



# Universitätsklinikum Bonn

## Zentrum für Kinderheilkunde

## Medizinische Einrichtungen

Allgemeine Pädiatrie und Poliklinik: Prof. Dr. M.J. Lentze

Hämatologie-Onkologie: Prof. Dr. U. Bode

Kardiologie: PD Dr. J. Breuer (Komm. Direktor)

Neonatologie: Prof. Dr. Dr. P. Bartmann

PD Dr. C. Roth

Endokrinologie/Diabetologie

Tel. +49-228- 2873333

Fax +49-228-2873444

Abteilung für Allgemeine Pädiatrie und Poliklinik · Adenauerallee 119 · D - 53113 Bonn

## Research Report ESPE Sabbatical leave Christian L. Roth, MD 09-01-2003 to 01-31-2005

Host: **Sergio R. Ojeda, Division of Neuroscience, Oregon National Primate Research Center/Oregon, Health & Science University, 505 N.W. 185th Avenue, Beaverton, Oregon 97006; e-mail: [ojedas@ohsu.edu](mailto:ojedas@ohsu.edu), Phone: +1 (503)690-5303, Fax: +1 (503)690-5384**

Grants: ESPE Sabbatical leave, German Research Foundation (DFG RO 2220/3-1)

### **Performed Studies:**

#### **I Identification of candidate genes of mammalian puberty using DNA arrays.**

To identify the initial building blocks of a hypothetical “puberty-inducing gene cluster” we used DNA microarrays. Global changes in gene expression that occur in the female rhesus monkey hypothalamus at the time of puberty were assessed using cDNA arrays containing 8,400 genes (Gene MicroArray Shared Resource, Oregon Health and Science University). We compared 5 juvenile (1-2 year-old) monkeys to 3 early pubertal (2-3-year old) and 5 midpubertal (3-4 year-old) animals. Upon merging the data (Ommivis software), the genes showing changes in expression were ranked according to consistency of the change across biological replicates, and genes showing a relative consistent developmental profile (increase or decrease across puberty) in at least 5 of the 8 biological replicates were selected for further study. Among 68 genes showing increased expression, specifically in MBH but not in cerebral cortex in pubertal monkeys compared to juveniles, were 11 tumor-related genes –6 tumor suppressor genes (TSGs) and 5 oncogenes. We have validated the microarray results by real-time PCR and confirmed the increase for selected genes. In additional real-time PCR studies we have also found increased mRNA levels for the prominent TSG *p53*, and *SynCAM*, encoding an adhesion tumor suppressor molecule involved in lung cancer. Hybridization histochemistry showed neuronal and glial expression of the studied tumor-related genes mainly in the hypothalamic periventricular region and the arcuate nucleus. The overrepresentation of tumor-related genes suggests that these genes are expressed in mediobasal hypothalamus as components of a regulatory gene network that executes and integrates cellular and cell-cell communication programs required for the initiation of puberty.

#### **II Study of the role of tumor suppressor genes in regulating the onset of puberty using gene knock-down by silencing RNAs**

In this study we try to identify members of the tumor suppressor gene regulating network using the technique of tumor suppressor gene knock-down by silencing RNAs in

- 2 -

hypothalamic fragments. During the last 5 months in Oregon, I started to work on this project and identified siRNAs which are effective in suppressing expression of tumor suppressor genes in neuronal cell cultures. The experiments are not yet finished and will be continued.

### **III Quantitative proteomics in female rat puberty**

We then used quantitative proteomics to complement this genomic approach, employing the novel technique of isotope-coded affinity tags (ICAT) to identify proteins that may be involved in the neuroendocrine control of puberty. We selected the ICAT method to be able to both quantify and identify individual proteins within a complex protein mixture. Proteins were extracted from the hypothalamus of juvenile (25-day-old) and peripubertal (first proestrus, LP) female rats. After derivatizing cysteine-containing proteins from juvenile or LP samples with the isotopically light- or heavy form of the ICAT reagent, the labeled peptides were separated and analyzed by microcapillary reversed-phase liquid chromatography/tandem mass spectrometry. Five proteins involved in glutamate metabolism were identified as differentially expressed. Two of them were selected for further analysis. One, glutamate dehydrogenase, catalyzes glutamate synthesis; its hypothalamic content increased at first proestrus. The other, glutamine synthetase, catalyzes the metabolism of glutamate to glutamine; its content decreased in proestrus. Western blot analysis verified these results. Glutamatergic neurons coordinate the facilitatory transsynaptic and astroglial inputs to GnRH neurons. Excitatory neurotransmitter activation is indeed a major event underlying the pubertal activation of GnRH secretion. Our results show that the increased glutamatergic inputs to the GnRH neuronal network at time of puberty are associated with characteristic changes of hypothalamic protein contents of glutamate regulating enzymes.

### **IV Mouse model of conditional neuronal GnRHR KO**

We are working on a neuronal conditional GnRHR knock-out model using the Cre-lox technique. I was involved in this collaborative study performing Southern Blot technique and nested PCRs to identify positive embryonic stem cell clones.

### **V Studies of appetite regulating peptides in serum of obese children**

Furthermore, I have also had the chance to collaborate with the obesity research group (Pablo Enriori, Michael Cowley) at the Primate Center, Oregon Health & Science University West Campus. We have been studying factors involved in the control of food intake such as peptide-YY and pancreatic polypeptide (PP), as evidenced in serum samples from obese children treated in two German obesity studies (Bonn, and Datteln – Dr. Reinehr). I am grateful that I had the opportunity in learning the technique of non-commercially available radioimmunoassays.

#### **Methods - Summary:**

- Computerized gene studies (Bioinformatics)
- RNA and protein extraction from tissues
- Conventional PCR techniques and quantitative RealTime PCR
- Quantitative proteomics using ICAT approach and Western Blot
- Tissue slice preparation and tissue culture
- Cell culture experiments
- Gene transfer, stable and transient transfections.
- DNA cloning and bacterial cultures

- Preparation of nucleotide samples for In situ hybridization (ISH)
- 3 -
- Gene knock-down experiments using siRNAs
  - Southern blots and 5'labelling of oligonucleotides for detection of gene constructs in embryonic stem cells
  - Radioimmunoassays

### References:

*Henning-Andersen-Award of the European Society for Paediatric Endocrinology (ESPE), entitled: Roth C, Mastronardi C, Mungenast A, Heger S, Jung H, Ojeda SR "Gene Expression Profiling of the Nonhuman Primate Hypothalamus at the Time of Female Puberty Reveals Activation of Tumor Suppressor Gene Expression". Horm Res 2004; 62 (Suppl 2)*

*Ojeda SR, Lomniczi A, Mungenast A, Mastronardi C, Parent AS, Roth C, Prevot V, Heger S. Towards understanding the neurobiology of mammalian puberty: genetic, genomic and proteomic approaches. (2005) In Kordon et. al: Hormones and the brain. Springer Berlin Heidelberg, pp 47-59*

*Roth CL, Enriori PJ, Harz K, Woelfle J, Cowley M, Reinehr T. Factors Regulating Peptide YY<sub>3-36</sub> in Obese Children before and after Weight Loss. The Endocrine Society's 87<sup>th</sup> Annual Meeting, San Diego June 2005, OR47-3*

*Ojeda SR, Roth C, Heger S, Mastronardi C, Mungenast A, Lomniczi A, Jung H, Dissen GA. New Genes Involved in the Hypothalamic Control of Female Puberty. Abstract ESPE meeting Lyon, Sep 2005*

*Heger S, Mastronardi C, Lomniczi A, Cabrera A, Roth C, Sippell WG, Jung H, Dissen GA, Ojeda SR. Role of a novel gene (Enhanced at Puberty, EAP-1) in the regulation of female puberty. Abstract ESPE meeting Lyon, Sep 2005*

*Roth CL, Enriori P, Harz K, Cowley MA, Reinehr T. Regulation of Pancreatic Polypeptide (PP) in Obese Children. Abstract ESPE meeting Lyon, Sep 2005*

*Roth CL, McCormack AL, Lomniczi A, Mungenast A, Ojeda SR. Quantitative proteomics using isotope-coded affinity tags (ICAT) identifies a major change in hypothalamic glutamate metabolism at the time of female puberty. Abstract ESPE meeting Lyon, Sep 2005*

*Roth CL, Ojeda SR. Molecular aspects of abnormal puberty of central origin. Submitted to Ped Endocrinol Review 2005*

### Summary

During my stay in Oregon working in the laboratory of Sergio Ojeda I gained insight in basic molecular genetics and learned various important techniques which are very useful for my future projects. I think highly of Sergio Ojeda and his team colleagues. They are excellent researchers but also always ready to help. In addition, Maria Ojeda, Dr. Ojedas wife, supported me and my family (my wife and two daughters) with some helpful advice especially at the beginning of our stay. In the lab there was always a warm and very encouraging atmosphere. I will recommend this laboratory to other ESPE members to perform basic molecular research.

Finally I would like to thank the ESPE for supporting my sabbatical leave.

Bonn, 2005-07-30

Christian L. Roth