Biochemical Pharmacology, Vol. 31. No. 1, pp.133-136, 1982.
Printed in Great Britain.

0006-2952/82/010133-04$03.00/0

DIFFERENT NUMBERS OF ß-RECEPTORS IN HUMAN LYMPHOCYTE SUBPOPULATIONS

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(Received 20 August 1981; accepted 19 October 1981).

In the past few years drug-receptor binding assays have been introduced to measure the number of ß-receptors in human lymphocytes (1,2,3) and to explore pathophysiological mechanisms (4,5,6). The interpretation of these studies is complicated by the possibility of different numbers of binding sites in subpopulations of lymphocytes. Mammalian lymphocytes show well defined differences in surface markers and immunological function, leading to a subdivision into B- and T-lymphocytes (7). Determinations of binding sites in human lymphocytes, first performed by Williams et al. (1) with ^{3}H-DHA showed 75 fmoles/mg protein in broken cells or 2000 sites/cell and no differences between B- and T-lymphocyte fractions (8). To investigate the number of binding sites in intact human lymphocytes and their subpopulations, we have used a new radiolabelled ligand, ^{125}I-Cyanopindolol (^{125}I-CYP) (9), which has the advantage over the tritiated ligand of a 65 times higher specific radioactivity and a higher affinity constant for ß-receptors and over ^{125}I-hydroxybenzylpindolol to have less non-specific binding and less α-adrenergic affinity (9). This last observation is probably due to the lack of the hydroxybenzyl group (10).

Methods: Approximately 180 ml heparinized whole blood from healthy persons - 2 men and 1 woman - was centrifuged over a Ficoll-Isopaque density gradient to obtain a leucocyte fraction consisting of 90-94 % lymphocytes (monocytes 6-10 %). These cells were washed three times in buffer A (140 mM NaCl, 5.5 mM Glucose, 10 mM Hepes, pH 7.45). The last pellet was dissolved in buffer B (6.7 mM phosphate buffer, 138 mM NaCl, pH 7.2). Separation of lymphocyte subpopulations by free-flow continuous electrophoresis was performed according to Hannig (11). Three subpopulations were sepa-

rated by charge and classified as H-, M-, and L-fractions. The L-fraction contained 30 % B-lymphocytes, the M- and H-fraction 70 % T-lymphocytes (12); monocytes with the slowest electrophoretic mobility (13) were discharged. 90 % viability was determined by trypan blue exclusion at the end of the separation procedure.

Binding assay: Lymphocytes, before and after separation, were incubated for 75 min at 23°C in a total volume of 0.2 ml, 1.0-3.0 x 10^6 cells/tube, in the presence of 50 pM ^{125}I-CYP and buffer B. Incubation in the presence of 10^{-6} M (-)propranolol was determined as unspecific binding (40-60 % of specific binding) (14). Incubation was terminated by rapid vacuum filtration through Gelman AE glass fibre filters (9).

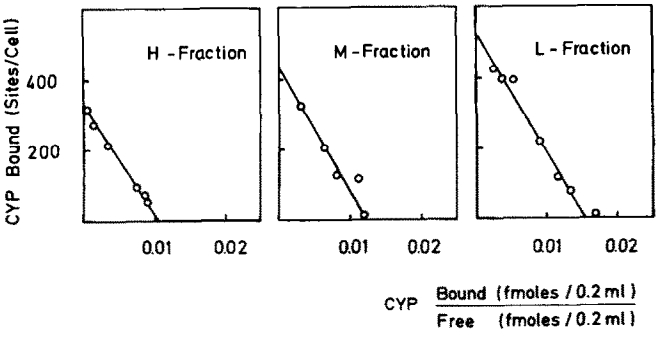

Fig. 1: Specific binding of ^{125}I-CYP to the H-, M-, L-fraction of freshly incubated human lymphocytes is plotted according to Scatchard (15). The number of total ^{125}I-CYP binding sites is different in each fraction.

Results and discussion: In order to quantitate the maximal CYP binding sites per cell, experiments with increasing concentrations of ^{125}I-CYP ($5x10^{-11}$- $5x10^{-8}$ M) were performed. The values were plotted according to Scatchard (15) for each separated lymphocyte subpopulation (Fig. 1). The plots very clearly show the highest number of binding sites in the L-fraction, and the lowest number in the H-fraction. The dissociation constants stay within the range of experimental error. In a previous report (14) stereospecific binding was shown to be essential for specific binding. Therefore, stereospecific binding was demonstrated in each subpopulation (Fig. 2). The results obtained from Scatchard plots in lymphocytes from three different healthy persons before fractionation and of the three fractions are summarized in Table 1. Even though there are large differences in absolute values of binding sites per average cell between individuals, there is clear evidence for the existence of lymphocytes with different numbers of binding sites.

The number of ^{125}I-CYP binding sites in whole lymphocytes of 10 healthy persons was determined. The values indicate large interindividual variation (Fig. 3). In agreement with our results, Abrass et al. (2) recently showed a similar mean number of

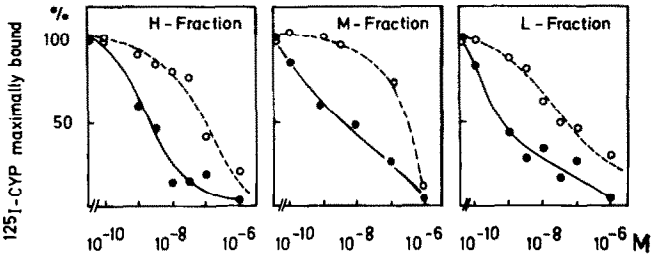

Fig. 2: Specific ^{125}I-CYP binding to the H-, M-, L-fractions of human lymphocytes is inhibited by addition of (-)propranolol •-• or (+)propranolol o-o in the indicated concentrations. The curves demonstrate the stereospecificity of the binding sites (100 % correlates with about 1800 cpm and 0 % with 900 cpm).

			H		M		L		I	
	sex	age	BS	K_D	BS	K_D	BS	K_D	BS	K_D
1	♂	37	330	4.1×10^{-10}	430	5.6×10^{-10}	529	3.3×10^{-10}	255	4.0×10^{-10}
2	♀	38	215	1.0×10^{-10}	399	1.9×10^{-10}	860	5.0×10^{-10}	431	1.8×10^{-10}
3	♂	28	180	4.5×10^{-10}	348	4.0×10^{-10}	1168	8.0×10^{-10}	575	4.0×10^{-10}

Table 1: The number of ^{125}I-CYP binding sites (BS) and their dissociation constants (K_D) are given for isolated lymphocytes (I) and for their H-, M-, L-fractions from 3 individuals.

^{3}H-DHA binding sites in whole lymphocytes with an interindividual variation of 200 to 1600 sites per cell. One reason for this large variation might be a different relationship between lymphocyte subpopulations each with different numbers of binding sites. In tonsil lymphocytes Pochet et al. (3) measured 600 sites in B-lymphocytes and 200 sites in T-lymphocytes, whereas in unfractionated lymphocytes the number of sites ranged up to 500 per cell. Our data indicate a higher number of ß-adrenergic receptors in the L-fraction. This result is contradictory to the data of Bisphopric et al. (8). The separation technique used in our laboratory was based on charge, whereas Bispopric et al. (8) used an immunological method. Different separation techniques may induce separation of different subpopulations besides the known and characterized B- and T-cells.

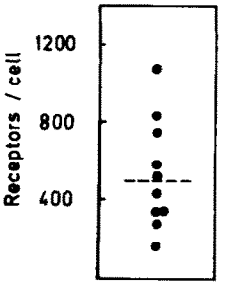

Fig. 3: Determination of binding sites in unfractioned whole human lymphocytes of 10 healthy persons. The mean value was 530 ± 91 SEM ^{125}I-CYP binding sites per cell. The intraindividual variation was about ± 10 %.

Acknowledgement: We are grateful to Dr. Engel, Sandoz AG, Basel, for his generous supply of CYP.

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