The peptide strategy has in recent years been one of the most intensively developed directions in molecular endocrinology, biochemistry, and physiology and is widely used for studies of the molecular mechanisms of hormone signal transmission in cells and for creating highly selective and highly efficient regulators of hormone signal systems. The main tasks of the peptide strategy are to find and develop peptides whose primary structures correspond to functionally significant regions of signal proteins. Peptides derived from the extracellular loops of receptors of the serpentine type have evoked extensive interest in recent years. These have been used to study the molecular bases of the interactions of receptors with ligands, fundamentally new approaches for the creation and testing of highly selective agonists and antagonists, and analysis of the etiology and pathogenesis of human and animal diseases associated with the development of autoimmune responses to extracellular receptor loops. Peptides corresponding to the extracellular loops of receptors and antibodies specific to them have been shown to regulate the activity of hormone signal systems in in vitro and in vivo conditions and can be regarded as functional probes for studies of physiological functions in health and pathology. This review summarizes and analyzes data obtained in recent years on the structures, functions, mechanisms of action, and applications of peptides derived from the extracellular loops of serpentine-type receptors. The potential of using these agents in basic biology and applied medicine is discussed.

Keywords: antigen, extracellular loops of receptors, cardiomyopathy, peptide, serpentine-type receptors.

The peptide strategy has in recent years been one of the most intensively developed directions in molecular endocrinology, biochemistry, and physiology and is widely used for studies of the molecular mechanisms of hormone signal transmission in cells and for creating highly selective and highly efficient regulators of hormone signal systems. The main tasks of the peptide strategy are to find and develop peptides whose primary structures correspond to the functionally significant regions of signal proteins – hormone receptors, heterotrimeric G-proteins, enzymes generating second messengers, and adaptor proteins. These signal proteins mediate the specific binding of hormone molecules, the transmembrane transmission of the signals generated to intracellular effector proteins, and transformation of the signal to an appropriate cellular response. Along with peptides which precisely repeat regions of the primary structure of signal proteins, their derivatives have been synthesized with substitution of amino acid residues and containing additional membrane-active sequences mediating the efficient transport of the peptide across the membrane, including hydrophobic radicals or regions of transmembrane domains. Peptides have been used to create cyclic and branched constructs, as well as chimeric molecules including regions from different signal proteins.

Most studies of hormonal signal systems using the peptide strategy approach have addressed heterotrimeric G-protein-linked serpentine-type receptors which cross the membrane seven times. Hundreds of chemically different signal molecules act on cells via these receptors, controlling basic cellular processes such as growth, metabolism, differentiation, apoptosis, and mobility. Serpentine-type receptors appeared in prokaryotic organisms (bacterial rhodopsin), are
widely distributed in single-celled eukaryotes, and developed to structural-functional diversity in higher eukaryotes [7, 65]. These receptors are transmembrane proteins with seven hydrophobic helical domains penetrating the plasma membrane and forming a transmembrane channel, within which most receptors contain a ligand-binding site. The N-terminal domains of serpentine-type receptors are located in the extracellular space and in many cases, such as in receptors for hypophyseal glycoprotein hormones, may include several hundred amino acid residues; three intracellular loops are also extracellular, take part in the specific binding of signal molecules, and are responsible for the immunogenic properties of receptor molecules. The C-terminal domains of serpentine-type receptors are located in the intracellular space, along with three cytoplasmic loops which take part in the binding and activation of heterotrimeric G-proteins and play a key role in transmitting signals from the hormone-activated receptor to the intracellular signal cascades [1, 2, 42, 46, 61, 86].

Most attention in studies of serpentine-type receptors using the peptide strategy has traditionally focused on the cytoplasmic loops and intracellular C-terminal domains and the adjacent parts of the transmembrane channel [4, 5, 62, 64, 74, 80]. As we and other authors have demonstrated, synthetic peptides whose primary structures correspond to regions of the cytoplasmic domains of receptor and their interfaces with the transmembrane areas can, in the absence of a hormonal stimulus activate heterotrimeric G-proteins and trigger the signal cascade mediated by the homologous receptor [6, 8, 9, 16, 24, 63, 66, 67]. It has also been demonstrated that these peptides highly selectively and efficiently affect transmission of the hormone signal via the receptors from which they are derived, acting as agonists and antagonists of the hormone signal pathways, activity being seen not only in vitro, but also in vivo [9, 16, 63, 66]. Existing data indicate that peptides derived from the cytoplasmic parts of receptors can be used to create fundamentally new intracellular regulators of hormone systems, which find wide use both in basic biochemistry and physiology and in applied medicine. The potential for using these peptides in medicine is evidenced by early successes in their application to the treatment of diseases associated with deficiencies in blood clotting, for controlling arterial blood pressure, and in various types of oncological disease [22, 43, 74].

However, attention in recent years has increasingly been directed to using the peptide strategy to study the initial stages of signal transduction, including the recognition and specific binding of hormone molecules by receptors, and using synthetic peptides derived from the extracellular regions of receptors and their adjacent interfaces with the transmembrane channels to clarify the etiology and pathogenesis of diseases associated with the production of antibodies to the antigenic determinants of the receptors. These determinants are located at specific loci of the extracellular loops of receptor molecules [53]. Data have been obtained showing that peptides derived from the extracellular loops of chemokine receptors can be used to block the binding of infectious agents, including human immunodeficiency virus, with cell membranes, preventing them from infecting the body [47, 84]. This review is the first attempt to generalize and systematize the results of investigations and applications of synthetic peptides derived from the extracellular loops of serpentine-type receptors and to assess the potential for their use in basic biology and medicine.

Use of Peptides in Studies of the Spatial Organization of the Extracellular Loops of Receptors

Studies of the molecular mechanisms of the specific binding of hormones and hormone-like substances with receptors and identification of the molecular determinants responsible for this process represent one of the key problems in contemporary molecular endocrinology and pharmacology and are required for the creation of highly selective and effective regulators of biochemical and physiological processes in the body. A significant contribution to resolving this problem has been made by recent studies of the structural organization of peptides corresponding to areas of the extracellular loops of receptors and constructs based on them.

Investigations of the structural organization of the second and third extracellular loops of rhodopsin from the bacterium Halobacterium halobium, which is a member of the most ancient family of serpentine-type receptors, were undertaken by synthesizing the peptides GALTKVYSYRFWVA119–133 and SAYPVVWLIGSEGAGIVPLNIE177–200, whose structures correspond to these loops; the secondary structures of these peptides were determined by nuclear magnetic resonance [36]. This established that both peptides formed U-shaped structures with two short helices corresponding to the N- and C-terminal segments of the peptides, connected together by a β-type turn. These results were subsequently fully confirmed by studies of bacterial rhodopsin by x-ray structural analysis [73]. The consistency of the results obtained with synthetic peptides derived from relatively short parts of receptor proteins with those obtained by x-ray structural analysis using full-size intact proteins is of great importance from several points of view. Firstly, it is evidence that the amino acid sequences in the extracellular loop completely encode the spatial structure, which thus either depends weakly or does not depend at all on the structural organization of the other domains of the receptor. Consequently, synthetic peptides whose structures correspond to extracellular loops significantly mimic their biologically active conformations in the full-size protein. Secondly, the programmability of spatial structure in the amino acid sequences of relatively small structural units of receptors, i.e., extracellular loops, is an important factor determining the correct packaging of receptor molecules in the plasma membrane. Thirdly, x-ray structural analysis is difficult or even impossible for most serpentine-type receptors. As a result, the good correspondence between the structure obtained using the peptide strategy and