The relation between fibrinolytic system and the renin-angiotensin system

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Abstract: Several lines of evidence point to an interrelation of the renin-angiotensin system (RAS) with the endogenous fibrinolytic system. This study was planned to examine the effect of salt depletion as a method of activation of the endogenous RAS on plasma fibrinolytic balance in 10 healthy human subjects in the presence and absence of angiotensin converting enzyme inhibitor-ACEi (captopril). Activation of the RAS during low salt intake was documented by a significant increase in serum aldosterone concentration. The data suggest that activation of the RAS results in increased plasminogen activator inhibitors (PAI-I) antigen and that interruption of the RAS with the ACE inhibitor captopril significantly lowers PAI-I antigen without lowering tissue-type plasminogen activator (t-PA) antigen. In conclusion, this study provides an evidence of a direct functional link between the RAS and the fibrinolytic system in humans and these findings may help to elucidate possible mechanisms by which ACE inhibition exerts vasculo protective effects and reduces the risk of atherothrombotic events.

Introduction

Impaired fibrinolytic function, characterized by increased plasminogen activator inhibitor type 1 (PAI-1) levels and decreased tissue plasminogen activator (t-PA) activity, has been found in patients with hypertension and may account in part for the increased risk of atherosclerosis and its clinical complications in these patients (1, 2). The balance between plasminogen activators and plasminogen activator inhibitors is a major determinant of net fibrinolytic activity (3, 4). Although the regulation of PAI-1 in vitro has been studied (5 - 7), factors that control the production and secretion of PAI-1 in vivo are less well characterized. The renin-angiotensin and fibrinolytic systems both play critical roles in cardiovascular homeostasis. Angiotensin II is involved mainly in blood pressure control and the handling of salt and water, thereby preventing the deleterious effects of hemorrhage. Angiotensin converting enzyme (ACE) inhibition has clearly been shown to improve prognosis in patients with congestive heart failure (8) and following myocardial infarction (9, 10).

This interrelationship may involve mechanisms other than changes in arterial blood pressure. In addition to various possible interactions, accumulating evidence suggests that the renin-angiotensin system is involved in the regulation of the fibrinolytic system (11). The mechanisms through which activation of the RAS increases or ACE inhibition reduces the risk of ischemic cardiovascular events in selected populations are not known. Angiotensin II has been suggested to mediate this interrelationship because this peptide was shown to stimulate plasminogen activator inhibitor-1 (PAI-1) in experimental settings (12). However, evidence from studies in man regarding effects of Angiotensin II on fibrinolytic function remains controversial. To test this hypothesis, this study was planned to examine the effect of salt depletion on tissue-type plasminogen activator antigen and plasminogen activator inhibitor-1 antigen in normotensive subjects in the presence and absence of ACEi (captopril).

Materials and methods

Ten healthy male normotensive volunteers aged 30 - 45 years and with mean body mass index 30 ± 2.1 Kg/m^2 were used in this study. All subjects underwent a complete history and physical examination before investigation. Subjects with cardio-vascular, renal, endocrine or pulmonary disease were excluded. Written informed consent was obtained. Each subject was provided with high (200 mmol/d) salt, caffeine-free, and alcohol-free diet for 5 days. At 10 a.m. of the 5^{th} day of diet, a catheter was placed in the anticubital vein and blood was drawn through the catheter. Then each subject was provided with a low (10 mmol/d) salt for another 5 days then another blood sampling is obtained. Then subjects were maintained on the 25 mg BID dose of captopril for an additional 14 days (13). They were then provided a 10 mmol sodium diet for the last 5 days of captopril treatment. On the 5^{th} day of the diet blood sampling were repeated at the end of the study. All blood samples were obtained at 10 a.m. to avoid the diurnal variation of the measurements and placed on ice and immediately centrifuged. Blood for measurement of PAI-1 and tPA was collected in standard evacuated tubes containing 0.105 mol/L sodium citrate. The separated plasma or serum were frozen and stored at - 70 ^{0}C until the time of assay.

Plasma samples were assayed for t-PA antigen and PAI-1 antigen using two site enzyme linked immunosorbent assay (kits purchased from Biopool AB, Umea Sweden). Serum Aldosterone was measured by radio-immunoassay technique. The PAI-1 and tPA mass ratio was determined by dividing plasma concentrations (ng/ml) by the molecular weights of the 2 proteins, with a value of 70.000 g/mol used for tPA and a value of 50.000 g/mol for PAI-1 (14)

Statistical analysis: All data were expressed as mean ± SD (standard deviation) and comparison of the data in different groups was performed by applying the paired student-T test. $p < 0.05$ was the criterion for statistical significance.

Results

Low salt intake was associated with significant increase in PAI-1 antigen level in relation to the level in high salt intake ($p < 0.001$). Also captopril significantly decreased PAI-1 antigen level measured under low salt conditions ($p < 0.001$). (Table 1, Figure 1) tPA increase with low salt intake than in high salt intake but there is no effect of ACE inhibition on tPA antigen during salt depletion (Table 1 and Figure 2). So, PAI-1/tPA molar ratio was significantly lower with ACE inhibition than during low salt alone. Also low salt intake was associated with high PAI-1/tPA molar ratio than with high salt intake ($p < 0.001$) (Table 1 and Figure 3). Low salt intake was associated with increased aldosterone compared with high salt intake ($p < 0.001$), ACE inhibition decreased the aldosterone level ($p < 0.001$) under low salt conditions, but the aldosterone level remained significantly higher than under high salt intake ($p < 0.05$). (Table 1 and Figure 4) There was a highly significant positive correlation between PAI-1 antigen levels and serum aldosterone under low salt conditions ($r = 0.8812$) ($p < 0.001$). In contrast, there was no significant correlation between PAI-1 and aldosterone under high salt conditions ($r = 0.3961$, $p > 0.05$).

Table 1: Effect of salt intake and ACE inhibition on fibrinolytic parameters and serum aldosterone level

Parameter	High salt	Low salt	Low salt + captopril
PAI-1antigen (ng/ml)	15.26 ±1.32	23.69 ±2.08***	15.71 ±1.16###
tPA antigen (ng/ml)	4.67 ±0.54	5.79 ±0.66***	5.55±0.55***
PAI-1/tPA	4.8 ±0.78	6.37 ±0.86***	4.0 ±0.39** ##
Serum aldosterone conc.(ng/dl)	10.33 ±1.05	23.81±2.66***	12.72 ±2.73* ###

*P < 0.05, **P < 0.01, ***P < 0.001 vs. high salt ##P < 0.01, ###P < 0.001 vs. low salt

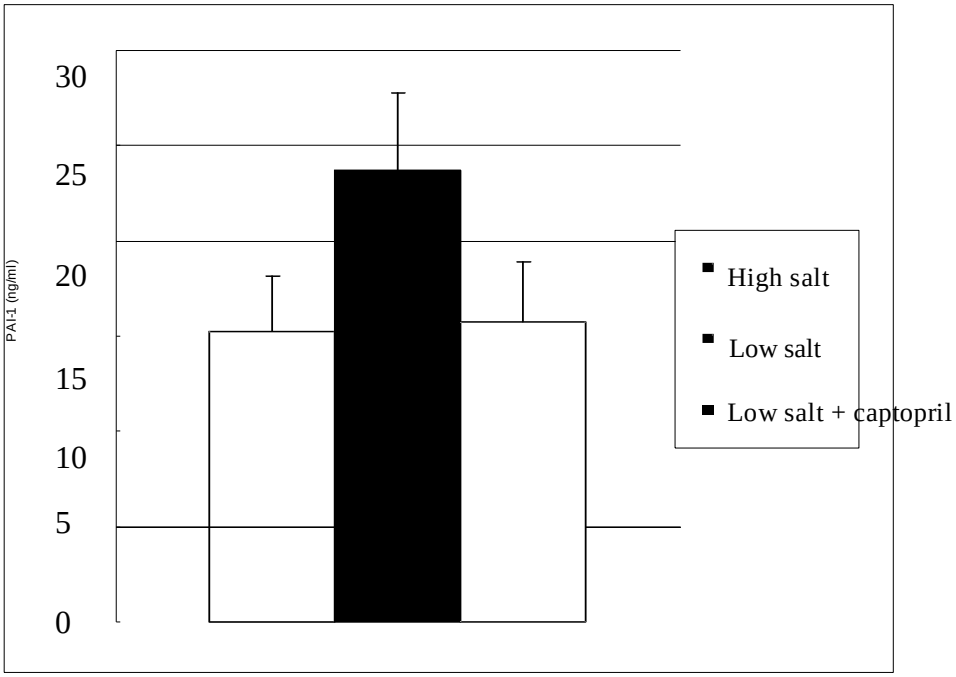

Figure 1: Effect of salt intake and ACE inhibition on PAI-1 antigen (ng/ml) inhibition on tPA antigen (ng/ml).

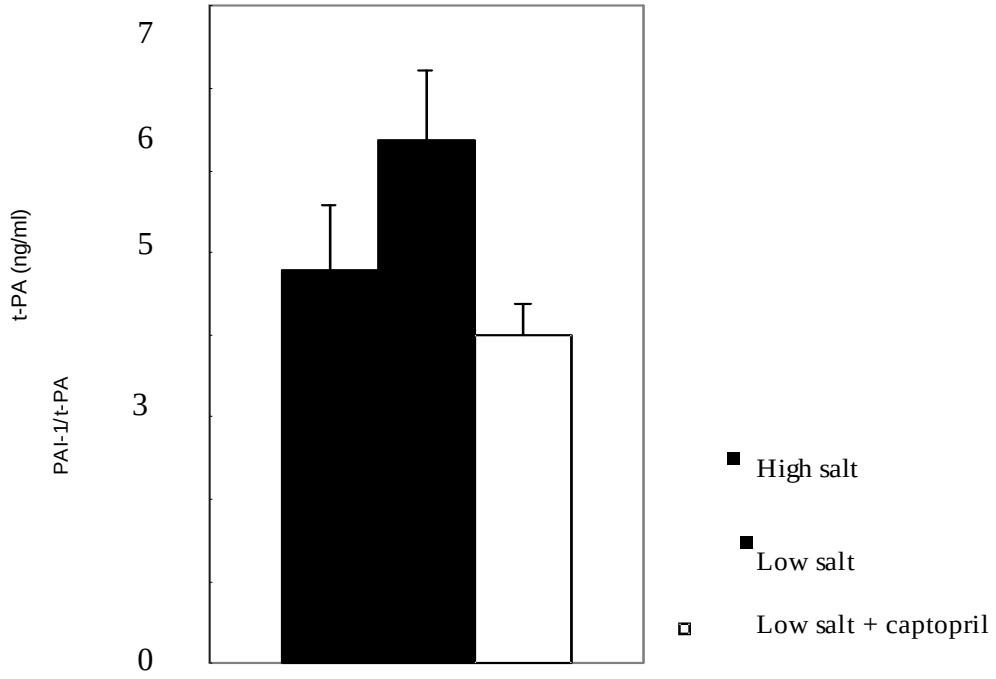

Figure 2: Effect of salt intake and ACE inhibition on tPA antigen (ng/ml).

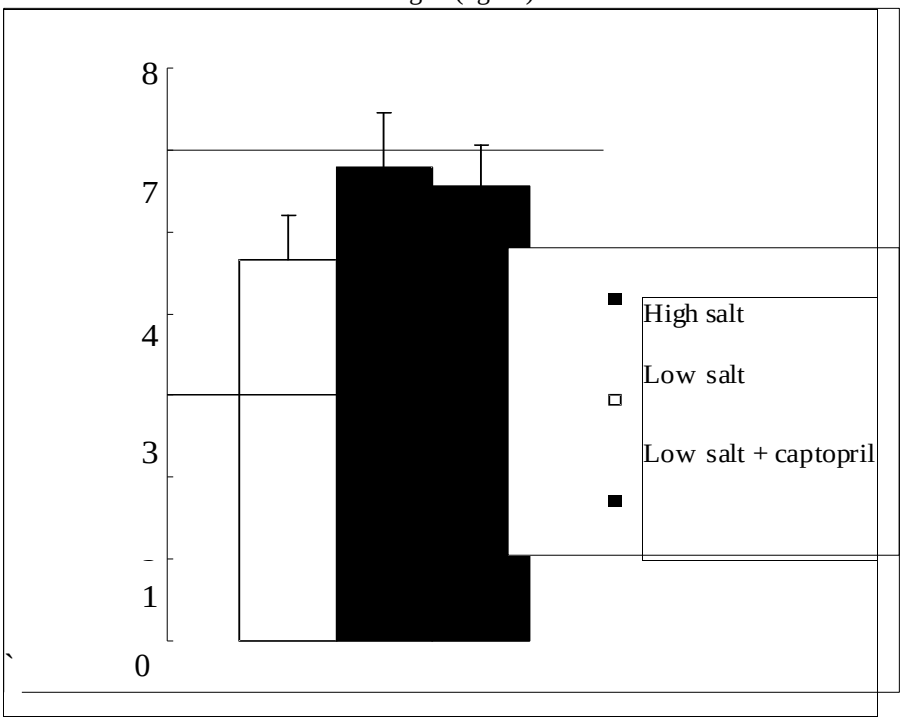

Figure 3: Effect of salt intake and ACE inhibition on PAI-1 /tPA mass

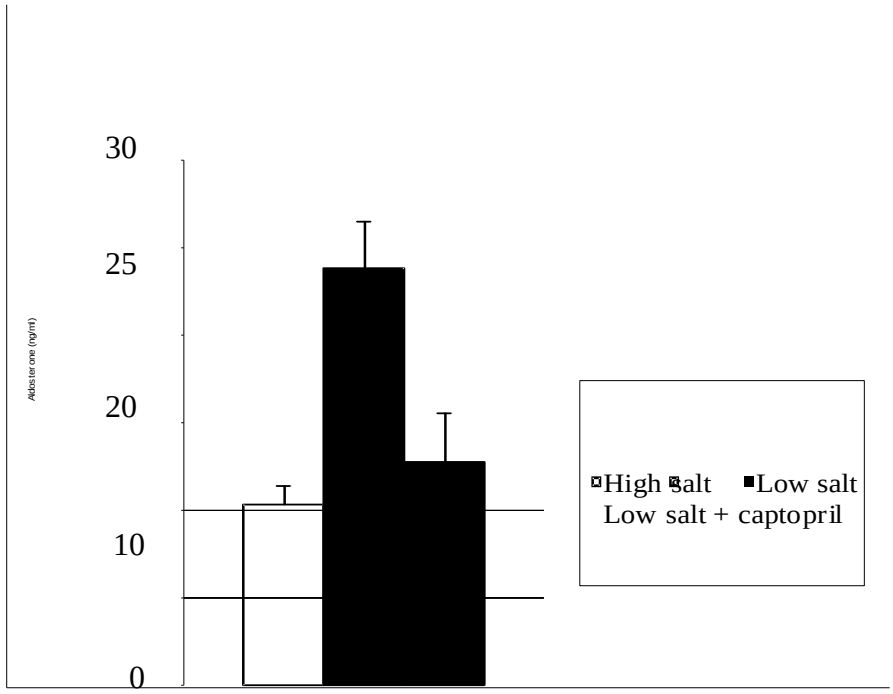

Figure 4: Effect of salt intake and ACE inhibition on aldosterone concentration (ng/dl).

Discussion

This study examined the effect of activation of the endogenous RAS on plasma fibrinolytic balance in healthy human subjects. The data suggest that activation of the RAS by low salt intake results in increased PAI-I antigen and that interruption of the RAS with the ACE inhibitor captopril significantly lowers PAI-I antigen without lowering tPA antigen. In this study, activation of the RAS was documented by significant increases in serum aldosterone concentration. The correlation between PAI-I antigen and serum aldosterone concentrations observed in this study further supports an interaction between the RAS and fibrinolytic system. The sensitivity of the adrenal cortex to Ang II varies with sodium intake such that conditions of salt depletion, Ang II are the major determinant of aldosterone (15. The highly statistically significant correlation between serum aldosterone and PAI-I antigen under low salt conditions and the lack of such a correlation under high salt conditions supports the hypothesis that Ang II regulates vascular PAI-I levels.

Sechi et al. (16) stated that a strong and independent association exists between renin, aldosterone, plasma fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) levels and this relationship might contribute to the development of hypertensive organ damage. ACE inhibitors have been shown to reduce progression of atherosclerosis in several animal models (17, 18) and to reduce the vascular expression of PAI-I in normal and balloon-injured vessels (19). The

present study suggests a mechanism whereby ACE inhibitors could alter the incidence of ischemic cardiovascular events in the setting of an activated RAS. A lack of effect of ACE inhibition on tPA antigen in the present study may simply reflect the effects of ACE inhibition on the kallikrein-kinin system as well as the RAS. ACE inhibitors not only decrease the production of Ang II but also decrease the degradation of bradykinin. Bradykinin has been shown to be a potent stimulus for tPA secretion *in-vitro* and *in-vivo* (20).

So, this study is in agreement with previous study by Brown et al. (20) that provides evidence for a direct functional link between the RAS and fibrinolytic system in humans. It suggests a mechanism through which ACE inhibitors could favorably alter progression of vascular disease, particularly in the setting of clinical states associated with activation of the tissue RAS.

Moreover, Ridker et al. (21) found that intravenous angiotensin II dose-dependently increased plasma PAI-1 antigen levels in healthy volunteers, whereas t-PA concentrations were unaffected or tended to be reduced. Also, in a clinical trial by Pfeiffer et al. (8) has demonstrated that the administration of ACE inhibitors to patients with left ventricular dysfunction reduces the incidence of recurrent myocardial infarction by approximately 25%. The positive effect of ACE-I on the fibrinolytic system has been related to: 1) inhibition of angiotensin II, which stimulates PAI-1 expression;
2) Inhibition of degradation of bradykinin, a potent stimulus for tPA production; and 3) improvement of insulin sensitivity (4). However, Larsson et al. (22) found that in healthy volunteers a short-term infusion of angiotensin II increased t-PA activity and antigen levels in plasma, suggesting that angiotensin II enhances fibrinolysis under these experimental conditions. There may be several explanations for these apparently conflicting results of Larsson. One might be the duration of angiotensin II infusion, or due to the hemodynamic effects caused by angiotensin II. Another possible consideration is that the clearance of t-PA may have been reduced during angiotensin II infusion.

Vaughan (23) reported that individuals with reduced fibrinolytic activity are at increased risk for ischemic cardiovascular events, and reduced fibrinolysis may underlie some of the pathological consequences of reduced nitric oxide availability. Within the vasculature, angiotensin II stimulates the release of PAI-1, thereby reducing fibrinolytic activity. Thus the plasminogen activator system is largely controlled by the renin-angiotensin system (RAS). In accordance with this finding; treatment with angiotensin converting enzyme inhibitors is associated with substantial reductions in the incidence of ischemic cardiovascular events. Taken together, these findings raise the possibility that Ang II may contribute to the development of a prothrombotic state at least in part by increasing plasma levels of PAI-1, thereby reducing the net activity of the endogenous fibrinolytic system. This potential relation between the renin-angiotensin system and fibrinolytic function may have important clinical and therapeutic consequences.

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