BIOMEDICAL ASPECTS OF Ca2+/CAMP REGULATION OF SIGNAL SYSTEMS BY PHYTOESTROGEN FERUTININ

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Estrogen deficiency in women in the climacteric period leads to the development of a number of diseases associated with lowering the level of Ca^{2+},in particular cardiovascular diseases and postmenopausal osteoporosis. Estrogen therapy leads to some normalization of the hormonal status and correction of the pathological condition, however, there are a number of limitations to the use of estrogen in HRT associated with the risk of breast cancer. Despite the long-term practical application of ferutinin in medical practice, the mechanism of its action at the cellular and molecular level remains insufficiently studied. At the same time, it is known that pharmacological preparations possess cellular specificity and their effects largely depend on protein-lipid composition, a set of specific receptors and specific regulatory mechanisms in specific types of cells. At the same time, it was shown at BLM that ferutinin exhibits activity of electrogenic Ca^{2+}-ionophore and causes an increase in the concentration of cytosolic calcium in different types of cells - thymocytes, neurons, hepatocytes and T-lymphocytes presumably realizing the Ca^{2+}-ionophore properties.

Objective of research. The aim of this work is investigation biomedical aspects of Ca2+/camp regulation of signal systems by phytoestrogen ferutinin

Method of research. Ferutinin used in this study was isolated from *Ferula tenuisecta* (Institute of Plant Substances Chemistry, Academy of Sciences of Uzbekistan).The study used donor human blood from middle-aged men (25-35 years old), the aortic fragments were isolated from outbred white rats. ***Isolation of platelets and determination of cytosolic calcium content by spectrofluorimetric method.*** Whole blood in a volume of 12 ml was collected in plastic tubes containingas an anticoagulant 0.11 M sodium citrate 9:1, with 1 μMPGE$_1$ and centrifuged at 190 g for 12 min at 37°C. Platelet load in enriched plasma of Ca^{2+}-sensitive probe FURA-2AM 2μM:4ml was carried out at 37°C in the dark for 30 minutes. 1μM PGE$_1$ was added to the plasma and centrifuged again at 2000 g for 12 min at 37 ° C. The cells were resuspended in 4 ml of Tyrode buffer containing 134 μMNaCl; 12 μM $NaHCO_3$; 2.9 μMKCl; 0.34 μM Na2HPO$_4$; 1 mM $MgCl_2$; 10 mM HEPES; 5mM glucose, pH = 4.5. The number of cells was determined spectrophotometrically at A_{800} against Tyrode buffer to a final concentration of 3×10^4. Fluorescence registration of cells loaded with FURA-2AM was performed at 37°C atexcitation wavelengths of 340/380nm and fluorescence at 490nm. The data

obtained with the help of the Ca^{2+}-sensitive probe Fura-2AM are presented as a ratio of fluorescence intensities excited at 340 nm and 380 nm (F_{340}/F_{380}). The measurements were carried out on a LS50B spectrofluorimeter (Perkin Elmer, UK).
Results. Aggregation of platelets causes a large number of stimulating agonists, in this connection, we estimated the platelet aggregation level in human whole blood in the presence of a classical inducer of 5μM ADP, which was 90.1±2.5 ref. units of aggregation and accepted for comparison control. However, despite a significant increase in the content of intra-platelet Ca^{2+}, induced by ferutinin, the level of platelet aggregation was insignificant and was expressed in 8.3 ±0.6 ref. units ofaggregation.The next stage of our research was the study of the effect of ferutinin on the activation of adenylate cyclase in rat's heart membrane preparations. The activation of adenylate cyclase with ferutinin was judged by the formation of [α-32P] cAMP. We found that ferutinin in the concentration range (50-100 μM) causes activation of adenylate cyclase in a dose-dependent manner. Thus, at a concentration of 50 μM, ferutinin caused activation of adenylate cyclase by 29.7% ± 6.8, with respect to the basal level, at 75 μMferutinin-induced activation of adenylate cyclase was 174.9% ± 11.0. The maximum activation of adenylyl cyclase induced by ferutininwas observed at a concentration of 100 μM and was 257.0% ± 41.5 with respect to the control.
Conclusion. Based on the results obtained, we assume that the inhibition of platelet aggregation in high ferutinin-induced intracellular mobilization of Ca^{2+}in platelets is due to estrogen-mediated activation of adenylate cyclase in platelets, since in intact cells, the realization of the effects of estrogens is multifactorial in nature involving both genomic and non-genomic intracellular processes, including effects on calcium homeostasis, activation of adenylate cyclase. A wide spectrum of biological effects of ferutinin is associated both with the realization of its estrogenic activity and with Ca^{2+}-ionophore properties. The obtained results supplement available representations about the mechanism of action applied in preparations of plant origin on the basis offerutinin.