Session IV- Animal Models of PNDs Kathryn Swoboda, MD-Chair

Summary by Kathryn J. Swoboda, MD

A number of animal models are currently available which provide us with additional insights into the underlying pathophysiology of the various neurotransmitter deficiency disorders. Topics in this session included an overview of mouse models of catecholamine defects, by Xiaoxi Zhuang; a summary of new therapeutic insights relating to the SSADH knockout mouse, by K. Michael Gibson; and an overview of insights gained from the hph-1 mouse, a model of GTP cyclohydrolase deficiency, by Keith Hyland.

Dr. Zhuang reviewed the strategies involved in the creation of gene deletion (knockout) animal models and gene addition (transgenic) models. Both strategies have been used in the creation of animal models of catecholamine defects, involving numerous components of the dopaminergic synapse. These include TH (tyrosine hydroxylase), VMAT2 and DAT (dopamine transporters), D1 - D5 dopamine receptors, and Golf. The TH knockout animal proved to be an embryonic lethal. However, rescue of TH activity in norepinephrine neurons allowed the generation of dopamine deficient mice who were hypoactive and aphagic, with early postnatal lethality. The mice showed long-term survival with L-DOPA injection with an enhanced response to D1 or D2 dopamine receptor agonists manifest by hyperactivity and stereotyped movements. The DAT knockout mouse, in which dopamine reuptake into the presynaptic dopaminergic terminal is impaired, is hyperactive, with anterior pituitary hypoplasia and dwarfism. Evaluation of a number of psychostimulants in this model, including methylphenidate, amphetamine, cocaine, and nisoxetine, induce a paradoxical calming effect, measured by decreased horizontal activity in cm per unit time. A DAT "knockdown" mouse model has also been created via insertion of additional sequences within the DAT gene construct. No growth retardation is seen in the dopamine transporter "knockdown" mice. Compensatory changes observed in the DAT knockdown mouse include increased extracellular dopamine concentrations but decreased total tissue dopamine concentration. Marked slowing of uptake of dopamine is balanced in part by a significant decrease in dopamine release. Increased dopamine receptor sensitivity has also been observed in these mice in response to quinpirole and SKF-81297. In summary, the DAT knockdown mice have reduced DAT level compared to controls, a 30% reduction in dopamine clearance, approximately 50% tissue dopamine levels, a 200% increase in extracellular dopamine levels, and a 50% reduction in tyrosine hydroxylase. They demonstrate normal postsynaptic receptor expression density for D1 and D2 receptors, but approximately 50% reduction in the expression of presynaptic D2 receptors. However, the postsynaptic activity of the D1 and D2 receptors is reduced. In terms of behavioral analysis, they demonstrate increased locomotor activity and decreased habituation in an open field test. Additional testing on these mice evaluating prepulse inhibition of acoustic startle revealed a trend towards increased inhibition at lower prepulse intensities, and decreased inhibition at increased prepulse intensities. Working memory, as measured in a radial arm maze test, revealed no significant differences from wild-type animals. Impulsivity, as measured in a differential-reinforcement-of-low-rate paradigm, revealed a significant increase in the first 15 second epoch, followed by a decrease in the second 15 second epoch. Dr. Zhang also reviewed strategies available for creating spatial and temporal resolutions in gene knockout paradigms. Knocking out a gene at a specific developmental stage is termed an inducible knockout, while knocking out a gene in specific organs and tissues is termed a tissue specific knockout. Creating a tetracycline inducible system requires several steps. In the specific

paradigm that Dr. Zhang provides for this model, the first step is to knock in a neo-stop-tetO site just after the promoter of the gene of interest. This creates a knockout paradigm by preventing transcription. A brain specific promoter is then attached to a tet-transactivator, allowing rescue of the phenotype. Addition of doxycycline to this system aborts the rescue, creating a knockout of the gene at a specific point during development. He also outlined the steps involved in creation of a tissue specific knockout. These include making a tissue-specific Cre recombinase mouse, tagging the gene of interest with Cre recognition targets, and deleting the gene of interest by Cre recombinase. In this way, dopamine and serotonin neuron-specific Cre mice can be created. In summary, it is possible to manipulate mouse models in a variety of ways to better understand the pathophysiology of the catecholamine defects. There are a variety of methods available to analyze the resulting compensatory changes in the system. It is possible to do fairly sophisticated behavioral analyses on mice, and to turn on or turn off a gene of interest in specific organs or tissues at various times in development to explore questions regarding the pathophysiology of these disorders.

Dr. Gibson provided an overview of a murine model of succinic semialdehyde dehydrogenase deficiency (SSADH) deficiency. The first clinical identification of patients with SSADH deficiency was in 1983, followed by the development of an isotope dilution assay for GHB in 1990, an enzyme assay in 1991, the first prenatal diagnosis in 1994, and the cloning of the human gene in 1995. In 2000, the first knockout mouse was created, and ongoing efforts over the past two years have explored various therapeutic interventions in the mouse model. SSADH is involved in the catabolism of succinic semialdehyde, which is derived from GABA, an inhibitory neurotransmitter. Succinic semialdehyde is catabolized via SSADH into succinic acid, which enters the Kreb's cycle. Alternatively it is converted to 4-hydroxybutyric acid. This is the compound that accumulates in the setting of SSADH deficiency, resulting in at least some of the clinical manifestations of the disorder. Clinical features are variable and include psychomotor retardation, delayed or absent speech, hypotonia, ataxia, behavioral problems, seizures and EEG abnormalities. Metabolic abnormalities include marked elevations in urine, plasma and cerebrospinal fluid (CSF) GHB, as well as modest elevations in CSF GABA. Dr. Gibson reviewed the mechanism of action of Vigabatrin, an irreversible inhibitor of GABA transaminase, which should theoretically result in lower levels of succinic semialdehyde and its metabolites. Vigabatrin is typically used as an anticonvulsant. A pilot trial in patients with SSADH deficiency, at a dosage of 25-250 mg/kg/day, resulted in no obvious change in urinary GHB output and only modest improvements in ataxia, hypotonia and speech. It was discontinued in most patients due to lack of apparent benefit. It is unclear at present why patients failed to demonstrate benefit, and why a reduction in urine GHB was not noted. The mouse mutant shows significant growth retardation compared to its wild type counterpart. SSADH activities in tissue homogenates were reduced to essentially zero in the brain and heart, with less than 1% residual activity in the liver and kidney. GABA and GHB levels in urine, as well as tissue GHB concentrations, were markedly elevated. Brain total GABA was significantly elevated, and was specifically elevated in hippocampus, frontal cortex, parietal cortes and cerebellum. A number of abnormalities of specific amino acid concentrations were noted in mutant vs wild type animals. Glutamine levels in brain were significantly reduced, and were specifically reduced in hippocampus, cerebellum, frontal and parietal cortex. Arginine, aspartate and ornithine were significantly increased in frontal and parietal cortex. Serine was substantially elevated in hippocampus, frontal cortex and cerebellum,

and glycine was elevated in frontal and parietal cortex. Cystathionine and alanine were elevated in hippocampus, frontal and parietal cortex, and glutamate was elevated in hippocampus. It is known that GHB acts primarily by inhibiting presynaptic dopamine release in vivo. Potential therapies for SSADH deficiency include naltrexone, an opioid receptor antagonist;NCS-382, a selective GHB receptor antagonist which blocks striatal dopamine release following GHB administration; and taurine, an essential amino acid which is found in high quantities in breast milk. Survival curves in mutant mice given various agents at the time of weaning were reviewed. Administration of Vigabatrin, taurine or CGP 35348 increase the percent of animals surviving to age 50 days compared to untreated animals. The backbone of the compound NCS-382 strongly resembles that of GHB. NCS-382 reveals the highest percent survival to date of any of the compounds tested. NCS-382 appears to work via antagonism of GHB for its high affinity reaction with the GHB receptor. CGP 35348 interferes with the lower affinity reaction of GHB with the GABA B receptor. The poor clinical response with treatment with Vigabatrin in humans may be explained in part by the animal model. Although Vigabatrin increases brain GABA levels by 50%, GHB is not lowered. An adenoviral mediated liver gene therapy paradigm has been used in an attempt to rescue the phenotype in the mouse model by administering the construct at day of life 10, prior to weaning. The percent survival in the treated animals significantly exceeds that of the untreated animals after 20 days of life. This data is preliminary and additional studies are needed with regard to these therapeutic interventions. However, therapeutic interventions appear feasible based on the above data. Additional questions remain regarding whether or not SSADH deficiency is associated with oxidative damage in light of the reduction of the Krebs cycle intermediate succinic semialdehyde, and whether or not rescue of liver function alone will have a significant impact on the neurologic phenotype.

Dr. Hyland provided an overview of the hph-1 mouse, a model of autosomal dominant GTP cyclohydrolase deficiency (Segawa's disease). He reviewed what is known about the pattern of biochemical abnormalities in the human disorder, in which GTPCH deficiency results in decreased neopterin, tetrahydrobiopterin (BH4), homovanillic acid, and 5-hydroxyindoleacetic acid. He pointed out the important role of BH4 as a cofactor in the function of the hydroxylases phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and tryptophan hydroxylase (TRYPH), critical in the generation of the neurotransmitter precursors L-DOPA and 5-HTP. A number of unanswered questions remain regarding Segawa's disease, including the observed diurnal variation in symptoms, increased penetrance in females, variability of the phenotype, and mechanism of decreased amounts of TH in the striatum. As the underlying defect in the mouse model is unknown (no mutation in the GTP cyclohydrolase gene has been identified as yet), which raises questions regarding the validity of the mouse model in understanding the human condition. From a biochemical standpoint, however, it appears to be an excellent model, as manifest by decreased BH4 in striatum, as well as decreased brain amines and amine metabolites. The mechanism of decreased neurotransmitter metabolites had been initially presumed to be due to the decreased amount of available cofactor, BH4, resulting in sub-saturating concentrations for the hydroxylases. In vivo activities of TH and TRYPH, and in vitro striatal TH activities in the animal model were reduced, while in vitro AADC activity was preserved. In vitro liver PAH activity was also significantly decreased, with preserved AADC activity. This raised the question of whether the mechanism of decreased neurotransmitter metabolism is a general phenomenon relating to BH4 requiring enzymes. The mechanism of decreased hydroxylase activities could theoretically be due to an effect of BH4 on stability or

synthesis of the proteins. To address this question, correction of peripheral and central BH4 levels was attained via intraperitoneal injection of high dose (200 mg/kg) BH4. BH4 brain levels remained elevated for at least 10 hours above controls. Following correction of BH4 levels, striatal TH activity showed a progressive increase in the hours post injection. Western blots of striatal TH and liver PAH before and after BH4 injection revealed a substantial increase in measurable protein levels. Liver PAH activity also demonstrated a substantial increase in the hours post injection. TH and PAH gene expression, as assessed by TH and PAH cDNA levels, also substantially increased in the hours post injection. This data suggests that BH4 controls the steady state concentration of TH and PAH by a mechanism involving activation of gene expression, and that the decrease in striatal TH occurs because of decreased gene expression. Given the similarities between the mouse model and human disease, Dr. Hyland concludes that the hph-1 mouse is a good model to study pathophysiological mechanisms in Segawa di