Serum vasopressin-degrading activity is related to blood total cholesterol levels in men but not in women

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Introduction: The precise role of vasopressin (AVP) in the pathophysiology of cardiovascular disease is controversial, but this peptide hormone is important for several reasons: circulating concentrations of AVP are elevated in hypertension; AVP is synthesized also in peripheral tissues, where acts as a paracrine hormone; and AVP has vasoconstrictor, mitogenic, hyperplastic and renal fluid retaining properties which may have deleterious effects. Furthermore, cholesterol blood levels are associated with hypertension.

Objectives: To analyze the relationship between blood total cholesterol levels and serum AVP-DA in healthy humans, and the differences between men and women.

Material and Methods: We used blood samples from 44 women and 31 men. Cholesterol levels were measured using commercially available kits. AVP-DA activity was determined in triplicate in a fluorometric assay using cystyl-ß-naphthylamide as the substrate. We used two-tailed t-test to analyse differences between men and women. Linear correlation coefficients were calculated to test relationships between AVP-DA activity and blood total cholesterol levels.

Results: Sex differences were observed for AVP-DA (P <0.05), being this activity higher in men than in women. According to the linear model of the regression analysis, AVP-DA activity showed a significant negative correlation with blood total cholesterol levels in men (r =-0.5303, P <0.01), whereas no correlation was observed in women.

Discussion: Several studies demonstrate the existence of greater plasma AVP concentrations in men compared to women, which could explain the gender-differences observed in the present work in relation with AVP-DA. However, AVP-DA activity is related (inversely) to blood cholesterol levels only in men. This could indicate that the risk of high cholesterol-related hypertension is more probable in men that in women.

Conclusions: Although AVP-DA misregulation could be involved in the pathogenesis of hypertension, its relation with cholesterol levels appears only in men, but not in women. Therefore, other factors are prevalent in the risk of hypertension in women.

INTRODUCTION

The precise role of vasopressin (AVP) in the pathophysiology of cardiovascular disease is controversial, but this peptide hormone is important for several reasons. Firstly, circulating concentrations of AVP are elevated in heart failure and some forms of hypertension. Secondly, there is evidence that AVP is synthesized not only in
the hypophyseal–pituitary axis but also in peripheral tissues including the heart where it acts as a paracrine hormone. Thirdly, AVP has vasoconstrictor, mitogenic, hyperplastic and renal fluid retaining properties which, by analogy with angiotensin II, may have deleterious effects when present in chronic excess [Burrel et al., 2000; Szczepanska-Sadowska, 1996]. AVP is metabolized by vasopressin-degrading cystyl-aminopeptidase activity (AVP-DA) [Barret and Rawlings, 1998]. Furthermore, cholesterol blood levels are associated with hypertension, although the underlying mechanism is not known.

OBJECTIVES
To analyze the relationship between blood total cholesterol levels and serum AVP-DA in healthy humans, and the differences between men and women.

MATERIAL AND METHODS

Subjects.
We used blood samples, obtained by venipuncture without additives, from 44 women aged 31 to 70 years and 31 males aged 31 to 63 years. All participants were ambulatory subjects who participate for health screening. The samples were centrifuged at 4°C for 10 min at 3000 × g, and the sera were analysed the same day. Hemolytic, icteric, or turbid samples were discarded. None of the subjects had any known disease. When subjects came to the hospital for health screening, they were required to inform whether or not they had some known disease or were taking drugs, and were also asked about alcohol consumption.

Vasopressin-degrading activity (AVP-DA) assay.
AVP-DA was measured fluorimetrically using cystyl-ß-naphtylamide (CysNNap) as substrate, as previously described by us [García-López et al., 2003]. Thus, ten microliters of sample were incubated in triplicate for 30 min at 37 °C with 1 ml of the substrate solution containing 100 µM CysNNap, 1.5 mM bovine serum albumin (BSA) and 0.65 mM dithiothreitol (DTT) in 50 mM of phosphate buffer, pH 7.4. After this time, the reaction was stopped adding 1 ml of acetate buffer 0.1 M, pH 4.2.

The amount of ß-naphthylamine released as the result of the enzymatic activity was measured fluorimetrically at 412 nm emission wavelength with an excitation wavelength of 345 nm using a Perkin-Elmer instrument. Proteins were quantified in triplicate by the method of Bradford using BSA as a standard. Specific AVP-DA was expressed as nanomoles of CysNNap hydrolysed per min per mg of protein, by using a standard curve prepared with the latter compound under corresponding assay conditions. The fluorogenic assay was linear with respect to time of hydrolysis and protein content.

Total cholesterol assay.
Blood total cholesterol was determined colourimetrically using a commercial kit (Sigma 352-50).

Statistical analysis.
We used two-tailed t-test to analyse differences between men and women. Linear correlation coefficients were calculated to test relationships between AVP-DA activity and blood total cholesterol levels. All comparisons with P values below 0.05 were considered significant.

RESULTS
Total blood cholesterol levels in women (237.00 ± 5.93 mg/dL) were significantly higher (p<0.05) than in men (212.25 ± 6.08 mg/dL) (Mean ± SEM in both cases). Moreover, significant gender differences were observed for AVP-DA ( p <0.05), being this activity higher in men than in women. Values (mean ± SEM) of specific AVP-DA in serum of healthy men and women are presented in figure 1. Furthermore, according to the linear model of the regression analysis, AVP-DA activity showed a significant negative correlation with blood total cholesterol levels in men ( r =-0.5303, P <0.01) (figure 2), whereas no significant correlation was observed in women (figure 3).
Figure 1: Specific serum vasopressin degrading activity (AVP-DA) in healthy men and women. Results are expressed in nanomoles of cystyl-ß-naphthylamide hydrolyzed per min and per mg of protein (Mean ± SEM; n=31-44; * p <0.05).
Figure 2: Linear regression analysis between blood total cholesterol levels and vasopressin-degrading activity (AVP-DA) in men. AVP-DA values are expressed in nanomoles of cystyl-β-naphthylamide hydrolyzed per min and per mg of protein; Total blood cholesterol values are expressed in mg/dL (n=31; r=-0.531; p=0.0021).
DISCUSSION
Several studies in humans demonstrate the existence of greater plasma AVP concentrations in normal men compared to normal women, which could explain the gender-differences observed in the present work in relation with AVP-DA. Similar results have been found by us in mice. This gender-differences in the levels of AVP-DA in serum described here could be a physiological response to maintain blood pressure at physiological levels. However, we have found here that AVP-DA activity is related (inversely) to blood cholesterol levels in men but not in women, although in our hands, women showed higher blood cholesterol levels than men. This could indicate that the risk of high cholesterol-related hypertension is more probable in men than in women. Experiment of our group in mice fed a high cholesterol diet showed that both in male and female mice, the cholesterol enriched diet significantly increased AVP-DA, which also could be explained as a physiological response to the potential high levels of AVP which could be induced by angiotensin II due to cholesterol [Reaux et al., 2001; Ramírez-Expósito et al., 2001], to maintain blood pressure at physiological levels, at least at short-term. In any case, the differences observed here between human and rodents could be due only to specie differences in the metabolism of cholesterol.

CONCLUSIONS
Although AVP-DA misregulation could be involved in the pathogenesis of hypertension, its relation with cholesterol levels appears only in men, but not in women. Therefore, other factors are prevalent in the risk of hypertension in women.

Figure 3: Linear regression analysis between blood total cholesterol levels and vasopressin-degrading activity (AVP-DA) in women. AVP-DA values are expressed in nanomoles of cystyl-ß-naphthylamide hydrolyzed per min and per mg of protein; Total blood cholesterol values are expressed in mg/dL (n=44; Analysis not significant)
BIBLIOGRAPHY
