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GPCR-Peptides: Prospective Use in Biology and Medicine

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The superfamily of G Protein-coupled Receptors (GPCRs) comprises a majority of cell surface receptors capable of binding various signal molecules, including amino acids and their derivatives, nucleotides, peptides, proteins and odorants. GPCRs play a pivotal role in the regulation of cell growth, differentiation, metabolism, motility, communication, and many other biochemical and physiological events [1-3]. They share the same topology and contain seven helical Transmembrane regions (TM) forming transmembrane channel, extracellular N-terminal Domain (NTD), intracellular C-terminal Domain (CTD), three Extracellular (ECLs) and three intracellular loops (ICLs). The membrane-proximal regions of ICLs interact with heterotrimeric G proteins and β-arrestins, both responsible for activation of the enzymes generating the second messengers and G protein-gated ionic channels. In a majority of GPCRs the second and third ICLs are involved in the interaction with G-proteins and the third ICL and CTD with β-arrestins. ECLs, primarily the second ECL, are responsible for the recognition of ligand and are involved in the formation of high affinity orthosteric site located in the transmembrane channel of receptor.

The pharmacological action of over 40% of the currently used drugs is carried out through GPCRs [4,5]. This is due to the fact that GPCRs and intracellular signaling cascades they regulate contribute to a large number of diseases, including widespread endocrine dysfunctions such as metabolic syndrome, obesity and diabetes mellitus [6,7]. As a consequence, the development of new selective and effective regulators of GPCRs is one of the actual problems of molecular endocrinology and biochemistry.

In the last two decades the evidence was obtained that the synthetic peptides corresponding to functionally important regions of ICLs and ECLs of GPCRs can affect *in vitro* and *in vivo* the activity of cognate receptors and signaling pathways regulated by them [8-14]. The biological activity of GPCR-peptides depends on the integrity of molecular determinants in the primary structure which are responsible for ligand recognition and binding in the case of ECL-peptides and for interaction with G proteins and β-arrestins in the case of ICL-peptides, and on the similarity of three dimensional structure of GPCR-peptide and the region homologous to them in the cognate receptor. Since ligand-binding regions of ECLs and G protein- and β-arrestin-interacting regions of ICLs are located primarily in the membrane-proximal segments of these loops, their conformation in the full-size receptor is stabilized by the adjacent hydrophobic TM and by the interaction between N- and C-terminal segments of loops. Due to this, the modification of GPCR-peptides by TM segments or hydrophobic radicals simulating TM, as well as the design of cyclic forms of GPCR-peptides mimicking the conformation of loops in native receptor allow a significant increase of their effectiveness [15-17]. Note that ICL-peptides modified by TM segments of about 2/3 of the entire TM in length or by fatty acid radicals with physicochemical properties similar to these segments are capable of penetrating the plasma membrane and interacting with the intracellular targets, i.e. intracellular regions of the cognate GPCR, and receptor-interacting regions of G protein α subunits. It was found that ICL-peptides modified by hydrophobic radicals, primarily palmitoyl radical, discovered by Covic et al. [9] in 2002 and designated as pepducins [9], selectively influence the transduction of hormonal signal via homologous GPCR, acting as intracellular agonists and antagonists, and are also capable of triggering the appropriate cell response in the absence of hormonal stimulus [18-24]. As the main molecular mechanism of action of pepducins includes their interaction with complementary regions of the cognate GPCR, the regulatory effects of pepducins are receptor specific; they have no influence even on the closely related receptors and are active only in the tissues where there are receptors homologous to them [25,26].

Pepducins derived from Protease-activated Receptors (PAR) of the types 1, 2 and 4 have influence on platelet aggregation, inflammation, angiogenesis, cancer and metastasis [27]. The drug PZ-128 developed on the basis of pepducin P1pal-7, a derivative of N-terminal region of the third ICL of PAR1, and found to inhibit significantly PAR1-mediated platelet aggregation and arterial thrombosis in guinea pigs and monkeys can be a good alternative to the low-molecular PAR1-antagonists used to treat arterial thrombosis [27]. The data was obtained that PAR1-derived pepducins suppress the tumor growth and metastasis, and reduce the cell viability in breast, ovarian and lung carcinoma cells [28]. The antitumor effect of PAR-derived pepducins comprises the increase of apoptosis of malignant cells, the inhibition of platelet aggregation and chemotaxis, and the suppression of tumor angiogenesis, which leads to a decrease of tumor survival and growth. It was shown that the PZ-128, alone and in combination with Taxotere, an anti-cancer chemotherapy drug, inhibited growth of breast tumor xenografts and their metastasis to the lung [28]. This pepducin, in addition, significantly reduced PAR-mediated migration of lung cancer cells isolated from patients with lung tumors and decreased lung tumor growth by 75 %. Its effectiveness was comparable with that of monoclonal VEGF-directed antibody drug Bevacizumab, an antitumor agent and angiogenesis inhibitor [29,30]. Pepducins corresponding to ICLs of the chemokine receptors CXCR1, CXCR2 and CXCR4, like those mentioned above, possessed potent antitumor activity [23,24,31,32]. CXCR4-derived pepducins both in monotherapy and in combination with rituximab, the monoclonal antibody to B-cell-associated antigen CD20, effectively suppressed the survival and metastasis of disseminated lymphoma xenografts, which can be used as basis of a new treatment strategy for lymphoid malignancies [24].

PAR2-derived pepducins influenced acute pancreatitis [33]. Possessing antagonistic activity, pepducin P2pal-18S, a derivative of the third ICL of PAR2, protected acinar cells against injury induced by bile acid *in vitro* and reduced the severity of experimental biliary pancreatitis in mice when administered before or 2 h after bile acid infusion *in vivo*. It follows that PAR2-derived pepducins may be successfully used in the clinical management of patients at risk for developing acute biliary pancreatitis.

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Many data are available now on ICL-peptides as regulators and modulators of functioning of endocrine system in the *in vitro* and *in vivo* conditions [19-22,34-38]. C-palmitoylated pepducin 612-627-K(Pal)A corresponding to C-terminal part of the third ICL of thyroid-stimulating hormone (TSH) receptor *in vitro* stimulated the basal activity of adenylyl cyclase and GTP-binding capacity of G_s-proteins, the components of TSH-sensitive adenylyl cyclase signaling system, and reduced the stimulating effects of TSH on this system in the thyroidal membranes [22]. The 5-days treatment of rats with intranasally administered 612-627-K(Pal)A resulted in a significant and sustained increase of free T_4 level and a decrease of TSH level [37]. These data furnish evidence that TSH receptor-derived pepducins can be used to develop new regulators of the thyroid functions.

GPCR-peptides, the derivatives of ECLs, are a promising tool for the study of pathogenesis of diseases induced by autoantibodies against extracellular regions of GPCR, such as cardiomyopathy, complex regional pain syndrome, chronic Chagas' disease, cognitive dysfunctions, Sjögren's syndrome [39-41]. The treatment of rats with peptide corresponding to the NTD of melanocortin receptor (MCR) of the type 4, led to the increase of food intake, weight gain, and insulin and triglycerides levels in the blood plasma, like in the case of blockade of hypothalamic MCRs [42]. In rats treated with peptide, a derivative of the third ICL of type 3 MCR, possessing MCR3-antagonistic activity there was the increase of body weight, elevated blood pressure, decreased locomotor activity, the increase of triglycerides, insulin, and leptin levels, like in the case of metabolic syndrome and type 2 diabetes mellitus [43]. ECL-peptides can also be used to inhibit binding of infectious agents, such as viruses, to the cell membrane. Peptide 1-27 corresponding to the NTD of GPR1 receptor and the antibodies against the peptide blocked the infection induced by HIV-1 that uses GPR1 as a co-receptor [44]. It is assumed that the administration of ECL-peptides causes not only the production of antibodies with antiviral activity, but also inhibits in a competitive manner the interaction of the virus with GPCR. The specific binding between ECL-peptides and a large number of chemicals can be the basis for a search of highly selective GPCR ligands [13,45,46]. The study of conformational change in ECL-peptides is carried out by easily accessible spectroscopic methods; therefore the screening of the ligands can be automated.

The progress achieved in the development of GPCR-peptides opens up prospects for their wide application, on the one hand, in medicine as drugs to treat endocrine, cardiovascular and other diseases, and on the other hand, in theoretical biology as functional probes to study the structural-functional organization of the hormonal signaling systems and molecular mechanisms of interactions of GPCRs with ligands and the downstream regulatory and adaptor proteins. Multiple modifications of GPCR-peptides can change their specific biological activity and influence their selectivity, efficiency, bioavailability and stability, which allow unlimited expanding of the area of the use of peptides. It should be noted that at present intensive studies are carried out on the peptides derived from receptor tyrosine kinases, G-proteins, enzymes generating the second messengers, and other signal proteins [12].

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