to prostaglandin inhibitors. The finding in this syndrome of values for urinary kinin one-third that of normal despite a very high urinary kallikrein is unexpected. This inverse relationship between urinary kallikrein and kinins was persistent: during prostaglandin synthetase inhibition kinin excretion rose abruptly as urinary kallikrein fell, and when treatment was stopped kinin excretion fell rapidly as urinary kallikrein slowly returned to control values. This inverse relationship between kallikrein and kinin suggests that other factors such as prostaglandins or prostaglandin-dependent renal changes control kinin excretion in this syndrome.

Patients with persistent psychogenic vomiting, hypokalemic alkalosis, hyperreninemia and vascular hyporesponsivity to angiotensin II show excessive production of prostaglandin E₂ (PGE₂) (46) and low urinary kinins (unpublished observations). It is possible that hypokalemia per se and its associated renovascular and hormonal changes (47), which are partly prostaglandin-dependent (47), accounted for the subnormal kinin excretion in this syndrome. It is unlikely, however that prostaglandins per se mediated the observed changes in kinin excretion in Bartter's syndrome as prostaglandin inhibition in normal subjects had no effect on kinin excretion, but they may have done so via changes in delivery of kininogen to the distal tubule where kallikrein is located, or in destruction by kininases or in the reabsorption of kinins.

Prostaglandin synthetase inhibition in these seven patients with Bartter's syndrome resulted in mean hormonal and metabolic changes that were similar to those in the four patients reported previously (5). The abrupt decrease in iPGEV on the 1st day of treatment was associated with a decrease in creatinine clearance, retention of sodium, and gain in body weight. None of these changes were observed in normal subjects. As blood pressure did not change, renal vasoconstriction, which has been suggested to explain the decrease in renal blood flow observed to occur in response to inhibition of prostaglandin synthetase (48), probably explains the decrease in creatinine clearance in patients with this syndrome. Such changes in renal hemodynamics, may have increased the proximal tubular reabsorption of sodium (49, 50). The recent demonstration of PGE₂ inhibits the transport of sodium in the collecting tubule of the rabbit (51) suggests that, if such an action exists in patients with Bartter's syndrome an increase in distal tubule reabsorption of sodium may also have contributed to the decrease in sodium excretion associated with prostaglandin inhibition. Whereas the data do not allow an assessment of the roles of proximal and distal tubular reabsorption of sodium in the retention

of sodium, the observation that the excretion of potassium closely paralleled that of sodium (Fig. 1) could be explained as a result of changes in flow of tubular fluid past the potassium secretory site (52) produced by changes in glomerular filtration, proximal tubular reabsorption, or both.

The period of escape from the significant antinatriuretic and antikaliuretic effects of prostaglandin inhibition occurred after only 2 days of treatment. One determinant of this phenomenon may have been marked expansion of extracellular fluid which may have led to a decrease in proximal tubular reabsorption of sodium (53). Another factor may have been the coincident increase in urinary kinins which occurred on the 3rd day of treatment. If kinins contributed to the escape from the antinatriuretic effects of prostaglandin inhibition, it is possible that they did so by inhibition of sodium reabsorption. Somewhat against the notion that this might occur in the distal tubule is the observation that distal fractional sodium chloride reabsorption during water diuresis in patients with Bartter's syndrome was not changed by treatment with indomethacin (46).

Finally, the information presented can be used to address the possible pathophysiologic mechanisms of Bartter's syndrome. Although many of the hormonal abnormalities in Bartter's syndrome are directionally similar to those observed in sodium-depleted normals, it is unlikely that volume depletion is a major factor in the pathogenesis of this disorder because marked expansion of the extracellular fluid volume with saline or albumin does not correct the hormonal or metabolic derangements. Also, patients with Bartter's syndrome can conserve sodium normally in response to a low sodium intake but do continue to lose potassium (3). Whereas treatment of this disorder with prostaglandin inhibitors dramatically improved the hormonal and metabolic abnormalities it failed to fully correct the hypokalemia. Indeed, long-term treatment with these inhibitors does not completely correct the renal loss of potassium or hypokalemia, even when it restores plasma renin, aldosterone, and U_iPGEV to normal (54). These findings suggest that hypokalemia may cause rather than be caused by hyperreninemia and aldosteronism. In keeping with this formulation is the observation that bilateral adrenalectomy does not correct the hypokalemia in patients with Bartter's syndrome (55, 56). Furthermore, hypokalemia in dogs caused increased excretion of prostaglandin E, elevated PRA, and hyporesponsivity to angiotensin II infusions; indomethacin restored these abnormalities toward normal (47). In addition, as mentioned previously, patients with persistent psychogenic vomiting, who were hypokalemic also showed increased excretion of PGE₂, hyper-

reninemia, aldosteronism, low urinary kinins, and hyposensitivity to angiotensin II. Thus, the increased synthesis of prostaglandins in Bartter's syndrome is probably not the primary abnormality but secondary to hypokalemia.

Whereas hypokalemia is probably the most proximal abnormality in the pathogenesis of Bartter's syndrome, it is unlikely that it results from renal loss per se for two reasons: (a) primary potassium deficiency tends to cause sodium retention (57), which does not occur in Bartter's syndrome, and (b) it is difficult to conceive of a mechanism which would allow the kidney to excrete inappropriately large quantities of potassium without an increase in distal tubular flow (52) as may occur with an inappropriately low fractional reabsorption of sodium chloride in the proximal tubule, in the loop of Henle, or in both. Thus, the findings in Bartter's syndrome are most consistent with a defect in the tubular reabsorption of sodium chloride as the most proximal cause of the syndrome. Indeed, recent evidence, based on free water clearance studies (46), suggests the presence of a defect in chloride reabsorption in the ascending loop of Henle. This defect may promote potassium loss by enhancing distal tubular flow which increases potassium exchange and loss (46, 52, 58).

Addendum. While this manuscript was being reviewed the following articles appeared on this subject (59, 60).

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