Calculation of the content of β_1- and β_2-adrenergic receptors in cells. The content of β1- and β2-adrenoreceptors in cells was calculated on the basis of cell-bound radioactivity as described in [*Shevelev*]. The calculation took into account that the binding of ^{125}I-CYP with cells bearing both types of adrenergic receptors can be decomposed into the following components:

$$P = B_1 + B_2 + B_{other} + B_{nonspecific}$$

where P is the amount of total radioactivity bound; B_1 is the specific binding to β_1-adrenoreceptors; B_2 is the specific binding to β_2-adrenoreceptors, B_{other} is the specific binding to other cellular receptors (particularly serotonin receptors), and $B_{nonspecific}$ is a completely nonspecific adhesion of ^{125}I-CYP to the cell that is not displaced by high concentrations of unlabeled ligand.

The values of B_{other} and $B_{nonspecific}$ should be the same in the first three cases, as they are unaffected by inhibitor ligands. Assuming that the B_1 value reflects the binding of ^{125}I-CYP to β_1-adrenoreceptors in the absence of inhibitor ligands (measurement I), in case II, this binding would be attenuated to $k_1 \times B_1$, where k_1 is the fraction of residual binding to β_1-adrenoreceptors in the presence of ICI 118,551. For the binding to β_2-adrenoreceptors in case II, we will have $k_2 \times B2$, where k_2 is the fraction of residual binding to β_2-adrenoreceptors in the presence of ICI 118,551. Similarly, in case III, we obtain binding levels $k_3 \times B1$ and $k_4 \times B2$ for β_1 and β_2-adrenoreceptors, respectively (k3 and k4 are the fractions of residual binding to β_1 and β_2-adrenoreceptors in the presence of ICI 118,551 and CGP 20712). The values of k_1-k_4 coefficients were determined experimentally earlier in [1] : k1 = 0.82, k2 = 0.029, k3 = 0.08, k4 = 0.029 .

If we denote the amount of radioactivity in cpm that bound to cells under the various conditions described in the subsection "Binding Reaction with ^{125}I-CYP" as P1, P2, P3, and P4, then we obtain the following system of equations:

$$\begin{aligned} P_1 &= B_1 + B_2 + B_{other} + B_{nonspecific} \\ P_2 &= k_1 B_1 + k_2 B_2 + B_{other} + B_{nonspecific} \\ P_3 &= k_3 B_1 + k_4 B_2 + B_{other} + B_{nonspecific} \\ P_4 &= B_{nonspecific} \end{aligned}$$

From these linear equations, formulas were determined to calculate B_1, B_2 and B_{other} from the data on bound radioactivity, taking into account the k_1-k_4 coefficients.

To calculate the number of adrenergic receptors of each type, approximate formulas corresponding to the chemical equilibrium in the ligand-receptor system were used:

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

where R_1 and R_2 are the amounts of β_1 and β_2-adrenoreceptors, K_1 and K_2 are the respective binding constants determined previously (K_1 = 40 pM, K2 = 15 pM), L is the concentration of the PC fraction of ^{125}I-CYP: 400000 cpm/ml (at gamma counter efficiency of 79%) or 114 pM under standard assay conditions, α is the conversion factor from cpm to number of molecules (in units), which is 172,000 units/cpm. The content of β_1 and β_2-adrenoreceptors per cell (N_1 and N_2, respectively) is calculated by dividing R_1 and R_2 by the number of cells n in the sample:

$N_1 = R_1/n$; $N_2 = R_2/n$

Reference:

1. Shevelev A.Y. et al. Radioligand binding assay for the simultaneous determination of β1- and β2-adrenergic receptors in human blood cells. Bioorganicheskaya Khimiya/Russian Journal of Bioorganic Chemistry, 48, 551-560 (In Russ) (2022).