α1-Adrenergic Receptor Subtypes in Human Peripheral Blood Lymphocytes

Alberto Ricci, Elena Bronzetti, Andrea Conterno, Stefania Greco, Paolo Mulatero, Marina Schena, Domenica Schiavone, Seyed K. Tayebati, Franco Veglio, Francesco Amenta

Abstract—We investigated the expression of α1-adrenergic receptor subtypes in intact human peripheral blood lymphocytes using reverse transcription–polymerase chain reaction (RT-PCR) and radioligand binding assay techniques combined with antibodies against the three subtypes of α1-adrenergic receptors (α1A-, α1B-, and α1D). RT-PCR amplified in peripheral blood lymphocytes a 348-bp α1A-adrenergic receptor fragment, a 689-bp α1D-adrenergic receptor fragment, and a 540-bp α1D-adrenergic receptor fragment. Radioligand binding assay with [3H]prazosin as radioligand revealed a high-affinity binding with a dissociation constant value of 0.65 ± 0.05 nmol/L and a maximum density of binding sites of 175.3 ± 20.5 fmol/10^6 cells. The pharmacological profile of [3H]prazosin binding to human peripheral blood lymphocytes was consistent with the labeling of α1-adrenergic receptors. Antibodies against α1A-, α1B-, and α1D-receptor subtypes decreased [3H]prazosin binding to a different extent. This indicates that human peripheral blood lymphocytes express the three α1-adrenergic receptor subtypes. Of the three different α1-adrenergic receptor subtypes, the α1D is the most represented and the α1B, the least. Future studies should clarify the functional relevance of α1-adrenergic receptors expressed by peripheral blood lymphocytes. The identification of these sites may represent a step for evaluating whether they represent a marker of α1-adrenergic receptors in cardiovascular disorders or for assessing responses to drug treatment on these receptors. (Hypertension. 1999;33:708-712.)

Key Words: lymphocytes ■ receptors, adrenergic, alpha ■ receptor subtypes ■ receptor antibodies

In the past few years, α- and β-adrenergic receptors have been demonstrated in peripheral blood lymphocytes with the use of radioligand binding assay techniques. The majority of information is available on β-adrenergic receptors, which were investigated primarily in essential hypertension, impaired left ventricular function, and acute stress.1-5 Data on the expression of α-adrenergic receptors in peripheral blood lymphocytes are less extensive, with the α2-receptor subtype being the most extensively investigated.6-7 Some studies on the α1-adrenergic receptor have not found its expression in human peripheral blood lymphocytes8,9 and some studies have.10

α1-Adrenergic receptors mediate some cardiovascular sympathetic responses, such as arteriolar smooth muscle constriction11 and cardiac contractility,12 and are involved in a variety of cardiovascular disorders, including hypertension, heart failure, and cardiac hypertrophy.13 α1-Adrenergic receptors represent a class of heterogeneous receptors.14 Cloning studies have identified three distinct α1-adrenergic receptor subtypes, named α1A-, α1B-, and α1D-adrenergic receptors.14-17 At present, α1-adrenergic receptor subtypes are defined as α1A (α1A), α1B (α1B), and α1D (α1D), with uppercase and lowercase subscripts being used to designate native or recombinant receptor, respectively.14,18

In view of the difficulty of investigating α1-adrenergic receptors in vivo or in vitro using human samples of cardiovascular system, circulating blood cells may represent a model for the study of the cardiovascular α1-adrenergic receptor system.

In this article we have characterized α1-adrenergic receptor subtypes expressed by human peripheral blood lymphocytes by reverse transcription–polymerase chain reaction (RT-PCR) to detect mRNA expression and by radioligand binding assay techniques combined with antibodies against different subtypes of α1-adrenergic receptors.

Methods

Subjects and Peripheral Blood Lymphocyte Separation

Blood samples (40 mL) were drawn from 30 healthy subjects (15 men and 15 women; age, 26 to 48 years), after informed consent had been obtained, for peripheral blood lymphocyte isolation. Cells were diluted 1:3 with phosphate-buffered saline (0.85% NaCl and 6.7 mmol/L potassium phosphate, pH 7.2) and separated on a Ficoll-Hypaque gradient. The layer of mononuclear cells was re-

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From the Department of Cardiovascular and Respiratory Sciences (A.R., E.B., S.G.), “La Sapienza” University, Rome; Department of Medicine and Experimental Oncology, Chair of Internal Medicine (A.C., P.M., M.S., D.S., F.V.), University of Turin; Section of Human Anatomy, Department of Pharmacological Sciences and Experimental Medicine (S.K.T., F.A.), University of Camerino, Italy.

Correspondence to Francesco Amenta, MD, Dipartimento di Scienze Farmacologiche e Medicina Sperimentale, Via Scalzino, 3-62032 Camerino, Italy. E-mail amenta@cambio.unicam.it

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708
moved and resuspended. Cells (10×10^6) were sedimented by centrifugation in Eppendorf tubes, lysed with 1 mL RNAfast by repetitive pipetting, and then stored at −80°C until further analysis. Cells used for radioligand binding assay after separation were collected in polypropylene tubes and immediately frozen at −80°C.

**Reverse Transcription–Polymerase Chain Reaction**

Total RNA was extracted using the RNAfast method. RT was performed using 1 μg total RNA, according to the manufacturer’s instructions (Perkin-Elmer). PCR was carried out in an automated DNA thermal cycler (Perkin-Elmer) using the following specific primers: α_1A_ sense and antisense, 5′-ACTACATCTGCAACCTGGCGG-3′ and 5′-TGATCTGGCACAGATGGTCTC-3′, respectively; α_1B_ sense and antisense, 5′-TCGGTGCGCCTGCAACCGGCACCTG-3′ and 5′-ATGCCCAAGGTTTGGCTGCTTTCTT-3′, respectively; α_1D_ sense and antisense, 5′-GTGGTGAGTGGTACGGGCTG-3′ and 5′-CATGACCGCCATGGCAGGT-3′. The amplification protocol was 90 seconds at 94°C, 2 minutes at 60°C, and 2 minutes at 72°C, with extension of 1 second per cycle for 30 cycles. PCR products were electrophoresed on a 1.5% agarose gel containing 0.5 mg/mL ethidium bromide. After gel electrophoresis, the amplified fragments of α_1-adrenergic receptor subtypes were blotted to nylon filters and hybridized with a specific nested oligonucleotide for each subtype of α_1-adrenergic receptor, and were labeled by T4 kinase with [32P]-ATP. Specific nested oligonucleotides used for α_1A, α_1B, and α_1D_ were 5′-AACCATGTCAACCCAGAGGA-3′, 5′-ATGGAACTCTGGGGTGTG-3′, and 5′-CTTATGGCCGTGGCAGGTAA-3′, respectively. A specific activity of 4×10^6 cpm/mL was added to the hybridizing solution (5× SSPE, 0.5× SDS, 5× Denhardt’s solution), and the filters were incubated for 2 hours at 42°C. Filters were then washed twice in 2× SSPE at room temperature and once in 5× SSPE at 52°C and exposed for 24 to 48 hours at −80°C.

**Radioligand Binding Assay**

Three hundred microliters of a lymphocyte suspension containing 1×10^6 cells was incubated in triplicate for 60 minutes at 25°C with increasing concentrations of [3H]prazosin (0.05 to 4 nmol/L) in 50 mmol/L Tris-HCl containing 150 mmol/L NaCl, 5 mmol/L EDTA, 100 μmol/L pargyline, and 3 μmol/L ascorbic acid (pH 7.4). Nonspecific binding was defined by incubating lymphocytes with [3H]prazosin in the presence of 10 μmol/L phentolamine.

The pharmacological specificity of [3H]prazosin binding to human peripheral blood lymphocytes was assessed by incubating suspensions containing 1×10^6 cells with 0.5 nmol/L [3H]prazosin in the presence of increasing concentrations (0.001 nmol/L to 1 mmol/L) of compounds active on adrenergic receptors, dopamine, and serotonin receptors.

At the end of incubation, cells were isolated onto Whatman GF-B glass fiber filters with the use of a manifold filtration apparatus. Filters were washed rapidly twice with ice-cold incubation buffer and transferred into scintillation vials for radioactivity measurement.

**Experiments With Antibodies Against α_1-Adrenergic Receptor Subtypes**

Suspensions of peripheral blood lymphocytes (1×10^6 cells/mL) were preincubated for 1 to 2 hours at 25°C with increasing concentrations (0.05 to 2 μg/mL) of goat polyclonal immunoglobulins raised against α_1A-, α_1B-, and α_1D-adrenergic receptors. Immunoglobulins were used alone, in association (anti-α_1A_ and anti-α_1B_, anti-α_1A_ and anti-α_1D_, anti-α_1B_ and anti-α_1D_), and all together. For control purposes, peripheral blood lymphocytes were incubated with immunoglobulins preadsorbed with 10 μg/mL of synthetic peptides used for raising antibodies. After preincubation, [3H]prazosin binding was assayed according to the above protocol with a radioligand concentration of 0.5 nmol/L.

**Data Analysis**

Data from binding and competition experiments were calculated with the Radlig program.19 In experiments performed with antibodies against α_1-adrenergic receptor subtype, [3H]prazosin binding in the

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**Figure 1.** RT-PCR analysis (A panels) of RNA in human peripheral blood lymphocytes and autoradiography of α_1-adrenoceptor subtype PCR products, hybridized with a nested oligonucleotide. [3P]P end-labeled by a T4 kinase reaction (B panels). The α_1A-, α_1B-, and α_1D-mRNA transcripts were detected as 348-, 689-, and 540-bp fragments, respectively. Lane 1 (present in A panels only), DNA size markers; lanes 2 to 7, samples from normal volunteers.
presence of preabsorbed antibody was considered as total binding. Binding values obtained in the presence of antibodies against \( \alpha_1 \)-, \( \alpha_2 \)-, and \( \alpha_3 \)-adrenergic receptor concentrations causing maximal inhibition of radioligand binding to human peripheral blood lymphocyte (0.5 \( \mu \)g/mL) were considered to represent nonspecific retention of radioligand to lymphocyte preparation or to be the result of labeling sites other than \( \alpha_1 \)-adrenergic receptors. The difference between total binding and nonspecific radioligand retention was considered as the specific binding defined immunologically.

**Chemicals**

\([^{3}H]\)Prazosin (specific activity, 78.0 Ci/mmol) was purchased from the Amersham Radiochemical Center. Polyclonal anti-\( \alpha_1 \)- (No. Sc-1474), anti-\( \alpha_2 \)- (No. Sc-1476), and anti-\( \alpha_3 \)- (No. Sc-1477) adrenergic receptor subtype immunoglobulins and \( \alpha_1 \)- (No. Sc-1474P), \( \alpha_2 \)- (No. Sc-1476P), and \( \alpha_3 \)- (No. Sc-1477P) blocking peptides were purchased from Santa Cruz Biotechnology. Immunoglobulins used did not cross-react with other \( \alpha \)- or \( \beta \)-adrenergic receptor subtypes (data not shown). (\(-\)\(\text{trans}\)-Mephendioxan\(\text{H}^{+}\)) was a gift of Dr. W. Quaglia of the University of Camerino. Other chemicals were obtained from Sigma Chemical Co. and Research Biochemicals.

**Results**

**Reverse Transcription–Polymerase Chain Reaction**

RT-PCR amplified a 348-bp \( \alpha_1 \)-adrenergic receptor fragment, a 689-bp \( \alpha_2 \)-adrenergic receptor fragment, and a 540-bp \( \alpha_3 \)-adrenergic receptor fragment (Figure 1, panels labeled A). \( \alpha_1 \)-Adrenergic receptor mRNA was expressed with higher abundance than \( \alpha_2 \)- and \( \alpha_3 \)-adrenergic receptor mRNAs (Figure 1, panels labeled A). The agarose gel was then blotted onto a nylon filter and hybridized with the nested oligonucleotides to increase the sensitivity of detection (Figure 1, panels labeled B).

**Radioligand Binding Assay**

\([^{3}H]\)Prazosin was bound specifically to human peripheral blood lymphocytes. The binding was concentration dependent, with a \( K_d \) value of 0.65±0.05 nmol/L (Figure 2A) and \( B_{max} \) of 175.3±20.5 fmol/10^6 cells (Figure 2B).

Analysis of data on the pharmacological profile of \([^{3}H]\)prazosin binding to human peripheral blood lymphocytes showed that the most powerful displacers of \([^{3}H]\)prazosin were antagonists of different subtypes of \( \alpha_1 \)-adrenergic receptors (spiperone\(\text{H}^{+}\)\(-\)\(\text{trans}\)-mephendioxan\(\text{H}^{+}\)\(+\)-niguldipine\(\text{H}^{+}\)) or nonselective \( \alpha_1 \)- or \( \alpha \)-adrenergic receptor antagonists (prazosin\(\text{H}^{+}\)phenolamine) (Table). The \( \alpha_1 \)-adrenergic receptor agonist clonidine, norepinephrine, the \( \beta \)-adrenergic receptor antagonist pindolol, and compounds active on dopamine (dopamine) and serotonin receptors (serotonin and methysergide) (Table) were much less potent than with compounds with an \( \alpha \)-adrenergic receptor profile or were ineffective as displacers of \([^{3}H]\)prazosin binding.
Pharmacological Specificity of [H]Prazosin Binding to Human Peripheral Blood Lymphocytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Receptor Selectivity</th>
<th>Kᵦ, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine</td>
<td>α₁-Adrenergic receptor agonist</td>
<td>2750±1416</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Dopamine receptor and adrenergic receptor agonist</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>Methysergide</td>
<td>Serotonin receptor antagonist</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>(+/-)-Niguldipine</td>
<td>Ca²⁺ channel blocker; α₁α₂&gt;α₁β&gt;α₂γ-adrenergic receptor antagonist</td>
<td>72.3±4.64</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Adrenergic receptor agonist</td>
<td>1300±112</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>Nonselective α-adrenergic receptor antagonist</td>
<td>24±1.8</td>
</tr>
<tr>
<td>Pindolol</td>
<td>β-Adrenergic receptor antagonist</td>
<td>721±38</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Nonselective α₁-adrenergic receptor antagonist</td>
<td>8.6±0.41</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Serotonin receptor agonist</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>Spiperone</td>
<td>Dopamine D₂-like receptor antagonist</td>
<td>α₁γ-adrenergic receptor antagonist</td>
</tr>
<tr>
<td>(-/-)-Trans-Mephendioxan</td>
<td>α₁α₂-adrenergic receptor antagonist</td>
<td>56.4±3.03</td>
</tr>
<tr>
<td>5-Methyl-urapidil</td>
<td>α₁α₂&gt;α₁β&gt;α₂γ-adrenergic receptor antagonist and SHT₁a receptor agonist</td>
<td>83.2±5.11</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHT indicates 5-hydroxytryptamine.

Anti-α₁γ-, anti-α₁β-, and anti-α₁δ-adrenergic receptor immunoglobulins decreased [H]prazosin binding to human peripheral blood lymphocytes progressively from an immunoglobulin concentration of 0.1 to 0.2 μg/mL (Figure 2C). The maximal inhibition of [H]prazosin binding was observed at an immunoglobulin concentration of 0.5 μg/mL (Figure 2C). No further decrease of binding was noticeable at higher immunoglobulin concentrations (Figure 2C). Analysis of inhibition of [H]prazosin binding by single antibodies against α₁-adrenergic receptor subtypes or by immunoglobulins in association showed that about 28% of sites labeled by [H]prazosin corresponded to α₁α₂-adrenergic receptors, 40% to α₁γ-adrenergic receptors, and 18% to α₁δ-adrenergic receptors (Figure 2C). Immunoglobulins preadsorbed with synthetic peptides used for raising antibodies did not affect [H]prazosin binding to human peripheral blood lymphocytes (Figure 2C).

Discussion

Elevated sympathetic tone and imbalance of α- and β-adrenergic receptor function are important factors in the pathogenesis of essential hypertension. α₁-Adrenergic receptors are important mediators of the sympathetic neuroeffector system, mediate positive inotropic effects of epinephrine in rat ventricular strips, and participate in vascular smooth muscle tone regulation. α₁-Adrenergic receptor activation contracts isolated blood vessels and increases blood pressure in vivo. On the basis of these observations α-adrenergic receptor blockade was introduced for the treatment of hypertension. The development of selective α₁-adrenergic receptor antagonists (prazosin and its derivatives) has led to the widespread use of these drugs as antihypertensive agents for many years.

The α₁-adrenergic receptor subtype responsible for vascular contraction may depend on both the vascular bed and the species investigated. The α₁-adrenergic receptor subtype(s) responsible for blood pressure regulation in the intact individual has been not identified yet. The most probable reasons for the limited amount of information on the topic are the complexity of investigating sympathetic cardiovascular activity in vivo and the difficulty of obtaining samples of human vascular tissue suitable for pharmacological and functional analysis in vitro.

It has been hypothesized that analysis of peripheral blood lymphocyte adrenergic receptor expression may represent a model for studying the cardiovascular adrenergic receptor system. The suitability of techniques so far proposed for demonstrating α₁-adrenergic receptor expression in normal lymphoid cells is still controversial. α₁-Adrenergic receptors were demonstrated in human peripheral blood lymphocytes using [H]yohimbine and [H]clonidine as radioligands. Radioligand binding studies performed with [H]prazosin were unable to identify α₁-adrenergic receptors in normal or leukemic human lymphocytes or reported the expression of these receptors in peripheral human natural killer cells. In view of these discrepancies we have reinvestigated the topic using both molecular biology and radioligand binding assay techniques. Currently, truly selective agonists or antagonists for discriminating α₁-adrenergic receptor subtypes are not available. In view of this, we used antibodies against α₁α₂-, α₁β-, and α₁δ-adrenergic receptor subtypes for investigating the receptor subtypes labeled by [H]prazosin in blood lymphocytes.

The present study has shown, with the use of different techniques, such as RT followed by nested PCR and radioligand binding assay in association with specific antibodies against α₁-adrenergic receptor subtypes, that human peripheral blood lymphocytes express the three α₁-adrenergic receptor subtypes so far identified (α₁α₂, α₁β, and α₁δ). The density of α₁-adrenergic receptors assayed in the present study is rather high (175.3±20.5 fmol/10⁶ cells). This suggests that human peripheral blood lymphocyte α₁-adrenergic receptors may have functional relevance. Comparative analysis of the density of α₁-adrenergic receptors measured in this work with data reported for lymphocyte α₁-adrenergic receptors showed a ratio of α₁ to α₂-adrenergic receptor of approximately 7.9 in human peripheral blood lymphocytes. The role of lymphocyte α₁-adrenergic receptors has not been clarified yet. It has been suggested that α₁-adrenergic receptors modulate lymphohemopoiesis. The association of [H]prazosin radioligand binding assay with antibodies raised against the different α₁-adrenergic receptor subtypes has shown that α₁β receptor is the α₁-adrenergic receptor subtype most represented in human peripheral blood lymphocytes and α₁δ receptor, the least. These radioligand binding data are consistent with semiquantitative analysis of mRNA levels tested by RT-PCR. Future studies
should clarify the relevance of this uneven density of α1-adrenergic receptor.

In view of the involvement of α1-adrenergic receptors in the regulation of cardiovascular homeostasis, α1-adrenergic receptor subtype expression in human peripheral blood lymphocytes may represent a marker of the status of α1-adrenergic receptors in cardiovascular disorders or may be useful for monitoring responses of these receptors to drug treatment. In line with this hypothesis are our preliminary results suggesting a downregulation of lymphocyte α1-adrenergic receptors in patients with essential hypertension.

References
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