**Pharmacological manipulation of cardiovascular responses to lower body negative pressure**

**Abstract** To evaluate influences on blood volume distribution, atrial natriuretic peptide concentrations (ANP) and thoracic and leg electrical impedance at 2.5 (TI2.5 and LI2.5, respectively) and 100 kHz (TI100 and LI100, respectively) were monitored during administration of ketanserin, noradrenaline and trimetaphan combined with lower body negative pressure (LBNP) in 12 subjects. Administration of clinically relevant doses of ketanserin alone did not induce changes in mean arterial pressure (MAP) or in the central blood volume, as electrical impedance and ANP concentrations did not change. During continued infusion of ketanserin an increase in MAP from a mean of 90 (range 83-108) to 113 (range 98-138) mmHg was induced by noradrenaline, but TI2.5 [mean 45.6 (range 39.3-54.2)] and TI100 [mean 33.8 (range 27.5-38.5)] remained stable until ganglionic blockade and LBNP were applied, when they increased by a mean of 3.1 (range 2.0-6.1) and 2.7 (range 1.1-4.2) Ω, respectively (P < 0.05). Conversely, LI2.5 [mean 79.6 (range 74.1-89.4)] and LI100 [mean 56.7 (range 52.4-63.3)] decreased by a mean of 3.2 (range 1.2-8.0) and 2.3 (range 0.9-3.9) Ω, ANP from a mean of 27.7 (range 10.2-62.7) to 12.7 (range 7.1-27.5) pmol·1^-1 and MAP fell to a mean of 62 (range 42-70) mmHg (P < 0.05). The heart rate was a mean of 75 (range 69-77) beats·min^-1 and did not change until LBNP, when it increased to a mean of 102 (range 78-104) beats·min^-1, as presyncope symptoms appeared. The data indicated that serotonergic blockade by ketanserin and α-sympathetic stimulation by noradrenaline did not affect blood volume distribution in normal humans, but that ganglionic blockade combined with LBNP reduced the central blood volume as leg volume increased; during central hypovolaemia tachycardia induced by ganglionic blockade did not prevent the fall in MAP, and thereby the appearance of presyncope symptoms.

**Key words** Atrial natriuretic peptide · Central hypovolaemia · Leg impedance · Thoracic impedance

**Introduction**

The circulation is monitored to secure the central blood volume (CBV) in situations where, e.g. bleeding, positioning and the use of anaesthetics contribute to reduced venous return. Changes in mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), pulmonary arterial mean and wedge pressures are used to assess the circulation. However, it has been found that these variables are not well correlated to CBV (Shoemaker 1975; Jacobsen et al. 1993; Pawelczyk et al. 1994), while changes in thoracic electrical impedance (TI) correlate closely with the blood volume of animals (Berman et al. 1971; Hill et al. 1976; Patterson et al. 1978; Jacobsen et al. 1993), healthy subjects (Ebert et al. 1986; Matzen et al. 1990, 1991) and surgical patients (Perko et al. 1991; Jönsson et al. 1995). Also it has been found that changes in TI correlate to a hormonal indicator of atrial stretch, atrial natriuretic peptide concentration (ANP; Matzen et al. 1990; Perko et al. 1994). It has been shown that during head-up tilt in humans, TI increases, while impedance decreases over the leg reflecting the volume redistribution (Matzen et al. 1991; Perko et al. 1993, 1995). It has been reported that determination of electrical impedance at two frequencies allows an estimate of the shift in fluid volume as opposed to changes in blood volume (Thomasset 1965; Larsen et al. 1987; Matzen et al. 1991; Perko et al. 1991). Cell membranes act as capacitors that insulate the intracellular space at low frequencies and it is
predominantly the extracellular fluid that is assessed. At higher frequencies the cell membranes become permeable to current and therefore both the extra- and intracellular spaces are evaluated.

Impedance cardiography is a noninvasive technique for determination of cardiac stroke volume based on the measurement of transthoracic electrical impedance. In the absence of left-to-right shunts and valvular insufficiency, this method has provided reliable and reproducible estimates of absolute (Baker et al. 1971; Denniston et al. 1976; Edmunds et al. 1982) as well as relative (Judy et al. 1969; Smith et al. 1970; Boer et al. 1979) values for cardiac output in humans.

Application of negative pressure to the lower body (LBNP) has been used as a means of sequestering blood from the circulation to induce central hypovolaemia (Murray et al. 1968). Progressive central hypovolaemia is characterized by a normotensive, tachycardic stage followed by a reversible, hypotensive stage with slowing of HR. However, the administration of atropine prior to LBNP has been shown to cause the initial increase in HR to be maintained during the subsequent decrease in MAP (Sander-Jensen et al. 1988). Patients are often treated with vaso-active drugs, which may have an independent influence on blood volume distribution. It could be expected that vasodilatation would deplete CBV due to diminished venous return to the heart, and conversely that venoconstriction would increase CBV.

The purpose of this study was to evaluate the effects of the serotonergic blocking agent ketanserin, α-adrenergic stimulation by noradrenaline and ganglionic blockade by trimetaphan combined with LBNP on CBV as monitored by TI and ANP. At the same time HR, stroke volume (SV) and MAP were recorded, and arterial blood samples for haematocrit obtained.

Methods

Subjects

Six male and six female healthy volunteers [mean age 33 (range 22–56) years, body mass mean 1.87 (range 1.50–2.26) and mean height 1.74 (range 1.62–1.88)] cm participated in this study after informed consent and approval by the Ethics Committee of Copenhagen.

Experiment protocol

The subjects lay supine for 30 min to obtain resting values. Then ketanserin (Sufrexal, Janssenpharma AS, Birkerod, Denmark) was infused as a 10-mg i.v. bolus followed by 6 mg h⁻¹. After 1 h, noradrenaline (Central Pharmacy, Herlev, Denmark) was administered i.v. as a saline solution at a rate of 5–15 µg·min⁻¹ until MAP had increased by 30%. To allow for a steady state to be established, a rest of 10 min followed the infusion. Immediately thereafter, infusion of trimetaphan camsylate (Arfonad, Roche, Basel, Switzerland) was started at rates of 0.4–0.9 mg·min⁻¹, and simultaneously LBNP was applied to shorten the period until the appearance of presyncopal symptoms and a decrease in MAP. The legs and lower abdomen was encased in a LBNP chamber (Murray et al. 1968). The subjects were requested to reduce movement of the legs to a minimum to allow accumulation of blood and fluid. Negative pressures of 5–25 mmHg were gradually intensified by venting the output of a commercial vacuum cleaner until the appearance of presyncopal symptoms.

A two-lead electrocardiogram was used for recording of HR. A Bentley transducer (Uden, Holland) was used to determine MAP through a 1-mm ID (20-gauge) teflon catheter placed in the left radial artery and connected to an Athena pressure monitor (S and W, Copenhagen, Denmark), which integrated pressures and HR over 6 s.

The TI and leg impedance (LI) were determined by a body impedance monitor BLM 2000 (Aqua Medico, Denmark) employing 10 µA at 2.5 and 100 kHz and by a Minnesota impedance cardiograph 304 b (Instrumentation for Medicine Corporation, Greenwich, Conn., USA) which used 4 mA at 100 kHz. For TI two electrodes were placed on the right sternocleid muscle and two on the lower left ribs in the midaxillary line (Matzen et al. 1990, 1991). For LI two electrodes were placed on the pelvis: one at the lateral portion of the left inguinal ligament and one 5 cm above the first electrode, and two were placed on the left limb. one on the head of the tibia and one 5 cm below. The outer electrodes served for current and the inner electrodes for voltage sensing. Values were recorded continuously as the mean of 100 single determinations each minute. In six subjects TI was measured by an impedance cardiograph (S and W, Copenhagen, Denmark; Madsen et al. 1993). The monitor calculated SV from pulsatile changes in TI (dz/dt). The incoming signal was automatically filtered by a software routine and the mean value of basal impedance Z₀ for 1 min was digitally presented. To eliminate respiratory variations in the impedance signal, an ensemble-averaging technique synchronized by the R-wave of the electrocardiogram was used (Sheps et al. 1982; Muza et al. 1985; Ekman et al. 1990). Cardiac output (CO) was derived from SV and HR. Total peripheral resistance (TPR) was calculated as the ratio of MAP to CO.

Arterial blood samples for ANP were taken at rest before and after 1 h of the ketanserin administration, at the end of noradrenaline administration, during LBNP just before the return to the atmospheric pressure and 3 min thereafter. For ANP, blood samples were collected on ice in polypropylene tubes containing heparin (15 IU·ml⁻¹) and aprotinin (500 KIU·ml⁻¹). Samples were centrifuged immediately at 5°C and plasma was stored at −20°C until assayed. The ANP was extracted from plasma using C-18 Sep-Pak cartridges and determined by radio-immunoassay (Schütten et al. 1987). Synthetic human (α-ANP 1–28 (Peninsula Laboratories Inc.) served as the reference preparation. The recovery of the extraction was 95%–100% and the sensitivity of the assay was 2 pg per tube. A dilution curve of plasma was parallel with the standard curve. Endogenous immunoreactive ANP was eluted in one peak by high pressure liquid chromatography (HPLC) and synthetic ANP was eluted in the same position. The intra- and interassay coefficients of variation were 3.8% and 4.7%, respectively (Schütten et al. 1987).

Statistics

Friedman's test was used to evaluate whether variables changed with time, and if proved significant, Wilcoxon's test was used for location of deviating values. A value of P < 0.05 was considered significant.

Results

Before the appearance of presyncopal symptoms with a concomitant decrease in MAP, the mean time of