Physiol. Res. 45: 241 – 243, 1996

SHORT COMMUNICATION

Lack of Evidence for the Interaction Between Renin-Angiotensin-Aldosterone System and Endothelin in Vivo

J. WIDIMSKÝ Jr., J. DVOŘÁKOVÁ, J. HLADOVEC[1], J. KOPECKÁ

Third and [1]Fourth Department of Medicine, Charles University, Prague, Czech Republic

Received February 5, 1996
Accepted March 12, 1996

Summary

Aim of the study was to reveal the possible factors regulating plasma endothelin (ET) levels *in vivo* in patients with essential hypertension (EH) by the simultaneous determination of plasma renin activity (PRA) and plasma aldosterone (ALD). In addition, the possible relationship between ET and circulating endothelial cells as a marker of endothelial damage was also investigated. The postural test revealed a significant increase of ET levels (26.7±9 vs 11.5±3 fmol/ml, $p<0.05$) in the upright position. Captopril administration did not change plasma ET levels. No significant correlation was found between ET and PRA or ALD. Although a tendency to a positive correlation between ET and circulating endothelial cells (as the marker of endothelial perturbation) was found, it did not attain statistical significance. Our data do not support the suggestion that the renin-angiotensin-aldosterone system plays a major role in the regulation of ET secretion *in vivo* in EH. Postural stimulation of ET secretion may be caused by other factors than renin-angiotensin-aldosterone system.

Key words
Endothelin – Secretion – Renin – Aldosterone

Endothelin (ET) is a vasoactive peptide recently isolated from cultures of endothelial cells (Yanagisawa *et al.* 1988). The term endothelin refers to a family of 21 amino acid peptides comprising ET-1, ET-2 and ET-3 (Simonson and Dunn 1991). Although a number of data are available regarding its synthesis and release *in vitro* (Davenport *et al.* 1990, Golfman *et al.* 1993), little information is available on the regulatory mechanisms governing endothelin secretion *in vivo*.

The present study was designed to show the possible stimulatory role of renin-angiotensin-aldosterone system on ET secretion *in vivo*. Since ET may be considered to be a marker of endothelial perturbation (Lerman *et al.* 1990), we also tried to reveal a possible relationship between ET and endothelial cells by the simultaneous determination of circulating desquamated endothelial cells.

We studied a group of 11 patients with essential hypertension of the IInd degree according to WHO criteria, mean age 42.5±5 years. All subjects were investigated at the Third Department of Medicine during hospitalization. Previous antihypertensive therapy was withdrawn at least 2 weeks before the study. In all subjects the diagnosis of secondary hypertension was excluded by previous examinations.

We measured plasma ET levels, plasma renin activity (PRA) as a marker of angiotensin II and plasma aldosterone (ALD) levels during the postural test (after a night's rest in bed and after 3 hours of walking). ET, PRA and ALD were also determined during the captopril test (after a night's rest in bed and 60 min following 25 mg captopril administration). In addition, the same dose of captopril was repeated at 1900 h to provide long-lasting ACE inhibition and then blood was withdrawn again for the determination of

ET, PRA and ALD after an overnight rest in bed 0700 h of the following day. The circulating carcasses of endothelial cells were counted before and 60 min after captopril administration.

Plasma ET-1,2 levels were determined using a ^{125}I-endothelin assay system (Amersham) (Davenport *et al.* 1990). PRA and ALD were determined by radioimmunoassay. The circulating carcasses of desquamated anuclear bodies of endothelial cells were concentrated by high-speed centrifugation and counted in a Bürker's chamber (Hladovec and Rossman 1973). Differences between the groups were assessed by the t-test with $p<0.05$ taken as significant. Means ± S.E.M. are presented.

The average values of blood pressure measured with a mercury sphygmomanometer were 158±11/101±8 mm Hg. Mean plasma creatinine values were 92±10 μmol/l.

Figure 1 summarizes the results of the postural test. Assumption of upright posture with 3 hours of mild walking significantly increased plasma ET levels, which paralleled the expected increase in PRA and ALD. However, no significant correlation between individual values of ET and these parameters was found ($r=0.37$ and $r=0.29$, respectively).

Captopril administration did not have any significant effect on ET levels (Fig. 2), but surprisingly decreased the endothelial cell concentration. As expected, captopril markedly elevated PRA (before: 3.0±1.9, after: 4.8±1.7 nmol/l/h, $p<0.05$) and this was accompanied by a mild decrease of ALD (before: 231±55 pg/ml, after: 200±59 pg/ml). Similar results without significant changes in ET were detected on the next day following the second captopril dose (data not shown).

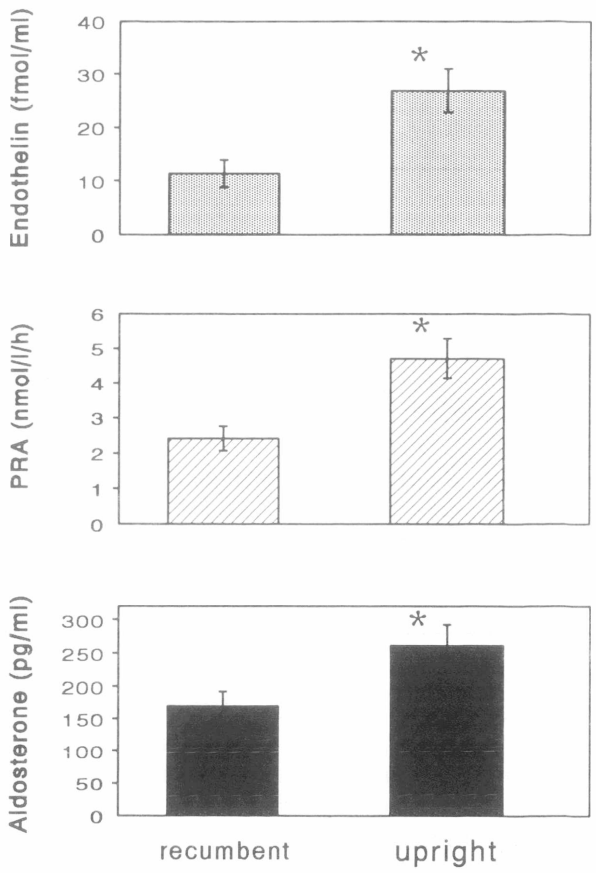

Fig. 1
*Humoral postural changes (recumbent and upright position after 3 hours of mild walking) in plasma endothelin, plasma renin activity (PRA) and plasma aldosterone ($n=11$, * $p<0.05$).*

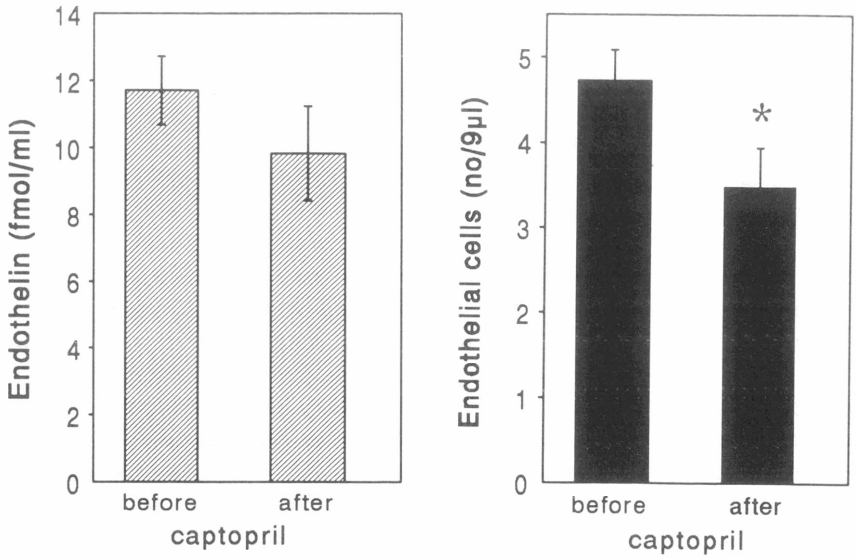

Fig. 2
Plasma endothelin and endothelial cells in captopril test ($p<0.05$).*

Our study reveals the important influence of the upright position on the circulating ET pool. The absence of significant correlation between ET and PRA and/or ALD suggests that other factors (possibly plasma catecholamines mediating also the increase of PRA in the upright position) are responsible for the ET increase. The absence of the potential modulatory effect of renin/angiotensin II and/or ALD on ET secretion is further substantiated by the lack of significant ET changes after blockade of angiotensin II production by ACE inhibition. We did not evaluate the potential increase of bradykinin and/or of prostaglandin production following ACE inhibition, which could potentially influence ET levels. Since most of ET is secreted abluminally (Yoshimoto *et al.* 1990), we cannot, however, evaluate possible alterations of ET release and/or production in the abluminal compartment. The perturbed endothelial cells may release increased amounts of ET into the circulation (Lerman *et al.* 1990) and thus substantially modulate the circulating pool of this peptide. Although there was a tendency to a positive correlation between ET and endothelial cells ($r=0.52$), it did not attain statistical significance. This discrepancy may reflect the relatively small group of subjects, possible differences in the site of origin of ET and endothelial cells and/or short half-life of plasma ET (Lüscher *et al.* 1992). The reason for captopril-induced decrease in endothelial cells levels is not clear and further studies are clearly needed. In conclusion, our data do not support a major role of renin-angiotensin-aldosterone system in the modulation of ET secretion into the circulation in essential hypertension. Posture-induced increment in plasma ET levels may be considered as a part of homeostatic regulatory mechanisms serving to maintain adequate blood pressure and organ perfusion levels.

Acknowledgement
This work was supported by a grant No. 2314-3 of Czech Ministry of Health.

References

DAVENPORT A.P., ASHBY M.J., EASTON P., ELLA S., BEDFORD J., DICKERSON C., NUNEZ D.J., CAPPER S.J., BROWN M.J.: A sensitive radioimmunoassay measuring endothelin-like immunoreactivity in human plasma: comparison of levels in patients with essential hypertension and normotensive control subjects. *Clin. Sci.* **78:** 261–264, 1990.

GOLFMAN L.S., HATA T., BEAMISH R.E., DHALLA N.S.: Role of endothelin in heart function in health and disease. *Can. J. Cardiol.* **7:** 635–653, 1993.

HLADOVEC J., ROSSMAN P.: Circulating endothelial cells isolated together with platelets and the experimental modification of their counts in rats. *Thromb. Res.* **3:** 665–668, 1973.

LERMAN A., HILDEBRAND F. L., MARGULIES K.B, O'MURCHU B., PERRELLA M.A., HEUBLEIN D.M., SCHWAB T.R., BURNETT J.C.: Endothelin: a new cardiovascular regulatory peptide. *Mayo Clin. Proc.* **65:** 1441–1455, 1990.

LÜSCHER T.F., BOULANGER CH.M., DOHI Y., YANG Z.: Endothelium-derived contracting factors. *Hypertension* **19:** 117–130, 1992.

SIMONSON M. S., DUNN M. J.: Endothelins: A family of regulatory peptides. *Hypertension* **17:** 856–863, 1991.

YANAGISAWA M., KURIHARA H., KIMURA S., MITSUI Y., KOBAYASHI M., WATANABE T.X., MASAKI T.: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332:** 411–415, 1988.

YOSHIMOTO S., ISHIZAKI Y., MORI A., SASAKI T., TAKAKURA K., MUROTA S.: The role of cerebral microvessel endothelium in regulation of cerebral blood flow through production of endothelin-1. *J. Vasc. Med. Biol.* **2:** 178, 1990.

Reprint Requests
J. Widimský Jr., Third Department of Medicine, U nemocnice 1, 128 00 Prague 2, Czech Republic.