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BETA ADRENOCEPTORS OF HUMAN RED BLOOD CELLS, DETERMINATION OF THEIR SUBTYPES

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Abstract—The characteristics of beta adrenergic receptors of human erythrocyte membranes were investigated using $(-)^{125}$iodocyanopindolol as a radioligand.

Inhibition of $(-)^{125}$iodocyanopindolol specific binding was checked using either atenolol and metoprolol as $beta_1$ selective antagonists or ICI 118551 and IPS 339 as $beta_2$ selective antagonists. The results showed non linear Hofstee's plots suggesting that both $beta_1$ and $beta_2$ adrenergic receptors are present.

Analysis of the data yielded a $beta_1$/$beta_2$ adrenergic receptor ratio of approximately 33/67. Thus it is concluded that $beta_2$ subtype is predominant on human erythrocyte membranes.

The presence of beta receptors on the membranes of human red blood cells (RBC) is still debated. Some authors have documented their existence [1–5] whereas others think that there is no evidence for such receptors [6, 7]. However, strong arguments obtained in animals support the existence of these receptors in man too. Various ligands known as agonists or antagonists of beta receptors, bind to the RBC and to the corresponding membranes in other animal species [8–13]. However, in man, the difficulty of the characterization of this binding lies probably in the very low number of such RBC binding sites. So, in order to ascertain their existence, it is required to use both a ligand of a high specific radioactivity and a relatively large amount of RBC to collect enough membranes, i.e. beta receptors. It seems also that this preparation must not be contaminated by other blood cells, since lymphocytes and granulocytes were shown to possess a rather large number of such sites [14, 15]. Considering that beta adrenoceptors have already been assessed in many other species [8–13] their existence seems likely on human RBC. Moreover a stimulation of those receptors in man leading to an increase in adenylate cyclase activity [3–6], strongly suggests that human erythrocyte membranes (HEM) have a biological function, mediated by those receptors perhaps in the cell deformability [16]. It was also demonstrated that a plasma component may increase the number of RBC beta receptors [1]; for Sager they are mainly of $beta_2$ subtype [5].

This study was performed to assess the existence of beta receptors on HEM and to define their subtypes using a ligand, $(-)^{125}$iodocyanopindolol [$(-)^{125}$ICYP], with a very high specific radioactivity (≥2000 Ci/mmole).

$(-)^{125}$ICYP is characterized by its specificity for beta-receptors associated with a high affinity constant and by a lack of selectivity for either $beta_1$ or $beta_2$ subtype. Its binding parameters, concentration of binding sites per mg of protein (B_{max}) and the corresponding dissociation constant (K_d) were measured after incubation with membrane preparations, either alone, or with agonists or antagonists selective for one receptor subtype.

MATERIALS AND METHODS

Materials

$(-)^{125}$ICYP was supplied by Amersham International. Several agonists, (−)isoproterenol, (−)epinephrine, (−)norepinephrine, purchased from Sigma Chemical Company. Different beta adrenoceptors antagonists were selected; (±)ICI 118551 $beta_2$ selective [17, 18], (±)atenolol $beta_1$ selective and (±)propranolol not selective were obtained from ICI Pharma, (±)metoprolol $beta_1$ selective from Ciba Geigy and (±) IPS 339 $beta_2$ selective, was kindly supplied by Pr G. Leclerc (Department of Pharmacology, Faculty of Medicine, F-67085, Strasbourg).

A suspending medium (A) containing NaCl (121 mM), $NaHCO_3$ (25.3 mM), $CaCl_2$ (1.3 mM) was adjusted at pH 7.8 according to Sager [4]. A buffer (medium B) containing NaCl (14 mM), KCl (5.4 mM), $CaCl_2$, (1.8 mM), $MgCl_2$ (0.8 mM) and Tris (25 mM) was adjusted at pH 7.4 with 1 N HCl at 37° for incubation experiments and at 0° for washing the membrane preparations.

Methods

Preparation of HEM. Blood was collected from young healthy subjects in polypropylene tubes containing heparin at a final concentration of 10 IU per ml. The blood was centrifuged at 750 *g* for 15 min at 0–4°. Plasma and buffy coat were aspirated to remove polynuclear and mononuclear cells and erythrocytes were resuspended in ice-cold medium A to 50% hematocrit. The supernatant was removed after centrifugation at 750 *g* for 15 min.

After three washings the composition of the preparation was checked using a cell differential counter (Coulter). The selected samples were devoid of lymphocyte or granulocyte. If not, additional washings and centrifugations were made until the erythrocyte suspension was pure. Then, the cells were lysed

in ice-cold distilled water for 30 min with a gentle shaking. HEM were obtained by homogenization of the lysate in a Potter-Elvehjem glass homogenizer with three strokes by the motor driven Teflon pestle. The homogenate was centrifuged at 30,000 *g* for 30 min. The pellet was washed in medium A four or five times. By this procedure more than 95% of haemoglobin was removed. Total protein concentration was determined according to Lowry [20]. Membrane protein concentration was 8 mg/ml; HEM were stored at −35°.

Beta-adrenergic receptor binding assay. Binding experiments were performed in medium B. Membranes were at a final concentration of 0.1 mg protein per ml. Nineteen different concentrations of $(-)^{125}$ICYP ranging from 10^{-12} to 6.10^{-11} M, with or without 10^{-6} M propranolol were used in a final volume of 1 ml. The equilibrium was obtained after 75 min at 37°. Reaction was then stopped by immersing all tubes in ice and samples were rapidly filtered under vacuum through Whatman GF/F glass fiber filters. Each filter was washed twice with 10 ml of medium B used as washing buffer. Radioactivity was determined in a scintillation spectrometer Packard Tricarb 460 CD. Specific binding was defined as total bound radioactivity minus the radioactivity not displaced by 10^{-6} M propranolol. This non specific binding was approximately 50% of total binding at K_d value. The two parameters B_{max} and K_d were estimated using a previously published method [21]: it consisted in fitting simultaneously total and non specific binding data. The non-linear regression based upon a uniformly weighted sum of squares was performed using a Gauss Newton iterative minimization method.

Determination of K_I *values.* To determine the efficacy of the various drugs in inhibiting $(-)^{125}$ICYP specific binding, samples were incubated with increasing concentrations of each drug in the medium B. In order to saturate all specific binding sites we used a concentration of $(-)^{125}$ICYP superior to the K_d value determined in the preceding section. Correspondant data were processed according to the general mass action law for competitive inhibition:

$$B = \frac{N_1 F}{K_{d_1} + \left(\frac{K_{d_1}}{K_{I_1}} \cdot I\right) + F} + \frac{N_2 F}{K_{d_2} + \left(\frac{K_{d_2}}{K_{I_2}} \cdot I\right) + F} \quad (1)$$

Where B is the concentration of $(-)^{125}$ICYP bound to the two receptors subtypes, F the concentration of free $(-)^{125}$ICYP and I the concentration of the inhibitor. N_1 and N_2 are the concentrations of the $beta_1$ and the $beta_2$ adrenergic receptors respectively.

In the absence of inhibitor, K_{d_1} and K_{d_2} are the respective dissociation constants of $(-)^{125}$ICYP. In equation (1), $K_{d_1} = K_{d_2}$ as this ligand is devoid of any selectivity for one subtype. When an inhibitor is added, K_{I_1} and K_{I_2} are the respective dissociation constants characterizing its interaction with each receptor subtype on HEM.

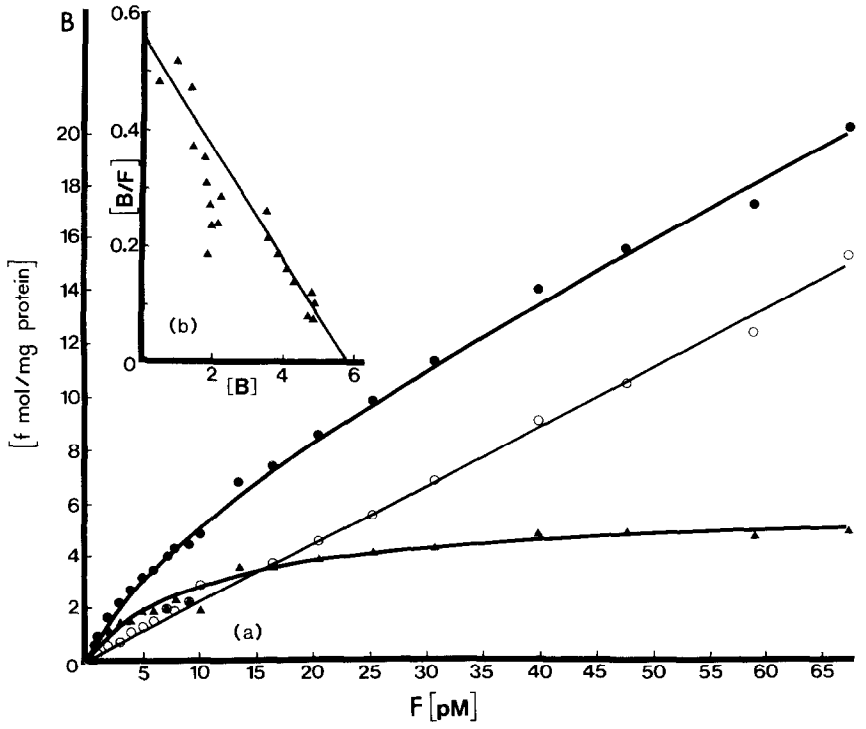

Fig. 1. Equilibrium binding of $(-)^{125}$ICYP to human erythrocyte membranes. (a) Specific binding (▲–▲) was calculated by difference between total (●–●) and non-specific (○–○) bindings in presence of 10^{-6} M (±) propranolol. Binding studies were performed at 37° for 75 min. Each value represents the mean of three experiments. (b) Scatchard plot of the only $(-)^{125}$ICYP specific binding, showing one class of binding sites. B is expressed in fmole/mg protein, $(B)/(F)$ in (ml)/(mg protein).

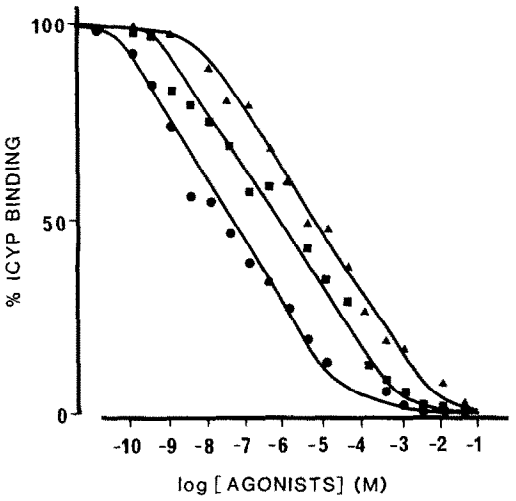

Fig. 2. Inhibition of $(-)^{125}$ICYP specific binding by selected agonists. Binding of $(-)^{125}$ICYP to HEM was measured with increasing concentrations of either (−)isoprenaline (●–●), either (−)epinephrine (■–■) or (−)norepinephrine (▲–▲). $(-)^{125}$ICYP concentration was 8.10^{-12} M ($K_d = 6.10^{-12}$ M for this subject), B_{max} value was 4.27 fmoles/mg protein. The results are represented as percentage of $(-)^{125}$ICYP specifically bound and represent the mean of three experiments.

Parameters N_1, N_2, K_{d_1}, K_{d_2}, K_{I_1} and K_{I_2} were estimated by fitting the equation (1) to the experimental data: a non linear regression technique based upon a Gauss-Newton type minimization algorithm was used.

RESULTS

$(-)^{125}$ICYP binding to human erythrocyte membranes. Typical binding experiments are shown on Fig. 1. Scatchard analysis of the specific binding data gave linear plots suggesting a single class of binding sites, mean K_d value for the 6 subjects was $1.91 \pm 1.44 \times 10^{-11}$ M and the B_{max} was 4.32 ± 1.48 fmoles/mg protein.

Table 1. Dissociation constants (K_I) of selected agonists calculated from competition binding studies to HEM

Drug	K_I (M)
(−)Isoproterenol	2.27×10^{-8}
(−)Epinephrine	4.5×10^{-7}
(−)Norepinephrine	3.2×10^{-6}

The inhibition of $(-)^{125}$ICYP specific binding was determined for each agonist as described in Fig. 2. K_I was calculated from competition binding according to Cheng and Prusoff [22].

Inhibition of $(-)^{125}$ICYP binding by non selective beta-adrenergic agonists. The influence of various adrenergic agonists on specific binding of $(-)^{125}$ICYP to HEM is presented in Fig. 2. The corresponding K_I values are shown in Table 1. The decreasing order of potency was IPR, EPI and NE with a difference of about one order between these three agonists.

Inhibition of $(-)^{125}$ICYP specific binding by selective beta-adrenergic antagonists. (±)Atenolol (Fig. 3a) and (±)metoprolol were being used as $beta_1$ selective antagonists, (±) ICI 118551 (Fig. 3b) and (±) IPS 339 as $beta_2$ selective antagonists. The transformation of the corresponding inhibition curves by Hofstee's representation (Fig. 4) showed two distinct straight lines for each drug indicating the presence of two binding sites, one of high affinity (the first half of the points) and the other of low affinity (the second half of the points) [23].

An interindividual variability of K_I was observed for the 6 subjects (Table 2).

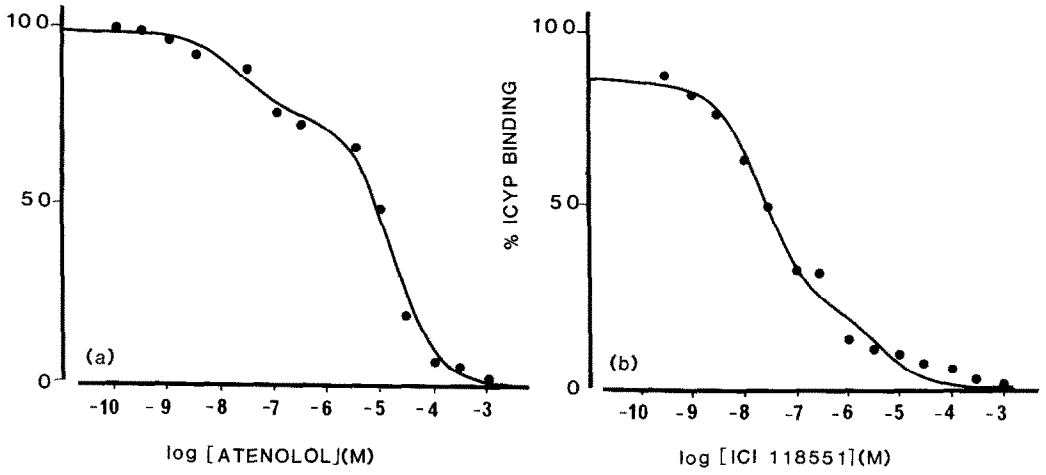

Fig. 3. Inhibition of $(-)^{125}$ICYP specific binding by selective antagonists. Binding of $(-)^{125}$ICYP to HEM was performed with increasing concentrations of either atenolol $beta_1$ selective (a), or ICI 118551, $beta_2$ selective (b). $(-)^{125}$ICYP concentration was 30.10^{-12} M ($K_d = 12.69 \times 10^{-12}$ M) the B_{max} value was 5.12 fmole/mg protein). For the subject N.4 $beta_1/beta_2 = 23/77$ for the displacement with atenolol and 27/73 with ICI 118551. The results are expressed as percentage of $(-)^{125}$ICYP specifically bound and represent the mean of three experiments.

Table 2. K_I of $beta_1$ and $beta_2$ selective drugs for their respective receptors on HEM

	Atenolol		Metoprolol		ICI 118551		IPS 339	
	K_{I_1} (10^{-9} M)	K_{I_2} (10^{-5} M)	K_{I_1} (10^{-9} M)	K_{I_2} (10^{-5} M)	K_{I_1} (10^{-5} M)	K_{I_2} (10^{-9} M)	K_{I_1} (10^{-5} M)	K_{I_2} (10^{-9} M)
1	7.02 ± 4.01	1.45 ± 0.43	ND	ND	14.06 ± 0.29	1.52 ± 0.32	0.09 ± 0.05	0.84 ± 0.20
2	ND	ND	344.71 ± 83.90	7.86 ± 6.17	4.78 ± 3.51	45.98 ± 17.3	ND	ND
3	71.45 ± 25.90	39.39 ± 19.7	ND	ND	1.15 ± 0.20	6.78 ± 1.07	0.95 ± 0.34	11.85 ± 3.40
4	5.72 ± 4.76	0.44 ± 0.07	5.97 ± 2.70	0.161 ± 0.06	0.129 ± 0.10	6.43 ± 2.21	0.45 ± 0.24	1.85 ± 0.95
5	1.25 ± 1.00	2.92 ± 1.10	ND	ND	1.92 ± 1.06	10.11 ± 1.90	0.29 ± 0.05	2.34 ± 0.25
6	ND	ND	26.85 ± 24.50	22.40 ± 9.40	3.58 ± 1.83	28.60 ± 16.7	0.48 ± 0.27	2.73 ± 1.32

The inhibition of $(-)^{125}$ICYP specific binding was determined for each antagonist selected as described in Fig. 3. Computer analysis of the resulting Hofstee plots yielded the apparent K_I value of each drug for each compound on HEM. Each value is the mean of three separate experiments.
(ND means not determined).

Table 3 showed the ratio between the B_{max} characterizing the sites of high affinity and low affinity, which suggests the predominance of $beta_2$ receptor subtype.

DISCUSSION

An indirect method was used to identify the HEM beta adrenoceptors. This technique, also less precise than direct binding experiments was used because $(-)^{125}$ICYP was the only available radioligand. Two unlabelled displacers, respectively $beta_1$ (atenolol, metoprolol) and $beta_2$ antagonists (ICI 118551, IPS 339) were systematically used in order to minimize the non entire selectivities for each subtype. Indeed, the selectivity of most antagonists and of those selected here is always relative. It can be measured by their efficacy, i.e. the ratio of the corresponding association constant ($K_a = 1/K_d$), they exhibit for each subtype:

$$\text{the efficacy } beta_1/beta_2 = \frac{K_{a_1}}{K_{a_2}} \text{ was } 22$$

for metoprolol and 30 for atenolol, was 0.016 for ICI 118551 and 0.04 for IPS 339 (calculations drawn from references 11, 24). Furthermore, as the respective efficacies of selective antagonists of the same subtype are not equivalent, two ligands of each group were used.

For ethical reasons, it was difficult to collect more than 80 ml blood from each volunteer. Thus, when HEM recovery was low it was impossible to check all the displacing agents. For this reason the HEM of each subject was studied systematically in a cross-over manner at least with one $beta_1$ and one $beta_2$ antagonist. Keeping in mind that lymphocytes and granulocytes discarded in the buffy coat by several washings, thus low B_{max} value can be attributed only to HEM. Moreover any contamination would lead to an increase of $beta_2$ adrenergic receptors as it is the only subtype of these cells [14, 15]. So, one may consider that only HEM beta-receptors can account for our results.

Characteristics of HEM beta adrenergic receptors. K_I values obtained with the selected agonists are close to those previously reported by Sager [3]. Thus the presence of beta-adrenergic receptors on HEM with a $beta_2$ subtype tendency is confirmed. When $beta_1$ or $beta_2$-selective antagonists were used, computer analyses of the competition curves clearly indicated the existence of two populations sites. The percentages of low- and high-affinity sites were about the same as for $beta_1$-selective drugs atenolol and metoprolol, i.e. 25–45% for high affinity sites and 55–75% for low-affinity sites (Table 3). Comparison of the affinities of these antagonists for their respective sites with those reported in the literature [23–25], also strongly suggests, that the high-affinity sites correspond to the $beta_1$ receptor subtype and that the low affinity sites correspond to the $beta_2$-receptors. These results were confirmed with the use of $beta_2$ selective antagonists, ICI 118551 and IPS 339. They showed the presence of 67% $beta_2$- and 33% $beta_1$-receptors (Table 3). Thus as expected by Sager [3–5], $beta_2$ subtype is the predominant HEM

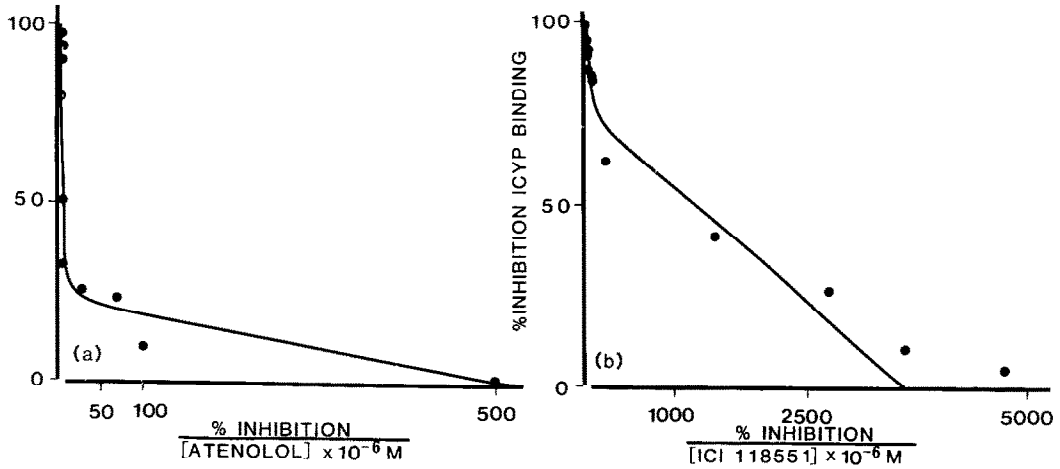

Fig. 4. Hofstee plot of the inhibition of $(-)^{125}$ICYP specific binding by selective antagonists. The amount of competiting antagonist bound (expressed as percentage of specific inhibition of $(-)^{125}$ICYP binding) was plotted on the ordinate. The same value divided by the concentration of competiting drug, was plotted on the abscissa: (a) atenolol added; (b) ICI 118551 added as competiting antagonists. The data represent the mean of three experiments performed on HEM.

adrenergic-receptor. Physiological and pharmacological data suggest that beta-adrenergic receptors can be classified into subtypes that may coexist in the same organ [23, 26–28]. Moreover, it is also proved that distinct subclasses of beta-adrenergic receptors can coexist in a single cell type [29].

In retrospect, it seems somewhat surprising that the majority of such studies have been performed mainly on non mammalian erythrocyte preparations. Initial characterization of the avian and amphibian erythrocyte receptors had suggested that these systems may possess $beta_1$- and $beta_2$-adrenergic receptors respectively. However, it was proved that for instance chick, frog [12] and turkey [10] erythrocyte membranes exhibit receptor characteristics different from those of mammalian $beta_1$ and $beta_2$ adrenergic receptors.

Our results as compared to those reported in different mammalian tissues, are in relatively good accordance. Nevertheless, we observed a high inter-individual variation between the K_I values of the different antagonists. Even if we accept Furchgott's [30] guidelines stating that drug affinities would differ more than 3-fold between membrane preparations, it is significant that K_I values of our subjects deviate from that expected for either receptor subtype in a range from 1 to 100. We have no explanation for these variations. As K_d values of $(-)^{125}$ICYP vary only in a relatively small range between the 6 subjects ($1.91 \pm 1.44\ 10^{-11}$ M), it seems that the large differences observed in the corresponding K_I values for the antagonists, are not relevant to methodological errors but more likely to physiological variation.

The significance of HEM beta-adrenergic receptors. In human erythrocytes, cAMP basal and maximal levels as observed after isoproterenol stimulation, represent respectively accumulations of 90 and 300 molecules per sec and per cell [5]. These variations can probably explain some biological effects as membrane deformation [16].

In rat erythrocytes, during maturation, the guanine nucleotide regulatory protein decreases [31]. This protein acts as a cofactor of beta adrenergic stimulation and is stimulated by guanine nucleotide. Thus the effect of catecholamine stimulation on adenylate cyclase activity decreases with maturation and explains the low activity in mature RBC. These observations suggest a functional role of HEM beta-adrenergic receptors as in other tissues.

$Beta_2$ adrenergic receptors are often considered as

Table 3. Relative concentrations of $beta_1$- and $beta_2$-adrenergic receptors in HEM

Subject No.	Atenolol	Metoprolol	ICI 118551	IPS 339	Mean
1	45/55	ND	15/85	25/75	29/71
2	ND	42/58	20/80	ND	31/69
3	46/54	ND	44/56	32/68	40/60
4	23/77	45/55	27/73	20/80	29/71
5	38/62	ND	26/74	36/64	33/67
6	ND	35/65	50/50	42/58	42/58
					33/67

The inhibition of $(-)^{125}$ICYP specific binding was determined for each antagonist selected as described inf Fig. 3. Computer analysis of the resulting Hofstee plots resulted in the relative percentages of each component on HEM. Each value is the mean of three separate experiments (ND means not determined).

the former subtype specially adapted to epinephrine. During evolution, the progressive transformation from $beta_2$ to $beta_1$ receptors may be the result of an adaptation to noradrenaline [32]. So the ratios of norepinephrine/epinephrine and $beta_1/beta_2$ are often compared [33]. As in human plasma norepinephrine levels, 500 pg/ml, are far higher than epinephrine levels, 50 pg/ml [34], a predominance of $beta_1$ receptors on HEM was expected.

However, the use of those two catecholamine levels seems too simplistic to anticipate the receptor subtypes distribution, since our results show a $beta_1$-$beta_2$ ratio of 33/67. Thus the regulation of erythrocyte beta-adrenoceptors, if any, may be either partially linked to the plasma catecholamines levels or even totally independent. In addition in other circulating cells with beta-adrenoceptors such as lymphocytes and granulocytes, $beta_2$ subtype is predominant [14, 15]. This point needs further investigations.

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REFERENCES

1. G. Sager and S. Jacobsen, *Biochem. Pharmac.* **28**, 2167 (1979).
2. T. Tsukamoto and M. Sonenberg, *J. clin. Invest.* **64**, 534 (1979).
3. G. Sager, *Biochem. Pharmac.* **31**, 99 (1982).
4. G. Sager, S. Noraas, S. Jacobsen, Ø. Stenerud and I. Aakesson, *Biochem. Pharmac.* **32**, 1943, (1983).
5. G. Sager, *Biochem. Pharmac.* **32**, 1946, (1983).
6. T. B. Nielsen, P. M. Lad, M. S. Preston and M. Rodbell, *Biochim. biophys. Acta.* **629**, 143 (1980).
7. C. M. Mendel and R. R. Almon, *Gen. Pharmac.* **10**, 31 (1979).
8. J. P. Bilezikian and D. Aurbach, *J. biol. Chem.* **248**, 5577 (1973).
9. C. Mukherjee and R. J. Lefkowitz, *Molec. Pharmac.* **13**, 291 (1977).
10. K. P. Minneman, G. A. Weiland and P. B. Molinoff, *Molec. Pharmac.* **17**, 1 (1980).
11. K. Dickinson, A. Richardson and S. R. Nahorski, *Molec. Pharmac.* **19**, 194 (1981).
12. K. Dickinson and S. R. Nahorski, *Eur. J. Pharmac.* **94**, 43 (1981).
13. A. De Lean, A. A. Hancock and K. J. Lefkowitz, *Molec. Pharmac.* **21**, 5 (1982).
14. L. T. Williams, R. Snyderman and R. J. Lefkowitz, *J. clin. Invest.* **57**, 149 (1976).
15. S. P. Galant, S. Underwood, L. Duriseti and P. A. Insel, *J. Lab. clin. Med.* **92**, 613 (1978).
16. Y. Yawata, H. S. Jacob, N. Matsumoto and J. White, *J. Lab. clin. Med.* **88**, 555 (1976).
17. A. Bilski, S. Dorries, J. D. Fitzgerald, R. Jessup, H. Tucker and J. Wale, *Br. J. Pharmac.* **69**, 292 P (1980).
18. S. R. O'Donnell and J. C. Wanstall, *Life Sci.* **27**, 671 (1980).
19. J. L. Imbs, F. Miesch, J. Schwartz, J. Velly, G. Leclerc, A. Mann and C. G. Wermuth, *Br. J. Pharmac.* **60**, 352 (1977).
20. O. H. Lowry, H. T. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. R. Zini, I. Gault, S. Ledewyn, Ph. d'Athis and J. P. Tillement, *Biochem. Pharmac.* **32**, 3375 (1983).
22. Y. C. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
23. K. P. Minneman, L. R. Hegstrand and P. B. Molinoff, *Molec. Pharmac.* **16**, 34 (1979).
24. K. P. Minneman, A. Hedberg and P. B. Molinoff, *J. Pharmac. exp. ther.* **211**, 502 (1979).
25. A. Heitz, J. Schwartz and J. Velly, *Br. J. Pharmac.* **80**, 711 (1983).
26. A. Hedberg, K. P. Minneman and P. B. Molinoff, *J. Pharmac. exp. ther.* **212**, 503 (1980).
27. K. P. Minneman and P. B. Molinoff, *Biochem. Pharmac.* **29**, 1317 (1980).
28. P. Robberecht, M. Delhaye, G. Taton, P. De Neef, M. Waelbroeck, J. M. De Smet, J. L. Leclerc, P. Chatelain and J. Christophe, *Molec. Pharmac.* **24**, 169 (1983).
29. V. Homburger, M. Lucas, E. Rosenbaum, G. Vassent and J. Bockaert, *Molec. Pharmac.* **20**, 463 (1981).
30. R. F. Furchgott, in *Catecholamines Handbook of Pharmacology*, (Eds. H. Blaschko and E. Muscholl), Vol. 33, p. 283. Springer, Berlin (1972).
31. L. E. Limbird, D. M. Gill, J. M. Stadel, A. R. Hickey and R. J. Lefkowitz, *J. biol. Chem.* **255**, 1854 (1980).
32. E. J. Ariens and A. M. Simonis in *Receptors and Receptors Mechanisms in β-Adrenoceptor Blocking Agents* (Ed. P. R. Saxena and R. P. Forsyth), p. 3. North Holland, Amsterdam (1976).
33. S. R. Nahorski, *Eur. J. Pharmac.* **51**, 199 (1978).
34. P. Trunet, F. Lhoste, J. C. Ansquer, S. Kestenbaum, C. Sabatier, J. P. Tillement and M. Rapin, *Metabolism* **33**, 101 (1984).