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The German Research Unit "Neuronal and glial P2 receptors; molecular basis and functional significance"

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Introduction

A highly successful initiative in the field of purinergic signalling was launched in 2007 in Germany with generous support of Deutsche Forschungsgemeinschaft (DFG). Leading scientists of high international calibre from several centres in Germany were included in the venture and together they have made substantial contributions. I asked Torsten Schöneberg and Peter Illes to write a feature article describing the history and development of this outstanding combination of research groups, which is published below.

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Purine research in Germany has had for many years a high priority in biomedical sciences, with physiologists/pharmacologists as its pioneers. These scientists were interested in the function of extracellular adenosine, especially in the brain (Ulrich Schwabe, Heidelberg, Germany; Peter Schubert, Munich, Germany), cardiovascular system (Jürgen Schrader, Düsseldorf, Germany), and kidney (Hartmut Osswald, Tübingen, Germany). From the early 1990s onward, the emphasis of the scientific activities notably shifted from adenosine to ATP. The immediate impetus for such a change in interest was the discovery of purinergic co-transmission by Geoffrey Burnstock and soon afterward the classification and cloning of purinergic receptors. New centers of purine research arose in Freiburg (Klaus Starke, Ivar von Kügelgen, and Peter Illes) and Frankfurt (Herbert Zimmermann, Günther Schmalzing, and Günther Lambrecht) engaged in deciphering the neuronal effects of nucleotides.

Scientists in Germany first coordinated their work, when Herbert Zimmermann attempted to set up in 1995 a "Collaborative Research Center" of the German Research Council (DFG) on "Nucleotides, a new and universal class of extracellular signal substances." This initiative was not funded but laid the basis for further attempts, which eventually in 2007, became successful with the establishment of the Dislocated Research Unit "Neuronal and glial P2 receptors; molecular basis and functional significance." The Correspondent for the first 3 years of funding was Peter Illes (located in Leipzig), his deputy Günther Schmalzing (located in Aachen), and the Correspondent of the second intended period of another 3 years of funding, Torsten Schöneberg (Leipzig). The purpose of the Research Unit was and is to strengthen the importance and international visibility of German P2 receptor research,

with an emphasis on purinergic mechanisms in the central nervous system, e.g., synaptic transmission, trophic and proliferative effects, activation of astrocytes and microglia, and neuron-glia cross-talk.

In addition to the scientific aims of the Unit, there was a second organizational purpose, namely to build up enduring structures for a nucleotide network coordinating research in Leipzig (Heike Franke, Peter Illes, Andreas Reichenbach, and Torsten Schöneberg), Frankfurt (Heinrich Betz, Annette Nicke, Jürgen Rettinger, and Herbert Zimmermann), Aachen (Günther Schmalzing), and Berlin (Helmut Kettenmann). The Reviewing Panel, assigned by the DFG to make a decision on our application, consisted basically of German scientists, with competence in the non-purine field, but relying heavily on the advice of a foreign member, who fortunately happened to be Geoffrey Burnstock. During the on-site reviewing procedure, it was decided to support the establishment of the Unit. However, at the same time, it was suggested to strengthen Leipzig as a national center of nucleotide research.

The scientific aims of the Unit were considered to be seminal and related to the mainstream of nucleotide research. In fact, we realized that because of the high redundancy and ligand promiscuity of P2 receptors, pharmacological approaches to elucidate the individual functions of P2 receptors are hard to control in vivo. Therefore, we established a unique collection of P2-receptor gene-deficient/or gene-modified mouse lines. Eleven single gene-deficient or gene-modified mouse lines (knockouts and transgenes) are, in fact, currently under joint investigation. In addition, we started to investigate deficiencies in a combinatory manner. One double KO mouse line was obtained from an outside source ($P2Y_1$/$P2Y_2$), and several other combinations are planned. This may help to unmask phenotype/relevance invisible in single gene-deficient mice and will be a task for future experiments.

In the meantime and just before the next panel meeting deciding about the future DFG support for our Research Unit, it may be concluded that all individual projects produced a number of relevant results during the past 3 years. These were the following: (1) the role of $P2X_3$ receptors in nociception and their interplay with P2Y receptors and TRPV1 were analyzed with considerable success. Further, mutagenesis studies aiming at the characterization of the agonist-binding site revealed groups of amino acids as potential determinants for ligand interaction. (2) The molecular mechanisms of $P2X_1$ and $P2X_2$ activation using affinity labeling methods were clarified. The results showed the ATP-binding kinetics and found that three ATP molecules are required to desensitize the receptor complex. (3) Fluorophore- and epitope-tagged $P2X_2$/$P2X_3$ transgenic mice were generated. These tools are essential to investigate receptor distribution, multimerization, and regulation as well as electrophysiological studies on primary cells expressing these P2X receptors. (4) A $P2X_7$ spice variant with an alternative transmembrane domain 1, which escapes gene inactivation in $P2X_7$ KO mice, was identified. Further, the group showed that, in contrast to most P2X receptors, $P2X_7$ forms only homotrimeric complexes. (5) The relevance of a new class of $P2Y_{12}$-like receptors was investigated. Phylogenetic and structure–function relationship studies revealed that, in contrast to previous claims, there are no residues which assign a GPCR as a nucleotide receptor. Two gene-deficient mouse lines, GPR34 and GPR82, were intensively characterized, and the involvement of these receptors in neuroinflammation and nociception were proven. (6) The relevance of P2 receptors in the glial cells of the retina was investigated. This study on P2 receptors contributed significantly to the understanding of hypoosmotic retinal swelling. These findings will have a great impact on potential therapeutic interventions for diabetic retinal edema and inflammation. (7) Gene-deficient mice, CD39 and CD73 were used to study the role of these ectonucleosidases in ATP-mediated microglia migration. The group showed that accumulation of microglia in the penumbra after experimental ischemia is reduced in CD39-deficient mice. (8) The role of nucleotides in proliferation and differentiation of fetal neural stem cells was investigated. It was shown that stimulation of $P2Y_1$ and $P2Y_2$ increases the number of dopaminergic neurons. The data indicate that nucleotides are significantly involved in differentiation and maintenance of dopaminergic neurons in embryonic CNS development.

There is a broad methodological competence available in the Research Unit, including cell biological, electrophysiological, biochemical, molecular biological, and pharmacological methods. The Medical Faculty Leipzig provides service units for DNA technologies (high throughput sequencing and microarray analysis), peptide synthesis, and signaling technologies (laser scanning and flow cytometers) as well as the so-called AlphaScreen technique (second messenger measurements, protein–protein, and protein-DNA interactions), which can be used by our groups. In addition, confocal laser scanning microscopes are available for immunohistochemistry, Ca^{2+} imaging, and the release of caged compounds by UV lasers. Further, a huge compound library for P2 ligand screening is made available, which will be performed using pipetting robots and measurement platforms.

In the past 3 years, the collaboration of the participating groups has produced about 50 publications with 10 more papers in the pipeline. Many contributions have been published in highly prestigious journals, and almost half of them are joint publications. The members of the

Research Unit are well recognized and invited as experts to contribute overviews and opinions to the major reviewing journals.

We organized in the spring of every year an informal gathering in either Leipzig, Frankfurt, or Aachen, where each group presented its recent data; lively discussions followed each presentation. A second more formal meeting of the Research Unit was organized in the autumn and fitted into the framework of an International Symposium ("2nd Italian–German Purine Club Meeting," Leipzig, 2007; "Ligand-gated cationic channels and G protein-coupled receptors in the nervous system," Leipzig, 2009) or complemented by lectures of a few invited outside speakers (Aachen, 2008). The number of participants was between 80 and 140 at the two Leipzig meetings. Further, the Research Unit contributed significantly to the organization of the "3rd Italian–German Purine Club Meeting" in Camerino, Italy, 2009 (Congress Chairperson: Gloria Cristalli).

After 3 years of successful operation, at the end of January 2010, the Research Unit will be once more evaluated. In our new concept, we followed the advice of the past reviewing panel and restricted ourselves almost completely to the Leipzig/Halle area while preserving the existing ties with Aachen and Berlin. As planned, Torsten Schöneberg will take over the position of Correspondent from Peter Illes who, however, continues to be a member of the program committee and the co-applicant of a project. We very much hope that our unique initiative, which has an enormous potential for the further development of nucleotide research in Germany, will be able to continue its work and thereby contribute to ongoing international activities.