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 α1B-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 α1A is the most represented and the α1D, 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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moved and resuspended. Cells (1×10^6) 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: \( \alpha_{1A} \) sense and antisense, \( 5'-ACTACATCGTCAACCTGGCG-3' \) and \( 5'-TGATCTTGGCAGATGGTCTCG-3' \), respectively; \( \alpha_{1B} \) sense and antisense, \( 5'-TCGCTGGCCTGCACCGGCAC-3' \) and \( 5'-ATGCCCAGTTTTGCGTCTTCTT-3' \), respectively; \( \alpha_{1D} \) sense and antisense, \( 5'-GTGGTGAAGTGCTCGGAGGC-3' \) and \( 5'-GATGACCAGCAGTGGCAGGT-3' \). 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 \( \alpha_1 \)-adrenergic receptor subtypes were blotted to nylon filters and hybridized with a specific nested oligonucleotide for each subtype of \( \alpha_1 \)-adrenergic receptor, and were labeled by T4 kinase with \([\text{32P]}\)ATP. Specific nested oligonucleotides used for \( \alpha_{1A} \), \( \alpha_{1B} \), and \( \alpha_{1D} \) were \( 5'-AACCCTGCTACCCGAGAGA-3' \), \( 5'-ATGGAACCTCTGGGTTGTTGTT-3' \), and \( 5'-CTATGGCAGGTACGTT-3' \), respectively. A specific activity of \( 4\times10^6 \) 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 for 60 minutes at 25°C with increasing concentrations of \([\text{3H}]\)prazosin (0.05 to 4 nmol/L) in 50 mmol/L Tris-HCl containing 150 nmol/L NaCl, 5 mmol/L EDTA, 100 μmol/L pargyline, and 3 μmol/L ascorbic acid (pH 7.4). Non-specific binding was defined by incubating lymphocytes with \([\text{3H}]\)prazosin in the presence of 10 μmol/L phentolamine.

The pharmacological specificity of \([\text{3H}]\)prazosin binding to human peripheral blood lymphocytes was assessed by incubating suspensions containing 1×10^6 cells with 0.5 nmol/L \([\text{3H}]\)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 \( \alpha_1 \)-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 \( \alpha_{1A} \)-, \( \alpha_{1B} \)-, and \( \alpha_{1D} \)-adrenergic receptors. Immunoglobulins were used alone, in association (anti-\( \alpha_{1A} \), anti-\( \alpha_{1B} \), anti-\( \alpha_{1A} \), and anti-\( \alpha_{1B} \), anti-\( \alpha_{1D} \), 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, \([\text{3H}]\)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. In experiments performed with antibodies against \( \alpha_1 \)-adrenergic receptor subtype, \([\text{3H}]\)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 \( \alpha_1 \)-adrenoceptor subtype PCR products, hybridized with a nested oligonucleotide, \( \text{3P} \) end-labeled by a T4 kinase reaction (B panels). The \( \alpha_{1A} \), \( \alpha_{1B} \), and \( \alpha_{1D} \) 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.
Lymphocyte α₁-Adrenergic Receptors

Femtomoles per 10⁶ cells. The axis of the ordinate shows the specifically bound to peripheral blood lymphocytes expressed in femtomoles per 10⁶ cells. The three immunoglobulins together (●) decreased radioactivity to nonspecific retention of the radioligand. The three immunoglobulins preabsorbed with 10 μg/mL of synthetic peptides used for raising antibodies (▲) did not affect [³H]prazosin binding to human peripheral blood lymphocytes. Data of [³H]prazosin specifically bound are expressed femtomoles per 10⁶ cells. Points are mean±SEM of triplicate determinations.

Results

Reverse Transcription–Polymerase Chain Reaction
RT-PCR amplified a 348-bp α₁a-adrenergic receptor fragment, a 689-bp α₁b-adrenergic receptor fragment, and a 540-bp α₁d-adrenergic receptor fragment (Figure 1, panels labeled A). α₁b-Adrenergic receptor mRNA was expressed with higher abundance than α₁a- and α₁d-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
[³H]Prazosin was bound specifically to human peripheral blood lymphocytes. The binding was concentration dependent, with a Kᵢ value of 0.65±0.05 nmol/L (Figure 2A) and Bₘᵦ of 175.3±20.5 fmol/10⁸ cells (Figure 2B).

Analysis of data on the pharmacological profile of [³H]prazosin binding to human peripheral blood lymphocytes showed that the most powerful displacers of [³H]prazosin were antagonists of different subtypes of α₁-adrenergic receptors (spiperone>(−)-trans-mephendioxan>(++)-niguldipine>5-methyl-urapidil) or nonselective α₁- or α₁-adrenergic receptor antagonists (prazosin>phenolamine) (Table). The α₁-adrenergic receptor agonist clonidine, norepinephrine, the β₁-adrenergic receptor antagonist pindolol, and compounds active on dopamine (dopamine) and serotonin receptors (serotonin and methysergide) (Table) were much less potent than compounds with an α₁-adrenergic receptor profile or were ineffective as displacers of [³H]prazosin binding.

Concentrations. The three immunoglobulins together (●) decreased radioactivity to nonspecific retention of the radioligand. The three immunoglobulins preabsorbed with 10 μg/mL of synthetic peptides used for raising antibodies (▲) did not affect [³H]prazosin binding to human peripheral blood lymphocytes. Data of [³H]prazosin specifically bound are expressed femtomoles per 10⁶ cells. Points are mean±SEM of triplicate determinations.
Pharmacological Specificity of [3H]Prazosin Binding to Human Peripheral Blood Lymphocytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Receptor Selectivity</th>
<th>$K_a$, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine</td>
<td>$\alpha_2$-adrenergic receptor antagonist</td>
<td>2750 ± 416</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Dopamine receptor and adrenergic receptor antagonist</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>Ca$^{2+}$ channel blocker; $\alpha_3$, $\alpha_4$, $\alpha_5$, $\alpha_7$, $\alpha_12$-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 $\alpha$-adrenergic receptor antagonist</td>
<td>24 ± 1.8</td>
</tr>
<tr>
<td>Pindolol</td>
<td>$\beta$-Adrenergic receptor antagonist</td>
<td>721 ± 38</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Nonselective $\alpha_1$-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 $D_3$-like receptor antagonist; $\alpha_3$, $\alpha_4$, $\alpha_5$, $\alpha_7$, $\alpha_12$-adrenergic receptor antagonist</td>
<td>8.5 ± 0.21</td>
</tr>
<tr>
<td>(-)-trans-Mephendioxan</td>
<td>$\alpha_3$, $\alpha_4$, $\alpha_5$, $\alpha_7$, $\alpha_12$-adrenergic receptor antagonist</td>
<td>56.4 ± 3.03</td>
</tr>
<tr>
<td>5-Methyl-urapidil</td>
<td>$\alpha_3$, $\alpha_4$, $\alpha_5$, $\alpha_7$, $\alpha_12$-adrenergic receptor antagonist and SHT$\alpha_1$ receptor agonist</td>
<td>83.2 ± 5.11</td>
</tr>
</tbody>
</table>

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

Anti-$\alpha_1\gamma$, anti-$\alpha_1\delta$, and anti-$\alpha_1\omega$-adrenergic receptor immunoglobulins decreased [3H]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 [3H]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 [3H]Prazosin binding by single antibodies against $\alpha_1$-adrenergic receptor subtypes or by immunoglobulins in association showed that about 28% of sites labeled by [3H]Prazosin corresponded to $\alpha_1\alpha$, $\alpha_1\delta$, $\alpha_1\omega$, 40% to $\alpha_1\omega$, and 18% to $\alpha_1\psi$-adrenergic receptor subtypes (Figure 2C). Immunoglobulins preadsorbed with synthetic peptides used for raising antibodies did not affect [3H]Prazosin binding to human peripheral blood lymphocytes (Figure 2C).

Discussion

Elevated sympathoadrenal tone and imbalance of $\alpha$- and $\beta$-adrenergic receptor function are important factors in the pathogenesis of essential hypertension. $\alpha_1$-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. On the basis of these observations $\alpha$-adrenergic receptor blockade was introduced for the treatment of hypertension. The development of selective $\alpha_1$-adrenergic receptor antagonists (prazosin and its derivatives) has led to the widespread use of these drugs as antihypertensive agents for many years.

The $\alpha_1$-adrenergic receptor subtype responsible for vascular contraction may depend on both the vascular bed and the species investigated. The $\alpha_1$-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 $\alpha_1$-adrenergic receptor expression may represent a model for studying the cardiovascular adrenergic receptor system. The suitability of techniques so far proposed for demonstrating $\alpha_1$-adrenergic receptor expression in normal lymphoid cells is still controversial. $\alpha_2$-Adrenergic receptors were demonstrated in human peripheral blood lymphocytes using [1H]yohimbine and [1H]conitine as radioligands. Radioligand binding studies performed with [1H]Prazosin were unable to identify $\alpha_1$-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 $\alpha_1$-adrenergic receptor subtypes are not available. In view of this, we used antibodies against $\alpha_1\alpha$, $\alpha_1\delta$, and $\alpha_1\omega$-adrenergic receptor subtypes for investigating the receptor subtypes labeled by [3H]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 $\alpha_1$-adrenergic receptor subtypes, that human peripheral blood lymphocytes express the three $\alpha_1$-adrenergic receptor subtypes so far identified ($\alpha_1\alpha$, $\alpha_1\delta$, and $\alpha_1\omega$). The density of $\alpha_1$-adrenergic receptors assayed in the present study is rather high (175.3 ± 20.5 fmol/10⁶ cells). This suggests that human peripheral blood lymphocyte $\alpha_1$-adrenergic receptors may have functional relevance. Comparative analysis of the density of $\alpha_1$-adrenergic receptors measured in this work with data reported for lymphocyte $\alpha_2$-adrenergic receptors showed a ratio of $\alpha_1$ to $\alpha_2$-adrenergic receptor of approximately 7.9 in human peripheral blood lymphocytes. The role of lymphocyte $\alpha_1$-adrenergic receptors has not been clarified yet. It has been suggested that $\alpha_1$-adrenergic receptors modulate lymphohematoiopsis. The association of [1H]Prazosin radioligand binding assay with antibodies raised against the different $\alpha_1$-adrenergic receptor subtypes has shown that $\alpha_1\alpha$ receptor is the $\alpha_1$-adrenergic receptor subtype most represented in human peripheral blood lymphocytes and $\alpha_1\omega$ receptor, the least. These radioligand binding data are consistent with semi quantitative 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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