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Effect of Chronic Inhibition of Converting Enzyme on Renal Handling of Salt and Water: A Study on a Pediatric Population

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Key Words
Angiotensin-converting enzyme inhibition · Renal salt and water balance · Pediatric patients

Abstract
Background/Aims: The effect of angiotensin-converting enzyme inhibition (ACEi) is amply documented in several pathological conditions. However, there are few reports about the effect of chronic ACEi on salt and water balance. The present work evaluates the effect of chronic ACEi on salt and water balance in a population of children receiving enalapril chronically in order to reduce albuminuria elicited by a uremic hemolytic syndrome. ***Methods:*** Nine children aged from 9 to 19 years with normal glomerular filtration rate, normotension and with urinary concentration capacity preserved were treated with enalapril with doses ranging between 0.1 and 0.30 mg/kg/day. Diuresis, urinary absolute and fractional excretion of Na^+, K^+ and urea, creatinine clearance, osmolal clearance and tubular water reabsorption were measured under three experimental procedures: (1) with free access to water; (2) with a water load and (3) with water restriction. In the last group urinary antidiuretic hormone (ADH) was measured. These tests were performed in a paired way, just before starting ACEi treatment and after 6 months of enalapril treatment. ***Results:*** Enalapril treatment diminished the urinary concentration capacity without affecting Na^+ and K^+ urinary excretion. Creatinine clearance was not modified except in the condition of water load where a fall in it was found after ACEi. ADH increased after enalapril treatment in children under water restriction. ***Conclusion:*** In these children chronic ACEi decreases urinary concentration capacity.

Introduction

Angiotensin II (Ang II) is one of the most potent endogenous vasoconstrictors and plays an important role in regulating blood pressure and sodium and water balance [1]. It actively participates in the control of renal hemodynamics and is one of the agents modulating Na-H exchange in the brush border of the proximal tubule [2, 3] and in the medullary thick ascending loop.

In addition to its action on the kidney, Ang II is a potent dipsogen, and systemic administration also triggers a marked release of antidiuretic hormone (ADH) [4]. The release of Ang II in response to hypovolemia [5] could contribute to the normalization of blood volume by increasing the release of ADH in parallel with its systemic and renal effects.

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Inhibition of angiotensin-converting enzyme not only reduces Ang II, but also increases kinins, which have a dipsogenic action. In rats chronically treated with captopril, this effect is reversed by HOE 140, a β_2-receptor antagonist [6]. Moreover, the acute administration of bradykinin in rats pretreated with captopril induced a dose-dependent increase in water intake, without any significant effect on water excretion [7].

The effect of chronic inhibition of the renin-angiotensin system in pathophysiological conditions is extensively documented, but little is known about the effects of inhibition for prolonged periods on the salt and water balance. In this paper we describe some parameters of water balance in children under chronic treatment with angiotensin-converting enzyme inhibition (ACEi). These patients had had an episode of hemolytic uremic syndrome 10 years prior to the time of the test, and they were without clinical signs of permanent renal damage.

Material and Methods

Selection of Children

Children with minimal renal alterations without hypertension or impaired glomerular filtration rate and with intact capacity to concentrate urine when submitted to a water restriction test were selected. The children had suffered hemolytic uremic syndrome [8]. Nineteen patients were recruited and 10 out of them were excluded, 2 for having proteinuria higher than 5 mg/kg day, 3 with creatinine clearance lower than 90 and higher than 80 ml/min/1.72 m^2, 1 did not reach the urine concentration required, 1 for pregnancy, 2 for failing to follow diet instructions and 1 for allergic dermatitis. No child refused to participate in the test. The remaining group of 9 children was composed by 5 males and 4 females who were enrolled for 18 months. At the time of the study the children showed minimal alterations in kidney function, with albuminuria higher than 14 and lower than 50 $\mu g/min \cdot m^2$. These patients were prescribed enalapril with doses ranging between 0.1 and 0.30 mg/kg/day to decrease the proteinuria to 50% of the pretreatment value. These children and their parents were informed about the nature of the study and the risks involved in this protocol. The protocol was approved by the ethical committee of the Children's Hospital of the City of Buenos Aires.

Inclusion Criteria

The patients were over 9 and under 19 years old. The lower level of 9 years was chosen because it ensured compliance with the protocol. The family and the child must understand the instructions for the proper execution thereof. The patients selected were eutrophic with adequate diuresis and euhydration; they were normotensive according to the tables for children in a normal population of the same age and sex [9]. The children had normal renal function and a creatinine clearance, with cimetidine premedication [10, 11], greater than 90 ml/min/1.73 m^2. The concentration capacity was normal with urinary osmolality greater or equal than 850 mosm/kg H_2O which is the lower level observed in our population of healthy children [12].

Exclusion Criteria

Patients with alteration of renal concentration capacity or receiving drugs that interfere with the renin-angiotensin system and/or prostaglandins, proteinuria greater than 5 mg/kg/day or hyperkalemia were excluded. Also were removed from the study those children that after ACE inhibition showed an increase in serum creatinine concentration above 30% of the pretreatment value, the serum potassium concentration above 5.2 mEq/l and hypotension (reduction of mean arterial pressure to 50–65 mm Hg) [13].

Diet

Two months before the functional test, the patients received a protein diet according to age and sex as recommended by the ~~recommended daily allowance~~ with appropriate caloric supply [14]. Daily protein intake was estimated by urea urinary excretion on a 24-hour sample prior to the study [15]. This diet was continued during the whole study.

Experimental Test

Three experimental approaches were chosen to evaluate the effect of ACEi on water balance in these patients. In the first approach, the children had free access to water. The second one consisted in a concentration test with water restriction and the last approach was a water load. This experimental design allowed for evaluating the basal condition (free access to water), urinary concentration and diluting capacities (water restriction and water overload).

The water loading test was carried out perorally, avoiding the continuous infusion of hypotonic intravenous solution.

The following functional tests were performed in the same group of children before and after being treated with enalapril for 6 months.

Free Drinking Water

These experiments were performed in children with free access to water and minimal protein intake, as given by the recommended daily allowance. Urine samples were taken spontaneously during 24 h from 8.00 p.m. to 8.00 p.m. The blood sample was extracted after previous fasting for 8 h. A small urine aliquot was separated and placed at –20°C for determination of albuminuria.

Water Restriction

Solid food was allowed all the time (24 h), with dry diet plus water restriction to 20 ml/kg for the first 12 h, and total restriction of fluid intake during the last 12 h. The water restriction was not so extreme as to jeopardize the studied population. However, we note that this is the usual protocol used in pediatrics [16]. We collected overnight urine, from 0.00 p.m. to 8.00 p.m., and a blood sample was extracted. An aliquot of urine was placed at –20°C for the determination of ADH.

Water Load

An oral load of 20 ml/kg body weight of water to drink in 20 min was delivered. Urine samples were taken hourly, starting when the children finished drinking the water. Blood samples were taken when the urinary osmolality was around 100 mosm/kg H_2O (Chaimovitz test modified) [17].

Table 1. Effect of chronic ACEi on different parameters related to salt and water balance in 9 children submitted to three experimental maneuvers: free access to drinking water (FW), water restriction (WR) and water load (WL), without (–E) or with enalapril (+E)

	FW		WR		WL	
	–E	+E	–E	+E	–E	+E
C_{Cr}, ml/min/1.73 m^2	89 ± 8	90 ± 8	67 ± 11	95 ± 9	103 ± 11	65 ± 6*
$U_{Na}V$, μEql/min	82 ± 11	102 ± 14	59 ± 7	72 ± 11	81 ± 12	124 ± 23
FE_{Na}	0.73 ± 0.13	0.90 ± 0.16	0.75 ± 0.14	0.73 ± 0.12	2 ± 0.07	1.69 ± 0.29
U_KV, μEql/min	24 ± 4	22 ± 3	16 ± 1	28 ± 5	81 ± 14	64 ± 13
FE_K	8.9 ± 1.9	7.7 ± 1.3	24.0 ± 5.0	17.0 ± 1.4	26.4 ± 5.8	24.9 ± 3.7
U_UV	8.8 ± 0.5	11.4 ± 1.8	13.0 ± 2.0	12.0 ± 1.7	7.8 ± 1.3	8.3 ± 1.7
FE_U	51 ± 8	43 ± 4	42 ± 2.8	53 ± 7	35 ± 8	46 ± 9
C_{osm}, ml/min/100 ml C_{Cr}	1.6 ± 0.2	2.2 ± 0.2	2.6 ± 0.4	2.0 ± 0.4	1.8 ± 0.2	3.0 ± 0.4*
U_{osm}, mosm/kg	611 ± 40	591 ± 29	983 ± 25	805 ± 54*	96 ± 10	82 ± 8
C_{H_2O}, ml/min/100 ml C_{Cr}	–0.71 ± 0.08	–0.87 ± 0.07	–1.80 ± 0.41	–1.35 ± 0.29	3.83 ± 0.55	7.50 ± 1.06*
P_{osm}, mosm/kg	293 ± 1	293 ± 2	296 ± 1	295 ± 1	281 ± 2	278 ± 2
Vm, ml/min	0.7 ± 0.05	0.94 ± 0.08*	0.39 ± 0.02	0.62 ± 0.07*	5.83 ± 0.89	10.49 ± 1.13*

C_{Cr} = Creatinine clearance; $U_{Na}V$ = urinary sodium excretion; FE_{Na} = fractional sodium excretion; U_KV = urinary potassium excretion; FE_K = fractional potassium excretion; U_UV = urinary urea excretion; FE_U = fractional urea excretion; C_{osm} = osmolal clearance; U_{osm} = urinary osmolality; C_{H_2O} = free water clearance; P_{osm} = plasmatic osmolality; Vm = diuresis. Data are means ± SEM. * $p < 0.05$.

Laboratory Measurements

Plasma and urine creatinine concentrations were measured by an enzymatic method based on the reaction of creatinine in the presence of glutamate dehydrogenase. Plasma and urine urea were measured using the urease method. The reading of creatinine and urea was performed in an autoanalyzer (Roche/Hitachi 917).

Plasma and urine concentrations of Na and K were measured with ion-selective electrodes also in an autoanalyzer Roche/Hitachi 917.

Plasma and urine osmolalities were measured in a vapor pressure osmometer (Wescor, Vapro 5200).

Urinary ADH values were determined using a radioimmunoassay with ^{125}I-labeled ADH (Diagnostic System Laboratories Inc., USA). Proteinuria was measured with the pyrogallol red method. Albuminuria was determined by a nephelometric method in urine kept in a freezer at –20°C. We used a 3100 Beckman Array nephelometer.

Formulas and Calculations

$$C_{H_2O} = V - C_{osm} \quad (1)$$

$$T^c_{H_2O} = C_{osm} - V \quad (2)$$

where: V = diuresis, C_{osm} = osmolal clearance, $T^c_{H_2O}$ = free water reabsorption, C_{H_2O} = solute-free water clearance, all of them measured in milliliters per minute.

Statistical Analysis

The results are expressed as means ± SEM. Values before and after treatment with ACEi were compared using Student's t test for paired samples. There were no outliers. Statistical analysis was performed using the Prism program, version 3.0.

Results

Results are summarized in table 1.

Children with Free Access to Water

Children with free access to water showed an increased diuresis after treatment with enalapril. All the other parameters were without modifications.

Urine Concentration Test

Urinary concentration capacity was diminished, and diuresis was larger after enalapril treatment. Urinary ADH before enalapril treatment was 25 ± 5 pg/min·ml, and after treatment it was 63 ± 7 pg/min·ml ($p < 0.05$). When normalized by urinary osmolality (urinary ADH/urine osmolality) it was 0.019 ± 0.004 pg/min/mosm before enalapril and 0.059 ± 0.001 pg/min/mosm after chronic enalapril treatment ($p < 0.05$).

Urinary Dilution Test

The data correspond to the period of maximum diuresis. Diuresis and C_{osm} increased significantly in children after treatment with enalapril. Enalapril induced a fall in the creatinine clearance.

Discussion

Experiments on human beings are logically restricted to particular groups in which the use of certain drugs is mandatory. In the present work we dealt with 9 children who were under enalapril treatment as a consequence of proteinuria elicited by a history of hemolytic uremic syndrome, without signs of renal damage – except the minimal albuminuria – with normal glomerular filtration rate and adequate concentration capacity. The primary objective of treating these normotensive children with ACEi was to reduce microalbuminuria at least 50%. This group allowed a unique opportunity to evaluate the effect of chronic inhibition of ACE since we could compare its effect using a paired t test. Blood sample withdrawal and cimetidine premedication were the more invasive procedures. The effect of either acute or chronic inhibition of the renin-angiotensin system on several aspects of renal and cardiac function has been extensively documented, both in human and animal models. However, the effect of chronic ACEi on water balance in humans is poorly documented. Neither the systolic blood pressure nor the diastolic blood pressure was modified by treatment with ACE inhibitors. This is not surprising given that physiological control systems are often redundant; thus, the controlled variable is subject to more than one controller [18].

The treatment for 6 months with ACE inhibitors significantly altered diuresis in children with the protocol of free water intake. Although urine osmolality was similar, the amount of osmotically active solutes removed with this protocol was greater at the expense of increased diuresis. Although not reaching levels of significance, the C_{osm} tended to be higher in children after treatment with enalapril. In human adults, the increase in C_{osm} induced by acute administration of an ACE inhibitor, with captopril, has been described [19].

The effect of chronic inhibition in rats has recently been described showing that AT1 receptor blockade decreases vasopressin-induced water reabsorption and aquaporin 2 levels in NaCl-restricted rats [20]. Ang II stimulates the activity of the cotransporter $Na^+ K^+ 2Cl^-$ in the medullary thick ascending limb of Henle [21]. It is therefore possible that chronic inhibition of the formation of Ang II increases the supply of solutes to the medullary collecting duct, which is the C_{osm}. This raises the question whether ACE inhibitors should be considered as an osmotic diuretic like acetazolamide, mannitol and furosemide. However, ACE inhibitors did not increase urinary potassium excretion [22, 23], as observed in this work, while the other osmotic diuretics do.

In the protocol with water restriction in children, a number of observations appeared that must be analyzed. First, the urinary minute volume after the dry diet in patients treated with enalapril was not reduced as before it. Second, urinary osmolality, resulting from the ability to concentrate, seemed affected as the maximum osmolality achieved in children treated with enalapril was less compared to that observed before treatment with an ACE inhibitor, and third, the urinary excretion of ADH is higher in patients treated with enalapril. This result is not consistent with the fact that Ang II stimulates the production of this hormone [24, 25]. Our results suggest that treatment with enalapril induces a latent state of dehydration so that when children are subjected to the stress of water deprivation, they have an excessive response to the release of ADH. Moreover, if we consider that the urine osmolality was lower, we must consider other factors in the response to water restriction. It is possible that treatment with ACE inhibitors leads to a medullary washing, which, coupled with the relatively low protein intake (these children had their protein diet restricted to anabolism) plus the likely increase in renal prostaglandin production, Ang 1–8, kinins and other vasodilator peptides, should affect the ability to concentrate [26]. This is consistent with the observations made in children with free access to water, which detects a significantly higher diuresis. The concentration of ADH corrected by osmolality found that after treatment with enalapril there is a higher degree of ADH per osmole generated in the process of urine concentration. Assuming that in the tubular fluid ADH comes exclusively from glomerular filtration, it could be concluded that the plasma concentration of ADH is increased by the effect of enalapril. The present results suggest that the response to ADH is less effective after treatment with ACE inhibitors [16, 27, 28]. It has been suggested that ACE inhibitor therapy affects the ability to concentrate urine, and the results presented here suggest that children under treatment showed a slight decrease that was evident under conditions of water restriction. After the overload of water we observed a decline of creatinine clearance. Edwards et al. [29] found no effect of ADH on isolated rabbit afferent arterioles but a vasoconstrictor effect on the efferent arteriole through V1 receptors. Thus, an inhibition of ADH production could lead to a decrease in efferent arteriolar resistance, a fall in glomerular capillary pressure and consequently the fall in glomerular filtration rate [24]. The ability of the kidneys of children treated with ACE inhibitors to get rid of fluid overload is clearly more effective. The generation of a larger positive solute-free water clearance was clearly

observed. This must be reflecting the increased inhibition of solute transport in the segments before the collecting duct [30]. Solute delivery to the corticomedullary junction and Ang II stimulate the reabsorption of solutes in this segment [31]. Thus, inhibition of Ang II synthesis may contribute to this increase in C_{osm}. If Ang II is involved in maintaining the unique effect, inhibition of its formation can lead to an increase in the dissipation of the osmotic gradient, affecting water reabsorption in the medullary collecting duct [30].

In conclusion, patients with chronic ACEi showed a slight reduction in the ability to concentrate the urine, with larger levels of ADH and an apparent reduction in the efficiency of ADH to reabsorb water. No changes were seen in other renal function parameters, except the decrease in glomerular filtration rate with fluid overload and decreased excretion of potassium in a water stress situation. Interestingly, this could explain the increased risk of hyperkalemia in states of prolonged oliguria [unpubl. obs.].

As it was shown in this paper, ACEi induces a state of relative water imbalance with propensity to reduce, under certain circumstances, the capacity to concentrate urine. These observations should be kept in mind in populations at risk like children and aged people exposed to diseases that can affect water and salt balance. This is particularly relevant in those conditions in which ACEi is used in combination with nonsteroidal anti-inflammatory drugs.

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References

1 Anderson S, Rennke HG, Brenner BM: Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. J Clin Invest 1986;77:1993–2000.
2 Heeg JE, de Jong PE, van der Hem GK, de Zeeuw D: Reduction of proteinuria by angiotensin converting enzyme inhibition. Kidney Int 1987;32:78–83.
3 Brown GP, Douglas JG: Angiotensin II binding sites on isolated rat brush border membranes. Endocrinology 1983;112:2007–2014.
4 Fitzsimons JT: Angiotensin, thirst, and sodium appetite. Physiol Rev 1998;78:583–686.
5 Gross PM: The subfornical organ as a model of neurohumoral integration. Brain Res Bull 1985;15:65–70.
6 MacLaughlin M, Monserrat AJ, Muller A, Matoso M, Amorena C: Role of kinins in the renoprotective effect of angiotensin-converting enzyme inhibitors in experimental chronic renal failure. Kidney Blood Press Res 1998;21:329–334.
7 Fregly MJ, Rowland NE: Bradykinin-induced dipsogenesis in captopril-treated rats. Brain Res Bull 1991;26:169–172.
8 Gallo EG, Gianantonio CA: Extrarenal involvement in diarrhoea-associated haemolytic-uraemic syndrome. Pediatr Nephrol 1995;9:117–119.
9 Update on the 1987 Task Force report on high blood pressure in children and adolescents: a working group report from the National High Blood Pressure Education Program. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. Pediatrics 1996;98:649–658.
10 Dieguez S, Ayuso S, Brindo M, Osinde E, Cánepa C: Renal functional reserve evolution in children with a previous episode of hemolytic uremic syndrome. Nephron Clin Pract 2004;97:c118–c122.
11 Hilbrands LB, Artz MA, Wetzels JF, Koene RA: Cimetidine improves the reliability of creatinine as a marker of glomerular filtration. Kidney Int 1991;40:1171–1176.
12 Medel R, Dieguez S, Brindo M, Ayuso S, Canepa C, Ruarte A, Podesta ML: Monosymptomatic primary enuresis: differences between patients responding or not responding to oral desmopressin. Br J Urol 1998;81(suppl 3):46–49.
13 Tobias JD: Controlled hypotension in children: a critical review of available agents. Paediatr Drugs 2002;4:439–453.
14 Subcommittee on the Tenth Edition of the RDAs Food and Nutrition Board Commission on Life Sciences: National Research Council Recommended Dietary Allowances, ed 10. Washington, National Academy Press, 1989.
15 Maroni BJ, Steinman TI, Mitch WE: A method for estimating nitrogen intake of patients with chronic renal failure. Kidney Int 1985;27:58–65.
16 Edelmann CM Jr, Barnett HL, Stark H, Boichis H, Soriano JR: A standardized test of renal concentrating capacity in children. Am J Dis Child 1967;114:639–644.
17 Chaimovitz C, Levi J, Better OS, Oslander L, Benderli A: Studies on the site of renal salt loss in a patient with Bartter's syndrome. Pediatr Res 1973;7:89–94.
18 Schmaier AH: The kallikrein-kinin and the renin-angiotensin systems have a multilayered interaction. Am J Physiol Regul Integr Comp Physiol 2003;285:R1–R13.
19 Usberti M, Di Minno G, Ungaro B, Cianciaruso B, Federico S, Ardillo G, Gargiulo A, Martucci F, Pannain M, Cerbone AM, et al: Angiotensin II inhibition with captopril on plasma ADH, PG synthesis, and renal function in humans. Am J Physiol 1986;250:F986–F990.
20 Kwon TH, Nielsen J, Knepper MA, Frokiaer J, Nielsen S: Angiotensin II AT1 receptor blockade decreases vasopressin-induced water reabsorption and AQP2 levels in NaCl-restricted rats. Am J Physiol Renal Physiol 2005;288:F673–F684.
21 Kovacs G, Peti-Peterdi J, Rosivall L, Bell PD: Angiotensin II directly stimulates macula densa Na-2Cl-K cotransport via apical AT(1) receptors. Am J Physiol Renal Physiol 2002;282:F301–F306.
22 Preston RA, Baltodano NM, Alonso AB, Epstein MJ: Comparative effects on dynamic renal potassium excretion of ACE inhibition versus angiotensin receptor blockade in hypertensive patients with type II diabetes mellitus. Clin Pharmacol 2002;42:754–761.
23 Husted RF, Laplace JR, Stokes JB: Enhancement of electrogenic Na^+ transport across rat inner medullary collecting duct by glucocorticoid and by mineralocorticoid hormones. J Clin Invest 1990;86:498–506.
24 Kaczmarczyk G, Vogel S, Krebs M, Bunger H, Scholz A: Vasopressin and renin-angiotensin maintain arterial pressure during PEEP in nonexpanded, conscious dogs. Am J Physiol 1996;271:R1396–R1402.

25 Sadowski J, Badzynska B: Intrarenal vasodilator systems: NO, prostaglandins and bradykinin: an integrative approach. J Physiol Pharmacol 2008;59(suppl 9):105–119.
26 Gabrielsen A, Bie P, Holstein-Rathlou NH, Christensen NJ, Warberg J, Dige-Petersen H, Frandsen E, Galatius S, Pump B, Sørensen VB, Kastrup J, Norsk P: Neuroendocrine and renal effects of intravascular volume expansion in compensated heart failure. Am J Physiol Regul Integr Comp Physiol 2001; 281:R459–R467.
27 Agnoli GC, Borgatti R, Cacciari M, Lenzi P, Marinelli M, Stipo L: Renal function and urinary prostanoid excretions in salt-depleted women: comparative effects of enalapril and indomethacin treatments. Prostaglandins Leukot Essent Fatty Acids 1999;60:87–93.
28 Wong NL, Tsui JK: Upregulation of vasopressin V2 and aquaporin 2 in the inner medullary collecting duct of cardiomyopathic hamsters is attenuated by enalapril treatment. Metabolism 2002;51:970–975.
29 Edwards RM, Trizna W, Kinter LB: Renal microvascular effects of vasopressin and vasopressin antagonists. Am J Physiol 1989; 256:F274–F278.
30 Geibel J, Giebisch G, Boron WF: Angiotensin II stimulates both Na^+/H^+ exchange and Na^+/HCO_3^- cotransport in the rabbit proximal tubule. Proc Natl Acad Sci USA 1990;87: 7917–7920.
31 Burton R: Clinical Physiology of Acid Base and Electrolyte Disorders, ed 5. New York, McGraw-Hill, 2001.