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Radioligand Binding Assay for the Simultaneous Determination of β_1- and β_2-Adrenergic Receptors in Human Blood Cells

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Abstract—In this study, we propose a method for the separate determination of both types of adrenergic receptors based on radioligand binding analysis using ^{125}I-iodocyanopindolol, comprising three measurements: (1) without competing ligands, (2) in the presence of selective ligand ICI 118,551 (0.25 μM), and (3) in the presence of two selective ligands, ICI 118,551 and CGP 20712 (0.25 μM each). The technique was tested on a model system of two transgenic cell lines ADL-7A and A2R9 with the expression of recombinant β_1- and β_2-adrenergic receptors. If the ratio of the number of β_1-adrenergic receptors to β_2-adrenergic receptors is 1 : 10, the measurement error is about 15%. Analysis of nine cell lines representing different types of blood cells showed the presence of β_2-adrenergic receptors in Daudi, Raji, Dami, K-562, HL-60, U-937, and THP-1 cells and their absence in Jurkat and MOLT-4 cells. β_1-Adrenergic receptors are reliably recorded only in THP-1 cells of monocytic origin. In other cell lines, with the exception of Dami, the number of β_1-adrenergic receptors was found below the detection limit, estimated as 250 molecules per cell. Measurements performed on the peripheral blood mononuclear cells of seven healthy donors showed the presence of β_2-adrenergic receptors in the range from 1000 to 2500 molecules per cell, while the content of β_1-adrenergic receptors in all cases appeared to be on the border or beyond the detection limit.

Keywords: β_1- and β_2-adrenergic receptors, radioligand binding assay, human cells
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INTRODUCTION

An important role in pathogenesis of cardiovascular and obstructive pulmonary diseases—the two most widely spread noninfectious pathologies—is played by adrenergic mechanisms. People suffering from these diseases often are to be administered β_1- and β_2-adrenoreceptor-affecting drugs. The major targets of β-agonists and β-blockers are heart and lung tissues poorly available for laboratory analyses. However, a high rate of correlation (~0.9) between the content of β-adrenergic receptors in blood lymphocytes and their content in auricle tissues has been demonstrated in a number of studies [1, 2]. Therefore, there is a possibility to assess the processes occurring in these tissues under the effect of treatment by observing the dynamics of β-adrenergic receptor behavior in peripheral blood cells.

On surface of blood leukocytes, mainly β_2-adrenergic receptors are presented [3, 4]. No comparative studies of β_2-adrenergic receptor content in blood cells of patients with cardiovascular or pulmonary obstructive diseases have been reported earlier. Therefore, experimental data on the dynamics of β_2-adrenergic receptor behavior under the effect of drugs targeting the adrenoreceptor system are fragmentary and accidental.

Analysis of β_1-adrenergic receptors is rather hampered due to extremely low representation thereof on blood cells. In most people, the content of β_1-adrenergc receptors in leukocytes is ~20 times lower than the content of β_2-adrenergic receptors [5]. There is indirect evidence that the presence of β_1-adrenergic receptors on T lymphocytes can sharply increase in some cardiovascular pathologies or under the pressure of some other factors [5].

The content of β_1- and β_2-adrenergic receptors is usually measured using the ^{125}I-labeled ligand iodocyanopindolol, which is capable of equally efficient

Abbreviations: FBS, fetal bovine serum; PBMC, peripheral blood mononuclear cells.

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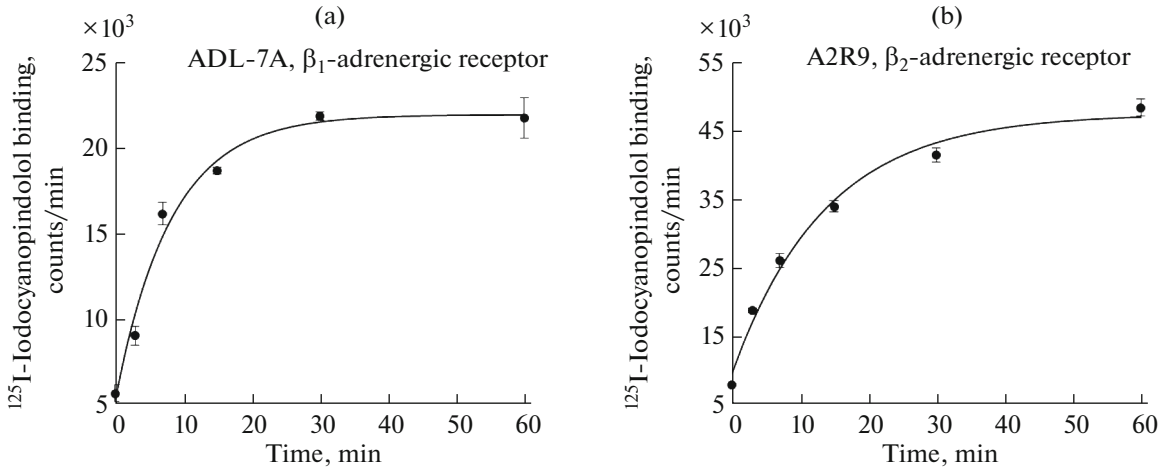

Fig. 1. Kinetics of the direct binding reaction of ^{125}I-iodocyanopindolol with ADL-7A (a) and A2R9 (b) cells at 37°C at a ligand concentration of 114 pM.

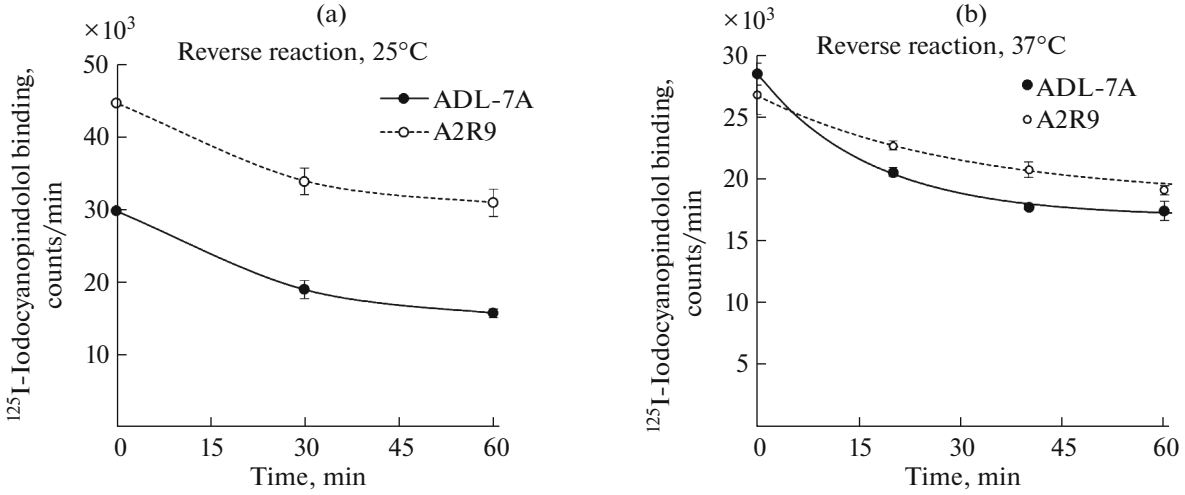

Fig. 2. Kinetics of the reverse reaction of dissociation of ^{125}I-iodocyanopindolol at 25 (a) and 37°C (b).

binding with both types of the receptors. The presence of the dominant quantities of β_2-adrenergic receptors on blood leukocytes is an additional factor preventing determination of the minor fraction of β_1-adrenergic receptors.

Current work is the continuation of our studies on modification of radioligand analysis for determination of β-adrenergic receptors in blood of individual patients [5, 7].

The aim of the work was the development of the method for radioligand analysis allowing for simultaneous and independent determination of the content of β_1- and β_2-adrenergic receptors on the surface of human blood cells.

RESULTS AND DISCUSSION

Kinetic parameters of the binding reaction. Correct setting of radioligand analysis experiments implies such a choice of conditions when the reaction of ligand binding to the receptor approaches equilibrium [8]. Figure 1 presents the kinetics of direct reaction of ^{125}I-iodocyanopindolol binding with transgenic cells ADL-7A and A2R9 expressing recombinant β_1- and β_2-adrenergic receptors, respectively, at 37°C and ligand concentration of 114 pM (400 000 counts/min mL). Evidently, 30-min incubation is sufficient for both binding curves to reach a plateau.

The kinetics of the reverse reaction determined at temperatures 25 and 37°C (Fig. 2) demonstrates the fact that although the loss of associated radioactivity due to dissociation of the ligand occurs, its fraction does not exceed 15% over the time required for washings (~30 min).

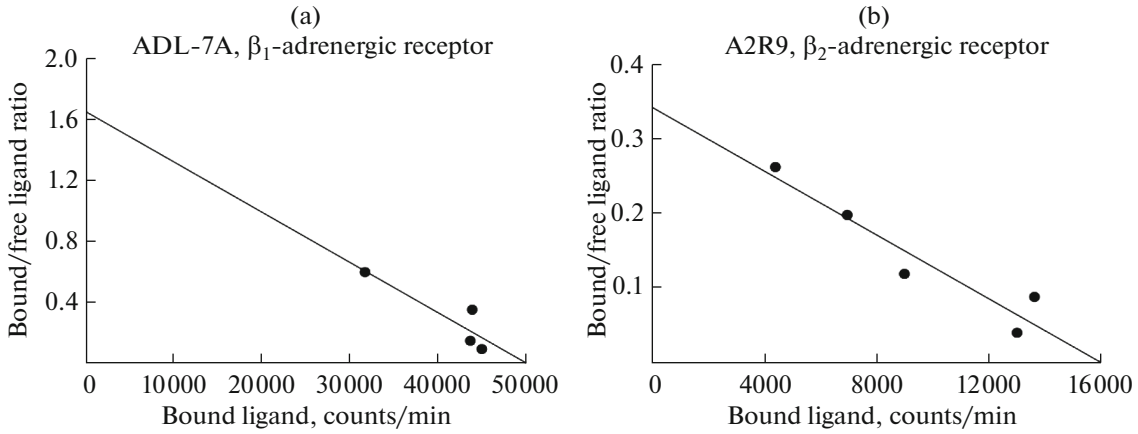

Fig. 3. Skatchard plots for ^{125}I-iodocyanopindolol binding to ADL-7A (a) and A2R9 (b) cells.

Binding constants and the number of receptors per cell. Binding constants of ^{125}I-iodocyanopindolol with β_1- and β_2-adrenergic receptors (K_d) and the number of receptors per cell of ADL-7a or A2R9 line (the B_{max} value) were determined using the Scatchard plots (Fig. 3). The results are presented in Table 1.

Inhibition with selective ligands. Agapova and coauthors [7] have previously demonstrated the possibility to determine β_2-adrenoreceptors in T lymphocytes using the difference of binding signals with ^{125}I-iodocyanopindolol in the presence or absence of a highly selective competing ligand ICI 188,551. However, the attempts to directly apply a similar approach to the determination of β_1-adrenergic receptors, using CGP 20712 as a competing ligand instead of ICI 118,551, failed. The reason is that the selectivity of CGP 20712 is not absolute: at concentrations of the ligand that are sufficient to inhibit binding of ^{125}I-iodocyanopindolol with β_1-adrenergic receptors, a low yet detectable inhibition of binding of ^{125}I-iodocyanopindolol with β_2-adrenergic receptors was recorded. Since the content of β_2-adrenergic receptors in blood cells normally exceeds that of β_1-adrenergic receptors by an order of magnitude, the rising interference prevents the isolation and identification of the difference in signals that is caused by the presence of the very β_1-adrenergic receptors.

Table 1. Dissociation constants and content of β_1- and β_2-adrenergic receptors in model transgenic cells

Model cells	Adrenergic receptor type	K_d, pM	Number of receptor molecules per cell (B_{max})
ADL-7A	β_1	40	4.3×10^6
A2R9	β_2	16	3.7×10^6

To minimize the noise caused by the presence of excessive amounts of β_2-adrenergic receptors, we proposed the scheme of analysis including three measurements of ^{125}I-iodosyanopindolol binding with cells: (1) in the absence of competing ligands; (2) in the presence of the ICI 118,551 ligand at a concentration that inhibits β_2-adrenergic receptor binding down to almost zero; and (3) in the presence of two ligands ICI 118,551 and CGP 20712. Under conditions when binding with β_2-adrenergic receptors is nearly blocked, even a small difference between the second and the third measurements should reflect the content of β_1-adrenergic receptor in the system. Of course, in calculations it should be taken into account that high concentrations of ICI 118,551 block binding of ^{125}I-iodocyanopindolol with β_1-adrenergic receptors to a certain degree.

In frames of the stated problem, binding of ^{125}I-iodocyanopindolol with cells carrying both types of adrenoreceptors can be expressed as a sum of three independent components: B_0, background binding not affected by selective ligands; B_1, specific binding with β_1-adrenergic receptors, and B_2, specific binding with β_2-adrenergic receptors. Background binding includes both completely nonspecific sticking of labeled cyanopindolol on the cell surface, not replaced by high concentrations of unlabeled ligand, and specific binding with other cell receptors, particularly, serotonin receptors [9]. In the presence of ICI 118,551 (second measurement), binding with β_1-adrenergic receptors should be weakened to the level k_1B_1, and with β_2-adrenergic receptors, to the level of k_2B_2, where k_1 and k_2 are coefficients characterizing the levels of residual binding of ^{125}I-iodocyanopindolol with β_1- and β_2-adrenergic receptors, respectively. In the presence of both selective ligands specific binding of labeled iodocyanopindolol should be described by similar values k_3B_1 and k_4B_2. According to the Cheng–Prusoff formula [10], coefficients k_1–k_4 depend on both concentrations of competing ligands and con-

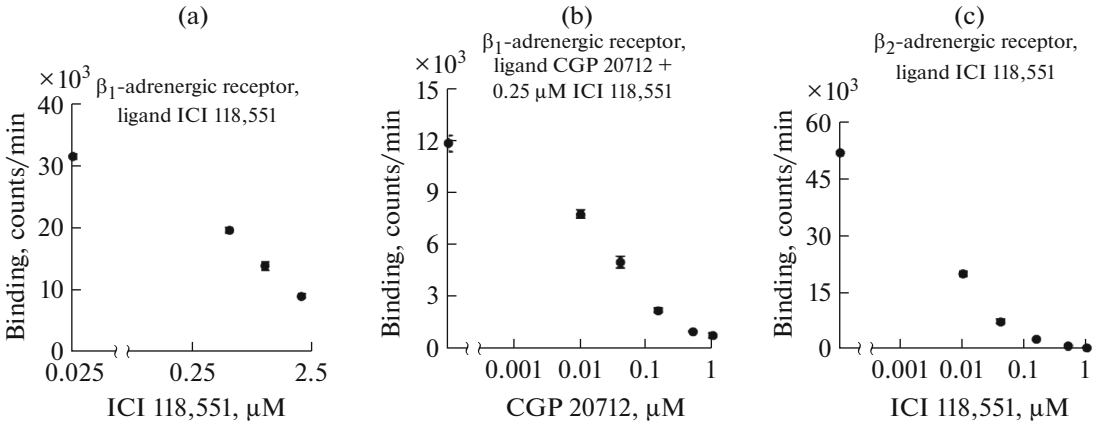

Fig. 4. Inhibition curves of ^{125}I-iodocyanopindolol binding to β_1- (a, b) and β_2-adrenergic receptors (c) by competing ligands.

centration of ^{125}I-iodocyanopindolol available for binding in the sample; thus, these concentrations must be fixed during measurements.

A sum of three linear equations can be written for the three abovementioned measurements. Knowing the coefficients k_1–k_4, the system can be solved for unknown B_0, B_1, and B_2. Under condition when the fraction of bound ^{125}I-iodocyanopindolol is small compared to its total amount in the system, the numbers of adrenoreceptors of each type can be calculated using approximate formulas:

$$R_1 = B_1(1 + K_1/L); \quad R_2 = B_2(1 + K_2/L),$$

where R_1 and R_2 are the numbers of β_1- and β_2-adrenergic receptors, K_1 and K_2 are relevant binding constants, L is the concentration of "bindable" ^{125}I-iodocyanopindolol in the sample: 400 000 counts/min mL or 114 pM under standard conditions of the analysis.

Working concentrations of selective ligands ICI 118,551 and CGP 20712—0.25 μM each—were selected basing on the analysis of inhibition curves (Fig. 4). Coefficients k_1–k_4 are presented in Table 2.

Model mixing of cells. The described scheme of analysis was tested using the ADL-7A and A2R9 cells that were mixed in various proportions. The ratio of model cells was chosen to imitate the situation typical of blood cells: the content of β_1-adrenergic receptors is significantly lower than the content of β_2-adrenergic receptors. The mixtures were subjected to the analysis according to the proposed scheme and based on the data, the amount of ADL-7A and A2R9 cells in the mixture were calculated. The feasibility of the method was evaluated by how close the data are to true numbers of both types of cells used to make the mixtures.

The results of three typical experiments are presented in Fig. 5. These and other similar experiments showed that if the ratio of β_1- and β_2-adrenergic receptors is ~1 : 10, the error of the analysis lies within 15%. If the fraction of β_1-adrenergic receptors decreases to 1 : 40, the error of determination thereof reaches 40%, which makes the results of the analysis a qualitative estimate.

Ligand binding on suspension cell cultures and blood mononuclear cells from healthy donors. The developed method was applied to assess the content of β_1- and β_2-adrenergic receptors in a number of cell lines originating from different types of human blood cells. The results of the analysis are presented in Table 3. β_2-Adrenergic receptors are barely represented in cells derived from T and B lymphocytes (Jurkat, MOLT-4, Daudi, and Raji) and are relatively highly represented in cells of monocytic origin THP-1 and U-937. As for β_1-adrenergic receptors, their presence was reliably detected in THP-1 cells and, doubtfully, in Dami cells. For most of the remaining cell lines studied, the content of β_1-adrenergic receptors turned out to be below the detection threshold, which on average can be estimated at the level of 250 molecules per cell.

It makes sense to compare our data on suspension cell cultures with the results of similar studies by other groups of authors. Sager and coauthors [11] measured the content of β_2-adrenergic receptors in HL-60 cells.

Table 2. Coefficients of residual binding of ^{125}I-iodocyanopindolol in the presence of selective inhibitor ligands

Competing ligands	β_1-Adrenergic receptor, ADL-7A cells	β_2-Adrenergic receptor, A2R9 cells
0.25 μM ICI 118,551	$k_1 = 0.76$	$k_2 = 0.032$
0.25 μM ICI 118,551 + 0.25 μM CGP 20712	$k_3 = 0.11$	$k_4 = 0.032$

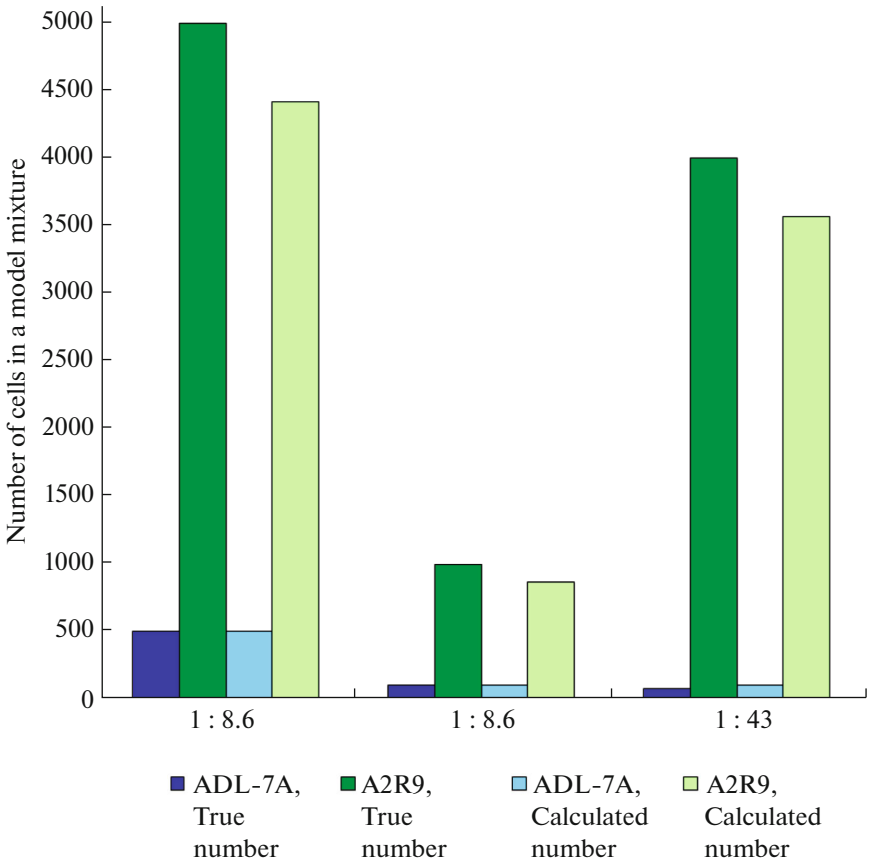

Fig. 5. Model mixing of different amounts of cells expressing β_1-adrenergic receptor (ADL-7A) and β_2-adrenergic receptor (A2R9) in different proportions. The three groups of columns are the results of three independent experiments.

Their data (~2000 molecules per cell) are comparable in the order of magnitude to the value of 4000 obtained in this work. Mäki and coauthors [12] carried out a radioligand analysis of the content of β-adrenergic receptors (without differentiation by type) in membrane fractions of cells of a number of lines, including HL-60, U-937, K-562, and Raji. As expected, β-adrenergic receptors were found in HL-60 and U-937 cells, and in comparable amounts. However, the presence of β-adrenergic receptors in K-562 and Raji cells was not recorded by the authors, which disagrees with the results of our study. Apparently, the detection threshold in the work by Mäki et al. passed higher and did not allow the detection of 5–10 times lower amounts

Table 3. Content of β_1- and β_2-adrenergic receptors in cell lines

Cells	Origin	Number of receptor molecule per cell	
		β_1-adrenergic receptors	β_2-adrenergic receptors
Jurkat	T lymphocytes	<50	<30
MOLT-4	T lymphoblasts	<150	<120
Daudi	B lymphocytes	<30	130 ± 20
Raji	B lymphocytes	<100	230 ± 60
Dami	Megakaryocytes	480 ± 230	250 ± 120
K-562	Granulocytes	<300	480 ± 200
HL-60	Promyelocytes	<500	4000 ± 300
U-937	Macrophages	<800	6900 ± 900
THP-1	Monocytes	23000 ± 3000	6200 ± 1800

of β_2-adrenergic receptors, which, according to our data, are contained in these cells. We found the largest number of β_1- and β_2-adrenergic receptors in the cells of a monocytic line THP-1. Grisanti and coauthors [6] and Talmadge and coauthors [13] also showed that THP-1 cells produce significant amounts of β_1- and β_2-adrenergic receptors using immunoblotting and radioligand analysis. Thus, testing the method we developed on cell lines gave results close to those reported in the literature.

A similar analysis of the content of β_1- and β_2-adrenergic receptors in peripheral blood mononuclear cells (PBMC) of seven healthy donors showed (Table 4) that the number of β_2-adrenoreceptors in these cells varies in the range of 1000–2500 molecules per cell. The presence of β_1-adrenergic receptors in PBMC cells could not be reliably recorded in any of the donors.

Discussion of the results. Currently, three types of β-adrenergic receptors are known: β_1, β_2, and β_3. In human leukocytes, β_3-adrenergic receptor is not found, β_2-adrenoreceptor is found in almost all types of white blood cells. Numerous studies have been devoted to the study of the representation of β-adrenergic receptors in the blood cells of sick and healthy people [4, 5, 8, 14–17]. It is quite difficult to compare the results of these studies, because analyses were carried out according to different methods and were often not verified quantitatively by other methods. Despite the absence of rigorous metrological verification, in some cases it was possible to identify very interesting and clinically significant changes in the representation of β_2-adrenergic receptors associated with respiratory pathologies [17]. In this work, the number of receptors was determined by the amount of radioactivity bound to cells in counts/min per 1 million cells. Obviously, direct conversion of the amount of the radioactive ligand bound to the cells into the number of receptors exposed on the surface of these cells, without taking into account K_d, leads to some underestimation of the measurement results. Therefore, the data of Agapova and coauthors [17] should be considered rather as estimates.

Nevertheless, analyzing the results of various studies, we can conclude that the content of β_2-adrenergic receptors in peripheral blood mononuclear cells varies in the range of 400–2500 molecules per cell, which is quite consistent with our data.

In most human organs and tissues, β_1- and β_2-adrenergic receptors are present together; therefore, the task of their separate determination is relevant. In their work, Bundkirchen and coauthors [18] presented one of the possible solutions to this problem using a mathematical analysis of the ^{125}I-iodocyanopindolol binding curves depending on the concentrations of inhibitory ligands ICI 118,551 and CGP 20712. The construction of such curves requires a large amount of experimental material, since it involves at least 40 measurements. The simpler three-dimensional analysis scheme we proposed has the advantage that ~6 million cells are sufficient for its implementation, which can be obtained from 10–20 mL of blood.

Table 4. Content of β_1- and β_2-adrenergic receptors in peripheral blood mononuclear cells of healthy donors

Donor	Number of receptor molecules per cell	
	β_1-adrenergic receptors	β_2-adrenergic receptors
1	<200	2370 ± 120
2	(60 ± 60)	1320 ± 60
3	<70	1260 ± 60
4	(140 ± 160)	1890 ± 150
5	<250	1890 ± 170
6	(240 ± 440)	1130 ± 240
7	(220 ± 460)	2030 ± 270

In parentheses, the number of cases when measurement error exceeds the measured value.

As a rule, simultaneously with the content of receptors, the corresponding K_d of iodocyanopindolol is assessed. In a number of studies, K_d was measured on model cells expressing one or another type of adrenoreceptors [19, 20]. K_d for both types of the receptors seem to be close to each other and range between 17 and 53 pM. Similar values, 40 pM for β_1-adrenergic receptor and 16 pM for β_2-adrenergic receptor, were also obtained in our work.

The question of how many β_1-adrenergic receptors are normally found in different types of blood cells remains open today. A communication by Smolyakova et al. [5] about the high level of β_1-adrenergic receptors in the blood cells of some patients with cardiac pathologies was possibly due to some special circumstances and needs additional experimental confirmation. We were only able to show that the number of β_1-adrenergic receptors in the total fraction of mononuclear cells in seven healthy people does not exceed 250 molecules per cell and cannot be reliably measured using the proposed method.

EXPERIMENTAL

Cell lines and culture conditions. We previously obtained the ADL-7A cell line [21] by transfecting HEK293 human embryonic kidney cells (from the collection of the Institute of Experimental Cardiology, Chazov National Medical Research Center for Cardiology, Moscow) with the pMC4IPW-ADRBopt plasmid encoding the optimized human β_1-adrenergic receptor gene under the control of the cytomegalovirus early gene promoter. The A2R9 cell line was obtained by transfection of the original HEK293 cells with the pC4IPW-hADRB2-EGFP plasmid encoding

the β_2-adrenergic receptor gene fused at the *C*-terminus to the green fluorescent protein, which served as a marker for identifying the transfected clones. The cells of these lines were cultured in the DMEM medium containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin (all Invitrogen reagents, United States), in an atmosphere of 5% CO_2 at 37°C.

To carry out the binding reaction with ^{125}I-iodocyanopindolol, the cells were removed from the surface of the flasks by treatment with trypsin, the proteolysis reaction was stopped by adding a culture medium with 10% FBS, the cells were precipitated by centrifugation for 15 min at 1100 rpm (200 g), washed twice with phosphate-buffered saline (PBS) at room temperature, and suspended in PBS to a final concentration of 10^1–10^6 cells/mL (HEK293) or 10^6 cells/mL (ADL-7A, A2R9).

Suspension human cells of the Raji, Daudi, Jurkat, K-562, U-937, MOLT-4, Dami, HL-60, and THP-1 lines from the collection of the Institute of Experimental Cardiology, National Medical Research Center for Cardiology, were cultured in the RPMI-1640 medium containing 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. For radioligand analysis, cells were pelleted by centrifugation and washed twice with PBS as described above.

Human peripheral blood mononuclear cells (PBMC). Blood sampling from volunteers was carried out in the laboratory of the Institute of Experimental Cardiology of the National Medical Research Center for Cardiology. Venous blood from seven healthy volunteers was collected in vacuum BD Vacutainer CPT tubes (Becton, Dickinson and Company, United States) and processed according to the manufacturer's instructions. Mononuclear cells were transferred to other tubes and washed twice with PBS.

Ligands. The following ligands were used in the work: cyanopindolol chemifumarate (Bio-Techne Corporation, United States), ICI 118,551 (Sigma-Aldrich, United States), and CGP 20712 (Bio-Techne Corporation, United States).

^{125}I-Iodocyanopindolol. The radioactive isotope of iodine in the composition of the Na^{125}I molecule (2000 Ci/mmol) was obtained from JSC Khlopin Radium Institute (St. Petersburg, Russia). The introduction of the ^{125}I atom into the cyanopindolol molecule using chloramine T was carried out by the method of Greenwood and coworkers [22] with some modifications. For the labeling reaction, 1 μg of cyanopindolol chemifumarate and 1 mCi of Na^{125}I were taken in 50 μL of 0.2 M potassium phosphate buffer, pH 7.0. The reaction was initiated by adding 10 μL of chloramine T solution (5 mg/mL). The reaction mixture was incubated at room temperature for 30 s; the reaction was stopped by adding 10 μL of sodium thiosulfate (20 mg/mL). The mixture was kept for 5 min at room temperature, after which 1 μL of a "cold" sodium iodide solution (6 mg/mL) was added.

The target product was purified by high-performance liquid chromatography on a Diasorb 130 column (5 μm, 4 × 150 mm; Elsiko, Russia) in the ion-pair mode with elution in a concentration gradient of 0–90% acetonitrile in 10% acetic acid. Fractions containing ^{125}I-iodocyanopindolol were combined, evaporated to dryness, dissolved in 70% ethanol, and stored at –20°C. The proportion of radioactivity in the fraction capable of binding to adrenoreceptors ("bindable" fraction) was evaluated in preliminary experiments with excess ADL-7A or A2R9 cells.

^{125}I-Iodocyanopindolol Binding Reaction. The model cells were mixed in such a way that 100 μL of the suspension contained 2×10^5 HEK293 cells acting as a carrier, and the dosed number of ADL-7A and/or A2R9 cells, as a rule, was 100–5000 cells. In the case of suspension cell lines, 2×10^5 to 2×10^6 cells per 100 μL were used, PBMC cells were taken in an amount of 5×10^5 to 10^6 per 100 μL of suspension. To reduce nonspecific binding, casein concentrate CBC2 (Stereospecific Detection Technologies, Germany) was added to the suspension at a dilution of 1 : 10.

Then, 100 μL of the cell mixture, 20 μL of PBS, or 20 μL of a 2.5 μM solution of the selective ligand ICI 118,551, or 20 μL of a mixture of 2.5 μM ICI 118,551 and 2.5 μM CGP 20712 were sequentially added to Eppendorf-type tubes. After that, 80 μL of a solution of PBS ^{125}I-iodocyanopindolol in PBS with 10% CBC2 was added. In standard experiments, radioactivity level in the bindable fraction was 80000 counts/min per sample. The tubes with the reaction mixture were incubated for 30 min at 37°C with stirring on a shaker.

At the end of incubation, the tubes with samples were centrifuged for 10 min at 2000 g. The supernatant was removed, the pellet was suspended in 200 μL of PBS, centrifuged for 10 min at 2000 g, after which the pellet was resuspended in 200 μL of PBS, centrifuged for 5 min at 10000 g, and the supernatant was removed. The amount of radioactivity bound to the cells was determined using a 2470 Wizard2 (Perkin-Elmer, United States) gamma counter with a counting efficiency of 79%. All measurements were carried out in three or four parallels. The deviation from the average value did not exceed 20%.

In kinetic experiments, after the incubation time had elapsed, unlabeled cyanopindolol was added to the samples up to 10 μM to completely stop the binding reaction.

CONCLUSIONS

In this study, a method for the simultaneous independent determination of β_1- and β_2-adrenergic receptors based on radioligand analysis using ^{125}I-iodocyanopindolol was developed and verified, the detection

threshold for β_1-adrenergic receptors was determined, and methodological issues of comparing the results of the analysis are discussed. The method was verified using model mixing of transgenic ADL-7A and A2R9 cell lines in various proportions and subsequent separate determination of the amount of β_1- and β_2-adrenergic receptors in the resulting mixtures. When the ratio of the number of β_1-adrenergic receptors to β_2-adrenergic receptors is 1 : 10, the measurement error does not exceed 15%, however, with a decrease in the proportion of β_1-adrenergic receptors to 1 : 40, the measurement error increases sharply and can reach 40%.

The content of β_1- and β_2-adrenergic receptors in nine cell lines (different types of blood cells), as well as in peripheral blood mononuclear cells of seven healthy donors, was analyzed using the developed method.

The measurement of the number of β_1- and β_2-adrenergic receptors in mononuclear blood cells of healthy donors, described in this article, is rather a demonstration of the possibility of using the technique we developed and cannot claim serious biomedical conclusions due to the clearly insufficient number of blood samples studied.

An important achievement of the proposed method can be considered the possibility of using this analysis for an individual person (patient), which makes the method very attractive for applied medical purposes. However, given the high dynamics of the human receptor apparatus under the influence of various factors (pathology, stress, drugs, etc.), the data obtained should be treated with caution. Apparently, such studies on the dynamics of changes in adrenoreceptor activity in response to the action of drugs will be more informative and more correct from a medical point of view.

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in human research comply with the ethical standards of the institutional and/or national research ethics committee and the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards.

Informed consent was obtained from each of the volunteer donors participating in the study.

Conflict of Interests

The authors declare no conflicts of interest.

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