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A Case of Secondary Aldosteronism Similar to Bartter's Syndrome with No Abnormality in Renal Chloride Reabsorption

KAZUHISA TAKEUCHI, YUTAKA IMAI, KEN OMATA, HIROSHI SATO, TAKAO SAITO, KOZO OTA, TOKIHISA KIMURA, KAORU YOSHINAGA and KEISHI ABE

The Second Department of Internal Medicine, Tohoku Universiry School of Medicine, Sendai 980

TAKEUCHI, K., IMAI, Y., OMATA, K., SATO, H., SAITO, T., OTA, K., KIMURA, T., YOSHINAGA, K. and ABE, K. *A Case of Secondary Aldosteronism Similar to Bartter's Syndrome with No Abnormality in Renal Chloride Reabsorption.* Tohoku J. Exp. Med., 1993, **169** (2), 141-157 —— We had a 20-year-old male patient of secondary aldosteronism similar to Bartter's syndrome, which had proved to be evident after the remission of nephrotic syndrome. In the patient, hypokalemic alkalosis and hyperreninemic hyperaldosteronemia were observed, although the blood pressure was normal. Hyperplasia of juxtaglomerular cells was observed and no abnormalities indicating either glomerulonephritis or renal artery stenosis were found; the pressor response to intravenously infused angiotensin (ang) II was markedly decreased; urinary prostaglandin (PG) E2, kallikrein and kinin excretion were elevated. The inhibition of PG synthesis with indomethacin decreased renal PG production and partially corrected both hypokalemia and pressor responsiveness to ang II. Thus, this case is considered to be a case of Bartter's syndrome. Contrary to the previously reported observations, the effective fractional chloride reabsorption rate in the renal distal tubules was normal (>80%) and not changed by PG inhibition. Plasma atrial natriuretic peptide level was normal. An interaction between renin-angiotensin and PG systems appears to play a prior role in this case. To explain the pathophysiology, we have hypothesized an abnormal function of ang II receptor signal transduction which excessively stimulates PLA2, resulting in overproduction of PG synthesis in tissues. ———— prostaglandin; nephrotic syndrome; angiotensin II receptor; kallikrein-kinin system; atrial natriuretic peptide

Secondary aldosteronism refers to an appropriately increased production of adrenal aldosterone in response to activation of the renin-angiotensin (R-A) system, while primary aldosteronism is due to an adrenal adenoma autonomously secreting mineralocorticoids. Secondary aldosteronism, therefore, occurs in such a pathophysiologic condition as a decrease in effective plasma volume (i.e.,

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Address reprints: K. Takeuchi, M.D., the Second Department of Internal Medicine, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan.

congestive heart failure, massive hemorrhage, repetitive vomiting, low proteinemia, salt-depletion, etc.) or a decrease in renal perfusion pressure (i.e., renal artery stenosis), which stimulates R-A system. Secondary aldosteronism is also caused by administration of excessive amount of steroid possessing mineralocoiticoid activity or by an increase in renin substrate which is observed in pregnancy or in administration of exogenous estrogen. A rare renin-producing tumor is another cause of this condition. Hypokalemia are usually observed in secondary aldosteronism due to the mineralocorticoids involved. Hypertension is also observed due to the increased synthesis of pressor hormone, angiotensin (ang) II or mineralocorticoids involved. Edema is often accompanied depending on the primary diseases (i.e., liver cirrhosis, nephrotic syndrome, congestive heart failure, etc.).

Bartter's syndrome, a form of secondary aldosteronism with hypokalemia, however, lacks either hypertension or edema. Bartter et al. (1962) first described this syndrome which was characterized as hyperreninemic hyperaldosteronemia with hypokalemia lacking hypertension. Several etiological hypotheses on this disorder have been described such as the hyperplasia of renal juxtaglomerular cells (Brackett et al. 1968); impairment of pressor responsiveness to ang II (Bartter et al. 1962; Fujita et al. 1982); impairment of renal distal chloride reabsorption (Gill and Bartter 1978) or renal proximal sodium reabsorption (Goodman et al. 1969); excessive production of vasodepressor prostaglandin (PGs), kallikrein and kinin (Gill et al. 1976; Vinci et al. 1978); generalized defect in membrane sodium transport (Gardner et al. 1970); hyperplasia of renal interstitial cells (which produce PG) (Verberckmoes et al. 1976); impairment of renal magnesium reabsorption (Cushner et al. 1990). And, recently, involvement of atrial natriuretic peptide (ANP) has been suggested (Yamada et al. 1986). The etiology, however, is still controversial.

For the diagnosis of Bartter's syndrome, secondary aldosteronism mimicking Bartter's syndrome due to repetitive vomiting or diarrhea, chronic anorexia or a prolonged excessive intake of diuretics should be ruled out. The finding of blunted vasopressor responsiveness to ang II is necessary and the diagnostic treatment with PG inhibitors is helpful.

We here report a case with secondary aldosteronism symptomatically identical to Bartter's syndrome. The patient had been suffered from the nephrotic syndrome, which had resulted in complete remission by chemotherapy before his visit in our hospital. Full examinations were performed to characterize this case in reference to the previously reported cases of Bartter's syndrome.

Case Report

A 20-year-old man (H.K.) was admitted to our hospital in March 1986 because of persistent hypokalemia.

October 1983, when the patient was a high school student, mild proteinuria was

reported on the annual school health examination. In a year, pretibial edema gradually proved to be evident. In October 1984, he visited a hospital. Laboratory examinations indicated a ⋕ test for urine protein, hypoproteinemia (total protein, 4.0 g/100 ml) and hypercholesterolemia (total cholesterol, 320 mg/100 ml). Nephrotic syndrome was, therefore, suspected and the patient was admitted to a hospital for the renal biopsy and treatment. On admission, marked proteinuria (urinary protein excretion, >10 g/day), hypoproteinemia and hypercholesterolemia were confirmed. The renal function and blood pressure were within normal limits and no hypokalemia (the potassium, 3.6 mmol) was observed. Microscopic examination of the renal biopsy pecimen showed no glomerulonephritis lesion. Other laboratory examinations showed no particular findings. Nephrotic syndrome (minimal change type) was finally diagnosed and the treatment was started. As shown in Fig. 1, the patient was treated with a combination of steroid (prednisolone) and

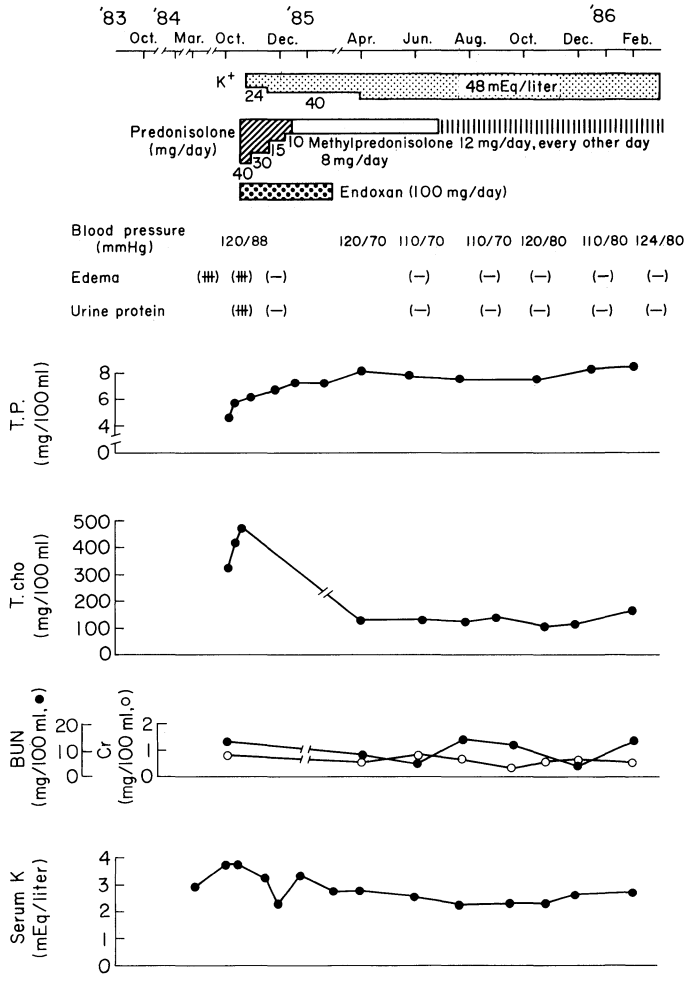

Fig. 1. Occurrence of secondary aldosteronism in the treatment of nephrotic syndrome. T.P., total protein; T. cho, total cholesterol; BUN, blood urea nitrogen; Cr, creatinine.

cyclophosphamide. Potassium chloride was also supplemented because of a gradual decrease in plasma potassium level. In response to the treatment, clinical symptoms were progressively improved and the nephrotic syndrome resulted in the complete remission (Fig. 1). Hypokalemia, however, gradually aggravated in spite of potassium supplementation. In September 1985, eight months after the admission, the patient was discharged and follow-up therapy was started with methylprednisolone (12 mg per alternate days) against nephrotic syndrome and potassium (48 mmol per day) against hypokalemia. Hypokalemia still persisted despite of the potassium administration. During this course, high blood pressure was not observed and there was no self-administration of either diuretics or mineralocorticoids (and the mimetic, licorice) and no episode of either diarrhea, vomiting or chronic anorexia.

Requiring further intensive examinations of hypokalemia, the patient was referred to Tohoku University Hospital, February 1986. In the outpatient clinic, the treatment with methylprednisolone was discontinued. One month later, hypokalemia (the potassium, 2.5 mmol) was still observed even by the administration of potassium (48 mmol per day). Neither proteinuria nor high blood pressure was observed. The patient was then admitted and further examinations were performed. The patient was a well developed man: the height and weight were 176 cm and 67.5 kg, respectively. His father died of an unknown disease accompanied with edema at forty four years of age. He had no brother. His mother had been healthy. The patient sometimes felt mild paralysis in extremities. The blood pressure was 120/60 mmHg, and the pulse 68 and regular. Neither edema nor outer abnormalities in the body and skin were observed. The urine was normal. Urinary excretions of β_2-microglobulin and N-acetyl-glucosamine were normal. The hematocrit was 44.3 percent; the white cell count 12,700; the platelet count 357,000 and the erythrocyte sedimentation rate 4 mm per hour. Urea nitrogen was 14 mg/100 ml, creatinine 0.6 mg/100 ml, total protein 7.7 g/100 ml, total cholesterol 153 mg/100 ml. The sodium was 141 mmol, the potassium 2.5 mmol, the chloride 100 mmol, the calcium 8.6 mg/100 ml, the phosphorus 5.0 mg/100 ml, and the magnesium 2.0 mg/100 ml. The blood gas analysis showed that the pH of blood was 7.5, the arterial carbon dioxide partial pressure (PCO_2) 42.5 mmHg, the oxygen (PO_2) 98.0 mmHg, the carbonate (HCO_3^-) 32.1 mmol, and the base excess +9.1 mmol. On endocrine examinations, PRA was 173 ng ang I/ml/6 hr (normal; 5-30) and PAC 24.3 ng/100 ml (normal: 2-12). Daily urinary excretions of 17-hydroxycortico-sterones and 17-ketosteroids were 5.9 mg per day and 7.9 mg per day (the mean of three measurements), respectively. Daily urinary calcium excretion was 0.203± 0.011 g/day (the mean of ten measurements ± S.E. in the base line period). Levels of growth hormone, vasopressin, thyroxine, thyronine, parathyroid hormone, cortisol and androgen were all within normal limits. The x-ray films of the chest and abdomen were normal. The computed tomography scan and echosonography showed no abnormal findings in the abdomen. Either the intravenous pyelogram, renogram and scans (using ^{123}I-hippuric acid) or renal scintigram (using ^{99}Tc-DPTA) showed normal findings. The creatinine clearance (using 24-hr urine creatinine excretion) was 86 ml/min. In summary, the patient had hypokalemia, metabolic alkalosis, hyperreninemia, hyperaldosteronemia, mild hyperreninemia, mild leukocythemia and moderate thrombocythemia. The complete remission of nephrotic syndrome was confirmed. Normal blood pressure was observed in spite of marked elevation of either renin or aldosterone. Bartter's syndrome was, therefore, suspected and the following examinations were performed.

Methods

During the admission, sodium chloride intake was restricted to be 10 g per day. The 24-hr urine was collected and the body weight was measured before the breakfast.

Analytical techniques. PRA was determined by the method established in our laboratory (Abe et al. 1975). PAC was measured by radioimmunoassay (RIA) using a commer-

cially available kit (Dinabot, Tokyo). Urinary prostaglandin was measured by the previously reported method (Takeuchi et al. 1991a), based on the method established in our laboratory (Abe et al. 1978; Sato et al. 1983). Briefly, 1 ml of urine sample was acidified with hydroxychloride to pH 3.0 and PG was extracted with ethylacetate. The extract was then lyophilized at 37°C. The sample was dissolved in acetone (1 ml) and lyophilized, again. The lyophilized sample was dissolved in 0.2 ml of solvent I (toluene : ethyl acetate : methanol = 60 : 40 : 10) and then filled up to 1.0 ml with solvent II (toluene : ethyl acetate = 60 : 40). To purify PGE2, the sample was then applied to silicic acid column chromatography. After washing the column with solvent II, the sample (1 ml) was applied. In order to extract PGA and PGB, 5 ml of solvent II was applied to the column and then the extracted solution was discarded. To obtain PGE and PGF, 5 ml of solvent I (toluene : ethyl acetate : methanol = 60 : 40 : 20) was next applied and the extract was saved, lyophilized and stored at −20°C until future PGE_2 measurement by RIA. Recovery rate of PGE_2 was estimated to be 60%. In RIA, the sample was dissolved in phosphate buffered solution. RIA was performed using a highly specific antibody against PGE_2 obtained from Pasteur Institute, Paris, France and tritiated PGE_2 from New England Nuclear, Boston, MA, USA. The lower limit of PG determination was 3 pg/ml. Urinary kallikrein was measured based on the previous method (Abe et al. 1978) by measuring kinin forming activity using the crude kininogen (substrate of kallikrein) obtained from bovine serum. Total kallikrein activity in the serum after trypsinization was also determined. Kinin and ANP was measured by RIA as previously reported (Abe et al. 1978 and Kimura et al. 1986, respectively). Measurements of PRA, PAC, PGE_2, kallikrein, kinin and ANP were determined in triplicate.

Renal biopsy. Renal biopsy was performed by the percutaneous puncture guided by echosonography. An enzyme histochemistry method was adopted to stain renin with human renin antibody (provided by K. Murakami, Tsukuba).

Ang II- infusion test. In order to examine the vascular responsiveness to ang II, ang II-infusion test was performed based on the method of Kaplan and Silah (1964) using synthetic ang II (Hypertensin®, Ciba). Blood pressure (BP) was monitored with an automated BP measurement apparatus (Nihon Kolin, Hamamatsu) every 30 sec. To confirm the normal control value, another set of ang II-infusion test was simultaneously carried out in a healthy volunteer (30-year-old man, K.T.). The test was performed either before or at the last day of the indomethacin treatment. PRA and PAC were also determined just before and after the infusion period.

Captopril test. The test was performed to examine the negative-feedback mechanisms in R-A system. After one-hour rest at supine position, captopril (50 mg) was administered. The blood and urine were collected every hour. PRA, PAC, urinary PGE_2 concentration, urinary active and total kallikrein concentrations and urinary kinin concentration were determined.

PG inhibition. Because of a diagnostic treatment, a PG inhibitor, indomethacin (150 mg per day) was administered. Urine volume and urinary excretion of either sodium, potassium, PGE_2, kallikrein or kinin were determined before, during and after the treatment period. For a long-term treatment, another PG inhibitor, sulindac, was substituted, because sulindac is known to be safe for scarity of adverse effect on gastrointestinal tracts.

Water loading. To examine renal electrolyte metabolism in the renal distal tubules, water loading test was performed by the previously reported method (Ota et al. 1984) in the patient and a healthy volunteer. Twenty ml per kg (body weight) of water was orally administered. The urine sample was consecutively collected every 30 min. Effective fractional reabsorption of chloride in the distal tubules was calculated based on the data obtained when the fractional water excretion reached the maximum, according to the following formula:

$$\text{Fractional reabsorption of chloride } (\%) = \frac{C_{H_2O}}{C_{H_2O} + C_{Cl}} \times 100,$$

where C_{Cl} and C_{H_2O} are abbreviations of chloride clearance and water clearance, respectively.

C_{Cl} was calculated on the formula: $C_{Cl}=\frac{U_{Cl}V}{P_{Cl}}$

where V is an abbreviation of urine volume; P_{Cl}, plasma chloride concentration; U_{Cl}, urine chloride concentration.

C_{H_2O} was calculated on the formula: $C_{H_2O}=V-\frac{UosmV}{Posm}$

where Uosm and Posm are abbreviations of urine and plasma osmolarity, respectively. The test was performed before and during the indomethacin treatment period. In this study, plasma ANP level, PRA and PAC were also determined.

Saline and low-molecular-weight dextran infusion. Saline infusion and dextran infusion tests were also performed. One liter of saline or low-molecular-weight dextran solution was intravenously infused for one hour. The blood was collected every 30 min and plasma ANP, PRA and PAC were measured. Both infusion tests were carried out either before or during the indomethacin treatment.

Resurts

Renal biopsy. The microscopic picture of renal specimens were stained with hematoxylin and eosin (HE) or by an enzyme immunohistochemistry method with human renin antibody, as shown in Fig. 2A or B, respectively. No findings of either glomerulonephritis or interstitial abnormality (including interstitial hyperplasia) but the moderate hyperplasia of juxtaglomerular (JG) cells were observed (Fig. 2A). Fig. 2B has shown enhanced renin staining in JG cells.

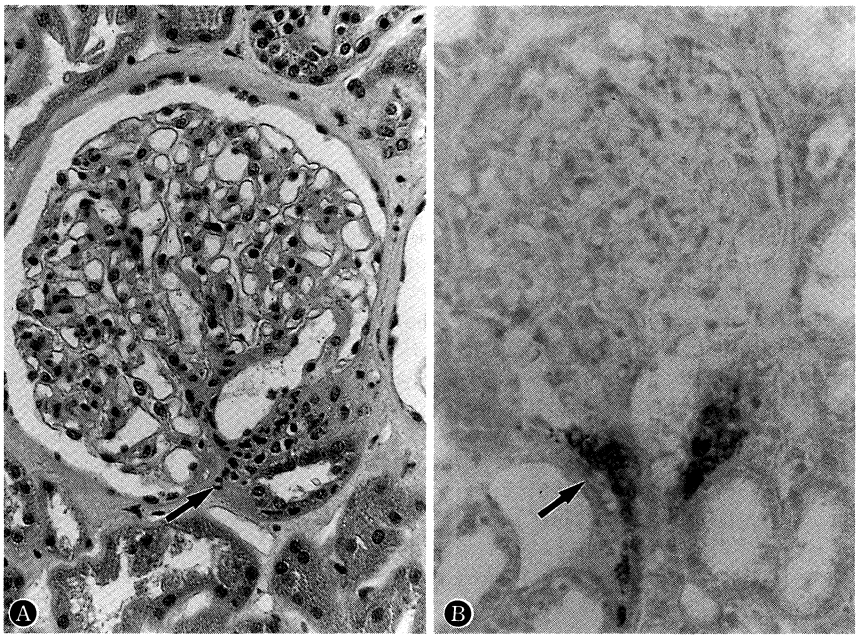

Fig. 2. Microscopic examination of renal tissue. (A): hematoxylin-eosin (HE) staining, (B): renin staining.

PG inhibition and time-course changes in clinical findings. Fig. 3 shows time-course changes in body weight, plasma potassium level, daily urine volume, daily urinary excretions of potassium and sodium, a ratio between urinary sodium and potassium concentration (Na/K), PRA and PAC. In the control period, the potassium was 2.2 mmol and no edema was observed. PRA and PAC showed

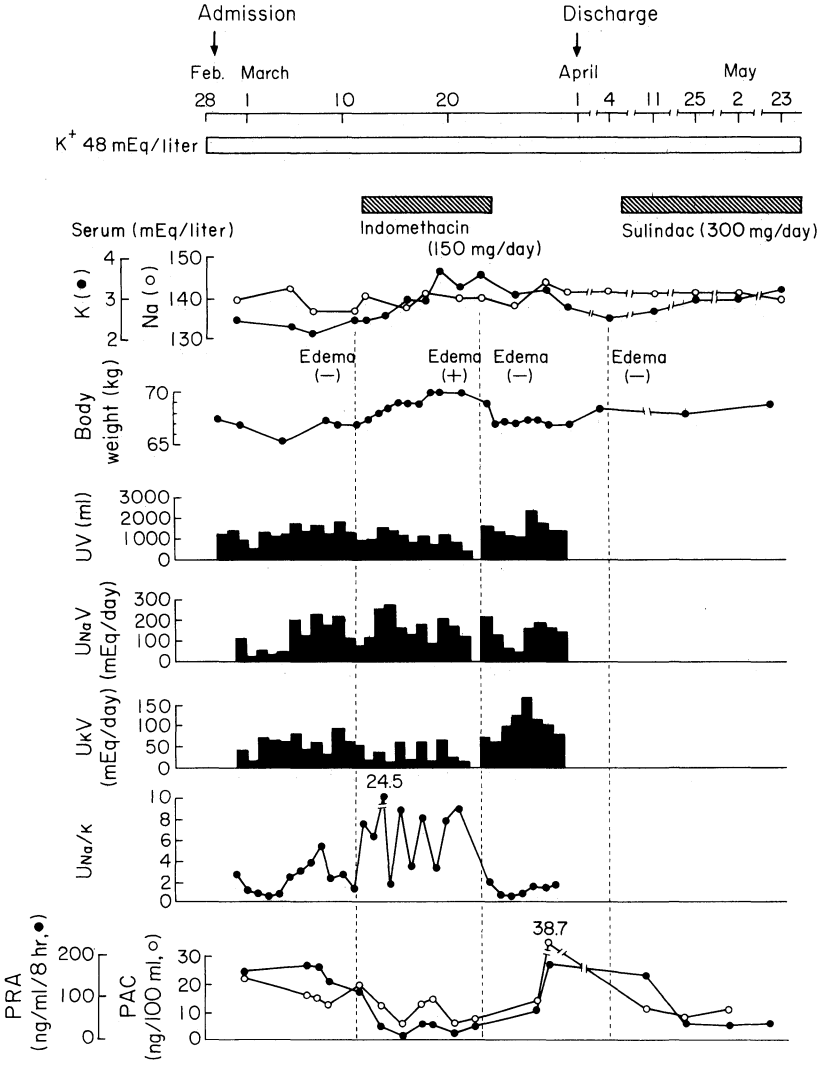

Fig. 3. The effect of prostaglandin inhibition on the potassium, sodium, body weight, total urine volume (UV), urinary excretion of sodium (UNaV) and potassium (UkV), the ration between urinary sodium and potassium concentrations (UNa/K), plasma renin activity (PRA) or plasma aldosterone concentration (PAC). Prostaglandin production was inhibited by indomethacin (150 mg/day), as shown in Fig. 3, and sulindac (300 mg/day). Normal values of PRA and PAC are 5-30 ng ang I/ml/6 hr and 2-12 ng/100 ml, respectively.

abnormally high levels. In the indomethacin treatment, the potassium was increased up to 3.4 mmol with an increase in body weight and edema. Urine volume and urinary excretion of potassium decreased, while there was no change in urinary excretion of sodium, resulting in high Na/K values. PRA and PAC were decreased, but the values still remained above normal limits. After discontinuation of indomethacin, the plasma potassium level returned to the baseline level; edema disappeared; urine volume and urinary excretion of potassium increased; Na/K, PRA and PAC returned to the basel levels.

Fig. 4 shows effects of indomethacin on urinary PGE_2, kallikrein and kinin excretion. Urinary PGE_2 excretion was significantly decreased. Urinary total kallikrein, active kallikrein and kinin excretions were significantly decreased by indomethacin. These changes were reversed by discontinuation of indomethacin.

The effect of another PG inhibitor, sulindac, was also shown in Fig. 3.

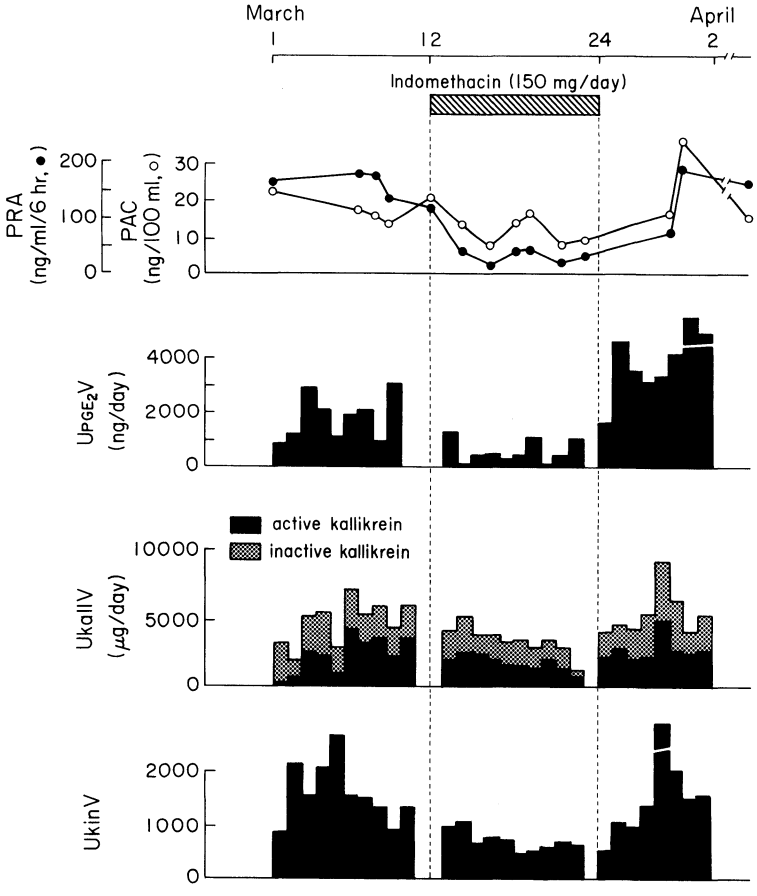

Fig. 4. The effect of indomethacin on urinary excretion of prostaglandin E_2 ($UPGE_2V$), kallikrein (UkallV) or kinin (UkinV). PRA, plasma renin activity; PAC, plasma aldosterone concentration.

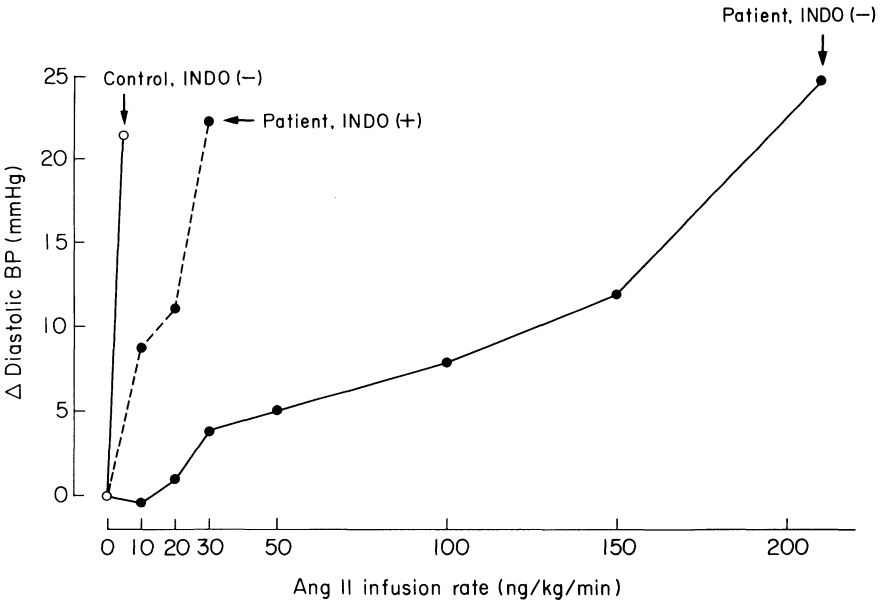

Fig. 5. Angiotensin (ang) II infusion test. Ang II infusion was performed in a normal healthy male (control); the patient when treated with indomethacin, 150 mg/day, (INDO (+)) and the patient when untreated with INDO (INDO (−)). Normal value to increase diastolic blood pressure by 15 mmHg is less than 10 ng/kg/min.

Sulindac decreased PRA and PAC. The potassium increased up to 3.2 mmol.

Ang II-infusion test. The ang II infusion rate necessary to elevate diastolic BP by 15 mmHg was more than 150 ng/kg/min in the patient, whereas, in the control subject, 10 ng/kg/min (Fig. 5). By the indomethacin treatment, the infusion rate was decreased to 25 ng/kg/min, which was still abnormally high. PRA and PAC were increased by ang II infusion in the control subject (PRA 7.2 to 5.3 ng/ml/6 hr (levels from before to after ang II infusion); PAC 7.8 to 9.0 ng/100 ml); in the patient treated with indomethacin (PRA 21.5 to 15.6; PAC 7.7 to 15.6) and in the patient without indomethacin (PRA 186 to 173; PAC 16.6 to 21.3).

Captopril test. Basal PRA and PAC were abnormally high. Captopril induced a six-fold increase in PRA in an hour and the increase lasted for another hour (Fig. 6A). PAC was reduced by 30% (Fig. 6B). Fractional urinary concentration of PGE_2 was increased by three-fold in one hour (Fig. 6C). Fractional urinary kallikrein concentration was increased by two-fold (Fig. 6D). Fractional urinary kinin concentration was increased by 2.5-fold one hour after the administration of captopril (Fig. 6E). In the indomethacin treatment, captopril-induced a similar change in PRA, PAC, fractional urinary PGE_2 or urinary kallikrein concentration, respectively, but the magnitude of change was smaller

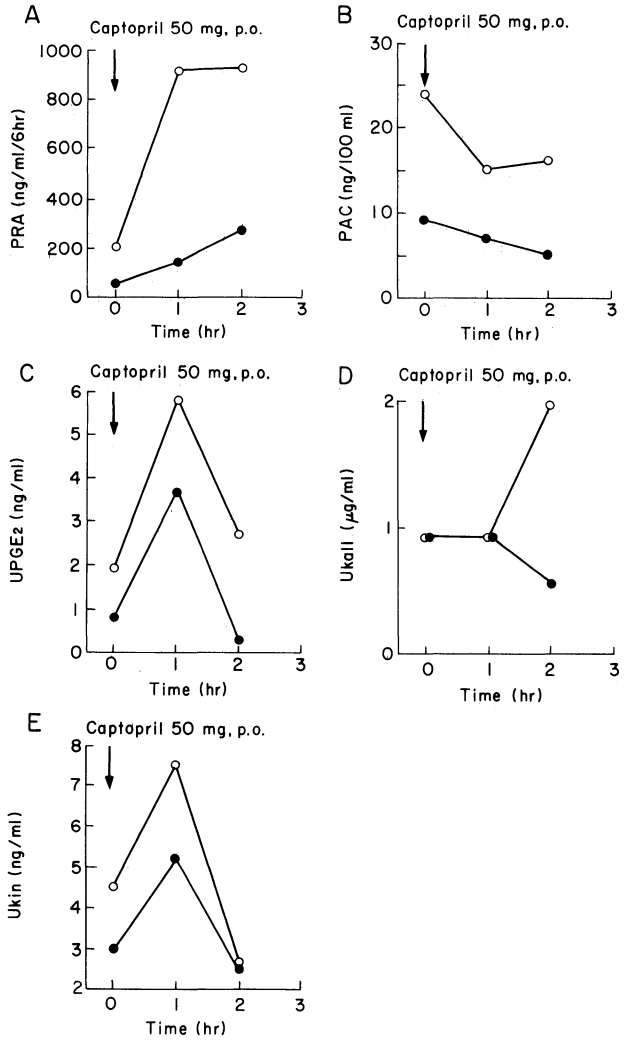

Fig. 6. The effects of angiotensin converting enzyme inhibition on (A): plasma renin activity (PRA), (B): plasma aldosterone concentration (PAC), (C): urinary prostaglandin excretion ($UPGE_2$), (D): urinary kallikrein excretion (Ukall) or (E): urinary kinin excretion (Ukin). The tests were performed with captopril 50 mg p.o. in the patient when treated with indomethacin, 150 mg, (INDO (+) ●) and when untreated with with INDO (INDO (−) ○).

than that in the absence of indomethacin.

Fractional reabsorption of chloride in the distal tubules. Table 1 summarizes the results. The values were determined when C_{H_2O} reached the maximum level: $C_{H_2O}=9.66$ ml/min, in the control period and 9.56 ml/min in the indomethacin treatment. The values were normal and there was no difference between in the control period and in the indomethacin treatment.

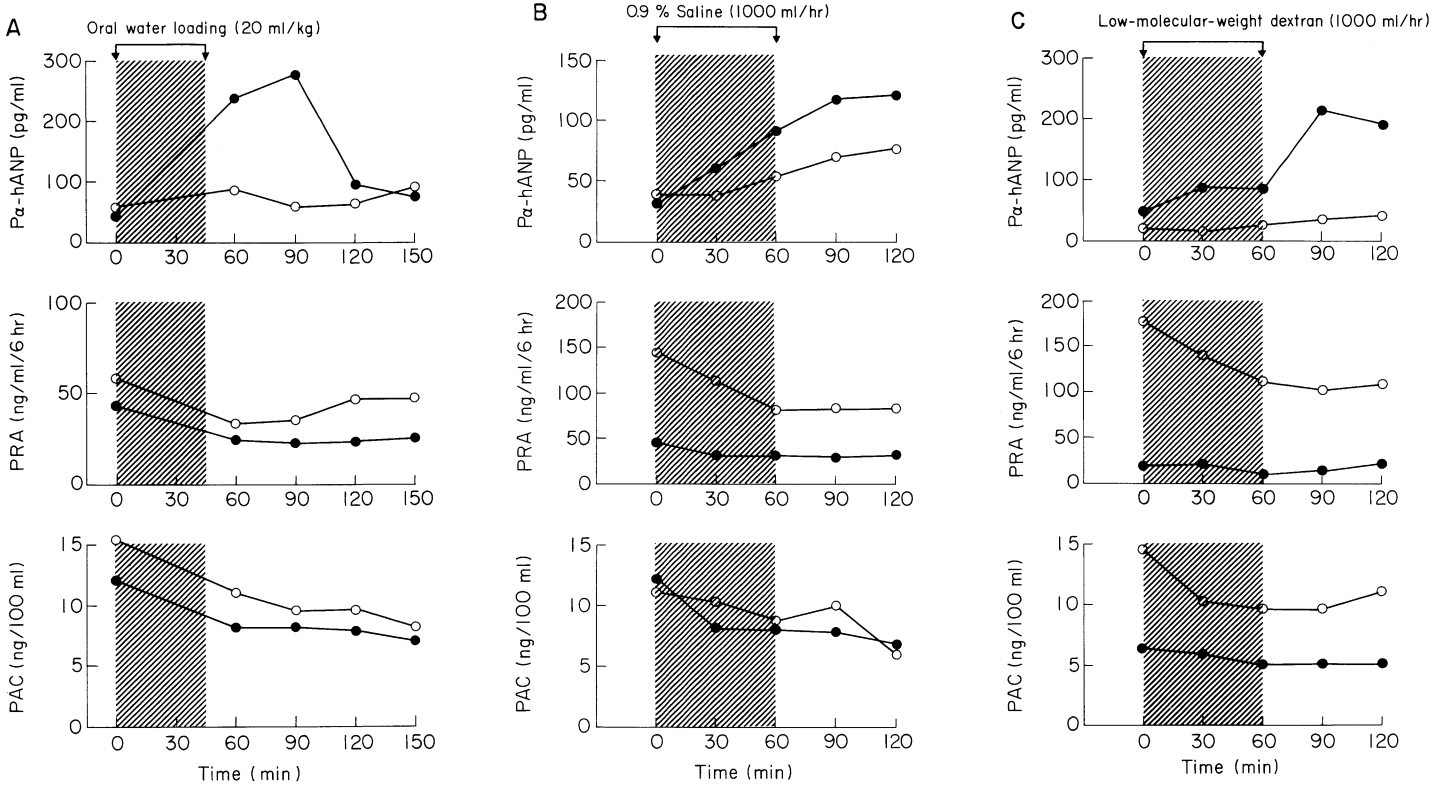

Fig. 7. Effect of volume loading on plasma level of atrial natriuretic peptide (ANP). Volume loading was performed by (A): oral intake of water, (B): intravenous infusion of 0.9% saline or (C): low-molecular-weight dextran solution. Each examination was performed in the patient when treated with indomethacin, 150 mg/day, (INDO (+) ●) and the patient when untreated with INDO (INDO (−) ○). The infusions were performed during the shadowed periods.

TABLE 1. *Effective fractional reabsorption of chloride at the distal tubules*

Normal	Patient	
	Control	+Indomethacin
85%	83%	81%

Effective fractional reabsorption of chloride ($\%EF_{Cl}$) was measured when fractional free water clearence reached the maximum level. Indomethacin, 150 mg/day, was administered. Normal $\%EF_{Cl}$ was obtained in a healthy volunteer.

Changes in ANP level, PRA and PAC by water, saline and dextran solution loadings. Plasma ANP level was measured after loadings of water, saline and low-molecular-weight dextran solution (Fig. 7A, B and C, top panels, respectively). The ANP was not changed by loading of either water or dextran solution, whereas ANP was significantly increased by saline loading. In the indomethacin treatment, however, the ANP was markedly increased by loadings of water, dextran solution and saline solution. The basal PRA and PAC were decreased by PG inhibition. PAC and PRA were inhibited by either water, saline or dextran loading in the control period as well as in the PG inhibition period.

DISCUSSION

The secondary aldosteronism and Bartter's syndrome. The patient had suffered from nephrotic syndrome, which sometimes causes secondary aldosteronism. The nephrotic syndrome, however, had resulted in the complete remission, when secondary aldosteronism proved to be evident. Therefore, nephrotic syndrome was ruled out as a cause of the secondary aldosteronism. Similar to previouly reported cases of Bartter's syndrome, we observed hypokalemic alkalosis; hyperreninemic hyperaldosteronemia without hypertension; JG cell hyperplasia, blunted vascular responsiveness to ang II; improvement of symptoms by PG inhibition. These observations are sufficient for the diagnosis of Bartter's syndrome (Bartter et al. 1962). In an etiological point of view, there has been a prevailing hypothesis that Bartter's syndrome is due to a reduced reabsorption of sodium and chloride in renal distal tubules (Gill and Bartter 1978). In the present case, however, we did not observe the abnormality when renal cholide reabsorption was examined by oral water loading. Norby et al. (1976), Kurtz et al. (1984) and Seyberth et al. (1985) have also reported similar cases of Bartter's syndrome lacking an abnormality in the distal tubular function. As discussed in literatures (Kurtz et al. 1984; Stein 1985), the variables of renal distal tubular function are influenced easily by the procedure adopted (i.e., oral water loading vs. intravenous saline infusion) or the volume as well as endocrine status of the patient. The hypothetical distal tubular dysfunction may not necessarily explain the cause of Bartter's syndrome.

In addition, idiopathic hypercalciuria mimicking Bartter's syndrome (Houser et al. 1984) has been reported to have no abnormality of chloride absorption in the distal tubules. Because of a lack of either hypercalciuria or nephrocalcinosis, our case diffrers from this case.

This is the first report of a case of secondary aldosteronism similar to Bartter's syndrome, which has been manifested after the complete remission of nephrotic syndrome. What has triggered the abnormality ? A drug-induced onset should be noted in this case. A few reports have suggested that symptoms mimicking Bartter's syndrome would have been induced either by the chemotherapy against pulmonary tuberculosis (Holmes et al. 1970) or by the chemotherapy (CHOP-Bleo) against malignant tumor using drugs including cyclophosphamide and steroid (prednisolone) (Lieber et al. 1984). In the present case, cyclophosphamide and steroid (prednisolone) had been used for the treatment of nephrotic syndrome. It would be speculated that the chemotherapy triggers the manifestation of an abnormality which had been clinically silent.

Implication of ANP in humoral homeostasis. In the present case, we did not observe a significant effect of PG inhibition on basal ANP level. In agreement with our observation, no involvement of ANP has been reported in Bartter's syndrome (Cushner et al. 1990). Gene analysis has also indicated no linkage of ANP gene with Bartter's syndrome (Graham et al. 1986). These observations do not support a primary involvement of ANP in the pathogenesis of Bartter's syndrome.

ANP level has been considered to be an indicator of volume status (Kimura et al. 1986). Consistent with these observations, volume loadings increased ANP level in our case and the ANP increase was enhanced by PG inhibition. These observations have further indicated that ANP level is a good indicator of volume status and suggest that PG plays a prior role in the volume regulation in this case.

R-A-A system, kallikrein-kinin system and PG. In the present case, PRA and PAC were markedly increased. However, the feedback mechanisms of R-A system appear to be intact, because the ang II infusion decreased PRA and increased PAC ; volume loadings also inhibited R-A system and angiotensin converting enzyme (ACE) inhibition increased PRA and decreased PAC, and ACE (as a kininase inhibitor) induced an increase in urinary kinin concentration. These observations indicate that negative-feedback mechanisms of R-A systiem function in their normal directions.

Consistent with the previous reports (Gill et al. 1976 ; Halushka et al. 1977 ; Vinci et al. 1978 ; Sasaki et al. 1980), PG inhibition with indomethacin decreased urinary PGE_2 excretion and decreased PRA and PAC, indicating that PG plays a stimulatory role in R-A system. Urinary kallikrein excretion was also decreased by PG inhibition. It has been suggested that PG may have directly stimulated kallikrein formation as previously reported (Nishimura et al. 1980). The increase in renal kallikrein production observed in our case may have also

been stimulated by an enhanced aldosterone synthesis, since aldosterone is a stimulator of kallikrein synthesis (Margolius et al. 1974). In any event, the kallikrein-kinin system appears to be secondarily influenced by PG and R-A systems.

In summary, PG stimulates the R-A system and possibly the kallikrein-kinin system. The negative-feedback mechanism of R-A system appears to function in a normal direction. The negative-feedback function, however, is not sufficient to correct the abnormality in R-A system. An intercation between PG and R-A system via ang II is suggested to be chiefly involved in this case.

A hypothesis : ang II receptor signal transduction abnormality. As previously described in the literature (Dunn 1981), an abnormal effect of ang II on humoral homeostasis appears to be involved in Bartter's syndrome. Recently, the cellular mechanism of of vascular ang II receptor signal transduction system has been getting clear. In vascular smooth muscle cells (one of the target tissues of ang II), ang II has been shown to induce an increase in cytosolic free calcium mediated by an inositol phosphate, a product of hydrolysis of phosphoinositide via phospholipase C (Griendling and Alexander 1990; Takeuchi et al. 1992). Molecular structure of vascular ang II (ATla) receptor has verified a link of this receptor with phospholipase C via a GTP-binding protein (Bergsma et al. 1992; Murphy et al. 1992). On the other hand, ang II has been shown to stimulate vasodilator PG synthesis in vascular smooth muscle (Alexander and Gimbrone 1976; Hassid and Williams 1983; Takeuchi et al. 1985) via PLA_2/cyclo-oxygenase. We have also shown that ang II simultaneously stimulates an increase in cytosolic free calcium and PG (prostacyclin) synthesis, indicating that ang II receptor can be linked to both PLC and PLA_2 pathways at the same time (Takeuchi et al. 1991b). Moreover, ang II is also known to induce a biphasic vascular response of initial contraction (due to an increase in cytosolic calcium (Ca^{2+})) and subsequent dilatation (due to production of vasodilator PG) (Blumberg et al. 1977). Thus, either PLC or PLA_2 pathway is considered to be involved in vasoconstriction or vasorelaxation, respectively. As mentioned previously, R-A and PG system via ang II is suggested to be chiefly involved in this case. In the vasculature, the pressor responsiveness to ang II was markedly decreased and the abnormality was improved by PG inhibition. Considering the knowledge of vascular ang II receptor signal transduction pathway, the vascular abnormality may possibly be explained if PLA_2 is abnormaly sensitive to ang II receptor, leading to excesive production of vasodilator PG, which decreases vascular pressor response to ang II. On the other hand, the linkage between ang II receptor and PLC may be preserved, since ang II induced-pressor responsiveness was still observed. Thus, ang II induced-excessive PG production is postulated to be involved in the abnormal vascular responsiveness to ang II. The pathway of ang II receptor signal transduction may be altered. This hypothesis may be generalized in other tissues and should be tested in future cases of Bartter's

syndrome.

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