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Modesti, Pietro Amedeo, Ilaria Cecioni, Alessandra Naldoni, Angela Migliorini, and Gian Gastone Neri Serneri. Relationship of renin-angiotensin system and ET-1 system activation in long-lasting response to postural changes. *Am. J. Physiol.* 270 (*Heart Circ. Physiol.* 39): H1200–H1206, 1996.—The present study was performed in seven healthy subjects (aged 22–35 years) to investigate *1*) whether plasma and urinary endothelin-1 (ET-1) are involved in the response to postural changes and *2*) the relationship between ET-1 formation and the renin-angiotensin system (RAS). Six hours of standing caused a prompt but very short-lasting increase in plasma ET-1 concentration (59% after 5 min, 12% after 1 h) and a notable and sustained enhancement of urinary ET-1 excretion (from 0.59 + 0.10 to 1.43 + 0.28 pg/min, or 142%; $P < 0.001$). Plasma renin activity increased by 169% after 1 h of standing. A parallel contraction of urinary volume (−62%), sodium excretion (−55%), and free water reabsorption (−24%) occurred. The return to the supine position after 6 h of orthostasis caused a reduction to baseline values of the ET-1 urinary excretion and urinary volume within 2 h. Inhibition of angiotensin-converting enzyme blunted, but did not eliminate, the orthostasis-induced increase in ET-1 urinary excretion (100%, $P < 0.002$) and changes in the renal functions. The present results indicate that renal ET-1 is involved in the hemodynamic long-lasting responses to postural changes and that its increase is partially controlled by RAS and suggest that ET-1 might play a role in the regulation of renal function in humans.

posture; water-electrolyte balance; blood volume; natriuresis; angiotensin-converting enzyme inhibitors

ENDOTHELIN-1 (ET-1) is a vasoconstrictor peptide synthesized by endothelial cells (36) and other cell types, including those of lung, kidney, gut, and neurons of the paraventricular nucleus of the hypothalamus (14, 20, 26, 36). The ET-1 peptide and the mRNA of its precursor, preproendothelin-1, are widespread in the endothelium of the renal vascular bed and in the inner medullary collecting ducts (20, 23, 27, 33, 37). Immunocytochemical studies suggest that renal ET-1 secretion occurs at the vascular endothelium of arcuate arteries, veins, glomerular capillaries and arterioles, peritubular capillaries, and vasa recta of distal nephron segments (34). In addition to the vascular endothelium, different nephron segments and especially renal tubules secrete abundant amounts of ET-1 (28). Endothelial secretion is highly compartmentalized in tubules, with the following secretion hierarchy: inner medullary collecting ducts > medullary thick ascending limb > cortical collecting ducts ≫ proximal tubule (28). ET-1 receptors have been located in the renal artery and afferent and efferent glomerular arterioles (ET_A receptor), in glomerular endothelium and possibly mesangium (ET_B receptor), and in the inner medullary collecting ducts (5, 10, 16, 30).

In humans the experimental increase or decrease in blood volume was associated with changes of both ET-1 concentration in plasma and ET-1 urinary excretion (22), suggesting ET-1 involvement in the regulation of blood volume. The assumption of a standing position is associated with important changes in central blood volume, sympathetic activity reflex, cardiac dynamics, and the tension of the vascular walls of peripheral capacity vessels (1). Renal plasma flow and glomerular filtration rate (GFR) decrease, leading to a reduction in urinary volume and sodium excretion. There is strong evidence that changes of water and mineral excretion are induced by changes in baroreceptor activity, with the participation of hormonal factors including angiotensin (ANG) II, aldosterone, and antidiuretic hormone (1). ET-1 is provided with important regulatory activities of renal function. When infused in animals, a low dose of ET-1 causes renal vasoconstriction and decreases renal blood flow and GFR (9, 13, 35). These effects are associated with reduced sodium excretion (3) and activation of renin and aldosterone release (18, 21). Moreover, ET-1 plasma concentration has been reported to undergo a prompt but short-lasting increase during 30-min tilting test (12, 29). Together, these results suggest that ET-1 may be involved in the adaptative responses to postural changes.

The present study was aimed at investigating whether plasma and/or renal ET-1 are involved in the response to postural changes and the relationship between ET-1 renal production and the renin-angiotensin system (RAS) in the orthostasis-induced changes in renal functions.

METHODS

Subjects Investigated

Seven healthy subjects aged 22–35 years (4 males and 3 females) were studied. Experimental procedures and the purposes of the study were explained to the subjects and all gave their informed consent. No subject was a smoker, had taken any drug for at least 4 wk, or had any positive family history of hypertension or diabetes.

All the subjects were first examined for serum electrolytes, fasting plasma glucose, and creatinine clearance, and urinalysis was performed. No pathological finding was detected, and creatinine clearance ranged within commonly accepted normal values.

Experimental Protocol

All subjects received a diet containing 108 meq/day NaCl (normal sodium diet) throughout the study period. All sub-

jects abstained from caffeine, alcohol, and smoking during all the experimental days.

The study was divided into two parts: first, the study of the changes of ET-1 in relation to postural changes, and second, the study of the relationship between ET-1 formation and RAS.

Changes of ET-1 in relation to posture. The subjects, after overnight fasting and recumbency, remained supine or assumed orthostasis for different durations. During each experimental period all the subjects were given hourly fluid replacement with tap water equal in volume to the sum of blood withdrawn, the insensible loss (30 ml/h), and the previous hour's urine output.

At 8 AM on the first day of the experiment, subjects were requested to remain in the supine position for another 12 h (until 8 PM). On the second day of the experiment, at 8 AM, subjects were requested to assume the orthostatic position and to maintain it until 8 PM. During this day all subjects were allowed to walk in the hospital setting. While remaining in the standing position they consumed a light lunch and dinner at 12 noon and 7 PM, respectively. During the third day of the experiment, subjects assumed the upright position from 8 AM to 2 PM and then returned to bed in the supine position until 8 PM. The sequence of these three periods was randomized.

Blood withdrawal was performed at baseline (8 AM) and repeated after 5, 10, 20, 40, and 60 min and then every 2 h for each experimental day. Plasma samples for the measurements of plasma renin activity were collected at baseline and after 1, 2, 6, and 12 h. Urinary samples for ET-1 and creatinine determination were taken at baseline (from 8 PM to 8 AM) and then every hour during each day of the experiment.

In addition, the clearances of creatinine, lithium, and free water, urinary volume, and sodium excretion were assessed on two separate days at 8 AM and at 2 PM after 6-h periods in the supine and in the standing positions. The day before the study all the subjects were administered lithium carbonate (500 mg po) at 11 PM.

Relationship between ET-1 formation and RAS. To investigate the relationship between ET-1 formation and the activity of RAS, the same subjects previously investigated were treated with ramipril (5 mg po at 7 AM), an inhibitor of angiotensin-converting enzyme (ACE), whose activity was reported to last for ~24 h after dosing (11).

ET-1 plasma concentration as well as ET-1 urinary excretion, the clearances of creatinine, lithium, and free water, urinary volume, and total sodium excretion were measured on two separate days at baseline (8 AM) and after 6 h in the supine or in the standing position.

Sampling and Extraction Procedures

Sample collection and extraction of ET-1 were performed as previously described (22). Briefly, samples of blood (10 ml) for ET-1 measurement were withdrawn in polypropylene tubes chilled to 0°C containing 0.5 ml of an inhibitory solution (final concentrations in blood: 1 mg/ml EDTA, 500 kallikrein inhibitor units/ml aprotinin; EDTA, Carlo Erba, Milan, Italy; Trasylol, Bayer, Germany). The blood specimens were then centrifuged at 3,000 *g* for 10 min at 4°C; the plasma was immediately frozen and stored at −80°C, and extraction was performed within 2 days. Urine samples were collected, immediately frozen, and stored at −80°C. The urine was also extracted within 2 days.

For extraction, plasma (5 ml) was applied to a Sep-Pak octadecyl-silane-C_{18} disposable column (Waters, Milford, MA) previously activated by consecutive washings with 3 ml of 100% methanol, 3 ml of 10% methanol, 3 ml of 20% methanol, 3 ml of 85% methanol, and 4 ml of 10% methanol. The column was then washed with 4 ml of 20% methanol, and the adsorbed peptide was eluted with 3 ml of 85% methanol. The eluates were evaporated until dry in a centrifugal concentrator (CT60e, Heto, Birkerod, Denmark) and stored at −80°C until the radioimmunoassay (RIA) was performed.

A 5-ml aliquot of each urine sample was run on a Sep-Pak octadecyl-silane-C_{18} disposable column previously activated with 10 ml of acetonitrile-0.1% trifluoroacetic acid (99.9:0.1 vol/vol). The column was then washed with 10 ml of water, and the adsorbed peptide was eluted with 2 ml of acetonitrile-0.1% trifluoroacetic acid (60:40). The elution fraction was lyophilized, and the dried residue was stored at −80°C until assayed.

The ET-1 recovery rates, calculated by the addition of different concentrations of cold ET-1, were 70 ± 9% from plasma and 90 ± 5% from urine.

High-Performance Liquid Chromatography of ET-1

To assess changes in the elution profile of plasma and urinary ET-1 induced by orthostasis, plasma- and urine-extracted samples were chromatographed as previously described (22). Briefly, vacuum-dried plasma (10 ml) and urine (10 ml) extracts were dissolved in 0.5 ml of 70% mobile phase A (0.1% trifluoroacetic acid in water)/30% mobile phase B (0.1% trifluoroacetic acid in acetonitrile) and centrifuged at 1,700 *g* for 10 min. A 250-μl aliquot of supernatant was then injected with a Hamilton syringe (Hamilton, Bonaduz, Switzerland) in a model U6K injector (Waters, Milford, MA) equipped with a 2,000-μl loop and connected to a reverse-phase μBondapak C_{18} column (3.9 × 300 mm, Waters, Milford, MA). The column was previously calibrated with pure standard unlabeled ET-1 and ^{125}I-labeled ET-1 (^{125}I-ET-1; 1,300 Ci/mmol, Peninsula, Belmont, CA). The purity of the ^{125}I-ET-1 was preliminarily checked by injection into the high-performance liquid chromatography (HPLC) system. The HPLC equipment consisted of two model 6000A pumps, a model 660 solvent programmer (Waters, Milford, MA), and a fraction collector (7000 Ultrorac LKB, Bromma, Sweden). The chromatography was performed by isocratic elution with 70% mobile phase A (0.1% trifluoroacetic acid in water)/30% mobile phase B (0.1% trifluoroacetic acid in acetonitrile) from 0 to 10 min followed by a linear gradient to 40% mobile phase A/60% mobile phase B up to 40 min at a flow rate of 1.0 ml/min. The experiments were performed at room temperature. The eluate was collected in 1-min fractions in polypropylene tubes.

The concentrations of ^{125}I-ET-1 in the HPLC fractions were measured in a gamma counter (Beckman, Fullerton, CA). The concentrations of ET-1 were measured by RIA.

RIA of ET-1

ET-1 immunoreactivity was measured by a specific RIA for ET-1 using rabbit polyclonal anti-ET-1 serum as previously described (22). Briefly, the extracted samples were resuspended in phosphate buffer immediately before the assay was performed. The standard ET-1 (Peninsula, Belmont, CA) or plasma and urine samples (100 μl) were mixed with 100 μl of rabbit anti-ET-1 serum (Peninsula, Belmont, CA), diluted with RIA buffer at a final dilution of 1:24,000 for urine and 1:72,000 for plasma and HPLC fractions. Antibody cross-reactivities were 100% with ET-1 (porcine, human), 7% with endothelin-2 (human), 7% with endothelin-3 (rat, human), 35% with Big endothelin (porcine), 17% with Big endothelin (human), and 3% with sarafotoxin S6b. There was no cross-reactivity with Big endothelin 22–38 (human), alpha-human

atrial natriuretic peptide, brain natriuretic peptide (porcine), ANG I, ANG II, ANG III, vasopressin, adrenocorticotropic hormone, or vasoactive intestinal peptide. The minimum detectable concentration was 0.1 pg/tube. The coefficients of intra-assay and interassay variations were 4% (n = 11) and 10% (n = 11) for plasma and 3% (n = 11) and 12% (n = 11) for urine, respectively. Results were expressed as picograms per milliliter for plasma and picograms per minute for urine.

Analytical Methods and Calculations

Plasma and urine were assayed for electrolytes (P_{Na}, plasma sodium; U_{Na}, urinary sodium; P_{Li}, plasma lithium; U_{Li}, urinary lithium) by an ion-sensitive electrode (Instrumentation Beckmann Astra, Beckmann Instruments, Brea, CA) and for creatinine by the Jaffe reaction (Instrumentation Beckmann Astra). Osmolar clearance (C_{osm}), lithium clearance (C_{Li}), and creatinine clearance (C_{cr}) were calculated with the use of standard formulas. Free water clearance (C_{H_2O}) was calculated as

$$C_{H_2O} = (1 - U_{osm}/P_{osm}) \times V$$

where U_{osm} (mosmol/kgH_2O) and P_{osm} (mosmol/kgH_2O) are the simultaneous urinary and plasma osmolalities, respectively, and V (ml/min) is the simultaneous rate of urinary flow. Negative values of C_{H_2O} occur when the urine is hypertonic to the plasma, and, by convention, these negative values are referred to as free water reabsorption (T_{H_2O}) where

$$T_{H_2O} = -C_{H_2O}$$

GFR was estimated by the clearance of endogenous creatinine. Delivery of proximal tubular fluid to the distal nephron, i.e., distal delivery filtrate (DDF), was approximated by V factored by GFR (4, 27). The relationship between T_{H_2O} and DDF in supine and standing positions was calculated.

Blood samples for plasma renin activity were collected in ice-cold tubes and immediately centrifuged. Plasma samples were then stored at −20°C. Plasma renin activity was then measured by RIA.

Statistical Analysis

If not otherwise indicated, data are presented as means ± SD. Comparisons of a single observation between groups were made with Student's t-test. Repeated observations over the experimental days were analyzed by analysis of variance for repeated measures (ANOVA). The relationship between T_{H_2O}/GFR and V/GFR was evaluated by simple regression analysis. All statistical analyses were performed with BMDP statistical software (BMDP Statistical Software, Los Angeles, CA).

RESULTS

Changes of ET-1 in Relation to Posture

Plasma concentration and urinary excretion of ET-1 in the supine position remained steady throughout the 12-h study period (Tables 1 and 2, Fig. 1). During the first 6 h, the mean ET-1 urinary excretion was 0.59 ± 0.10 pg/min. The parameters of the renal function measured after the 6-h supine position are reported in Table 3.

After standing position was assumed, ET-1 concentration in plasma significantly increased after 5 min (from 0.51 ± 0.12 at baseline to 0.81 ± 0.20 pg/ml, $P < 0.05$), but the increase was very short-lasting, and the differences in comparison with baseline and supine position were no longer significant for all the remaining study period (Table 1). On the contrary, during the first 6-h orthostasis, ET-1 urinary excretion increased and passed from 0.59 ± 0.10 in supine position to 1.43 ± 0.28 pg/min (+142%, $P < 0.001$)(Table 3). When ET-1 urinary excretion was measured hourly, ET-1 renal formation was significantly increased from the first hour of orthostasis and gradually rose, plateauing after 3 h and reaching a value twofold higher than baseline (from 0.59 ± 0.19 to 1.52 ± 0.31 pg/min, an increase of +158%, $P < 0.0001$)(Fig. 1, Table 2) without any further significant hour-to-hour variations ($P < 0.001$ vs. supine by ANOVA test). Analysis of the reverse-phase HPLC profile of ET-1 immunoreactivity in urine samples pooled during the fourth and fifth hour after the assumption of the standing position did not show any significant changes in comparison with baseline (Fig. 2). During the period of standing position, total urinary volume and sodium excretion significantly decreased (Table 3).

Table 1. *Endothelin-1 (ET-1) concentration in plasma and percent changes vs. baseline during three experimental periods*

	Supine		Standing		Standing-Supine	
Hours	ET-1 concn, pg/ml	%Change	ET-1 concn, pg/ml	%Change	ET-1 concn, pg/ml	%Change
8	0.55 ± 0.10		0.51 ± 0.12		0.50 ± 0.10	
9	0.58 ± 0.14	5	0.57 ± 0.13	12	0.59 ± 0.13	18
10	0.56 ± 0.21	2	0.51 ± 0.18	0	0.59 ± 0.12	18
12	0.58 ± 0.11	5	0.55 ± 0.10	8	0.55 ± 0.12	10
14	0.69 ± 0.18	25	0.59 ± 0.16	16	0.52 ± 0.24	4
16	0.54 ± 0.13	−2	0.55 ± 0.11	8	0.53 ± 0.13	6
18	0.57 ± 0.19	4	0.52 ± 0.15	2	0.52 ± 0.15	4
20	0.59 ± 0.07	7	0.58 ± 0.06	14	0.58 ± 0.07	16

Values are means ± SD.

Orthostasis was associated with important changes in plasma renin activity and renal functions (Table 3). Plasma renin activity rose from 0.64 ± 0.19 to 1.72 ± 0.48 and 2.31 ± 0.74 ng ANG $I \cdot h^{-1} \cdot ml^{-1}$ ($P < 0.003$) after 1 and 2 h, respectively, and remained elevated for the entire observation period (2.29 ± 0.57 ng ANG $I \cdot h^{-1} \cdot ml^{-1}$ after 6 h, $P < 0.002$, and 1.7 ± 0.31 ng ANG $I \cdot h^{-1} \cdot ml^{-1}$ after 12 h, $P < 0.01$). Free water reabsorption and lithium clearance were significantly reduced during orthostasis (Table 3). GFR as expressed by creatine clearance slightly, but not significantly, decreased (Table 3).

Free water reabsorption as a fraction of GFR (T_{H_2O}/GFR) was unaffected by orthostasis [not significant (NS)](Table 3). Urinary flow rate measured hourly significantly decreased from the first hour of orthostasis, from 0.99 ± 0.18 at baseline to 0.60 ± 0.15 ($P < 0.005$) and 0.44 ± 0.10 ml/min ($P < 0.0005$ vs. baseline) after 1 and 2 h standing, respectively (Table 4). No further significant hour-to-hour variation was found during the succeeding 4 h of observation ($P < 0.001$ vs. supine by ANOVA test)(Table 4, Fig. 1). Urinary flow as a fraction of GFR (V/GFR) was significantly reduced

Table 2. *ET-1 hourly urinary excretion and percent changes vs. baseline during three experimental periods*

	Supine		Standing		Standing-Supine	
Hours	Hourly urinary excretion, pg/min	%Change	Hourly urinary excretion, pg/min	%Change	Hourly urinary excretion, pg/min	%Change
8	0.63 ± 0.15		0.59 ± 0.19		0.58 ± 0.11	
9	0.57 ± 0.11	−10	1.10 ± 0.30	86*‡	1.09 ± 0.26	87*‡
10	0.57 ± 0.13	−10	1.31 ± 0.35	123†‡	1.29 ± 0.23	123†§
11	0.54 ± 0.16	−14	1.52 ± 0.31	158†§	1.45 ± 0.16	150†§
12	0.65 ± 0.13	3	1.59 ± 0.31	169†§	1.45 ± 0.20	150†§
13	0.62 ± 0.07	−1	1.50 ± 0.31	154†§	1.43 ± 0.18	147†§
14	0.58 ± 0.09	−8	1.58 ± 0.29	167†§	1.43 ± 0.26	147†§
15	0.63 ± 0.10	0	1.57 ± 0.35	166†§	0.91 ± 0.09	56*§
16	0.61 ± 0.12	−3	1.61 ± 0.27	173†§	0.74 ± 0.17	28
17	0.57 ± 0.09	−10	1.61 ± 0.22	173†§	0.65 ± 0.16	13
18	0.64 ± 0.11	1	1.58 ± 0.27	167†§	0.60 ± 0.16	4
19	0.66 ± 0.10	5	1.57 ± 0.36	166†‡	0.63 ± 0.20	8
20	0.66 ± 0.13	4	1.53 ± 0.35	160†‡	0.66 ± 0.16	13

Values are means ± SD. *$P < 0.05$; †$P < 0.001$ vs. baseline (8 AM). ‡$P < 0.05$; §$P < 0.001$ vs. supine position.

during standing ($P < 0.003$)(Table 3). After the standing position was assumed, the relationship of T_{H_2O}/GFR as a function of urinary flow linearized (Fig. 3).

When the subjects returned to the supine position after 6 h of orthostasis, plasma ET-1 did not significantly change, whereas urinary ET-1 excretion promptly decreased, and after 2 h it was no longer different from that observed at the same time during the 12-h supine period (Table 2, Fig. 1). Urinary flow rate increased from 0.60 ± 0.21 to 0.81 ± 0.28 ml/min after 1 h ($P <$ 0.05 vs. orthostasis) and to 0.94 ± 0.14 ml/min after 2 h ($P < 0.01$)(Table 4). During the remaining period in the supine position no significant differences were found in comparison with the values observed during the 12-h period in the supine position (Table 4, Fig. 1).

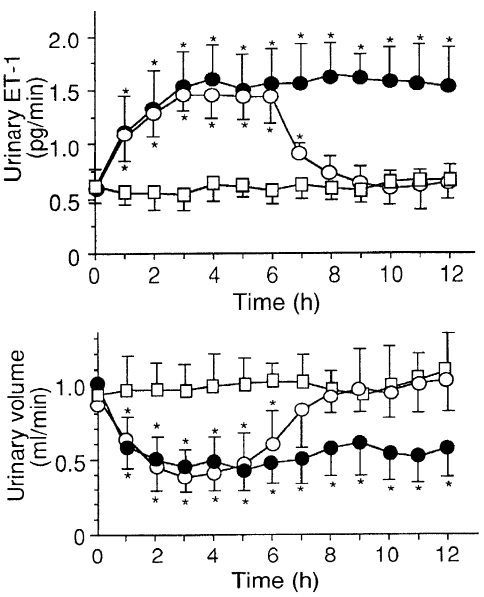

Fig. 1. Hourly urinary endothelin-1 (ET-1) excretion and urinary volumes during 12 h of supine position (□), 12 h of upright position (●), and 6 h of orthostasis followed by 6 h of supine position (○) in healthy subjects ($n = 7$). *$P < 0.05$ vs. supine position.

Relationship Between ET-1 Formation and RAS

Ramipril administration to subjects in supine position caused the expected inhibitory effects on RAS. Plasma renin activity increased from 0.78 ± 0.28 to 1.12 ± 0.38 ng ANG I·h^{-1}·ml^{-1} ($P < 0.01$). Inhibition of RAS in the supine position did not change ET-1 concentration in plasma or ET-1 urinary excretion in comparison with placebo. On the contrary, the 6-h urinary flow and sodium excretion rates significantly increased to 1.94 ± 0.64 ml/min (+48%, $P < 0.05$) and 0.24 ± 0.04 meq/min (+33%, $P < 0.05$), respectively. Creatine clearance slightly but nonsignificantly decreased (106 ± 25 ml/min, −14%; NS). Lithium clearance and free water reabsorption increased on average ~48 and ~59% ($P <$ 0.02 and $P < 0.03$, respectively)(Table 3). T_{H_2O}/GFR and V/GFR were both significantly increased ($P < 0.01$ and $P < 0.02$, respectively) in comparison with the supine position after placebo. Ramipril did not alter the relationship between T_{H_2O}/GFR and V/GFR in the supine position.

The increase in ET-1 urinary excretion induced by orthostasis was attenuated but not eliminated by ramipril administration. In fact, the mean ET-1 urinary excretion, which was 1.43 ± 0.28 pg/min during orthostasis with placebo, decreased to 1.04 ± 0.20 pg/min during orthostasis with ramipril ($P < 0.01$), with a mean increase of 100% over ET-1 excreted during the 6-h supine position after ramipril administration (0.52 ± 0.18 pg/min, $P < 0.002$)(Table 3). Also, the changes in renal function induced by orthostasis were attenuated but not eliminated by the administration of ramipril (Table 3, Fig. 3). More precisely, urinary flow and sodium excretion rates decreased to 0.62 ± 0.21 ml/min (−68%, $P < 0.05$) and 0.16 ± 0.06 meq/min (−33%, $P < 0.05$), respectively. GFR did not significantly change (101 ± 12 ml/min, −5%; NS), whereas lithium clearance and free water reabsorption decreased to 21 ± 3 ml/min (−44%, $P < 0.02$) and 1.05 ± 0.17 ml/min (−46%, $P < 0.05$), respectively (Table 3). T_{H_2O}/GFR significantly decreased ($P < 0.01$) and V/GFR similarly decreased ($P < 0.007$)(Table 3). The slope of

Table 3. *Renal response to 6-h standing after placebo and ramipril (5 mg) administration*

	Placebo			Ramipril		
	Supine	Standing	%Difference	Supine	Standing	%Difference
ET-1 excretion rate, pg/min	0.59 ± 0.10	1.43 ± 0.28†	142	0.52 ± 0.18	1.04 ± 0.20*‡	100
V, ml/min	1.31 ± 0.50	0.50 ± 0.27*	−62	1.94 ± 0.64‡	0.62 ± 0.21*	−68
U_{Na}, meq/min	0.18 ± 0.06	0.08 ± 0.03*	−55	0.24 ± 0.04‡	0.16 ± 0.06*‡	−33
GFR, ml/min	123 + 25	102 + 21	−17	106 ± 25	101 ± 12	−5
C_{Li}, ml/min	25 ± 5	15 ± 5†	−39	37 ± 12‡	21 ± 3*‡	−44
C_{osm}, ml/min	2.54 ± 0.64	1.44 ± 0.60*	−43	3.89 ± 0.94‡	1.67 ± 0.34*‡	−57
C_{H_2O}, ml/min	−1.2 ± 0.3	−0.9 ± 0.3*	24	−1.9 ± 0.8‡	−1.1 ± 0.2*‡	46
T_{H_2O}, ml/min	1.22 ± 0.32	0.93 ± 0.32*	−24	1.94 ± 0.82‡	1.05 ⊥ 0.17*‡	−46
T_{H_2O}/100 ml GFR, ml/min	1.00 ± 0.28	0.96 ± 0.43	−4	1.85 ± 0.52‡	1.05 ± 0.20*	−43
V/100 ml GFR, ml/min	1.11 ± 0.56	0.52 ± 0.34*	−53	1.83 ± 1.04‡	0.63 ± 0.28*	−65

Values are means ± SD. V, urinary volume flow rate; U_{Na}, urinary sodium excretion; GFR, glomerular filtration rate; C_{Li}, lithium clearance; C_{osm}, osmolar clearance; C_{H_2O}, free water clearance; T_{H_2O}, free water reabsorption. *$P < 0.05$; †$P < 0.001$ standing vs. supine. ‡$P < 0.05$ ramipril day vs. placebo day.

this relationship differed between placebo and ramipril (Fig. 3).

Thus the inhibition of RAS did not eliminate either the increase of ET-1 excretion or the changes of renal function induced by orthostasis.

DISCUSSION

The present results indicate that urinary ET-1 excretion significantly increases when the subjects assume the standing position, whereas ET-1 plasma concentration substantially does not change. An important corollary arising from this completely different pattern is new evidence that plasma and urinary ET-1 are the expression of two different functional systems (22).

The very short-lasting increase in ET-1 plasma concentration after orthostasis agrees with a previous observation (29) of a rapid but unsustained increase in plasma ET-1 concentration after 5 min of upright tilting. The absence of any significant increase in ET-1 plasma concentration during prolonged standing, as in the present study, indicates that ET-1 arising from vascular endothelial cells plays a minor role in the occurrence of the complex cardiovascular adjustments to postural changes. However, a definite conclusion cannot be drawn, because ET-1 may work in a paracrine way and its variations in plasma concentration might not be adequate to demonstrate the formation and release of ET-1 (8).

Renal ET-1 seems to be involved in the prolonged response to postural changes. The assumption of upright position was associated with a marked increase in the excretion of urinary ET-1, which remained high throughout the duration of standing. When the subjects returned to the supine position, ET-1 urinary excretion returned to baseline values.

The increased urinary ET-1 excretion independent of the ET-1 plasma concentration supports a renal source of urinary ET-1. A growing body of evidence has shown that both cortex and renal medulla are important sites of ET-1 synthesis (25). Immunoreactive ET-1-like material and preproendothelin-1 mRNA have been found in human renal medulla, in capillary network cells of vasa recta, and in medullary collecting ducts (15, 23, 24).

The modifications of ET-1 urinary excretion related to postural changes were consistent with those of plasma renin activity, and when the RAS was inhibited by ramipril administration the ET-1 urinary excretion significantly lowered, indicating that the RAS operates an important control on the renal ET-1 formation. Thus this finding seems to confirm in humans the observation that ANG II stimulates ET-1 formation (6, 17, 38) in cultured endothelial and mesangial cells.

The control of RAS on ET-1 renal formation was more evident when the RAS was activated, as occurs during orthostasis, because the inhibition of RAS in the supine position caused only a slight, nonsignificant (−12%)

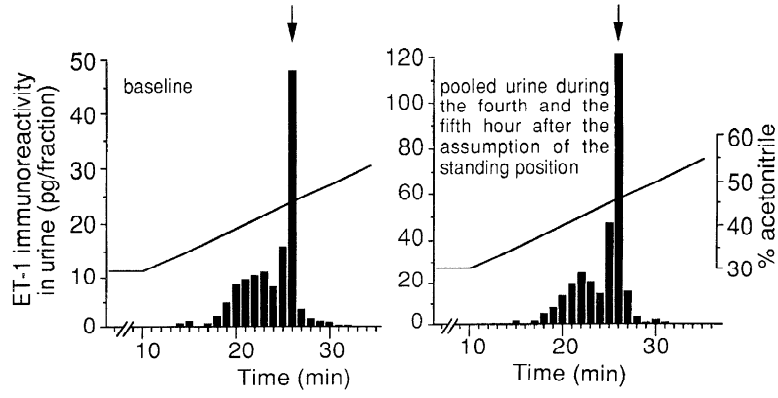

Fig. 2. Reverse-phase high-performance liquid chromatography profile of ET-1 immunoreactivity in human urinary extracts before and after 4–5 h of upright position. Filled columns indicate concentrations of ET-1 immunoreactivity in each fraction. *Arrows*, elution position of standard ET-1. *Solid line*, a linear gradient of acetonitrile from 30 to 60%.

Table 4. *Hourly urinary flow rate (V) and percent changes vs. baseline during three experimental periods*

	Supine		Standing		Standing-Supine	
Hours	V, ml/min	%Change	V, ml/min	%Change	V, ml/min	%Change
8	0.93 ± 0.14		0.99 ± 0.18		0.91 ± 0.07	
9	0.96 ± 0.21	3	0.60 ± 0.15	−39*‡	0.61 ± 0.16	−33*‡
10	0.96 ± 0.17	3	0.44 ± 0.10	−56†§	0.44 ± 0.15	−52†§
11	0.96 ± 0.16	3	0.45 ± 0.11	−55†§	0.40 ± 0.12	−56†§
12	0.99 ± 0.20	6	0.49 ± 0.15	−51†§	0.42 ± 0.13	−54†§
13	1.01 ± 0.16	9	0.47 ± 0.13	−53†§	0.47 ± 0.19	−48*§
14	1.02 ± 0.12	10	0.48 ± 0.14	−52*§	0.60 ± 0.21	−34*‡
15	1.01 ± 0.18	9	0.49 ± 0.15	−51†§	0.81 ± 0.28	−11
16	1.00 ± 0.11	8	0.57 ± 0.17	−42†§	0.94 ± 0.14	3
17	0.91 ± 0.17	−2	0.61 ± 0.20	−38*‡	1.00 ± 0.27	10
18	0.99 ± 0.18	6	0.54 ± 0.17	−45†§	0.98 ± 0.26	8
19	1.01 ± 0.18	9	0.51 ± 0.16	−48†§	1.02 ± 0.22	12
20	1.03 ± 0.21	11	0.57 ± 0.19	−42*‡	1.08 ± 0.24	19

Values are means ± SD. *$P < 0.05$; †$P < 0.001$ vs. baseline (8 AM). ‡$P < 0.05$; §$P < 0.001$ vs. supine position.

reduction in ET-1 urinary excretion, whereas the inhibition of RAS during orthostasis was associated with a significant reduction (−27%) in ET-1 urinary excretion. However, it is important to stress that the ET-1 urinary excretion markedly increased during standing (+100%), even in the presence of RAS inhibition. Thus the involvement of ET-1 in the postural changes parallels but is largely independent of the RAS activation. Changes in renal functions following standing are characterized by reduction in creatinine and lithium clearances and by the contraction of urinary volume and sodium excretion associated with an increased free water clearance. These functional changes agree with the well-known changes in plasma volume following the standing position (7, 19), considered as a consequence of the redistribution of blood to the arms and legs (32) and renal blood flow decrease (1, 32). The inhibition of RAS by ramipril administration did not cancel these changes but reset them to a lower level. The evident changes in renal function following the assumption of standing position were associated with a marked increase of urinary ET-1 excretion (+100%), suggesting a participation of renal ET-1 in renal functional adaptation to postural changes independently of the activity of RAS. In this context, the effects of renal ET-1 on renal functions are quite similar to (3) but largely independent of those of RAS (2).

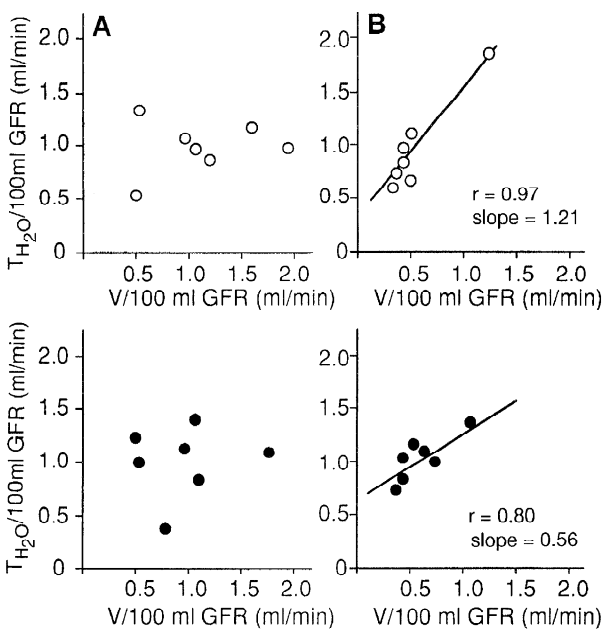

Fig. 3. Free water reabsorption (T_{H_2O}) plotted as a function of distal delivery of filtrate, estimated by urine flow rate (V), using data from placebo (○) and ramipril day (●) in supine (*A*) and erect posture (*B*). T_{H_2O} and V are factored by glomerular filtration rate (GFR, creatinine clearance) to correct for small variations in filtration rate.

The present results do not allow insight into the mechanism(s) responsible for the increased ET-1 renal formation. ET-1 concentration in renal venous blood was not measured, so we are unable to determine whether orthostasis, by reducing renal blood flow (1), may stimulate ET-1 formation by vascular endothelium. Upright posture has been reported to stimulate arginine vasopressin peptide (AVP)(1, 12). The redistribution of relationship between free water reabsorption and urinary flow rate induced by orthostasis suggests a release of AVP (31). The reduced slope of the plot during orthostasis after ramipril may suggest a reduced AVP activity consequent to RAS inhibition (2). Specifically designed investigations are needed to clarify this issue. Moreover, the present results are not able to establish whether renal ET-1 is responsible for the renal functional adaptations to orthostasis because these might be consequent to both the modifications in baroreceptor activity and neurohormonal changes related to postural changes (1). Only by the use of specific ET-1 antagonists can this problem be clarified.

In conclusion, the present findings indicate both that renal ET-1 excretion is augmented in the standing position and that ET-1 renal formation is partially controlled by RAS. The renal functional adaptations to postural changes remain very clear even after RAS inhibition and are associated with a consistent increase in renal ET-1.

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