Annual Report 2001

Pharmakologisches Institut (PKI)
der Universität Bern

Department of Pharmacology
of the University of Bern

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1. Introduction

1.1. Vorwort

Dies ist der erste umfassende Jahresbericht des Pharmakologischen Instituts der Universität Bern. Die Notwendigkeit für einen Jahresbericht ergibt sich aus den veränderten gesellschaftlichen Rahmenbedingungen. Neben einer Abrechnung der erbrachten Leistungen gegenüber der öffentlichen Hand und unserer Sponsoren bietet eine Zusammenfassung unserer Tätigkeit auch viele Chancen. Mit unseren Jahresberichten wollen wir über unsere Erfahrungen und Interessen in Lehre und Forschung informieren, um neue Partner aus akademischen Einrichtungen und der Industrie zu gewinnen.

Die Pharmakologie hat eine Brückenfunktion zwischen biologischer Grundlagen- und klinischer Forschung. Das Pharmakologische Institut ist deshalb sehr an Kontakten zu den Kliniken des Inselspitals und zu anderen Forschungseinrichtungen der Universität Bern interessiert. Damit wollen wir helfen, die klinische Forschung als auch die Weiter- und Fortbildung am Inselspital zu stärken. Zum anderen sind wir an der Zusammenarbeit mit Firmen interessiert, wie die weiter hinten aufgeführten gegenwärtigen Kontakte der einzelnen Forschungsgruppen zeigen.

Die DozentInnen und OberassistentInnen des Pharmakologischen Instituts übernehmen traditionell viel Lehrverpflichtungen. Zusätzliche Lehrbelastungen im Berichtsjahr erwuchsen aus der Aufgabe der gesamten Medizinischen Fakultät das 3. Studienjahr Medizin zu reformieren. Seit Wintersemester 2001/2002 ist das neue System „Problem-based Learning (PBL)“ im Einsatz. In der Kerngruppe zur Planung und Umsetzung des PBL-Systems ist Herr Prof. Porzig, als Pharmakologie-Fachvertreter in den einzelnen Themenblöcken sind die Herren Proff. Honegger, Porzig, Sigel und Simon vertreten. Gemeinsam mit Dozenten von Pharmakologischen Instituten anderer Universitäten arbeiten wir gegenwärtig an einem gesamt-schweizerischen Lernzielkatalog für Pharmakologie. Die Ausbildung der Zahnmedizinstudenten in Pharmakologie erfolgte weiterhin im klassischen Stil (verantwortlich: Herr Prof. Stucki). Herr Prof. Honegger wurde zum Ortspräsidenten für Pharmazie des BAG gewählt und sicherte in dieser Eigenschaft die Möglichkeit des Pharmaziestudiums an der Universität Bern in den unteren 4 Semestern ab. Eine weitere wichtige Aktivität im Rahmen der Lehre stellt unsere Arbeit innerhalb des

Programms für die Interfakultäre Ausbildung des Forschungsnachwuchses der Universität Bern (PIAF) dar.

In der Forschung erzielten die Mitarbeiter und Mitarbeiterinnen des Pharmakologischen Instituts gute Resultate. Insgesamt wurden 24 Originalarbeiten in internationalen Fachzeitschriften publiziert (Summe der „impact factors“ über 100). MitarbeiterInnen des Pharmakologischen Instituts wurden zu insgesamt 50 Vorträgen bzw. Seminaren eingeladen. Herr Dr. Zühlke wurde die Venia docendi verliehen und 4 Forscherinnen und Forscher schlossen die Doktorarbeit ab. Gegenwärtig werden 6 Mitarbeiter mit namhaften Beiträgen des Schweizerischen Nationalfonds unterstützt.

An meine Berufung an das Pharmakologische Institut war eine spezielle Aufgabe geknüpft: Die schwerpunktmässige strategische Ausrichtung der Forschung in Richtung Entzündungspharmakologie/Immunpharmakologie. Mit dem Aufbau eines funktionsfähigen Labors, welches auf diesem Gebiet aktiv arbeitet, ist ein entscheidender Schritt getan worden. Andererseits war es wichtig, die bereits vorhandenen Forschergruppen nicht einzuengen, sondern die Arbeitsbedingungen und die Infrastruktur insgesamt weiter zu verbessern. Hier ist in den nächsten Jahren noch einiges zu tun, wobei wir hoffentlich von aussen Unterstützung finden werden. In der gegenwärtigen Vielfalt unserer Forschungsinteressen sehen wir sowohl Vorteile als auch Nachteile. Die mittelfristige Forschungsstrategie des Instituts muss eine grössere Konzentration der vorhandenen personellen und finanziellen Ressourcen beinhalten.

Um in der Lehre und in der Forschung internationalen Massstäben zu genügen, müssen grosse Anstrengungen unternommmen werden. Ich danke allen Mitarbeitern und Mitarbeiterinnen für diesen Einsatz. Gleichzeitig danke ich allen Sponsoren und den Mitgliedern der Fakultät, die mir den Start in Bern erleichtert haben. Ich möchte hier den Vizedekan für Forschung, Herrn Prof. R. Friis, besonders erwähnen. Herrn Drollinger (Kantonales Hochbauamt) danke ich für die Bereitschaft das Institut im Rahmen der vorhandenen Möglichkeiten zu renovieren.

Prof. Dr. med. Hans-Uwe Simon Bern, Februar 2002
Direktor

1.2. Foreword

This is the first comprehensive report of the Department of Pharmacology of the University of Bern. The need for an annual report is the result of the changing conditions of our society. An annual report does not only summarize the results of the performed work, it also offers the chance to inform potential new partners from academic and industrial institutions about our teaching and research activities.

Pharmacology fulfils functions in both biological basic science and clinical research. The Department of Pharmacology wants to succeed in both directions and is therefore very much interested in intense contacts to the clinics of the University Hospital (Inselspital) as well as to the different research institutes of the University of Bern. We hope to help to strengthen both research and teaching at the Inselspital. On the other hand, we are very much interested in collaborating with the industry on new developments. Current activities are listed in this report.

The scientific staff of the Department of Pharmacology has traditionally taken over a considerable teaching burden of the Medical Faculty. An additional burden was the reformation of the third study year of medical students, one of the current central tasks of the Faculty. Since October 2001, we teach within the new „Problem-based Learning (PBL)“-system. One of the principal investigators of the institute, Prof. Porzig, is a member of the core group. As specialists for Pharmacology, the professors Honegger, Porzig, Sigel, and Simon contribute in all of the thematic teaching blocks. Besides the work on the study material for the PBL-system at the University of Bern, we currently work together with colleagues from the other Swiss universities on a catalog, which defines the teaching goals in Pharmacology for medical students. The teaching of dental medical students in Pharmacology was performed in the classical style, but a reformation of this course is also in progress. Responsible teacher here is Prof. Stucki. Prof. Honegger has been appointed as the local president of the Pharmacy society and helped to maintain the undergraduate study in Pharmacy at the University of Bern at least within the first two study years. Another important teaching activity is demanded within the graduation program for MD/PhD students of the University of Bern (PIAF).

The Department of Pharmacology has also successfully performed research in 2001. Together, we published 24 original articles in international peer-reviewed journals (the sum of the “impact factors” is greater than 100). Co-workers of the institute were invited to 50 lectures or seminars. Dr. Zühlke received the „Habilitation“ and 4 young scientists received the PhD degree. These numbers speak for themselves and demonstrate the high standard of the research. Thanks to the good work, 6 co-workers are currently supported by grants of the Swiss National Science Foundation.

My appointment to the Department of Pharmacology was associated with a special task. The Medical Faculty wishes to establish a new main direction of research in our department and this is „Pharmacology of inflammation“ as part of a strategic plan to strengthen research in inflammation and immunology in Bern. We have created a fully functioning and active laboratory in this research direction as a first step to fulfil this goal. On the other hand, it has been important not to restrict the already established research groups. Instead, we managed to further improve the working conditions for all groups. However, we still absolutely require some expensive equipment to continue with internationally competitive research. In the variety of our current research interests, we see both advantages and disadvantages. The strategy for the future development of the department includes a higher degree of concentration in our research topics.

In order to meet international standards in teaching and research, great efforts, and efficient work are required from our co-workers. I thank all co-workers for their hard work. I also thank all the sponsors as well as those members of the Medical Faculty, who helped me in performing a good start in Bern. In particular, I would like to mention the Vice Dean for Research, Prof. Friis. We are indebted to the Cantonal Office for reconstruction, Mr. Drollinger, for their readiness to renovate our building.

Prof. Hans-Uwe Simon, MD, PhD Bern, February 2002
Director

2. Staff 2001

Director

Prof. Dr. Hans-Uwe Simon, MD, PhD

Deputy Director

Prof. Dr. Hartmut Porzig, MD

Permanent Members

Prof. Dr. Ulrich E. Honegger, PhD
Prof. Dr. Hartmut Porzig, MD
Prof. em. Dr. Harald Reuter, MD
Prof. Dr. Erwin Sigel, PhD
Prof. Dr. Hans-Uwe Simon, MD, PhD
Prof. Dr. Jörg Stucki, PhD

Scientific Staff

Frank Altznauer, PhD student
Dr. Kurt Baltensperger, PhD
Roland Baur, head technician
Ralf Baumann, PhD student
Dr. Sabine Baumann, PhD*
Charlotte Becker, head technician (until June 2001)
Dmytro Berezhnoy, PhD student (since October 2001)
PD Dr. Andreas Buhr, PhD (until January 2001)*
Dr. Sibylle Bürgi, PhD
Kristien De Cock, PhD student (since November 2001)*
Gian-Franco De Marquis, MD student*
Karin Kirschner, PhD student
Ivana Kotevic, PhD student (since May 2001)*
Evelyne Kozlowski, technician (since July 2001)*
Sibylla Martinelli, PhD student (since April 2001)*
Dr. Frédéric Minier, PhD (since December 2001)*
Yves Nyfeler, M.Sc. student (until September 2001)*
Susanne Probst, technician*
Dr. Claes Ruedeberg, PhD, consultant*
Martin T. Schärer, PhD student (until September 2001)
Inès Schmid, head technician
PD Dr. Alessandra L. Scotti, PhD (until August 2001)*
Olivier Thomet, PhD student (until March 2001)*
Heleen van Hees, technician (until November 2001)
Ekaterina Vassina, PhD student
Anton Vichalkovski, PhD student (since July 2001)*
Dr. Clemens Wagner, PhD
Raphael Wirth, MD student*
Adrian Wirz, PhD student*

Karl Wittwer, MD student (since September 2001)*
Dr. Shida Yousefi, PhD
PD Dr. Roger D. Zühlke, PhD (until April 2001)

External University Teachers

PD Dr. Armand Cachelin, MD, PhD*
PD Dr. Stefan Mühlebach, PhD*
PD Dr. Uwe Zangemeister-Wittke, PhD (since October 2001)*
PD Dr. Roger D. Zühlke, PhD (since October 2001)*

External Computer Support
Faton Shala*

Guest Scientists

Dr. Bernard Foucaud, PhD, Strassburg, France (September 2001)*
Dr. Daniel Aeberli, MD, Dept. of Rheumatology, Inselspital, Bern (since Sept. 2001)*
Tuomas Hulkko, MD student, Oulu, Finland (July 2001)*

Office

Erika Fritsche, head secretary
Peggy Shala, secretary
Franziska Marti*, secretary to Prof. Reuter

Workshop

Hans Andres

House Keeping

Maria Di Loreto
Esther Weber

*paid from external sources, mostly research grants

3. Teaching Activities
3.1. Lectures

Lectures for medical students

Date	Lecturer	Titel of the lecture
Jan 08, 2001	Prof. Hans-Uwe Simon	Respirationstrakt: Bronchodilatoren, Glukokortikoide
Jan 09, 2001	Prof. Ulrich E. Honegger	Schmerzmittel: Analgetika, Antiphlogistika (I)
Jan 09, 2001	Prof. Ulrich E. Honegger	Schmerzmittel: Analgetika, Antiphlogistika (II)
Jan 15, 2001	Prof. Hartmut Porzig	Schmerzmittel: Lokalanästhetika
Jan 16, 2001	Prof. Hans-Uwe Simon	Respirationstrakt: Husten
Jan 17, 2001	Prof. Ulrich Honegger	Antiparkinsonmittel
Jan 17, 2001	Prof. Ulrich Honegger	Antiepileptika
Jan 30, 2001	Prof. Hans-Uwe Simon/ Prof. Hartmut Porzig/ Prof. Ulrich Honegger	Allgemeine Pharmakologie
Feb 21, 2001	Prof. Hans-Uwe Simon	Überempfindlichkeit
Feb 21, 2001	Prof. Hans-Uwe Simon	Zytokine, Zelltherapie, Immunstimulantien
Mar 07, 2001	Prof. Hartmut Porzig	Diuretika (I)
Mar 07, 2001	Prof. Hartmut Porzig	Diuretika (II)
Apr 11, 2001	Prof. Ulrich Honegger	Neuropharmakologie: Grundlagen
Apr 11, 2001	Prof. Ulrich Honegger	Psychopharmaka: Wirkungsmechanismen
Apr 17, 2001	Prof. Ulrich Honegger	Neuroleptika
Apr 18, 2001	Prof. Ulrich Honegger	Anxiolytika
Apr 18, 2001	Prof. Ulrich Honegger	Sedativa, Hypnotika
Apr 27, 2001	Prof. Ulrich Honegger	Antidepressiva

Apr 27, 2001	Prof. Ulrich Honegger	Psychopharmaka: Zusammenfassung
May 09, 2001	Prof. Hartmut Porzig	Toxikologie – Übersicht, Testmethoden
May 14, 2001	Prof. Hartmut Porzig	Vergiftungen und Gegenmassnahmen
May 14, 2001	Prof. Hartmut Porzig	Spezielle Toxikologie
May 16, 2001	Prof. Hans-Uwe Simon/ Prof. Hartmut Porzig/ Prof. Ulrich Honegger	Spezielle Pharmakologie
May 21, 2001	Prof. Hartmut Porzig	Spezielle Toxikologie (I)
May 21, 2001	Prof. Hartmut Porzig	Spezielle Toxikologie (II)
May 23, 2001	Prof. Hans-Uwe Simon/ Prof. Hartmut Porzig/ Prof. Ulrich Honegger	Spezielle Pharmakologie, Toxikologie
May 29, 2001	Prof. Hans-Uwe Simon	Interessante neue Pharmaka
May 30, 2001	Prof. Hans-Uwe Simon/ Prof. Hartmut Porzig/ Prof. Ulrich Honegger	Spezielle Pharmakologie, Toxikologie
Oct 25, 2001	Prof. Hans-Uwe Simon	Grundlagen der Arzneimittel-anwendung, Pharmakodynamik
Oct 29, 2001	Prof. Ulrich Honegger	Einführung in die Pharmakokinetik
Nov 01, 2001	Prof. Hartmut Porzig	Toxikologische Grundbegriffe, Vergiftung
Nov 05, 2001	Prof. Hans-Uwe Simon	Entzündungshemmung
Nov 13, 2001	Prof. Hartmut Porzig	Autonomes Nervensystem (2. Studienjahr)
Nov 23, 2001	Prof. Ulrich Honegger	Pharmakotherapie im Alter (4. Studienjahr)
Nov 29, 2001	Prof. Hans-Uwe Simon	Pharmakotherapie bei Lungenkrankheiten

Dec 06, 2001	Prof. Hartmut Porzig	Antithrombotische Therapie
Dec 13, 2001	Prof. Hartmut Porzig	Pharmakologie des sympathischen Nervensystems
Dec 13, 2001	Prof. Hartmut Porzig	Wirkprinzipien von Antihypertonika
Dec 20, 2001	Prof. Hartmut Porzig	Antiischämische und antiatherogene Substanzen

Lectures for dental students (Coordinator: Prof. Dr. J. Stucki)

Date	**Lecturer**	**Title of the lecture**
Jan 08, 2001	PD Dr. Armand Cachelin	Starke Analgetika
Jan 10, 2001	PD Dr. Roger D. Zühlke	Lokalanästhetika (I)
Jan 15, 2001	PD Dr. Roger D. Zühlke	Lokalanästhetika (II)
Jan 17, 2001	PD Dr. Roger D. Zühlke	Insulin, Orale Antidiabetika
Jan 22, 2001	Prof. Erwin Sigel	Anxiolytika, Hypnotika
Jan 24, 2001	Prof. Ulrich Honegger	Psychopharmaka
Jan 29, 2001	PD Dr. Armand Cachelin	Immunsuppressiva
Jan 31, 2001	Prof. Ulrich Honegger	Antihistaminika
Feb 05, 2001	Prof. Erwin Sigel	Antikoagulantien, Plättchenhemmer
Feb 07, 2001	Prof. Ulrich Honegger	Lokale Präparate
Feb 12, 2001	PD Dr. Stefan Mühlebach	Antibiotika (I)
Feb 14, 2001	PD Dr. Stefan Mühlebach	Antibiotika (II)
Feb 19, 2001	PD Dr. Stefan Mühlebach	Antibiotika (III)
Feb 21, 2001	Prof. Jörg Stucki	Repetitorium
Oct 29, 2001	Prof. Jörg Stucki	Rezeptoren, Signalwege
Oct 31, 2001	Prof. Jörg Stucki	Dosis-Wirkungskurven
Nov 05, 2001	Prof. Jörg Stucki	Antagonisten

Nov 07, 2001	Prof. Jörg Stucki	Barrieren, Absorption, Verteilung
Nov 12, 2001	Prof. Jörg Stucki	Bioavailability, Ausscheidung
Nov 14, 2001	Prof. Jörg Stucki	Arzneimittelmetabolismus
Nov 19, 2001	Prof. Jörg Stucki	Gesamtkinetik, Dosierung
Nov 21, 2001	Prof. Jörg Stucki	Toleranz, Abhängigkeit
Nov 28, 2001	Prof. Jörg Stucki	Chronopharmakologie
Dec 03, 2001	Prof. Jörg Stucki	Ergänzungen, Repetitorium
Dec 05, 2001	Prof. Erwin Sigel	Schwache Analgetika (I)
Dec 10, 2001	Prof. Erwin Sigel	Schwache Analgetika (II)
Dec 12, 2001	Dr. Kurt Baltensperger	Sympathikus
Dec 17, 2001	Dr. Kurt Baltensperger	Kreislaufpräparate (I)
Dec 19, 2001	Dr. Kurt Baltensperger	Kreislaufpräparate (II)

3.2. Coordination PBL Medical Students, 3. year (2001/2002)

Core group:
Prof. Hartmut Porzig

Representatives of Pharmacology in teaching blocks:
Prof. Ulrich E. Honegger (blocks V and VI)
Prof. Hartmut Porzig (blocks II and III)
Prof. Erwin Sigel (block IV)
Prof. Hans-Uwe Simon (blocks I and VII)

3.3. Tutorials (study year 2001/2002)

Medical students 2. year:
Dr. Kurt Baltensperger

Medical students 3. year:
Dr. Sibylle Bürgi
Prof. Ulrich E. Honegger
Prof. Erwin Sigel
Prof. Hans-Uwe Simon
PD Dr. Uwe Zangemeister-Wittke

3.4. Seminars of Invited Speakers

Date	Teacher	Title of the seminar
Jan 10, 2001	PD Dr. A. Walz University of Bern	Function of CXC chemokines in acute inflammation
Jan 17, 2001	Prof. Dr. D. Bertrand University of Geneva	Nicotinic receptors in genetically transmissible neurologic disorders and consequences of chronic nicotine exposure
Jan 24, 2001	Prof. Dr. I. Mansuy ETH Zurich	Genetic manipulation of learning, memory and synaptic plasticity with calcineurin and its inhibitor
Jan 31, 2001	Prof. Dr. B. Erni University of Bern	The bacterial phosphotransferase system (PTS): an interface between energy and signal transduction
Feb 14, 2001	PD Dr. A. Wodnar-Filipowicz University of Basel	Transduction of cord blood hematopoietic progenitors with lentiviral vectors expressing GFP and natural killer cell activatory receptors
May 02, 2001	Prof. Dr. G. Folkers ETH Zurich	Computational and experimental biophysics in the design of genetic switches
May 16, 2001	PD Dr. U. Zangemeister-Wittke University of Zurich	The antisense approach to facilitate tumor cell apoptosis: the promise of targeting apoptosis inhibitors
May 30, 2001	Dr. C. Fromond Novartis, England	Long-term protective and specific effect of heatkilled mycobacterium vaccine on the allergic pulmonary inflammation in immunized mice
Jun 06, 2001	Prof. Dr. K. Hofbauer University of Basel	Careers in drug discovery: requirements and perspectives
Jun 13, 2001	Dr. S. Kellenberger University of Lausanne	Potential mechanisms of ion permeation and gating of the epithelial Na channel EnaC
Jun 20, 2001	Prof. Dr. D. Hoessli University of Geneva	Rafts/microdomains: functional platforms at the lymphocyte surface
Jun 28, 2001	PD Dr. T. Hartung University of Konstanz	Bakterielle Immunstimuli – Mechanismen der Zellaktivierung und Immunmodulation

Jun 28, 2001	Dr. I. Diterich University of Konstanz	Immunmodulation durch Borrelien – Mechanismen und klinische Konsequenzen
Aug 06, 2001	Dr. H. Toplak University of Graz	Typ-2 Diabetes mellitus bei Kindern: Eine neue Herausforderung
Sep 19, 2001	Prof. Dr. F. Levi-Schaffer University of Jerusalem	Mast cells/eosinophils interactions in allergic and non-allergic diseases
Nov 21, 2001	Prof. Dr. C. Müller University of Bern	Role of TNF in immunity
Nov 28, 2001	Prof. Dr. J.-M. Dayer University of Geneva	Controlling the production of IL-1 and TNF during cell-cell interaction
Dec 06, 2001	Prof. Dr. K.-H. Krause University of Geneva	Stem cells and cell therapy: facts, concepts and questions
Dec 19, 2001	Dr. Y. Arsenijevic University of Lausanne	Adult neural stem cells: a potential for the clinic?

In addition, the scientific staff of the institute meets to discuss ongoing research projects and recently published work each Tuesday at 5 pm.

3.5. Academic Degrees

Roger Zühlke, PD Dr. pharm.
Habilitation (Privatdozent) in Pharmacology
University of Bern, April 2001

Olivier Thomet, Dr. pharm.
Thesis: „Antiinflammatory effects of Petasites hybridus (Ze339) and its compounds in vitro and evidence for in vivo efficacy in allergic rhinitis patients“.
University of Bern, March 2001

Sabine Baumann, Dr. phil. nat.
Thesis: „$GABA_A$ receptors: Subunit arrangement and novel interaction partners“.
University of Bern, May 2001

Sibylla Bürgi, Dr. phil. nat. ETHZ
Thesis: „Antidepressiva-induzierte β-Adrenoceptor-Downregulation. Biochemische, molekularbiologische und konfokalmikroskopische Untersuchungen“.
University of Bern and ETH Zürich, July 2001

Martin Schärer, Dr. phil. nat.
Thesis: „Characterization of a protein associated with the $GABA_A$ receptor“.
University of Bern, November 2001

Yves Nyfeler, lic. phil. nat.
Thesis: „Zur Aufklärung der Struktur der Benzodiazepin-Bindungsstelle“.
University of Bern, October 2001

4. Research Activities

4.1. Research Projects and Publications

Group Prof. Ulrich E. Honegger

Group members: Dr. Sibylle Bürgi, PhD
Adrian Wirz, PhD student
Raphael Wirth, MD student
Gian-Franco De Marquis, MD student
Susanne Probst, technician
Dr. Claes Ruedeberg, PhD, consultant

Our main interests focus on antidepressant drugs in particular on the elucidation of their modes of action. We have for many years concentrated on classical well established tricyclic compounds but have lately expanded our research efforts to the pharmacology of plant extracts. Studies are performed in *in vitro*-systems including cultured cells and brain slices of rats. Beside of this main research line we are using fibroblasts cultured from skin biopsies of patients to investigate signal pathways as a diagnostic mean. Cell culture models are also used for the study of COX-inhibitory properties of plant extracts and for the investigation of the kinetic behaviour of lipophilic, persistent polyhalogenated compounds.

A closer look to the antidepressant-induced mechanism of β-adrenoceptor downregulation

S. Bürgi, K. Baltensperger, U.E. Honegger
(Collaboration with Prof. Dr. G. Folkers, ETH Zurich)

Long term use of most antidepressants leads to a reduction in the number of functional β–adrenoceptors_ in the central postsynaptic membranes and in cultured cells. The effect timely coincides with the clinical improvement of depressive symptoms in humans. The exact mechanism of β–adrenoceptor down-regulation is currently unknown. We have previously shown that chronic antidepressant exposure of cultured rat astrocytoma cells impair cellular phospholipid (PL) metabolism and specifically change membranous PL-composition. The aim of this study was to investigate whether the drug-induced changes in the membrane properties could influence β–adrenoceptor endocytosis and recycling. An increased rate of receptor endocytosis and/or an inefficient receptor recycling could cause a reduced β–adrenoceptor density in the plasma membrane of chronically tricyclic antidepressant-treated astrocytoma cells. To detect drug-induced changes in β–adrenoceptor endocytosis and recycling not only in radioligand binding studies but also by laser scanning confocal microscopy, a β1–adrenoceptor – green fluorescent protein (β1–AR-GFP) fusion construct was stably expressed in astrocytoma cells. Confocal fluorescence microscopy revealed that in antidepressant-treated cells agonist-induced (β1–AR-GFP internalization was no longer reversible in contrast to internalized receptors in untreated control cells. Since the recycling of transferrin receptors was not altered in antidepressant treated cells we could conclude that

drug-induced changes in β–_adrenoceptor recycling were receptor specific and not a consequence of an impaired membrane recycling. A closer look to the β–_adrenoceptor molecule in antidepressant-treated cells revealed changes in the amount of phosphorylation and glycosylation which might interfere with recycling. It is our current and future interest to investigate the role of these molecular changes for recycling and thus down-regulation.

Chronic antidepressant-induced changes in recycling of serotonin receptor subtypes

A. Wirz, S. Bürgi, U.E. Honegger

From our studies on antidepressant-induced alterations in the recycling of β1–_adrenoceptors we know that this phenomenon is rather receptor-specific than a general one. From *in vivo*-studies with serotonin receptors it has been shown that the 5-HT2 subtype is down-regulated following chronic antidepressant treatment while number and characteristics of the 5-HT1 subtype are not affected by the antidepressant drug treatment. In analogy to the β1–_adrenoceptor – green fluorescent protein fusion construct we are currently transfecting COS cells transiently and astrocytoma cells permanently with 5-HT receptors-GFP fusion constructs. This should allow us to study functionality and trafficking of these receptor types in control and antidepressant exposed cells. The aim will be to distinguish the differences in behavior of these receptor subtypes in response to antidepressants and to correlate it with differences in molecular alterations such as phosphorylation or glycosylation.

Pharmacology of St.John's wort (hypericum perforatum) extracts, their fractions and individual constituents

S. Probst, C. Ruedeberg, U.E.Honegger

St. John's wort extracts are widely and successfully used in the treatment of mild and moderate forms of depression. *In vitro*-test systems are routinely used in our laboratory to investigate antidepressant effectiveness. A model to simulate the acute effects of antidepressant compounds on neurotransmitter reuptake is the use of freshly prepared rat brain slices. The constantly oxygenated slices are incubated with radioactively labelled 3H-norepinephrine or 3H-serotonin in the presence and the absence of hypericum extract, fractions and individual compounds of it, such as hypericine or hyperforine and with 10 μM imipramine as a measure for 100% uptake inhibition. The extracts showed a dose-dependent inhibition of neurotransmitter uptake which was equally efficient in the case of serotonin but less efficacious for norepinephrine. It was of interest to see that both purified compounds, suspected to be part of the active principle were without any effect on neurotransmitter uptake. Fractions of the whole extract were the more potent the more apolar the contents were. A very similar picture was seen when the chronic effects were investigated on down-regulation of β–adrenoceptors of extract-exposed astrocytoma cells. The plant extracts their fractions and the individual compounds showed a similar efficacy on receptor down-regulation as on neurotransmitter uptake inhibition.

Cultured skin fibroblasts of a patient with an Albright syndrom (Albright hereditary osteodystrophy, AHO) as a mean to localise the molecular impairment of the G protein-dependent cAMP formation

R. Wirth, U.E. Honegger

(Collaboration with Prof. Dr. U. Wiesmann, Children Hospital Bern)

AHO patients are characterised by a form of hypoparathyreoidism which involves an impaired formation of cAMP following the stimulation of PTH. Since these patients suffer from multiple symptoms indicating a general defect that influences many physiological functions we assumed a possible fault at the level of the G-protein. Skin fibroblasts were extensively used in our laboratory to investigate β-adrenoceptor-dependent cAMP formation, we studied this signal pathway in cells of an AHO-patient. We compared the cAMP-response in cultured skin fibroblasts of the patient with that of a sex and age-matched healthy person. cAMP-formation following isoprenaline stimulation was drastically reduced in the patient's cells compared to that in the control fibroblasts. The same was true for the prostaglandin-E2 stimulated cAMP-formation. It was, however, of interest to see that adenylyl cyclase activity was more responsive to the direct enzyme stimulation by forskoline. Thus cAMP-formation in cells of AHO patients seems to be affected by a general impairment of the stimulating G-protein.

Cultured human skin fibroblasts as a model system to test drug-induced COX inhibition

S. Probst, U.E. Honegger
(Collaboration with Prof. Dr. K. Hostettmann, Institut de Phytochimie et Pharmacognosie, Université de Lausanne)

We know from own previous work that human skin fibroblasts and in particular foreskin cells possess an active cyclooxygenase system. It can be induced by interleukin-1 to express the inducible COX-2 subtype beside of the constitutive COX-1 form. The enzymes can be stimulated by bradykinin to form prostanoids which can be measured by means of ELISA kits. Induced and non-induced foreskin fibroblast cultures are used to screen potential subtype-selective COX-inhibitory plant extracts and to compare their effectiveness with synthetic analgesic and antiphlogistic compounds.

Cellular kinetics of persistent compounds with endocrine effectiveness

S. Probst, S. Mühlebach, U.E. Honegger
(Collaboration with PD Dr. S. Mühlebach, Kantonsspital Aarau)

Persistent lipophilic compounds are relevant representatives of environmental contaminants. They have entered the biosphere partly unintended from waste deposits, through leakage from closed systems or as residues of incineration due to resistance to high temperature. They show chemical blockage by chloro- or bromo-substitution of metabolically vulnerable positions in the molecule, e.g. of lipophilic aromatic ring systems normally degradable by cytochrome P_{450} enzymes as shown in PCB or DDT derivatives. The global distribution and marked bioaccumulation of such compounds through the food chain is a consequence of their extreme lipophilicity and high level of metabolic resistance leading to persistence in fat deposits eventually in man. There is little knowledge on mechanisms of fat storage and release of such compounds nor is a simple test method available to screen new chemical entities for their potential of bioaccumulation. To study more thoroughly ecotoxicological aspects of such compounds their kinetic behaviour has to be characterised in defined test models such as cell culture systems using well-defined and reproducible conditions. Apart from methodological studies to establish useful screening or test systems with representative cell lines to imitate important uptake and storage organs like fat, brain or skin, such cell culture systems allow to study toxicokinetics of selected model compounds using varying experimental conditions. Specific interactions with defined receptors may be investigated which may have relevance for acute or long-term

effects. An ultimate goal will be to establish structure-effect correlations for a better ecotoxicological risk assessment of new chemical compounds developed and released into defined technical application fields. The aim of this study was to define methodological and experimental conditions in single and multiple (sector) cell culture systems (lit) using fibroblasts, adipocytes (differentiated 3T3 cells) and astrocytoma C6 cells. Selected model compounds with different molecular size and degree of halogenization were investigated. From our present results we can conclude that *in vitro*-cell culture systems are useful tools for the pharmacokinetic screening of highly persistent lipophilics. A correct and stable solution of these compounds in the culture media can best be achieved after incorporation into liposomes. The use of different cell types with distinct properties allows to detect differences in cell-specific kinetics and storage of lipophilics. The combination of up to four sectors covered with monolayers of different cell types in one plate represents a simple *in vitro*-system to analyze competitive cellular uptake of persistent lipophilic contaminants. Extents of uptake and accumulation were drug- and cell-specific. Rates of uptake were fast and reached equilibrium within 15 minutes.

Original publication

1. U. Kientsch, S. Bürgi, C. Ruedeberg, S. Probst, U.E. Honegger:
St.John's wort extract Ze 117 (Hypericum perforatum) inhibits norepinephrine and serotonin uptake into rat brain slices and reduces_β–adrenoceptor numbers on cultured rat brain cells.
Pharmacopsychiatry 34 (2001), 56-61.

Review articles

1. U.E. Honegger:
Vom Mauerblümchen zum Kraut der Hoffnung.
Praxis 90 (2001), 1131.

2. U.E. Honegger, U. Kientsch, S. Probst, C. Ruedeberg:
In vitro Untersuchungen zum Wirkungsmechanismus von Johanniskraut-Extrakt ZE 117.
Praxis 90 (2001), 1137.

3. U.E. Honegger:
Phytopharmaka im Wechselbad der Zulassungsbehörden.
Phytotherapie 1 (2001) 26-27.

Books

1. A. Seidenberg, U. Honegger (translation: I. Gebele)
Metadona Heroina y Otros Opioides
Verlag Diaz de Santos, Madrid, Spain (2001).

2. A. Seidenberg, U. Honegger (translation: T. Will)
Méthadone, Héroïne et autres Opioides.
Verlag Médecine & Hygiène Genève, Paris (2001).

3. J. Schöpf, U. Honegger
Interaktionen in der Psychopharmakotherapie.
Steinkopff Verlag, Darmstadt (2001).

Group Prof. Hartmut Porzig

Group members: Dr. Kurt Baltensperger, PhD[1]
Karin Kirschner, PhD student
Ivana Kotevic, PhD student (since May 2001)
Anton Vichalkovski, PhD student (since July 2001)

[1]In addition, independent research work with own Swiss National Science Foundation projects.

The research interests of our group center on mechanisms regulating proliferation and differentiation of human hematopoietic, in particular erythroid progenitor cells. In principle, during blood cell formation there are three major problems that have to be solved: (1) maintain a constant pool of undifferentiated stem cells, (2) regulate proliferation and lineage commitment according to the overall needs of the body, (3) maintain a constant number of terminally differentiated blood cells. To reach these objectives, a host of humoral signals participate in determining the fate of hemato-poietic progenitor cells. Best known among these is the cytokine family of peptide growth factors acting via stimulating cellular tyrosine kinases. In recent years it became increasingly clear that the effects of cytokines are modulated by signals that act via G protein-linked receptors. This latter group includes, among others, chemokines, thrombin, purines and lipids and constitutes the focus of our most recent research projects. The cross-talk between cytokine - and G protein-coupled receptors – induced signal transduction pathways is independently investigated by Dr. K. Baltensperger. Of particular interest in this respect is the development of new strategies for the treatment of malignant diseases, such as leukemia.

A second line of ongoing research deals with functional aspects of the sodium/ calcium exchanger protein in cardiac cells and the expression pattern of its three major subtypes in primary neuronal cell cultures. This membrane transport system is an important element in maintaining cellular Ca^{2+} homeostasis in excitable cells.

The interaction of G proteins and protein kinase C with BCR/ABL tyrosine kinase-dependent signaling in human leukemia cells

K. Kirschner, K. Baltensperger, H. Porzig

Typically, leukemic cells are resistant towards physiological signals inducing terminal differentiation in hematopoietic cells because they have acquired a mechanism providing autonomous and irreversible stimulation of cell proliferation. One such mechanism is the induction of BCR/ABL tyrosine kinase activity in chronic myelogenous leukemia generated by a chromosome translocation. This kinase permanently promotes cell division by stimulating key intermediates within the signaling pathway of cytokine growth factors. Earlier studies in our lab have identified

mechanisms that seem capable of enforcing cytokine-independent differentiation via the hematopoietic cell-specific G protein, $G_{\alpha 16}$, and protein kinase C [PKC]. In the present project, we investigate whether this alternative pathway is still functional in BCR/ABL-transformed cells and could be used as a target for drugs to initiate terminal differentiation and consequently, to block malignant cell growth. Most of the work is done in a human erythroleukemia cell line that does not express BCR/ABL and can still be induced to differentiate with cytokines or by activating the G_{16}-dependent pathway. After co-transfection of these cells with BCR/ABL and dominant active mutants of Gα16 and PKC, we shall monitor the resultant changes in rate and extent of differentiation with a reporter gene assay. The difficult task of transfecting hematopoietic cells with high efficiency has been solved by adapting the lentivirus-based transduction method developed by Dr. D. Trono and coworkers in Geneva.

Modulation of cytokine signaling by thrombin and SDF-1 during growth and differentiation of hematopoietic progenitor cells

A. Vichalkovski, H. Porzig

While hematopoietic progenitor cells express more than ten different G protein-coupled receptors, their functional role in cell development has remained obscure. We focus on signaling mechanisms linked to receptors for SDF-1 and thrombin because both agonists are already known to modulate growth responses in some cell types possibly via pathways including protein kinase C (PKC) and/or the small GTPase Rho. Thrombin has been shown to inhibit erythropoietin (Epo) and stem cell factor (SCF)-dependent cell proliferation, while SDF-1 was reported to enhance hematopoietic cell growth. Based on our previous experience we currenly explore to what extent the cytokines TPO, SCF, IL-3, GM-CSF and Epo affect the function of thrombin and SDF-1 during lineage commitment and differentiation of erythroid and granulocyte/macrophage differentiation. Multipotent hematopoietic progenitors that carry the CD34 antigen and that we isolate from peripheral blood (in cooperation with the Department of Hematology, University of Bern) or from cord blood (in cooperation with the Woman's Hospital, University of Bern) are kept in suspension culture and grow in the presence of different cytokine combinations. In parallel to the described studies in primary cells which are usually in short supply, we are using established hematopoietic cell lines (HEL, MB-02, HL-60) to study specific aspects of SDF-1 mediated signal transduction. First results using ^{14}C-Thymidine incorporation in suspension culture of primary cells after a standardized starvation protocol, suggest that SDF-1 exerts its growth promoting effect via a G protein of the G_{12} family and the small GTPase RhoA, but, surprisingly, unlike most cytokines and G protein-coupled receptor ligands, does not seem to target PKC. Moreover, cytokine responsiveness and SDF-1 or thrombin sensitivities appear strongly interrelated. Stem cell factor(SCF)-dependent early progenitors show strong SDF-1 responses while thrombin affects almost exclusively erythropoietin-dependent terminally differentiating cell populations. Similarly, overexpression of the SDF-1 receptor CXCR4 in HEL cells using the retroviral vector mentioned above, strongly down-regulates the thrombin-sensitivity of these cells. In view of the antagonistic effects of SDF-1 and thrombin on cell proliferation, such mutual exclusive expression of G protein-dependent signaling pathways might point to a novel regulatory principle in progenitor cell development.

Immunohistochemical analysis of sodium/calcium exchanger expression in rat hippocampus cultures

H. Porzig
(Collaboration with K.D. Philipson, UCLA, Los Angeles, CA)

Na^+/Ca^{2+} exchange activity is known to be expressed throughout the brain in both glial and neuronal tissue. mRNA of all three major subtypes of the mammalian Na^+/Ca^{2+} exchanger protein (NCX1, NCX2, NCX3) has been detected in most brain areas, albeit at varying densities. However, for lack of subtype specific labels, the cellular expression pattern of this transport protein has remained largely unknown. We have used three subtype-specific antibodies, two monoclonal and one polyclonal, to identify the cellular distribution of the exchanger subtypes in rat hippocampus cell cultures. Surprisingly, we found little overlap for the expression of this membrane protein in different cell types. NCX1 labeled almost exclusively the membranes of neuronal cells and their associated dendritic network. It was found in nearly all neuronal cells of the population growing in culture. NCX2 was predominantly localized in various types of glia cells. Only occasionally it was detected in membranes of neuronal cell bodies but never in the dendritic network. In addition to labeling membranes, the NCX2 antibody strongly cross-reacted with an unidentified glial fibrillar protein. NCX3 expression appeared very low in hippocampus cultures and was restricted to a small subpopulation of neuronal cells. It was never detected in glia cells. Our results provide novel information on the cell-specific expression of the three Na^+/Ca^{2+} exchanger subtypes (NCX1, NCX2 and NCX3) in mammalian brain. These data may reflect functional differences among the subtypes that are not obvious from studies in recombinant cell lines and hence, may help to understand the functional role of specific glia-associated Ca^{2+} transport systems.

Remodeling of the Na^+-Ca^{2+} exchange activity in rat cardiac myocytes after infarction

A.M. Gomez, B. Schwaller, H. Porzig, G. Vassort, E. Niggli, M. Egger
(Collaboration with E. Niggli, Dept. of Physiology, University of Bern, and B. Schwaller, Dept. Histology and General Embryology, University of Fribourg)

Hypertrophied and failing cardiac myocytes show prolonged $[Ca^{2+}]_i$ transients. While it is widely accepted that the sarcoplasmic reticulum (SR) Ca^{2+} pump function is decreased in hypertrophied myocytes, the possible involvement of the Na^+-Ca^{2+} exchange (NCX) in the $[Ca^{2+}]_i$ transient lengthening remains uncertain. The cardiac NCX is one important mechanism for Ca^{2+} extrusion and cell relaxation, together with the SR Ca^{2+} pump. We analyzed the NCX function in rat ventricular myocytes 5-6 months after experimental myocardial infarction, produced by left coronary artery ligation in rats. Caged Ca^{2+} was dialyzed into the cytoplasm via a patch-clamp pipette and released by flash photolysis to activate NCX and measure the associated current (I_{NaCa}), while $[Ca^{2+}]_i$ changes were simultaneously recorded with a confocal microscope. Cells from post-myocardial infarction (PMI) rats had a 2.2 fold larger membrane surface and a more marked (about 2.75 fold) myocyte volume increase compared to sham-operated animals (SO). I_{NaCa} density activated by $[Ca^{2+}]_i$ jumps of comparable amplitudes was significantly higher in myocytes from PMI rats (263.9%). This increase was also reflected at the level of total NCX protein expression, where values were higher (180±11% of control) in PMI myocytes. Surprisingly, although the I_{NaCa} in PMI cells was larger, the Ca^{2+} transport rate was maintained. Interestingly, PMI and SO myocytes presented virtually identical NCX Ca^{2+} transport rates per cell volume. We conclude that the increase in NCX density may constitute an adaptive

response rather than a causal change, intended to maintain the required Ca^{2+} extrusion from a larger volume, to allow for adequate relaxation.

G protein-dependent signal transduction and induction of differentiation in hematopoietic cells

I. Kotevic, K. Baltensperger

Our studies are aimed at identifying “upstream” and “downstream” signaling elements of G_{16}, a G protein exclusively expressed in hematopoietic cells. A mutant GTPase-deficient and thus constitutively activated α-subunit, $G_{\alpha16}$R186C, shows a strong differentiating effect in MB-02 cells. We therefore tested whether stimulation of $G_{\alpha16}$, through a receptor that is endogenous to MB-02 cells, could also induce cellular differentiation and whether interaction of receptors with $G_{\alpha16}$ could be directly demonstrated. Two major findings resulted from this analysis of upstream signaling to G_{16},: i) Chronic stimulation of the fMLP receptor, a known activator of G_{16}, may induce cellular differentiation in the factor-dependent erythroleukemia cell line, MB-02. ii) The P_2Y_2 receptor, which was functionally identified as an activator of G_{16}, in an earlier study, directly interacts with $G_{\alpha16}$, as shown by fluorescence resonance energy transfer on a confocal fluorescence microscope. These data support the hypothesis that stimulation of endogenous G protein-coupled receptors may induce cellular differentiation in leukemic cells, and that this stimulation may involve $G_{\alpha16}$.

A joint project with Dr. S. Bürgi (group of Prof. U.E. Honegger) on the downregulation of β-adenoceptors by tricyclic antidepressants is described elsewhere (page 16).

Original publication

1. T. Thurneysen, D.A. Nicoll, K.D. Philipson, H. Porzig:
Immunohistochemical detection of the sodium/calcium exchanger in rat hippocampus cultures using subtype-specific antibodies.
Ann. NY Acad. Sci. (2001), in press.

Group Prof. em. Harald Reuter (until June 2001)

Group members: Charlotte Becker, head technician (until June 2001)
PD Dr. Alexandra Scotti, PhD (until August 2001)
Heleen van Hees, technician (until April 2001)
PD Dr. Roger D. Zühlke, PhD (until April 2001)[1]

[1]In addition, independent research work with own Swiss National Science Foundation projects.

Voltage dependent L-type Ca^{2+}-channels are essential for several vital functions of organs in human and animal bodies. For example, Ca^{2+}-influx through these channels triggers contraction of the heart, controls hormone secretion from endocrine cells, and initiates transcriptional events in neurons that are involved in memory and learning. In addition, Ca^{2+} ions moving through the channels regulate their own activity by either promoting closure or enhanced opening of the channels. Our recent work indicated that both forms of autoregulation, Ca^{2+}-dependent inactivation and facilitation, involve the Ca^{2+}-binding protein calmodulin (CaM). Together with a group (headed by Prof. R.W. Tsien) at Stanford University we could show that CaM binding to a consensus amino acid sequence in the C-terminal cytoplasmic tail of the pore-forming α1C subunit of L-type Ca^{2+}-channels is essential for both types of autoregulation. When Ca^{2+} rises, the C-terminal lobe of CaM binds preferentially to the IQ-motif, thus accelerating inactivation. Although these results could explain the role of CaM in promoting Ca^{2+}- dependent inactivation, its role in facilitation is still unclear, but probably involves CaM-kinase II.

In a second project we have studied the time course of expression of $GABA_A$ receptors in cultured hippocampal neurons. We could show that the receptors in dendrites occur before presynaptic nerve endings are fully established.

Molecular basis of calmodulin tethering and Ca^{2+} - dependent inactivation of L-type Ca^{2+} Channels

G.S. Pitt, R.D. Zühlke, A. Hudmon, H. Schulman, H. Reuter, R.W. Tsien
(collaboration with the Departments of Molecular and Cellular Physiology and Neurobiology, Stanford University Medical School, CA, USA)

Ca^{2+} - dependent inactivation (CDI) of L-type Ca^{2+} channels plays a critical role in controlling Ca^{2+} entry and downstream signal transduction in excitable cells. Ca^{2+} - insensitive forms of calmodulin (CaM) act as dominant negatives to prevent CDI, suggesting that CaM acts as a resident Ca^{2+} sensor. However, it is not known hown the Ca^{2+} sensor is constitutively tethered. We have found that the tethering of Ca^{2+} - insensitive CaM was localized to the C-terminal tail of α_{1C}, close to the CDI effector motif, and that it depended on nanomolar Ca^{2+} concentrations, likely attained in quiescent cells. Two stretches of amino acids were found to support the tethering and

to contain putative CaM-binding sequences close to or overlapping residues previously shown to affect CDI and Ca^{2+} - independent inactivation. Synthetic peptides containing these sequences displayed differences in CaM-binding properties, both in affinity and Ca^{2+} dependence, leading us to propose a novel mechanism for CDI. In contrast to a traditional disinhibitory scenario, we suggest that apoCaM is tethered at two sites and signals actively to slow inactivation. When the C-terminal lobe of CaM binds to the nearby CaM effector sequence (IQ motif), the braking effect is relieved, and CDI is accelerated.
See original publication No. 1

Synaptic and extrasynaptic γ-aminobutyric acid type A receptor clusters in rat hippocampal cultures during development
A. L. Scotti, H. Reuter
We have simultaneously measured the expression of postsynaptic $GABA_A$ receptor clusters and of presynaptic boutons in neonatal rat hippocampal cultures between days 1 and 30. $GABA_A$ receptors were labeled with antibodies recognizing the extracellular domains of β2/3 and γ2 subunits. Boutons were visualized by activity dependent uptake of the styryl dye FM4-64, or by antibodies against the presynaptic vesicular protein SV2 or the GABA synthesizing enzyme glutamic acid decarboxylase (GAD). $GABA_A$ receptor clusters could be seen in living neurons already 6 hours after culturing, much before presynaptic markers could be identified in nerve terminals. The densities of receptor clusters that contained the β2/3_subunits were constant between days 10 and 30 in culture, while γ2 subunit containing clusters fluctuated and reached a maximum on day 20. SV2 and GAD staining could be measured from day 2 onwards. Clustering of GAD in presynaptic terminals and FM4-64 uptake were observed only at day 5 and afterwards. SV2 staining and FM4-64 uptake increased in parallel between days 5 and 20 and remained constant thereafter. GAD stained boutons were fewer than those labeled with other, less specific, presynaptic stains. They reached a maximum on day 20 and fell again toward day 30. Double labeling of $GABA_A$ receptors and of presynaptic boutons in neurons during differentiation showed that even after 30 days in culture, large fractions of $GABA_A$ receptor clusters containing β2/3 and/or γ2 subunits remained extrasynaptic.
See original publication No. 2

Original publications

1. G.S. Pitt, R.D. Zühlke, A. Hudmon, H. Schulman, H. Reuter, R.W. Tsien:
Molecular basis of calmodulin tethering and Ca^{2+} - dependent inactivation of L-type Ca^{2+} channels.
J. Biol. Chem. 276 (2001), 30794-30802.

2. A.L. Scotti, H. Reuter:
Synaptic and extrasynaptic γ-aminobutyric acid type A receptor clustern in rat hippocampal cultures during development.
Proc. Natl. Acad. Sci. USA 98 (2001), 3489-3494.

Review article

1. T.A. Ryan, H. Reuter:
Measurements of vesicle recycling in central neurons.
News Physiol. Sci. 16 (2001), 10-14.

Group Prof. Erwin Sigel

Group members: Roland Baur, head technician
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Heleen van Hees, technician (May-November 2001)

The $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the mammalian nervous system. They are integral membrane proteins consisting of five pseudosymmetrically arranged subunits surrounding a central chloride ion selective channel. Subtle modulation of their function influences our state of vigilance, anxiety and muscle tension. They represent the molecular targets of the frequently used tranquilizers of the benzodiazepine type (Valium). Our projects are concerned with the major adult isoform of the receptor $\alpha1\beta2\gamma2$. We are interested in finding novel natural and chemically synthesized modulators of the receptor, in the receptor architecture, in receptor associated proteins and in the mode of its channel gating. In addition, we look for human mutations affecting $GABA_A$ receptors. For this purpose, we use point mutation and expression of recombinant proteins in HEK-293 cells (transient transfection) and Xenopus oocytes (mRNA microinjection), pharmacological (radioactive ligand binding studies), electrophysiological (2-electrode-voltage clamp, patch-clamp), biochemical, and molecular biology techniques.

Architecture of $GABA_A$ receptors

S.W. Baumann, K. De Cock, E. Sigel

The $GABA_A$ receptor is the major inhibitory neurotransmitter receptor and contains an integral chloride ion selective channel. The major isoform of the $GABA_A$ receptor is thought to be composed of 2α, 2β and 1γ subunit. These five subunits surround the ion pore in an unknown arrangement. We are now fusing subunits at the DNA level to form dimeric and trimeric subunits. Functional expression in the Xenopus oocyte of exclusively the correct combination of a dimer and a trimer will mimic the electrophysiological properties of the wild type receptor. Using these techniques we have identified $\beta\alpha\gamma\beta\alpha$ as the correct arrangement. The establishment of the receptor architecture and of linked subunits allows to introduce point mutations in α and β subunits for the first time in a defined position. The consequence of point mutations affecting the binding of agonists is presently investigated by comparing $\beta2\alpha1\gamma2/\beta2\alpha1$, with those carrying a point mutation homologous to position $\alpha1$F64, but located in a defined location ($\beta2$F62L$\alpha1\gamma2/\beta2\alpha1$, $\beta2\alpha1$F64L$\gamma2/\beta2\alpha1$, $\beta2\alpha1\gamma2/\beta2$F62L$\alpha1$ and

β2α1γ2/β2α1F64L). Similarly, receptors carrying two α subunit isoforms, α1 and α6 in defined positions will be compared (β2α6γ2/β2α1 and β2α1γ2/β2α6). This will open the way for the screening of potentially useful substances at defined $GABA_A$ receptor subtypes.

See original publication No. 4

Architecture of the benzodiazepine binding site on $GABA_A$ receptors

D. Berezhnoy, Y. Nyfeler, E. Sigel

(Collaboration with Dr. M. Goeldner, University of Strasbourg, France).

The $GABA_A$ receptor is the molecular target of the frequently used tranquilizers of the benzodiazepine type. We want to identify parts of ligands of the benzodiazepine binding sites in contact with specific amino acid residues in the binding pocket. For this purpose, we are looking for a covalent interaction between cysteine reactive ligands of the benzodiazepine binding pocket and appropriate cysteine point mutants of the receptor. This should give information on the structure of the benzodiazepine binding pocket. The project will be extended to a comparison of different allosteric modulators of the receptor in order to get information on their relative position and overlapping, and also to different isoforms of the $GABA_A$ receptor.

FRET for the study of conformational changes in the $GABA_A$ receptor

F. Minier, S.W. Baumann, E. Sigel

$GABA_A$ receptor subunits will be each N-terminally and C-terminally modified with either the yellow or the cyan derivative of green fluorescent protein (GFP). Subunits labelled with the two colors will be complemented with an appropriate triple subunit construct to give a functional receptor. FRET will be used to see whether the relative conformation of two neighboring subunits is affected by agonists, competitive antagonists or benzodiazepines.

A 34kD protein as an interaction partner of $GABA_A$ receptors

M.T. Schärer, K. Kannenberg, S.W. Baumann, E. Sigel

(Collaboration with Dr. P. Hunziker, Institute of Biochemistry, University of Zurich)

The $GABA_A$ receptor is the major inhibitory neurotransmitter receptor and contains an integral chloride ion selective channel. Many proteins must be transiently or permanently associated to this receptor during biosynthesis, mature function and during degradation. Using immunoprecipitation, subsequent purification and protein microsequencing, we identified a 34kD protein adhering to $GABA_A$ receptors. Immunoprecitation using an antibody directed against this protein in combination with dissociation experiments showed that the mature $GABA_A$ receptor interact with this protein with nM affinity. Immunopreciptation of the protein could be achieved with antibodies against the $GABA_A$ receptor. Using the yeast-2-hybrid system, subunits of the $GABA_A$ receptor and subunit regions responsible for the interaction were established.

See original publication No. 3

A novel residue in M2 of the $GABA_A$ receptor affecting gating by GABA and picrotoxin affinity

A. Buhr, E. Sigel

(Collaboration with Drs. K. Fuchs and W. Sieghart, Brain Research Institute, Vienna, Austria, and Dr. C. Wagner at this institute)

The transmembrane segment M2 is known to line the ion channel in $GABA_A$ receptors. Point mutated α1β3 receptors were expressed in *Xenopus* oocytes and

analyzed using the electrophysiological techniques. The point mutation L253 in the β3 subunit co-expressed together with the α1 subunit led to small spontaneous picrotoxin-sensitive currents in the absence of GABA. Upon wash-out of picrotoxin, a huge transient inward current was observed. The amplitude of the inward current was strongly dependent on the picrotoxin concentration used and on the duration of its application. The point mutated channel showed a strong decrease in picrotoxin affinity indicating an important role of residue β3L253 in the interaction with picrotoxin. A kinetic model is presented that mimics the gating behavior of the mutant receptor.

See original publication No. 1

A point mutation in the β3 subunit of the human $GABA_A$ receptor might be linked to inherited sleep problems

A. Buhr, R. Baur, E. Sigel

(Collaboration with Drs. M.T. Bianchi, D.J. Hinkle and R.L. Macdonald, Departments of Neurology, Molecular Physiology and Biophysics and Pharmacology, Vanderbilt University School of Medicine, Nashville, USA; Drs. P. Courtet, V. Pignay and J.P. Boulenger, Services Universitaires de Psychiatrie, CHU Montpellier, INSERM, Montpellier, France; and Prof. Dr. S. Gallati, Dept. of Pediatrics, Inselspital, University of Bern)

We screened 124 human individuals for single nucleotide polymorphisms of the α1, β3 and γ2 genes of the $GABA_A$ receptor, in the regions corresponding to the ligand binding domains on the protein level. In a patient with chronic insomnia a point mutation was found in the gene of the beta3 subunit. This mutation results in the substitution of the amino acid residue arginine for histidine in position 192 (β3R192H). Functional analysis of human α1β3(R192H)γ2S $GABA_A$ receptors using ultra fast perfusion techniques revealed a slower rate of the fast phase of desensitization compared to α1β3γ2S $GABA_A$ receptors. Additionally, current deactivation (a major determinant of IPSC duration) was faster in the mutated receptors. This raises the possibility of decreased GABAergic inhibition contributing to insomnia, as some members of the family of the blood donor also have sleep disorders. The mutation β3R192H might therefore be linked to this condition. The intron/exon boundaries of the α1 subunit gene were also established and three additional variants were found in the α1 and β3 genes.

Novel positive allosteric modulators of the $GABA_A$ receptor

U. Thomet, R. Baur, E. Sigel

(Collaboration with Drs. R. Razet, A. El Hadri, P. Poitier and R.H. Dodd, Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, Gif-sur-Yvette Cedex, France; and Drs. R. Furtmüller, F. Jursky and W. Sieghart, Brain Research Institute, University of Vienna, Austria)

Two families of novel compounds were synthesized that act in electrophysiological experiments as positive allosteric modulators of recombinant $GABA_A$ receptors. It was shown that most of them do not interact with the benzodiazepine binding site. Most interestingly, some of them were shown to act in a $GABA_A$ receptor isoform specific way. Both families were patented. Presently, negotiations for licensing are carried out with a large pharmaceutical company.

See original publication No. 2 and patents No. 1 and 2

The relative amount of cRNA coding for γ2 subunits affects stimulation by benzodiazepines in $GABA_A$ receptors expressed in *Xenopus* oocytes

R. Baur, E. Sigel

(Collaboration with Drs. A.J. Boileau, L.M. Sharkey and C. Czajkowski, University of Wisconsin, Madison, USA)

Very variable values for the extent of stimulation by benzodiazepines of recombinant $GABA_A$ receptors expressed in Xenopus oocytes have been reported. We have found, that by increasing the ratio of genetic information coding for the γ2 subunit relative to the α1 and β2 subunits, current stimulation can be made larger, more reproducible and is stabilized over expression time.

Wogonin isolated from *Scutellaria baicalensis Georgi* acts as a selective anxiolytic

R. Baur, E. Sigel

(Collaboration with Drs. K.M. Hui and H. Xue, Dept. of Biochemistry, Hong Kong University of Science and Technology, Hong Kong, China)

The monoflavonoid wogonin, purified from *Scutellaria baicalensis* Georgi inhibited [^{3}H]flunitrazepam binding to the benzodiazepine receptors (BDZ-Rs). In electrophysiological studies, wogonin enhanced the GABA-activated current. In *Xenopus* oocytes, half-maximal stimulation of currents elicited by GABA was observed at about 3 μM wogonin. Maximal stimulation by wogonin amounted to about 37 % of that by 0.3 μM diazepam. The enhancement was partially reversed by the co-application of the BDZ-R antagonist Ro15-1788. We conclude that wogonin is a naturally occurring partial positive allosteric modulator of the $GABA_A$ receptor acting at the BDZ binding.

Functional charcterization of human mutations in a muscle chloride channel leading to Myotonia Congenita

M.T. Schaerer, E. Sigel

(Collaboration with Drs. Li Chen, D. Lang, J. Fritschi, L. Kappeler and J. Burgunder, Laboratory of Neuromorphology, Departments of Neurology and Clinical Research, University of Bern; and Drs. F. Joncourt and S. Gallati, Laboratory of Molecular Genetics, Children's Hospital, University of Bern; and Prof. Dr. J. Weis, Division of Neuropathology, Institute of Pathology, University of Bern)

Myotonia congenita is a group of inherited muscular diseases characteristically involving muscle stiffness. The chloride channel, ClC-1 which is encoded by the gene *CLCN1*, is a major voltage dependent ion channel in the skeletal muscle. In this study, exon 17 skipping was identified at the DNA level. This was confirmed by RT-PCR amplification. The functional significance of this exon skipping was then investigated by expressing mutagenised *CLCN1* with exon 17 deletion in *Xenopus* oocytes. No measurable chloride current could be detected in these oocytes, indicating the expression of a non-functional ClC-1. On the other hand, a normal function could be achieved when both the mutant and wild-type ClC-1 were co-expressed. These data are compatible with the fact that exon 17 skipping leads to a recessive inheritance of the disease.

Original publications

1. A. Buhr, C. Wagner, K. Fuchs, W. Sieghart and E. Sigel:
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Differential crosstalk of ROD compounds with the benzodiazepine binding site.
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Interaction between $GABA_A$ receptor β subunits and the multifunctional protein gC1q-R.
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Subunit arrangement of γ-aminobutyric acid type A receptors.
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1. E. Sigel:
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Patents

1. R.H. Dodd, R. Razet, P. Poitier, W. Sieghart, F. Jursky, R. Furtmüller, U. Thomet, E. Sigel:
1-(2-butyrolactones et 2-thiobutyrolactones)-isoquinolines N-substituées utile comme stimulant de l'activité de l'acide γ-aminobutyrique et dans le traitement des troubles nerveux et leur procédé de préparation.
PCT WO 01/29030 A1 (2001) Centre National de la Recherche Scientifique (CNRS), Innovationsagentur GmbH - BU Technology (Vienna) et Unitectra Technologietransfer (Bern).

2. R.H. Dodd, A. El Hadri, P. Poitier, W. Sieghart, F. Jursky, R. Furtmüller, U. Thomet, E. Sigel:
3-amino-2,2-di-c-alkyl-1,4-butyrolactones and 1,4-thiobutyrolactones N-substitués utiles comme stimulant de l'activité de l'acide γ-aminobutyrique et dans le traitement des troubles nerveux et leur procédé de préparation.
PCT WO 01/29017 A2 (2001) Centre National de la Recherche Scientifique (CNRS), Innovationsagentur GmbH - BU Technology (Vienna) et Unitectra Technologietransfer (Bern).

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We are interested in the precise features of chronic inflammatory responses. Several diseases serve as models to study such processes. In particular, we investigate pathogenic mechanisms of the following diseases: Bronchial asthma, atopic dermatitis, idiopathic eosinophilia, idiopathic eosinophilic esophagitis, cystic fibrosis, rheumatoid arthritis, chronic obstructive pulmonary disease, and cytokine-producing cancers. Our research goal is the identification of new drug targets for future therapeutic approaches in these diseases. Besides the pathogenic aspects of our research, we have developed several in vitro and in vivo test systems to determine potential effects of a given drug on the immune system. Our research requires a network with physician-scientists from many different clinics. Such a network, mostly involving clinics of the University Hospital in Bern (Inselspital), has been established within the last year. First results of these collaborative interactions are seen in the following abstracts, which briefly describe our research activities in 2001.

Death receptors bind SHP-1 and block cytokine-induced anti-apoptotic signaling in neutrophils

I. Daigle, S. Yousefi, M. Colonna, D.R. Green, H.-U. Simon
(Collaboration with the Basel Institute for Immunology, Basel, Switzerland, and the La Jolla Institute for Allergy and Immunology, San Diego, USA)

Death domain-containing receptors of the tumor necrosis factor (TNF)/ nerve growth factor (NGF) family can induce apoptosis upon activation in many cellular systems. Here, we provide evidence for a tyrosine-based inhibition motif present within the death domain of these receptors. Src homology (SH) 2 - containing tyrosine or inositol phosphatases, SHP-1, SHP-2, and SHIP, can bind in a caspase-independent manner to this motif, suggesting that inhibitory signals might be delivered following cross-linking of death receptors. Indeed, stimulation of death receptors is associated with the disruption of antiapoptosis pathways initiated by survival factors. For instance, Fas, TNF, or TRAIL receptor stimulation abrogates granulocyte/ macrophage colony-stimulating factor (GM-CSF)-, granulocyte colony-stimulating factor (G-CSF)-, and interferon (IFN)-γ-mediated antiapoptosis in neutrophils. In these cells, activation of the tyrosine kinase Lyn is prevented, most likely via

association with activated SHP-1. Thus, we demonstrate molecular and functional evidence for negative signaling by death receptors.
See original publication No. 12

Role for caspases in eosinophil and neutrophil apoptosis
I. Daigle, H.-U. Simon
Apoptosis is a necessary process to maintain cell numbers in multicellular organisms. In some chronic inflammatory diseases, reduced cell death of different types of granulocytes is one important mechanism for cell accumulation. Here, we studied the underlaying intracellular mechanisms of normal and delayed eosinophil and neutrophil apoptosis, in particular the expression and function of caspases. The caspases belong to a family of cystein proteases thought to be central as effectors of apoptosis in most systems. We observed that caspases 1 and 3 are involved in the regulation of spontaneous neutrophil apoptosis in vitro. In contrast, these two caspases did not appear to play a major role in spontaneous eosinophil apoptosis. However, broad range caspase inhibitors prevented eosinophil death, suggesting that caspases are also involved within the apoptosis machinery of eosinophils. We further investigated possible initiators of the constitutive caspase activation in granulocytes. These studies excluded the possibility that spontaneous apoptosis of neutrophils or eosinophils is the consequence of Fas ligand / Fas receptor molecular interactions.
See original publication No. 10

Alternative functions for TRAIL receptors in eosinophils and neutrophils
I. Daigle, H.-U. Simon
Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in many tumor cells but only rarely in normal cells. The receptors of TRAIL belong to the superfamily of tumor necrosis factor (TNF) / nerve growth factor (NGF) receptors. Here, we investigated TRAIL receptors expression and function in eosinophils and neutrophils. Granulocytes were isolated from human blood and purified using standard protocols. Receptor expression was analyzed by reverse transcription (RT)-PCR and flow cytometry. Cell death was analyzed by ethidium bromide exclusion test. Apoptosis was determined by analyzing phosphatidyl serine (PS) surface exposure and morphologic evaluation. Freshly purified eosinophils and neutrophils expressed TRAIL-R1, TRAIL-R3, and TRAIL-R4, but not TRAIL-R2 surface proteins. Stimulation of eosinophils with TRAIL resulted in either inhibition of apoptosis or no effect depending on the individual from whom the cells were isolated. In neutrophils, TRAIL stimulation did not influence apoptosis. In eosinophils and neutrophils that demonstrated no effect upon TRAIL stimulation alone, TRAIL partially blocked cytokine-mediated antiapoptosis. Both eosinophils and neutrophils express functional TRAIL receptors on their surface. In contrast to tumor cells, TRAIL does not induce apoptosis in granulocytes, but rather induces survival in eosinophils from approximately 50% of the donors. Alternatively, TRAIL may limit cytokine-mediated antiapoptosis under certain inflammatory conditions.
See original publication No. 9

Functional CD137 receptors are expressed by eosinophils from patients with IgE-mediated allergic responses but not with non-IgE-mediated eosinophilic disorders

I.V.W.M. Heinisch, C. Bitzer, W. Volgger, H.-U. Simon

(Collaboration with the High-Altitude Clinic Davos-Wolfgang and the Clinic for Dermatology and Allergy, Davos, Switzerland)

CD137 (ILA/4-1BB), a member of the TNF/nerve growth factor (NGF) receptor superfamily, has previously been suggested to be involved in T cell activation and differentiation. The aim of this study was to investigate expression and potential function of CD137 in eosinophils. Eosinophils were isolated from normal control individuals as well as patients with bronchial asthma, atopic dermatitis, and idiopathic eosinophilia. CD137 expression was analyzed by reverse transcription (RT)-PCR and flow cytometry. The in situ expression of CD137 on eosinophils in nasal polyp and skin tissues was analyzed by using immunohistochemistry. To examine whether CD137 regulates eosinophil death and apoptosis, cells were stimulated with a plate-bound anti-CD137 antibody in the presence or absence of survival cytokines. Cell death was measured by ethidium bromide exclusion test. Apoptosis was determined by analysing phosphatidylserine (PS) surface exposure. Blood and tissue eosinophils from patients with IgE-mediated allergic responses (atopic dermatitis, extrinsic asthma) express CD137. In contrast, eosinophils from normal control individuals and patients with non-IgE-mediated eosinophilic inflammatory responses (intrinsic asthma, idiopathic eosinophilia) do neither express detectable levels mRNA nor protein for CD137. Expression of CD137 in eosinophils was induced in vitro by stimulating the cells with supernatants derived from in vivo- or in vitro-activated T cells, suggesting that a soluble T cell-derived factor might be responsible for the observed phenomenon. Although CD137 expression was associated with increased IgE levels, IL-4 and IL-13 did not induce CD137 gene expression in eosinophils. Activation of CD137 abrogated both GM-CSF- and IL-5-mediated antiapoptosis in CD137-expressing but not CD137-deficient eosinophils. In contrast, the survival effect of IFN-γ was not affected by anti-CD137 treatment. Our data indicate that CD137 activation may limit GM-CSF- and IL-5-mediated antiapoptosis of eosinophils. The absence of this potential anti-inflammatory mechanism may further increase eosinophil numbers at inflammatory sites of patients with intrinsic asthma and idiopathic eosinophilia. The T cell-derived factor that induces CD137 expression in eosinophils remains to be identified.

See original publication No. 5

Idiopathic eosinophilic esophagitis is associated with a T-helper 2-type allergic inflammatory response

A. Straumann, M. Bauer, B. Fischer, K. Blaser, H.-U. Simon

(Collaboration with the Department of Gastroenterology, Kantonsspital Olten, Olten, Switzerland)

Idiopathic eosinophilic esophagitis (IEE) is a chronic-inflammatory disorder of the esophagus of unknown origin. The established cornerstone of diagnosis is a dense infiltration of the esophagus with eosinophils, but neither the precise pattern of inflammatory cells infiltration nor mechanisms, which likely contribute to induction and maintenance of the inflammatory response have been described. The intention of this study was to characterize the esophageal inflammatory infiltrate and the expression of cytokines in the esophagus of patients with this disease. In addition, we searched for immunological abnormalities of blood leukocytes to exclude major primary hypo- or hyperreactive conditions of the immune system. The infiltration of inflammatory

cells in esophagus, stomach, and duodenum was analyzed by immunohistochemistry using monoclonal antibodies against lineage-associated molecules. Cytokine expression was measured by enzyme-linked immunosorbent assay and immunohistochemical analysis. Lymphocyte subpopulations in blood were determined by flow cytometry. High eosinophil infiltration into the esophageal squamous epithelium was observed in IEE patients but not in control individuals. Interestingly, increased T-cell and mast cell numbers were also found within the epithelium of these patients. In contrast, the numbers of inflammatory cells were not increased in stomach and duodenum of IEE patients, suggesting a specific inflammatory process within the esophagus. Moreover, increased expression of interleukin-5 and tumour necrosis factor-α was observed in esophageal epithelial biopsies. The distribution of lymphocyte subsets in the peripheral blood and their capacity to generate cytokines did not reflect the changes observed at the inflammatory site. Taken together, IEE is a selective inflammatory response of the esophagus. T-cells, interleukin-5, eosinophils, and IgE-mediated mechanisms appear to be involved, giving rise to the possibility that allergic reactions might play a role in the pathogenesis of the disease.

See original publication No. 11

Inhibition of inflammatory effector functions of granulocytes by petasins

O.A.R. Thomet, U.N. Wiesmann, K. Blaser, H.-U. Simon

(Collaboration with the Dept. of Metabolic Diseases, Children's Hospital, University of Bern, Bern, Switzerland)

Recently, new drugs acting on the leukotriene pathway has been shown to be useful in the treatment of allergic and asthmatic disorders. Here, we compared the effects of the 5-lipoxygenase inhibitor Zileuton with an extract of Petasites hybridus (Ze 339) and it’s active compounds Petasin, Isopetasin and Neopetasin, respectively, on freshly purified human eosinophils and neutrophils. Priming of isolated cells with GM-CSF and subsequent stimulation with C5a and PAF was associated with a rapid production of cysteinyl-leukotrienes in eosinophils or LTB_4 in neutrophils, respectively. Moreover, the same stimulation conditions were associated with the release of eosinophil cationic protein (ECP) in the eosinophil system. A significant inhibition of either cysteinyl-leukotrienes in eosinophils or LTB_4 synthesis in neutrophils was observed by using Zileuton, Ze 339, or it’s active compounds. In contrast, ligand-induced ECP release was blocked by Ze 339 and Petasin but not Zileuton, Isopetasin and Neopetasin, suggesting different mechanisms of drug actions. Indeed, C5a- or PAF-induced intracellular calcium changes were abrogated by Ze 339 and Petasin but not Zileuton, Isopetasin and Neopetasin. This suggests that Petasin and Ze 339 may inhibit inflammatory effector functions in granulocytes by disrupting signaling events proximal to intracellular calcium mobilization. These data indicate that Petasin and Ze 339 may represent compounds that target new molecules suitable for the treatment of inflammatory disorders.

See original publications No. 3 and 7

Eosinophils express functional interleukin-13 in eosinophilic inflammatory diseases

P. Schmid-Grendelmeier, F. Altznauer, B. Fischer, C. Bitzer, A. Straumann, G. Menz, K. Blaser, B. Wüthrich, H.-U. Simon
(Collaboration with the Allergy Unit, Dept. of Dermatology, University of Zurich, Zurich; High-Altitude Clinic Davos-Wolfgang, Davos, and Dept. of Gastroenterology, Kantonsspital Olten, Olten, Switzerland)

Interleukin (IL)-13 is an immunoregulatory and effector cytokine in allergic diseases such as bronchial asthma. A variety of immune and non-immune cells are known as IL-13 producers. In this study, we investigated whether and under which conditions human eosinophils generate IL-13. Freshly isolated highly purified peripheral blood eosinophils from patients with several eosinophilic inflammatory diseases and from normal control individuals were investigated. We observed that blood eosinophils from patients suffering from bronchial asthma, atopic dermatitis, parasitic infections, hypereosinophilic syndrome, and idiopathic eosinophilic esophagitis expressed IL-13 as assessed by enzyme-linked immunosorbent assay (ELISA), ELISpot-assay, flow cytometry, and immunocytochemistry. By using nasal polyp tissues and immunohistochemistry, we demonstrated IL-13 expression in eosinophils under in vivo conditions. In contrast, blood eosinophils from normal control individuals as well as blood neutrophils from both eosinophilic and control patients did not produce detectable IL-13 levels. However, when blood eosinophils from normal control individuals were stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-5 in vitro, they generated IL-13 mRNA and protein, suggesting that IL-13 expression by eosinophils observed under inflammatory conditions is also a cytokine driven process. Addition of eotaxin to blood eosinophils containing IL-13 resulted in a rapid release of this cytokine. Eosinophil-derived IL-13 was functional as it increased surface expression of the low-affinity IgE receptor (CD23) on purified B cells. In conclusion, human eosinophils are able to produce and release functional IL-13 in eosinophilic inflammatory responses.

Clinical and immunological effects of low-dose interferon-alpha treatment in patients with corticosteroid unresponsive asthma

H.-U. Simon, H. Seelbach, R. Ehmann, M. Schmitz
(Collaboration with the High-Altitude Clinic Davos-Wolfgang, Davos, Switzerland)

Interferon-α (IFN-α) is a cytokine that possesses potent anti-viral and immunoregulatory activities. We aimed to assess clinical and immunological effects of low-dose IFN-α in patients with severe corticosteroid unresponsive asthma with and without Churg-Strauss syndrome. There is currently no efficient pharmacological treatment available for this group of patients. We studied 10 patients with corticosteroid unresponsive asthma, in which 3×10^6 IU/d IFN-α were administrated in addition to the prednisone dose given already before introduction of the cytokine therapy. The prednisone dose was gradually reduced dependent on the clinical situation and used as a clinical readout to evaluate the efficacy of the cytokine therapy. To distinguish between IFN-α - and prednisone - mediated immunological changes, the corticosteroid dose was kept constant for at least 2 weeks upon introduction of the cytokine therapy in 7 patients. The effects of treatment on clinical and immunological parameters were measured at weeks 2-4 and months 5-10 depending on the availability of the patient. IFN-α treatment rapidly improved the clinical situation as assessed by lung function parameters and required prednisone dose. Important immunological changes included: Decreased leukocyte numbers, increased relative numbers of CD4+ T cells, increased differentiation of T helper 1

(Th1) cells, and increased expression of interleukin-10 (IL-10) in peripheral blood mononuclear cells (PBMC). Taken together, IFN-α treatment was associated with dramatic improvements in the condition of patients with corticosteroid unresponsive asthma with and without Churg-Strauss syndrome. Potential mechanisms of action include the establishment of correct Th1/Th2 balance and the induction of the anti-inflammatory IL-10 gene.

Immunological effects of competitive versus recreational sports in cross-country skiing

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(Collaboration with the Thurg.-Schaffh. Höhenklinik, Sportsmedicine Center of the Swiss Olympic Committee, Davos, Switzerland)

For a period of two months during the competitive season, the effects of endurance training in cross-country skiers were evaluated, so as to compare the adaptive and innate immune systems between 10 competitive athletes, 10 moderately trained athletes, and 10 untrained healthy controls. The main results were the following: The peripheral T lymphocyte count of the competitive athletes was decreased. In contrast, the numbers of peripheral blood NK cells were increased in this group. These data imply a diminuation of the adaptive immune system by repeated bouts of intense exercise and contemporaneous reinforcement of the innate immune response. Moreover, the inducible IL-12 expression following monocyte stimulation was significantly decreased in competitive athletes. Compared with the other two groups, the moderately trained athletes showed a significantly increased production of IFN-γ upon T cell stimulation in vitro. These data suggest that the immune system may profit from moderate endurance training by an increased capacity to generate IFN-γ, while the immune situation by repeated exhausting exercise of competitive athletes tends to deteriorate through downregulation of IFN-γ and IL-12.

See original publication No. 8

Peripheral blood mononuclear cells from extrinsic and intrinsic atopic dermatitis patients demonstrate increased capacity of generating interleukin-13 but differ in their potential of synthesising interferon-γ

D. Simon, S. Borelli, L.R. Braathen, H.-U. Simon

(Collaboration with the Department of Dermatology, University of Bern, Bern, and the Clinic for Dermatology and Allergy, Davos, Switzerland)

A subgroup of patients with atopic dermatitis (AD) are known to have normal total and specific IgE levels and negative skin prick tests towards common environmental allergens. This form of the disease has been termed intrinsic AD (IAD). Although allergic mechanisms appear to be important, the pathogenesis of both extrinsic and intrinsic forms of the disease is unknown. We have compared the cytokine production pattern of peripheral blood mononuclear cells (PBMC) from extrinsic AD (EAD), IAD, and normal control individuals. PBMC were stimulated with anti-CD3 and/or anti-CD28 monoclonal antibodies (mAb) and cytokine production was measured by immunoassays in supernatants of 24-h cultures. Compared to healthy subjects and IAD patients, stimulated PBMC from EAD patients produced less interferon (IFN)-γ. However, stimulated PBMC from both EAD and IAD patients produced more interleukin (IL)-13 than PBMC from control individuals. Moreover, IL-5 production was significantly increased in IAD but not in EAD patients. Therefore, the underlying mechanism leading to increased differentiation of T helper (Th) 2 cells may involve a deficient capacity in producing IFN-γ in EAD but not in IAD patients. IL-13 may be a

key cytokine in the pathogenesis of both EAD and IAD.
See original publication No. 13

High serum TARC levels in the lymphocytic variant of the hypereosinophilic syndrome
A. de Lavareille, F. Roufosse, P. Schmid, A.-S. Roumier, L. Schandene, E. Cogan, H.-U. Simon, M. Goldman
(Collaboration with the Department of Immunology, University of Brussel, Brussels, Belgium)
The idiopathic hypereosinophilic syndrome is secondary to expansion of an IL-5-producing T cell subset in a subgroup of patients. As this variant is associated with an increased risk for development of T cell lymphoma, early identification of such patients is critical to adequate management. Although the Th2-like cells often bear an aberrant surface phenotype and can be readily detected by flow cytometry, we now show that lymphocyte phenotyping may be normal in some cases. In contrast, measurement of serum TARC levels consistently shows increased values in patients with this condition compared to other patients with the IHS, and could therefore represent a useful diagnostic tool.

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10. I. Daigle, H.-U. Simon:
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Peripheral blood mononuclear cells from IgE- and non-IgE associated allergic atopic eczema/dermatitis syndrome (AEDS) demonstrate increased capacity of generating interleukin-13 but differ in their potential of synthesising interferon-γ.
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Book chapter

1. H.-U. Simon, F. Levi-Schaffer:
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In: New Trends in Allergy V (Eds. J. Ring, H. Behrendt, D. Vieluf); Springer-Verlag, Berlin, Heidelberg, 2002, in press.

Group Prof. Jörg Stucki

Group member: Dr. Clemens Wagner, PhD

The introduction of methods originating from nonlinear dynamics in the analysis of brain waves (electroencephalograms, EEG) goes back to the pioneering work of Walter Freeman. Nonlinear time series analysis of the EEG of the olfactory system[1] revealed that the dynamics of neuronal activity is low dimensional, though unpredictable. This is a characteristic property of a deterministic chaotic system in contrast to the dynamics of a stochastic process. The former system typically collapses after a transient to a low dimensional attractor whereas the dynamics of the latter remains high dimensional. Deterministic chaos in neural networks has not only been observed at the network level but also at the level of a single neuron. Already the Hodgkin-Huxley model of the squid axon, developed in the 50ties, showed a parameter range where chaotic dynamics appears. Since these early discoveries much effort has been devoted to devise sophisticated methods to establish the idea of chaos in the brain. However, the determination of chaotic dynamics from time series analysis is a subtle task, mainly due to the presence of noise in experimental systems. Thus, whether chaos is indeed present in the brain or if its detection is just an artifact, due to the applied methods, is still an open question. Moreover, the significance of chaotic dynamics in neural systems has not yet been elucidated. The goal of our work is to explore possible operational benefits of deterministic chaos at different levels of network organization (single neuron, neuromodules, neural networks). We study typical functions of neuronal networks like memory formation and information processing under the evolution of chaotic dynamics.

Intermediate Level (Neuromodules)

Artificial Memory based on Unstable Periodic Orbits of a Chaotic Attractor

We started our studies at the intermediate level (a few neurons constitute a neuromodule) and constructed an artificial memory which is based on the properties of a chaotic system **[see original publication No. 1]**. Unstable periodic orbits (UPO) form the skeleton of a chaotic attractor. Each UPO is uniquely defined and we used these orbits to store simple processes. This can be achieved by applying a chaos control algorithm to the system keeping it on the orbit during the read out-time. After the recall of the orbit the system relaxes back to the resting state. The selection of UPO's takes place via the input, which moves the system from the resting state to the associated orbit (targetting algorithm). In essence, remembering therefore is not a recall of a stored bit pattern, but rather it is a reconstruction of a dynamic process.

[1] The main parts of the central olfactory system are the bulb, anterior nucleus, and prepyriform cortex.

Learning of periodic orbits is achieved e.g. by properly adjusting axonal delay constants and synaptic weights. Since we explore the properties of chaotic dynamics we neglected the details of neurons and used the logistic map (a paradigm for deterministic chaos) as global network dynamics to compute the time evolution of the system. This simple model has the following advantages: first, high capacity (in principle infinite number of UPO's), second, simple and fast switching between different orbits and third, stable functioning under noise.

Chaos Control Method

One of the crucial processes of the above-described artificial memory is the learning routine that finally controls the system on the orbit. A time delay algorithm has the drawback that it also allows for the control of spurious orbits, which are not UPO's of the chaotic attractor. A recently described experiment led to the derivation of the "limiter control" algorithm where the trapping on spurious orbits could be eliminated **[see original publication No. 2]**. In the optimal case the limiter control method is represented by a flat topped map (chopping off the top of the map).

Renormalization and Scaling of flat topped maps

Due to the functional properties at the top the flat topped map stands in between the tent map and the logistic map. The tent map shows a "smooth" transition to chaos whereas the logistic map follows a period doubling bifurcation route. Surprisingly, the bifurcation diagram of the flat topped map also shows a period doubling tree despite of the fact that the system never moves on a chaotic trajectory (height of the map used as bifurcation parameter). By applying the renormalization group approach we derived the values of the scaling parameters for the period doubling cascade. The analysis revealed that the tree collapses close to the accumulation point resulting in the Feigenbaum parameter $\delta=0$. In contrast, the openings of subsequent bifurcations remain constant in the vicinity of the transition point ($\alpha=1$) **[see original publication No. 3]**. The above described scaling is universal and valid for all one dimensional flat topped maps. Beyond the accummulation point the bifurcation diagram of flat topped maps shows two typical structures called "stars" and "windows". We showed that the scaling of these structures depend on the derivative of the map at the origin and are therefore not universal **[see book chapter]**.

Extension to Flow Systems

A natural extension of the map model is the representation of neurons by more realistic flow systems (continuous rather than discrete time functions). On the one hand this can be implemented using the programs "Genesis" or "Neuron" which allow simulations of single neurons and nets of neurons to any desired level of complexity, whereby the practical limits are set by the available computer hardware. On the other hand we can simulate an array of neurons by using an analogue computer which simulates the behaviour of neuronal models (FitzHugh-Nagumo, Morris-Lecar) in real time. The available analogue hardware in our group is limited to 4 neuronal units. This approach is fast and efficient, because it allows to change parameters via potentiometers and to observe the results immediately on an oscilloscope.

The combination of these two computational methods enabled us to find regions in the parameter space of the Morris-Lecar model (supplemented by inhibitory feedback) which resulted in chaotic dynamics. Moreover, introduction of feedback-delay allowed stabilization of the dynamics of this model to well characterized periodic orbits. Thus the map properties of our initial logistic model can be reproduced also in a neuromodule of flow systems resembling realistic neurons. Biologically delay feedback in a brain can be implemented via the length of interneuron connections and changes of synaptic weights by long term potentiation

(LTP) and long term depression (LTD). Our model suggests that the learning brain could use both of these mechanisms.

Single Neuron Level

Chaotic Neurons

Single neurons in brain slices and single neurons in the intact brain show very different behaviour. In response to a constant current input the former exhibits a spike train of constant frequency whereas the spike train of the latter to the same input is well described by a Poisson process. The integrate and fire model nicely represents the properties of neurons in brain slices due to constant firing for a constant input. In order to model neurons of the intact brain by an integrate and fire neuron one introduced a variable threshold which follows a Poisson process.

In order to construct a chaotic integrate and fire neuron (which responds with a chaotic spike train to a constant current input) we simply replaced the Poisson process by i.e. the logistic map. We then compared the performance of a stochastic neuron (Poisson, γ-distribution) to a chaotic neuron using a discrimination task of static stimuli. The parameters of the neurons were chosen in such a way that the coefficients of variations of the output spike train remained equal. The performance of the neurons was judged by the area under the curve of the receiver operating characteristics. The simulations revealed an enhanced information transfer of the chaotic neuron compared to the stochastic neuron.

In order to further elaborate these studies we will compare the performance of chaotic neurons to stochastic neurons using time varying inputs with and without noise. Furthermore, we will examine the properties of a leaky chaotic integrate and fire neuron. Another interesting question is how efficient information is transmitted if we synaptically couple chaotic neurons.

Network Level

Synchronization

Coherent collective behaviour is a very general phenomenon observed in many disciplines of natural sciences. In neurobiology, synchronized activity in distributed areas of the brain is thought to be involved in binding of information. Increased synchronized network activity is also observed during expectation and attention. The ability of a network to synchronize depends, beside other properties (input, coupling strength, dynamics of individual neurons), on its connectivity. The brain is dissected into areas with different connection densities. The visual cortex V1 is divided in orientation selective columns with high connectivity within the columns and low connectivity in between. A similar architecture was described for the layer IV barrels in the rodent somatosensory cortex.

We study the potential of a network to synchronize using different architectures. Again, we ignore the details of the individual neurons and use chaotic oscillators as basic elements. Starting from a globally coupled map lattice (GCML) we reduce the number of connections to explore random coupled map lattices (RCML). Further architectures of interest are small world networks and networks with nearest neighbour coupling.

Clustering

GCML do not only show synchronized behaviour, rather they also show the capability of clustering which might be related to the formation of cortical maps. Depending on the coupling strength different clusters are formed during evolution whereby synchronization occurs within the clusters. We will investigate how far this structure forming process is conserved under the different architectures. Due to the difficulty of

the analytical treatment of RCML we will devise a learning algorithm which explores the huge parameter space of synaptic weights.

Information Transfer

It is thought that information transfer is optimal in synchronized cortical neurons. We start to examine this issue using the above mentioned architectures whereby an input and an output layer has to be defined. In order to analyze the information transfer we will use a replica system (without input but equal initial conditions) and study the difference between the two networks. This method allows not only the derivation of an input-output relationship but also the analysis of the velocity and the spatial spreading of information in the system.

Noise

Including noise into the dynamics of these systems will open a further scope of investigations. As already has been shown in GCML noise has a focussing effect on the mean field of the network. RCML of high connectivity have very similar properties as a noisy GCML since the missing inputs can be interpreted as noise. However, if the mean field of RCML also reveals the same features remains to be elucidated. Considering information transfer in these networks different sources of noise have to be distinguished. On the one hand we will examine the effect of noisy input (external noise) on the performance of the network and on the other hand the noise of individual oscillators (internal noise) will be taken into account to estimate the network reliability.

Original publications

1. C. Wagner, J.W. Stucki:
Construction of an associative memory using unstable periodic orbits of a chaotic attractor.
J. Theor. Biol. (2002), in press.

2. C. Wagner, R. Stoop:
Optimized chaos control with simple limiters.
Phys. Rev. E 63 (2001) 017201.

3. C. Wagner, R. Stoop:
Renormalization approach to optimal limiter control in 1-D chaotic systems.
J. Stat. Phys. 106 (2002) 97-107.

Book chapter

1. C. Wagner, R. Stoop:
Universal scaling behavior of flat topped maps.
In: Proceedings of the Conference "Progress in Nonlinear Science, Control of Oscillations", in Honour of the 100. Birthday of A.A. Andronov (Ed. V.Shafeev), 2001, in press.

4.2. Congress Invitations

Prof. Ulrich E. Honegger

Pharmacokinetic interactions, a factor for the choice of an antidepressant.
International symposium “Anxiety & Depression”, Damascus (Syria), May 12-15, 2001

Drug – induced obesity.
11. European Congress on Obesity (EASO). Vienna (Austria), May 30 – June 2, 2001

Alter als Faktor für die Kopfweh-Pharmakotherapie.
17. Schweizerische Kopfwehtagung, Basel (CH), August 23-24, 2001

Relevante physiologische Veränderungen im höheren Lebensalter.
Symposium “Epilepsien im höheren Lebensalter“ Kloster Eberbach (Germany), September 28-30, 2001

Prof. Hartmut Porzig

Cellular distribution of Na^{+}/Ca^{2+} exchanger isoforms in rat hippocampus cultures.
American Physiological Society Conference: Cellular and molecular physiology of sodium-calcium exchange, Banff, Alberta (Canada); October 10-14, 2001

Prof. Harald Reuter

New insights into regulation of L-type calcium channels by calmodulin.
42th Spring Meeting of the German Society of Experimental and Clinical Pharmacology, Mainz (D); March 13-15, 2001

Involvement of calmodulin in L-type Ca-channel regulation.
1st Joint Symposium Tschira Foundation/Academia Europaea, “Imaging and Models of Brain Function“, Heidelberg (D); March15-17, 2001

Calmodulin as a regulator of L-type Ca-channels.
International Titisee Conference on “Ion Channels in Health and Disease“, March 21-25, 2001

Calcium-effector coupling in neurons and other excitable cells.
XXXIV International Congress of Physiological Sciences, Christchurch (New Zealand); August 26-31, 2001

Prof. Hans-Uwe Simon

Difficult-to-treat asthma.
Davoser Gespräche, Hochgebirgsklinik Davos-Wolfgang, Davos (CH); January 11-13, 2001

Novel signalling pathways and cellular functions initiated by death receptor activation – role in inflammation.
American Academy of Allergy Asthma & Immunology, 57th Annual Meeting, New Orleans (USA); March 16-21, 2001

Control of eosinophilia by long-term T cell clones.
American Academy of Allergy Asthma & Immunology, 57th Annual Meeting, New Orleans (USA); March 16-21, 2001

Apoptosis as a key factor for Th2 predominance in allergic disorders.
American Academy of Allergy Asthma & Immunology, 57th Annual Meeting, New Orleans (USA); March 16-21, 2001

T-cell clones in idiopathic eosinophilia.
American Academy of Allergy Asthma & Immunology, 57th Annual Meeting, New Orleans (USA); March 16-21, 2001

Eosinophils in health and disease.
Basic and Clinical Allergy; Imperial College of Science, Technology and Medicine, London (UK); April 2-6, 2001

Genetics and biology of asthmatic inflammation.
European Academy of Allergology and Clinical Immunology, XXth Congress, Berlin (D); May 9-13, 2001

Role of neutrophils and eosinophils in interstitial lung diseases.
International WASOG Conference on Diffuse Lung Diseases, Venedig (I); June 7-9, 2001

Apoptosis in normal and malignant haematopoiesis.
ESO/ESMO/EHA Course: Leukaemias and lymphomas, clinical and molecular problems, Ascona (CH); June 24-26, 2001

Management des schwierig zu behandelnden Asthmas.
Wissenschaftliches Symposium: "100 Jahre Hochgebirgsklinik Davos Wolfgang", Davos (CH); June 30, 2001

Zytokine: Klassifikation, Rezeptoren, Wirkungsmechanismen.
Immunologie-Tag „Zytokine und Antizytokin-Therapie“, Bern (CH); September 20, 2001

Eosinophil apoptotic mechanisms.
European Respiratory Society, Annual Congress, Berlin (D); September 22-26, 2001

Hypereosinophilia.
XXIII National Congress of the Italian Society of Allergology and Clinical Immunology, Sorrento (I); October 10-14, 2001

Factors and pathways regulating apoptosis in inflammation.
Dr. Josef Steiner Cancer Foundation/Swiss Society for Oncology, Bern (CH); November 2-3, 2001

Physiologie des Immunsystems.
Allergologie-Kurs für Augenärzte, Hannover (D); November 16-17, 2001

Immunologische Grundlagen allergischer Erkrankungen.
Allergologie-Kurs für Augenärzte, Hannover (D); November 16-17, 2001

In-vitro-Diagnostik allergischer Erkrankungen.
Allergologie-Kurs für Augenärzte, Hannover (D); November 16-17, 2001

Pathophysiologische Grundlagen allergischer Erkrankungen.
Dermato-Allergologischer Kurs der Schweizerischen Gesellschaft für Dermatologie und Venerologie (SGDV), Bern (CH); November 23-24, 2001

4.3. Seminar Invitations

Prof. Ulrich E. Honegger

Behandlung von spastischen Zuständen bei Kindern.
AerztInnen und Pflegende der regionalen Heime für behinderte Kinder, Bern (CH); January 29, 2001

Medikamenten Ge-Brauch und Miss-Brauch.
Naturforschende Gesellschaft Bern, Bern (CH);
guest of Prof. Dr. R. Weingart; April 23, 2001

Do we still need phytotherapy in the age of COX-2 inhibitors?
Université de Lausanne et pharmacien/nes du canton de Vaud, Lausanne (CH);
guest of Prof. Dr. K. Hostettmann; April 24, 2001

Pharmakologie der Analgetika, im Besonderen von COX-2 Hemmern.
Interdisziplinärer Experten Round-Table, Zürich (CH); May 30, 2001

Ueberblick über die Wirkungsweisen von Psychopharmaka.
Universität Basel, Kinder- und Jugendpsychiatrische Uniklinik, Basel (CH);
guest of Prof. Dr. D. Bürgin; June 13, 2001

COX-2 Hemmer, Erwartungen, Erfahrungen, offene Fragen.
Berner Rheumatologen, Bern (CH); guest of Prof. Dr. P. Villiger.

Apotheker des Kantons Bern, Biel und Bern (CH); June 2001

Behandlung von Drogenabhängigen. Wirkungen von Psychopharmaka.
Regionalgericht Bern+Laupen, Gerichtspräsidenten und RichterInnen,
Amtshaus Bern, Bern (CH); July 2, 2001

Hyperforine: Principe actif ou source d'interactions.
Université de Lausanne, 3ème cycle en sciences pharmaceutiques, Zermatt (CH);
guest of Prof. Dr. K. Hostettmann; October 1-5, 2001

Feasibility of pharmacodynamic models in bioavailability testing of plant extracts.
Université de Lausanne, 3ème cycle en sciences pharmaceutiques, Zermatt (CH);
guest of Prof. Dr. K. Hostettmann; October 1-5, 2001

Neue Wege in der Raucherentwöhnung.
Continuation weekend for neurologists, Sils-Maria (CH); October 12-14, 2001

Partielle Dopamin-Agonisten als Neuroleptika. Grundlagen und Bedeutung für die Therapie.
Advisory Board of a drug company, Bern (CH); December 3, 2001

Prof. Hartmut Porzig

Beyond cytokines: A role for protein kinase C and trimeric G proteins in the proliferation and differentiation of human hematopoietic cells.
University of Zurich, Seminarreihe "Aktuelle Probleme der Krankheitsforschung",
Zurich (CH); guest of PD Dr. U. Zangemeister-Wittke; June 16, 2001

Prof. Erwin Sigel

Towards the architecture of $GABA_A$ receptors.
Brain Research Insitute, University of Vienna, Vienna (A);
guest of Prof. Dr. W. Sieghart; June 1, 2001

Prof. Hans-Uwe Simon

Hypereosinophiles Syndrom.
University of Bern, Institut für Medical Onkologie, Inselspital, Bern (CH);
guest of Prof. Dr. M.F. Fey; January 11, 2001

Role of apoptosis in inflammation.
University of Frankfurt, Institut für Pharmakologie und Toxikologie, Frankfurt (D);
guest of Prof. Dr. J. Pfeilschifter; March 01, 2001

Effector functions of eosinophils in allergic inflammation.
Breakfast seminar, American Academy of Allergy Asthma & Immunology,
57th Annual Meeting, New Orleans (USA); March 16-21, 2001

Role of apoptosis in allergic inflammation.
Breakfast seminar, American Academy of Allergy Asthma & Immunology,
57th Annual Meeting, New Orleans (USA); March 16-21, 2001

Steroid-resistant asthma.
Lunch seminar, European Academy of Allergology and Clinical Immunology, XXth Congress, Berlin (D); May 9-13, 2001

Caspase-independent signalling via death receptors.
Institute for Research in Biomedicine (IRB), Bellinzona (CH);
guest of Prof. Dr. M. Thelen; May 28, 2001

Hypereosinophilie.
University of Bern, Dermatologische Klinik, Inselspital, Bern (CH);
guest of Prof. Dr. L. R. Braathen; June 21, 2001

New drug targets in antiinflammatory therapy.
EiRx Therapeutics Ltd., Cork (Irland);
guest of Dr. I. Hayes; September 12, 2001

New aspects of apoptosis regulation: From death receptors to phosphatases, proteases, and beyond.
University of Bern, Departement für Chemie und Biochemie, Bern (CH);
guest of Prof. Dr. B. Erni; October 29, 2001

Molecular mechanisms controlling granulocyte apoptosis-role in inflammation.
University of Zurich, Seminarreihe “Aktuelle Probleme der Krankheitsforschung“, Zurich (CH); guest of PD Dr. U. Zangemeister-Wittke; December 17, 2001

4.4. Organization of Meetings and Courses

Dr. Kurt Baltensperger

Course "Introduction to Confocal Microscopy".
Bern, March 5-9, 2001.

Prof. Ulrich E. Honegger

Symposium: Medizinische Bedeutung von Johanniskraut als Antidepressivum.
Bern, February 15, 2001

Satellite symposium: Johanniskraut als modernes Phytotherapeutikum.
Bern, February 15, 2001

Forum Medizin: Topics in Medicine of general interest for the public (4 seminars).
Wohlen, April-June, 2001

Prof. Erwin Sigel

Practical course "Functional Analysis of Living Cells" for PhD students of the Medical Faculty (3rd course).
Bern, February 28 – March 2, 2001.

Prof. Hans-Uwe Simon

Course in Allergology (together with M. Zierhut and A. Schapowal).
Davos, March 30-31, 2001.

International Workshop on G-CSF and IFN-α.
Bern, June 27, 2001.

Fall meeting of the Swiss, German, and Austrian Societies of Pharmacology and Toxicology (scientific coordinator).
Bern, October 1-2, 2001.

9th Euroconference on Apoptosis (member of the advisory board).
Vienna, October 13-16, 2001.

4.5. Chairperson at Congresses

Prof. Hans-Uwe Simon

13. Mainzer Allergie-Workshop, German Society for Allergology and Clinical Immunology; Session „Granulocytes"; Mainz (D), March 2-3, 2001.

Annual meeting of the Swiss Society for Allergology and Clinical Immunology; Workshop "Free Communication I"; Lausanne, April 5-6, 2001.

9th Euroconference on Apoptosis, European Cell Death Society (ECDO); Session "Keynote lecture"; Vienna, October 13-16, 2001.

4.6. Referee Work for Peer-Reviewed Journals

Prof. Hartmut Porzig

N-S Arch Pharmacol
Cell Physiol Biochem

Prof. Harald Reuter

Nature
Proc Natl Acad Sci USA
J. Neurosci.

Prof. Erwin Sigel

Biochim Biophys Acta
Brain Res
Cell+Tissue Research
Eur J Neurosci
FEBS Lett
J Biol Chem
J Membrane Biol
J Neurochem
J Pharmacol Exp Ther
J Physiol (London)
Mol Pharmacol
Neurochem Int
Neuropharmacology
Pflügers Arch
Proc Roy Soc B
Trends Pharmacol Sci (TIPS)

Prof. Hans-Uwe Simon

Allergy
Apoptosis
Biochem Pharmacol
Blood
Cell Death Differ
Clin Exp Allergy
Clin Exp Immunol
Cytokine
Eur J Immunol
Eur Respir J
Int Arch Allergy Immunol
Int J Hyg Environ Health
J Allergy Clin Immunol
J Hepatol
J Immunol
J Invest Dermatol
J Leukocyte Biol
J Pharm Pharmacol
Life Science
Oncogene
Proc Natl Acad Sci USA
Swiss Med Wkly

4.7. Referee Work for Grant Bodies

Prof. Erwin Sigel

Swiss National Science Foundation
Wellcome Trust
Medical Research Council (MRC)
Austrian Foundation for the Advancement of Science

Prof. Hans-Uwe Simon

Wellcome Trust
Medical Research Council (MRC)
British Asthma Campaign
BONFOR-Program, University of Bonn (D)

4.8. Awards

Prof. Harald Reuter

Corresponding Member of the “Académie Royal de Médecine de Belgique“, Bruxelles, May 2001

Ernst Jung-Medaille für Medizin in Gold. Jung-Stiftung für Wissenschaft und Forschung (Jung-Foundation for Science and Research), Hamburg (D), December 2001

Prof. Hans-Uwe Simon

Recognition of the Italian Society for Allergology and Clinical Immunology, Sorrento (I), October 2001

Dr. Olivier Thomet

Alfred Vogel Preis 2001, Alfred Vogel Stiftung, Baden (CH), November 2001

5. Administrative, Advisory, and Honorary Posts

Dr. Kurt Baltensperger

Coordinator of the Confocal Microscopy Facility of the Dept. of Clinical Research at the PKI

Information Technology Coordinator at the PKI

Roland Baur

Coordinator for radioactive work at the PKI

Prof. Ulrich E. Honegger

Ortspräsident Pharmazie des BAG, Prüfungssitz Bern

Mitglied der Subkommission Pharmazie des Leitenden Ausschusses des BAG. Verantwortlich für die Universitäten Bern, Neuchâtel und Fribourg (BENEFRI)

Mitglied der Kommission Grundstudium der Medizinischen Fakultät der Universität Bern

Mitglied der Arzneimittelkommission des Schweizerischen Apothekerverbandes

Wissenschaftlicher Beirat des Apothekervereins des Kantons Bern

Präsident der Kommission für Fakultätsexamen in Pharmazie der Medizinischen Fakultät der Universität Bern

Member of the GlaxoSmithKline Advisory Board for Epilepsy

Member of the Zeller Medical Advisory Board

Prof. Hartmut Porzig

Member of the steering committee for the curriculum reform of 3rd year medical studies

Member of the working Group: “Interfakultäre Gradate School” and Curriculum “Medizinische Biologie”

Member of the Swiss Working Group: “Nachwuchsförderung/Akademische Qualifikationen”

Member of the Editorial Board of Naunyn-Schmiedebergs Archives of Pharmacology

Prof. Harald Reuter

Chairman of the Advisory Board of the “Biocenter“, University of Basel
Chairman of the “Committee on Human Rights“ of the Council of Swiss Academies

Member of the “International Human Rights Network of Academies and Scholarly Societies“

President of the “Schweizerische Stiftung für medizinisch-biologische Stipendien“ (Swiss foundation for medical-biological stipends)

Obmann (chairman) and Senator for the “Section Physiology and Pharmacology/Toxicology“ of the “Deutsche Akademie der Naturforscher Leopoldina“

Prof. Erwin Sigel

Biosafety Coordinator for the PKI

Member of the committee supervising the “Programm für die Interfakultäre Ausbildung des Forschungsnachwuchses der Universität Bern (PIAF) (Interfaculty Doctorate and PhD of the Medical Faculty)

Prof. Hans-Uwe Simon

Director of the curriculum “Pharmacology” within the program for interfaculty education of graduate students at the University of Bern

Treasurer, European Cell Death Society (ECDO)

Member of the management committee, European Commission Research Area: structural aspects, COST action 844 “Apoptosis and programmed cell death: molecular mechanisms and applications in Biotechnology and Agriculture”

Member of the Executive Committee of the European Academy of Allergology and Clinical Immunology

Fellow of the American Academy of Allergy, Asthma and Immunology (AAAAI)

Member of the Board, Swiss Academy for Medical Ethics

Section Editor, Apoptosis

Member of the Editorial Board, International Archives of Allergy and Immunology

Member of the Editorial Board, Allergy

Member of the Scientific Board, Allergologie

Member of the Editorial Board, Clinical and Experimental Allergy

Member of the Editorial Board, Int. Journal of Hygiene and Environmental Health

Member of the Advisory Board, Allergo-Journal

6. Services

6.1. Confocal Microscopy

The facility hosts a Zeiss laser scanning microscope (LSM410), which may be used by members of the Dept. of Clinical Research at no charge. For subsequent image handling a state-of-the art computing station was purchased during the past year. The coordinator (Dr. K. Baltenperger) provides training for new users, and technical and scientific support. During the past year the confocal microscope has been used by a total of 26 different users, and was in operation for over 600 hours with an average session time of 3 hours.

6.2. Flow Cytometry

A service is provided for analyzing potential pathogenic mechanisms of eosinophilic disorders and other chronic inflammatory diseases. Monitoring of patients under immunomodulatory therapy is also included. The costs are currently covered by research grants of the coordinator (Prof. H.-U. Simon), who can also be consulted for scientific support. Usage of the flow cytometer by non-members of the institute within collaborative projects is also possible.

7. Public Work

All members of the PKI

Exhibition of the Department of Pharmacology within the Festival “Science et Cité”, Bern, May 2001

8. Sponsors

8.1. Research Grants

Dr. Kurt Baltensperger

Swiss National Science Foundation (grant No. 31-059124.99)

Stiftung zur Förderung der wissenschaftlichen Forschung an der Universität Bern

Prof. Ulrich E. Honegger

Bundesamt für Umwelt, Wald und Landschaftsschutz (BUWAL)

Zeller Medical AG, Romanshorn (CH)

GlaxoSmithKline Beecham AG, Schönbühl (CH)

Prof. Hartmut Porzig

Schweizerische Krebsliga (together with K. Baltensperger, grant No. SKL 778-2-1999)

Sandoz Foundation, Basel (CH)

Prof. Harald Reuter

Swiss National Science Foundation (grant No. 31-045093.95/2)

Prof. Erwin Sigel

Swiss National Science Foundation (grant No. 31-64789.01)

UroGenesys, Los Angeles (USA)

GENION, Hamburg (D)

Prof. Hans-Uwe Simon

Swiss National Science Foundation (grant No. 31-58916.99)

Helmut Horten Foundation, Madonna del Piano (CH)

Novartis Foundation, Basel (CH)

EMDO-Foundation, Zurich (CH)

OPO-Foundation, Zurich (CH)

Stiftung zur Krebsbekämpfung, Zurich (CH)

Prof. Jörg Stucki

Swiss National Science Foundation (grant No. 31-49745.96)

Dr. Clemens Wagner

Josephine Clark Foundation, Bern (CH)

Dr. Shida Yousefi

Stiftung zur Förderung der wissenschaftlichen Forschung an der Universität Bern

PD Dr. Roger D. Zühlke

Swiss National Science Foundation (grant No. 31-056906.99)

8.2. Meetings

Zeller Medical AG, Romanshorn (CH)
Symposium: Medizinische Bedeutung von Johanniskraut als Antidepressivum. (organization: U.E. Honegger); Bern, February 15, 2001

Zeller Medical AG, Romanshorn (CH)
Satellite symposium: Johanniskraut als modernes Phytotherapeutikum (organization: U.E. Honegger); Bern, February 15, 2001

Roche Pharma Schweiz AG, Reinach (CH) and Aventis Pharma AG, Zurich (CH)
International Workshop on G-CSF and IFN-α _(organization: H.-U. Simon);Bern, June 27, 2001.

Alcon Pharmaceuticals Ltd., Cham (CH) and Alcon Pharma GmbH, Freiburg (D)
Course in Allergology (organization: H.-U. Simon together with M. Zierhut and A. Schapowal); Davos, March 30-31, 2001.

8.3. Travel Support

USGEB
Support of K. Kirschner, Spring meeting of the German Society of Experimental and Clinical Pharmacology and Toxicology, Mainz, March 2001.

Zeller Medical AG, Romanshorn (CH)
Support of O. Thomet, SSAI meeting in Lausanne, April 2001.

Essex Chemie AG, Lucerne (CH)
Support of H.-U. Simon, ERS meeting in Berlin, Sept. 2001.

COST action 844
Support of F. Altznauer and H.-U. Simon, 9. Euroconference on Apoptosis in Vienna, Oct. 2001.

8.4. Other Support

Novartis AG, Basel (CH)
Computer for the lecture room of the institute.

Novartis AG, Basel (CH)
Support of the PKI exhibition during the festival „Science et Cité“.

Bürgi fund
Seminar series at the institute.

AstraZeneca AG, Zug (CH)
Booklet with case reports for the symposium: "100 Jahre Hochgebirgsklinik Davos Wolfgang".

Essex Chemie AG, Lucerne (CH)
Reprints of review article No. 1 on page 41.