PURINES 2014: Nucleotides, nucleosides and nucleobases - international conference on signalling, drugs and targets

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Nucleotides, Nucleosides and Nucleobases *International Conference on Signalling, Drugs and Targets*

July 23-27, 2014

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Purines 2014 was held in Bonn, Germany, from July 23 to 27, 2014. This meeting continued a series of conferences, the first of which was held in Banff, Alberta, Canada in 1978 and entitled “International Conference on Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides”. At that time increasing evidence had been provided for ATP as a signal substance. The observation that adenosine can either inhibit or stimulate adenylate cyclase had just led to the

distinction of A_1 and A_2 adenosine receptors. Since then the field of purinergic (and pyrimidinergic) signaling has envisaged tremendous advances. Much of the previous evidence was based on pharmacological and physiological experiments. In the 1980s first sequences of ectonucleotidases were obtained and P2X and P2Y receptors could be differentiated. In the 1990s first sequences of adenosine receptors and of P2X and of P2Y receptors were reported, but only the new millennium envisaged the elucidation of the first atomic structures by X-ray crystallography of ectonucleotidases, of the first adenosine receptor, the A_{2A} receptor, and of the first P2X (P2X₄) and P2Y receptor (P2Y₁₂). Furthermore G protein-coupled receptors for the nucleobase adenine were introduced as a new class of purine receptors (AdeR).

The conference in Bonn emphasized that in spite of nearly half a century of intensive research the field of purinergic signaling continues to grow rapidly. The meeting revealed many trends for future research including, for example, in the field of the structure-based development of purinergic drugs, the imaging of purinergic

receptors in human brain, the development of transgenic mouse models, the striking role of purinergic signaling in a considerable variety of central and peripheral diseases including inflammation and cancer, the emerging field of purinergic signaling in plants, the discovery of novel purine receptors and related receptors, the expanding field of transporters and enzymes involved in purinergic signaling, the impact of bioinformatics and modern sequencing on purine research and many others. More than 550 delegates from at least 33 different countries and from 6 continents attended the conference. Seven plenary lectures focused on specific aspects of purinergic signaling and the four daily parallel sessions hosted 44 symposia.

The conference was held in the city center within the main building of the Rheinische Friedrich-Wilhelms-University Bonn, previously the castle of the king elector of Cologne. It was opened by Christa Müller (congress chairperson), Herbert Zimmermann (president of the German Purine Club), the vice chancellor of the University of Bonn (Jürgen von Hagen) and the Mayor of the city of Bonn (Jürgen Nimptsch).

purines 2014 in bonn

www.Purines2014.de Sign up for email list to receive updates, such as opening of registration, at purines2014@coventus.de

Nucleotides, Nucleosides and Nucleobases International Conference on Signalling, Drugs and Targets

Plenary Speakers:

Geoffrey Burnstock
University College London, UK

Elizabeth Hartland
University of Melbourne, Parkville Victoria, Australia

Kenneth A. Jacobson
NIH, Bethesda, USA

Fiona Marshall
Heptanes Therapeutics Ltd., Hertfordshire, UK

Maiken Nedergaard
University of Rochester, Rochester, USA

Michail Sitkovsky
Northeastern University, Boston, USA

Norbert Sträter
University of Leipzig, Leipzig, Germany

Francesco di Virgilio
University of Ferrara, Ferrara, Italy

Date: July 23–27, 2014

Venue: University of Bonn
Main Building (Castle)
Am Hof 1
53113 Bonn, Germany

Congress Chairpersons:

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The stage was set by the enchanting lecture of Geoffrey Burnstock, the founder of the concept of purinergic signalling, entitled “The field of purinergic signaling is expanding in many different directions”. Burnstock provided a detailed account of the physiological and pathological significance of purinergic signaling and its molecular players. His lecture illustrated that we are only beginning to understand how

purinergic signaling affects the multiple physiological and in particular pathological states of essentially every organ system. It highlighted the diverse and novel experimental approaches that were subsequently further detailed during the conference. Geoffrey Burnstock was awarded honorary membership of the German Purine Club for his numerous seminal contributions to the field of purinergic signaling.

Geoffrey Burnstock

Another highlight of the first evening was the “Burnstock Lecture” presented by Francesco di Virgilio, University of Ferrara, Italy. Francesco di Virgilio had provided fundamental insight into the function of P2X7 receptors in inflammation and cancer. Amongst others he had developed a novel recombinant plasma membrane-targeted luciferase (pmeLUC) that allows the visualization of extracellular ATP in intact animals. It is now widely used to detect ATP release by tumor tissue *in situ*. In his lecture he addressed the complex signaling network mediated by ATP and adenosine, generating an array of inhibitory and stimulatory responses that affect immune cell function, tumor growth, and metastatic dissemination. The plenary lecture on the next morning was dedicated to John W. Daly (1933–2008) who had made fundamental contributions to the pharmacology and medicinal chemistry of adenosine receptors. It was held by Kenneth A. Jacobson, National Institutes of Health, Bethesda, USA. Ken Jacobson, the leading medicinal chemist in the field, summarized his recent success in developing novel ligands for adenosine and P2Y receptors including drug-like small molecules and fluorescent probes. His work had significantly contributed to the elucidation of the X-ray structures of the A_{2A} and P2Y₁₂ receptors, which are important for drug development allowing virtual screening approaches. Moreover he reported the development

of novel, fluorescent-based receptor assays for adenosine receptors.

The following morning, four additional plenary lectures attracted full audience. Michail Sitkovsky from the Northwestern University Boston in his inimitable ostensive way addressed the Hellström paradox, the peaceful coexistence of tumors and anti-tumor killer cells in the same cancer patient. Tumors can create a hostile—immunosuppressive microenvironment that prevents their destruction by anti-tumor T cells. Therapeutic manipulation of hypoxia-A₂-adenosinergic suppression should redirect the immune response involving a novel approach of using anti-hypoxia-A₂-adenosinergic immunological co-adjuvants.

Paracrine purinergic signaling in the central nervous system was the focus of Maiken Nedergaard, University of Rochester Medical Center. Using FACS-purified astrocytes, microglia, oligodendrocytes, endothelial cells and pericytes and a brain transcriptome databank, her group performed a molecular analysis of the cell type-specific pathways involved in purinergic signaling, including the cellular sources of ATP, ADP and adenosine, the purine receptors, ectonucleotidases and nucleoside transporters. This approach permitted the analysis of the cells in their “*in vivo*” state and revealed novel insight into the cellular compartmentalization of the molecules involved in purinergic signaling. In cortex, ectoenzymatic

sources of adenosine were found to be strictly compartmentalized to oligodendrocytes and an ATP-to-adenosine pathway was not ubiquitously present. Cross-talk among adenosine pathways would thus very much depend on anatomical signaling niches and the cell type-specific mosaic expression of purinergic molecular components.

Norbert Sträter, University of Leipzig, Germany, provided an overview of the structure and functions of ectonucleotidases, a field pioneered by his group. Particular emphasis was placed on ecto-5'-nucleotidase (CD73), an extracellular AMP-hydrolyzing enzyme with homologs in bacteria and other microorganisms. The catalytic cycle of ecto-5'-nucleotidase involves a large and unique domain motion. In the open form, the nucleotide binding pocket is accessible for substrate binding and product leaving. In its closed form the substrate binds between the two domains. The atomic structures of CD73 now provide the basis for rational inhibitor design.

Finally, the A, B and C of structure-based inhibitor design of G protein-coupled receptors was illuminated by Fiona Marshall, Heptares Therapeutics Ltd, Herfordshire, UK. Having solved X-ray structures of GPCRs for all the major sub-classes of the GPCR superfamily, structure-based drug design can be applied for the first time across the entire GPCRome. Fiona Marshall illustrated how insights derived from new ligand-receptor complexes and a variety of computational methods can be used to analyze druggability and to drive drug design. This included the A_{2A} adenosine receptor and also a variety of receptors from other subclasses of G protein-coupled receptors and highlighted the challenges in the discovery of small molecule modulators or negative allosteric modulators.

Forty-six symposia and the 231 related posters covered essentially the entire field of purinergic signaling including novel technical developments and therapeutic approaches.

Poster Session at Purines 2014 in Bonn

Purinergic receptors represented a major topic of the conference. Nine symposia were devoted to the various aspects of receptor structure and function, including new technologies in the study of purinergic receptors, the pathological implications of receptor polymorphisms, structure and function of purine receptor heteromers, recent advances in the study of transgenic models, in situ imaging of purine receptors, platelet receptors, and the emerging fields of intracellular purine receptors. Three symposia presented exciting advances in the analysis of X-ray structures and molecular modeling of P1 and P2 receptors and ectonucleotidases. Among the novel receptors discussed were the G-protein-coupled adenosine receptors,

guanosine and potential guanine receptors and the recently discovered plant receptor for ATP.

Another important topic of the meeting was purine-based drug development. Novel strategies for drug development and lessons learned from previous approaches were highlighted in a special Roundtable devoted to the “Challenges of purinergic drugs” organized by Maria-Pia Abbracchio. Speakers from industry (Jeff Zablocki, Gilead Sciences; Anthony Ford, Afferent Pharmaceuticals), academia (Jian-Fan Chen, Christa Müller) and from a patients’ organization foundation (Paola Zarin, Italian Multiple Sclerosis Foundation) presented their views followed by a lively discussion. It was concluded that a close

collaboration between all players including industry, academia, patients, and foundations will be most powerful in developing the medicines of the future and bring them to market and to the patients. This subject was also addressed in four symposia that dealt with potential clinical candidates for purine receptors, medicinal chemistry and drug development of P1 and P2 receptors, and inhibitors of the various ectonucleotidases.

The molecular components involved in release of nucleosides and nucleotides represent an important constituent of the purinergic purinome. One symposium centered around the release of nucleosides and nucleobases whereas another one addressed the yin and yang of the mechanisms and functional implications of vesicular uptake and exocytotic release of nucleotides on the one hand and pannexin1-mediated release on the other hand.

Inflammatory illnesses impact many organ systems. Both, purinergic receptors and ectonucleotidases, are central constituents of inflammatory processes and might be targeted to prevent, halt or even reverse progression of inflammatory diseases. Three symposia covered the role of nucleotides and their metabolizing enzymes in inflammation and immunology, the role of purines in neuroinflammation and the regulation of the immune system by purines.

The topic cancer is closely related. Exciting progress is being made in the development of immunotherapeutics for the treatment of cancer. Novel targets in this field include ectonucleotidases, in particular ecto-5'-nucleotidase (CD73) generating adenosine, adenosine receptors that cause dysregulation of the hypoxic tumor microenvironment and the P2X7 receptor that has been shown to regulate immune responses and promote tumor engraftment, growth and vascularization. Two symposia addressed novel insights and therapeutic approaches, one on purinergic regulation of tumor growth and metastasis, and one on purine-based therapeutic approaches in cancer therapy.

Purinergic signaling plays an important role in communication in the nervous system. This includes not only communication between neurons but also between the various types of glia and neurons and between glia. Physiological and pathological mechanisms of purinergic signaling were discussed in three symposia on ATP-mediated crosstalk between microglia, astrocytes and neurons, purine signaling and therapeutic interventions in pain, and the role of adenosine and ATP in sleep, highlighting the role of adenosine as a homeostatic sleep factor. Purine signaling and therapeutic applications in the sensory stems were addressed in a separate symposium. It highlighted recent insight into the functional role of purines in the inner ear, the eye, taste buds and the olfactory bulb.

Purinergic mechanisms are involved in a large variety of functional disorders of the nervous system and in neurodegenerative diseases. They therefore represent very important targets for therapy. Four symposia uncovered novel mechanisms in the etiology of neurodegenerative diseases, disease models, and clinical trials. The symposia included purines and neurodegeneration, purine receptors in neuroinflammation

and neurodegeneration, adenosine receptor antagonism and Parkinson's and Alzheimer's disease, and neuroprotective functions for purinergic receptors in the brain.

Stem cells and the subsequent differentiation processes represents another novel field where purinergic signaling is involved. This concerns the embryonic and mature nervous system equally well as mesenchymal stem cells or hematopoietic stem cells. Therefore, one symposium addressed the role of purines in embryonic development and in adult stem cell growth and differentiation.

Purines/pyrimidines presumably represent the most ubiquitous signal system in the mammalian body. It is therefore not surprising that they play a major role in the physiology and in particular also in the pathology of essentially every organ system. Eight symposia covered the impact of purinergic signaling in the various organ systems, their pathological implications and the corresponding therapeutic approaches. These included purinergic signaling in the cardiovascular system, roles of purines in gastrointestinal physiology and disease, pancreatic purinergic signaling in health and disease—exocrine and endocrine, purinergic signaling in the musculoskeletal system, ATP and P2 receptors in the regulation of renal transport and blood pressure, regulation of lipid and glucose metabolism by purines, and purines in tissue fibrosis and wound healing. In many of these processes ectonucleotidases and purinergic receptors were found to be involved. But the pathologic implications of vesicular release mechanisms remain to be studied. The importance of ectonucleotidases in several physiological and pathological processes and their potential role as biomarkers was discussed in a separate symposium on the role of ectonucleotidases in nucleotide signaling.

Purinergic signaling plays an important role in parasite-host interaction of a variety of bacterial and protozoan parasites. This involves both ectonucleotidases and purinergic receptors. A special symposium on purines in host pathogen interaction addressed the cellular mechanisms of pathogenesis as well as possible strategies against infection and for therapy, including *Leishmania*, *Toxoplasma gondii*, *Trichomonas vaginalis*, and *Legionella*.

Finally, Purines 2014 for the first time hosted a symposium on nucleotide signaling in plants. Higher plant genomes apparently lack equivalents of the P2 purinoceptors found in animals. Yet, plant cells can release ATP and other nucleotides into their extracellular matrix under conditions of stress and hydrolyze them extracellularly. ATP and ADP can induce increases in intracellular calcium and the production of reactive oxygen species and nitric oxide. Recently, a plant ATP receptor has been identified that - in contrast to the P2 receptors - is a lectin receptor kinase. It is expected that this is the beginning of a new field that will uncover the role of extracellular nucleotides in many physiological and pathological processes in plants.

The gala dinner further emphasized the familiar atmosphere of the meeting and the conference took to the

floor. During the dinner the certificates for the Burnstock lecture and John Daly lecture were presented to Francesco di Virgilio and Kenneth A. Jacobson, respectively and the five poster prizes were awarded to

Christoph Döhler (Leipzig/Germany), Lucy Robinson (Cambridge/UK), Damien Lemoine (Illkirch/France), Philipp Burow (Halle/Germany), and Takahiro Masuda (Fukuoka/Japan).

Kenneth A. Jacobson delivering the John Daly Lecture, and Francesco di Virgilio who presented the Burnstock Lecture

At the end of the conference the next Purine meetings were announced. The Purines 2016 conference entitled "Purinergic Signaling" will be held from January 24–29 at the Fairmont Hotel Vancouver in Vancouver, British Columbia, Canada. It will be sponsored by the Keystone Symposia (see <http://www.keystonesymposia.org/16J5>). The organizers are Joel Linden,

Kenneth A. Jacobson, Mark J. Smyth, and Simon C. Robson. Purines 2018 will take place in Foz do Iguaçu in Brasil. The Organizing Committee and the authors of this report express their gratitude to all scientists - senior or junior - for their excellent contributions and their enthusiasm that made Purines 2014 a great success.

Some of the organizers and participants of PURINES 2014

See you again at the next PURINES conference!