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Abstract: OBJECTIVE: The objective of our work was to analyze if changes in the expression of beta-adrenoceptors (b-ARs) and G-protein-coupled receptor kinases (GRKs) in human lymphocytes - a practical surrogate for myocardial or vascular cells - are related to the hypertensive state and its clinical consequences. METHODS: real time quantitative RT-PCR was employed to evaluate the expression of the three b-ARs (b1, b2, b3) and three GRKs (GRK2, GRK3, GRK5) in human lymphocytes obtained from both normotensive and hypertensive subjects, some of whom had been treated with blockers of the renin-angiotensin system. Office blood pressure, 24-hour ambulatory blood pressure, urinary albumin excretion and serum biochemical profile were also recorded. RESULTS AND CONCLUSIONS: b1-AR expression levels were higher in circulating lymphocytes from hypertensive patients ($2^{-\Delta\Delta Ct}$ =2.135 ± 0.4252*, vs control group), but this difference was not observed when these subjects were treated with blockers of the renin-angiotensin system. b1- AR levels directly correlated (r2=0.5711, P=0.0185) with urinary albumin excretion in microalbuminuric patients, which relates alterations of this receptor to cardiovascular risk. An inverse correlation was observed between the expression levels of b2-AR and diastolic blood pressure (r2=0.2078, P=0.0031), suggesting that b2-AR-levels in lymphocytes mirror their expression in vascular cells, in which b2-AR-mediated relaxation regulates vascular resistance. mrRNA levels for GRK3 were inversely correlated with systolic and diastolic blood presure (day, night and 24h), which suggests a protective role for GRK3 in the regulation of human blood pressure, as supported by previous findings in transgenic mice.

*Condensed abstract

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CONDENSED ABSTRACT: β_1-AR mRNA levels were higher in lymphocytes from hypertensive patients, but this difference was not observed when they were treated with blockers of the renin-angiotensin system. β_1 AR levels directly correlated with urinary albumin excretion in microalbuminuric patients. An inverse correlation was observed between the expression levels of β_2-AR and diastolic BP, suggesting that β_2-AR-levels in lymphocytes mirror vascular cells, in which β_2-AR-mediated relaxation regulates vascular resistance. mrRNA levels for GRK3 were inversely correlated with systolic and diastolic blood pressure, which suggests a protective role for GRK3 in the regulation of human blood pressure

***Submission letter & conflict of interests**

This piece of the submission is being sent via mail.

ABBREVIATIONS DEFINITION LIST

ACEIs = angiotensin converting enzyme inhibitors

ARBs = angiotensin receptor blockers

β-ARs = β-adrenoceptors

BP = blood pressure

DBP = diastolic blood pressure

EDTA = ethylene diamino tetraacetic acid

GRKs = G-protein-coupled receptor kinases

RT-PCR = quantitative reverse transcription polymerase chain reaction

SBP = Systolic blood pressure

UAE = urinary albumin excretion,

β-ADRENOCEPTOR AND GRK3 EXPRESSION IN HUMAN LYMPHOCYTES IS RELATED TO BLOOD PRESSURE AND URINARY ALBUMIN EXCRETION .

Short Title: **β-ADRENOCEPTORS AND GRK3 IN HYPERTENSION**

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ABSTRACT

OBJECTIVE: The objective of our work was to analyze if changes in the expression of β-adrenoceptors (β-ARs) and G-protein-coupled receptor kinases (GRKs) in human lymphocytes - a practical surrogate for myocardial or vascular cells - are related to the hypertensive state and its clinical consequences. **METHODS:** real time quantitative RT-PCR was employed to evaluate the expression of the three β-ARs (β_1, β_2, β_3) and three GRKs (GRK2, GRK3, GRK5) in human lymphocytes obtained from both normotensive and hypertensive subjects, some of whom had been treated with blockers of the renin-angiotensin system. Office blood pressure, 24-hour ambulatory blood pressure, urinary albumin excretion and serum biochemical profile were also recorded. **RESULTS AND CONCLUSIONS**: β_1 AR expression levels were higher in circulating lymphocytes from hypertensive patients ($2^{-\Delta\Delta Ct}$ =2.135 ± 0.4252*, *vs* control group), but this difference was not observed when these subjects were treated with blockers of the renin-angiotensin system. β_1 AR levels directly correlated (r^2=0.5711, *P*=0.0185) with urinary albumin excretion in microalbuminuric patients, which relates alterations of this receptor to cardiovascular risk. An inverse correlation was observed between the expression levels of β_2-AR and diastolic blood pressure (r^2=0.2078, *P*=0.0031), suggesting that β_2-AR-levels in lymphocytes mirror their expression in vascular cells, in which β_2-AR-mediated relaxation regulates vascular resistance. mrRNA levels for GRK3 were inversely correlated with systolic and diastolic blood presure (day, night and 24h), which suggests a protective role for GRK3 in the regulation of human blood pressure, as supported by previous findings in transgenic mice.

Key words: hypertension, β-adrenoceptors, GRK3, urinary albumin excretion, ACE inhibitors

INTRODUCTION

It is well established that three subtypes of β-adrenergic receptors (β_1, β_2 and β_3) are expressed in the cardiovascular system and represent essential targets in the control of its function [1]. After agonist binding, activated β adrenoceptors (β-ARs) interact with a stimulatory G-protein (Gs) that activates adenylyl cyclase and increases intracellular levels of cAMP, which mediate most of the responses characteristic of β-AR stimulation. However, the agonist-occupied conformation of β-AR promotes its phosphorylation by a family of serine-threonine kinases named G-protein coupled receptor kinases (GRKs), which uncouples the receptor from its G-protein and promotes its internalization, thereby undermining its function. Therefore, the efficiency with which β-ARs interact with G-proteins depends on the phosphorylation of the receptor by GRKs, which are responsible for agonist-promoted desensitization [2]

An impairment of β-adrenergic vasodilatation, together with increased function and protein expression of one member of the GRK-family, GRK2, has been observed in both the lymphocytes of hypertensive patients and in animal models of hypertension [3-6]. However, lymphocytes from hypertensive patients have been reported to exhibit a higher β_2-adrenoceptor density than those of normotensive controls [7-9]. In this way, increased expression of GRK2 may be the consequence of a rise in β-AR expression, and the subsequent impairment of β-AR-mediated vasodilatation could be due to more complicated mechanisms, including different β-AR subtypes and GRKs. In fact, GRK3 and, in particular, GRK5 predominate in the heart, and may also participate in the phosphorylation of β-ARs, thereby modulating their desensitization process [2] .

As summarized above, different studies of hypertensive patients have analyzed changes in the expression of β-ARs or GRK2 . However, β-AR subtypes and GRKs in the same

group of patients have not been assessed to date. The objective of our work was to analyze if changes in the expression of β-ARs and GRKs in human lymphocytes - a practical surrogate for myocardial or vascular cells [3-10] - are related to the hypertensive state and its clinical consequences. For this purpose, we employed real time quantitative reverse transcription polymerase chain reaction (RT-PCR) to evaluate the expression of the three β-ARs (β_1, β_2, β_3) and three GRKs (GRK2, GRK3, GRK5) in human lymphocytes obtained from both normotensive and hypertensive subjects.

METHODS

Selection of study participants

Subjects were selected from patients attending an outpatients' clinic, and consisted of 40 individuals (21 males and 19 females) who were grouped as follows: 10 healthy volunteers with normal BP (group I), 5 patients with isolated office hypertension (group II), 15 patients with hypertension (group III) and 10 hypertensive patients who had been receiving treatment with angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) over the course of at least one month (group IV). Patients in the hypertension and isolated office hypertension groups had not undergone any previous antihypertensive therapy. The inclusion criteria were: a) diastolic blood pressure (DBP) in the range of high normal to moderate essential hypertension (defined as between 90 and 114 mmHg; Korotkoff phase V, sitting position) on 3 visits at 1-month intervals in the absence of specific causes; b) age 18 to 65 years old; c) no previous treatment for hypertension with ACEIs or ARBs. Patients with established cardiovascular or renal diseases were excluded. After enrolment, and according to clinical criteria, patients

received a non-pharmacological treatment consisting of moderate salt restriction and a low-calorie diet if they were deemed to be overweight. A control group was composed of healthy normotensive subjects, who were age and sex matched. All patients who fulfilled the inclusion criteria were invited to participate and their written consent was requested. The Ethical Committees of the Hospital of Alzira and the University of Valencia approved the study.

Clinical procedures

All patients underwent a complete clinical workup to rule out secondary hypertension. Serum biochemical profile, lipids, UAE, office blood pressure and 24-hour ambulatory blood pressure were recorded in the outpatient setting. The latter parameter was measured using a mercury sphygmomanometer, with the patient in a sitting position, after resting for five minutes in a quiet environment, following the recommendations of the British Hypertension Society [11]. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Korotkoff phase I and phase V, respectively) were defined as the average of three readings taken at 10 minute intervals. Office hypertension was defined as systolic blood pressure ≥ 140 mm Hg and / or diastolic blood pressure ≥ 90 mmHg. Ambulatory blood pressure was monitored on a regular working day using an oscillometric monitor (Spacelabs 90202 or 90207). Following the standard protocol, recording began between 08:30 and 09:00 am, with readings being taken every 20 minutes from 06:00 am until midnight and every 30 minutes from midnight to 6:00 am. Prior to initiating the study, the accuracy of the blood pressure values measured with the monitor was confirmed by contrasting them with measurements simultaneously recorded with a mercury sphygmomanometer. Differences of less than 5 mmHg were permitted. Irregularities in blood pressure readings were rejected automatically when: 1) SBP >270 mmHg or <70

mmHg; or 2) DBP >160 mmHg or <40 mmHg. Patients with recordings showing an error rate of >25% of the total readings were excluded from the study.

For analysis, the averages of systolic and diastolic BP and heart rate (HR) were calculated for the following time periods: a) 24 hours, b) day/activity period (08:00 am until 10:00 pm), c) night/sleep period (midnight to 06:00 am). Ambulatory hypertension was defined according to the highest recorded values of daytime blood pressure (≥135 mmg Hg systolic BP and ≥85 mmHg diastolic BP).

On the basis of blood pressure, patients were classified as: a) normotensive (normal office and ambulatory blood pressure); b) with isolated office hypertension (office hypertension and normal ambulatory blood pressure); c) with hypertension (office and ambulatory hypertension); or d) with treated hypertension (ambulatory hypertension treated with ACEIs or ARBs).

Blood samples for biochemical determination were obtained in the morning, after a minimum of 8 hours fasting. Ten mL of each sample was anticoagulated with ethylene diamino tetraacetic acid (EDTA) in order to perform lymphocyte isolation, for which the Lymphoprep™ method was employed following the manufacture's conditions. Cells were immediately stored at -80ºC until further analysis. Serum biochemical profiles were measured using the Roche-Hitachi Modular Autoanalyzer (Roche Diagnostics, Mannheim, Germany). The glomerular filtration rate was estimated based on the clearance of endogenous creatinine with respect to body surface (ml/min/1.73m^2).

Urinary albumin excretion (UAE) of two urine collections separated by a 24 hour interval was measured using an immunonephelometric assay (Boehring Institute), as previously described [12]. The UAE of each patient was considered to be the mean of the values obtained from the two urine collections. If the difference between these two values was greater than 25% of the higher value, or if creatinine excretion over 24 hours was

lower than expected for body size and sex, a third sample was obtained. In this way, an additional sample was collected from 5% of the patients. Microalbuminuria was defined as UAE 30-300 mg/24 hours

Real-time quantitative RT-PCR

Total RNA was obtained from each sample as previously described [13]. Total RNA (1–2 µg) and oligo(dT)16 primer (250 ng) in DEPC-treated water were preheated to 70°C and cooled on ice for cDNA synthesis. Reactions (25 µl) contained 50 mM Tris ·HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, 40 U of RNAsin® Ribonuclease Inhibitor (Promega Corp., Madison USA), 2 mM of each deoxynucleoside triphosphate, and 300 U of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT, Promega Corp., Madison, USA), and were incubated at 42°C for 60 min.

mRNAs encoding the three β-adrenoceptors (β_1, β_2 and β_3) and the three GRKs and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard were quantified by TaqMan® real-time RT-PCR with a GeneAmp 5700 sequence-detection system (Applied Biosystems, USA). We analyzed (in duplicate reactions) a 10-fold dilution of the RT reaction of each sample using the TaqMan™ Gene Expression Assays (Applied Biosystems, USA). The seven specific primer-probe sets were β_1-AR (Hs00265096_s1), β_2-AR (Hs00240532_s1), β_3-AR (Hs00609046_m1), GRK2 (Hs00176395_m1), GRK3 (Hs00178266_m1), GRK5 (Hs00178389_m1) and GAPDH (Hs99999905_m1) (Applied Biosystems, USA). Real-time PCR reactions were set following the manufacture's instructions, as previously described [6].

The threshold cycle values (Ct) obtained for each gene were referenced to GAPDH and converted to the linear form using the term $2^{-\Delta Ct}$ as a value directly proportional to the copy number of mRNA. To compare the mRNA levels of the target genes of the groups,

expression was also assessed using the comparative method ($2^{-\Delta\Delta Ct}$), with the normotensive patients constituting the control group [14].

Statistical Analysis

All data were expressed as mean ± standard error mean (sem). A t-test and one-way analysis of variance (ANOVA) were performed using Graph Pad Prism 4 Software (Graph Pad Software Inc, San Diego, CAL, USA). Simple linear regression analyses were employed to establish associations between variables. Hypothesis of homoscedasticity, and normality and independence of residuals was previously checked by means of the Levene's test, Shapiro-Wilks' test and Durbin-Watson statistic, respectively. Sigma Plot v.8.02 statistical graphing software was employed (Systat Software, Chicago, IL, USA). Statistical significance was considered to be $P < 0.05$

UAE data were analyzed in 2 ways. First, they were categorized into normo-albuminuric and microalbuminuric groups, as previously defined. Second, a continous variable with logarithmic transformation (log UAE) was employed.

RESULTS

Clinical characteristics of the study population

The study population was distributed into four groups according to office blood pressure and 24-hour ambulatory blood pressure values. The general characteristics and risk factors for cardiovascular disease of each group are presented in Table 1. As expected, significant differences in blood pressure values were observed in the three groups of hypertensive patients with respect to those of healthy volunteers. However, no

relevant differences were observed between the groups in terms of age, gender, heart rate, body mass index, lipid profile, fasting glucose levels or other biochemical variables, confirming the absence of other pathologies or alterations that could have been acting as confounding factors. It is interesting to note the relatively young age of the study population and the fact that all patients were at an initial stage of hypertension (borderline or mild hypertension), during which no significant increases in the level of circulating catecholamines (noradrenaline, adrenaline or dopamine) had yet been detected. In fact, a slight but significant decrease in adrenaline circulating levels was observed in the hypertensive group (Table 1).

When UAE was determined, subjects with microalbuminuria (UAE 30-300 mg/24h) were identified among hypertensive (n= 6) and treated hypertensive patients (n=3). The individual determinations of this parameter are represented in Figure 1 and the mean value for each group of patients is shown in Table 1.

Expression levels of β-ARs and GRKs in human lymphocytes

To determine the steady-state levels of mRNAs for the three β-adrenoceptor subtypes and the three kinases (GRK2, GRK3 and GRK5) in circulating human lymphocytes, quantitative RT-PCR reactions were performed using GAPDH as a housekeeping gene. In normotensive subjects (n = 10), the highest expression level among the three β-AR subtypes was that of β_2 AR (827±139 expressed as $2^{-\Delta Ct}$ *vs* GAPDH), followed by that of β_1 AR (15±3). On the other hand, the mRNA level of β_3 AR was almost undetectable. Similar results have previously been reported by other authors [15]. In the case of the GRKs, the most abundant was GRK2 (1005±124 expressed as $2^{-\Delta Ct}$ *vs* GAPDH), followed by GRK3 (302±16) and GRK5 (260±24), both of which were expressed at similar levels in human lymphocytes from normotensive subjects.

When the whole study population was considered, the same distribution was detected for β-ARs and GRKs, and no differences were observed between the expression levels of males (n = 21) and females (n = 19) (Figure 2).

Changes in the expression levels of β-ARs and GRKs in hypertensive patients.

A comparative analysis of the mRNA levels of each gene was performed for each of the hypertensive states evaluated. The results are summarized in Figure 3. A higher expression level was observed for β_1-AR among the hypertensive patients. This difference only reached statistical significance in the case of patients with high normal or moderate essential hypertension without treatment (25±5, n = 15). Slight but statistically insignificant changes were observed in the hypertensive patients when the expression levels of GRK5 (increased) and of β_2-AR, GRK2 and GRK3 (decreased) were evaluated (Figure 3).

Correlation between expression levels of β-ARs and GRKs in human lymphocytes and clinical variables

To assess the clinical significance of changes in the expression levels of β-ARs and GRKs, the relationship between lymphocyte expression of the majoritary of genes and each of the clinical variables summarized in Table 1 was evaluated using linear regression analysis .

Lymphocyte expression of β_2AR significantly and inversely correlated with 24h diastolic blood pressure (Figure 4) and diurnal diastolic blood pressure (results not shown). Accordingly, a higher expression level of β_2AR in lymphocytes, which could reflect the expression of this receptor in the heart and vessels, was related to lower values of diastolic blood pressure. Another significant relationship was revealed between GRK3 and

values of systolic and diastolic ambulatory blood pressure determined during either 24 h (results not shown) or day and night periods in the four groups of subjects (Figure 5)

UAE data were analyzed in 2 ways. First, they were categorized into normo-albuminuric and microalbuminuric groups, as previously defined (Figure 1). Second, a continous variable with logarithmic transformation (log UAE) was employed. Linear regression analysis of gene expression and log UAE was insignificant in the total population, but did reach statistical significance in the microalbuminuric group, thus providing evidence that lymphocyte expression of β_1AR significantly and directly correlated with urinary albumin excretion in these patients (Figure 6).

None of the other clinical variables evaluated (see Table 1) showed a significant correlation with gene expression in lymphocytes (results not shown).

DISCUSSION

β-ARs and hypertension

Our results obtained using real time quantitative RT-PCR confirm previous data acquired via a semiquantitative method, demonstrating the coexistence of the three β-AR subtypes (β_1, β_2 and β_3) in human lymphocytes [15]. The highest level of expression was that of β_2, followed by that of β_1, which was significantly lower. Due the very low expression of the β_3 subtype in human lymphocytes we excluded it from the comparative analysis of the different groups of patients.

A weak but statistically insignificant decrease in the mRNA levels of β_2-AR was observed in the group of hypertensive patients. The lack of a significant change in the

mRNA levels of β_2-AR contrast with previous reports of an increased density of this subtype in lymphocytes from hypertensive patients [7-9]. This apparent discrepancy could be attributed to the fact that the authors assumed that β_2-AR is the only subtype present in lymphocytes and used a non-subtype selective radioligand to quantify the receptors. In the present study, rather than a higher expression of β_2-ARs, we observed an increase of mRNA levels of β_1-AR that was slight in the lymphocytes of patients with isolated office hypertension and reached statistical significance in those of hypertensive patients. This observation is in accordance with our previous report of an increased expression of β_1-ARs in the rat aorta, which preceded the hypertensive state and was maintained during it [6]. Interestingly, this increase was attenuated in the hypertensive patients treated vith ACEIs or ARBs, suggesting that inhibition of the renin-angiotensin system directly or indirectly ajusts β_1-AR expression.

The β-AR mRNA level in lymphocytes could reflect levels in the heart and vessels, as indicated by previous reports [10,15,16]. If this is so, the clinical variables characteristic of the hypertensive state and dependent on β-AR expression in the heart and vessels may be related to the mRNA levels observed in the lymphocytes of our study population. Based on this assumption, we assessed a possible correlation between β-ARs and the clinical variables determined in each patient (see Table 1). The characteristics of the patients included in the present study permitted this evaluation, since all were relatively young, exhibited normal values of the biochemical parameters determined and did not suffer any concomitant pathology, all of which helped to rule out confounding factors.

No significant correlation was found between β_1-AR expression and ambulatory blood pressure values, thereby excluding a direct role for this receptor in the modulation of blood pressure. However, a major finding of this analysis is the positive relationship

between β_1 adrenoceptor expression in lymphocytes and urinary albumin excretion (log UAE) in the group of microalbuminuric patients. Elevated UAE depends on the control of haemodynamic and metabolic factors [12], and is associated with the development of hypertension [17] and cardiovascular risk [18], as it is closely related with endothelial damage [19,20], greater arterial stiffness [21], maladaptive vascular remodelling [22] and impaired aortic elasticity [23]. However, the exact mechanisms underlying the link between microalbuminuria and cardiovascular risk are not fully defined. Assuming that the expression of β_1-AR in circulating lymphocytes mirrors that in organs and/or vessels [7-10], our present findings are of great relevance. Given the positive correlation of β_1 AR expression with UAE, an increase in β_1 AR levels in human vessels and/or kidney could be related to subclinical vascular damage and alterations of kidney function. Our observation that ACEIs and ARBs tend to normalize the increased β_1-AR expression in lymphocytes may explain clinical evidence that renin-angiotensin system blockade reduces albuminuria and that combined treatment with β-blockers causes an additional reduction of UAE [24].

The other major finding of our analysis is that expression levels of β_2-AR, which were slightly lower among hypertensive patients, significantly and inversely correlated with 24h diastolic blood pressure values. This suggests a direct participation of this subtype in the regulation of diastolic blood pressure, and conversely, that changes in β_2-AR expression are a consequence of alterations of diastolic blood pressure. If we consider that β-adrenergic vasodilatation (mainly mediated by the β_2 subtype) is a key mechanism of the modulation of vascular resistance [1-3], the most feasible interpretation of this correlation is that a higher expression of β_2-AR in vessels (mirrored in lymphocytes) facilitates vasodilatory mechanisms, thereby decreasing vascular resistance and lowering diastolic blood pressure values.

GRKs and hypertension

Lymphocytes expressed not only GRK2, but also GRK3 and GRK5, as previously described [16,25,26]. The three GRKs share certain characteristics but are distinct non-redundant enzymes with specific functional and regulatory properties [2,27,28]. While the role of GRK2 in the regulation of β-ARs has been extensively studied, the specificities of the other isoforms for human GPCRs are unclear. Previous studies in mice reveal that myocardium-targeted overexpression of GRK2 and GRK5 results in the attenuation of the contractile response to β-AR, whereas cardiac overexpression of GRK3 is characterized by normal β-AR activity [29], suggesting a specific desensitization of cardiac β-ARs (mainly the β_1 subtype) that is mediated by GRK2 and GRK5, but not by GRK3.

Increased GRK2 protein expression in lymphocytes, which is not related to an increase in the steady-state levels of mRNA, has been described in human models of hypertension [30]. Our results confirm this, since no significant changes in the level of GRK2 mRNA were observed in the lymphocytes of hypertensive patients. No change, or at most a weak but insignificant decrease in the mRNA levels of GRK3 and a non-significant increase in the mRNA levels of GRK5 were also observed in this group of patients. Given that the subjects of our study had high normal to moderate essential hypertension, further studies are required to analyze whether or not these slight changes are more pronounced in patients with severe hypertension.

Although significant changes in the expression levels of GRKs were not observed in hypertensive patients, our study reveals that lymphocyte expression of GRK3 significantly and inversely correlates with values of systolic and diastolic ambulatory blood pressure, indicating that this kinase is involved in the regulation of human blood pressure. The opposite – namely, that GRK expression is modulated by blood pressure

– is not supported by previous findings in transgenic mice, in which cardiac-restricted inhibition of endogenous GRK3 resulted in a phenotype with elevated systolic and diastolic blood pressure and without alterations of heart rate, parameters that were determined by different methods in conscious and unrestrained mice [29].

If we assume that changes in GRK3 expression in lymphocytes reflect changes in the heart and/or vessels, as previously demonstrated for GRK2 and GRK5 [26,31,32], ours is the first report of a pathophysiological role for GRK3 in the human cardiovascular system, and could contribute to a better understanding of the position of the GRK3 gene in a locus on a chromosome associated with left ventricular mass and contractility [33]. The exact significance of our findings must be confirmed by further experiments to determine the G-protein-coupled receptor modulated by GRK3, and to directly implicate it in the regulation of blood pressure. Based on evidence that changes in GRK3 expression do not alter the biochemical signalling and functional role of myocardial β-ARs but that responses mediated by α_{1B}-AR are profoundly altered when GRK3 expression is modified [29, 34-36], future experiments may reveal a role for GRK3 in the control of blood pressure by acting on α_{1B} AR or another G-protein coupled receptor.

Taking the abovementioned results into consideration, the close correlation observed between GRK3 expression and blood pressure values suggest an important role for this kinase in the desensitization process of an unknown G-protein coupled receptor (or a family of receptors) directly related to the control of human blood pressure. The clinical significance of this observation is to be determined by future research.

In conclusion, we present three main findings in the present report: 1) β_1 AR expression levels are higher in circulating lymphocytes from hypertensive patients, but are returned to normal by treatment with ACEIs or ARBs. 2) β_1 AR expression levels directly

correlate with UAE in microalbuminuric patients, which relates changes of this receptor to cardiovascular risk and highlights the need for future work in this area. 3) The expression levels of β_2-AR are inversely correlated with diastolic blood pressure, and those of GRK3 are inversely correlated with systolic and diastolic blood pressure, confirming the importance of β_2-AR-mediated vasodilatation in the control of vascular resistance and suggesting a protective role for GRK3 in the regulation of cardiovascular homeostasis.

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Legends for figures

Figure 1. Comparative analysis of urinary albumin excretion (UAE) in healthy human volunteers (controls), patients with isolated office hypertension, hypertensive patients and hypertensive patients treated with ACEIs or ARBs.

Microalbuminuria was defined as UAE 30-300 mg/24h (values above dotted line)

Horizontal continous lines represents the mean of n values in each group

Figure 2. mRNA levels of the β_1, β_2, β_3 adrenoceptors and GRK2, GRK3 and GRK5 in circulating lymphocytes obtained from male (white circles, n =21) and female (black circles, n = 19) subjects, expressed as $2^{-\Delta Ct}$ with GAPDH as a housekeeping gene.

Horizontal line represents the mean of n values. Independent samples t-test and one-way analysis of variance (ANOVA) were performed but no significant differences were found between groups

Figure 3. Comparative analysis of the expression of the β_1 and β_2 adrenoceptors and GRK2, GRK3 and GRK5 in circulating lymphocytes obtained from healthy human volunteers (controls), patients with isolated office hypertension, hypertensive patients and hypertensive patients treated with ACEIs or ARBs.

Values were expressed as $2^{-\Delta\Delta Ct}$ with respect to the control group and are the mean + s.e.m,

Independent samples t-test and one-way analysis of variance (ANOVA) were performed, * $P < 0.05$

Figure 4. Graphical representation of the linear regression observed between the mRNA levels of β_2-AR in human lymphocytes and ambulatory diastolic blood pressure (BP) values

Figure 5. Graphical representation of the linear regression observed between the mRNA levels of GRK3 in human lymphocytes and ambulatory systolic (continous line) or diastolic (dotted line) blood pressure (BP) values, determined as described in the Methods section during day (top graph) or night (bottom graph). In the case of diastolic blood pressure at night, the Shapiro-Wilk test does not allow us to ensure the normality of residuals.

Figure 6. Graphical representation of the linear regression observed between the mRNA levels of β_1-AR in human lymphocytes from normoalbuminuric (white circles and dotted line) and microalbuminuric (black circles and line) patients and urinary albumin excretion expressed as a continous variable with logarithmic transformation (log UAE).

TABLE 1. Demographic and biochemical characteristics of each study group

	Control	Isolated Office Hypertension	Hypertension	Treated hypertension
Age, y	49 ± 4	45 ± 5	49 ± 3	48 ± 4
Male/female, n	7 /3	2/3	8/7	4/6
Body mass index, kg/m^2	29 ± 2	30 ± 2	28 ± 1	26 ± 1
Office BP, mmHg				
Systolic	123 ± 2	151 ± 7***	145 ± 2***	136 ± 4**
Diastolic	76 ± 1	90 ± 6**	92 ± 2***	85 ± 3*
BP 24 h, mmHg				
Systolic	122 ± 3	128 ± 3	131 ± 2*	129 ± 4
Diastolic	76 ± 3	80 ± 2	83 ± 1*	79 ± 2
BP day , mmHg				
Systolic	125 ± 4	133 ± 3	136 ± 3*	135 ± 4
Diastolic	79 ± 3	84 ± 1	87 ± 2*	83 ± 2
BP night, mmHg				
Systolic	113 ± 3	113 ± 6	118 ± 2	115 ± 3
Diastolic	69 ± 2	69 ± 4	72 ± 1	67 ± 3
Heart rate, bpm				
24 h	75 ± 3	74 ± 2	75 ± 3	75 ± 3
day	78 ± 3	78 ± 2	79 ± 3	80 ± 3
night	70 ± 4	64 ± 2	66 ± 3	69 ± 3
Total cholesterol, mg/dL	204 ± 9	200 ± 15	216 ± 12	205 ± 11
HDL cholesterol, mg/dL	58.0 ± 4.8	52.8 ± 5.4	55.2 ± 2.9	56.4 ± 6.1
LdL cholesterol, mg/dL	115 ± 4	104 ± 9	138 ± 9	135 ± 10

Triglyceride, mg/dL	125 ± 21	128 ± 27	114 ± 15	163 ± 33
Insuline, μU/mL	18.3 ± 5.6	22.0 ± 4.6	12.2 ± 0.8	14.0 ± 2.9
Fasting blood sugar, mg/dl	100 ± 3	104 ± 6	97 ± 2	118 ± 10
Haemoglobin, g/L	14.4 ± 0.2	14.8 ± 0.5	15.0 ± 0.43	14.0 ± 0.34
HbA1c (%)	5.47 ± 0.12	5.87 ± 0.23	5.83 ± 0.09	6.46 ± 0.42*
Adrenaline, pg/mL	52.9 ± 7.7	50.0 ± 7.5	32.5 ± 3.8*	38.0 ± 4.7
Noradrenaline, pg/mL	396 ± 78	304 ± 41	481 ± 103	469 ± 73
Dopamine, pg/mL	30.7 ± 6.9	31.7 ± 7.8	32.6 ± 2.7	24.2 ± 3.9
Creatinine, mg/dL	0.89 ± 0.04	0.77 ± 0.07	0.82 ± 0.04	0.82 ± 0.05
CRCL, ml/min/1.73m^2	123 ± 14	117 ± 13	118 ± 12	128 ± 16
UAE, mg/24h	5.57 ± 0.19	5.20 ± 1.20	64.07 ± 34.79	86.22 ± 38.99

BP indicates blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycosylated haemoglobin; CRCL, creatinine clearance; UAE, urinary albumin excretion

Values are expressed as mean ± s.e.m

P < 0.05, ** P< 0.01, *** P< 0.001 *vs*. control group

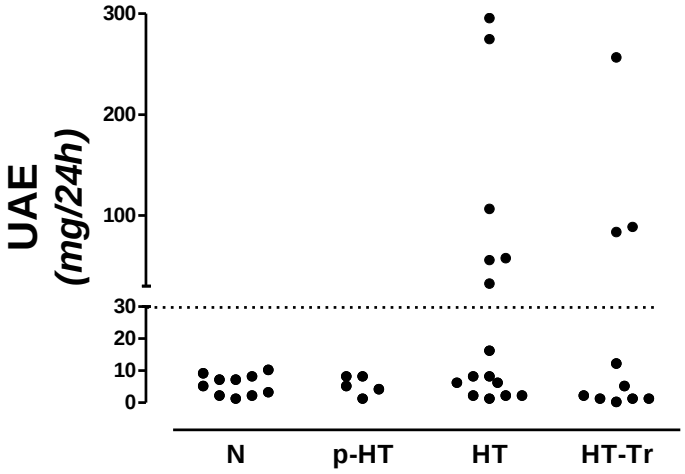


Figure 1

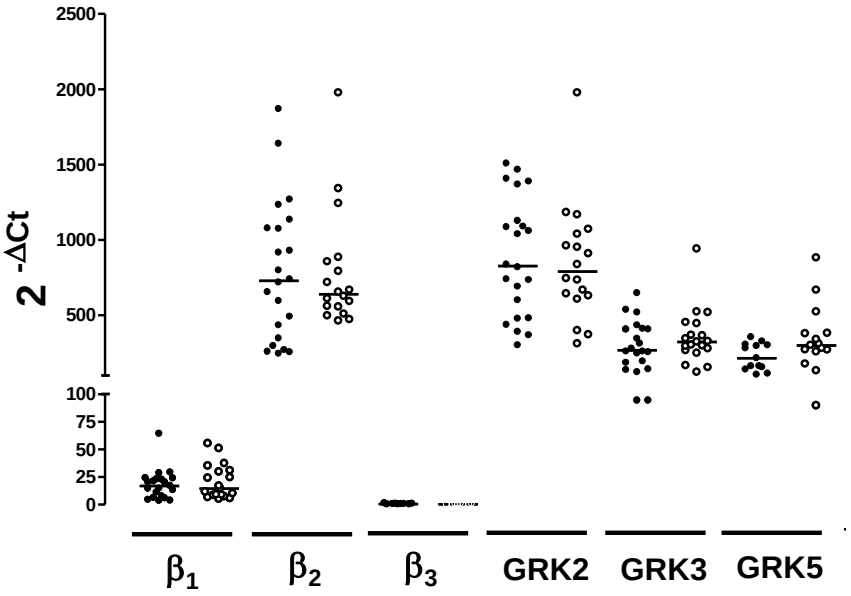

2 -ΔCt
2500
2000
1500
1000
500
100
75
50
25
0
β1
β2
β3
GRK2
GRK3
GRK5

Figure 2

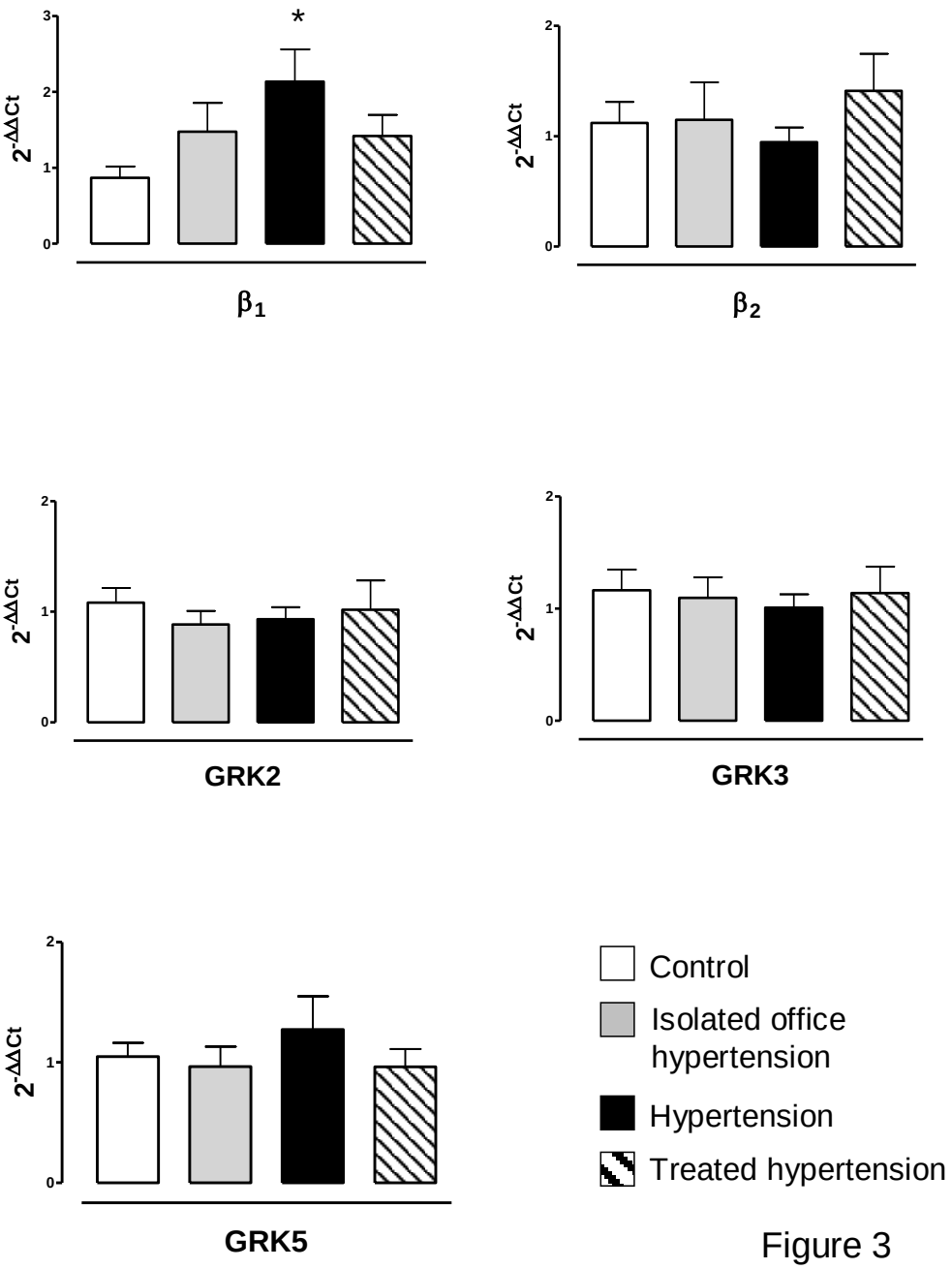

*
2-ΔΔCt
β1
β2
GRK2
GRK3
GRK5
Control
Isolated office hypertension
Hypertension
Treated hypertension

Figure 3

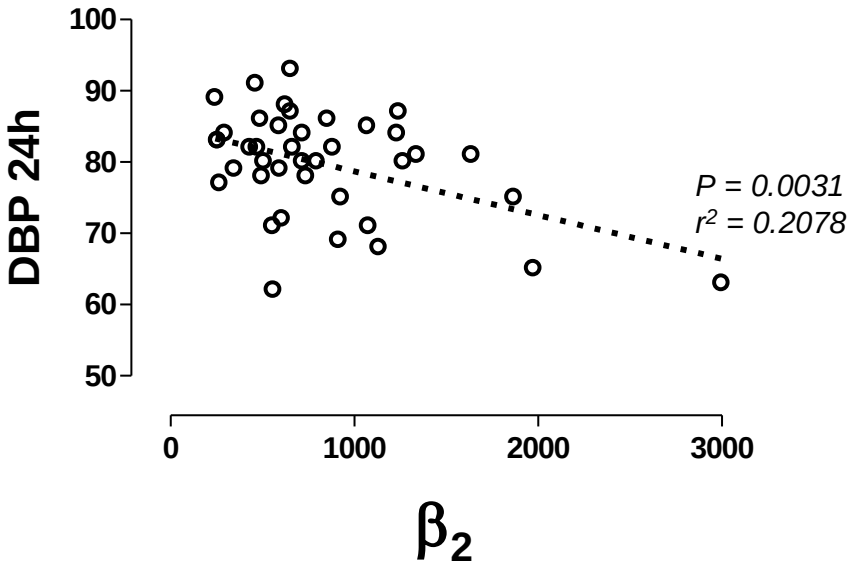
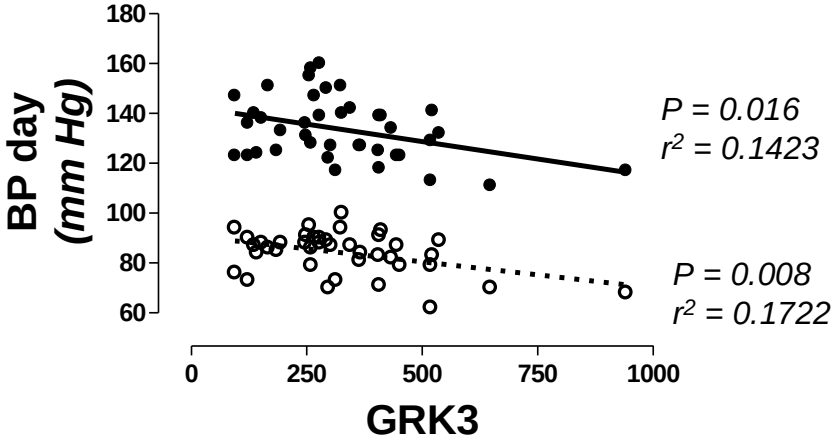


Figure 4


BP day
(mm Hg)
180
160
140
120
100
80
60
0
250
500
750
1000
GRK3
P = 0.016
r2 = 0.1423
P = 0.008
r2 = 0.1722

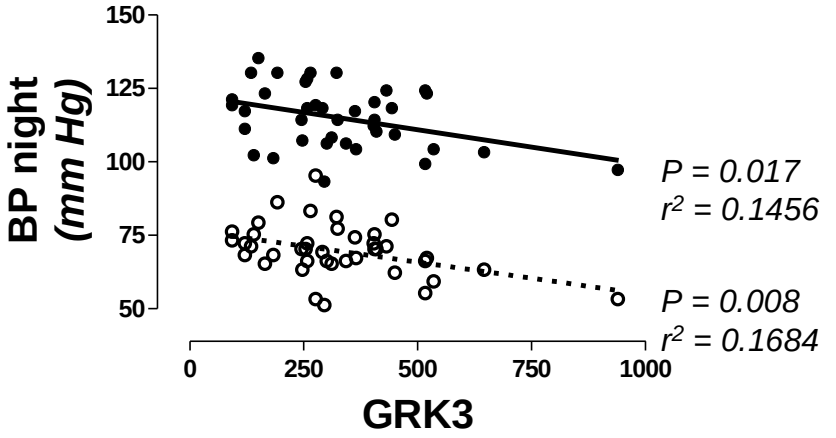

BP night
(mm Hg)
150
125
100
75
50
0
250
500
750
1000
GRK3
P = 0.017
r2 = 0.1456
P = 0.008
r2 = 0.1684

Figure 5

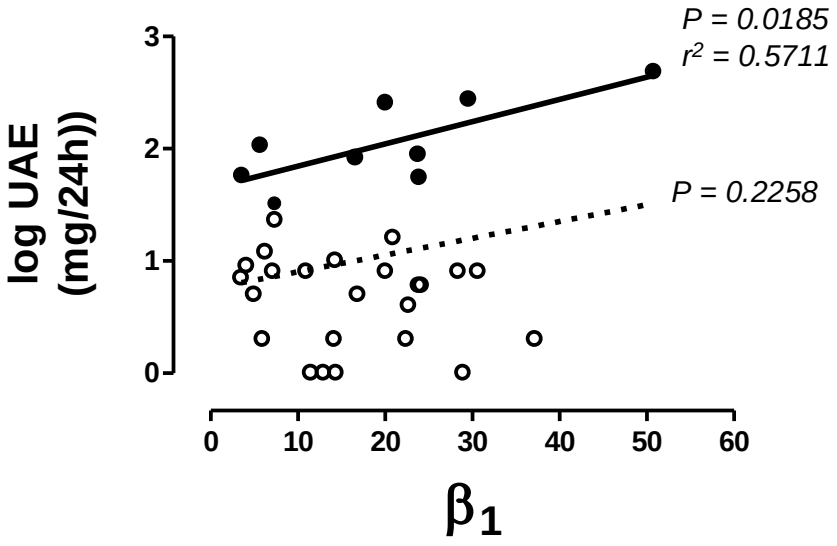


Figure 6