The Effect of Dietary Sodium on the Concentrations of Vasoactive Intestinal Peptide in Plasma and Lung*

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Study objectives: In this study, we sought to determine whether changes in the concentration of vasoactive intestinal peptide (VIP) in the lung might explain the increase in bronchial reactivity associated with high sodium diets.

Design: Male Sprague-Dawley rats, eight in each group, were placed on low-sodium, normal-sodium, or high-sodium diets and distilled drinking water ad libitum for 7 days. On the day of study, blood was sampled to determine plasma VIP concentration and the lungs were harvested and snap frozen in liquid nitrogen. VIP was measured in plasma and tissue extracts by radioimmunoassay.

Results: The VIP concentrations in both lung and plasma varied with dietary sodium. Plasma VIP level was significantly higher in the rats that had received the low-sodium diet (51.45 ± 7.35 pmol L⁻¹) than in the rats that had received the high-sodium diet (29.84 ± 6.83; p < 0.05). In the lung, VIP level was greater in the rats that had received the normal-sodium diet (378.13 ± 41.68 fmol/g) than in rats that had received either the low-sodium diet (137.30 ± 26.11 fmol/g; p < 0.0005) or the high-sodium diet (182.64 ± 28.63 fmol/g; p < 0.005).

Conclusions: The lower plasma and pulmonary concentrations of VIP observed in rats that had received a high-sodium diet suggest that VIP may play a role in the increased bronchial reactivity reported with this diet.

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VIP = vasoactive intestinal peptide

Key words: asthma; dietary sodium; vasoactive intestinal peptide

Epidemiologic studies have demonstrated that the prevalence of asthma is greater in western societies.¹ Although atmospheric pollution has been implicated in this increase, other environmental factors such as dietary sodium intake may also contribute. Regional population studies in England and Wales have correlated mortality from asthma with sales of table salt.² More specifically, the response to inhaled histamine has been shown to correlate inversely with the 24-h urinary excretion of sodium.³ Lower histamine doses provoked greater bronchoconstriction in subjects who had greater 24-h urinary sodium excretions. Further, the bronchoconstrictor response to histamine challenge has been shown to increase following an increase in daily sodium ingestion.⁴ Although the exact mechanism is not understood, it has been postulated that increased dietary sodium potentiates the activity of the cell membrane pump on bronchial smooth muscle, thereby increasing bronchial reactivity.⁵

Other work has suggested that changes in the concentration of bronchodilators such as vasoactive intestinal peptide⁶ (VIP) and bronchoconstrictors such as endothelin⁷ and substance P⁸ may be important in the pathogenesis of asthma. VIP is a powerful bronchodilator that also inhibits bronchial smooth muscle cell proliferation.⁹ In addition, it has been reported to inhibit some cell membrane sodium pumps.¹⁰ Further, we have demonstrated that dietary sodium intake affects both the rate at which VIP is cleared from the circulation by the lungs¹¹ and its secretion into the circulation.¹² Tissue concentrations of VIP might be expected, therefore, to vary with the dietary sodium intake.

In this study, we sought to determine whether the concentration of VIP in the lung varies with dietary sodium thereby providing a link between sodium intake and reduced pulmonary VIP concentrations.

Methods and Materials

Male Sprague-Dawley rats weighing 250 to 300 g were placed on low- (0.008%), normal- (2.2%), or high-sodium (4.4%) diets (Jans Chemicals, Forbes, New South Wales). To accentuate the effects of sodium depletion, a fourth group was studied after 1 week of receiving the low-sodium diet and furosemide (1 mg/kg/d) administered in the drinking water. The diets used in these studies were identical apart from their sodium chloride content that was calculated as percentage dry weight and contained all essential nutrient elements for adequate nutrition. The rats were permitted ad libitum access to diet and distilled water and allowed to achieve sodium balance over 7 days. In the 48 h prior to study, the rats were placed in metabolic cages and urine was collected to determine urinary sodium excretion.

On the day of experiment, the rats were anesthetised using halothane (2.5%) in nitrous oxide (1.0 L min⁻¹) and oxygen (0.5 L

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Truncal blood was collected into aprotinin (Trasylol) (500 KIU/mL) and heparin (50 U/mL) for determination of plasma VIP concentration. The lungs were then harvested, weighed, snap frozen in liquid nitrogen, and stored at −80°C until use.

**Tissue Extraction and Determination of VIP Concentrations**

Frozen lungs from each rat were pulverized using a stainless steel hammer and anvil precooled with liquid nitrogen. The pulverized tissue was placed in 10 mL of 0.1N hydrochloric acid containing 100 pmol/L of edetic acid and 0.01% sodium metabisulfite. It was then heated in a water bath at 100°C for 10 min. After cooling on ice, the tissue was homogenized using an homogenizer (OMNI 1000) at speed 3 for 30 s. The homogenate was centrifuged at 1,000g, 4°C for 30 min. The supernatant was decanted, aliquotted, and stored at −20°C until radioimmunoassay.

The concentrations of VIP in pulmonary extracts and plasma were then determined by radioimmunoassay using a polyclonal antibody and 125I-VIP purified by reverse-phase high-pressure liquid chromatography as described previously.13 Recoveries of exogenous VIP added to lung preparations prior to homogenization were 65 to 70%.

**Statistical Methods**

Initial comparisons among the four groups for each parameter were made by analysis of variance. Individual pairs of comparisons were by t test using a pooled variance estimate (CSS Statistica); p values of less than 0.05 were considered significant.

**Results**

Twenty-four-hour urinary sodium excretions are shown in Figure 1. There were significant differences among the four study groups in both the plasma and pulmonary concentrations of VIP (plasma: p<0.05; pulmonary: p<0.0005). In the plasma, the concentration of VIP was greatest in the animals that had been maintained on the low-sodium diet prior to study and least in those rats that had received furosemide and a low-sodium diet. The plasma VIP concentration in the animals that had received the low-sodium diet (51.45±7.35 pmol L⁻¹) was significantly greater than rats maintained on the high-sodium diet (29.84±6.83 pmol L⁻¹; p<0.05) and the rats given furosemide in addition to a low-sodium diet (12.48±5.76 pmol L⁻¹; p<0.01) (Fig 2).

In contrast to the plasma, the concentration of VIP in the lungs was greatest in the rats that had received the normal diet. However, the lowest concentration of VIP was again observed in the rats given furosemide and the low-sodium diet before study. The concentration of VIP in the lungs for the rats that had received

**Figure 1.** Twenty-four-hour urinary sodium excretions of rats fed low-sodium diet plus furosemide, low-sodium diet, normal diet, and high-sodium diet. Asterisk and number sign= p<0.0005; denotes differences between furosemide and normal and high sodium groups, respectively. Double asterisk and double number sign= p<0.0005; denotes differences between low sodium and normal and high sodium groups, respectively.

**Figure 2.** Plasma concentrations of VIP, values are mean±SEM for n=8 in each group. Asterisk=p<0.05; denotes difference between low and high sodium dietary groups; double asterisk=p<0.01; difference between low sodium and low sodium plus furosemide groups.

**Figure 3.** Concentrations of VIP in the lungs of each of the four experimental groups. Values are mean±SEM for n=8 in each group. Asterisk=p<0.005; high compared with normal sodium diet; double asterisk=p<0.005; furosemide/low sodium compared with normal, low sodium compared with normal; number sign=p<0.05; low sodium compared with furosemide/low sodium; double number sign=p<0.005; high sodium compared with furosemide/low sodium.
the normal-sodium diet (378.13±41.68 fmol/g of tissue) was significantly greater than that of rats that had received either the high-sodium (182.64±28.63 fmol/g of tissue; p<0.005) or the low-sodium (137.30±26.11 fmol/g of tissue; p<0.0005) diets. It was also significantly greater than that of the rats given furosemide and a low-sodium diet (78.47±4.98 fmol/g of tissue; p=0.0005) (Fig 3). The pulmonary VIP concentrations of the rats that had received both the low- and high-sodium diets were also significantly greater than that of the rats given furosemide in addition to the low-sodium diet (low: p<0.05; high: p<0.005).

**Discussion**

This study demonstrates that the amount of sodium ingested in the diet can influence both the plasma and pulmonary concentrations of VIP. In animals that were fed the high-sodium diet, the pulmonary concentrations of VIP were significantly lower than in animals given the normal-sodium diet. In addition, the plasma concentration was lower in the high-sodium group, although this decrease did not reach statistical significance. This lower pulmonary concentration of VIP may explain the increase in bronchial reactivity that is seen in asthmatics with increased levels of dietary sodium intake.

Interestingly, decreasing the dietary sodium intake to almost negligible amounts does not appear to confer a benefit by increasing the concentration of VIP in the lung. This was significantly lower in the low-sodium diet group than in the normal-sodium group. However, in the low-sodium diet group, the plasma concentration of VIP was increased significantly, which may act to offset the lower pulmonary concentrations. In other work, we have demonstrated that the plasma concentration of VIP increases in response to an oral sodium load when it is given against a background of a low-sodium diet but not against a background of a normal-sodium diet. Thus, the low-sodium diet may protect against the effects of a short-term increase in the amount of sodium that is ingested.

The mechanism by which sodium affects VIP production is not known, and could involve both direct and indirect mechanisms such as the renin-angiotensin system. It would appear, however, that a direct effect is the more likely. We have shown previously that alterations in dietary sodium intake affect both the rate of removal from and secretion of VIP into the circulation. In contrast, angiotensin II, independent of dietary sodium, affects the clearance rate of VIP from the circulation but does not affect its secretion.

Both the plasma and pulmonary concentrations of VIP were lowest in the group given furosemide in addition to a low-sodium diet. Although furosemide is a bronchodilator, it is better known as a powerful natriuretic and diuretic agent and in combination with a low-sodium diet results in sodium depletion. VIP is natriuretic and like furosemide increases sodium excretion by the loop of Henle, probably by interaction with the sodium potassium chloride cotransporter. Thus, the lower concentrations of VIP in the group receiving furosemide and the low-sodium diet appear appropriate and may reflect a response to sodium depletion per se or alternately the presence of a competing agonist.

We conclude, therefore, that changes in the level of sodium ingested result in variations in the concentration of VIP in lung and plasma. Further, the direction of these changes suggests that a reduction in VIP may mediate the increased bronchial reactivity that has been reported with increased dietary sodium intake.

**References**

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