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Human Red Cell Content of Cyclic Nucleotides and Cations upon β-adrenergic Blockade

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ABSTRACT

The content of cyclic nucleotides (cAMP, cGMP) and Mg^{2+}, Zn^{2+}, Na^+ and K^+ was determined in human erythrocytes and after beta-adrenergic blockade in healthy individuals. The figures for cAMP and cGMP were 4.20 and 3.36 nmoles/l intracellular volume, respectively. Only slight effects on the cyclic nucleotide content as well as on cation content were seen upon beta-blockade in the erythrocytes. It is concluded that the single determination of red cell cAMP is not a sufficient indicator of beta-adrenergic blockade. The determination of cAMP in combination with cGMP, Zn^{2+} and Na^+ might reflect a betaadrenergic blockade.

Receptors for many hormones and other transmitters are localized at the outer surface of the plasma membrane. It is not known how combination of the diverse ligands with their receptors can produce vast changes in the function and structure of target cells. An improvement in the efforts to characterize the molecular basis of hormonal action was the detection by Sutherland (13) of the adenylate cyclase system.

The adenylate cyclase system is present in most cells of the human organism (11). This system is responsible for the transformations in cellular metabolism induced by numerous hormones and transmitters. Evidence exists that the adenylate cyclase system is composed of several types of subunits, including hormone receptors, catalytic unit and nucleotide regulatory components that selectively bind GTP. It has long been discussed whether the enzyme system is present in the human erythrocyte membrane. Also in other respects the human red cell may not be regarded as representative of a cell since it lacks typical cell characteristics such as subcellular organelles and therefore also the mitotic cycle. On the other hand, both avian erythrocytes and human leukocytes having these organelles have long been known to contain the adenylate cyclase system. However, it has recently been claimed that adenylate cyclase is also present in human erythrocyte membranes (12).

The aim of the present investigation was therefore to determine the quantity of human red cell cyclic nucleotides, and to study the possible manipulation of the cyclic nucleotides *in vivo* via the receptor subunit. The adrenergic beta-receptor blockers reduce the cyclic nucleotide in their target cells (9). The question then arises whether the human erythrocytes also respond as target cells in this respect. If such were the case it would offer a simple model to indicate the degree of beta-blockade instituted, instead of more circumstantial methods e.g. the inhibition of isoprenaline-induced tachycardia or analysis of tissue biopsy material.

SUBJECT AND METHODS

13 healthy volunteers, 8 males and 5 females, age range 18–50 years, took part in the study. None had known hypertension. Blood samples were drawn on two occasions; initially without any preceding treatment, and after 14 days of treatment with beta-receptor blockers, receiving 40 mg propranolol (Inderal®) b.i.d. orally or an equivalent dose of pindolol (Viskén®) or methoprolol (Seloken®), and all completed the trial. A significant pulse retardation was induced by the beta-adrenergic blockade in all of the volunteers and registered at the end of the trial.

Blood samples were also drawn from 29 normotensive blood donors not given any treatment. The blood was collected in heparinized tubes and immediately chilled in ice-water. The plasma and buffy coat layer were removed and the red cells were washed twice in an isotonic medium containing 130 mM NaCl and 25 mM KCl. For intracellular Na^+ and K^+ determinations the last two washings were instead performed in 0.11 M magnesium chloride. Intracellular volume was determined for the packed red cells by estimation of wet and dry weight and correcting for plasma trapping with the ^{125}J-albumin method as described by Fortier et al. (5).

The packed erythrocytes were haemolysed by adding

Table I. *Human erythrocyte content of cAMP, cGMP, Mg^{2+}, Zn^{2+}, Na^+ and K^+ in two categories of normals*

Figures in brackets denote one standard deviation

	n	cAMP (nmol/l)	cGMP (nmol/l)	Mg^{2+} (mmol/l)	Zn^{2+} (μmol/l)	Na^+ (mmol/l)	K^+ (mmol/l)
Blood donors	29	4.04 (±1.42)	3.32 (±0.47)	1.20 (±0.13)	101.8 (±17.3)	6.9 (±0.9)	107.9 (±5.3)
Volunteers	13	4.43 (±1.09)	3.40 (±0.94)	1.26 (±0.11)	100.7 (±11.7)	6.3 (±0.7)	99.2 (±6.0)

0.5 ml distilled water to 1.5 ml packed cells. Twenty microlitres of saponin solution (2 g of saponin in 10 ml of distilled water) were also added to the blood–water mixture. The solution was mixed thoroughly and rapidly frozen to −20°C in a dry ice–ethanol solution. The frozen material was either thawed immediately or kept frozen for at most 14 days before analysis.

During thawing 0.5 ml of 40% (w/v) trichloroacetic acid (TCA) were added, giving a total volume of 2.5 ml of the haemolysate. The mixture was centrifuged twice and the clear supernatant obtained was analysed as regards cAMP, cGMP, Mg^{2+}, Zn^{2+}, Na^+ and K^+.

The quantity of cyclic nucleotides was determined using a radiometric immunoassay system including an acetylation stage (7). The reagents involved were all purchased in a kit from New England Nuclear Company, Boston, Mass., USA. Mg^{2+} and Zn^{2+} were determined in a lanthanum chloride solution by atomic absorption emission spectrophotometry with an IL 353 instrument with Sr included as internal standard. Na^+ and K^+ analysis were performed by flame photometry.

RESULTS

Table I illustrates that the two categories of normals, i.e. the volunteers before treatment and the blood donors, displayed about the same mean values for both cAMP and cGMP. Thus the mean value for cAMP was 4.43(±1.09), 4.04(±1.42) (nmol/l) and for cGMP 3.40(±0.94), 3.32(±0.47) (nmol/l) for the volunteers and blood donors, respectively. The values for monovalent and divalent cations for the two categories also agreed with each other.

Table II demonstrates the intracellular concentration of cAMP and cGMP in the erythrocytes before and after beta-adrenergic receptor blockade. A slight effect on the cAMP content by the different beta-blockers is seen, and a discrepancy between the intracellular content of cGMP and Na^+ before and after treatment may be discerned. The results of the study concerning the other monovalent and divalent cations in erythrocytes are also given in the table. As is seen, intracellular Mg^{2+} and K^+ are not affected by the beta-blockade. The intracellular Zn^{2+} concentration displayed again a weak tendency to decrease after beta-blockade treatment. Statistically, however, there was no significant difference in any of the parameters studied.

Table II. *Effect of beta-adrenergic blockade on erythrocytic content of cyclic nucleotides and cations*

Figures in parentheses denote one standard deviation

	n	cAMP (nmol/l)	cGMP (nmol/l)	Mg^{2+} (mmol/l)	Zn^{2+} (μmol/l)	Na^+ (mmol/l)	K^+ (mmol/l)
Before treatment	13	4.43 (±1.09)	3.40 (±0.94)	1.26 (±0.11)	100.7 (±11.7)	6.3 (±0.7)	99.2 (±6.0)
After treatment	13	4.93 (±1.19)	3.83 (±0.83)	1.25 (±0.13)	94.7 (±11.7)	7.1 (±1.1)	96.6 (±5.4)
t independent[a]		1.07	1.39	0.23	1.29	1.66	0.79
p		0.15–0.10	0.10–0.05	0.45–0.40	0.15–0.10	0.10–0.05	0.25–0.20

[a] $$\frac{\bar{x}-\bar{y}}{\sqrt{\left[\frac{(n_x-1)\,\text{S.D.}_x{}^2+(n_y-1)\,\text{S.D.}_y{}^2}{n_x+n_y-2}\right]\left[\frac{1}{n_x}+\frac{1}{n_y}\right]}}$$

DISCUSSION

The mature human erythrocyte is a highly differentiated cell and has lost several cellular characteristics during its differentiation at the expense of its haemoglobin-carrying function. Hence, it is unable to undergo mitosis and some receptors at the cell's surface for amino acid transport have disappeared during differentiation to the mature cell (2). Similarly, Kaiser et al. (8) reported on the presence of an adrenergic beta-receptor effector system in the membrane of rat reticulocytes, while during erythrocyte maturation several components of this system were rapidly lost. Consequently, the weak effect in the present study of the beta-receptor blockade on the intra-erythrocytic content of cyclic nucleotides might be explained by defective regulator unit function at the mature human red cell surface. This may depend in turn upon a reduced capacity of the particular receptor on the mature human red cell surface to be redistributed upon hormonal stimulation due for instance to inappropriate receptor density on the mature human erythrocyte, or changed association of the receptor with other molecules such as lipids of varying degrees of saturation and fluidity and submembrane microfilaments.

In this context it is also worth mentioning the possibility of a specific refractoriness of the adenylate cyclase system (cf. ref. 1). This means that a sudden increase in the intracellular level of cyclic AMP is initiated on hormonal stimulation. However, this elevated level soon declines despite the continued presence of the stimulating hormone. Thus, the human erythrocytes might become refractory to the hormone after a possible transient sharp increase ("spiking") in the cyclic AMP level. Such a refractoriness has indeed been observed in various tissues, among them avian erythrocytes (6, 10).

However, Rodan et al. (12) reported a 30–50% increase in the adenylate cyclase activity after epinephrine (15 μg/ml) and norepinephrine (10 μg/ml) stimulation (in an *in vitro* experimental system). However, these hormonal concentrations used exceed manyfold the physiological plasma concentration of these hormones (4). These authors did not report any results with lower hormonal concentrations. Therefore the possibility exists that a rudimentary receptor function is connected with the adenylate cyclase system of the human red cell not reacting under *in vivo* conditions but only reacting at these unusually high concentrations *in vitro*.

One might argue that the duration of beta-blockade treatment was too short and the dosage used too small. However, a clinical response to the beta-blockade was registered in the form of a significant reduction in the pulse rate. Furthermore, in a recent publication (14) it is claimed that the pulse rate regulation is mediated via cyclic AMP in the sino-atrialnode.

We conclude that the determination of cAMP alone in human erythrocytes, although offering an easily available specimen, is not an adequate measure of the tissue response on beta-adrenergic blockade. On the other hand, the determination of erythrocytic cAMP in combination with cGMP, Na^+ and Zn^{2+} may reflect an response on beta-adrenergic blockade.

Of the two divalent cations studied, the Mg^{2+} concentration remained constant, while Zn^{2+} showed a weak tendency to decrease during beta-blockade. However, the material is too small to permit of any definite conclusion. An attractive hypothesis would be that an elevated intracellular Zn^{2+} concentration is in some way connected with hypertension (cf. ref. 15). This could be explained on the basis of Zn^{2+} being an inhibitor of the diesterase (3). This hypothesis would be advantageously tested on hypertensive patients given beta-blockade as a single treatment and simultaneously following the Zn^{2+} concentration in their red cells. Such a study is under progress at present.

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