UNIVERSITÄT OSNABRÜCK

2. Westerberger Herbsttagung

Perspektiven der molekularen Neurobiologie:

Molecular Physiology of Neurodegenerative Diseases

Friday, October 8, 2004, 9:00 am
Faculty of Biology and Chemistry, Barbarastr. 11, Main Lecture Hall

Human disease and animal models:

Jürgen Götz
(Universität Zürich, Division of Psychiatry Research, Schweiz)

Christian Schultz
(Klinikum der J.W. Goethe-Universität, Klinische Neuroanatomie, Frankfurt am Main)

Anne Eckert
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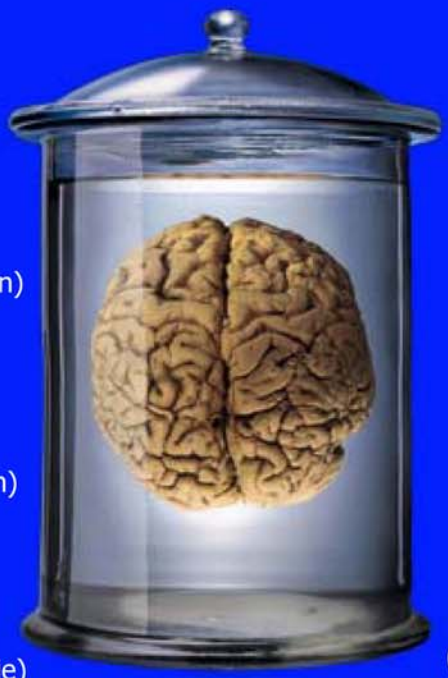

Advanced culture models:

Jochen Walter
(Universität Bonn, Abteilung Neurobiologie)

Luc Buee
(C.N.R.S, Neuroendocrinologie et physiophatologie neuronale, Lille, France)

Hans-Hinrich Althaus
(MPI für experimentelle Medizin, Göttingen)

Neelam Shahani
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Carina Weissmann
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sowie der Universitätsgesellschaft Osnabrück

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2nd Westerberger Herbsttagung
"Molecular Physiology of Neurodegenerative Diseases"

Friday, October 8, 2004, 9:00 am
Faculty of Biology and Chemistry, Barbarastr.11, Main Lecture Hall

9:00 - 9:15	Introduction (Roland Brandt, Osnabrück)
9:15 - 9:30	Cognitive Science in Osnabrück (Peter König, Osnabrück)
	Session I: Human disease and animal models (Chair: M. Hundelt)
9:30 - 10:15	Lecture 1 (Christian Schultz, Frankfurt) "Age-related tau pathology in nonhuman primates"
10:15 - 11:00	Lecture 2 (Anne Eckert, Basel) "Mitochondria failure in the pathogenesis of Alzheimer's disease"
11:00 - 11:30	- Coffee break -
11:30 - 12:15	Lecture 3 (Jürgen Götz, Zürich) "Functional Genomics applied to transgenic animal models of Alzheimer's disease"
12:15 - 12:45	Progress talk 1 (Kerstin Niggemann, Osnabrück und Münster) "Age-related changes of the microtubule-associated protein tau in nonhuman primates"
13:00 - 14:00	- Lunch break (Buffet) -
	Session II: Advanced culture models (Chair: G. Jeserich)
14:00 - 14:45	Lecture 4 (Hans-Hinrich Althaus, Göttingen) "Multiple Sclerosis: Signaling for Remyelination"
14:45 - 15:30	Lecture 5 (Jochen Walter, Bonn) "Molecular mechanisms in the regulation of Alzheimer's disease-associated secretases"
15:30 - 16:00	- Coffee break -
16:00 - 16:45	Lecture 6 (Luc Buee, Lille) "Neuronal death and Alzheimer's disease: apoptosis or neurofibrillary degeneration?"
16:45 - 17:15	Progress talk 2 (Carina Weissmann, Osnabrück) "Living cells on the spot: study of τ dynamics within living neuronal cell models by means of photoactivatable constructs"
17:15 - 17:45	Progress talk 3 (Neelam Shahani, Osnabrück) "Functional analysis of wild type and pseudohyperphosphorylated tau proteins in organotypic hippocampal slices"
17:45 - 18:00	Concluding remarks (Roland Brandt, Osnabrück)
Starting at 18:30	Speakers' dinner

Introduction

Two years ago Prof. Jeserich and I organized the first Symposium on *"Perspectives of Molecular Neurobiology"* on the occasion of the establishment of the Department of Neurobiology here in Osnabrück. At that time our aim was to give all interested an overview about the activities of the groups within the Department. In addition we wanted to place our work in the context of the exciting research which is going on in Molecular Neurobiology and invited four external speakers (the booklet is still available on the homepage of the Department at "archives", http://www.biologie.uni-osnabrueck.de/Neurobiologie/neurobiol/Neurobiology_home.html). After our last Meeting we got a lot of positive response from different sides - from students, from local teachers, from clinicians at local hospitals and from visitors from neighbouring universities. This encouraged us, to organize a follow-up Symposium this year, and, perhaps, to repeat such a Symposium in a biyearly manner. In contrast to our first Meeting, we decided to focus the topic this year much more. And since one of the major topics of the work of our Department is in neurodegenerative diseases we decided to focus on the *"Molecular Physiology of Neurodegenerative Diseases"* with a special emphasis on Alzheimer's disease.

Why Alzheimer's disease? Since the time when this disease has been described for the first time by the physician Dr. Alois Alzheimer at a Scientific Symposium in Tübingen in 1906 it has become evident that this disease is not a somewhat "exotic disease" but is the most frequent cause for dementia in elderly. In fact, worldwide more than 20 millions suffer from the disease - in Germany alone there are about 1 million of patients - and the numbers are increasing. Until now, there is still no causal therapy available. It is clear, that this disease is a heavy burden for family members. And it is also clear, that it is a heavy burden for the national health systems. In Germany alone there are an estimated 1 billion of Euro for Alzheimer's disease related costs per year.

Already Dr. Alois Alzheimer was aware from the analysis of the brain of his first patient Auguste D., that the disease is characterized by two abnormal protein aggregates in the brain. One are the neurofilbrillary tangles and the other are senile plaques. The contribution of these abnormalities to the disease progression is still not fully unterstood. But there is lots of evidence that both aggregates have an important role in the disease and may provide a target for therapeutic interventions. To analyze these aggregates in disease progression, research has concentrated on the development of suitable models. However no perfect model exists so far. But there is hope.

In the morning session of this symposium several speakers will give an overview about features of Alzheimer's disease and will present latest results in their research on the development of potential animal models. In the afternoon session, specialized culture models to analyze neurodegenerative mechanisms will be in the center of interest. With this symposium we aim to present an update about the current state of research in this exciting field and about potential developments in the future.

Currently, the Department of Neurobiology at the Faculty of Biology and Chemistry consists of two groups which are headed by Prof. Jeserich and me. In addition to the main lectures which are given by speakers from German, Swiss and French universities and research institutes, several

members of our Department will give short "progress reports" . These progress reports should give you a flavor about what is going on here in our Department and how our work relates to the general research programme.

I would like to mention that the Department contributes to the education of the students of biology, especially in the Bachelor and Master program "Biology of the Cell". In addition the Department also provides teaching for Bachelor students of "Cognitive Sciences". Cognitive Sciences started at the University of Osnabrück in 1998 with an international Bachelor program as the first Cognitive Sciences undergraduate programme in Germany.

Since we consider our Department as a "bridge" between Biology on one side and "Cognitive Sciences" on the other, we have invited Prof. Peter König, Chair of the Department of "Neurobiopsychology" at the Cognitive Science Institute to give an overview about Cognitive Sciences in Osnabrück. We are also planning to organize the "3rd Westerberger Fall Symposium" as a joint symposium between the Neurobiology and the Neurobiopsychology.

I would like to thank the co-organizers of this meeting, Dr. Monika Hundelt - chairperson of the morning session - and Prof. Gunnar Jeserich - chairperson of the afternoon session. I also thank all Members of the Department for their help in preparing coffee, booking hotels, choosing the buffet, taking photographs etc. In addition I would like to thank the local "Sonderforschungsbereich 431", the local "Graduate college" and the "Universitätsgesellschaft Osnabrück" for financial support. Of course, a special thank goes to all speakers for coming here and for contributing to an exciting Meeting on the *"Molecular Physiology of Neurodegenerative Diseases"*.

R. Brandt
Head of the Department of Neurobiology
at the University of Osnabrück

Lecture 1

Age-related tau pathology in nonhuman primates

Christian Schultz, MD

Alzheimer's disease (AD) is a progressive neurodegenerative and dementing disorder that can be detected clinically only in its end phase. AD is the most widespread type of dementia and affects about 10% of individuals older than 65 years and about 40% of individuals older than 80 years of age. The earliest sign of AD is a subtle decline in memory functions in a state of clear consciousness. A definitive diagnosis of AD based on clinical observations is impossible and requires confirmation by post mortem examination.

One of the neuropathological hallmarks of AD is comprised of extracellular precipitations of the ß-amyloid peptide. The second acknowledged neuropathological hallmark of AD is the presence of neurofibrillary inclusions composed of an abnormally phosphorylated and aggregated microtubule-associated tau protein (Braak 1991). The lesions develop in the form of neurofibrillary tangles (NFTs) and neuropil threads (NTs). Such neurofibrillary pathology is not unique to AD but is seen in other diseases that are collectively designated as "tauopathies." In these different brain areas are affected and abundant tau-positive inclusions in glial cells are present (Ikeda 1998). The destructive process that underlies the neurofibrillary pathology in AD evolves according to a predictable topographic sequence with little variation among individuals (Braak 1991).

Owing to the limitations imposed on experimental studies of the human brain, the question whether AD-related changes can be investigated in experimental animals is of considerable importance. Amyloid plaques can be induced in transgenic mice that express APP mutations causing autosomal dominant forms of AD in humans (Hock 2001). These transgenic mice provide insights into the pathogenesis and possible treatment of ß-amyloid deposition. Recent studies have also succeeded in generating authentic NFTs in transgenic mice (Götz 2002).

All of the transgenic mouse models are inherently limited by the large phylogenetic gap that exists between the murine brain and that of humans. Nonhuman primate models could help to narrow this gap. Recently, we have described a conspicuous pattern of tau pathology in baboons (Schultz 2000, 2002). The tau pathology in these nonhuman primates preferentially affects neurons and glial cells in the medial temporal lobe. In some of the older animals, a specific pattern of tau pathology was noted in the entorhinal cortex resembling an early stage of AD-related pathology. The aged baboon thus provides a potentially valuable nonhuman primate model for studies of the pathogenesis of selective neuronal tau pathology as it is characteristic of all human tauopathies, including AD. Experiments on both transgenic mice and nonhuman primates may complement one another, thereby helping to pinpoint pathogenic factors that underlie the neurofibrillary pathology in the aging human brain.

References

Braak H., Braak E.: Acta Neuropathol (1991) 82:239–259
Götz J., et al.: Science (2001) 293:1491-1495
Hock B.J., and Lamb B.T.: Trends Genet (2001) 17:S7-12
Ikeda K., et al.: Neurobiol Aging (1998) 19: S85-91
Schultz C., et al.: J Neuropathol Exp Neurol (2000) 59: 39-52
Schultz C., et al.: Interdiscipl Top Gerontol (2002) 31:118-129

Lecture 2

Mitochondria failure in the pathogenesis of Alzheimer's disease

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Increasing evidence suggests an important role of mitochondrial dysfunction in the pathogenesis of Alzheimer's Disease (AD). Activity changes in mitochondrial enzymes including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase have been described in AD brain. In addition, patients with AD showed impaired cytochrome c oxidase activity in the CNS and even in other tissues including platelets. The Swedish double mutation in the amyloid precursor protein (APP) gene (K670M/N671L) results in six- to eightfold increased amyloid-beta (Aß) production compared to human wildtype APP cells (APPwt). We have previously shown that the APPsw mutation enhances the vulnerability to secondary insults, e.g. oxidative stress, finally leading to apoptotic cell death through the activation of the c-Jun N-terminal kinase (JNK) and caspases, e.g. caspase 3 and 9. The latter observation provided evidence that mitochondria-mediated apoptosis might play an important role in Aß-mediated intracellular damage. Thus, we investigated effects of acute and chronic exposure to increasing concentrations of Aß on mitochondrial function and nitric oxide (NO) production in vitro and in vivo. Our data demonstrate that PC12 cells and HEK cells bearing the Swedish double mutation in the APP gene (APPsw), exhibiting substantial Aß levels, have increased NO levels and reduced ATP levels. The inhibition of intracellular Aß production by a functional γ-secretase inhibitor normalizes NO and ATP levels indicating a direct involvement of Aß in these processes. Extracellular treatment of PC12 cells with comparable Aß concentrations only leads to weak changes, demonstrating the important role of intracellular Aß. In 3-month-old APP tg mice, which exhibit no plaques but already detectable Aß levels in the brain, reduced ATP levels can also be observed showing the in vivo relevance of our findings. Moreover, we could demonstrate that APP is present in mitochondria of APPsw PC12 cells. This presence might be directly involved in the impairment of cytochrome c oxidase activity and depletion of ATP levels in APPsw PC12 cells. In addition, APPsw HEK cells, which produce 20-fold increased Aß levels compared to APPsw PC12 cells, and APP tg mice already show a significantly decreased mitochondrial membrane potential under basal conditions. We suggest a hypothetical sequence of pathogenic steps linking mutant APP expression and amyloid production with enhanced NO production and mitochondrial dysfunction finally leading to cell death.

Lecture 3

Functional Genomics applied to transgenic animal models of Alzheimer's disease

Jürgen Götz
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Alzheimer's disease (AD) is characterized clinically by a progressive loss of memory and other cognitive functions, resulting in dementia. The cognitive decline is associated with neuron loss and the accumulation in brain of both extracellular β-amyloid (Aβ42) containing plaques and intracellular neurofibrillary tangles (NFT). The latter contain hyperphosphorylated tau protein as major proteinaceous component. Tau is subject to additional posttranslational modifications. Their role, however, in tau aggregation and nerve cell degeneration is not well understood. Tau pathology is a central neuropathological characteristic not only of AD but also of many other neurodegenerative disorders that are characterized by dementia.
To model tau aggregation and NFT formation *in vivo*, we generated transgenic mice which express human tau together with the pathogenic mutation P301L which is associated with FTDP-17, an inherited form of dementia with similarities to AD. To test the amyloid cascade hypothesis, that claims a role of β-amyloid in NFT formation, we stereotaxically injected β-amyloid into brains of transgenic and control mice. β-Amyloid induced a fivefold increase in NFT in the amygdala of P301L transgenic, but not control mice. NFT formation was associated with phosphorylation of tau at S422. Mutagenesis of this epitope in a human tissue culture system abrogated both the Aβ42-mediated reduction in tau solubility and tau filament formation. Amygdala-dependent tasks revealed that P301L mice had anxiety levels not different from wild-types, but their exploratory behavior was significantly increased. Acquisition of a fear response to tone and context as well as taste aversion was comparable to wild-types. However, extinction of a conditioned taste aversion (CTA) was significantly accelerated. To gain insight into pathogenic mechanisms, we used proteomic and transcriptomic approaches. Differentially expressed genes were identified with Affymetrix chips followed by real-time quantitative PCR, *in situ* hybridization analysis of brain sections, and Northern blots. One of the upregulated genes, glyoxalase I, is part of a cellular detoxification pathway and prevents the formation of advanced glycation end-products (AGEs). We found accumulation of glyoxalase I protein in tangle-shaped neurons in AD brain. The proteomic approach identified proteins involved in detoxification, mitochondrial function and neurite outgrowth. Together, we aim to identify genes involved in filament formation and neurodegeneration, to dissect patho-cascades and to develop treatment strategies designed to prevent or halt AD and related disorders.

Lecture 4

Multiple Sclerosis: Signaling for Remyelination

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Axons of vertebrates are enwrapped by a compact, lipid-rich multilamellar membrane, the myelin sheath, which is interrupted along the length of axons by nodes of Ranvier. This achievement enabled vertebrates to propagate impulses much faster via a saltatory mode in contrast to a continuous conduction. However, the reverse of the achievement is a breakdown of function, when the integrity of the myelin sheath is not ensured. Diseases such as multiple sclerosis are characterised by a focal loss of myelin and oligodendrocytes (OL), the myelin producing cells, which goes along with neurological deficits. Intrinsic attempts of myelin repair do occur, but remain insufficient. To improving remyelination constitutes a major therapeutical challenge not only for restoring conduction velocity but also to prevent naked axons to degenerate. We have isolated and cultivated OL from young-adult pig brains to get information about their capabilities. We could demonstrate that OL can furnish myelin in vitro, when carbon fibres were added. Protein kinases appeared to play an important role in OL process regeneration and in the synthesis of myelin proteins. To our surprise, OL responded to nerve growth factor (NGF), which belongs to the family of neurotrophins. We could show that OL express the neurotrophin receptors TrkA and p75NTR, and that the subsequent signaling cascade is functioning. Cultured human OL also expressed NGF receptors, which made these findings more relevant. In an extension of the in vitro studies, in vivo experiments were initiated, in which demyelinated areas were produced under anesthesia by stereotactically injecting lysolecithin into the centrum semiovale of the mini-pig brain. Alzet mini-pumps were implanted allowing a superfusion of lesioned areas with either buffered NGF or the buffer (PBS) alone. Histochemical stainings revealed that PBS superfused areas remained largely demyelinated. In contrast, an extensive remyelination could be observed for NGF superfused areas. These results and those of other laboratories, which gave evidence for an anti-inflammatory and protective effect of NGF, indicate that neurotrophins should be considered as potential therapeutics for multiple sclerosis (1).

References

1. Althaus HH (2004): Remyelination in multiple sclerosis: a new role for neurotrophins? Progr. Brain Res. 146: 415-432

Lecture 5

Molecular mechanisms in the regulation of Alzheimer's disease-associated secretases

Jochen Walter
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Alzheimer's disease is characterized by the accumulation of neurofibrillary tangles and ß-amyloid plaques. While ‚tangles' are intraneuronal aggregates of the microtubule-associated protein tau, plaques are extracellular deposits that contain fibrillar forms of the amyloid ß-peptide (Aß). Aß derives from the ß-amyloid precursor protein (ßAPP) by proteolytic processing involving two membrane bound proteases, called ß- and γ-secretase. ß-Secretase was identified as the aspartic protease BACE1. The catalytically active domain of this protease is directed towards the lumen of intracellular compartments or the cell surface. BACE1 also contains a single transmembrane domain and a small cytoplasmic tail. Cleavage of ßAPP by BACE1 results in the secretion of the ßAPP ectodomain and the generation of a membrane-bound C-terminal fragment (CTF) that contains the Aß domain. This CTF represents a substrate for γ-secretase and is cleaved within the transmembrane domain resulting in the release of Aß into extracellular fluids. The γ-secretase consists of four membrane proteins named, presenilins, nicastrin, pen-2 and aph-1 that assemble into high molecular weight protein complexes. Accumulating evidence indicates that the presenilins represent the catalytically active components in these complexes. Beside the cleavage of ßAPP and other type I membrane proteins, presenilins are also involved in the regulation of ß-catenin signaling and apoptosis, independent of their proteolytic activity.
We demonstrated that overexpression of BACE1 significantly increased the generation of Aß. In contrast, BACE2, a close homologue of BACE1, revealed distinct substrate specificity. BACE2 can cleave ßAPP within the Aß domain, thereby precluding the generation of Aß. We also found that the subcellular trafficking of BACE1 is regulated by phosphorylation of its cytoplasmic domain by casein kinase-1. Phosphorylation of BACE1 facilitates the binding to proteins of the GGA (Golgi-localized Gamma ear containing ARF-binding) family and regulates the retrograde transport of this protease from endosomal compartments to the trans-Golgi network.
Phosphorylation also is involved in the regulation presenilin functions. We found that phosphorylation of presenilins occurs at the recognition site for caspases and identified the respective kinases. The phosphorylation inhibits cleavage of presenilins by caspases and modulates the progression of apoptosis. In addition, phosphorylation also regulates the binding of presenilin-1 to ß-catenin and the degradation of this protein. The functional implications of these regulatory mechanisms in Alzheimer's disease will be discussed.

Lecture 6

Neuronal death and Alzheimer's disease: apoptosis or neurofibrillary degeneration?

Luc Buee
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Abnormal Tau phosphorylation is likely to be a critical mechanism involved in Tau aggregation and neurofibrillary degeneration in Alzheimer's disease. Furthermore, each abnormal phosphorylation site may have its own impact on Tau aggregation. Indeed, correlation between specific epitopes of phosphorylated Tau and the severity of neuropathological stage in Alzheimer's disease has been reported. Like that, mitotic Tau phosphorylation is detected in early stages of pre-neurofibrillary tangles, and probably may be critical to the initiation of early conformational changes leading to Tau aggregation. However, the significance of such phosphorylation in Tau aggregation remains to be established. Understanding the role of mitotic Tau phosphorylation in NFTs formation and neurofibrillary degeneration requires the identification of kinases leading to these specific epitopes (TG-3), and relies on the development of appropriate cellular models.
Cdk5 is of particular interest since it is a Pro-directed phosphorylation kinase that belongs to the cyclin-dependent protein kinase family. However, its usual activators do not share any cyclin consensus sequence and are referred to as p35 and p39. Interestingly, p35 is usually anchored to the membrane. The p35-Cdk5 complex is abundantly found in the adult brain and Cdk5 activity is increasing in neurons during development. P35 can be proteolysed by calpains following changes in calcium homeostasis into a cytosolic C-terminal fragment referred to as p25. This latter is more stable than p35 and binds tighter to Cdk5 leading to a hyperactive p25-Cdk5 complex with a mislocation. Such cleavage of p35 to p25 was reported in Alzheimer's disease. Furthermore, exposure of primary cortical neurons to various insults like Aß peptide also leads to p25 formation and cell death. It's worth noting that in processes where p25-Cdk5 was likely involved, an aberrant cell cycle deregulation was reported.
In the present study, we used an inducible expression system that allowed investigation of Tau phosphorylation by p25/Cdk5 kinase complex in proliferating neuroblastoma Tau-SY5Y cells and in differentiated neuronal ones, independently of any effect of p25-induced toxicity. Our results showed that p25/Cdk5 complex is linked to Tau phosphorylation at the mitotic epitope TG-3 in proliferating as well as in neuronal cells. Beside TG-3 labelled Tau, p25/Cdk5 also generated mitotic reactive nucleolin in post mitotic differentiated cells. Several studies, mostly based on analysis of mitotic epitopes, support the idea of a reactivation of mitotic mechanisms in AD. The present data strongly suggest that p25/Cdk5 may be responsible for the generation of mitotic phosphoepitopes, reported as markers of early stages preceding neurofibrillary tangle formation, thus arguing for a pathological role of Cdk5 activity in AD. Further studies indicated that this kinase is likely to be involved in the reported expression of cell cycle markers in AD.
Interestingly, it was suggested that a reactivation of the cell cycle is a key step toward apoptosis. In fact, neurons of the adult brain are in G0: they do not divide and are differentiated. Post-mitotic neuronal cells coming out of G0 into G1 are usually stopped at the G1/S checkpoint and then undergo into either re-differentiation or apoptosis. Therefore, the deregulation of cell cycle proteins may be considered as pathological. It should be noted that re-expression of G1/S markers is the best correlated to the appearance of apoptosis in neurons. It is characterized by the formation of the cyclin D1-Cdk4/6 complex, phosphorylation of Rb, dissociation of the Rb-E2F complex and activation of genes leading to apoptosis.
In this respect, we investigated the molecular events that precede p25-Cdk5 triggered neuronal death

using the same neuronal cell line expressing an inducible p25 protein. In this system, no sign of apoptotic feature was seen before 24 hours of p25 induction. Thus, at that time, analyses of cell cycle regulatory proteins were performed by immunoblotting. Cyclins A and B1, p27Kip1, and cdc2-p34 showed a significant deregulation. After time course experiments, the earliest feature correlated to p25 expression was the phosphorylation of the retinoblastoma protein (Rb). Indeed this phosphorylation was observed 6 h following p25 induction and was abolished in the presence of a specific cdk5 inhibitor roscovitine, which does not inhibit the usual Rb cyclin D-kinases, cdk4/6. Furthermore, co-immunoprecipitation assays suggested a direct interaction between Cdk5 and Rb. The present work provides support of a close link between Cdk5 deregulation and cell cycle dysfunction and showed that Rb phosphorylation is an early event in p25-Cdk5 induced neurotoxicity. Hence, Rb protein, which has a relevant function in neuronal survival, is likely to be an early target of p25-Cdk5 kinase complex.
Thus, Rb may represent an appropriate candidate, which may connect Cdk5 to cell cycle deregulation in Alzheimer's disease and neuronal cell death.

Progress talk 1

Age-related changes of the microtubule-associated protein tau in nonhuman primates

Kerstin Niggemann
Department of Neurobiology, University of Osnabrück and Department of Pathology, Covance Lab. GmbH in Münster

The defining neuropathological characteristics of Alzheimer's disease (AD) are abundant filamentous intracellular tau lesions and extracellular deposits of fibrillar amyloid β peptides. Neurofibrillar protein aggregates containing tau in the absence of β-amyloid deposits are also hallmarks of neurodegenerative tauopathies such as Argyrophilic grain dementia (AgD), Pick's disease (PiD), Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) or Progressive supranuclear palsy (PSP).
In normal cells, the microtubule-associated protein (MAP) tau stabilizes neuronal microtubules for their role in the development of cell processes, establishment of cell polarity and intracellular transport. In tauopathies such as AD, tau becomes abnormally high phosphorylated (hyperphosphorylated) at many sites, aggregates into paired helical filaments (PHFs) and looses its ability to maintain the microtubule tracks.
A major obstacle in the elucidation of the pathogenesis of AD has been the lack of a reliable animal model of cerebral aging. Naturally occurring neurofibrillary tangles (NFTs) composed of paired helical filaments and senile plaques composed of fibrillar β-peptides have been documented in aged specimens of Asiatic brown bear (Cork et al., *J. Neuropathol. Exp. Neurol.* 47:629-641(1988)) and a wolverine (Roertgen et al., *Neurobiol. Aging* 17: 243-247(1996)).
To examine the age-related biochemical and histological changes of tau in a nonhuman primate, we performed an analysis of 20 Cynomolgus monkeys (*Macaca fascicularis*) aged from 4 to more than 20 years. For that, we extracted the tau protein from one hemisphere of the brain for biochemical investigation and examined the pattern of tau-pathology from the remaining hemisphere.
The tau-pathology in the Cynomolgus monkey preferentially affects glial cells where structures were positively stained by the modified Gallyas-Braak method and immunostained for phosphorylated tau by AT8. The glial cells possessing the structures were negative for glial fibrillary acidic protein, a marker for astrocytes, indicating that the glial fibrillary tangles were present in oligodendrocytes.
On Western blots, soluble tau detected with various pan-tau and phospho-tau specific antibodies appears as three major bands at 59 to 74 kDa, insoluble tau appears as three major bands at 64 to 74 kDa. Dephosphorylation of soluble tau with alkaline phosphatase was used to show the phosphorylation state of soluble tau. Dephosphorylated tau shows three major bands at 50, 57 and 64 kDa.
These results suggest that the Cynomolgus monkey is an interesting model for the study of biochemical and histological changes of tau-pathology occurring in human neurodegenerative brains.

Progress talk 2

Living cells on the spot: Study of τ dynamics within living neuronal cell models by means of photoactivatable constructs

Carina Weissmann
Department of Neurobiology, University of Osnabrück

The tau protein, a MAP (Microtubule Associated Protein) principally located on the axon of nerve cells aggregates into intracellular filaments in some neurodegenerative diseases called tauopathies (Brandt, 2001; Shahani and Brandt, 2002). Until this date, most of the studies on the behaviour of this protein were based on *in vitro* experiments.
The aim of this presentation is to introduce the use of the photoactivatable GFP molecule (PAGFP) (Lippincott-Schwartz and Patterson, 2003) to measure the dynamics throughout time of different Tau constructs within living PC12 cells.
PC12 cells are derived from a rat pheochromocytoma. Under ordinary culture conditions the cells have properties similar to those of immature rat adrenal chromaffin cells, but when grown in the presence of nerve growth factor (NGF), extend neurites, become electrically excitable, become more responsive to exogenously applied acetylcholine, have increased numbers of calcium channels and increase their synthesis of several neurotransmitters, and therefore resemble sympathetic neurons.
Both mutated forms of tau as well as normally expressed isoforms were fused to PAGFP, transfected and expressed in PC12 cells grown in the presence of NGF.
Eventually, this approach will assess dynamic parameters for each PAGFP-tau constructs, which will vary as a result of tau interactions with other cell components.
A determination of functional interactions of tau constructs in living neuronal cell models could provide important data for understanding tau malfunction during neurodegenerative diseases.
This presentation will mostly concentrate on the technical aspects and preliminary results obtained during the initial stage of this project.
(supported by the DFG-Graduate College 612)

References

Brandt, R. (2001) Cytoskeletal mechanisms of neuronal degeneration, *Cell & Tissue Research* **305**:255-265.

Lippincott-Schwartz, J., Patterson, G.H. (2003) Development and use of fluorescent potein markers in living cells, *Science* **300**:87-91.

Shahani, N., Brandt, R. (2002) Functions and malfunctions of the tau proteins, *Cell. Mol. Life Sci.* **59**:1668-1680.

Progress talk 3

Functional analysis of wild type and pseudohyperphosphorylated tau proteins in organotypic hippocampal slices

N. Shahani
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Hyperphosphorylated tau is the major component of the paired helical filaments in neurofibrillary tangles found in Alzheimer's disease (AD) brain. To determine the potential role of tau hyperphosphorylation in AD, pseudohyperphosphorylated tau (PHP tau) that mimics key structural and functional aspects of AD-like hyperphosphorylated tau protein were expressed in organotypic hippocampal slices. We generated human adult and fetal tau constructs [for both wild type (wt) and PHP tau], which were aminoterminally tagged with enhanced green fluorescent protein (EGFP), and cloned in Sindbis virus-expression system. A high and efficient expression of EGFP-tau proteins (both adult and fetal) was observed exclusively in neurons of cultured hippocampal slices. Intense EGFP fluorescence was present in the cell body and the processes of infected neurons. Expression was detected as early as 7hrs post-infection and persisted until 6 days. Effect of tau overexpression on neurodegeneration was assessed by analysis of lactate dehydrogenase (LDH) release, caspase-3 activity and changes in nuclear morphology. PHP tau (both adult and fetal) expression in neurons resulted in an increased degeneration compared to wt tau or control (EGFP alone). In order to correlate the increased degenerative changes with the phosphorylation of PHP tau protein (at the sites not mutated), semi-quantitative analysis of phosphorylated tau was made by immunoblot analyses using phosphorylation- and conformation-dependent antibodies. PHP tau showed an increased phosphorylation at T181, T212, S214, S262 and S356 and decreased phosphorylation at T205 compared to wt tau. Interestingly, PHP tau was highly immunoreactive for Alz-50 and MC1 antibodies, which recognizes a conformation of tau typical of AD-tau. The data suggests that the hyperphosphorylation state of tau itself my represent a toxic insult for the neurons. Further, this study provides a novel model utilizing the expression of Alzheimer's disease-relevant tau constructs in organotypic hippocampal slices, to understand the 'toxic gain of function' of soluble tau as a result of structural changes that are induced by cumulative, high-stoichiometric tau phosphorylation.

Introduction: R. Brandt, Osnabrück

P. König, Abt. Neurobiopsychologie, Osnabrück

Chairperson morning session:
M. Hundelt

Lecture I:
C. Schultz, Frankfurt

Lecture II:
A. Eckert, Basel

Lecture III:
J. Götz, Zürich

Progress talk I:
K. Niggemann, Osnabrück, Münster

Lecture IV:
H.H. Althaus, Göttingen

Chairperson afternoon session: G. Jeserich

Lecture V:
J. Walter, Bonn

Lecture VI:
L. Buée, Lille

Progress talk II:
C. Weissmann, Osnabrück

Progress talk III:
N. Shahani, Osnabrück

Conversations during Coffee and Buffet

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Pressemitteilung der Universität Osnabrück

Gegen das Vergessen

Universität Osnabrück: Tagung über die Ursachen der Alzheimerschen Krankheit

Osnabrück, 29.9.2004
Nr. 193/2004

Die Alzheimersche Krankheit ist bei älteren Menschen die häufigste Ursache für Demenz. Weltweit leiden etwa 20 Millionen Patienten an dieser Erkrankung, wobei die Zahl aufgrund gestiegener Lebenserwartungen voraussichtlich weiter ansteigen wird. Dies stellt ein ungeheure Herausforderung für die öffentliche Gesundheitsversorgung dar. Das Fachgebiet Neurobiologie an der Universität Osnabrück erforscht die Ursachen der Alzheimerschen Erkrankung. Auf einer Tagung am Freitag, 8. Oktober, sollen neue Forschungsergebnisse diskutiert werden. Wissenschaftler aus der Schweiz, Österreich und Deutschland nehmen daran teil.

Bei der Alzheimerschen Erkrankung kommt es zu Proteinablagerungen im Gehirn. »Um die altersabhängige Bildung dieser Aggregate oder ihrer Vorstufen zu dokumentieren und eventuelle Präventions- oder Heilmittel zu testen, wurden Organismen gesucht, die diese Ablagerungen aufweisen. Dabei erwiesen sich vor allem genetisch veränderte Mäuse als besonders hilfreich«, erklärt der Osnabrücker Biologe Prof. Dr. Roland Brandt. Am Vormittag des Symposiums wird über menschliche neurodegenerative Erkrankungen und die Bedeutung von Tiermodellen für deren Erforschung referiert. Spezialisierte Zellkulturmodelle für die Aufklärung neurodegenerativer Mechanismen sollen am Nachmittag diskutiert werden. Sprechen werden unter anderem PD Dr. Jürgen Götz (Zürich), Dr. Luc Buée (Lille, Frankreich) und PD Dr. med. Christian Schultz (Frankfurt).

Alle Interessierten sind herzlich eingeladen. Die Tagung beginnt um 9 Uhr im Hörsaal der Biologie, Barbarastr. 11, 49076 Osnabrück. Eine Voranmeldung ist nicht erforderlich.

Weitere Informationen:

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dpa-Meldung, 2. Oktober 2004

301325 Sep 04 xmee008
Gesundheit/Medizin/Forschung/
(dpa-Gespräch)
Zunehmende Alzheimer-Erkrankungen - «Kosten in Milliardenhöhe» =

Osnabrück (dpa/lni) - Die Alzheimer-Krankheit stellt nach Experten-Ansicht eine wachsende wirtschaftliche und gesundheitspolitische Herausforderung der Zukunft dar. «Alzheimer verursacht Kosten in Milliarden-Höhe», sagte Professor Roland Brandt, Neurobiologe an der Universität Osnabrück, im Gespräch mit der dpa, im Vorfeld einer internationalen Tagung zu Ursachen der Krankheit am 8. Oktober in Osnabrück. Schon jetzt seien 40 Prozent aller über 80-Jährigen an Alzheimer erkrankt, rund eine Million Menschen bundesweit - Tendenz steigend.

«Hauptrisikofaktor für die Krankheit bleibt das Alter», erklärte Brandt. Hinzu komme ein genetisches Risiko. Möglichkeiten zur Vorbeugung gebe es kaum. «Einzelne Untersuchungen zeigen zwar, dass bestimmte Vitamine schützend wirken können, ebenso das tägliche Glas Rotwein oder frühe intensive Gehirnaktivität in Ausbildung und Studium», sagte Brandt. «Aber wenn die Krankheit erst einmal ausgebrochen ist, hilft es auch nicht, das Telefonbuch auswendig zu lernen.»

Gehirn-Untersuchungen bei Unfallopfern hätten gezeigt, dass mitunter 20-Jährige erste Anzeichen der Krankheit aufweisen. «Alzheimer braucht 50, 60 Jahre, um sich zu entwickeln», erklärte Brandt. «Zu Beginn verklumpen Proteine im Gehirn. Es kommt zu zwei Arten von Ablagerungen, später zum Absterben von Nervenzellen im Gehirn.» Die Folge seien die bekannten Krankheits-Symptome: Gedächtnis- und Orientierungsstörungen, Verlust der Sprachfähigkeit und des Urteilsvermögens, Veränderungen der Persönlichkeit.

Derzeit forscht die Abteilung für Neurobiologie an der Universität Osnabrück an den Ursachen von Alzheimer. «Es deutet viel darauf hin, dass eine bestimmte Sorte von Proteinablagerung für den späteren Zellentod im Gehirn verantwortlich ist», erklärte Brandt. «Das könnte ein neuer therapeutischer Ansatzpunkt sein.» Mit Hilfe von Versuchen an Mäusen wollen die Wissenschaftler ein Modell entwickeln, mit dem später mögliche Behandlungen getestet werden können. «Bisher ist Alzheimer nicht heilbar. Medikamente können den Krankheitsverlauf nur verlangsamen», sagte Brandt. «Voraussetzung ist, dass wir die Ursachen verstehen.»
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