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Frontiers in Medicinal Chemistry in Regensburg

Stefan Dove, Roland Seifert, Sigurd Elz, and Armin Buschauer*[a]

"Frontiers in Medicinal Chemistry" are not only the current state of the art in this area that highlight recent advances in the medical therapy of diseases, but also represent the interfaces with other fields such as molecular biology, biochemistry, and pharmacology. It was within this context that the annual joint conference between the Medicinal Chemistry Division of the German Chemical Society (GDCh) and the Pharmaceutical/Medicinal Chemistry Division of the German Pharmaceutical Society (DPhG) took place in Regensburg, March 2–5, 2008. These events have developed into internationally highly regarded "Highlight Meetings" that cover current main therapeutic issues, with the participation of prominent scientists from academia and industry. Because innovative therapeutic concepts are increasingly based on the discovery and analysis of biological target molecules and their function as well as on the investigation of the mode of action of potential drugs, the "Frontiers in Medicinal Chemistry" are interdisciplinary. For this reason the conference covered methods and results from different areas, presented by top scientists from Germany and abroad.

The University of Regensburg, the conference venue, has about a 30-year tradition in medicinal chemistry. Since 2002, the Faculty for Chemistry and Pharmacy has established medicinal chemistry for the first time among German universities as a core area in teaching and research with a four-semester advanced course (MSc) and a Deutsche Forschungsgemeinschaft (DFG) graduate training program (GRK 760). The scientific focus of the GRK on G-protein-coupled receptors (GPCRs) was pivotal for one of this year's conference topics.

GPCRs are involved in the regulation of virtually every function in the body and are targets for about 50% of currently marketed drugs. The long-awaited crystal structure of a human GPCR, the β_2 adrenoceptor, in complex with carazolol was published at the end of 2007, and will act as a catalyst in the development of future therapeutics.[1] In view of this milestone in GPCR research, it was a great honor for the conference that one of the authors, Prof. Dr. Gebhard Schertler (Medical Research Council, Cambridge, UK), presented the opening lecture. He gave an impressive report on the extremely difficult path taken, spanning more than 15 years from the initial attempts at receptor purification to the solved crystal structure. This included the solution of problems with which many research groups have previously faltered. Both protein engineering—covalent binding of the β_2 adrenoceptor to a Fab (fragment, antigen binding) and insertion of lysozyme into a shortened intracellular loop—in the laboratory of B. K. Kobilka (Stanford University, Palo Alto, USA) and further development of the microcrystallization technique at the MRC and in the research group of R. C. Stevens (Scripps Research Institute, San Diego, USA) contributed significantly to this success. The highlight of the lecture was the presentation of the as yet unpublished crystal structure of a turkey β_1 adrenoceptor (Figure 1). Its comparison with the β_2 structures and the differences between the adrenoceptors and bovine rhodopsin have led to new implications and hypotheses on ligand binding and activation mechanisms of GPCRs. The high similarity of the transmembrane regions even between the β adrenoceptors and rhodopsin is in contrast to the large differences of most intra- and extracellular domains. Surprisingly, the ligand binding sites of the β_1 and β_2 adrenoceptors are nearly identical. The selectivity of agonists and antagonists appears to be primarily a result of differences between extracellular regions through which the ligands approach the "final" binding sites.

Figure 1. Prof. Dr. Gebhard Schertler presents the crystal structures of the β_1 and β_2 adrenoceptors in his opening lecture. (*Photo: Miroslaw Lopuch*)

Other lectures in the GPCR field concerned the development of ligands, therapeutic concepts, and methodologies. Dr. Emily M. Stocking (Johnson & Johnson, La Jolla, USA) reported new histamine H_3 receptor antagonists with improved pharmacokinetics. Their pharmacological properties—increases in vigilance and learning memory without activation of other regions of the brain as with classical psychostimulants—suggest therapeutic applications for other diseases of the central nervous system (CNS), especially psychoses such as schizophrenia, could be better treated according to the principle of "one disease, multiple targets". In support of this concept, the high degree of homology between neurotransmitter GPCRs facilitates the binding of similar structural fragments (privileged scaffolds). Thus, dopamine D_3 receptor antagonists have been developed in the research group of Prof. Dr. Holger Stark (University of Frankfurt, Germany)

[a] *Prof. Dr. S. Dove, Prof. Dr. R. Seifert, Prof. Dr. S. Elz, Prof. Dr. A. Buschauer*
Institute of Pharmacy
University of Regensburg
93040 Regensburg (Germany)
Fax: (+49) 941-943-4820
E-mail: armin.buschauer@chemie.uni-regensburg.de

by the combination of "H_1 antihistaminic substructures" with D_3 pharmacophores.

GPCRs frequently form dimers or even oligomers. The functional significance of these GPCR quaternary structures is one of the most current research topics in medicinal chemistry and molecular pharmacology. Using the $GABA_B$ receptor as model, Dr. Jean-Philippe Pin (University of Montpellier, France) has investigated a class 3 GPCR in which dimerization is essential for function. Two homologous subunits, the $GABA_{B1}$ and the $GABA_{B2}$ receptor, of which the former binds GABA and the latter activates the G protein, associate to the functional heterodimer. The formation of larger oligomers was detected by fluorescence resonance electron transfer (FRET) experiments. Dr. Pin also reported that conventional FRET methods for the detection of receptor dimers are susceptible to artifacts, and for this reason new approaches based on the detection of dimers with antibodies and SNAP-tag labeling were developed.

Associated with the discovery of GPCR dimers was the idea that a suitable ligand molecule can bind simultaneously to both (identical) subunits. Such so-called bivalent ligands contain two identical or similar active structures (pharmacophores) separated by a spacer. Prof. Dr. Peter Gmeiner (University of Erlangen, Germany) investigated this area using dopamine D_2 receptor ligands as model. The structure–affinity relationships of compounds with two identical (homodimers) or different (heterodimers) pharmacophores and variable spacer lengths as well as of the underlying monomers showed that, with suitable spacers, the binding affinity increased in the order: monomer–heterodimer–homodimer. However, these data do not answer whether the bivalent ligands actually bind simultaneously to both receptor subunits.

Dr. Gayathri Swaminath (Amgen, San Francisco, USA) introduced new GPCR GPR40 agonists. This receptor is expressed on the β cells of the pancreas and stimulates insulin secretion following activation by fatty acids. A single oral dose of the agonist improved glucose clearance in healthy and diabetic rodents. Therefore, such compounds are potentially suitable for the treatment of type 2 diabetes. Apparently desensitization of GPR40 does not play a role in long-term therapy of diabetes mellitus. Future investigations will have to clarify whether activation of GPR40 is advantageous in comparison with direct (exenatide) or indirect (DPP IV inhibitors) activation of incretin receptors, similarly acting on insulin secretion. The combined activation of GPR40 and incretin receptors is potentially beneficial.

In addition to GPCRs, antiviral therapeutic approaches were discussed at the conference. Chemokine receptor ligands, for example, play an important role in inflammation as well as growth inhibition in human immunodeficiency viruses (HIV) and hepatitis C viruses (HCV). Prof. Dr. Mette M. Rosenkilde (University of Copenhagen, Denmark) has characterized the ligand binding sites for antagonists at the chemokine receptors CCR5 and CXCR4, which are involved in the entry of HIV into host cells. Her approach incorporates a combination of mutagenesis, structure–activity relationships of ligands, and molecular modeling. Such molecular–pharmacological effects were pivotal for the development of maraviroc, the first CCR5 antagonist authorized for HIV therapy.

Prof. Dr. Karin Mölling (University of Zürich, Switzerland) reported another innovative approach for the treatment of HIV infections: a 54-base-pair oligodeoxynucleotide, designated as a small interfering DNA (siDNA), was directed against the highly conserved PPT motif; it hybridizes with viral target RNA, and, by this, activates viral reverse transcriptase/RNase H activity. In this way the viral RNA is irreversibly destroyed before it can be rewritten into DNA. The treatment of HIV-infected cells with siDNA inhibits HIV release. The therapeutic effects of siDNA treatment were detected in vivo with an HIV mouse model. The first ex vivo investigations on HIV patient material with siDNA are currently underway. It is hoped that this approach can be used to prevent HIV transmission by sexual contact and through the placental barrier. Similarly, this principle can be used as post-exposure prophylaxis and in cases of infection with multidrug-resistant HIV strains. However, there are currently no detailed pharmacokinetic and toxicological data for verification.

Prof. Dr. Roland K. Hartmann (University of Marburg, Germany) presented an overview of RNA silencing (also known as RNA interference) by siRNAs (small interfering RNAs). This is a physiological mechanism that can be used pharmacologically in that specific siRNAs against a specific target RNA are inserted into cells. Hybridization of the siRNA with the target mRNA and its degradation then takes place. In this way the expression of any given gene can, in principle, be down-regulated. By specific chemical modification, siRNAs can be stabilized so that they penetrate the plasma membrane and act in the intact cell. An example presented by Professor Hartmann concerns the pathogenesis of human B cell lymphoma, the proliferation of which is regulated by the serine/threonine kinase Pim-1. He demonstrated siRNA-mediated knock-down of Pim-1 with associated inhibition of cell proliferation. It may be assumed that in the near future much research activity will be directed at the use of siRNA/DNAs in the treatment of viral infections and tumors. Improvements in metabolic stability and bioavailability remain significant challenges in the use of oligonucleotides as medicinal materials.

Dr. Anthony Wood (Pfizer, Sandwich, UK) reported on the development of new pyrazole-based HIV reverse transcriptase inhibitors. The special feature of these inhibitors is that they are not only active against the wild-type enzyme, but also against mutant transcriptase variants that normally lead to resistance. Thus, the spectrum of drugs available for the treatment of multidrug-resistant HIV infections is also expanded at this level. Additionally, the toxicological and pharmacokinetic properties of these new inhibitors are very favorable so that initial clinical studies can soon be started.

In the opening lecture on the topic of cardiovascular and metabolic diseases, Prof. Dr. Lutz Hein (University of Freiburg, Germany) gave a presentation on the pathophysiology and therapy of cardiac failure, one of the main causes of death in the industrialized world. It has been established that excessive activity

of sympathetic neurons plays a pivotal role in the pathogenesis of cardiac failure. For instance, mice that overexpress the β_1 adrenoceptor develop chronic cardiac failure, and β_1 adrenoceptor antagonists show therapeutic effects in the treatment of this disease. Noradrenaline release is inhibited through presynaptic $\alpha 2$ adrenoceptors. It is therefore not surprising that the symptoms of cardiac failure can be induced in $\alpha 2$ adrenoceptor knockout mice, but surprisingly, in addition to sympathetic neurons, the adrenal medulla plays a significant role in the pathogenesis of this disease. As there is still no causal therapy of cardiac failure, it is necessary to search for new targets. By means of gene array investigations in which thousands of genes are analyzed at the mRNA level, Professor Hein identified in his cardiac failure model the methyl-CpG binding protein MeCP2 as a new potential target for the development of drugs against cardiac failure, as this protein is less expressed in the models. The role of MeCP2 in the pathogenesis of cardiac failure, however, is very complex because an overexpression of MeCP2 in transgenic mice also leads to cardiac failure.

Prof. Dr. Wolfram Zimmermann (University of Hamburg, Germany) illustrated the possibilities that in vitro cultured cardiac tissue offers for the analysis of the pathophysiology and therapy of cardiac disease. Whereas such heart models have been available for some time for birds and rodents, it has only recently been possible to stimulate in vitro human cardiac stem cells to differentiate into functionally active cardiac tissue. This cardiac tissue shows electrical activity and contraction and can be stimulated through β_1 adrenoceptors. Therefore, many cardiovascular drugs can be investigated in this model before they are studied in animals or in humans. Another application of such functional cardiac cell tissues is to place them over areas of myocardial infarction, thus allowing the invasion of functional cardiomyocytes into the diseased tissue. It may be assumed from this that cardiac tissue engineering will play an increasingly important role for the development of drugs and the treatment of cardiovascular disease.

Capadenoson (BAY 68-4986) is the first nonpurine-like adenosine A_1 receptor agonist for the oral treatment of stable angina pectoris. Dr. Joachim Mittendorf (Bayer HealthCare AG, Wuppertal, Germany) reported on the development of this class of novel pyridine derivatives from which Capadenoson has emerged by final structure optimization. It decreases heart rate in various species, has a cardioprotective effect, and thus a promising activity profile for the treatment of coronary heart disease. Prof. Dr. Gerd Schnorrenberg (Boehringer Ingelheim, Biberach, Germany) provided some insight into the design of the potent and long-acting dipeptidylpeptidase-4 (DPP-4) inhibitor, BI 1356, a xanthine derivative. Due to DPP-4 inhibition, the degradation of the incretins GLP-1 and GIP is reduced. GLP-1 (glucagon-like peptide-1) in particular, has potent glucose-concentration-dependent insulinotropic and glucagon-release inhibitory activity. Professor Schnorrenberg demonstrated the process from screening hits with micromolar inhibitory activity to an antidiabetic drug with activity in the nanomolar range that is by now in phase Ib clinical trials. Insight into the binding mode was given by the crystal structure of DPP-4 in complex with BI 1356.

In a session on imaging techniques in drug research and medical diagnostics, Dr. Heribert Schmitt-Willich (Bayer–Schering Pharma AG, Berlin, Germany) described the development of new contrast agents for the imaging of structures by magnetic resonance, and Prof. Dr. Hans-Jürgen Wester (Technical University, Munich, Germany) and Dr. Rainer Kneuer (Novartis Pharma AG, Basel, Switzerland) discussed radioactive (PET, SPECT) and fluorescent probes for molecular imaging.

It has become a tradition to end the conference with a "highlight session" in which various innovative research results in the area of medicinal chemistry are presented. One focus for this session was the development of drugs for rare diseases (incidence $<1{:}10\,000$). High development costs are one reason that rare diseases are generally not prioritized as drug development projects by large pharmaceutical companies. Resources are instead focused on cancer, cardiovascular and metabolic diseases, the higher incidence of which secures greater profit stability. Dr. Hyder A. Jinnah (Johns Hopkins University, Baltimore, USA), a recognized expert in the area of dystonia, a prototypical rare disease, emphasized that although each rare disease does indeed represent in itself a rarity, the multitude of such diseases add up to a considerable social burden because of the lack of therapeutic possibilities and subsequent costs for nursing and care. Dr. Jinnah explained two principal strategies for the development of drugs for rare diseases. One strategy is based on analyzing the pathophysiology of the disease in question, and in this way finding a starting point for potential drugs. There are already suitable animal models for dystonia. The other strategy is based on testing empirically known drugs that are already marketed for other diseases, for new indications. The advantage for this approach is that the costs for preclinical drug development are saved. Dr. Jinnah emphasized that a very close partnership between university and company scientists is essential for the development of drugs for the treatment of rare diseases.

Dr. Heiner Glombik (Sanofi-Aventis, Frankfurt, Germany) reported a concept for the treatment of type 2 diabetes involving selective blockade of the renal sodium-dependent glucose transporter SGLT2. It is hoped that the inhibition of tubular glucose re-absorption mediated by SGLT2 results in increased renal glucose excretion and a subsequent decrease in blood glucose concentration. A new class of thiophene derivatives, the most prominent representative of which is AVE2268, has been developed from the experimentally long-used nonselective SGLT inhibitor phlorizin. Dr. Glombik confirmed the potential benefit of AVE2268 on the basis of numerous in vitro and in vivo investigations.

Another area barely addressed by large pharmaceutical companies is the development of oral male contraceptives. Unlike the companies, however, the US National Institutes of Health (NIH) and the World Health Organization (WHO) see a large need for research in this area. Consequently the NIH is generously supporting an interdisciplinary re-

search consortium directed by Dr. Joseph S. Tash (University of Kansas, USA) that is concerned with the development of male contraceptives. Consortium participants include university physiologists, medicinal chemists, pharmacologists, and biochemists. Dr. Tash demonstrated that soluble adenylyl cyclase (sAC), which is essential for sperm mobility, is an excellent pharmacological target for oral contraceptives, as it is expressed exclusively in the testes. Therefore, selective sAC inhibitors should exhibit minimal toxicity. Flavopiridol analogues that potently inhibit sAC and sperm motility were developed through combinatorial chemistry. Lonidamine derivatives, which induce infertility in animals with high potency and reversibility, were identified by high-throughput screening; interestingly it appears that a totally unexpected target, heat-shock protein 90, mediates the action of lonidamine.

Bradykinin-B_1 receptor antagonists as a new class of analgesics were the topic of the final plenary lecture, presented by Dr. Michael Wood (Merck, West Point, USA). B_1 receptors are constitutively expressed in the CNS, but occur at a particularly high density in injured tissue. They are thought to play an important role in chronic pain and in inflammatory processes, so that their blockade can lead to analgesia. Wood reported in detail on the critical phases and successes in the development of nonpeptidic antagonists from the class of 2,3-diaminopyridines that were obtained by systematic modification of initial lead compounds. It became clear that numerous tricks had to be used to fulfill all the requirements of potency, selectivity, oral bioavailability, CNS penetration, and safety profile that are required for modern analgesics.

The program of the conference was complemented by a series of short lectures, traditionally held by junior scientists. Additionally, the presentation of scientific posters provided an excellent opportunity for conference participants, especially undergraduates, post-graduates, and post-doctoral researchers to disseminate their own research results amongst their expert peers. In acknowledgement of its importance, much attention and time were devoted to this form of presentation throughout the entire conference. In particular, the 78 poster contributions were discussed in depth with the authors at a three-hour evening session. A committee selected three contributions for the poster awards. Dr. Eric Schneider (University of Regensburg, Germany) was honored for the discovery of a high-affinity, G-protein-independent agonist state of the human histamine H_4 receptor. Two related posters presented by Sanja Grünewald and Stephanie Kanzow (University of Kiel, Germany) concerned new information on the enzymatic reduction of N-hydroxylated amidines and guanidines, and the development of such compounds as prodrugs was awarded a joint prize. Valerie S. Hondorf (Max Planck Institute, Göttingen, Germany) was honored for her NMR spectroscopic investigations on the dynamics of the complex of p38α-MAP kinase with an inhibitor. The authors of the selected posters presented their results in five-minute lectures.

The award ceremony of the Prize for Innovation in Medicinal/Pharmaceutical Chemistry was an important event. This €5000 award, which is the highest endowed award of its type in Germany, was presented to Dr. Franz von Nussbaum, project leader of cardiovascular research at Bayer HealthCare AG, Wuppertal, for his work on katanosins, a still essentially unexplored class of antibiotics (Figure 2). A novel mode of action makes these compounds "resistance breakers", which show promise in the fight against multiresistant germs such as staphylococci and enterococci, the continued spread of which is particularly problematic in hospitals. The first two representatives of the katanosin antibiotic class are by now in the clinical development phase. In his award lecture, Dr. von Nussbaum presented the semisynthetic–enzymatic method he developed for the production of the bacterial lead lysobactin, a cyclic depsipeptide, and of other derivatives.

Figure 2. Dr. Franz von Nussbaum (center) was awarded with the prize for innovation of the two specialist areas. At left: Dr. Hans Ulrich Stilz, Chairman of the Medicinal Chemistry Division of the GDCh; at right: Prof. Dr. Bernd Clement, Chairman of the Division of Pharmaceutical/Medicinal Chemistry of the DPhG. (*Photo: Miroslaw Lopuch*)

The Frontiers in Medicinal Chemistry conference in Regensburg has shown once again that exciting and economically successful projects result from bringing together industrial and academic research at the interface of the fields of biology, chemistry, medicine, pharmacy, and pharmacology. Such projects also open attractive areas of research, particularly for junior scientists.

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[1] S. G. Rasmussen, H. J. Choi, D. M. Rosenbaum, T. S. Kobilka, F. S. Thian, P. C. Edwards, M. Burghammer, V. R. Ratnala, R. Sanishvili, R. F. Fischetti, G. F. Schertler, W. I. Weis, B. K. Kobilka, *Nature* **2007**, *450*, 383–387.

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