MENTAL RETARDATION AND DEVELOPMENTAL DISABILITIES
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Positron Emission Tomography Methods with Potential for Increased Understanding of Mental Retardation and Developmental Disabilities

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Positron emission tomography (PET) is a technique that enables imaging of the distribution of radiolabeled tracers designed to track biochemical and molecular processes in the body after intravenous injection or inhalation. New strategies for the use of radiolabeled tracers hold potential for imaging gene expression in the brain during development and following interventions. In addition, PET may be key in identifying the physiological consequences of gene mutations associated with mental retardation. The development of high spatial resolution microPET scanners for imaging of rodents provides a means for longitudinal study of transgenic mouse models of genetic disorders associated with mental retardation. In this review, we describe PET methodology, illustrate how PET can be used to delineate biochemical changes during brain development, and provide examples of how PET has been applied to study brain glucose metabolism in Rett syndrome, serotonin synthesis in autism, and $GABA_A$ receptors in Angelman's syndrome and Prader–Willi syndrome. Future application of PET scanning in the study of mental retardation might include measurements of brain protein synthesis in fragile X syndrome and tuberous sclerosis complex, two common conditions associated with mental retardation in which cellular mechanisms involve dysregulation of protein synthesis. Mental retardation results in life-long disability, and application of new PET technologies holds promise for a better understanding of the biological underpinnings of mental retardation, with the potential to uncover new treatment options.

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Numerous gene mutations are being identified as causal in conditions involving developmental delay and mental retardation. Technologies such as positron emission tomography (PET) may be key in identifying the physiological and biochemical consequences of these gene mutations. Understanding the physiological and biochemical impact of a genetic mutation or polymorphism is essential for devising interventions to change the course of abnormal brain development. These PET studies may include studies in human subjects and studies utilizing genetic animal models of human mutations resulting in mental retardation. Studies in transgenic mouse models of human genetic disorders are now possible using PET scanners designed for rodents [Massoud and Gambhir, 2003; Tai et al., 2003], and new developments in "micro" PET technology to obtain higher spatial resolution will increase the utility of this approach in the near future [Stickel and Cherry, 2005]. In this review, we begin by introducing the basics of the PET methodology. We discuss ethical issues constraining the implementation of PET technology in developmental disorders. We then review PET studies showing changes in glucose metabolism, serotonin synthesis, and $GABA_A$ receptor binding during human brain development, to demonstrate the impact of the timing of PET studies during development. We illustrate how these same tracers have been applied in different syndromes associated with mental retardation. Finally, we suggest how future studies of brain protein synthesis might be informative in elucidating altered developmental processes in two common causes of mental retardation for which genes have been identified and cellular mechanisms involve regulation of protein synthesis: Fragile X syndrome and tuberous sclerosis complex (TSC).

PET METHODOLOGY

PET is a technique that can measure and image the distribution of tracers that are designed to track biochemical and molecular processes in the body after intravenous injection or inhalation. This is accomplished by radiolabeling compounds of interest with a positron emitting radionuclide. These short-lived

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isotopes (most frequently used isotopes include fluorine-18 with a half-life of 110 min, carbon-11 with a half-life of 20 min, and oxygen-15 with a half-life of 2 min) emit positrons during nuclear decay that in turn collide with surrounding electrons, resulting in annihilation of both particles and the release of two high-energy (511 KeV) gamma rays. The two gamma rays generated by a single event travel in opposite directions and are recorded by multiple pairs of oppositely situated detectors that constitute the PET camera [Hoffman and Phelps, 1986; Ter-Pogossian, 1995]. Thus, the time course of changes in the distribution of the radiolabeled tracer in the body are recorded by the ring of detectors during the radioactive decay of the radionuclide. Using the dynamic tissue time–activity data obtained in this manner and appropriate kinetic modeling of tracer behavior, one can then calculate physiological parameters of interest (for example, receptor density, glucose metabolic rate, protein synthesis rate, etc.). Absolute quantification and estimation of relevant parameters of a given biochemical pathway in a noninvasive manner distinguishes PET from other similar technologies such as single photon emission computed tomography (SPECT) in which absolute quantification is difficult.

A large number of PET radiopharmaceuticals have been developed over the past 30 years for the study of normal physiological processes and for application to the study of many disorders. Indeed, more than 1,400 PET tracers have been produced, and the rate of development of new tracers seems to continue in the exponential growth phase even today [Iwata, 2004]. Radiolabeled tracers can be divided into several categories. The first group includes normal metabolic substrates or analogs of these substrates, such as glucose, amino acids, fatty acids, nucleotides, and oxygen [Shiue and Welch, 2004]. Another group of PET tracers includes ligands that bind to proteins such as receptors and transporters [Smith et al., 2003; Gjedde et al., 2005]. Finally, newer PET probes include antibodies, oligonucleotides, and tracers to image reporter genes to monitor gene therapy [MacLaren et al., 2000; Jain and Batra, 2003]. The traditional PET tracers were predominantly small molecules and were produced using synthetic chemistry techniques. These techniques have produced many specific tracers useful for clinical purposes. However, increasingly, PET radiopharmaceuticals are being produced by bioengineering techniques, paralleling similar developments in the drug industry [Weissleder and Mahmood, 2001]. The PET tracers produced by these techniques are typically large-molecular-weight "biotechnology probes." These techniques are often flexible enough to produce "designer" probes with the desired specificity, affinity, and other properties. Such targeted imaging can lead to imaging precise molecular abnormalities in humans in a relatively noninvasive manner. However, these new technologies have been predominantly applied to disorders outside the brain, since the blood–brain barrier bars entry of these larger probes. Several groups of investigators are working on strategies for facilitating brain entry of these large-molecular-weight tracers. For example, targeting tracers to endogenous brain endothelial transporters such as carrier-mediated transporters, active efflux transporters, or receptor-mediated transporters has been attempted to improve brain tracer delivery [for review, see Pardridge, 2005].

PET potentially can be used noninvasively to assess gene expression either at the mRNA or protein expression levels using specific molecular imaging probes to study the dynamic processes in vivo in real-time in a quantitative and noninvasive manner [Massoud and Gambhir, 2003]. For example, reporter gene imaging can be used to measure gene expression levels [Herschman et al., 2000; Doubrovin et al., 2004; Herschman, 2004]. These studies could be invaluable in genotype–phenotype correlations. When cloned into promoter/enhancer sequences or engineered into fusion proteins, imaging reporters can be used to study the expression levels of any gene product. While animal models have tremendously advanced our understanding of the molecular and pathological phenotypic changes for a given genetic abnormality, predicting the human behavioral phenotypes from the underlying genetic changes has been less successful. PET technology, when applied to the study of gene expression levels, is ideally suited to fill this gap. Most of the advances in PET using molecular imaging probes have been made in oncology, but similar strategies can be applied theoretically to the study of mental retardation and developmental disabilities.

ETHICAL ISSUES IN APPLYING PET TO CHILDREN

Given the large biochemical and physiological changes in the brain during development, the optimal design for PET studies in subjects with mental retardation would be to compare experimental results *over the course of development* from the group with mental retardation to those of an aged-matched normal control group. However, in the PET studies published thus far, normal control groups have not been available. The majority of the children were studied for a neurological (usually epilepsy) or neurodevelopmental condition, and comparisons were made among groups of children with different disorders. This is due the to current thinking that administration of the radiopharmaceutical necessary for PET scanning in normal children may be ethically unacceptable. Potential risks in performing imaging studies with PET include pharmacological effects of PET radiopharmaceuticals, the use of sedation, and the exposure to ionizing radiation. However, it should be emphasized that PET scanning involves the administration of a single dose of the radiopharmaceutical in *tracer* quantities, indicating that the pharmacological effects are unlikely to be of any concern. These tracer doses typically range from 10,000- to 1,000,000-fold lower than therapeutic doses and, in fact, typical doses used in PET are lower than quantities of toxins legally present in our drinking water [Barrio et al., 2004]. Sedation may need to be employed for many imaging procedures, particularly in young children. Several large studies indicate that the risk associated with sedation is small and that sedation of children can be done in a safe and highly efficacious manner in a hospital radiology department using a structured sedation program modeled after the guidelines of the American Academy of Pediatrics [Merola et al., 1995; Egelhoff et al., 1997]. As for radiation exposure, this is quite small in a typical PET study, particularly in comparison to the exposure obtained during many other clinical radiological procedures. Ernst et al. [1998] reviewed studies of low-level radiation exposure with large sample sizes and long follow-up and concluded that health risks from low-level radiation could not be detected above those of adverse events in daily life. Furthermore, they found no evidence that these levels of radiation were more harmful to children than to adults. The effective dose for a typical PET study conveys a lifetime cancer mortality risk of 0.0005. Given that the lifetime risk of dying from cancer is 23.66 for males and 19.99 for females, this additional risk of 0.0005 is negligible.

Given the small risk of PET scanning to the individual child, we raise the issue whether it is ethical to deny adequate access of PET technology for the study of life-changing disorders of childhood, including those associated with mental retardation. We suggest that the risk to normal children over the age of 8–10 years, in which sedation is not necessary, is minimal to the individual child.

Further, we propose screening of healthy children to identify and exclude those children who have had a large past medical exposure to radiation and strong family history of cancer. In the future, risk may be further minimized by prescreening for genes (such as mutations in DNA repair enzymes) that may put certain individuals at higher risk. Strategies for establishing normal values in young children may come from the development of PET instrumentation to allow a larger field of view and smaller aperture so that dynamic imaging of normal organs can be acquired during the same scanning period as the target organ. Mental retardation results in life-long disability, and carefully designed PET studies will not only enhance our understanding, but may also lead to new treatment strategies for disorders associated with mental retardation.

STUDIES OF BRAIN DEVELOPMENT WITH PET

Normal development depends upon the orchestrated execution of genetic developmental programs under optimal environmental conditions. Mental retardation occurs when there is disruption in normal brain development. To apply PET in the study of developmental disorders, it is necessary to first recognize normal patterns of brain maturation. For example, PET studies in humans have shown significant changes in glucose metabolism during normal brain development [Chugani et al., 1987]. Similarly, PET studies of neurotransmitter synthesis [Chugani et al., 1999] and receptor binding [Chugani et al., 2001] have also demonstrated significant changes during human brain development.

Glucose Metabolism

Studies of regional cerebral glucose metabolism in human infants using PET with the tracer 2-deoxy-2[^{18}F]-fluoro-D-glucose (FDG) have shown that the pattern of glucose utilization undergoes dramatic changes in the first postnatal year. A consistent pattern is seen in the newborn, with the highest glucose metabolic activity in primary sensorimotor cortex, thalamus, brain stem, and cerebellar vermis [Chugani and Phelps, 1986; Chugani et al., 1987; Chugani, 1994; Kinnala et al., 1996]. Intermediate levels of glucose metabolism are present in cingulate cortex, amygdala, hippocampus, and occasionally the basal ganglia [Chugani, 1996, 1998]. The major portion of cerebral cortex shows the lowest glucose metabolism. This neonatal pattern of glucose metabolism, largely confined to subcortical structures, is consistent with the less complex behavior of neonates compared to infants. Subsequently, the ontogeny of regional brain glucose metabolism appears to follow a phylogenetic order, with functional maturation of older anatomical structures preceding that of newer areas [Chugani and Phelps, 1986; Chugani et al., 1987; Chugani, 1994; Kinnala et al., 1996; Chugani, 1996, 1998]. Moreover, functional maturation of various brain regions as depicted by a rise in regional glucose metabolism correlates well with the maturation of behavioral, neurophysiological, and neuroanatomical events in the infant. As visuospatial and visuosensorimotor integrative functions are acquired in the second and third months of life [Bronson, 1974], and primitive reflexes become reorganized [Andre-Thomas et al., 1960; Parmelee and Sigman, 1983], increases in glucose metabolism are observed in parietal, temporal, and primary visual cortical regions, frontal eye fields (Brodmann area 8), basal ganglia, and cerebellar hemispheres. Increasing glucose metabolism in cerebral cortex during the second and third months of life presumably reflects maturation of the cortex and is consistent with the dramatic maturation of the electroencephalogram seen during the same period [Kellaway, 1979]. Between 6 and 8 months, the remaining frontal cortex begins to show a maturational rise in glucose metabolism, which continues until 1 year of age. Functional maturation of the frontal cortex begins in the lateral and inferior portions and later proceeds to include the medial and lastly the dorsal prefrontal areas. Functional maturation of these frontal cortical regions coincides with the emergence of higher cortical and cognitive abilities. By 1 year of age, the overall pattern of brain glucose metabolism is similar to that seen in adults. Measurement of the local cerebral metabolic rates of glucose utilization (LCMRglc) in children using PET has confirmed the findings of Kennedy and Sokoloff [1957] that children undergo a period during development when brain energy demand exceeds that of adults [Chugani et al., 1987]. Unlike the study of Kennedy and Sokoloff [1957], which measured global cerebral blood flow and oxygen utilization, PET measurements of LCMRglc showed that the magnitude of increase over adult values is most marked for the neocortex, intermediate for basal ganglia and thalamus, and is probably not present in brain stem and cerebellum. In other words, there appears to be a hierarchical ordering of structures in terms of the degree to which maturational increases in LCMRglc exceed adult values. The typically low neonatal values of LCMRglc, which are about 30% lower than adult rates, rapidly increase from birth and reach adult values by about the second year. Thereafter, LCMRglc values continue to increase and begin to exceed adult values during the third postnatal year. By about 3 years, a plateau is reached that extends until about 9–10 years; following this, there is a gradual decline in LCMRglc to reach adult values again by about 16–18 years [Chugani et al., 1987; Chugani, 1994, 1998]. The relative increase of LCMRglc over adult values, which is most pronounced in neocortical regions between 3 and 10 years, reaches a peak LCMRglc of over twice the LCMRglc levels seen in adults.

Serotonin Synthesis

Similar to developmental changes shown for cerebral glucose metabolism, ontogeny studies in nonhuman primates also demonstrate changes in neurotransmitter content and receptor binding [Goldman-Rakic and Brown, 1982; Lidow et al., 1991]. For example, in the macaque, there is a steep rise in cortical serotonin content beginning before birth and reaching a peak at 2 months of age, followed by a slow decline until about 3 years of age, when puberty occurs [Goldman-Rakic and Brown, 1982]. The same group of investigators has reported a similar time course for expression of serotonin receptors [Lidow et al., 1991]. We [Chugani et al., 1999] measured whole brain serotonin synthesis capacity at different ages using the tryptophan analogue α-[^{11}C]methyl-L-tryptophan (AMT) and PET in autistic children and a comparison group comprised of 8 healthy nonautistic siblings (6 males, 2 females, 2–14 years) of autistic children, as well as 16 children with epilepsy who were developing normally (9 males, 7 females, 3 months to 13 years). We obtained permission to study healthy siblings of children with autism in order to study the broader phenotype of autism present in many autism family members, thus providing potential benefit to the healthy siblings who participated. For this group of "control " children, serotonin synthesis capacity was >200% of adult values until the age of 5 years and then declined toward adult values. Serotonin synthesis capacity values declined at an earlier age in girls than in boys. These data suggest that humans undergo a period of high brain serotonin synthesis capacity during childhood followed by a decline toward adult values and that there are gender differences.

$GABA_A$ Receptors

An understanding of human $GABA_A$ receptor ontogeny is highly relevant in elucidating the pathophysiology of neurodevelopmental disorders in which GABAergic mechanisms play a role as well as in understanding age-related differences in the pharmacology of drugs acting on this system. We have measured age-related changes in the brain distribution of the $GABA_A$ receptor complex in vivo using PET in children with epilepsy under evaluation for surgical treatment [Chugani et al., 2001]. PET imaging was performed using the tracer [^{11}C]flumazenil (FMZ), a ligand that binds to α-subunits of the $GABA_A$ receptor. FMZ binding was quantified using a two-compartment model yielding values for the volume of distribution (VD) of the tracer in tissue. All brain regions studied showed the highest value for FMZ VD at the youngest age measured (2 years), and the values then decreased exponentially with age. Medial temporal lobe structures, primary visual cortex, and thalamus showed larger differences between age 2 years and adulthood (approximately 50% decrease) compared to basal ganglia, cerebellum, and other cortical regions (which showed 25–40% decreases from 2 years to adulthood). Furthermore, subcortical regions reached adult values earlier (14–17.5 years) compared to cortical regions (18–22 years).

EXAMPLES OF ALTERED GLUCOSE METABOLISM, SEROTONIN SYNTHESIS, AND $GABA_A$ RECEPTORS IN MENTAL RETARDATION

Altered Glucose Metabolism in Rett Syndrome

Rett syndrome is a progressive neurological disorder in which decelerated head growth in girls is accompanied by mental retardation [Rett, 1966]. In addition, girls with Rett syndrome have characteristic stereotyped hand movements, ataxia, seizures, and autistic traits. Girls with Rett syndrome have mutations in the methyl-CpG binding protein 2 (MECP2) gene located at chromosome Xq28. Villemagne et al. [2002] studied glucose metabolism with PET in six girls with Rett syndrome aged 3 to 15 years of age and compared their results to the studies described above [Chugani and Phelps, 1986; Chugani et al., 1987]. This study found relatively increased glucose metabolism in the frontal cortex of the younger girls (3–8 years of age) studied. The authors related this result to a postmortem report of increased *N*-methyl-D-aspartate receptors in superior frontal gyrus in young Rett subjects [Blue et al., 1999], given the coupling of cerebral glucose metabolism to glutamate transport [Magistretti et al., 1999]. In addition, glucose metabolism in Rett subjects was relatively *decreased* in visual association areas and relatively *increased* in the cerebellum. These results are consistent with the notion of developmental arrest in girls with Rett syndrome, since this pattern of glucose metabolism is found during development in children less than 1 year of age [Chugani and Phelps, 1986, Chugani et al., 1987].

Focal and Global Abnormalities of Serotonin Synthesis in Autism

Autism is a neurodevelopmental disorder, characterized by deficits in social interaction and communication (verbal and nonverbal) and associated with restricted, repetitive, or stereotyped behaviors, and is associated with mental retardation in approximate 75% of cases. To determine whether there are brain serotonergic abnormalities in children with autism, we have evaluated serotonin synthesis capacity in vivo with PET, using the tryptophan analogue α-[^{11}C]methyl-L-tryptophan (AMT) as the tracer. Our publications illustrate two fundamentally different types of serotonergic abnormality in children with autism [Chugani et al., 1997, 1999; Chandana et al., 2005]. The first is a difference in whole brain serotonin synthesis capacity in autistic children compared to age-matched nonautistic children. As described above, serotonin synthesis capacity was >200% of adult values until the age of 5 years and then declined toward adult values in nonautistic children. In contrast, serotonin synthesis capacity in autistic children increased gradually between the ages of 2 and 15 years to values 1.5 times the adult normal values [Chugani et al., 1999]. These data suggested that humans undergo a period of high brain serotonin synthesis capacity during early childhood and that this developmental process is disrupted in autistic children. The second type of abnormality we have reported relates to focal abnormalities in brain serotonin synthesis. Asymmetries of AMT uptake in frontal cortex, thalamus, and cerebellum were visualized in children with autism [Chugani et al., 1997]. In addition, we measured brain serotonin synthesis in a large group of autistic children (n = 117) with AMT PET and related these data to handedness and language function [Chandana et al., 2005]. Cortical AMT uptake abnormalities were objectively derived from small homotopic cortical regions using a predefined cutoff asymmetry threshold (>2 SD of normal asymmetry). Autistic children demonstrated several patterns of abnormal cortical involvement, including right cortical, left cortical, and absence of abnormal asymmetry. Groups of autistic children defined by presence or absence and side of cortical asymmetry differed on a measure of language as well as handedness. Autistic children with left cortical AMT decreases showed a higher prevalence of severe language impairment, whereas those with right cortical decreases showed a higher prevalence of left and mixed handedness. These results suggest that global as well as focal abnormally asymmetric development in the serotonergic system could lead to miswiring of the neural circuits specifying hemispheric specialization.

Decreased $GABA_A$ Receptor Binding in Angelman Syndrome and Prader–Willi Syndrome

Angelman syndrome and Prader–Willi syndrome result from deletions in chromosome 15q11-q13 [for reviews see Dykens et al., 2004; Soejima and Wagstaff, 2005]. Deletion of the maternal chromosome in this region results in Angelman syndrome, which is characterized by severe mental retardation, epilepsy, a puppet-like gait, and lack of speech. Deletion of the paternal chromosome 15q11-q13 results in Prader–Willi syndrome, which is characterized by mild or moderate mental retardation, hypotonia, obesity, and genital abnormalities. This region of chromosome 15 encodes $GABA_A$ receptor subunit genes GABRB3, GABRA5, and GABRG3 [Wagstaff et al., 1991; Menold et al. 2001, Buxbaum et al., 2002]. [^{11}C]Flumazenil PET has been used to examine whether there are $GABA_A$ receptor binding abnormalities in patients with Angelman syndrome and Prader–Willi syndrome. Angelman patients with a maternal deletion of 15q11–13 leading to the loss of β3 subunit of the GABA receptor showed significantly decreased binding of [^{11}C]flumazenil in frontal, parietal, hippocampal, and cerebellar regions compared to a patient whose deletion did not include the GABRB3 gene [Holopainen et al., 2001]. Lucignani et al. [2004] studied six adults with Prader–Willi syndrome and found decreased [^{11}C]flumazenil binding in insula and cingulate, frontal, and temporal neocortices compared to normal control subjects. These studies demonstrate the utility of PET in elucidating the functional consequence of specific genetic abnormalities.

FUTURE APPLICATIONS: ALTERED PROTEIN SYNTHESIS IN FRAGILE X SYNDROME AND TUBEROUS SCLEROSIS COMPLEX

Protein synthesis can be measured with PET using radiolabeled amino acids. Although a number of amino acids have been radiolabeled for PET studies in oncology, 1-[^{11}C]L-leucine is the most suitable amino acid for quantifying protein synthesis rate in brain because of its theoretically straightforward kinetic modeling. This method is based upon the 1-[^{14}C]L-leucine autoradiographic method described and validated by Smith et al. [1984, 1988]. This method was adapted for PET with 1-[^{11}C]L-leucine in humans 20 years ago [Phelps et al., 1984; Hawkins et al., 1989], but has not been widely applied due to difficulties with the chemical synthesis of the radiotracer and the lack of a method to estimate the fraction of amino acid pool derived from protein breakdown in the tissue [Smith et al., 1988], leading to an underestimation of the rate of protein synthesis. The estimate of the tissue-derived amino acid pool was particularly a problem for the study of developmental disorders, since the amount of brain amino acid recycling was reported to change as a function of age [Sun et al., 1995]. Both the chemistry and kinetic modeling problems have recently been resolved [Schmidt et al., 2005; Smith et al., 2005; Mu et al. 2005], and therefore measurement of protein synthesis in certain types of mental retardation involving genes regulating protein synthesis may provide important information about how these mutations affect protein synthesis at different points during brain development and in different brain regions.

Fragile X Syndrome

Fragile X syndrome, the most common genetic disorder associated with mental retardation, is caused by a repeat expansion of CGG trinucleotide in the 5′-untranslated region of the gene fragile X mental retardation-1 (Fmr1) [Verkerk et al., 1991]. The presence of the expanded repeats in Fmr1 results in methylation and transcriptional inactivation of the gene, leading to the reduction of fragile X mental retardation protein (FMRP). There is evidence that FMRP inhibits translation in synapses through interactions with mRNA [Li et al., 2001] and inhibition of the assembly of the 80S ribosome on mRNAs [Laggerbauer et al., 2001]. Qin et al. [2005] have studied cerebral protein synthesis in the Fmr1 null mouse model of fragile X using the quantitative 1-[^{14}C]L-leucine autoradiographic method. In this study, cerebral protein synthesis in Fmr1-null adult mice was compared to wild-type mice at 4 and 6 months of age. They found regional selective elevations in protein synthesis in the Fmr1 mice compared to wild-type mice at both ages, consistent with the role of FMRP as a suppressor of protein synthesis. Elevated protein synthesis was most pronounced in hippocampus, thalamus, hypothalamus, and brain stem. Qin et al. [2005] related their results to behavioral features in fragile X and in the Fmr1-null mouse, including learning and memory deficits, increased sensory sensitivity, autistic behaviors, parasympathetic dysregulation, hyperarousal, and hyperactivity. The examination of this mouse model with microPET would allow further insights into the role of FMRP during brain development. Moreover, microPET studies would allow the *longitudinal* study of the same animal during development. In addition, these animal experiments provide the rationale for measuring cerebral protein synthesis in humans with fragile X syndrome using 1-[^{11}C]L-leucine with PET.

Tuberous Sclerosis Complex

Tuberous sclerosis complex is an autosomal dominant inherited disorder, now known to result from mutations in at least two different genes, TSC1 [Fryer et al., 1987; van Slegtenhorst et al., 1997] and TSC2 [Kandt et al., 1992]. The neurological problems associated with TSC range from mild to severe, and include epilepsy, which is often resistant to pharmacological treatment, developmental delay, and cognitive problems of varying severity (for review, see Curatolo et al., 2002]. Molecular and biochemical studies have shown that tuberin and hamartin, the protein products of TSC1 and TSC2 genes, are regulators of the insulin signaling pathway. Specifically, there is evidence that tuberin and hamartin form a dimer, which negatively regulates Rheb (Ras homolog enriched in brain), a positive regulator of mTOR, which is a constituent of the insulin/phosphotidyl inositol 3-kinase (PI3K) pathway (for reviews see Manning and Cantley, 2003; Kwiatkowski, 2003]. Mutations of TSC1 and TSC2 result in a release of inhibition of mTOR resulting in an *increase in protein synthesis* and cell growth. Thus, there is rationale for studying cerebral protein synthesis in TSC, particularly in children during the period of maximal brain growth.

CONCLUSION

There are numerous probes for biochemical and molecular imaging with PET, yet only a small number of these probes have been applied to the study of mental retardation. Rational application of radiopharmaceuticals to the study of mental retardation can provide important insight into the functional consequences of certain gene mutations in the developing brain. Identifying the patterns and quantifying the physiological and biochemical abnormalities in the brains of mentally retarded subjects will yield important biological markers that can be used to monitor disease progression and response to therapy. It is also conceivable that new therapeutic strategies may emerge as a result of these studies with PET. ■

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