PREVIOUS AND ONGOING RESEARCH INTERESTS

Neuropharmacology (since 1988)

My interest in choline metabolism in the brain (from my postdoctoral work with Konrad Löffelholz) was extended to microdialysis studies which soon focused on acetylcholine release, first in rats, then in (transgenic) mice, e.g. in mouse models of Alzheimer´s disease (AD). In addition, the ischemia-induced release of choline lead to studies in ischemia and reperfusion which we combined with microdialysis to monitor brain metabolism *in situ*. The chance finding that bilobalide blocked ischemia-induced choline release led to a long-standing interest in the neuroprotective properties of this compound in experimental stroke; recent data demonstrate interactions between bilobalide, amino acid transmitters, and mitochondrial function. Finally, release of choline under receptor stimulation (e.g. by metabotropic glutamate receptors) was found to be due to phospholipase D whose role in signal transduction lead to investigations of lipid signalling in astrocyte proliferation. The blockade of the PLD pathway by ethanol gave rise to a hypothesis of ethanol action in fetal alcohol embryopathy. A current focus in my lab is energy metabolism in the brain, in the context of neurodegenerative disease (AD, stroke), anaesthesia, and epilepsy.

Central cholinergic systems. Release of acetylcholine in animal models (since 1997)

In recent years, we perfected the microdialysis technique in mice to investigate acetylcholine levels in brains of transgenic animals. We use this technique to characterize a variety of animal models including models of Alzheimer´s disease (APP- and tau-transgenic mice); animals grafted with neuronal transplants; or mice that are deficient for NO synthases.

Representative publications

- M.L. Buchholzer and J. Klein (2002) NMDA-induced acetylcholine release in mouse striatum: role of NO synthase isoforms. *J. Neurochem.* 82, 1558-1560.
- J. Hartmann, C. Erb, U. Ebert, K.H. Baumann, A. Popp, G. König and J. Klein (2004) Central cholinergic functions in human amyloid precursor protein knock-in/ presenilin-1 transgenic mice. *Neuroscience* 125, 1009-1017.
- J. Hartmann, C. Kiewert and J. Klein (2010) Acetylcholine release and energy metabolites in amyloid-bearing APP_{SWE} x PSEN1dE9 mice. *J. Pharmacol. Exp. Ther.* 332, 364-370.

Central cholinergic systems. Regulation of acetylcholine release after modulation of acetylcholinesterase (since 2000)

Acetylcholinesterase (AChE) inhibitors are mainstay drugs to treat Alzheimer´s disease. We investigate the effects of AChE modulation in mice in which AChE activities are genetically modified, i.e. transgenic mice that over-express or are deficient for AChE. Our data show that the extent of AChE inhibition that can clinically be reached by AChE inhibitor therapy is probably insufficient to enhance acetylcholine in the brain of Alzheimer´s disease patients.

Representative publications

- C. Erb, J. Troost, S. Kopf, U. Schmitt, K. Löffelholz, H. Soreq and J. Klein (2001) Compensatory mechanisms facilitate hippocampal acetylcholine release in transgenic mice expressing human acetylcholinesterase. *J. Neurochem*. 77, 638-646.
- J. Hartmann, C. Kiewert, E.G. Duysen, O. Lockridge, N.H. Greig and J. Klein (2007) Excessive hippocampal acetylcholine levels in acetylcholinesterase-deficient mice are moderated by butyrylcholinesterase activity. *J. Neurochem.* 100, 1421-1429.
- F. Mohr, M. Zimmermann and J. Klein (2013) Mice heterozygous for AChE are more sensitive to AChE inhibitors but do not respond to BuChE inhibition. *Neuropharmacology* 67, 37-45.

Non-neuronal cholinergic systems (since 2010)

This new interest has risen mainly from collaborations with colleagues at University of Giessen. We investigate the presence and role of acetylcholine in non-neuronal cells, e.g. in epithelial cells or in leukocytes.

Representative publications

- S.U. Schirmer, I. Eckhardt, H. Lau, J. Klein, Y.C. DeGraaf, K.S. Lips, C. Pineau, I.L. Gibbins, W. Kummer, A. Meinhardt and R.V. Haberberger (2011) The cholinergic system in rat testis is of non-neuronal origin. *Reproduction* 142, 157-166.
- S. Beyer, J. Klein and M. Diener (2014) Choline acetyltransferase and organic cation transporters are responsible for synthesis and propionate-induced release of acetylcholine in colon epithelium. *Eur. J. Pharmacol*. 733, 23-33.
- K. Deckmann, K. Filipski, G. Krasteva-Christ, M. Fronius, M. Althaus, A. Rafiq, T. Papadakis, L. Renno, I. Jurastow, L. Wessels, M. Wolff, B. Schütz, E. Weihe, V. Chubanov, T. Gudermann, J. Klein, T. Bschleipfer and W. Kummer (2014) Bitter triggers acetylcholine release from polymodal urethral chemosensory cells and bladder reflexes. *Proc. Natl. Acad. Sci. USA* 111: 8287-8292.
- A. Hecker, M. Küllmar, S. Wilker, K. Richter, S. Atanasova, T. Timm, A. Zakrzewicz, J. Klein, A. Kaufmann, S. Bauer, W. Padberg, W. Kummer, S. Janciauskiene, M. Fronius, E. Schweda, G. Lochnit and V. Grau (2015) Phosphocholine-modified macromolecules and canonical nicotinic agonists inhibit ATP-induced IL-1ß release via CHRNA9. J. Immunol., doi: 10.4049/jimmunol.1400974.

Energy metabolism in the brain (since 2010)

In the context of stroke, epilepsy, and anesthesia, we follow approaches that may protect the brain from damage under these conditions. We investigate both pharmacological as well as dietary approaches.

Representative publications

- T. Horn and J. Klein (2010) Lactate levels in the brain are elevated upon exposure to volatile anesthetics: a microdialysis study. *Neurochem. Int.* 57, 940-947.
- R. Samala, J. Klein and K. Borges (2011) The ketogenic diet changes metabolite levels in hippocampal extracellular fluid. *Neurochem. Int.* 58, 5-8.
- T. Horn and J. Klein (2013) Neuroprotective effects of lactate in brain ischemia: dependence on anesthetic drugs. *Neurochem. Int*. 62, 251-257.
- T. Schwarzkopf, T. Horn, D. Lang and J. Klein (2013) Blood gases and energy metabolites in mouse blood before and after cerebral ischemia: effect of anesthetics. *Exp Biol Med* 238: 84-89.
- T.M. Schwarzkopf, K. Koch and J. Klein (2015) Reduced severity of ischemic stroke and improvement of mitochondrial function after dietary treatment with the anaplerotic substance triheptanoin. *Neuroscience* 300: 201-209.

FINISHED PROJECTS

Regulation and function of phospholipase D in the brain: Role in astrocyte proliferation (1998-2014)

Our recent work on phospholipase D focused on its role in cell proliferation, particularly in astrocytes. The PLD pathway is activated by mitogenic agents but is disrupted in the presence of ethanol, a well-known teratogen causing reduced brain and body growth (alcoholic embryopathy). In the presence of ethanol, PLD forms phosphatidylethanol instead of phosphatidic acid, and thereby phosphatidic acid formation is suppressed. We presented evidence that the disruption of the PLD pathway is the cause for the ethanol-induced impairment of astroglial proliferation.

Representative publications

- K. Kötter and J. Klein (1999) Ethanol inhibits astroglial cell proliferation by disruption of phospholipase D-mediated signalling. *J. Neurochem.* 73, 2517-2523.
- B. Schatter, I. Walev und J. Klein (2003) Mitogenic effects of phospholipase D and phosphatidic acid in transiently permeabilized astrocytes: effects of ethanol. *J. Neurochem.* 87, 95-100.
- J. Klein (2005) Functions and pathophysiological roles of phospholipase D in the nervous system. J. Neurochem. 94, 1473-1487.
- B. Schatter, S. Jin, K. Löffelholz and J. Klein (2005) Ethanol-induced apoptosis in astrocytes: cross-talk between phosphatidic acid and ceramide. *BMC Pharmacology* 5:3 (11 pages).
- U. Burkhardt, B. Wojcik, M. Zimmermann and J. Klein (2014) Phospholipase D is a target for inhibition of astroglial proliferation by ethanol. *Neuropharmacology* 79: 1-9.

Cerebral ischemia: Neuropharmacology of plant products (1995-2014)

We have an ongoing interest in centrally acting plant extracts and have previously focused our studies on two major plant constituents, hyperforin from St. John´s wort and bilobalide from *Ginkgo biloba*. Our studies with hyperforin showed that hyperforin, at low doses, induced the release of acetylcholine by a depolarisation-type effect while high doses inhibit high-affinity choline uptake and ACh synthesis. Bilobalide was found to be a potent antagonist of the NMDA receptor-induced breakdown of phosphatidylcholine which is mediated *via* calcium influx and phospholipase A_2 (PLA_2) activation. Current work focuses on characterizing potential neuroprotective properties of bilobalide and Ginkgo extracts in models of neuronal cell death and edema formation (e.g., middle cerebral artery occlusion in mice).

Representative publications

- O. Weichel, M. Hilgert, S.S. Chatterjee, M. Lehr and J. Klein (1999) Bilobalide, a constituent of *Ginkgo biloba*, inhibits NMDA-induced phospholipase A_2 activation and phospholipid breakdown in rat hippocampus. *Naunyn-Schmiedeberg´s Arch. Pharmacol*. 360, 609-615.

- M. Buchholzer, C. Dvorak, S.S. Chatterjee and J. Klein (2002) Dual modulation of striatal acetylcholine release by hyperforin, a constituent of St. John´s wort. *J. Pharmacol. Exp.Ther*. 301, 714-719.
- A. Mdzinarishvili, C. Kiewert, V. Kumar, M. Hillert and J. Klein (2007) Bilobalide prevents ischemia-induced edema formation in vitro and in vivo. *Neuroscience* 144, 217-222.
- D. Lang, C. Kiewert, A. Mdzinarishvili, T.M. Schwarzkopf, R. Sumbria, J. Hartmann and J. Klein (2011) Neuroprotective effects of bilobalide are accompanied by a reduction of ischemia-induced glutamate release in vivo. *Brain Res*. 1425, 155-163.
- T. Schwarzkopf, K. Koch and J. Klein (2013) Neurodegeneration after transient brain ischemia in aged mice: beneficial effects of bilobalide. *Brain Res*. 1529: 178-187.

Cerebral ischemia: Ischemia and phosphatidylcholine breakdown (1995 to 2004)

Release of choline from phosphatidylcholine can be catalyzed by phospholipase A_2 or by phospholipase D. We find that PLA_2 is mostly responsible for choline release under pathological conditions such as hypoxia and ischemia. Choline release can serve as an indicator of membrane breakdown *in vitro* and *in vivo*, both in animal and in human studies. We have characterized metabolic changes in human skeletal muscle during surgery and have observed release of choline during ischemia. We have also reported increased phosphatidylcholine breakdown in Alzheimer´s disease, based on measurements of metabolite profiles in CSF of patients.

Representative publications

- J. Klein (2000) Membrane breakdown in acute and chronic neurodegeneration: focus on choline-containing phospholipids. *J. Neural Transm*. 107, 1027-1063.
- U. Korth, G. Merkel, F. Fernandez, O. Jandewerth, T. Koch, K. van Ackern, O. Weichel and J. Klein (2000) Tourniquet-induced changes of energy metabolism in human skeletal muscle monitored by microdialysis. *Anesthesiology* 93, 1407-1412.
- A. Walter, U. Korth, M. Hilgert, J. Hartmann, O. Weichel, M. Hilgert, K. Fassbender, A. Schmitt and J. Klein (2004) Glycerophosphocholine is elevated in cerebrospinal fluid of Alzheimer patients. *Neurobiol. Aging* 25, 1299-1303.

Regulation and function of phospholipase D in the brain: Glutamatergic regulation (1992-2000)

Phospholipase D is a receptor-regulated enzyme involved in signal transduction. In our early work on the phospholipase D signalling pathway, we characterized the activation of hippocampal phospholipase D (PLD) by glutamate which occurs *via* metabotropic glutamate receptors. We have also described modulations of synaptosomal PLD activity under depolarising conditions which suggest a role of the enzyme in neurotransmitter release.

Representative publications

- T. Holler, E. Cappel, J. Klein and K. Löffelholz (1993) Glutamate activates phospholipase D in hippocampal slices of newborn and adult rats. *J. Neurochem*. 61, 1569-1572.
- J. Klein, V. Chalifa, M. Liscovitch und K. Löffelholz (1995) Role of phospholipase D activation in nervous system physiology and pathophysiology. *J. Neurochem*. 65, 1445-1455.
- J. Klein, M. Iovino, M. Vakil, H. Shinozaki and K. Löffelholz (1997) Ontogenetic and pharmacological studies on metabotropic glutamate receptors coupled to phospholipase D activation. *Neuropharmacology* 36, 305-311.
- J. Klein, M. Vakil, F. Bergman, T. Holler, M. Iovino and K. Löffelholz (1998) Glutamatergic activation of hippocampal phospholipase D: Postnatal fading and receptor desensitization. *J. Neurochem*. 70, 1679-1685.
- M. Waring, J. Drappatz, O. Weichel, P. Seimetz, E. Sarri, I. Böckmann, U. Kempter, A. Valeva and J. Klein (1999) Modulation of neuronal phospholipase D activity under depolarizing conditions. *FEBS Lett*. 464, 21-24.

Central cholinergic systems: Synthesis of acetylcholine (1988-2002)

In my postdoctoral work with Konrad Löffelholz, we developed the hypothesis of the "homeostasis of brain choline" which claims that the extracellular concentration of choline in the brain is regulated within narrow limits by homeostatic mechanisms. Our data explained why the administration of choline (or lecithin) was not successful as a treatment for patients with central cholinergic dysfunction (e.g. in Alzheimer´s disease). Using *in vivo*-approaches such as microdialysis, we later found that the synthesis of acetylcholine can be facilitated in a synergistic manner by the combined application of precursors (choline, glucose). We suggested to test the combined administration of glucose plus choline in clinical studies. We also investigated the pharmacological and behavioral effects of nicotinamide, a compound that increases choline levels in the brain.

Representative publications

- J. Klein, A. Köppen and K. Löffelholz (1990) Small rises of plasma choline reverse the negative arterio-venous difference of brain choline. *J. Neurochem*. 55, 1231-1236.
- J. Klein, A. Köppen, K. Löffelholz and J. Schmitthenner (1992) Uptake and metabolism of choline by rat brain after acute choline administraton. *J. Neurochem*. 58, 870-876.
- A. Köppen, J. Klein, T. Holler and K. Löffelholz (1993) Synergistic effect of nicotinamide and choline administration on extracellular choline levels in the brain. *J. Pharmacol. Exp. Ther*. 266, 720-725.
- A. Köppen, J. Klein, C. Erb and K. Löffelholz (1997) Acetylcholine release and choline availability in rat hippocampus: effects of exogenous choline and nicotinamide. *J. Pharmacol. Exp. Ther*. 282, 1139-1145.

- S.R. Kopf, M.L. Buchholzer, M. Hilgert, K. Löffelholz and J. Klein (2001) Glucose and choline improve passive avoidance behaviour and increase hippocampal acetylcholine release in mice. *Neuroscience* 103, 365-371.

Toxicology: Metabolism of aromatic hydrocarbons (1985-1990)

In my doctoral work with Franz Oesch, I characterized a novel metabolic pathway for aromatic hydrocarbons which is catalyzed by dihydrodiol dehydrogenase (DDH). DDH is a family of enzymes which oxidize metabolically formed dihydrodiols to the respective catechols of polycyclic hydrocarbons such as benz(a)anthracene or benzo(a)pyrene. This pathway prevents the formation of highly carcinogenic dihydrodiol epoxides but gives rise to catechol structures that can induce oxidative stress by redox cycling.

Representative publications

- J. Klein, K. Post, H. Thomas, W. Wörner, F. Setiabudi, H. Frank, F. Oesch and K.L. Platt (1990) The oxidation of the highly tumorigenic benz(a)anthracene-3,4-dihydrodiol by rat liver dihydrodiol dehydrogenase. *Chem.-Biol. Interact.* 76, 211-226.
- J. Klein, H. Thomas, K. Post, W. Wörner and F. Oesch (1992) Dihydrodiol dehydrogenases activities of rabbit liver are associated with hydroxysteroid dehydrogenase and aldo-keto reductase. *Eur. J. Biochem.* 205, 1155-1162.
- J. Klein, K. Post, A. Seidel, H. Frank, F. Oesch and K.L. Platt (1992) Quinone reduction and redox cycling catalyzed by purified rat liver dihydrodiol / 3alpha-hydroxysteroid dehydrogenase. *Biochem. Pharmacol*. 44, 341-349.