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Original article

Changes in the expression of α_{1B}-adrenoceptor in peripheral mononuclear cells correlates with blood pressure and plasmatic homocysteine

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ABSTRACT

Human peripheral mononuclear cells (HPMC) have been suggested as a practical surrogate for myocardial or vascular cells. Present work analyses if changes in the expression of α_1-adrenoceptors (ARs) in HPMC are related to the hypertensive state and its clinical consequences. Quantitative RT-PCR was employed to evaluate the mRNA levels of the three α_1-ARs (α_{1A}, α_{1B}, α_{1D}) in HPMC isolated from normotensive and hypertensive patients, and also in tissues from two animal models of hypertension: spontaneously hypertensive rats (SHR) and hypertension induced by chronic treatment with L-NAME. In patients, 24-h ambulatory blood pressure and serum biochemical profile were also recorded. We found that α_{1B}-AR expression was higher in HPMC from hypertensive patients and correlated with blood pressure and plasmatic homocysteine. A rise in the α_{1B}-AR expression in kidneys, but not in heart from hypertensive animal models was also found. α_{1D}-AR did not change in HPMC, not in rat heart or kidney, but a significant correlation with plasmatic aldosterone was found. In conclusion, we have proved that α_1-ARs mRNA expression in HPMC correlates with clinical variables and could be used as a potential biomarker in hypertensive patients.

1. Introduction

The importance of the sympathetic nervous system in the pathophysiology of essential hypertension has been strengthened by evidences supporting its activation during rise in blood pressure [1]. Sympathetic stimulus is driven through the activation of adrenoceptors (AR) by catecholamine. Among the whole family of ARs, it has been demonstrated that α_1-ARs have a major role in the control of blood pressure [2]. Previous studies have shown an increased expression of α_1-ARs in vessels, heart and kidney [3] of hypertensive animals. More specifically, a major role of α_{1D}-ARs has been proposed by different groups and us as one of the changes involved in increase in vascular resistance in hypertensive state [4–9].

Investigating the role of α_1-ARs in human hypertension is limited by availability of samples. To solve this problem, indirect approaches have been previously suggested. As suggested by others, measuring α_1-AR's expression in human peripheral mononuclear cells (HPMC) could represent a valid model to extrapolate the expression among the cardiovascular system [10]. However, α_1- ARs have been poorly characterized in HPMC. There are few studies looking at α_1-AR's role in these blood cells, but controversy is still on this field. Some authors were not able to detect α_1-AR's protein [11,12], or mRNA expression in HPMC [11,12], while some others did it [10,13,14]. These discrepancies could be due to the different methodology employed. Regardless of α_1-AR's expression discrepancies, their role in the regulation/activation of immune cells had been studied but still needs to be clarified [15].

α_1-ARs have been proposed as a biomarker in cardiovascular diseases, as well as it was proposed for β-ARs by others [16]. β-ARs have been described to mirror the expression in tissues, and some

Abbreviations: AR, adrenoceptor; RT, reverse transcription; PCR, polymerase chain reaction; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Ct, threshold cycle; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto.

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of the clinical variables which are characteristic of the hypertensive state [17]. α_1-ARs family is composed by three different subtypes: α_{1A}, α_{1B}, and α_{1D}-AR. Both mRNA and protein for the three subtypes have been detected in HPMC suggesting to use them as a surrogate biomarker in cardiovascular diseases, specifically in those where ARs play a key role such in hypertension [13,14]. The use of α_1-ARs proposal has been proved in animal models where the expression of the three α_1-ARs in aortas from Spontaneously Hypertensive Rats (SHR) was mirrored in peripheral blood lymphocytes [10].

Based on these observations, we believe that a detailed study regarding changes in the expression of the α_1-AR subtypes in HPMC of hypertensive patients is needed. In the present study we examine by real time quantitative reverse transcription polymerase chain reaction (RT-PCR) the expression of the three α_1-ARs (α_{1A}, α_{1B} and α_{1D}) in HPMC from normotensive and hypertensive patients. The existence of a significant correlation between α_1-AR expression in HPMC and the relevant clinical variables characteristic of the hypertensive status (see Table 1) will support the fact that HPMC mirrors the expression in other tissues among the cardiovascular system. Then mRNA levels of α_1-ARs could be considered as a biomarker in cardiovascular diseases (as previously reported for β-ARs [16,17]).

In addition to the study in human samples, we perform a similar analysis in two animal models of hypertension, the spontaneously hypertensive rats (SHR) and the L-NAME induced hypertension, comparing them to their respective controls (WKY and Wistar).

2. Material and methods

2.1. Selection of study participants and clinical procedures

Patients included in the study were selected from an outpatient clinic. The overall study population consisted of 25 subjects (16 males and 9 females). The inclusion criteria were: a) diastolic blood pressure (DBP) in the range of high normal to moderate essential hypertension, defined as being between 90 and 114 mmHg (Korotkoff phase V, sitting position) for 3 visits at 1-month intervals in the absence of specific causes.; b) age 18 to 65 years; c) never previously treated for hypertension or treated with Angiotensin Converting Enzyme Inhibitors (ACEIs) or Angiotensin II Receptor Blockers (ARBs). Patients with established cardiovascular or renal diseases were excluded. A control group recruited were healthy normotensive subjects, and who were age and sex matched. All patients who fulfilled the inclusion criteria were invited to participate, and written consent was obtained. The Ethical Committees of the Hospital of Alzira (ref. CPMP/ICH/135/95) and the University of Valencia (Ref. H1396873322007) approved the study.

All patients had a complete clinical workup to rule out secondary hypertension. Serum biochemical profile, lipids, urinary albumin excretion, office blood pressure and 24-h ambulatory blood pressure monitoring were obtained in the outpatient setting. Blood pressure was measured using a mercury sphygmomanometer with the patient in sitting position after five minutes of rest in a

Table 1
Demographic and biochemical characteristics of each group.

	Control	Isolated Office Hypertension	Hypertension	Treated Hypertension
Age, y	45 ± 5	46 ± 9	48 ± 4	58 ± 2
Male/female, n	(6)/(1)	(2)/(3)	(5)/(2)	(3)/(3)
Body mass index, kg/m^2	28 ± 2	32 ± 3	27 ± 1	31 ± 2
BP 24 h, mmHg				
Systolic	119 ± 3	121 ± 2	135 ± 2***	129 ± 4
Diastolic	75 ± 2	74 ± 3	86 ± 1*	80 ± 4
BP day, mmHg				
Systolic	123 ± 3	122 ± 3	142 ± 3**	136 ± 4*
Diastolic	78 ± 2	74 ± 4	92 ± 2**	85 ± 3
BP night, mmHg				
Systolic	108 ± 4	115 ± 3	121 ± 3	115 ± 6
Diastolic	67 ± 3	68 ± 1	74 ± 2	70 ± 4
Heart rate, bpm				
24 h	70 ± 2	76 ± 6	78 ± 4	75 ± 3
day	75 ± 3	77 ± 6	84 ± 4	78 ± 3
night	64 ± 3	72 ± 7	68 ± 4	63 ± 1
Total cholesterol, mg/dL	206 ± 7	168 ± 15	228 ± 16	226 ± 18
HDL cholesterol, mg/dL	52.9 ± 4.3	55.4 ± 5.9	52.9 ± 5.0	52.2 ± 3.2
LdL cholesterol, mg/dL	126 ± 9	88 ± 11	152 ± 12	144 ± 16
Triglyceride, mg/dL	137 ± 26	117 ± 31	113 ± 29	148 ± 43
Insuline, μU/mL	18.3 ± 6.7	22.0 ± 8.0	12.5 ± 1.7	13.3 ± 5.2
Fasting blood sugar, mg/dl	102 ± 4	105 ± 7	95 ± 3	111 ± 11
Haemoglobin, g/L	14.7 ± 0.2	14.8 ± 0.6	15.8 ± 0.5	14.2 ± 0.5
HbA1c (%)	5.47 ± 0.15	5.87 ± 0.33	5.78 ± 0.17	6.20 ± 0.28
Adrenaline, pg/mL	50.0 ± 10.3	50.0 ± 9.2	28.0 ± 6.6	36.2 ± 7.2
Noradrenaline, pg/mL	409.3 ± 102.0	304.0 ± 49.9	459.5 ± 177.4	495.4 ± 111.1
Dopamine, pg/mL	27.6 ± 7.7	31.8 ± 9.5	33.0 ± 4.6	27.0 ± 5.2
Creatinine, mg/dL	0.91 ± 0.05	0.80 ± 0.07	0.84 ± 0.06	0.83 ± 0.06
CRCL, ml/min/1.73m2	124 ± 17	117 ± 14	109 ± 14	115 ± 17
Homocysteine, nmol/L	7.15 ± 0.71	6.97 ± 1.02	9.50 ± 2.06	11.00 ± 2.04
Aldosterone (pg/mL)	96.7 ± 7.7	151.3 ± 42.9	85.5 ± 10.1	96.0 ± 28.0

BP indicates blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycosylated haemoglobin; CRCL, creatinine clearance; Values are expressed as mean ± s.e.m.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$ vs. control group.