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# RAISED PLASMA ENDOTHELIN-I CONCENTRATION FOLLOWING COLD PRESSOR TEST

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Plasma concentration of immunoreactive endothelin-1 was measured by radioimmunoassay in 6 healthy subjects before and following cold pressor test by immersion of one fore-arm into ice-water. Mean (SEM) plasma endothelin-1 concentration rose from 1.2 (0.7) to peak value 8.4 (2.3) pg/ml in venous plasma from the immersed hand, and, reaching peak 2 minutes later, from 1.4 (0.5) to 4.6 (2.3) pg/ml in venous plasma from the contralateral hand. In 66 healthy control subjects, venous plasma concentration of endothelin-1was 2.9 ± 1.2 pg/ml (mean ± SD). Exposure to cold is associated with raised blood levels of endothelin-1, which points to a relation between endothelin-1 and vasoconstriction associated with low temperature. 

Endothelin-1 (ET-1) is a 21-residue peptide with powerful vasoconstrictive and blood-pressure rising properties produced by endothelial cells (1). Three distinct human endothelin related genes have been identified, coding for three peptides, ET-1, ET-2, and ET-3 (2).Only ET-1 is produced by endothelial cells and has been shown to be present in circulating blood (3,4). ET-1 is believed to act locally, causing contraction of myocytes of the vascular wall (1,4). Specific binding sites for ET-1, likely to be receptors, have been shown not only in blood vessel walls, but in a variety of tissues, including lung, heart, kidney, adrenal gland, and brain (4). Recently, low concentrations of ET-1 have been detected in venous plasma from healthy subjects (3,5,6). Raised plasma concentrations of ET-1 have been observed in patients with uraemia (7), following myocardial infarction (3,6), in cardiogenic shock (8), and subarachnoidal haemorrhage with vasospasm (9). Cultured endothelial cells release increased amounts of ET-1 when stimulated with angiotensin II(10) vasopressin (10), thrombin (1 ) or transforming growth factor ß(11). However, no physiological stimulus causing in vivo release of ET-1 has been reported.

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Abbreviations used in the text: ET-1, ET-2, ET-3, endothelin 1,-2,-3; HPLC, high power liquid chromatography.

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We have measured immunoreactive ET-1 by radioimmunoassay in plasma from healthy subjects. We here report increased concentrations of plasma ET-1 in venous blood following cold pressor test.

## SUBJECTS AND METHODS

*Cold pressor test.* Healthy subjects ( 3 females and 3 males) on free diet, were studied following 6 hours fast. While seated, cannulas were introduced into both antecubital veins 30 min before the test. One fore-arm was immersed into ice-water for 2 minutes, while the other served as a control. At 4 min before and 0, 2, 6, 10 , and 16 min after immersion, venous blood was drawn into ice-chilled tubes containing $Na_2EDTA$, 15 mM final concentration. Plasma was separated and stored at -20 °C until assayed for ET-1. Blood-pressure was recorded by mercury sphygmomanometer. Control plasma samples were obtained from 66 ambulatory, healthy subjects .

*Radioimmunoassay of ET-1.* Synthetic ET-1 corresponding to 10-20 μg (Peptide Institute, London, UK) coupled to keyhole limpet haemocyanin using glutaraldehyde condensation was used as an immunogen for monthly injections into popliteal lymph nodes, together with emulsified Freunds adjuvant. The antiserum chosen showed < 0.1% cross-reaction with big endothelin-1 1-38 and its 22-38 fragment, with the 20- 50, the 74-91 and the 171-201 sequences of preproendothelin (Peptide Institute) , atrial natriuretic peptide (Peninsula, London, UK ) , angiotensin II (Schwarz-Mann, St. Louis, Louisiana, USA), and $arg_8$-vasopressin (Ferring, Malmö, Sweden) . It cross-reacted 100 % with ET-2 and ET-3 (Peptide Institute). ET-1 was iodinated with 125-iodine (IMS 30; Amersham, Bucks, UK) using chloramine-T method . One ml plasma was acidified with 4% acetic acid and extracted using Bondelut- C18 OH analytical columns . Following wash with distilled water the absorbed peptide was eluted with 60% ethanol and 4% acetic acid. The eluted fraction was dried in an air-stream, and dissolved into assay buffer, 0.15 M phosphate, pH 7.4, containing 1.0 mg/ml of human serum albumin, 0.015 mM $Na_2EDTA$. Radioimmunoassay was performed using sequential incubation by adding 125-I-labelled ET-1 on the third day. Separation of bound label was performed on the fourth day using anti-rabbit IgG (Peninsula, London, UK ) second antibody technique. Sensitivity was 0.8 pg/tube. Recovery of synthetic ET-1 added to plasma was 80%.

*Chromatography.* Plasma was extracted as described above. The dried extract was dissolved into 0.1% trifluoroacetic acid and subjected to reverse phase high power liquid chromatography (HPLC) using Spherisorb Ods 2 column and acetonitrile gradient.
Student's t-test for paired or unpaired observations was used for statistical analysis.

## RESULTS

Immersion of one fore-arm into ice-water resulted in a rapid appearance of ET-1 immunoreactivity in venous plasma from the hand immersed, then, a few minutes later, from the opposite hand (Fig. 1). At 16 min plasma ET-1 values had returned to normal level. Both systolic and diastolic blood-pressure increased and remained rised for 20 min. Plasma concentrations of ET-1 in 66 healthy control subjects, indicated as normal range (NR; Fig. 1), were low, $2.9 \pm 1.2$ pg/ml (mean ± SD). Fractions of extracted human venous plasma, following HPLC-chromatography, showed ET-1 immunoreactivity closely corresponding to the elution pattern of synthetic ET-1 and to that of 125-I labelled ET-1, respectively (Fig. 2).

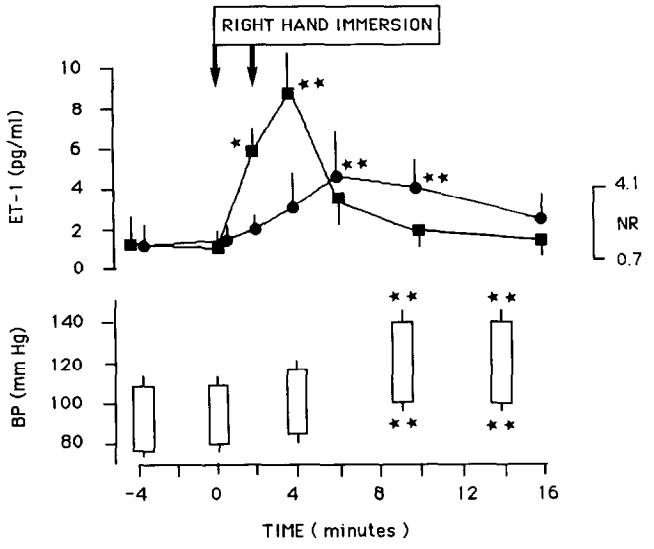


Figure 1. Effect of ice water immersion of the right hand (indicated by arrows) on venous plasma ET-1 concentration (upper panel ) in plasma from right ( ■ ) and left arm ( ● ), mean ± SEM, and on systolic and diastolic blood pressure ( BP; lower panel). NR = normal range; mean ± SD. Significance when compared with pre-immersion values: *) $p<0.05$; **)$p<0.01$.

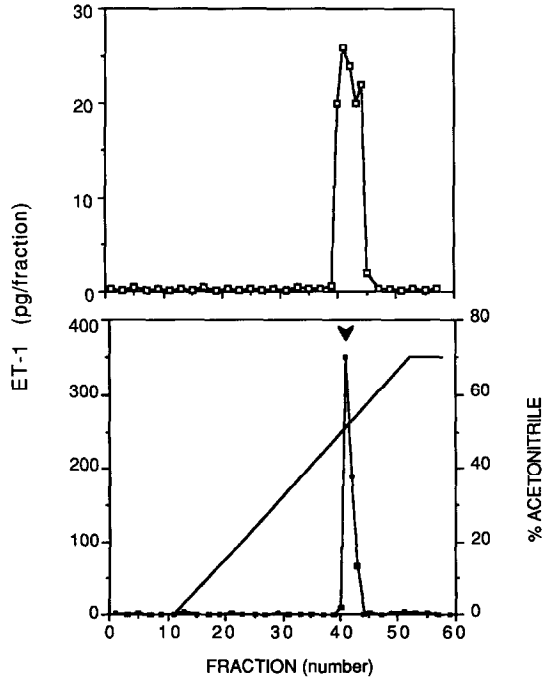


Figure 2. Immunoreactive ET-1 in high power liquid chromatography fractions of human venous plasma extract (upper panel) and synthetic ET-1 (lower panel). The elution peak of 125-I-ET-1 (arrowhead) and acetonitrile gradient (solid line, lower panel) are indicated.

## DISCUSSION

ET-1 was shown here to be present at low concentrations in human blood, and to be released into the circulation by a physiological stimulus, the cold pressor test. This confirms earlier observations that immunoreactive ET-1 is present in human blood (3,5-9) and provides evidence that rapid release of endothelin may be part of the physiological response to cold exposure. In view of the extreme vasoconstrictive potency of ET-1, about 10 times that of angiotensin II (1), even the low concentrations of ET-1 observed here may be of biological importance. It cannot be concluded from our data whether ET-1 contributes to the increase in blood-pressure observed in response to cold pressor test. It is of note, that while calcium entry blockers effectively block the vasoconstriction effect of ET-1 (1,4 ), nifedipine therapy completely abolished the blood-pressure rise and coronary vasospasm in cold pressor test, while propranolol treatment did not (12).

Only endothelial cells are known to produce ET-1 (1,4,10). Thus, the likely source of immunoreactive ET-1 in human plasma is vascular endothelium. The mechanism whereby cold pressor test causes increase of plasma ET-1 concentrations is unknown. The rapid release within minutes of ET-1 following cold exposure indicates release from tissue stores, presumably vascular endothelium. However, such release appears difficult to reconcile with the scarcity of secretory granulae in endothelial cells (1,4 ). The first peak of immunoreactive ET-1 appearing in blood from the immersed hand could be locally released, while the second one, from the opposite hand, could be due to systemic release, induced by factors secondary to cold exposure, such as stimulation of sympathetic nervous system (13).

The biochemical character of ET-1 immunoreactivity measured was only partly clarified here. Plasma extract subjected to HPLC co-eluted with synthetic ET-1, and with extract of human umbilical cord endothelial cell culture medium containing native ET-1 (unpublished data from our laboratory). This indicates that the immunoreactive material measured in circulating plasma was indeed ET-1, the only endothelin peptide so far demonstrated in mammalian blood (3,4). Because our antiserum did not cross-react with big endothelin-1 , shown to be present in human plasma following acute myocardial infarction (3), we cannot conclude whether big endothelin-1 is released following cold exposure.

The possible importance of immunoreactive ET-1 in circulating blood awaits further elucidation. ET-1 is likely to act in a paracrine way, being produced in endothelial cells and causing contraction of underlying smooth muscle cells(1,4 ). Raised plasma ET-1 concentrations may only reflect "spill-over" due to increased release of ET-1 in vascular beds. However, because ET-1 is present in circulating blood in concentrations within the range expected to be biologically active , circulating ET-1 may participate in the regulation of vascular tone and haemodynamics. "Leakage" of ET-1 into the circulation may prove to be of pathophysiological significance in certain diseases characterized by vasospasm. In fact, raised plasma concentrations of ET-1 reportedly occur in acute myocardial infarction (3,6) and in subarachnoidal haemorrhage with vasospasm (9). ET-1 may also be a mediator of coronary spasm occurring in some patients upon cold exposure (12). Thus, coronary spasm was demonstrated in patients with systemic sclerosis

upon cooling of the trunk with ice-bags (14). Moreover, cold-induced pulmonary vasoconstriction and oedema and subsequent development of hypertension reported in scuba divers (15) could be related to incresed ET-1 release following exposure to cold.

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