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New Approaches for G-protein Coupled Receptor Ligands Identification

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Abstract

Heimann A, Ferro E. New Approaches for G-protein Coupled Receptor Ligands I0dentification. Annu Rev Biomed Sci 2009;11:T95-T101. G protein-coupled receptors (GPCRs) represent the class of proteins with the highest impact from social, therapeutic and economic point of view. Today, more than 50% of drug targets are based on GPCRs and the annual worldwide sales exceeds 50 billion dollars. GPCRs are involved in all major diseases areas such as cardiovascular, metabolic, neurodegenerative, psychiatric, cancer and infectious diseases. The classical drug discovery process has relied on screening compounds, which interact favorably with the GPCR of interest followed by further chemical engineering as a mean of improving efficacy and selectivity. On the other hand, several new peptides with potential bioactivity are discovery every year. This review will focus on recent advancement on methods for identification of novel peptides with potential ability to activate GPCRs.

Keywords: G-protein coupled receptors, peptides, intracellular signaling, high-throughoutput screening, drug discovery.

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1. Introduction

In essence, the high-throughoutput screening (HTS) is used as a brute-force approach to collect a large amount of experimental data - usually observations about how some biological entity reacts when exposed to a variety of chemical compounds -in a relatively short time. For the past few years many approaches have been applied to study compounds effects in cellular disease-modeling systems. Recent scientific and technological advances have introduced new paradigms for drug discovery research. The availability of chemical libraries and robotic systems for bioassay allow synthesis and testing of hundreds or even thousands of compounds in a single day. These developments present great challenges and opportunities for assay automation and data analysis. As advances in molecular biology and bioinformatics identify more potential biochemical targets and combinatorial libraries provide a large number of compounds for testing, it is clear that methods for efficiently optimizing biochemical assays are of great importance. The vast amount of data that becomes available when libraries are tested against an array of molecular targets creates new opportunities for structure-activity relationship analysis and amplifies the need for effective statistical methods to ensure the integrity of the data and identify trends and relationships in the data.

2. G-protein Coupled Receptors as Drug Target

G protein-coupled receptors (GPCRs) represent the class of proteins with the highest impact from social, therapeutic and economic point of view. The GPCRs are divided into at least six families of GPCRs showing little or no sequence similarity: Class A Rhodopsin like, Class B Secretin like, Class C Metabotropic glutamate/pheromone, Class D Fungal pheromone, Class E cAMP receptors (Dictyostelium), Putative families, Orphans, Non-GPCR families: Class Z Bacteriorhodopsins.

Today, more than 50% of drug targets are based on GPCRs and the annual worldwide sales exceeds 50 billion dollars. GPCRs are involved in all major diseases areas such as cardiovascular, metabolic, neurodegenerative, psychiatric, cancer and infectious diseases (Menzaghi *et al.*, 2002). The classical drug discovery process has relied on screening compounds, which interact favorably with the GPCR of interest followed by further chemical engineering as a mean of improving efficacy and selectivity.

3. Antibody-Base Approaches to Screen for Drugs

Antibodies have proven to be exquisite investigation tools in the field of life sciences. They also constitute to one of the oldest and most successful biological products for diagnostics and therapeutics (Bohen *et al.,* 2003). In this context, technologies include ELISA (enzyme linked immunoassay) based assays, Western blot based assays, flow cytometry and confocal fluorescence microscopy analysis. The biggest advantage of using GPCR conformation-specific antibodies for screening is that it is possible to combine the specificity of the antibody for the receptor and the ability of the antibody to recognize the active state of the receptor. Using these antibody characteristics it is possible to screen for allosteric modulators of heterodimers and GPCR regulators (Gupta *et al.*, 2007; Gupta *et al.*, 2008). For an example, using anti-mu opioid conformation-specific antibody it is possible to identify the effect of several CB1 agonist on the agonist induced activation of mu opioid receptor (Gupta *et al*., 2008). The increase of DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin), a synthetic opioid peptide with high μ-opioid receptor specificity, activation response induced by HU-210 (1,1-Dimethylheptyl-11-hydroxy-tetrahydrocannabinol), a synthetic cannabinoid antagonist, and WIN 55,212-2 ((R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone) a potent cannabinoid receptor agonist, suggests an interaction between the cannabinoid and opioid receptors (Rios *et al.,* 2006). Although morphine is the drug of choice for the treatment of pain, its use is limited

by severe side effects. Thus, a major research focus has been to identify brain regions and molecular partners that could play critical roles in the development of major side effects such as tolerance and physical dependence. However, no definite model has emerged that could be used to design a new category of drugs as powerful as morphine but with less abuse potential. This is partly due to difficulties in distinguishing the brain regions that are targeted by a drug, which depend on the route of administration, dose of the drug and its bioavailability. The possibility of finding drugs combination (allosteric modulators) that allows morphine to have its maximum effect in a small dosage opens a new perspective to pain treatment. In this context, the use of GPCR conformation-specific antibody is a unique tool, because it is the only way to distinguish specific receptor activation in complex combination of receptors environment.

4. Antibody-Base Approaches as Drugs Themselves

Over the past decade, antibodies, mainly used for diagnostic and as research reagents, have been developed into effective therapeutic agents with broad applications in cancer, inflammation and infectious diseases. The first FDA-approved therapeutic monoclonal antibody was a murine IgG2a CD3 specific transplant rejection drug, OKT3 (also called muromonab) in 1986 (Emmons *et al.*, 1987). This drug found use in solid organ transplant recipients who became steroid resistant. Hundreds of therapies are undergoing clinical trials. Most are concerned with immunological and oncological targets. Up to now, there are 22 monoclonal antibodies approved for clinical use, more than 70 candidates in late stage clinical trials, and greater than 1,000 in preclinical development (for review, see Stockwin & Holmes, 2003). The majority of these antibody therapeutic candidates are derived from classical hybridoma technology. To reduce immunogenicity in humans, these mouse monoclonal antibodies are often humanized (Alegre *et al.*, 1992; Hsu *et al.*, 1999; Zhu *et al.*, 2009). The development of human antibody-based phage display libraries and human immunoglobulin transgenic mice has allowed the discovery of human antibodies (Hudson & Souriau, 2001; Kotlan & Glassy, 2009).

Protein-based drugs offer unique advantages over small-molecule drugs in terms of both discovery and therapeutic use. The advent of recombinant DNA technology enabled the production of recombinant proteins and the generation of partially or fully human monoclonal antibodies, and continued developments in molecular biology have provided powerful approaches to generate improved proteins with more drug-like features.

Antibodies are of crucial importance to the body's immune system. They are proteins, which have the ability to specifically recognize and bind to foreign, and potentially toxic, molecules or pathogens such as bacteria or viruses. Antibodies are produced naturally by a type of white blood cell known as B-cells. Each antibody is highly specific to its antigen, meaning it is capable of recognizing the antigen amongst thousands of others, often similar, molecules. Once the target antigen is recognized, the antibody binds to it tightly and aids its elimination from the body. These properties make antibodies a very attractive proposition as potential therapeutic agents.

Antibody therapeutics act by mimicking and harnessing the body's immune system with the antibody working as the drug molecule (Jones *et al.*, 2009; Shrivastava *et al.*, 2009). Monoclonal antibodies can be used in three main therapeutic approaches: blocking cell activity and/or modulating immune function to prevent certain cell responses – applicable in autoimmune disorders, inflammation, allergic reactions and cancer (O'Connor & Czuczman, 2008). Activating and modulating cell activity immune functions to stimulate a desired immune response applicable in, for example, certain oncology indications and in some Alzheimer's disease immunotherapy.

5. Delivering Molecules/Agents to Specific Cells and Tissues

It is well known that there are some pathological circumstances that lead to production of autoantibodies, e.g. antibodies that recognize only the tumor tissue. Some of these autoantibodies have a GPCR agonist effect that are crucial in the development of some diseases (e.g. activating angiotensin II type 1 auto-antibodies is found in women with preeclampsia and has great influence in renal-allograft rejection) (Dechend *et al.*, 2003). This finding suggests the possibility of using the GPCR conformation-specific antibodies itself as drugs, it is needed to investigate further more if GPCR conformation-specific antibodies have some effect by itself on the activation of the receptor, but its ability of recognizing the activate state of GPCRs opens an unique possibility of using it to address over active receptors conditions on some diseases, leading to treatments less aggressive than completely abolishment of all target receptor activity like antagonists actions.

6. Conformational-Sensitive Antibodies for Identification of GPCR Activity State

Many of the antibody-based approaches in high throughput screening (HTS) are designed for protein discovery or protein array. Although those are important tools, recently antibodies have been used in HTS for functional and kinetic based assays. There are several types of assays based on the detection of secondary signals such as calcium and inositol phosphates available to scientists studying the pharmacology of GPCRs. Distinct Ca^{2+}-indicator assay kits are available for comparison studies to achieve best results with weakly responding receptors. Also detection of down-stream events such as activation of phosphorylated ERK1/2, phosphorylated Akt, phospho-Src and phospho-STAT3 can be done using antibodies that are able to distinguish phosphorylation sites, which collectively provide a comprehensive solution for the analysis of GPCR signaling (Caunt *et al.*, 2006; Goldsmith & Dhanasekaran, 2007; McKay & Morrison, 2007; Aoki *et al.*, 2008). Recently, antibodies that recognize the active state of the GPCR itself has been developed, they have targeted the C-terminal phosphorylation sites and the N-terminal conformation changes induced by the agonists (Gupta *et al.*, 2007; Gupta *et al.*, 2008).

The use of conformational-sensitive antibodies to study GPCR activation, by identifying the conformational changes promoted by the agonist in an ELISA based assay, has been shown to represent a cheap, useful and direct approach compared to others that detects the GPCR activation using down-stream events (Gupta *et al.*, 2007). The conformational-sensitive antibodies assays seems to be applicable to the family A of GPCRs and opens the way to examine the localization of active receptors as well as the extent of modulation of receptor activity by cross-talk between receptors. It has been shown that this assay allows monitoring the activated receptors *in vitro* and most importantly on tissues of animals treated with a drug. The ability of conformational-sensitive antibodies to monitor such specific changes makes them ideal and powerful tools to study the spatial-temporal dynamics of GPCR activation in the brain (Gupta *et al.*, 2008). Therefore, conformational-sensitive antibodies represents a new, powerful, and hitherto technique to examine the duration and extent of activation of endogenous receptors as well as to screen for drugs including allosteric modulators of family A GPCRs, which could be of potential therapeutic value.

7. Identification of Hemopressin as an Inverse Agonist/Antagonist of Cannabinoid Type 1 Receptors

Hemopressin (PVNFKFLSH) is a novel bioactive peptide derived from the alpha1-chain of hemoglobin, originally isolated from rat brain homogenates (Rioli *et al.*, 2003). Hemopressin causes hypotension in anesthetized rats and is metabolized in vivo and in vitro by the thimet-oligopeptidase, neurolysin, and the angiotensin-converting enzyme (Rioli *et al.*, 2003; Dale *et al.*, 2005a). Hemopressin also exerts an antinociceptive action in experimental inflammatory hyperalgesia induced by carrageenin or bradykinin via a mechanism that is independent of opioids (Dale *et al.*, 2005b). Recently, using conformational-sensitive antibodies, hemopressin was characterized as an inverse agonist of cannabinoid type 1 (CB1) receptors (Heimann *et al.*, 2007). Since these antibodies are activation-state sensitive, the effect of hemopressin on the agonist-induced increase in antibody binding was examined and the CB_1 receptor antagonist, SR141716 (*Rimonabant*™) was used as a control. Our previously published data demonstrated that the agonist-induced increase in antibody recognition can be significantly blocked by hemopressin as well as by SR141716, suggesting that hemopressin acts similarly to SR141716 and also represents an antagonist for CB_1 receptors (Heimann *et al.*, 2007). To examine the selectivity of peptides such as the hemopressin for each of the receptors evaluated, the conformation sensitive antibodies to μ and δ opioid receptors, α_{2A} and $_2$ adrenoceptors, angiotensin II type 1 and type 2 receptors and CB_2 cannabinoid receptors were used. While all of these antibodies exhibited agonist-induced conformational selectivity, hemopressin was not able to modulate antibody recognition to any of these receptors except CB_1 receptors, supporting the idea that hemopressin is highly selective for CB_1 cannabinoid receptors.

8. Concluding Remarks

Although peptide drugs have been on the market for decades - insulin being the most prominent example - it was not until 10 to 15 years ago that the pharmaceutical industry really started to work seriously on the development of a new generation of peptide-based therapeutics, prompted by advances in the understanding of the genetics of disease. But initial enthusiasm was soon dampened by the realization that drug delivery technologies at that time were not up to the task of getting these relatively large compounds into the body effectively, while production could be complex and expensive (Watt, 2006). Now, with drug delivery seeing enormous strides forward, the stage seems to be set for a renaissance in peptide drugs (Otvos, 2008). Due to their high specificity and low toxicity profile, peptides have once again become central to the development of new drugs. Imagine a conversation between a small molecule and a peptide on a make-believe pharmacological playground. The small molecule would tout its virtues of small size, low price, oral availability, ability to cross membranes, and straightforward synthesis (Garber, 2005). The peptide would respond: "True, I may be bigger, more expensive to synthesize, and less stable than you. I may clear faster from the body and usually need to be injected rather than swallowed as a pill. But I can be much more potent, show higher specificity, and have few toxicology problems. I also don't accumulate in organs or face drug-drug interaction challenges like you do. So there." (Marx, 2005).

Although small molecules continue to dominate the discovery of drugs for receptors, transporters, ion channels and enzymes, biological drugs (for example, antibodies, proteins and peptides) are emerging as powerful adjuncts for discovering inhibitors of targets, such as protein-protein interactions, that are not readily pharmaceutically treatable by conventional approaches. With the exhaustion of many drug discovery pipelines, it is becoming increasingly important to

develop new means to access these non-classical targets, particularly because conventional targets represent a small minority of the disease-associated proteome.

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