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Author(s):Dr. Maria Jesus Ramirez-Exposito, Dr. Marce Arrazola, Dr. Jose Manuel Arias de Saavedra, Dr. Rafael Agesta, Dr. Maria Pilar Carrera, Dr. Maria Dolores Mayas, Dr. Jose Manuel Martinez-Martos

Corresponding Author:
Dr. Jose Manuel Martinez-Martos,
PhD, Health Sciences, University of Jaen, Campus Universitario Las Lagunillas, E-23071 - Spain

Submitting Author:
Dr. Jose Manuel Martinez-Martos,
PhD, Health Sciences, University of Jaen, Campus Universitario Las Lagunillas, E-23071 - Spain

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Abstract

The increase of blood pressure with development and ageing has been extensively reported. The aim of this study is to analyse the changes in the angiotensinase and vasopressin-degrading activities in men and women in various age groups. Aminopeptidase N (APN), aminopeptidase B (APB) and vasopressin-degrading activity (AVP-DA) were measured fluorometrically using arylamide derivatives as substrates. All the activities decreased during ageing. The correlation between aminopeptidase activities and age in males and females demonstrated only a highly significant inverse correlation for APB in females. These results could indicate the maintenance or the increase in Ang III and vasopressin in plasma, which may contribute to the increase in blood pressure observed during ageing.

Introduction

Determination of serum aminopeptidases (AP) is useful in clinical diagnosis for several human diseases. However, in physiological conditions, these enzymes also play a role in the regulation of circulating biologically active peptides. Therefore, hormonal changes in serum may thus be reflected in this enzymatic activity [1].

Development and ageing are characterised by a progressive increase in blood pressure in which plasma renin-angiotensin system (RAS) and arginine-vasopressin (AVP) may play a significant role. It has been described that human serum aminopeptidase A (AP A), the enzyme responsible for the hydrolysis of Ang II to Ang III, increases during development and ageing, and the nature of this increase differs in women and men [2]. Therefore, to better understand the role of AP in blood pressure control, we analysed serum Ang III- and AVP-degrading activities in women and men during development and ageing. We measured aminopeptidase N (APN; EC 3.4.11.2) and aminopeptidase B (APB; EC 3.4.11.6), reported as capable to hydrolyse Ang III [3], and AVP-degrading activity (AVP-DA; EC 3.4.11.3) [4], using arylamide derivatives as substrates, in various age groups.

Methods

We used blood samples, obtained by venipuncture without additives, from 148 females aged 2 to 87 years and 139 males aged 1 to 92 years. All participants were ambulatory subjects who came to the Hospital for health screening. The samples were centrifuged at 4ºC for 10 min at 3000 x *g*, and the sera were analysed the same day. When subjects came to the hospital for health screening, they were required to inform whether or not they had some known disease, taking drugs and also asked for alcohol consumption. Blood samples were separated into aliquots for determinations of aminopeptidase activities and total protein content. Haemolytic, icteric, and turbid samples were discarded. The selected subjects reported to have no known disease, taking no drugs and no alcohol consumption.

APN, APB and AVP-DA were measured fluorometrically as previously described [2] using Ala-, Arg- and Cys-ß-naphthylamide as substrates. Ten microliters of each supernatant was incubated during 30 min at 25ºC with 1 ml of the substrate solution (2.14 mg/100 ml Ala-ß-naphthylamide or 3.35 mg/ 100 ml Arg-ß-naphthylamide or 5.63 mg/100 ml Cys-ß-naphthylamide), 10 mg/100 ml bovine serum albumin (BSA), and 10 mg/100 ml dithiothreitol (DTT) in 50 mM of phosphate buffer, pH 7.4, for APN and APB; and 50 mM HCl-Tris buffer, pH 6, for AVP-DA. All the reactions were stopped by adding 1 ml of 0.1 mol/L acetate buffer, pH 4.2. The amount of ß-naphthylamine, released as a result of enzymatic activity, was measured fluorometrically at 412 nm emission wavelength with an excitation wavelength of 345 nm. This rapid fluorometric method permits quantify aminopeptidase activities in the order of 10^{-14} mole. Serum specific aminopeptidase activities were expressed as pmol of substrate hydrolysed per min

per mg of protein. Fluorogenic assays were linear with respect to time of hydrolysis and protein content.

For statistical analysis, to be able to compare sex, the male and female subjects were divided into five age-matched groups (Table 1) which included the main stages of development. We used one-way analysis of variance (ANOVA) to analyse differences between groups. Linear correlation coefficients were calculated to test relationships between two variables. All comparisons with *P* values below 0.05 were considered significant.

Results

Values (mean ± SEM) of specific aminopeptidase activities in serum of the different age groups in female and male subjects are showed in Table 2. Significant age-related changes were observed in males for APN ($P < 0.05$), in females for APB ($P < 0.05$) and in females for AVP-DA ($P< 0.05$). In general, all the activities decreased during ageing and reached statistical significance in the groups of 46-65 and > 66 years old. Sex differences were observed only for AVP-DA in the group of 16-45 years old ($P<0.05$), being higher in males than in females. In the whole population, according to the linear model of the regression analysis, APB ($r = -0.14$, $P < 0.01$) and AVP-DA ($r = -0.13$, $P < 0.02$) showed a significant inverse correlation with age, whereas no correlation was observed for APN ($r= -0.10$, $P < 0.08$). The correlation between AP activities and age in males and females considered separately demonstrated a highly significant inverse correlation for APB in females ($r=-0.24$. $P<0.0001$), whereas no correlation were observed for APN or AVP-DA. No correlation were demonstrated in males, but a borderline inverse correlation was found for AVP-DA ($r=-0.15$, $P=0.06$).

Discussion

Blood pressure, particularly systolic blood pressure, tends to increase progressively with age. Blood pressure increases more in males than in females among young people, but later increases more notably with age in females and values in elderly males and females are similar. This behaviour is similar in several international populations, as described by the Hypertension optimal treatment (HOT) [5] and other studies [6-8]. In the RAS, plasma renin activity and Ang II decline progressively with age. The decrease in plasma renin activity is exponential, being very rapid in early childhood and continuing into old age [9]. The contractile response to Ang II decrease also with age [10]. This age-related profile is the opposite of the one described previously for APA activity [2]. Therefore, the inverse correlation between peptide level and peptide-hydrolysing activity suggests a relation between high breakdown capacity and low level of substrate, which in turn supports a role for human plasma APA activity as an angiotensinase. However, these observations appear incompatible with the mentioned tendency of blood pressure to increase with age and for Ang II-induced contractility to decline [2]. Therefore, additional factors must be involved in this phenomenon, which could include changes with ageing that result in gradually increasing rigidity of vessels and the role of other vasoactive peptides such as Ang III or AVP.

Increased levels of APA [2] imply a high metabolism of Ang II and consequently an increase in the formation of Ang III, which is further converted to Ang IV by APB and/or APN. APN can lower blood pressure when is intracerebroventricularly infused in spontaneously hypertensive rats, probably as a result of the increased metabolism of Ang III. Pretreatment with the Ang II antagonist losartan significantly attenuated this hypotensive effect [11]. However, it has been suggested recently that a central component might be responsible for the sympathetic hyperactivity observed at early stages of hypertension [12]. In fact, hyperactivity of the brain RAS has been implicated in the development and maintenance of high blood pressure [13,14]. In the brain RAS, Ang III exhibits the same affinity for type 1 and type 2 Ang II receptors than Ang II, and both peptides cause similar increases in vasopressin release and blood pressure [15-17]. The inhibition of AlaAP induces vasopressin release by increasing the half-life of brain Ang III [3]. On the other hand, while ageing-related decreases in AVP concentration have been found in rat brain [18], an increase has been noted in rat [18] and human [19] plasma. This may be due in part, to the here reported decreasing AVP-DA during ageing.

According with the present results, there is no change or even a decrease in APN, APB and AVP-DA during ageing. Therefore, presumably due to their reduced metabolism, Ang III and AVP would maintain or increase their levels in plasma, which may contribute to the increase in blood pressure observed during ageing in male and female through central nervous system mechanisms. Our results also showed a significant decrease of APB activity in female but not in male at the age of 46-65. However, blood pressure in female is similar or lower than in male. This discrepancy could be explained by the existence of a

light (non significant) decrease on male APB, which could explain this similar blood pressure in female and male. The significant decrease on APB activity in female may be also related with other factors. In fact, it has been described an important influence of sex steroids on several serum aminopeptidase activities [1], and this female age-group (46-65) is characterized by important changes in sex hormone levels due to menopause.

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Illustrations

Illustration 1

Table 1. Age groups in males and females

	Males		Females	
Age (years)	n	mean ± SD	n	mean ± SD
1-6	18	3.3 ± 1.53	17	4.7 ± 1.65
7-15	19	11.5 ± 2.57	20	11.1 ± 2.82
16-45	44	27.4 ± 8.48	51	26.8 ± 7.50
46-65	25	55.4 ± 5.7	26	53.3 ± 4.85
> 66	33	77.3 ± 5.85	34	76.2 ± 5.07
Total	139	39.0 ± 6.13	148	38.1 ± 5.60

Illustration 2

Table 2. Age-related changes in human serum aminopeptidase activities (mean ? SD).

	APN		APB		AVP-DA	
Age(yrs)	Males	Females	Males	Females	Males	Females
≤ 6	352.8 ± 70.8	373.7 ± 50.2	141.3 ± 82.3	155.7 ± 48.2	41.5 ± 24.6	38.6 ± 7.0
7-15	420.5 ± 85.0	375.6 ± 71.0	154.5 ± 35.7	150.5 ± 49.2	43.2 ± 8.7	37.9 ± 8.0
16-45	404.3 ± 117.3	382.1 ± 79.2	157.7 ± 48.4	158.3 ± 89.9	42.3 ± 18.6	36.1 ± 9.3
46-65	397.5 ± 103.0	402.1 ± 61.2	127.2 ± 35.0	116.9 ± 20.9 a	34.5 ± 8.5	32.6 ± 5.6 a
≥ 66	343.8 ± 134.3 a	356.8 ± 85.7	143.7 ± 105.0	125.0 ± 33.2	37.3 ± 20.7	36.4 ± 9.9

Values are expressed as pmol of Ala-, Arg- and Cys-ß-naphthylamide hydrolysed per min per mg of protein in the different age groups of males and females (a $P<0.05$).

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