Effect of dietary salt restriction on urinary serotonin and 5-hydroxyindoleacetic acid excretion in man

Arya M. Sharma, Ulrike Schorr, Hans-Michael Thiede and Armin Distler

Objective: To determine the effect of dietary salt restriction on urinary excretion of serotonin and its principal metabolite 5-hydroxyindoleacetic acid (5-HIAA) in man.

Design: We studied 16 healthy male volunteers (age range 20–28 years) who ate a standard diet containing 20 mmol/day NaCl, to which either 220 mmol/day NaCl or placebo was added as a supplement for 1 week each, according to a randomized, single-blind crossover design.

Methods: Urinary excretion of serotonin, 5-HIAA, noradrenaline and vanillylmandelic acid (VMA) were measured during the low- and high-salt periods using reverse-phase high-performance liquid chromatography.

Results: During the low-salt diet, 24-h urinary excretion of serotonin increased by 42%, accompanied by a 52% rise in the excretion of 5-HIAA. Salt restriction also increased noradrenaline excretion by 77% and VMA excretion by 40%. Regression analysis revealed a strong positive relationship between the excretion of serotonin and of noradrenaline ($r = 0.84$, $P < 0.001$) and between that of 5-HIAA and of VMA ($r = 0.74$, $P < 0.001$).

Conclusions: Salt restriction stimulates the serotonergic system in man. Stimulation of this system, in conjunction with the sympathetic nervous system, may contribute to renal sodium conservation during dietary salt restriction in man.

Journal of Hypertension 1993, **11**:1381–1386

Keywords: Diet, sodium restriction, serotonin, 5-hydroxyindoleacetic acid, catecholamines, sodium homeostasis.

Introduction

Serotonin (5-hydroxytryptamine) is a potent vasoactive amine and its infusion into animals and man has been reported to decrease renal blood flow, glomerular filtration rate and urinary sodium and water excretion [1–6]. Serotonin is synthesized from the essential amino acid L-tryptophan. The first step of this synthetic pathway, the hydroxylation of L-tryptophan, is catalysed by the rate-limiting enzyme tryptophan hydroxylase; the product L-5-hydroxytryptophan is then decarboxylated by L-aromatic-amino acid decarboxylase to the active amine. High activities of both tryptophan hydroxylase [7] and L-aromatic-amino acid decarboxylase [8] are found in the renal proximal tubule, the principal site of sodium reabsorption, and the infusion of either tryptophan [9] or L-5-hydroxytryptophan [10] results in antidiuretic and antinatriuretic effects similar to those observed with serotonin. *In vivo* and *in vitro* studies have shown that these effects are due to intrarenal synthesis of serotonin from its precursors [10,11]. Serotonin is degraded primarily by the action of monoamine oxidase, which is present in abundance in the renal cortex [12], to 5-hydroxyindoleacetic acid (5-HIAA). Also, the kidney has the capacity to conjugate serotonin to the glucuronide or the sulphate [13]. Based on these findings, it has been hypothesized that an endogenous renal serotonergic system might play a role in the regulation

From the Department of Internal Medicine, Division of General Internal Medicine and Nephrology, Universitätsklinikum Steglitz, Free University of Berlin, Berlin, Germany.

Sponsorship: This study was supported by the Deutsche Forschungsgemeinschaft (research grant Sh 35/1-1).

Requests for reprints to: Dr A.M. Sharma, Medizinische Klinik, Klinikum Steglitz, Free University of Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany.

Date of receipt: 4 June 1993; revised: 25 August 1993; accepted: 31 August 1993.

of water and sodium excretion [10]. However, little is yet known concerning the physiological importance of the renal serotonergic system and the factors governing its activity.

Severe dietary salt restriction is a strong physiological stimulus, activating both the sympathetic and the renin–angiotensin–aldosterone systems [14,15], which are well known to contribute to renal sodium conservation. The objective of the present study was to determine the effect of dietary salt restriction on urinary excretion of serotonin and its principal metabolite 5-HIAA in healthy volunteers. Our reasoning was that if the serotonergic system plays a role in sodium conservation, it would be activated similarly by salt restriction. We also measured the excretion of catecholamines and their principal metabolite vanillylmandelic acid (VMA) to determine their relationship with the excretion of serotonin and 5-HIAA.

Subjects and methods

Subjects and protocol

The protocol of the study was approved by the ethics committee of Universitätsklinikum Steglitz. All participants gave informed consent. The study was performed in an ambulatory setting, but all meals were provided by the hospital kitchen.

Sixteen healthy male volunteers, age range 20–28 years, were recruited after a routine physical and laboratory examination to rule out hypertension, hyperlipidaemia, diabetes mellitus and hepatic or renal disease. The subjects ate a standard diet containing 20 mmol sodium, 60 mmol potassium and 20 mmol calcium per day for 14 days, adding either placebo or a 220 mmol/day NaCl supplement for 1 week each, according to a randomized, single-blind crossover design. The resultant daily intake of 240 mmol sodium during the high-salt period exceeds the average salt intake in West European societies by approximately 30%, but is still well within the normal range [16]. Total energy intake was estimated so as to keep body weight constant. On the last 3 days of each period the diet contained no foods known to have a high serotonin or VMA content. The subjects were advised to drink approximately 2 litres/day water and to refrain from smoking, drinking alcohol and unaccustomed exercise. Compliance was assessed throughout the study by measuring daily 24-h urinary sodium chloride excretion. Subjects were considered compliant when daily sodium and chloride excretion was <35 mmol or >220 mmol on the last 3 days of the low- and high-salt periods, respectively.

Urinary excretion of serotonin, 5-HIAA, catecholamines and VMA were measured in the last 24-h urine sample (day 7; 1 g ethylenediaminetetraacetic acid and 25 ml 4 mol/l perchloric acid was added to each 24-h urine sample as preservatives) under the low- and high-salt regimens. On the morning of the last day of each regimen the subjects were weighed and their blood pressure was recorded (with the subject supine) at 2-min intervals over a 1-h period using an automatic device (Tonoprint, Speidel and Keller, Jungingen, Germany). A venous blood sample was then drawn for the measurement of 5-HIAA. All samples were stored at –70°C until analysis.

Analytical methods

Urinary serotonin, 5-HIAA, catecholamines (adrenaline, noradrenaline and dopamine) and VMA excretions were measured by reverse-phase high-performance liquid chromatography with electrochemical detection (BCJM 1; ERC Gesellschaft für den Vertrieb wissenschaftlichter Geräte mbH, Alteglofsheim b. Regensburg, Germany). Using subsequent extraction and purification procedures, all of the above excretions were determined from a single 200-µl aliquot taken from the 24-h urine sample. Similarly, plasma 5-HIAA levels were measured using high-performance liquid chromatography with a slight modification of the method for urine measurements, described below. The following substances (all obtained from Sigma Chemie GmbH, Munich, Germany) were added to serve as internal standards: 20 ng *N*-methyl-5-hydroxytryptamine (standard against serotonin), 500 ng 5-hydroxyindolecarboxylic acid (standard against 5-HIAA), 10 ng 3,4-dihydroxybenzylamine (standard against catecholamines) and 500 ng iso-VMA (standard against VMA). For the measurement of sulphated amines, a 200-µl aliquot of the 24-h urine sample was incubated for 1 h in the presence of 250 mU sulphatase type VI (Sigma) before further analysis. After the addition of 50 µl 0.3% mercaptoethanol as an antioxidant, the sample was adjusted to pH 6.5 using 2.5 ml 0.2 mol/l ammonium acetate buffer. The sample was then passed over a cation-exchange column (Biorex 70, 100-mesh; Bio-Rad Laboratories GmbH, Munich, Germany). The eluate (I) was collected and processed further for measurement of 5-HIAA and VMA (see below). After washing with 10 ml water, 6 ml 2 mol/l NaCl was passed over the ion exchanger, and the eluate (II) was divided into two aliquots for the determination of serotonin (IIa) and catecholamines (IIb), respectively.

Serotonin

For the further extraction of serotonin, 1 ml aliquot IIa was passed through a C-18 column (Bond Elut; Analytichem International, Harbor City, California, USA) that had been preconditioned by washing with methanol and water. After washing the column twice with 1 ml water, the sample was eluted with 1 ml 4 mol/l formic acid in methanol. The sample was then dried and the residue was dissolved

in a 500-µl aliquot of the mobile phase [0.1 mol/l citrate–phosphate buffer (pH 4.5), 10% methanol and 10 mg/l octanesulphonic acid], of which 50 µl was injected into the high-performance liquid chromatography system, where it was passed through a steel column (150×3.9 mm) packed with novapack (4 µm; Waters Associates Inc., Milford, Massachusetts, USA) The flow rate was set at 0.5 ml/min and the detection potential at the glassy carbon electrode versus the Ag–AgCl reference electrode at 850 mV. A typical urine analysis is shown in Fig. 1.

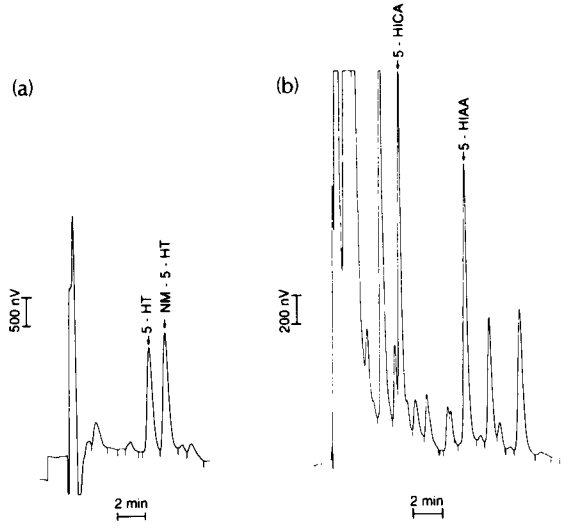

Fig. 1. Representative chromatograms of urine extracts separated by reverse-phase high-performance liquid chromatography showing endogenous (a) serotonin (5-HT) and (b) 5-hydroxyindoleacetic acid (5-HIAA) peaks. For sample preparation and chromatography conditions, see Analytical methods. Internal standards NM-5-HT, *N*-methyl-5-hydroxytryptamine; 5-HICA, 5-hydroxyindolecarboxylic acid.

Catecholamines

The other 5-ml aliquot (IIb) was adjusted to pH 8.5 with TRIS-buffer, and the catecholamines were extracted further by binding to acid-washed aluminium oxide. After washing twice with 7 ml water and eluting with 500 µl 0.1 mol/l perchloric acid, a 50-µl aliquot was injected into the high-performance liquid chromatography system, where the amines were separated by passing through a steel column (120×4 mm) packed with spherisorb ODS II (3 µm; H. Knauer GmbH, Berlin, Germany). The mobile phase consisted of a sodium citrate buffer (pH 4.5) containing 10% methanol and 100 mg/l octanesulphonic acid. The flow rate was set at 0.6 ml/min and the detection potential at 800 mV.

5-Hydroxyindoleacetic acid and vanillylmandelic acid

For the further extraction of 5-HIAA and VMA, 3-ml eluate I was passed over an anion exchanger (Serdolit AS 6, 200- to 400-mesh; Serva Feinbiochemica GmbH & Co., Heidelberg, Germany). After washing twice with 5 ml water and 2 ml methanol, the sample was eluted (III) with 8 ml 4 mol/l formic acid in methanol. For the measurement of 5-HIAA, a 1-ml aliquot of the eluate (IIIa) was dried in the presence of an antioxidant (10 µl 2% cystein solution), the residue was dissolved in 1 ml of the mobile phase and 10 µl was injected into the high-performance liquid chromatography unit. Mobile phase, columns, flow and detection conditions for the measurement of 5-HIAA were identical to those above for the measurement of catecholamines. A typical urine analysis of 5-HIAA is shown in Fig. 1. For the measurement of VMA, a further 2-ml aliquot of the eluate (IIIb) was dried and redissolved in 500 µl mobile phase [sodium phosphate buffer (pH 3.1) and 1% methanol] and was then passed over a C-18 column, conditioned as above (see Serotonin). After fractional elution with 3 ml mobile phase, a 50-µl aliquot containing the VMA was injected into the high-performance liquid chromatography unit, where it was passed through a steel column (120×4 mm) packed with spherisorb ODS II (3 µm; Knauer). The flow rate was set at 0.8 ml/min and the detection potential at 850 mV.

All of the samples were measured in duplicate. Recovery was in the range 60–70% (SEM <5%) for all substances. The detection limit was <20 pg for VMA and <5 pg for the other substances measured. Inter- and intra-assay variance was <10% (SEM <3%) for all substances measured. Urinary electrolytes were measured using standard laboratory techniques.

Statistical analysis

All values are expressed as means ± SEM. Total serotonin and noradrenaline are expressed as the sum of free and sulphated serotonin and noradrenaline, respectively. Differences between variables under the high- and low-salt diets were tested for significance by two-tailed Student's t-test for paired samples. Regression analysis was performed assuming linear regression. $P<0.05$ was considered statistically significant.

Results

Twenty-four-hour urinary excretion of both free and sulphated serotonin increased by 35% ($P=0.03$) and total serotonin excretion by 42% ($P=0.01$) under the low- compared with the high-salt regimen (Fig. 2). This increase in serotonin excretion was accompanied by a 50% increase in excretion of its principal metabolite 5-HIAA ($P<0.01$; Fig. 3). There was also a small but significant increase in plasma 5-HIAA from 6.2±0.5 (high-salt) to 6.8±0.5 ng/ml (low-salt; $P=0.03$). Under salt restriction, excretion of free noradrenaline was higher by 70%, excretion of sulphated noradrenaline was higher by 80% ($P<0.001$; Fig. 2) and excretion of VMA by 40% ($P=0.01$; Fig. 3) compared with under the high-salt diet, indicating activation of the sympathetic nervous

system. Although both free and sulphated urinary dopamine tended to be lower under the low-salt diet, the change was not statistically significant (data not shown). Similarly, adrenaline excretion was not affected significantly by the different dietary regimens (data not shown).

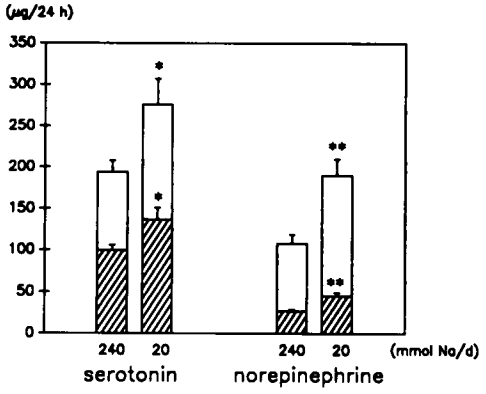

Fig. 2. Twenty-four-hour urinary excretion of free (▨) and sulphated (□) serotonin and noradrenaline (norepinephrine) during high- (240 mmol/day) and low-salt (20 mmol/day) diets in healthy young men (n = 16). Values are expressed as means ± SEM. *$P<0.05$, ***$P<0.001$, versus low-salt.

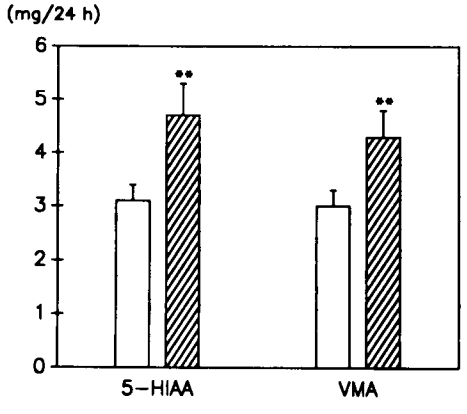

Fig. 3. Twenty-four-hour urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) and vanillylmandelic acid (VMA) during high- (□, 240 mmol/day) and low-salt (▨, 20 mmol/day) diets in healthy young men (n = 16). Values are expressed as means ± SEM. **$P<0.01$, versus low salt.

Table 1. Blood pressure and urinary variables during high- and low-salt diets in young normotensive men (n = 16).

Variable	High salt	Low salt
Blood pressure (mmHg)		
Systolic	111.4 ± 1.2	110.0 ± 6.2
Diastolic	56.0 ± 1.8	55.5 ± 1.5
Weight (kg)	70.7 ± 1.6	69.5 ± 1.6***
Urinary sodium excretion (mmol/day)	239.5 ± 11.9	15.6 ± 2.7***
Urine volume (ml/day)	1933 ± 182	1373 ± 141**

Values are expressed as means ± SEM. **$P=0.01$, ***$P<0.001$, versus high salt.

Regression analysis revealed a highly significant correlation between the excretion of serotonin and noradrenaline and the excretion of their respective

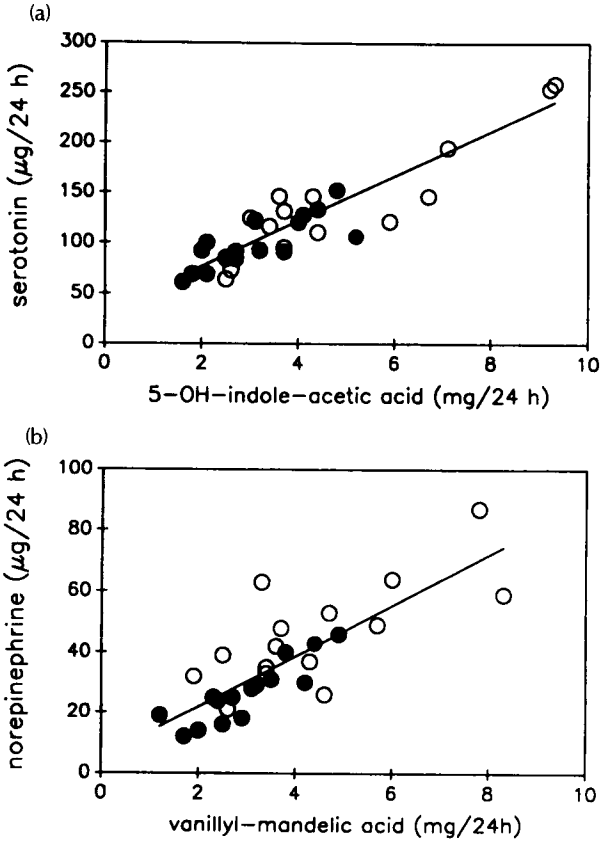

Fig. 4. Regression analysis between 24-h urinary excretion of (a) serotonin and (b) noradrenaline (norepinephrine) and their respective metabolites 5-hydroxyindoleacetic acid (5-OH-indole-acetic acid) and vanillylmandelic acid during high- (●) and low-salt diets (○) in healthy young men. (a) $r=0.91$, $P<0.001$; (b) $r=0.80$, $P<0.001$.

metabolites 5-HIAA ($r=0.91$, $P<0.001$) and VMA ($r=0.80$, $P<0.001$; Fig. 4). We also found strong positive correlations between serotonin excretion and noradrenaline excretion ($r=0.84$, $P<0.001$) and between 5-HIAA excretion and VMA excretion ($r=0.74$, $P<0.001$; Fig. 5).

Blood pressure was not significantly affected by salt intake (Table 1). As expected, body weight, urinary sodium excretion and urine volume were greater under the high- than the low-salt diet ($P<0.001$).

Discussion

In the present study we found a significantly greater urinary serotonin excretion during dietary salt restriction than under a high-salt diet. A concomitant increase in the excretion of the principal serotonin metabolite 5-HIAA indicated that serotonin production and subsequent metabolism was enhanced under the low-salt regimen. This suggests that the serotonergic system was activated by dietary salt restriction and that it could play a role in renal sodium conservation.

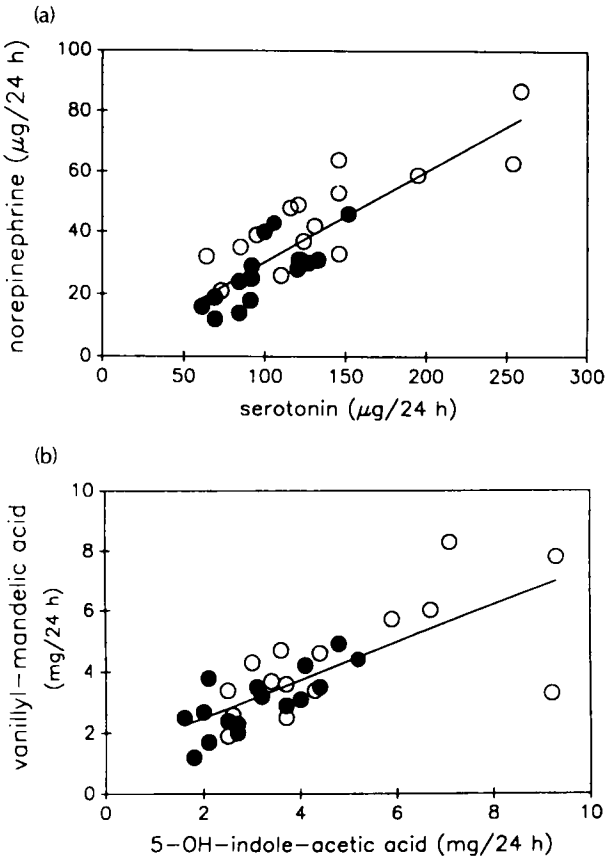

Fig. 5. Regression analysis between 24-h urinary excretion of (a) serotonin and noradrenaline (norepinephrine) and (b) 5-hydroxyindoleacetic acid (5-OH-indole-acetic acid) and vanillylmandelic acid during high- (●) and low-salt diets (○) in healthy young men. (a) $r=0.84$, $P<0.001$; (b) $r=0.74$, $P<0.001$.

Very little information is available concerning the effects of serotonin on renal function in man. In hypertensive men administered serotonin intravenously, renal plasma flow, urine flow and sodium excretion were decreased, but the effects on glomerular filtration rate were inconsistent [2]. These effects of serotonin were blocked by the administration of the benzyl analogue of serotonin, 1-benzyl-2,5-dimethylserotonin. Interestingly, oral administration of the serotonin precursor tryptophan to normal volunteers resulted in a marked reduction in glomerular filtration rate and urine flow, an effect which might be mediated by intrarenal synthesis of serotonin [9]. Most of our current knowledge of the role of the serotonergic system in renal physiology is derived from animal experiments. Intraperitoneal injection of tryptophan in rats caused a selective increase in sodium reabsorption without changing glomerular filtration rate [17]. Similar effects were observed after the infusion of L-5-hydroxytryptophan, and were accompanied by an increase in renal excretion of serotonin but no changes in plasma serotonin, suggesting that the effects of L-5-hydroxytryptophan were mediated by serotonin formation in the kidney [10,18]. Endogenous renal formation of serotonin from its precursors, and subsequent excretion of serotonin in the urine has been demonstrated in the rat both *in vivo* [10] and in the isolated perfused kidney [11], and high activities of both of the key enzymes required for serotonin synthesis (tryptophan hydroxylase and L-aromatic-amino acid decarboxylase) have been reported in the renal cortex [7,8]. L-Aromatic-amino acid decarboxylase is located primarily within renal tubular cells but is also present in autonomic nerve endings [7,18]. Morphological evidence indicates that extraneuronal serotonin formation from L-5-hydroxytryptophan occurs exclusively in the apical pole of cells located in the S1 and S2 segments of the proximal convoluted tubule [8], the site of maximum sodium reabsorption. These findings suggest that intrarenal formation of serotonin may influence salt and water excretion. The antidiuretic and antinatriuretic effects of serotonin are probably mediated by its action on 5-hydroxytryptamine type 1 receptors, as these effects are not blocked by the 5-hydroxytryptamine type 2 receptor antagonist ketanserin [6].

Activation of the serotonergic system by dietary salt restriction was, as expected [14], accompanied by an increase in noradrenaline excretion (Fig. 2), indicating activation of the sympathetic system. The present observation of increased excretion of the principal noradrenaline metabolite VMA during salt restriction indicates increased noradrenaline production and subsequent metabolism, a finding that is compatible with activation of the sympathetic system by salt restriction. Increased renal sympathetic activity is known to augment renal sodium conservation, both by increasing sodium reabsorption directly and by stimulating the release of renin. The strong positive correlations between serotonin and noradrenaline (Fig. 5a), and between their respective metabolites 5-HIAA and VMA (Fig. 5b), observed in the present study suggest that both the sympathetic and the serotonergic systems are activated by salt restriction and may therefore contribute synergistically to sodium conservation.

Several mechanisms might contribute to the postulated effect of serotonin on renal sodium conservation. Reduced glomerular filtration rate or renal blood flow, or both, mediated by serotonin directly or by augmentation of the vasoconstrictor response to noradrenaline and angiotensin II [19], could result in diminished sodium and water excretion. Serotonin has also been shown to raise glomerular cyclic AMP levels [20], increase calcium fluxes and phosphoinositol turnover, and activate protein kinase C [21]; effects which might stimulate tubular transport systems involved in sodium reabsorption such as the Na^+–H^+ exchanger [22]. Serotonin may also potentiate the effects of other neurohormonal substances, including noradrenaline, angiotensin II and insulin [19,21], on transmembrane fluxes involved

in sodium reabsorption. Also, serotonin is a potent aldosterone secretagogue [23].

The serotonergic system has recently been implicated in the pathophysiology of renal diseases including hypertension [19,24,25], diabetic nephropathy, glomerulonephritis and rejection of renal transplants [13], but whether the relationship between serotonergic activity and salt excretion is altered in any of these states is not yet known.

In summary, the present study shows that dietary salt restriction in man leads to an increase in urinary excretion of serotonin and 5-HIAA, indicating activation of the renal serotonergic system. This is accompanied by activation of the sympathetic system. These findings are compatible with the hypothesis that activation of the serotonergic system, in conjunction with activation of the sympathetic system, contributes to renal sodium conservation during dietary salt restriction in man. However, the pathophysiological role of the renal serotonergic system in states involving abnormal sodium and water homeostasis remains to be determined.

Acknowledgements

We thank K. Schlotter and B. Girresch for skilful technical assistance in analysing the plasma and urine samples.

References

1. PAGE EW, GLENDENING MB: Production of renal cortical necrosis with serotonin (5-hydroxytryptamine) [abstract]. *Am J Med* 1955, 19:285.
2. HOLLANDER W, MICHELSON AL: The effects of serotonin and antiserotonins in hypertensive man. *J Clin Invest* 1956, 35:712–713.
3. DEL GRECO F, MASSON G, CORCORAN A: Renal and arterial effects of serotonin in the anesthetized rat. *Am J Physiol* 1956, 187:509–514.
4. SPINAZZOLA AJ, SHERROD TR: The effects of serotonin (5-hydroxytryptamine) on renal hemodynamics. *J Pharmacol Exp Ther* 1957, 119:114–121.
5. PARK CS, CHU CS, PARK YS, HONG SK: Effect of 5-hydroxytryptamine on renal function of the anesthetized dog. *Am J Physiol* 1968, 214:384–388.
6. SHOJI T, TAMAKI T, FUKUI K, IWAO H, ABE Y: Renal hemodynamic responses to 5-hydroxytryptamine (5-HT): involvement of the 5-HT receptor subtypes in the canine kidney. *Eur J Pharmacol* 1989, 171:219–228.
7. SOLE MJ, MADAPALLIMATTAM A, BAINES AD: An active pathway for serotonin synthesis by renal proximal tubules. *Kidney Int* 1986, 29:689–694.
8. HAGEGE J, RICHET G: Proximal tubule dopamine histofluorescence in renal slices incubated with L-dopa. *Kidney Int* 1985, 27:3–8.
9. ARORA SP, SCHOMULLER JM, MODLINGER RS: Adverse effects of tryptophan on renal function [abstract]. *Clin Res* 1979, 27:598A.
10. STIER CTJ, ITSKOVITZ HD: Formation of serotonin by rat kidneys *in vivo*. *Proc Soc Exp Biol Med* 1985, 180:550–557.
11. STIER CTJ, MCKENDALL G, ITSKOVITZ HD: Serotonin formation in nonblood-perfused rat kidneys. *J Pharmacol Exp Ther* 1984, 228:53–56.
12. BLASCHKO HP: Enzymic oxidation of tryptamine derivatives. *J Physiol (Lond)* 1953, 122:403–408.
13. ABBOUD HE, DOUSA TP: Renal metabolism and actions of histamine and serotonin. *Miner Electrolyte Metab* 1983, 9:246–259.
14. LUFT FC, RANKIN LI, HENRY DP, BLACH R, GRIM CE, WEYMAN AE, *ET AL.*: Plasma and urinary norepinephrine values at extremes of sodium intake in normal man. *Hypertension* 1979, 1:261–266.
15. HOLLENBERG NK: The renin-angiotensin system and sodium homeostasis. *J Cardiovasc Pharmacol* 1984, 6 (suppl 1):S176–S183.
16. INTERSALT COOPERATIVE RESEARCH GROUP: Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *BMJ* 1988, 297:319–328.
17. HERKEN H, WEBER H: l-Tryptophan-induced increase of renal sodium reabsorption. *Arch Pharmacol* 1971, 271:206–210.
18. ITSKOVITZ HD, CHEN YH, STIER CTJ: Reciprocal renal effects of dopamine and 5-hydroxytryptamine formed within the rat kidney. *Clin Sci* 1988, 75:503–507.
19. VANHOUTTE PM: Does 5-hydroxytryptamine play a role in hypertension? *Trends Pharmacol Sci* 1992, 3:370–373.
20. ABBOUD HE, SHAH S, DOUSA TP: Effects of dexamethasone on cyclic nucleotide accumulation in glomeruli. *J Lab Clin Med* 1979, 94:708–717.
21. TAKUWA N, GANZ M, TAKUWA Y, STERZEL RB, RASMUSSEN H: Studies of the mitogenic effect of serotonin in rat renal mesangium cells. *Am J Physiol* 1989, 257:F431–F439.
22. GRINSTEIN S, ROTHSTEIN A: Mechanisms of regulation of the Na^+/H^+ exchanger. *J Membr Biol* 1986, 90:1–12.
23. HANING R, TAIT SA, TAIT JF: *In vitro* effects of ACTH, angiotensins, serotonin and potassium on steroid output and conversion of corticosterone to aldosterone by isolated adrenal cells. *Endocrinology* 1970, 87:1147–1167.
24. COLLIS MG, VANHOUTTE PM: Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. *Circ Res* 1977, 41:759–767.
25. TUNCER M, VANHOUTTE PM: Role of prostanoids in the increased vascular responsiveness and delayed tachyphylaxis to serotonin in the kidney of spontaneously hypertensive rats. *J Hypertens* 1991, 9:623–629.