Effect of cAMP on Peptide Formation in Human Erythrocytes Depending on Cell Age

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We found that exogenous and endogenous cAMP induces changes in the spectrum and amount of intraerythrocytic peptides in human erythrocytes of different age. Activation of cAMP formation with epinephrine leads to the appearance of peptides of the other type and to an increase in their total amount in young cells, while blockade of these β-adrenoreceptors with propranolol eliminates these effects. Inhibition of cAMP phosphodiesterase with imidazole in the absence of hormonal signals elevates the content of longer peptides in erythrocytes compared to the effect of exogenous cAMP. The degree of this elevation depends on erythrocyte age.

Key Words: *erythrocyte; cyclic adenosine monophosphate; adrenoceptors; hemoglobin; peptides*

Peptide bioregulation is one of the most complex and polyfunctional systems in living organisms [7]. Study of the mechanisms of peptide regulation provided the basis for a concept on the existence of a continuous collection of regulatory peptides mediating stimulation or inhibition of all vital processes [8]. Many regulatory effects of peptides and mechanisms of their interaction with cells via various types of receptors were discovered and analyzed [7]. However, the pathways of intracellular formation of peptide compounds are poorly studied. Apart from usual mechanisms of gene expression and synthesis of oligopeptides directly on ribosomes, the existence of hydrolytic production of peptides from functionally active proteins is hypothesized. This pathway seems to be most suitable for the formation of short bioactive peptides [8].

Hemoglobin is a precursor of a great number of bioactive molecules, some of them are probably synthesized in erythrocytes [1]. The process of peptide generation in erythrocytes includes primary specific endopeptidase cleavage followed by degradation of long oligopeptides by exopeptidase [6].

These data prompted us to revise our understanding of erythrocytes as simply organized cells. The presence of functionally active receptors, *e.g.* β-adrenoreceptors, insulin-like, and purine receptors, was demonstrated. Previous studies revealed regulatory mechanisms of erythrocyte apoptosis via activation of cyclooxygenase, generation of prostaglandin E_2, and activation of cationic channels, as well as via primary activation of phospholipase A_2 during erythrocyte compression, formation of platelet-activating factor, degradation of sphingomyelin, and production of ceramide [9].

These facts led us to a hypothesis on the existence of a complex signal system regulating the formation of bioactive peptides in erythrocytes, which plays an important physiological role.

Here we studied the effect of cAMP content in human erythrocytes on the formation of peptides.

MATERIALS AND METHODS

We studied purified erythrocyte fraction of donor human blood divided into young and old cells by the method of serial centrifugation. This methods prevents cell damage, but does not guarantees fraction purity. Therefore, preliminary verification of the obtained fractions was performed by the erythrocyte filterability index and glucose-6-phosphate dehydrogenase activity.

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The obtained erythrocyte fractions were placed into Ringer–Lock medium (1:1). The control samples contained no reactants. For evaluation of the effect of exogenous cAMP, epinephrine and propranolol in a final concentration of 10 μg/ml were added to the samples. Other samples were incubated with imidazole (43 μg/ml) at 37°C for 10 min for blockade of cAMP phosphodiesterase. Moreover, epinephrine solution (10 μg/ml) was added for a short time to the samples containing imidazole (43 μg/ml). cAMP concentration in erythrocytes was also elevated by their incubation with cAMP (100 μg/ml, ICN Biomedicals) at 37°C for 10 min.

For more rapid penetration of cAMP and imidazole into cells and for partial inactivation of reception and, hence, signal pathways, pore in the membrane were formed by adding Triton X-100 in a concentration of 20 μg/ml.

For arresting the action of signal molecules, double volume of physiological saline was added to the samples followed by centrifugation at 600*g* for 10 min. Then, the supernatant was removed and the proteins were precipitated with 10% trichloroacetic acid (TCA, 1:2 TCA-erythrocyte suspension ratio) and centrifugation at 1000*g* for 10 min. In each sample, the content of peptide compounds in the supernatant was measured by the method of Lowry and UV-absorption (254 nm) after adding phosphate buffered saline (pH 7.2) for neutralization of acid in the samples. Optical density was measured on a SF-46 spectrophotometer.

TCA extract (0.5 ml) of each sample of erythrocyte fractions was analyzed by gel-chromatography (8×190 mm column packed with sephadex G-10; 0.1 M phosphate buffer, pH 6.86, as the mobile phase, 0.5 ml/min elution rate). Peptide compounds in the eluate were detected on a SF-46 spectrophotometer at λ=254 nm.

The data were processed statistically using Student *t* test. After testing of the conformity of the experimental samples to normal distribution by Pearson test, calculation of asymmetry coefficient and excess, and comparison of sample variance using Fisher test. In all cases, the empirical samples conformed the normal distribution and sampling variances only little differed (for p=0.05).

RESULTS

Verification of the erythrocyte separation into the fraction of old and young cells showed that the index of filterability in these fractions differed by almost 2 times and activity of glucose-6-phosphate dehydrogenase in the fraction of old cells was lower by 26-27%, which attested to good separation of the initial erythrocyte population to fractions containing primarily young and old cells.

The exposure to high epinephrine concentrations (10 μg/ml) increased the content of peptides in young cells and UV absorption by 50 and 23%, respectively. After addition of both the ligand and antagonist (propranolol), the effect of the ligand was not observed (Table 1).

In the fraction of old erythrocytes, peptide content did not increase after addition of epinephrine in high concentrations, while UV absorption increased by 19% (p<0.01); comparison of all samples revealed a tendency similar to changes observed in young cells. This can be explained by impairment of metabolic processes in cells and disturbances in membrane receptors [6,7].

We previously compared the chromatograms of peptides isolated from erythrocytes and peptides obtained after incubation of hemoglobin with pepsin and proved hemoglobin origin of peptide compounds in erythrocytes [2,3].

TABLE 1. Content of Peptide Compounds and UV Absorption of TCA-Extracts of Erythrocyte Fractions ($M\pm m$; n=10)

Preparation	Young cells		Old cells	
	UV-absorption, arb. units	peptide content, μg/ml	UV-absorption, arb. units	peptide content, μg/ml
Control	400±16	200±11	386±15	220±14
Epinephrine	492±10***	300±15***	458±15**	234±16
Propranolol	410±12	198±26	384±16	210±20
Epinephrine+propranolol	437±14	240±26	422±18	232±15
Imidazole	460±17**	260±14**	474±15**	250±12
Epinephrine+imidazole	535±14***	357±17***	489±15***	285±12**

Note. Arb. units=optical density×10^3. **p<0.01, ***p<0.001 compared to the control.

These findings suggest that the processes of hydrolytic cleavage of hemoglobin can be activated by cAMP via cAMP-dependent protein kinases. However, incubation of erythrocytes with exogenous cAMP led to an increase in UV-absorption of the extracts from young and old cells by 66 and 34%, respectively ($p<0.01$). Measuring of peptide content by the method of Lowry did not detect the increase in the concentration of peptide compounds, which indirectly attested to the formation of shorter peptides in case of extracellular influence of cAMP. This agrees with previous report [6] on shortening of the peptides released from erythrocytes into the incubation medium.

The increase in cAMP concentration due to blockade of cAMP phosphodiesterase with imidazole (43 μg/ml) was accompanied by an increase in both UV absorption of erythrocyte extracts and content of peptides measured by the method of Lowry. Peptide content in the fractions of young and old erythrocytes increased by 30 and 14% and UV absorption increased by 15 and 23%, respectively ($p<0.01$).

Addition of epinephrine to samples containing imidazole increased both the content of peptide compounds and UV absorption. In young cells, peptide content increased by 37% compared to samples containing imidazole alone and by 19% compared to samples containing epinephrine alone ($p<0.01$).

Gel-chromatography of peptide compounds obtained after activation of adrenoreception and elevation of intracellular cAMP concentration confirmed these findings. Changes in the peptide spectrum in response to hormonal stimulus were accompanied by their considerable accumulation in cells (Fig. 1). An additional peak was detected in samples incubated with epinephrine. The second peak typical of all samples attested to an increase in UV absorption in samples with increased intracellular cAMP concentration by 24, 36, and 57%, respectively ($p<0.001$), compared to the effects of imidazole and epinephrine individually and in combination (Fig. 1).

Accelerated production of peptide compounds by erythrocytes under conditions of activation of adrenoreception as well as changes in their spectrum can attest to the existence of a complex mechanism of regulation of hydrolytic cleavage of hemoglobin in erythrocytes and in cell membrane. Further studies of this phenomenon, analysis of the spectrum of peptides produced by erythrocytes under conditions of activation and blockade of various signal pathways, and evaluation of their biological activity are of great theoretical and practical importance.

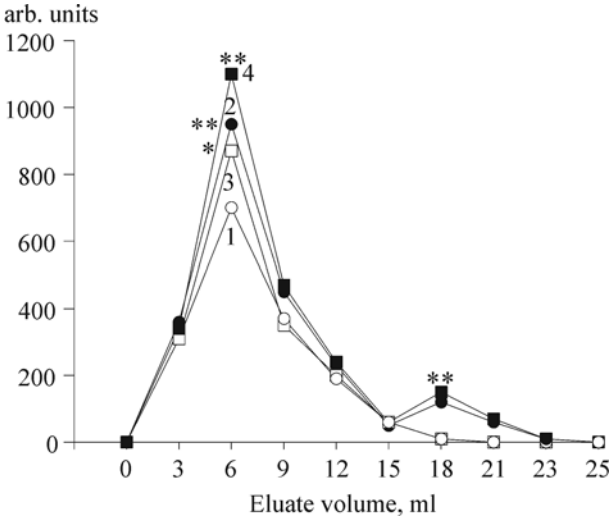

Fig. 1. Gel–chromatography of peptide extracts from the fraction of young erythrocytes after incubation with epinephrine and imidazole. *1*) control, *2*) epinephrine, *3*) imidazole, *4*) epinephrine+imidazole. *$p<0.01$, **$p<0.001$ compared to the control.

REFERENCES

1. A. A. Karelin, M. M. Filippova, and O. N. Yatskin, *Bioorg. Khimiya*, **24**, No. 4, 271-281 (1998).
2. N. A. Klenova and R. O. Klenov, *Bulletin of Samara State University, Natural Science Series. Biology*, No. 7, 75-79 (2006).
3. N. A. Klenova and R. O. Klenov, *Estestvoznanie Gumanism*, **3**, No. 4, 16-17 (2006).
4. E. V. Kolobova, S. A. Dvoryanskii, V. I. Tsyrkin, *et al., Fiziol. Cheloveka*, **24**, No. 3, 134-142 (1998).
5. G. P. Makshanova and I. M. Ust'yantseva, *Ibid*., **29**, No. 1, 95-99 (2003).
6. M. M. Filippova, A. A. Karelin, O. N. Yatskin, *et al., Bioorg. Khimiya*, **34**, No. 2, 160-170 (2008).
7. L. K. Shataeva, V. Kh. Khavinson, and I. Yu. Ryadnova, *Peptide Self-Regulation of Living Systems (Facts and Hypothesis)* [in Russian], St. Petersburg (2003).
8. V. T. Ivanov, A. A. Karelin, M. M. Filippova, *et al*., *Biopolimers*, **43**, No. 2, 171-188 (1997).
9. F. Lang, K. S. Lang, P. A. Lang, *et al*., *Acta Physiol. (Oxf.)*, **187**, Nos. 1-2, 191-199 (2006).