Blood Pressure and Endocrine Responses of Healthy Subjects in Cold Pressor Test after Acutely Increased Dietary Sodium Intake

Olli Arjamaa1), Tero Mäkinen2), Lauri Turunen1), Pirkko Hutunen3), Juhani Leppäluoto4), Olli Vuolteenaho5) and Hannu Rintamäki2)

1) Center of Arctic Medicine, University of Oulu, Oulu, Finland
2) Oulu Regional Institute of  Occupational Health, Oulu, Finland
3) Department of Forensic Science, University of Oulu, Oulu, Finland
4) Department of Physiology, University of Oulu, Oulu, Finland

Abstract  The objective of the study was to compare blood pressure and endocrine responses in a cold pressure test in young healthy subjects who had shown increased blood pressure during an acutely increased sodium intake. Subjects (n=53) added 121 mmol sodium into their normal diet for one week. If the mean arterial pressure had increased by a minimum of 5 mmHg compared to the control measure, they were selected for the experiments. The selected subjects (n=8) were given 121 mmol supplemental sodium d−1 for 14 days after which they immersed the right hand into a cold (+10 °C) water bath for 5 min. The blood pressure increased (P<0.05) during the test and was independent of the sodium intake. The plasma noradrenaline increased from 2.41 ± 0.38 nmol l−1 to 2.82 ± 0.42 nmol l−1 (P<0.05) with normal diet and from 1.85 ± 0.29 nmol l−1 to 2.40 ± 0.37 nmol l−1 (P<0.05) with high sodium diet. The starting concentrations and the endpoint concentrations were statistically similar. The plasma levels of natriuretic peptides (NT-proANP, ANP and BNP) did not change during the test, and the concentrations were independent of the sodium diet. To conclude, acutely increased sodium intake does not change blood pressure or hormonal responses in a cold pressor test in young healthy subjects.

Keywords: cold pressor test, dietary sodium intake, natriuretic peptides, noradrenaline

Introduction

A large number of studies have been published on the effects of dietary sodium intake on the resting blood pressure in humans (for review, see Muntzel and Drüeke, 1992). High prolonged sodium intake has been postulated to be associated with increased blood pressure with elevated risk of developing hypertension, although there are subjects whose blood pressure is sodium resistant (Weinberger, 1996). A recent study, based on 20 000 subjects who were followed for about 25 years showed, however, no relation of sodium intake to subsequent all-cause and cardiovascular-disease mortality in a general population (Alderman et al., 1998).

Low ambient temperature is a well-known environmental factor that increases blood pressure in man but its effects have not been studied as extensively as those of dietary sodium intake. Studies which have explored the combined effects of sodium intake and cold exposure on blood pressure are scarce. A previous report (Ditto et al., 1993) showed that in those using extra sodium (172 mmol sodium d−1, supplemental to normal diet, for two weeks) the diastolic blood pressure in a cold pressor test was significantly higher than in the controls while there was no difference in the systolic blood pressure increase. This finding was corroborated by a study of Arjamaa et al. (1999) in which men of various age were exposed to cold (−15°C, wind 3.5 m s−1, 15 min) after having consumed about 290 mmol sodium d−1 in total for two weeks. In young subjects, whose resting blood pressure had increased during acutely elevated sodium intake, high sodium intake did not, however, change the blood pressure response during a whole body cold exposure (Arjamaa et al., 2001).

The effects of cold exposure on blood pressure are not fully understood under acutely increased dietary sodium intake (Ditto et al., 1993; Arjamaa et al., 1999; Arjamaa et al., 2001). Thus, the purpose of this study was to clarify the cold pressor responses of subjects whose resting
blood pressure was dependent on sodium intake.

**Methods**

**Subjects**

Fifty-three healthy, non-smoking students (17 males and 36 females) volunteered for the study. Resting blood pressure was measured according to the Kaplan (1998). The subject was sitting in a quiet room at 22°C for 15 min and then the blood pressure was recorded automatically (ABPM Meditech, Meditech KFT, Hungary). Then the subjects added 121 mmol sodium d⁻¹ (tablets of 1 g) to the normal diet for one week after which the resting blood pressure was measured again. If the mean arterial blood pressure had increased at least 5 mmHg compared to the pressure before sodium loading the subject was selected for further experiments (Mattes and Falkner, 1999). These subjects (5 males and 3 females) were normotensive, the age ranged from 22 to 26 years, body mass was 65.1 ± 4.7 kg (mean ± SEM), height 174.3 ± 3.1 cm, and the body mass index 21.3 ± 0.9 kg m⁻². The experimental protocol was explained to them and a written consent was obtained from each subject before the study. The subjects were familiarized with measurements and experimental conditions, and they underwent a protocol before the study started. The protocol was approved by the ethical committee of Oulu University Central Hospital.

**Protocol**

A randomised-crossover protocol was used. Three months after the one-week sodium loading half (n=4) of the selected subjects were first exposed to cold while on the normal diet (group A) and the remaining half (n=4) were on sodium supplementation (group B). After a period of three weeks to allow “wash-out” of the sodium supplement, the group A was tested on the sodium supplementation and the group B with the normal diet. The supplemental sodium consisted of 121 mmol sodium d⁻¹ in a form of crystalline table sodium tablets (1 g per tablet) and it was divided in 3–4 doses and taken during 14 days before a cold pressor test. All the subjects kept a dietary record book for four days before the test from which the total energy consumption and dietary sodium intake were calculated. A 24-h urine sample was collected on the day before the cold pressor test to determine the ion excretion. Subjects were weighed and venous blood samples were drawn from an antecubital vein before and immediately after the test for hormonal and ion determinations.

**Cold pressor test**

The test was performed at 22°C and the subjects wore their normal indoor clothing. First the subject was sitting for 15 min in a noiseless room and the blood pressure was automatically recorded (ABPM-Meditech, Meditech KFT, Hungary). Then the subject put the right hand into a water bath at +10°C for 5 min. The blood pressure was recorded at an interval of 1 min and the subjects reported their thermal sensations (ISO 10551, 1995) before, during, and after the cold pressor test on a five point subjective scale. The heart rate was monitored three times before the test, at an interval of 5 min, and every min during the test.

**Analyses**

Dietary recordings were analysed by Micro-Nutrica program (v. 2.0, 1993). Blood samples were collected in 10 ml serum vacuum tubes (first kept at room temperature for 30 min) and in 10 ml EDTA tubes that were centrifuged (5 min, 2000 g) and stored at ~80°C until assayed, as were the urine samples. Haematocrit, haemoglobin, and the red cell count were immediately analysed with an automatic cell counter (Coulter T-540-series, Coulter Electronics LTD, UK). Changes on plasma volume were calculated according to the formulas of Dill and Costill (1974). Urine sodium was measured by a direct ion selective method (Microlyte 3+2 Ion Selective Analyzer, Kone Instruments, Finland).

**Radioimmunoassays**

ANP (atrial natriuretic peptide) was extracted from plasma using SepPak C₁₈ cartridges (Vuolteenaho et al., 1992). NT-proANP (N-terminal fragment of pro-ANP) was assayed directly from unextracted plasma. The radioimmunoassay protocols have been described previously for ANP (Vuolteenaho et al., 1985) and NT-proANP (Vuolteenaho et al., 1992). The sensitivities of the ANP and NT-proANP were 1.0 and 40 pmol l⁻¹ plasma, respectively. The within and between assay coefficients of variation in each assay were <10% and <15%, respectively. Both assays were specific for the particulate peptide. The assays, however, crossreacted fully with proANP. With these methods, the following plasma levels (mean ± SD) were detected in healthy adults aged 20–55 years: ANP 10.9 ± 4.0 pmol l⁻¹ and NT-proANP 227 ± 84 pmol l⁻¹.

BNP (brain natriuretic peptide) was extracted from plasma with the same method as described for ANP (Vuolteenaho et al., 1992) and the assay was performed with the same protocol as described previously for ANP (Vuolteenaho et al., 1985; Vuolteenaho et al., 1992). The sensitivity of the BNP assay was 1 pmol l⁻¹ plasma. The within and between assay coefficient of variation in the assay were <10% and <15%, respectively. The BNP antiserum cross-reacts less than 0.1% with ANP, NT-proANP and CNP (C-type natriuretic peptide).

Aldosterone measurements were performed using a clinical radioimmunoassay kit (ICN Pharmaceuticals Inc., CA 92626, USA) according to the instructions provided
by the manufacturer.

**Noradrenaline**

500 µl plasma was extracted into 30 mg Al₂O₃ in Tris-HCl buffer (pH 8.65). As an internal standard 3,4 dihydroxybenzylamide hydrobromide (Sigma, St. Louis, MO, USA) was used to correct absolute recovery variations in catecholamines. After washing four times with H₂O, the noradrenaline were eluted into 100 µl 0.2 M HClO₄ solution. Noradrenaline in the eluates were measured by high performance liquid chromatography with a multi-channel electrochemical detector (ESA, CoulArray, Model 5600, USA). An ESA Catecholamine HR-80 column (C₁₈ reversed phase column, 80.0 × 4.0 mm, 3 µm) and citric acid-monochloracetic acid-acetonitrile buffer (pH 3.4) as the mobile phase were used. Flow rate was 1.0 ml min⁻¹. For calibration purposes, known noradrenaline standards were treated in the same way as the samples and the peak height ratios (relative to the peak height of the internal standard) of unknown noradrenaline were compared with those of known synthetic standards (CoulArray Software 1.003).

**Statistics**

Results are shown as means ± SEM. Paired Student’s t-test, or repeated measures ANOVA followed by either Dunnett’s or Tukey’s multicomparison test was used in the analyses of the results. Significance was accepted at the P<0.05 level.

**Results**

According to the dietary record book, the normal daily sodium intake was 148 ± 6 mmol d⁻¹. When 121 mmol sodium was added in the daily diet the urinary sodium 24 h⁻¹ increased significantly (paired Student’s t-test; P<0.05), from 98 ± 13 to 207 ± 13 mmol d⁻¹, while serum sodium remained unchanged. The calculated energy consumption was 10200 ± 700 kJ d⁻¹. Plasma aldosterone did not change (P>0.05) when the sodium intake was increased: 222 ± 39 (normal sodium) and 186 ± 69 pmol l⁻¹ (high sodium). Sodium loading did not increase the body weight and the subjects estimated that their health status did not change during the two-week elevated sodium intake.

**Blood pressure**

Before the cold pressor test, the systolic blood pressure was 118 ± 3 mmHg and the diastolic blood pressure 74 ± 3 mmHg in group A (Fig. 1). In group B, the systolic blood pressure was 118 ± 5 mmHg and a diastolic blood pressure of 71 ± 2 mmHg. There was no statistically significant difference (repeated measures ANOVA; P>0.05) between the two groups in the systolic and diastolic blood pressures. The blood pressure increased significantly when the cold pressor test was started (repeated measures ANOVA with Dunnett-test; P<0.05). The blood pressure was at its highest after two min from the start of the cold pressure test and then the blood pressure decreased. Again, the systolic pressure was similar in both groups: 137 ± 8 mmHg (normal sodium) vs. 138 ± 5 mmHg (high sodium) (repeated measures ANOVA; P>0.05) and the diastolic blood pressure was 89 ± 4 mmHg in controls and 90 ± 3 mmHg in elevated sodium group (repeated measures ANOVA; P>0.05) (Fig. 1).

Heart rates (data not shown) were similar in both groups before and during the cold pressor test.

**Noradrenaline**

Plasma noradrenaline concentration increased as shown in Fig. 2. The change was statistically significant (repeated measures ANOVA with Tukey’s test; P<0.05) during normal diet and during elevated sodium diet. The starting concentrations and the end-point concentrations were similar.

**Natriuretic peptides**

The plasma concentrations of natriuretic peptides (ANP, NT-proANP and BNP) tended to be higher in the elevated sodium diet group than in the normal diet group, both before and after the cold pressor test. The natriuretic peptides did not change during the test, and the concentrations were independent of the sodium diet, Fig. 3.

**Haemoconcentration**

Haematocrit, haemoglobin and red blood cell count were within normal limits independent of the sodium intake and the test did not change significantly these parameters. The cold pressor test did not alter the plasma volume.
Cold Pressure Test and Sodium Intake

Discussion

We reported earlier (Arjamaa et al., 1999) that a two-week sodium load, in addition to the dietary sodium intake, did not increase the mean arterial pressure in cold in healthy men of various ages, a significant increase in diastolic blood pressure was observed. Later we repeated the experiments in young adults whose blood pressure had shown sensitivity to dietary sodium intake (Arjamaa et al., 2001). In a wind tunnel (–15°C, 3.5 ms\(^{-1}\), 15 min), the increase of the blood pressure of these subjects was independent of dietary sodium intake (Arjamaa et al., 2001). We also performed a cold pressor test with these subjects.

The haemodynamic responses of the cold pressor test have been well documented (Barnett et al., 1963; Harlan et al., 1964; Eich and Jacobsen, 1967; Falkner et al., 1981; Dlin et al., 1983; Wood et al., 1984; Thomas et al., 1985; Tanji et al., 1989; Kasagi et al., 1995; Loycke et al., 1995) while the effects of a whole body exposure to acute cold wind have received less attention (Arjamaa et al., 1999; Arjamaa et al., 2001; Mäkinen et al., 2000). It is well known that cold exposure increases blood pressure and that the effect is directly related to the duration and the amplitude of the exposure. It is also known that high dietary sodium intake will lead to elevated blood pressure, at least in sodium sensitive subjects (Muntzel and Drüeke, 1992). The combined effects of cold exposure and sodium loading on blood pressure, however, have been investigated in only three previous studies (Ditto et al., 1993; Arjamaa et al., 1999; Arjamaa et al., 2000).

In the present study, calculated from the dietary record book, the mean habitual sodium intake was 148 ± 5 sodium d\(^{-1}\). In 1997, the average sodium intake in Finland was 183 mmol d\(^{-1}\) in men and 122 mmol d\(^{-1}\) in women (Lahti-Koski, 1999). Judged from urine analyses, the additional sodium was almost all excreted in the urine demonstrating that the subjects had complied with the high-sodium diet and that they did not have any disease, which would have led to the accumulation of sodium in the body. Although we did not test the completeness of the urine collection by any marker we assumed it to be

---

**Fig. 2** Plasma noradrenaline before (1) and after (2) cold pressure test in subjects with normal (□) or two-week elevated dietary sodium intake (●). Means and SEM are given (n=8). Repeated measures ANOVA, followed by Tukey’s test, * = \( P<0.05 \).

**Fig. 3** Plasma natriuretic peptides before (1) and after (2) cold pressor test in subjects with normal (□) or two-week elevated dietary sodium intake (●). Means and SEM are given (n=8). Repeated measures ANOVA, \( P>0.05 \).
The subjects responded with a significant increase in blood pressure during the cold pressor test as expected. The response was similar independent of the sodium intake. Before the test, there were no significant differences in the systolic or diastolic blood pressure between the two groups (Fig. 1). This finding is in agreement with the results of our previous study in which we did not observe any increase in blood pressure in response to two-week sodium loading (Arjamaa et al., 1999; Arjamaa et al., 2001). The effects of sodium on blood pressure may be dose-dependent as a very high intake of sodium (about 1035 mmol sodium d⁻¹ in total for one week) in Japanese males caused vasodilatation of skin vessels after one week (Yoshimura et al., 1952).

The plasma levels of noradrenaline increased significantly in the cold pressor test (Fig. 2). The starting levels were similar independent of the sodium load. The sodium load did not change the response suggesting that the sodium load did not alter the function of autonomous nervous system. ANP, NT-proANP and BNP plasma concentrations, measured immediately after the cold pressor test, were similar when compared to the levels before the test although the blood pressure increased during the test (Fig. 3). It seems that either the cold pressor test did not increase the central venous pressure (CVP) or the increase was so small and short lasting that it did not increase the release of natriuretic peptides. An additional explanation is perhaps the dilution as the peripheral samples have significantly less ANP and NT-proANP than the samples collected directly from the heart atrial lumen (Arjamaa et al., 1996).

In conclusion, the blood pressure and endocrine responses were similar in the present study as compared to an earlier study in which these subjects were exposed to cold in a wind tunnel (Arjamaa et al., 2001). Although the resting blood pressure of the subjects had shown sodium dependence about three months before we could complete under these experimental conditions. The serum level of sodium was similar in both groups.

References


Harlan WR Jr, Osborne RK, Graybiel CA (1964) Prognostic value of the cold pressor test and the basal blood pressure based on an eighteen-year follow-up study. Am J Cardiol 13: 683-687


between cold pressor test and development of hypertension based on 28-year follow-up. Hypertension 25: 71-76

Received: December 11, 2000
Accepted: March 12, 2001
Correspondence to: Olli Arjamaa, Center of Arctic Medicine, PO Box 5000, 90014 University of Oulu, Finland
e-mail: Olli.Arjamaa@oulu.fi