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Research Article

Increased platelet alpha$_{2B}$-adrenergic receptor gene expression in well-controlled hypertensives: the effect of arterial stiffness

Maria E. Marketou, MD, PhD[a,*], Joanna E. Kontaraki, PhD[b], John A. Papadakis, MD[c], George E. Vrentzos, MD[c], Alexandros Patrianakos, MD, PhD[a], Konstantinos Fragkiadakis, MD[a], Panagiotis Tsiverdis, MD[a], Dimitris Lempidakis, MD[a], Gregory Chlouverakis, PhD[d], Panos E. Vardas, MD, PhD[a], and Fragiskos I. Parthenakis, MD, PhD[a]

[a]*Department of Cardiology, Heraklion University Hospital, Heraklion, Crete, Greece;*
[b]*Division of Biostatistics, Department of Social Medicine, Molecular Cardiology Laboratory, School of Medicine, University of Crete, Heraklion, Crete, Greece;*
[c]*Department of Internal Medicine, Heraklion University Hospital, Crete, Heraklion, Crete, Greece; and*
[d]*Division of Biostatistics, School of Medicine, University of Crete, Heraklion, Crete, Greece*

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Abstract

Catecholamines play a major role in atherothrombotic mechanisms in essential hypertension. Alpha$_{2B}$-adrenergic receptors (α2B-ARs) are implicated in the pathophysiology of platelet aggregation. In this study, we evaluated platelet α2B-AR gene expression levels in patients with well-controlled essential hypertension compared with normal individuals and investigated their association with increased arterial stiffness. Fifty-nine patients with well-controlled essential hypertension (34 men, mean age 65 $\pm$ 9 years) and 26 normotensives (19 men, mean age 64 $\pm$ 8 years) were included in the study. For each patient, carotid-femoral pulse wave velocity (PWV) and carotid-radial PWV were evaluated. In addition, blood samples were obtained and platelets were isolated. The α2B-AR gene expression levels in platelets were examined by real-time polymerase chain reaction for each participant. Well-controlled hypertensive patients showed significantly higher gene expression levels of α2B-Rs in platelets compared with normotensives (34.7 $\pm$ 29.5 vs 17.6 $\pm$ 12.5, respectively, $P = .005$). Interestingly, we found that carotid-femoral PWV and carotid-radial PWV were positively correlated with platelet α2B-R gene expression levels (r = 0.59, $P < .001$, and r = 0.39, $P = .002$, respectively).Platelet α2B-R gene expression levels are increased in patients with well-controlled essential hypertension compared with normotensives and are correlated with increased PWV in those patients. Our data indicate an association of arterial stiffness and platelet α2B-Rs gene expression and indicate the need for further research. J Am Soc Hypertens 2017;■(■):1–7.

Keywords: Essential hypertension; pulse wave velocity; thrombosis.

Introduction

Essential hypertension is associated with an increased incidence of atherothrombotic events, such as myocardial infarction and stroke.[1,2] The precise pathophysiological mechanisms that connect increased arterial pressure with thrombogenesis and thrombotic episodes still remain unclear at many levels. We know that, to some degree, the prothrombotic status in hypertension is associated with a number of factors, such as hemodynamic disturbances, vascular dysfunction, and an imbalance between procoagulant and fibrinolytic activity, which ultimately lead to platelet activation and thrombotic complications.[3,4] In addition, a significant factor in thrombogenic abnormalities, especially in younger patients, appears to be the activation of the

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*Corresponding author: Maria E. Marketou, MD, PhD, Department of Cardiology, Heraklion University Hospital, P.O. Box 1352, Heraklion 71110, Greece. Tel: +30 2810 394855; Fax: +30 2810 542055.

E-mail: maryemarke@yahoo.gr

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sympathetic nervous system (SNS). Overactivity of the SNS has already been demonstrated in patients with essential hypertension,[5] and this is widely acknowledged to be a critical component in the pathogenesis of thrombosis.[6]

Catecholamines play an important role in thrombogenesis, and their effects on platelet aggregation are exerted via the alpha2-adrenergic receptors (α2-ARs).[7] Alpha adrenergic activation is certainly a factor associated with the contraction of the arterial wall, which is innervated by the sympathetic nerves and may contribute to the control of arterial wall tone. It has been known for a long time that α-ARs can mediate vasoconstriction in most vascular beds and are implicated in the pathophysiology of hypertension.[8,9] In addition, previous studies have indicated an altered expression of α2-ARs in cardiovascular diseases, with an increased prevalence of stroke and coronary thrombosis and have highlighted the significant role of α2-ARs in platelet aggregation.[9] Notably, the alpha$_{2B}$-adrenergic receptor (α2B-AR) subtype in platelets is an important regulator of aggregation and α2B-AR inhibition has a significant antiaggregant effect. Interestingly, even in patients who were receiving dual antiplatelet therapy, the inhibition of α2B-ARs in platelets had an additional antiaggregant effect.[10]

On the other hand, arterial stiffness is a hallmark of vascular dysfunction and has been proposed as an independent risk factor for fatal and nonfatal cardiovascular events in patients with hypertension. It may also be implicated in the pathophysiology of increased thrombogenicity in hypertensives.

In the present study, we sought to evaluate α2B-ARs in the platelets of well-controlled hypertensive patients, compared with a normotensive population, and to investigate their association with arterial stiffness. For the measurement of arterial stiffness, we used pulse wave velocity (PWV), which is widely accepted as the ''gold standard'' measure for this purpose.[11]

Methods

Hypertensive patients with well-controlled essential hypertension from our outpatient hypertension clinic were included in the study. The diagnosis of hypertension was based on three outpatient measurements of blood pressure (BP) >140/90 mm Hg at intervals of no longer than 2 weeks, in accordance with the recommendations of the European Society of Hypertension/European Society of Cardiology.[12] To be eligible for inclusion in the study as well-controlled, patients had to have achieved the target BP of ≤140/90 mm Hg by their second visit, confirmed by 24-hour ambulatory BP monitoring showing a mean 24-hour BP < 130/80 mm Hg. These patients were compared with sex- and aged-matched individuals who visited our outpatient clinic either complaining about atypical chest pain, or having other cardiovascular risk factors, were not hypertensives and served as control group.

All patients underwent a complete physical examination and routine laboratory tests. Patients with any of the following characteristics were excluded: pregnant or lactating women; grade 3 hypertension or secondary hypertension; coronary artery disease; heart failure; cerebrovascular, liver or renal disease; albumin excretion >300 mg/24 h; history of drug or alcohol abuse; any chronic inflammatory or other infectious disease during the last 6 months; thyroid gland disease; body mass index (BMI) > 35 kg/m^2; or a history of any hematologic disease. Vascular or neoplastic conditions were ruled out in all participants by a careful examination of the history and routine laboratory tests. BMI was calculated as mass/height^2 (kg/m^2). A full echocardiographic examination was performed in all participants.

Once BP levels had been controlled, blood samples were taken for assessment of α2B-AR gene expression levels in platelets. More specifically, in all participants, after a rest of 20 minutes, blood was drawn from a superficial brachial vein via a 21-ga needle, with care to avoid stasis, hemolysis, and contamination by tissue fluids or exposure to glass.

The study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the local ethics committee. All patients gave informed consent to their inclusion in the study.

RNA Isolation and Platelet α2B-AR Messenger RNA Quantification

Blood samples were collected into citrated tubes and centrifuged at 150*g* for 20 minutes. To avoid leukocyte contamination, only the top 75% of the supernatant platelet-rich plasma was collected and platelets were isolated by centrifugation. Total RNA was isolated from platelets using the TRI-Reagent (Ambion; Life Technologies, Carlsbad, CA, USA) and reverse-transcribed using the PrimeScript RT reagent Kit (Takara Bio Inc, Otsu, Shiga, Japan). Measurements of messenger RNA levels were performed by quantitative real-time polymerase chain reaction (qPCR) using the Corbett Research 6000 detection system. qPCR assays were performed using the KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Woburn, MA, USA). All samples were performed in duplicates. The housekeeping gene *GAPDH* (*glyceraldehyde-3-phosphate-dehydrogenase*) was used as an endogenous reference gene. Primer sequences for the *alpha2B adrenoreceptor* were 5′-GAT TTG GAA GGG CAC CGA GGG A-3′ (sense) and 5′-GGC CAG CAG AGG GTC ACA GTC AG-3′ (antisense); those for GAPDH were 5′-CCA TCT TCC AGG AGC GAG-3′ (sense) and 5′-GCA GGA GGC ATT GCT GAT-3′ (antisense). The standard curve method was used for absolute quantification of the amplification products and specificity was determined by performing a melting curve analysis.