Published in final edited form as:

J Inherit Metab Dis. 2007 November ; 30(6): 865–879. doi:10.1007/s10545-007-0709-5.

Neurological implications of urea cycle disorders

A. L. Gropman,

Department of Neurology, Children's National, Medical Center and the George Washington, University of the Health Sciences, 111 Michigan Avenue, N. W., Washington, DC 20010, USA. Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, Maryland, USA

M. Summar, and

Department of Genetics and Metabolism, Vanderbilt University, Nashville, Tennessee, USA

J. V. Leonard

Institute of Child Health, London, UK

A. L. Gropman: agropman@cnmc.org

Summary

The urea cycle disorders constitute a group of rare congenital disorders caused by a deficiency of the enzymes or transport proteins required to remove ammonia from the body. Via a series of biochemical steps, nitrogen, the waste product of protein metabolism, is removed from the blood and converted into urea. A consequence of these disorders is hyperammonaemia, resulting in central nervous system dysfunction with mental status changes, brain oedema, seizures, coma, and potentially death. Both acute and chronic hyperammonaemia result in alterations of neurotransmitter systems. In acute hyperammonaemia, activation of the NMDA receptor leads to excitotoxic cell death, changes in energy metabolism and alterations in protein expression of the astrocyte that affect volume regulation and contribute to oedema. Neuropathological evaluation demonstrates alterations in the astrocyte morphology. Imaging studies, in particular ¹H MRS, can reveal markers of impaired metabolism such as elevations of glutamine and reduction of myoinositol. In contrast, chronic hyperammonaemia leads to adaptive responses in the NMDA receptor and impairments in the glutamate–nitric oxide–cGMP pathway, leading to alterations in cognition and learning. Therapy of acute hyperammonaemia has relied on ammonia-lowering agents but in recent years there has been considerable interest in neuroprotective strategies. Recent studies have suggested restoration of learning abilities by pharmacological manipulation of brain cGMP with phosphodiesterase inhibitors. Thus, both strategies are intriguing areas for potential investigation in human urea cycle disorders.

Introduction

The urea cycle disorders (UCDs) represent a group of rare inborn errors of metabolism that lead to accumulation of ammonia, a toxic product of protein metabolism. Due to an enzyme deficiency, individuals with UCDs have a reduced ability to metabolize ammonia, which accumulates (Fig. 1). These disorders may present at any age and the consequences are neurological and of varying severity (Table 1) (Bachmann 2003a; Batshaw 1994). Common to the majority of the UCDs is the presence of hyperammonaemia, and its effects on neurological functioning. Arginase deficiency differs in terms of its clinical sequelae, as the

major neurological presentation is that of spastic diplegia/quadruplegia, and rarely is there hyperammonaemia to the extent experienced in the other UCDs (Prasad et al 1997). In addition, HHH syndrome, due to a defect in the ornithine transporter, may present with spastic diplegia and evidence of pyramidal tract involvement. The reason for this distinctive difference in the neurological presentation is unknown.

The two most proximal enzyme defects, namely carbamyl-phosphate synthetase deficiency 1 (CPS I) and ornithine transcarbamylase deficiency (OTCD), tend to present with the highest risk for acute neurological injury. This is especially true for neonatal-onset disease in which the outcome, even with early recognition and treatment, has been uniformly poor (Bachmann 2002, 2003a, b, 2005; Gropman and Batshaw 2004; Msall et al 1984; Smith et al 2005). Late-onset UCDs still carry a potential risk of significant encephalopathy and neurological damage if not recognized and treated early in the course of disease (Smith et al 2005; Summar et al manuscript in preparation). At present, precise prediction of neurological outcome is not straightforward as there is no direct correlation between genotype, age of onset, peak ammonia level, imaging and/or phenotype (Brenningstall 1986; Nicolaides et al 2002). However, in general, the age of onset, duration and degree of hyperammonaemia may be used to predict the prognosis and the extent to which the neurological changes may be reversible (Bachmann 1992, 2003a; Enns et al 2007; Msall et al 1984; Picca et al 2001; Uchino et al 1998). Normal intelligence is possible after a hyperammonaemic event and appears to depend on the duration of coma (Batshaw et al 1982; Msall et al 1984, 1988; Nagata et al 1991). Damage to the central nervous system caused by elevated blood ammonia concentrations appears reversible when levels do not exceed 200–400 mg/dl; however, accumulating damage results in irreversible impairment. The highest mortality peaks for all urea cycle disorders occur very close to the initial presentation (Summar et al 2007, manuscript in review).

Our knowledge of the pathophysiology and neurological manifestations of hyperammonaemia derives from clinical experience, autopsy series of children with neonatal-onset disease, and the study of hepatic encephalopathy, the major nongenetic cause of both acute and chronic hyperammonaemia (Bachmann 2003b; Bachmann and Colombo 1984; Butterworth 1998, 2001, 2002; Butterworth et al 1987; Cohn and Roth 2004; Giguere and Butterworth 1984). Additionally, several animal-and cell-culture models in which acute hyperammonaemia is present or can be provoked have been studied to help understand the underlying mechanisms of neuronal dysfunction (Belanger et al 2002; Gushiken et al 1985; Neary et al 1987; Qureshi and Rao 1997; Spector and Mazzocchi 1983; Veres et al 1987). Despite this, the underlying mechanisms are not completely understood.

Clinical manifestations of acute hyperammonaemia

In the acute setting, the early neurological effects of increasing central nervous system (CNS) ammonia include anorexia and vomiting as well as changes in mental status. Progressive CNS dysfunction may ensue, reflecting ammonia-induced cell swelling, including lethargy, ataxia, seizures, asterixis, hypothermia and ultimately coma may follow (Bachmann 2003a; Smith et al 2005). Death may occur, or alternatively survival with significant neurological injury ensues. A decreased level of consciousness (lethargy, drowsiness, unresponsiveness, coma, and obtundation), and/or abnormal motor function (slurred speech, tremors, weakness, decreased or increased muscle tone, and ataxia), may be more frequent clinical findings with the first episode rather than with subsequent episodes of acute hyperammonaemia (Summar et al 2007, manuscript in review).

Patients with partial urea cycle enzyme deficiencies who manifest with late-onset presentations may have less obvious features including chronic encephalopathy, autism,

learning disorders, hyperactive and self-injurious behaviour, vomiting with changes in level of consciousness, stroke-like episodes (Fig. 2) and, in teens and adults, psychiatric symptoms including episodic psychosis, bipolar disorder and/or major depression (Batshaw et al 1980; Felig et al 1995; Gaspari et al 2003; Gilchrist and Coleman 1987; Mizoguchi et al 1990; Rimbaux et al 2004; Smith et al 2005). Often, upon investigation, there is a dietary history of protein aversion or self-selective vegetarianism (Gordon 2003).

Inadvertent protein overindulgence, illness/fasting with subsequent catabolism, surgery, pregnancy/postpartum period, or the use of medications such as sodium valproate (an anticonvulsant often used to treat atypical psychiatric disorders) may unmask a latent case of CPS I or OTCD (DiMagno et al 1986; Eather et al 2006; Enns et al 2005; Honeycutt et al 1992; Leao 1995; Oechsner et al 1998; Tokatli et al 1991; Tripp et al 1981).

Pathogenic mechanisms of acute hyperammonaemia

Pathogenic mechanisms may include glutamine-induced cerebral oedema, energy failure and neurotransmitter alterations. Ammonia is able to diffuse freely across the blood–brain barrier into the brain in amounts proportional to the arterial blood concentration and blood flow and as a result metabolic trapping can occur with the concentration in the brain tending to be higher than in peripheral blood (Felipo and Butterworth 2002a; Ott et al 2005). Ammonia in the bloodstream rapidly enters the brain and almost all is converted instantly to glutamine, offering short-term buffering of excess ammonia in patients with hyperammonaemia. This is accomplished in the astrocyte via glutamine synthetase (GS). Glutamine is osmotically active and can cause astrocytic swelling leading to cytotoxic oedema (Batshaw et al 1986; Bender and Norenberg 1996; Norenberg 1996; Norenberg et al 2005). Studies in which the GS inhibitor methionine sulfoximine is administered demonstrate reduced ammonia-induced brain oedema in both *in vivo* (Takahashi et al 1991) and *in vitro* models (Blei et al 1994; Norenberg and Bender 1994; Takahashi et al 1991). The astrocyte, therefore, is an important intermediate in the interactions of glutamine and ammonia via the glutamate–glutamine cycle (Fig. 3).

Although GS is responsible for most ammonia detoxification in the brain, some occurs by its combining with α -ketoglutarate to make glutamate. Potentially, this could lead to depletion of α -ketoglutarate and alterations in the Krebs cycle.

Although the plasma ammonia and glutamine levels are typically the parameters by which clinical management decisions are made, the correlation between plasma levels of ammonia and glutamine is quite poor and plasma glutamine levels may not reflect brain levels, which may be higher.

Acute hyperammonaemia also causes other dynamic changes, particularly increased blood–brain barrier permeability, depletion of intermediates of cell energy metabolism and the disaggregation of microtubules. Recent data suggest that axonal development may be adversely affected by exposure to ammonia, and that it can be normalized *in vitro* by creatine supplementation in developing mixed brain cell aggregate cultures, thus supporting the hypothesis of the impact of hyperammonaemia on energy metabolism in the developing brain (Braissant et al 2002).

Energy failure in urea cycle disorders

The metabolic rate of the brain decreases in the early stages of hyperammonaemia, and whole-brain creatine phosphate levels and whole-brain ATP content fall later in the development of the disease. High-energy phosphates decline, presumably due to secondary inhibition of the malate–aspartate hydrogen shuttle. High levels of ammonia interfere with

the Na,K-ATPase activity. Either energy failure or excitotoxic mechanisms disable the sodium–potassium pump, allowing extracellular sodium and water to enter the cell, leading to cell swelling, proteolysis, mitochondrial degradation and free-radical production. Histological and ultrastructural findings in liver biopsy specimens show extensive mitochondrial changes (Felipo and Butterworth 2002b).

Recent research brought into question whether hyperammonaemia alone accounts for all of the neurological manifestations seen in urea cycle defects, or whether glutamine, energy deficit, or other mechanisms play a supportive role (Albrecht and Norenberg 2006; Albrecht et al 2007; Felipo and Butterworth 2002a; Jayakumar et al 2006; Norenberg et al 2005; Zwingmann and Butterworth 2005). The ultimate effect of ammonia on the brain may be influenced by other factors, such as the developmental stage of brain development, as some of the symptoms of hyperammonaemia may be age-dependent and possibly reversible (Garcia et al 2003).

Neurotransmitter and receptor alterations in hyperammonaemia

Alterations of multiple neurotransmitter systems have been described in hyperammonaemic states (Butterworth 2000). In acute hyperammonaemia, involvement of *N*-methyl-D-aspartate (NMDA) receptors, glutamate, neuronal degeneration and death are seen. The proposed mechanisms have been reviewed by Albrecht (1998). The neurotoxicity that results from activation of NMDA receptor-mediated excitotoxicity may be further modulated by metabotropic receptors, which are inactivated in the face of hyperammonaemia. In pathological conditions, glutamate mediates neuronal cell injury and death through the activation of the NMDA receptor, leading to apoptosis. Equally damaging, glutamate binding to non-NMDA receptors also allows the entry of sodium into the postsynaptic neuron, leading to disruption of cell volume and subsequent cytotoxic oedema.

Ammonia also exerts effects on AMPA-receptor-mediated neurotransmission (Butterworth 2001), which subserves learning and memory, and it has been demonstrated that AMPA/kainate (AMPA/KA) receptor activation contributes to hypoxic-ischaemic white-matter injury in the adult brain (Tekkok et al 2007; Tekkok and Goldberg 2001).

In contrast, chronic hyperammonaemia induces adaptive changes in the NMDA receptors, with depression in excitatory transmission and impaired glutamate–nitric oxide (NO) and cGMP pathway (Izumi et al 2005; Kosenko et al 2003; Llansola et al 2007; Monfort et al 2005; Rodrigo and Felipo 2006). The difference between acute and chronic hyperammonaemia may be explained by neuromodulation of the glutamate receptors by glutathione.

Chronic hyperammonaemia induces astrogliosis with increases in GS (Felipo and Butterworth 2002a). Chronic hyperammonaemia leads to alterations of neurotransmitters and may result in an increased uptake of tryptophan into the brain (Bachmann and Columbo 1983, 1984; Szerb and Butterworth 1992). Tryptophan is metabolized to serotonin and anorexia, a serotonergic symptom, is typically seen early in the course of UCDs, and often precedes metabolic decompensation (Bergeron et al 1990). The uptake of tryptophan by the brain is further enhanced when plasma levels of branched-chain amino acids are low, which is often the case in these patients, who are on dietary protein restriction.

Hyperammonaemia can be experimentally induced in animal and cell culture models. In hyperammonaemic states, glial cell morphology may be altered and this may affect changes in protein synthesis or neurotransmitter uptake (Tanigami et al 2005). One of the observed changes is the increase in the GS expression in astrocytes located in glutamatergic areas of

the brain. The induction of GS expression may act to balance the consequences of increased ammonia and glutamate uptake and, potentially, may protect against neuronal damage.

The Spf mouse model, the result of a single base-pair mutation, leads to only 10% of normal OTC enzyme activity. As in patients, these mice suffer hyperammonaemia, and have elevated levels of glutamine, alanine, α -ketoglutarate, aspartate and lactate (Briand and Cathelineau 1982; DeMars et al 1976; Spector and Mazzocchi 1983). Neurotransmitter alterations have been found involving the cholinergic, serotonergic and glutamatergic neurotransmitter systems and there is a loss of medium spiny striatal neurons (Ratnakumari et al 1994). The Spf mouse model of OCTD shows neuropathological evidence of excitotoxic cell death, suggesting that brain damage in acute hyperammonaemia is partly mediated by NMDA-mediated excitotoxicity (Robinson et al 1995). These excitotoxic effects are potentiated by the increased synthesis of NO and may be inhibited by modulation of γ -aminobutyric acid GABAergic tone. Additionally ammonia leads to upregulation of astrocytic peripheral benzodiazepine receptors which are associated with synthesis of neurosteroids. One type of neurosteroid, pregnenolone, is able to modulate the GABA(A) receptor complex by increasing GABAergic tone (Albrecht 1998; Itzhak et al 1995; Rao et al 1994).

Alterations of neurotransmitters leads to long-term effects on cognition and learning

The molecular mechanisms of learning and memory are not well characterized. NMDA receptors are involved in particular types of learning and memory and long-term potentiation (LTP), which is thought to underlie memory formation and play a critical role in behavioural learning (Monfort et al 2005). The activation of NMDA receptors increases the amounts of calcium in postsynaptic neurons. This enables binding to calmodulin, activating neuronal nitric oxide (NO) synthase, and increasing NO, which activates guanylate cyclase. The activation of guanylate cyclase leads to increasing cyclic guanine monophosphate (cGMP), which may be important in learning and memory and induction of LTP (Monfort et al 2002, 2005).

If the urea cycle is impaired, the production of arginine or ornithine may become rate limiting. Arginine has several important functions in metabolism as it is a precursor of metabolically active components such as NO (via the enzyme nitric-oxide synthetase, NOS), ornithine, creatine and polyamines. The arginine recycling enzymes are induced in astrocytes by ammonia. The stimulated neuronal NOS (nNOS) may lead to production of O_2^- in the brain with formation of peroxynitrite, a neurotoxic compound, by combination of O_2^- with NO. Excess arginine may be neurotoxic via enhanced production of NO (Scaglia et al 2004).

Pathological changes in urea cycle disorders

There is a specific pattern of brain injury that has been observed in the proximal UCDs. Acute hyperammonaemia selectively affects the white matter of the brain, and initially may be seen as reversible changes involving the deep sulci of the insular and perirolandic regions which may be watershed territories (Fig. 4). There is also evidence that UCDs affect the basal ganglia, in particular the lentiform nuclei (Eather et al 2006). This correlates with cognitive difficulty in tasks that require attention and fine motor skills (Gyato et al 2004; Swillen et al 1999).

The degree of neuropathological damage is related to the duration of hyperammonaemia and whether it is acute or chronic in origin. Acute hyperammonaemia leads to alterations in

astrocyte morphology (swelling). In survivors of prolonged neonatal coma who live several months and then ultimately die, neuropathological findings consist of additional grey matter-based lesions including cortical atrophy, ventriculomegaly, gliosis with Alzheimer type II astrocytes (a marker of chronic hyperammonaemia), spongiform changes at the grey–white junction, ulegyria, and spongiform changes in the deep grey nuclei including the basal ganglia and thalamus (Fig. 5).

Neuroimaging studies performed months later in neonatal coma survivors are consistent with these pathological findings, correlating with hypomyelination of white matter, myelination delay, cystic changes of the white matter and gliosis of the deep grey-matter nuclei (Dolman et al 1988; Harding et al 1984; Kornfeld et al 1985).

Cognitive outcome of urea cycle disorders

The resultant cognitive effects of neonatal hyperammonaemia are well known (Gropman and Batshaw 2004; Msall et al 1988; Nicolaides et al 2002). Studies of children rescued from neonatal hyperammonaemia show significant risk of mental retardation and developmental disabilities. Overall, the mean IQ is 43 (Msall et al 1984). An inverse correlation has been found between the duration (not peak) of hyperammonaemia and cognitive outcome as well as an inverse linear correlation between the duration of hyperammonaemic coma and both IQ and cortical atrophy. In addition, the siblings of children with CPS I or OTCD who were treated prospectively retained IQs in the low normal range. Children with citrullinaemia or argininosuccinic aciduria (ASA) also have a significant risk of mental retardation despite early treatment.

Most recently, Gyato and colleagues (2004) compared neurocognitive indices with clinical status, mutation analysis and urea synthetic capacity in 19 women heterozygous for OTCD. Although they had average IQ scores, there was a specific neuropsychological phenotype that emerged, characterized by strengths in verbal intelligence, verbal learning, verbal memory and reading, and weaknesses in fine motor dexterity/speed and nonsignificant weaknesses in nonverbal intelligence, visual memory, attention/executive skills and mathematics, which suggests selective vulnerability of white matter versus grey matter.

Monitoring the brain during and after injury in urea cycle disorders

There are several methods for clinically evaluating brain function and the degree of neurological injury which may provide prognostic information or markers for response to therapeutic intervention.

The bedside EEG can provide a measurable and possibly quantifiable index of the degree of cerebral dysfunction, as well as information that is complementary to other sources of data including the physical examination, biochemical monitoring, and neuroimaging. This is especially true in cases where the degree of coma is severe and thus the value of the clinical/neurological examination is lost.

Although the EEG has little specificity as to aetiology, specific patterns such as triphasic waves that indicate hepatic (and renal) insufficiency and spindle coma are seen with severe brainstem dysfunction. The EEG may not correlate with the plasma levels of ammonia, but changes in the EEG in response to therapy, such as reversal of an initially flat EEG, may provide more encouraging prognostic indications to pursue aggressive management. Other features such as the background variability of the tracing as it occurs over several seconds to minutes, and reactivity to painful stimuli, can also indicate the degree of encephalopathy. A continuous or serial EEG provides monitoring of clinical changes and responses to therapy.

Previous reports have documented EEG changes in patients with OTCD, with multifocal independent spike and sharp wave discharges being the most common finding, although a nonspecific pattern consistent with a diffuse encephalopathy is more commonly observed (Batshaw 1984; Bourrier et al 1988; Brunquell et al 1999; Brusilow 1985; Engel and Buist 1985). In addition, EEG abnormalities have been reported in infants with OTCD in the absence of clinical seizures (Bogdanovic et al 2000; Brunquell et al 1999; Engel and Buist 1985; Verma et al 1984) and may be used to explain differential responses to intervention and outcome when the clinical examination appears similar. EEG patterns that fail to respond, either in amplitude or frequency, to noxious, auditory, or visual stimuli predict a poor prognosis for meaningful neurological recovery, whereas similar patterns with preserved reproducible reactivity imply the potential for some recovery and should be compared with recordings repeated several days later.

An EEG can also detect anomalies in adults with partial-onset disease who often present with psychiatric features, but in fact may have complex partial status epilepticus revealed by continuous semi-rhythmic sharp wave discharges as the cause of the encephalopathy (Bogdanovic et al 2000).

The use of neuroimaging for the *in vivo* study of urea cycle disorders

Neuroimaging is a powerful diagnostic and research tool that can provide information about the timing, extent, reversibility and possible mechanism of neural injury in a noninvasive manner. Magnetic resonance imaging (MRI) is well established as a primary technique allowing for anatomical and structural imaging that may assist in diagnosis, longitudinal assessment, treatment and patient management. However, several newer imaging modalities have shown promise in contributing to the knowledge of brain function, injury and recovery in metabolic disorders and are starting to be used in patients with UCDs. In addition, various modalities can be combined to provide complementary information. Some of the tools available to assess the effects of metabolic disorders on the brain include functional MRI (fMRI), which can assess the integrity of neural networks, diffusion tensor imaging (DTI), which measures microscopic white-matter integrity, and magnetic resonance spectroscopy (MRS), which provides biochemical information and allows a noninvasive measure of brain metabolism under different conditions (steady state, dynamic conditions) (Gropman 2005).

MRI and urea cycle disorders

In UCDs, the imaging signature of subacute effects may resemble hypoxic-ischaemic encephalopathy, with oedema affecting both grey and white matter and with T1 shortening of the grey matter (Bindu et al. 2007).

Adult patients with partial deficiencies may present with reversible signal abnormalities such as increased signal intensities on T2-weighted images in the cingulate gyri, frontal and temporal lobes and insular regions (Chen et al 2001; Gaspari et al 2003). During the acute episode, computed tomography (CT) and MRI reveal cerebral swelling and symmetrical parenchymal lesions, with sparing of the brainstem and cerebellar hemispheres. Chronic changes, as seen on follow-up studies, may demonstrate persistent foci of leukoencephalopathy or other white-matter changes (Takanashi et al 2003). Additionally, ischaemic infarcts in parenchymal areas not served by a specific vascular territory (Fig. 2) are suspicious for metabolic aetiology, and strokes have been reported in females with partial deficiency (Mamourian and du Plessis 1991).

fMRI, DTI and urea cycle disorders

Preliminary fMRI results in patients with partial OTCD suggest that there are altered neuronal networks in the frontal lobe. DTI in these patients suggest that there are white-

matter microstructural changes in the cingulum, superior frontal white matter, and the supplemental and motor cortex white matter (Gropman et al, in preparation).

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy investigations are performed to study brain chemistry and thus ascertain information regarding this disease from the spectra of ^1H -visible substances (Table 2). The noninvasive detection of elevated brain glutamine by ^1H MRS has been documented in chronic hepatic encephalopathy (Kreis et al 1992) as well as experimentally-induced hyperammonaemia (Bates et al 1989; Fitzpatrick et al 1989). ^1H MRS studies in UCDs, in particular OTCD, have demonstrated impaired metabolism with elevations in glutamine, and decreases in myoinositol and choline in symptomatic and clinically severely affected patients (Connelly et al 1993; Takanashi et al 2002, 2003). In addition, preliminary experience at 3 T suggests that decreases in myoinositol may be an early marker for impaired nitrogen handling in patients with partial OTCD (Gropman et al, manuscript in preparation). In addition, other biochemical changes distinguish subjects with OTCD from controls (Fig. 6). Magnetic resonance spectroscopy studies using ^{13}C - and ^{15}N -labelled compounds have allowed us to understand the time course of how the brain detoxifies ammonia and thereby synthesizes glutamate, glutamine and GABA (Kanamori and Ross 1993, 1995, 2006; Kanamori et al 1996, 1997; Shen et al 1998; Sibson et al 1997).

Absolute metabolite quantitation is the preferred method of MRS data analysis in UCDs, as metabolite ratios to creatine may be flawed by the assumption that creatine remains stable in this condition. Creatine (Cr) is often used as a reference for measuring metabolites on ^1H MR spectra that are acquired with a long echo time (TE). The imaging of Gln/Glu requires shorter TE; studies have shown creatine to be decreased in experimental models of hyperammonaemia (Bachmann et al 2004).

^1H MRS studies suggest that monitoring of the α -proton in glutamine could be useful for assessment of metabolic control. Reductions in brain concentrations of myoinositol, an important organic osmolyte, are linked to the development of ammonia-induced injury.

Potential interventions for the urea cycle disorders

Treatment strategies

The mainstay of treatment of the UCDs has been to reduce the production of nitrogenous waste and lower the ammonia levels as quickly as possible. This may be achieved by providing a low-protein diet supplemented with citrulline and/or arginine, and preventing endogenous catabolism through the provision of adequate nutrition and/or by exploitation of alternative pathways for excretion of waste nitrogen. Brusilow and colleagues (1979) first suggested the use of endogenous biosynthetic pathways to eliminate non-urea waste nitrogen as a substitute for defective urea synthesis. Alternative pathway therapy allows for the total body load of nitrogen to be decreased, even with abnormal function of the urea cycle, by promoting the synthesis of non-urea nitrogen-containing metabolites that can then be excreted at high rates.

However, given the unsatisfactory prognosis for neonatal-onset disease, and the uncertain prediction of neurological outcome in partial disorders, the possibility of neuroprotection for acute hyperammonaemia, and phosphodiesterase inhibitors for treatment of chronic encephalopathy, becomes a relevant topic. In addition, even with aggressive nutritional and dietary management, patients with UCDs are at risk for intercurrent hyperammonaemic episodes resulting from peripheral mobilization of nitrogen stores caused by the catabolic stress that may accompany illness, fasting, surgery or other stressors.

In other neurological conditions, several agents are being investigated in various models of injury including excitotoxic injury with agents that block the NMDA receptor, mediators of oxidative stress and mitochondrial dysfunction, anti-apoptotic agents and anti-inflammatory agents. Other avenues for intervention invoke changes in pathways for gene/protein regulation, peroxisome proliferator-activated receptor activators such as fenofibrate, anticonvulsants, neurotrophins and hypothermia (Alzaga et al 2006).

Hypothermia

Induced hypothermia has recently re-emerged as a neuroprotective strategy. Cooling reduces the activities of metabolic pathways. The reduction in energy demand, in the face of low energy supply, provides the rationale for its use. Hypothermia leads to decreased metabolic rates for glucose and oxygen (Chatauret et al 2003). Experimental models have shown reduced histological damage in response to ischaemic injury due to traumatic brain injury (TBI), stroke and neonatal hypoxic ischaemic encephalopathy (Arican et al 2006; Busto et al 1987; Dietrich et al 1993; Gluckman et al 2005; Reith et al 1996; Shankaran et al 2005; Slotboom 2007; Welsh et al 1990).

Cooling may have a membrane-stabilizing effect that prevents the release of excitatory neurotransmitters and may decrease apoptosis and inflammation. Mild cooling may result in a reduction of the delivery of ammonia to the brain (Inamasu and Ichikizaki 2002; Polderman 2004; Vaquero and Blei 2005; Vaquero et al 2005). In patients with severe hyperammonaemia due to hepatic encephalopathy without elevations of intracranial pressure (ICP), mild hypothermia appeared to reduce the risks of developing elevated ICP (Belanger-Quintana et al 2003; Jalan et al 2004; Zwingmann et al 2004).

Hypothermia has been used as an emergency treatment of neonatal hyperammonaemia due to CPS I deficiency when haemofiltration, alternative pathway metabolites, and glucose and insulin failed to lower plasma levels of ammonia below 2000 $\mu\text{mol/L}$ in a newborn (Whitelaw et al 2001). He was monitored by amplitude-integrated EEG, which showed low-amplitude background rhythm with frequent seizures that resolved after 36 h of cooling.

NMDA-receptor antagonists

NMDA-receptor blocking agents may play a role in UCDs although, as yet, their use in UCDs is unproven. Several antagonists have been used in trials of neurodegenerative disorders and include magnesium, memantine, MK 801, and others. Antagonists of these receptors of the signal transduction pathway enzymes such as nNOS could be beneficial in the treatment of CNS manifestations. There is no current experience with the use of these drugs in UCDs.

Magnesium sulfate, a naturally occurring electrolyte that is necessary for proper nerve and muscle function, acts at the NMDA receptor and has few side-effects, although in infants, hypotonia, respiratory depression and cardiovascular effects (arrhythmia, hypotension) may occur. Magnesium blocks NMDA receptor-gated calcium channels to reduce the cytotoxic effects of calcium in pathological cascades that lead to neuronal death. Magnesium also improves cerebral blood flow to salvageable regions in the injured brain (Natale et al 2007), by attenuating the production of reactive oxygen species, thus improving endogenous antioxidant levels and reducing oxidative stress contribution to secondary injury.

Memantine is a well-tolerated low-affinity, noncompetitive NMDA-receptor antagonist. In organotypic hippocampal slices or dissociated cultures, memantine protects neurons from direct NMDA-induced excitotoxicity (Volbracht et al 2006). Memantine works by open-channel blockade, which requires prior activation of the receptor, and is a unique mechanism among NMDA-receptor antagonists (Lipton 2006).

Phosphodiesterase inhibitors and cognitive function in hyperammonaemia

Decreased cGMP has been shown to impair learning and memory in chronic hyperammonaemia. Treatment with the phosphodiesterase inhibitor sildenafil normalized function of the glutamate-NO-cGMP pathway of the brain and restored normal learning in rats with chronic hyperammonaemia due to portacaval anastomosis (Erceg et al 2005).

Anti-inflammatory agents

The potential role of inflammation and cytokines in UCDs has prompted consideration of the many anti-inflammatory agents that have begun to appear in clinical trials of neurodegenerative disorders (Wang et al 2006). Cytokines act as mediators of protein catabolism via inhibition of anabolic hormones and also through the modulation of enzymes that are involved in protein synthesis and degradation. In fact, it has been suggested that inflammation may exacerbate the neuropsychological alterations caused by hyperammonaemia (Shawcross and Jalan 2005). Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin have been shown to prevent the development of ammonia-induced brain oedema in rats that have undergone portacaval anastomosis (Chung et al 2001). This and other studies raise the possibility of decreasing inflammation as a therapeutic approach to the treatment of hyperammonaemia.

Sex differences in neural injury and neuroprotection

The idea of a male ischaemia-sensitive phenotype has been suggested in animal studies (Yamori et al 1976). This may be due to differential utilization of molecular cell death pathways by males and females. In particular, this may be seen in neuronal isoforms of NOS and the DNA repair enzyme poly(ADP-ribose) polymerase (PARP-1) (Loihl et al 1999; McCullough et al 2005; Park et al 2004). Evidence suggests a role for PARP-1 in glutamate excitotoxicity. PARP-1 inhibitors have been found to be more effective in males (Rogers and Wagner 2006), suggesting that sex-specific strategies of inhibiting PARP-1 in NMDA-mediated neurotoxicity may offer acute and chronic neuroprotection.

Conclusions

The major consequences of the urea cycle defects are neurological. In the majority of cases this is related to the consequences of hyperammonaemia, although it is not clear whether ammonia alone is responsible for all the features seen, and whether glutamine toxicity or alterations in brain energy metabolism are contributory. Hyperammonaemia leads to behavioural/cognitive changes, neurotransmitter changes and presumed energy failure. Acute hyperammonaemia leads to NMDA-induced neuronal degeneration, whereas chronic hyperammonaemia has effects on the glutamate-nitric oxide-cyclic guanosine monophosphate pathway in brain and contributes to cognitive impairment. Much of our knowledge of the consequences of hyperammonaemic encephalopathy comes from clinical experience with UCDs and the study of hepatic encephalopathy, as well as from animal models including the rat urease model, the portacaval shunted rat, the sparse fur/ash mouse model of OTCD, and astrocyte cultures or other *in vitro* models, in which handling of ammonia is impaired. Monitoring of the CNS, including the use of EEG and neuroimaging, can provide important information that may be used for prognosis, following therapeutic interventions, and aid in clinical decision-making as well as to investigate mechanisms of CNS injury and recovery. Newer imaging modalities such as fMRI, DTI and MRS can be useful to study the long-term consequences of UCDs on brain organization, white-matter integrity, and metabolism. The mainstay of therapy has been ammonia-lowering through dietary restriction and/or alternative pathway treatments. However, the possibility of using various treatments in a neuroprotective realm, such as hypothermia, anti-inflammatory agents, and NMDA receptor blockers is being actively investigated in other fields such as

stroke, TBI and hypoxic ischaemic encephalopathy (HIE), and may prove to be potential useful agents in the treatment of UCDs. Likewise, drugs that may alter the chronic effects of ammonia on learning and memory offer additional avenues for investigation.

Acknowledgments

My thanks go to the many mentors during this project including Drs Marshall Summar, Brian Ross, Mendel Tuchman and Mark Batshaw. A. L. G. is supported by an NCRR career development award K12RR17613.

Abbreviations

UCD	urea cycle disorder
HHH syndrome	hyperornithinaemia–hyperammonaemia–homocitrullinuria syndrome
GS	glutamine synthetase
OTC	ornithine transcarbamylase
LTP	long-term potentiation
fMRI	functional MRI
MRS	magnetic resonance spectroscopy

References

- Albrecht J. Roles of neuroactive amino acids in ammonia neurotoxicity. *J Neurosci Res.* 1998; 51(2): 133–138. [PubMed: 9469566]
- Albrecht J, Norenberg MD. Glutamine: a Trojan horse in ammonia neurotoxicity. *Hepatology.* 2006; 44(4):788–794. [PubMed: 17006913]
- Albrecht J, Sonnewald U, Waagepetersen HS, Schousboe A. Glutamine in the central nervous system: function and dysfunction. *Front Biosci.* 2007; 12:332–343. [PubMed: 17127302]
- Alzaga AG, Salazar GA, Varon J. Resuscitation great. Breaking the thermal barrier: Dr. Temple Fay. *Resuscitation.* 2006; 69(3):359–364. [PubMed: 16750475]
- Arican N, Kaya M, Yorulmaz C, et al. Effect of hypothermia on blood–brain barrier permeability following traumatic brain injury in chronically ethanol-treated rats. *Int J Neurosci.* 2006; 116(11): 1249–1261. [PubMed: 17000527]
- Bachmann C. Ornithine carbamoyl transferase deficiency: findings, models and problems. *J Inherit Metab Dis.* 1992; 15(4):578–591. [PubMed: 1528018]
- Bachmann C. Mechanisms of hyperammonemia. *Clin Chem Lab Med.* 2002; 40(7):653–662. [PubMed: 12241009]
- Bachmann C. Outcome and survival of 88 patients with urea cycle disorders: a retrospective evaluation. *Eur J Pediatr.* 2003a; 162(6):410–416. [PubMed: 12684900]
- Bachmann C. Long-term outcome of patients with urea cycle disorders and the question of neonatal screening. *Eur J Pediatr.* 2003b; 162 (Supplement 1):S29–S33. [PubMed: 14634803]
- Bachmann C. Long-term outcome of urea cycle disorders. *Acta Gastroenterol Belg.* 2005; 68(4):466–468. [PubMed: 16433005]
- Bachmann C, Colombo JP. Increased tryptophan uptake into the brain in hyperammonaemia. *Life Sci.* 1983; 33(24):2417–2424. [PubMed: 6645806]
- Bachmann C, Colombo JP. Increase of tryptophan and 5-hydroxyindole acetic acid in the brain of ornithine carbamoyltransferase deficient sparse-fur mice. *Pediatr Res.* 1984; 18(4):372–375. [PubMed: 6201810]
- Bachmann C, Braissant O, Villard AM, Boulat O, Henry H. Ammonia toxicity to the brain and creatine. *Mol Genet Metab.* 2004; 81(Supplement 1):S52–57. [PubMed: 15050974]

- Bates TE, Williams SR, Kauppinen RA, Gadian DG. Observation of cerebral metabolites in an animal model of acute liver failure *in vivo*: a ^1H and ^{31}P nuclear magnetic resonance study. *J Neurochem*. 1989; 53(1):102–110. [PubMed: 2566653]
- Batshaw ML. Hyperammonaemia. *Curr Probl Pediatr*. 1984; 14(11):1–69. [PubMed: 6510017]
- Batshaw ML. Inborn errors of urea synthesis. *Ann Neurol*. 1994; 35(2):133–141. [PubMed: 7906500]
- Batshaw ML, Roan Y, Jung AL, Rosenberg LA, Brusilow SW. Cerebral dysfunction in asymptomatic carriers of ornithine transcarbamylase deficiency. *N Engl J Med*. 1980; 302(9):482–485. [PubMed: 7351973]
- Batshaw ML, Brusilow S, Waber L, et al. Treatment of inborn errors of urea synthesis: activation of alternative pathways of waste nitrogen synthesis and excretion. *N Engl J Med*. 1982; 306(23):1387–1392. [PubMed: 7078580]
- Batshaw ML, Hyman SL, Mellits ED, Thomas GH, DeMuro R, Coyle JT. Behavioural and neurotransmitter changes in the urease-infused rat: a model of congenital hyperammonaemia. *Pediatr Res*. 1986; 20(12):1310–1315. [PubMed: 3797124]
- Belanger M, Desjardins P, Chatauret N, Butterworth RF. Loss of expression of glial fibrillary acidic protein in acute hyperammonaemia. *Neurochem Int*. 2002; 41(2–3):155–160. [PubMed: 12020615]
- Belanger-Quintana A, Martinez-Pardo M, Garcia MJ, et al. Hyperammonaemia as a cause of psychosis in an adolescent. *Eur J Pediatr*. 2003; 162(11):773–775. [PubMed: 12942317]
- Bender AS, Norenberg MD. Effects of ammonia on L-glutamate uptake in cultured astrocytes. *Neurochem Res*. 1996; 21(5):567–573. [PubMed: 8726965]
- Bergeron M, Swain MS, Reader TA, Grondin L, Butterworth RF. Effect of ammonia on brain serotonin metabolism in relation to function in the portacaval shunted rat. *J Neurochem*. 1990; 55(1):222–229. [PubMed: 1693945]
- Bindu PS, Sinha S, Taly AB, et al. Extensive cortical magnetic resonance signal change in proximal urea cycle disorder. *J Child Neurol*. 2007; 22(2):238–239. [PubMed: 17621492]
- Blei AT, Olafsson S, Therrien G, Butterworth RF. Ammonia-induced brain oedema and intracranial hypertension in rats after portacaval anastomosis. *Hepatology*. 1994; 19(6):1437–1444. [PubMed: 8188174]
- Bogdanovic MD, Kidd D, Briddon A, Duncan JS, Land JM. Late onset heterozygous ornithine transcarbamylase deficiency mimicking complex partial status epilepticus. *J Neurol Neurosurg Psychiatry*. 2000; 69(6):813–815. [PubMed: 11080238]
- Bourrier P, Varache N, Alquier P, et al. Cerebral edema with hyperammonemia in valpromide poisoning. Manifestation in an adult, of a partial deficit in type I carbamylphosphate synthetase. *Presse Med*. 1988; 17(39):2063–2066. [PubMed: 2974563]
- Braissant O, Henry H, Villard AM, et al. Ammonium-induced impairment of axonal growth is prevented through glial creatine. *J Neurosci*. 2002; 22(22):9810–9820. [PubMed: 12427837]
- Breningstall GN. Neurologic syndromes in hyperammonemic disorders. *Pediatr Neurol*. 1986; 2(5):253–262. [PubMed: 3334203]
- Briand P, Cathelineau L. Sparse-fur mutation: a model for some human ornithine transcarbamylase deficiencies. *Adv Exp Med Biol*. 1982; 153:185–194. [PubMed: 6819764]
- Brunquell P, Tezcan K, DiMario FJ Jr. Electroencephalographic findings in ornithine transcarbamylase deficiency. *J Child Neurol*. 1999; 14(8):533–536. [PubMed: 10456765]
- Brusilow SW. Disorders of the urea cycle. *Hosp Pract (Off Ed)*. 1985; 20(10):65–72. [PubMed: 3930543]
- Brusilow SW, Valle DL, Batshaw M. New pathways of nitrogen excretion in inborn errors of urea synthesis. *Lancet*. 1979; 2(8140):452–454. [PubMed: 89510]
- Busto R, Dietrich WD, Globus MY, Valdes I, Scheinberg P, Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J Cereb Blood Flow Metab*. 1987; 7(6):729–738. [PubMed: 3693428]
- Butterworth RF. Effects of hyperammonaemia on brain function. *J Inherit Metab Dis*. 1998; 21(Supplement 1):6–20. [PubMed: 9686341]

- Butterworth RF. Hepatic encephalopathy: a neuropsychiatric disorder involving multiple neurotransmitter systems. *Curr Opin Neurol*. 2000; 13(6):721–727. [PubMed: 11148676]
- Butterworth RF. Glutamate transporter and receptor function in disorders of ammonia metabolism. *Ment Retard Dev Disabil Res Rev*. 2001; 7(4):276–279. [PubMed: 11754522]
- Butterworth RF. Pathophysiology of hepatic encephalopathy: a new look at ammonia. *Metab Brain Dis*. 2002; 17(4):221–227. [PubMed: 12602499]
- Butterworth RF, Giguere JF, Michaud J, Lavoie J, Layrargues GP. Ammonia: key factor in the pathogenesis of hepatic encephalopathy. *Neurochem Pathol*. 1987; 6(1–2):1–12. [PubMed: 3306479]
- Chatauret N, Zwingmann C, Rose C, Leibfritz D, Butterworth RF. Effects of hypothermia on brain glucose metabolism in acute liver failure: a H/C-nuclear magnetic resonance study. *Gastroenterology*. 2003; 125(3):815–824. [PubMed: 12949727]
- Chen YF, Huang YC, Liu HM, Hwu WL. MRI in a case of adult-onset citrullinemia. *Neuroradiology*. 2001; 43(10):845–847. [PubMed: 11688701]
- Chung C, Gottstein J, Blei AT. Indomethacin prevents the development of experimental ammonia-induced brain edema in rats after portacaval anastomosis. *Hepatology*. 2001; 34(2):249–254. [PubMed: 11481608]
- Cohn RM, Roth KS. Hyperammonaemia, bane of the brain. *Clin Pediatr (Phila)*. 2004; 43(8):683–689. [PubMed: 15494874]
- Connelly A, Cross JH, Gadian DG, Hunter JV, Kirkham FJ, Leonard JV. Magnetic resonance spectroscopy shows increased brain glutamine in ornithine carbamoyl transferase deficiency. *Pediatr Res*. 1993; 33(1):77–81. [PubMed: 8433866]
- DeMars R, LeVan SL, Trend BL, Russell LB. Abnormal ornithine carbamoyltransferase in mice having the sparse-fur mutation. *Proc Natl Acad Sci USA*. 1976; 73(5):1693–1697. [PubMed: 5727]
- Dietrich WD, Busto R, Alonso O, Globus MY, Ginsberg MD. Intracerebral but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats. *J Cereb Blood Flow Metab*. 1993; 13(4):541–549. [PubMed: 8314910]
- DiMagno EP, Lowe JE, Snodgrass PJ, Jones JD. Ornithine transcarbamylase deficiency—a cause of bizarre behaviour in a man. *N Engl J Med*. 1986; 315(12):744–747. [PubMed: 3748082]
- Dolman CL, Clasen RA, Dorovini-Zis K. Severe cerebral damage in ornithine transcarbamylase deficiency. *Clin Neuropathol*. 1988; 7(1):10–15. [PubMed: 3370859]
- Eather G, Coman D, Lander C, McGill J. Carbamyl phosphate synthase deficiency: diagnosed during pregnancy in a 41-year-old. *J Clin Neurosci*. 2006; 13(6):702–706. [PubMed: 16860720]
- Engel RC, Buist NR. The EEGs of infants with citrullinemia. *Dev Med Child Neurol*. 1985; 27(2):199–206. [PubMed: 3996777]
- Enns GM, O'Brien WE, Kobayashi K, Shinzawa H, Pellegrino JE. Postpartum “psychosis” in mild argininosuccinate synthetase deficiency. *Obstet Gynecol*. 2005; 105(5 Pt 2):1244–1246. [PubMed: 15863597]
- Enns GM, Berry SA, Berry GT, et al. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. *N Engl J Med*. 2007; 356(22):2282–2292. [PubMed: 17538087]
- Erceg S, Monfort P, Hernández-Viadel M, et al. Oral administration of sildenafil restores learning ability in rats with hyperammonemia and with portacaval shunts. *Hepatology*. 2005; 41(2):299–306. [PubMed: 15660436]
- Felig DM, Brusilow SW, Boyer JL. Hyperammonemic coma due to parenteral nutrition in a woman with heterozygous ornithine transcarbamylase deficiency. *Gastroenterology*. 1995; 109(1):282–284. [PubMed: 7797025]
- Felipo V, Butterworth RF. Neurobiology of ammonia. *Prog Neurobiol*. 2002a; 67(4):259–279. [PubMed: 12207972]
- Felipo V, Butterworth RF. Mitochondrial dysfunction in acute hyperammonaemia. *Neurochem Int*. 2002b; 40(6):487–491. [PubMed: 11850105]
- Fitzpatrick SM, Hetherington HP, Behar KL, Shulman RG. Effects of acute hyperammonaemia on cerebral amino acid metabolism and pH_i *in vivo*, measured by ¹H and ³¹P nuclear magnetic resonance. *J Neurochem*. 1989; 52(3):741–749. [PubMed: 2563756]

- Garcia MV, Lopez-Mediavilla C, Juanes de la Pena MC, Medina JM. Tolerance of neonatal rat brain to acute hyperammonaemia. *Brain Res.* 2003; 973(1):31–38. [PubMed: 12729950]
- Gaspari R, Archangeli A, Mensi S. Late-onset presentation of ornithine transcarbamylase deficiency in a young woman with hyperammonemic coma. *Ann Emerg Med.* 2003; 41(1):104–109. [PubMed: 12514690]
- Giguere JF, Butterworth RF. Amino acid changes in regions of the CNS in relation to function in experimental portal-systemic encephalopathy. *Neurochem Res.* 1984; 9(9):1309–1321. [PubMed: 6150451]
- Gilchrist JM, Coleman RA. Ornithine transcarbamylase deficiency: adult onset of severe symptoms. *Ann Intern Med.* 1987; 106(4):556–558. [PubMed: 3826955]
- Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet.* 2005; 365:663–670. [PubMed: 15721471]
- Gordon N. Ornithine transcarbamylase deficiency: a urea cycle defect. *Eur J Paediatr Neurol.* 2003; 7(3):115–121. [PubMed: 12788037]
- Gropman AL. Expanding the diagnostic and research toolbox for inborn errors of metabolism: the role of magnetic resonance spectroscopy. *Mol Genet Metab.* 2005; 86(1–2):2–9. [PubMed: 16276565]
- Gropman AL, Batshaw ML. Cognitive outcome in urea cycle disorders. *Mol Genet Metab.* 2004; 81(Supplement 1):S58–S62. [PubMed: 15050975]
- Gushiken T, Yoshimura N, Saheki T. Transient hyperammonaemia during aging in ornithine transcarbamylase-deficient, sparse-fur mice. *Biochem Int.* 1985; 11(5):637–643. [PubMed: 4091843]
- Gyato K, Wray J, Huang ZJ, Yudkoff M, Batshaw ML. Metabolic and neuropsychological phenotype in women heterozygous for ornithine transcarbamylase deficiency. *Ann Neurol.* 2004; 55(1):80–86. [PubMed: 14705115]
- Harding BN, Leonard JV, Erdohazi M. Ornithine carbamoyl transferase deficiency: a neuropathological study. *Eur J Pediatr.* 1984; 141(4):215–220. [PubMed: 6734670]
- Honeycutt D, Callahan K, Rutledge L, Evans B. Heterozygote ornithine transcarbamylase deficiency presenting as symptomatic hyperammonaemia during initiation of valproate therapy. *Neurology.* 1992; 42(3 Pt 1):666–668. [PubMed: 1549234]
- Inamasu J, Ichikizaki K. Mild hypothermia in neurologic emergency: an update. *Ann Emerg Med.* 2002; 40(2):220–230. [PubMed: 12140503]
- Itzhak Y, Roig-Cantisano A, Dombro RS. Acute liver failure and hyperammonemia increase peripheral-type benzodiazepine receptor binding and pregnenolone synthesis in mouse brain. *Brain Res.* 1995; 705(1–2):345–348. [PubMed: 8821768]
- Izumi Y, Izumi M, Matsukawa M, Funatsu M, Zorumski CF. Ammonia-mediated LTP inhibition: effects of NMDA receptor antagonists and L-carnitine. *Neurobiol Dis.* 2005; 20(2):615–624. [PubMed: 15935684]
- Jalan R, Olde Damink SW, Deutz NE, Hayes PC, Lee A. Moderate hypothermia in patients with acute liver failure and uncontrolled intracranial hypertension. *Gastroenterology.* 2004; 127(5):1338–1346. [PubMed: 15521003]
- Jayakumar AR, Rao KV, Murthy ChR, Norenberg MD. Glutamine in the mechanism of ammonia-induced astrocyte swelling. *Neurochem Int.* 2006; 48(6–7):623–628. [PubMed: 16517020]
- Kanamori K, Ross BD. ^{15}N n.m.r. measurement of the *in vivo* rate of glutamine synthesis and utilization at steady state in the brain of the hyperammonaemic rat. *Biochem J.* 1993; 293(Pt 2):461–468. [PubMed: 8102050]
- Kanamori K, Ross BD. *In vivo* activity of glutaminase in the brain of hyperammonaemic rats measured by ^{15}N nuclear magnetic resonance. *Biochem J.* 1995; 305(Pt 1):329–336. [PubMed: 7826349]
- Kanamori K, Ross BD. Kinetics of glial glutamine efflux and the mechanism of neuronal uptake studied *in vivo* in mildly hyperammonemic rat brain. *J Neurochem.* 2006; 99(4):1103–1113. [PubMed: 17081141]

- Kanamori K, Ross BD, Chung JC, Kuo EL. Severity of hyperammonemic encephalopathy correlates with brain ammonia level and saturation of glutamine synthetase *in vivo*. *J Neurochem*. 1996; 67(4):1584–1594. [PubMed: 8858943]
- Kanamori K, Bluml S, Ross B. Magnetic resonance spectroscopy in the study of hyperammonaemia and hepatic encephalopathy. *Adv Exp Med Biol*. 1997; 420:185–194. [PubMed: 9286434]
- Kornfeld M, Woodfin BM, Papile L, Davis LE, Bernard LR. Neuropathology of ornithine carbamyl transferase deficiency. *Acta Neuropathol (Berl)*. 1985; 65(3–4):261–264. [PubMed: 3976361]
- Kosenko E, Llansola M, Montoliu C, et al. Glutamine synthetase activity and glutamine content in brain: modulation by NMDA receptors and nitric oxide. *Neurochem Int*. 2003; 43(4–5):493–499. [PubMed: 12742096]
- Kreis R, Ross BD, Farrow NA, Ackerman Z. Metabolic disorders of the brain in chronic hepatic encephalopathy detected with H-1 MR spectroscopy. *Radiology*. 1992; 182(1):19–27. [PubMed: 1345760]
- Leao M. Valproate as a cause of hyperammonaemia in heterozygotes with ornithine-transcarbamylase deficiency. *Neurology*. 1995; 45(3 Pt 1):593–594. [PubMed: 7898728]
- Lipton SA. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat Rev Drug Discov*. 2006; 5(2):160–170. [PubMed: 16424917]
- Llansola M, Rodrigo R, Monfort P, et al. NMDA receptors in hyperammonemia and hepatic encephalopathy. *Metab Brain Dis*. 2007 Aug 15. [Epub ahead of print].
- Loihl AK, Asensio V, Campbell IL, Murphy S. Expression of nitric oxide synthase (NOS)-2 following permanent focal ischemia and the role of nitric oxide in infarct generation in male, female and NOS-2 gene-deficient mice. *Brain Res*. 1999; 830(1):155–164. [PubMed: 10350569]
- Mamourian AC, du Plessis A. Urea cycle defect: a case with MR and CT findings resembling infarct. *Pediatr Radiol*. 1991; 21(8):594–595. [PubMed: 1815186]
- Mizoguchi K, Utsunomiya H, Emoto H, Shimizu T. A case of ornithine transcarbamylase deficiency with acute and late onset simulating Reye's syndrome in an adult male. *Kurume Med J*. 1990; 37(2):105–109. [PubMed: 2255172]
- Monfort P, Munoz MD, Kosenko E, Felipo V. Long-term potentiation in hippocampus involves sequential activation of soluble guanylate cyclase, cGMP-dependent protein kinase, and cGMP-degrading phosphodiesterase. *J Neurosci*. 2002; 22(23):10116–10122. [PubMed: 12451112]
- Monfort P, Munoz MD, Felipo V. Molecular mechanisms of the alterations in NMDA receptor-dependent long-term potentiation in hyperammonaemia. *Metab Brain Dis*. 2005; 20(4):265–274. [PubMed: 16382337]
- Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED. Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. *N Engl J Med*. 1984; 310(23):1500–1505. [PubMed: 6717540]
- Msall M, Monahan PS, Chapanis N, Batshaw ML. Cognitive development in children with inborn errors of urea synthesis. *Acta Paediatr Jpn*. 1988; 30(4):435–441. [PubMed: 3150233]
- McCullough LD, Zeng Z, Blizzard KK, et al. Ischemic nitric oxide and poly (ADP-ribose) polymerase-1 in cerebral ischemia: male toxicity, female protection. *J Cereb Blood Flow Metab*. 2005; 25(4):502–512. [PubMed: 15689952]
- Nagata N, Matsuda I, Matsuura T, et al. Retrospective survey of urea cycle disorders: Part 2. Neurological outcome in forty-nine Japanese patients with urea cycle enzymopathies. *Am J Med Genet*. 1991; 40(4):477–481. [PubMed: 1746614]
- Natale JE, Guerguerian AM, Joseph JG, et al. Pilot study to determine the hemodynamic safety and feasibility of magnesium sulfate infusion in children with severe traumatic brain injury. *Pediatr Crit Care Med*. 2007; 8(1):1–9. [PubMed: 17251875]
- Neary JT, Norenberg LO, Gutierrez MP, Norenberg MD. Hyperammonaemia causes altered protein phosphorylation in astrocytes. *Brain Res*. 1987; 437(1):161–164. [PubMed: 2827864]
- Nicolaides P, Liebsch D, Dale N, Leonard J, Surtees R. Neurological outcome of patients with ornithine carbamoyl-transferase deficiency. *Arch Dis Child*. 2002; 86(1):54–56. [PubMed: 11806886]
- Norenberg MD. Astrocytic-ammonia interactions in hepatic encephalopathy. *Semin Liver Dis*. 1996; 16(3):245–253. [PubMed: 8989810]

- Norenberg MD, Bender AS. Astrocyte swelling in liver failure: role of glutamine and benzodiazepines. *Acta Neurochir Suppl (Wien)*. 1994; 60:24–27. [PubMed: 7526622]
- Norenberg MD, Rao KV, Jayakumar AR. Mechanisms of ammonia-induced astrocyte swelling. *Metab Brain Dis*. 2005; 20(4):303–318. [PubMed: 16382341]
- Oechsner M, Steen C, Sturenburg HJ, Kohlschütter A. Hyperammonaemic encephalopathy after initiation of valproate therapy in unrecognised ornithine transcarbamylase deficiency. *J Neurol Neurosurg Psychiatry*. 1998; 64(5):680–682. [PubMed: 9598692]
- Ott P, Clemmesen O, Larsen FS. Cerebral metabolic disturbances in the brain during acute liver failure: from hyperammonaemia to energy failure and proteolysis. *Neurochem Int*. 2005; 47(1–2):13–18. [PubMed: 15921824]
- Park EM, Cho S, Fry KA, et al. Inducible nitric oxide synthase contributes to gender differences in ischemic brain injury. *J Cereb Blood Flow Metab*. 2004; 26(3):392–401. [PubMed: 16049426]
- Picca S, Dionisi-Vici C, Abeni D, et al. Extracorporeal dialysis in neonatal hyperammonemia: modalities and prognostic indicators. *Pediatr Nephrol*. 2001; 16(11):862–867. [PubMed: 11685590]
- Polderman KH. Application of therapeutic hypothermia in the intensive care unit. Opportunities and pitfalls of a promising treatment modality—Part 2: Practical aspects and side effects. *Intensive Care Med*. 2004; 30(5):757–769. [PubMed: 14767590]
- Prasad AN, Breen JC, Ampola MG, Rosman NP. Argininemia: a treatable genetic cause of progressive spastic diplegia simulating cerebral palsy: case reports and literature review. *J Child Neurol*. 1997; 12(5):301–309. [PubMed: 9378897]
- Qureshi IA, Rao KV. Sparse-fur (spf) mouse as a model of hyperammonaemia: alterations in the neurotransmitter systems. *Adv Exp Med Biol*. 1997; 420:143–158. [PubMed: 9286431]
- Rao VL, Audet R, Therrien G, Butterworth RF. Tissue-specific alterations of binding sites for peripheral-type benzodiazepine receptor ligand [³H]PK11195 in rats following portacaval anastomosis. *Dig Dis Sci*. 1994; 39(5):1055–1063. [PubMed: 8174418]
- Ratnakumari L, Qureshi IA, Butterworth RF. Regional amino acid neurotransmitter changes in brains of spf/Y mice with congenital ornithine transcarbamylase deficiency. *Metab Brain Dis*. 1994; 9(1):43–51. [PubMed: 7914668]
- Reith J, Jorgensen HS, Pedersen PM, et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet*. 1996; 347(8999):422–425. [PubMed: 8618482]
- Rimbaux S, Hommet C, Perrier D, et al. Adult onset ornithine transcarbamylase deficiency: an unusual cause of semantic disorders. *J Neurol Neurosurg Psychiatry*. 2004; 75(7):1073–1075. [PubMed: 15201380]
- Robinson MB, Hopkins K, Batshaw ML, McLaughlin BA, Heyes MP, Oster-Granite ML. Evidence of excitotoxicity in the brain of the ornithine carbamoyltransferase deficient sparse fur mouse. *Brain Res Dev Brain Res*. 1995; 90(1–2):35–44.
- Rodrigo R, Felipe V. Brain regional alterations in the modulation of the glutamate-nitric oxide-cGMP pathway in liver cirrhosis. Role of hyperammonaemia and cell types involved. *Neurochem Int*. 2006; 48(6–7):472–477. [PubMed: 16517021]
- Rogers E, Wagner AK. Gender, sex steroids, and neuroprotection following traumatic brain injury. *J Head Trauma Rehabil*. 2006; 21(3):279–281. [PubMed: 16717505]
- Scaglia F, Brunetti-Pierri N, Kleppe S, et al. Clinical consequences of urea cycle enzyme deficiencies and potential links to arginine and nitric oxide metabolism. *J Nutr*. 2004; 134:2775S–2782S. [PubMed: 15465784]
- Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*. 2005; 353(15):1574–1584. [PubMed: 16221780]
- Shawcross D, Jalan R. The pathophysiologic basis of hepatic encephalopathy: central role for ammonia and inflammation. *Cell Mol Life Sci*. 2005; 62(19–20):2295–2304. [PubMed: 16158192]
- Shen J, Sibson NR, Cline G, Behar KL, Rothman DL, Shulman RG. ¹⁵N-NMR spectroscopy studies of ammonia transport and glutamine synthesis in the hyperammonemic rat brain. *Dev Neurosci*. 1998; 20(4–5):434–443. [PubMed: 9778582]

- Sibson NR, Dhankhar A, Mason GF, Behar KL, Rothman DL, Shulman RG. *In vivo* ^{13}C NMR measurements of cerebral glutamine synthesis as evidence for glutamate–glutamine cycling. *Proc Natl Acad Sci USA*. 1997; 94(6):2699–2704. [PubMed: 9122259]
- Slotboom J. Localized therapeutic hypothermia in the brain for the treatment of ischaemic stroke. *J Appl Physiol*. 2007; 102(4):1303–1304. [PubMed: 17255367]
- Smith W, Kishnani PS, Lee B, et al. Urea cycle disorders: clinical presentation outside the newborn period. *Crit Care Clin*. 2005; 21(4 Supplement):S9–S17. [PubMed: 16227115]
- Spector EB, Mazzocchi RA. The sparse fur mouse: an animal model for a human inborn error of metabolism of the urea cycle. *Prog Clin Biol Res*. 1983; 127:85–96. [PubMed: 6889405]
- Summar, ML.; Gargosky, S.; Brusilow, S.; Lee, B. Descriptions and outcomes of 316 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes. 2007. [Manuscript in review]
- Swillen A, Vandeputte L, Cracco J, et al. Neuropsychological, learning and psychosocial profile of primary school aged children with the velo-cardio-facial syndrome (22q11 deletion): evidence for a nonverbal learning disability? *Child Neuropsychol*. 1999; 5(4):230–241. [PubMed: 10925707]
- Szerb JC, Butterworth RF. Effect of ammonium ions on synaptic transmission in the mammalian central nervous system. *Prog Neurobiol*. 1992; 39(2):135–153. [PubMed: 1354386]
- Takahashi H, Koehler RC, Brusilow SW, Traystman RJ. Inhibition of brain glutamine accumulation prevents cerebral oedema in hyperammonemic rats. *Am J Physiol*. 1991; 261(3 Pt 2):H825–H829. [PubMed: 1679605]
- Takanashi J, Kurihara A, Tomita M, et al. Distinctly abnormal brain metabolism in late-onset ornithine trans-carbamylase deficiency. *Neurology*. 2002; 59(2):210–214. [PubMed: 12136059]
- Takanashi J, Barkovich AJ, Cheng SF. Brain MR imaging in neonatal hyperammonemic encephalopathy resulting from proximal urea cycle disorders. *AJNR Am J Neuroradiol*. 2003; 24(6):1184–1187. [PubMed: 12812952]
- Tanigami H, Rebel A, Martin LJ, et al. Effect of glutamine synthetase inhibition on astrocyte swelling and altered astroglial protein expression during hyperammonaemia in rats. *Neuroscience*. 2005; 131(2):437–449. [PubMed: 15708485]
- Tekkok SB, Goldberg MP. AMPA/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. *J Neurosci*. 2001; 21(12):4237–4248. [PubMed: 11404409]
- Tekkok SB, Ye Z, Ransom BR. Excitotoxic mechanisms of ischemic injury in myelinated white matter. *J Cereb Blood Flow Metab*. 2007; 27(9):1540–1552. [PubMed: 17299453]
- Tokatli A, Coskun T, Cataltepe S, Ozalp I. Valproate-induced lethal hyperammonaemic coma in a carrier of ornithine carbamoyltransferase deficiency. *J Inherit Metab Dis*. 1991; 14(5):836–837. [PubMed: 1779634]
- Tripp JH, Hargreaves T, Anthony PP. Sodium valproate and ornithine carbamyl transferase deficiency. *Lancet*. 1981; 1(8230):1165–1166. [PubMed: 6112522]
- Uchino T, Endo F, Matsuda I. Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. *J Inherit Metab Dis*. 1998; 21(Supplement 1):151–159. [PubMed: 9686352]
- Vaquero J, Blei AT. Mild hypothermia for acute liver failure: a review of mechanisms of action. *J Clin Gastroenterol*. 2005; 39(4 Supplement 2):S147–S157. [PubMed: 15758651]
- Vaquero J, Rose C, Butterworth RF. Keeping cool in acute liver failure: rationale for the use of mild hypothermia. *J Hepatol*. 2005; 43(6):1067–1077. [PubMed: 16246452]
- Veres G, Gibbs RA, Scherer SE, Caskey CT. The molecular basis of the sparse fur mouse mutation. *Science*. 1987; 237(4813):415–417. [PubMed: 3603027]
- Verma NP, Hart ZH, Kooi KA. Electroencephalographic findings in urea-cycle disorders. *Electroencephalogr Clin Neurophysiol*. 1984; 57(2):105–112. [PubMed: 6198150]
- Volbracht C, van Beek J, Zhu C, Blomgren K, Leist M. Neuroprotective properties of memantine in different *in vitro* and *in vivo* models of excitotoxicity. *Eur J Neurosci*. 2006; 23(10):2611–2622. [PubMed: 16817864]

- Wang JY, Wen LL, Huang YN, Chen YT, Ku MC. Dual effects of antioxidants in neurodegeneration: direct neuroprotection against oxidative stress and indirect protection via suppression of glia-mediated inflammation. *Curr Pharm Des.* 2006; 12(27):3521–3533. [PubMed: 17017945]
- Welsh FA, Sims RE, Harris VA. Mild hypothermia prevents ischemic injury in gerbil hippocampus. *J Cereb Blood Flow Metab.* 1990; 10(4):557–563. [PubMed: 2347886]
- Whitelaw A, Bridges S, Leaf A, Evans D. Emergency treatment of neonatal hyperammonaemic coma with mild systemic hypothermia. *Lancet.* 2001; 358(9275):36–38. [PubMed: 11454378]
- Yamori Y, Horie R, Handa H, et al. Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. *Stroke.* 1976; 7(1):46–53. [PubMed: 1258104]
- Zwingmann C, Chatauret N, Rose C, Leibfritz D, Butterworth RF. Selective alterations of brain osmolytes in acute liver failure: protective effect of mild hypothermia. *Brain Res.* 2004; 999(1): 118–123. [PubMed: 14746928]
- Zwingmann C, Butterworth R. An update on the role of brain glutamine synthesis and its relation to cell-specific energy metabolism in the hyperammonemic brain: further studies using NMR spectroscopy. *Neurochem Int.* 2005; 47(1–2):19–30. [PubMed: 15916833]

Fig. 1.
Diagrammatic depiction of the major enzymatic reactions in the urea cycle

Fig. 2.
CT scan demonstrating left-sided, posteriorly located area of decreased attenuation, consistent with infarction (arrow)

Fig. 3.

The glutamate–glutamine cycle. Neuronal glutamate is released from the nerve terminal and taken up by the glial cell where it is converted into glutamine, a less toxic molecule. The glutamine can be transferred to the neuron and allow glutamate to be resynthesized

Fig. 4.
T2-weighted MRI image, axial and coronal showing white-matter changes (arrows) seen in OTCD deficiency

Fig. 5. T1-weighted coronal (a) and autopsy specimen (b) of neonate with OTCD. Note the significant grey-matter involvement with severe cortical atrophy and cystic changes

Fig. 6. ^1H MRS acquisition from the thalamus of control subject (black) and one with OTCD (red). Note the elevation of glutamine and decreased level of myoinositol in the subject relative to control. In addition, there are differences in other metabolites, including decreased choline in the subject relative to control

Table 1

Neurological manifestations of urea cycle disorders

Classic proximal urea cycle defects	Partial enzyme deficiencies
Anorexia	Protein aversion
Vomiting	Hyperactive behaviour
Cognitive and motor deficits	Self-injurious behaviour
Lethargy	Stroke-like episodes
Ataxia	Psychiatric symptoms
Asterixis	
Brain oedema	
Cytotoxic and vasogenic oedema	
Hypothermia	
Seizures	
Coma	

Table 2Major compounds resolved by ^1H MRS

Compound	Abbreviation	Biological role
<i>N</i> -Acetyl aspartate	NAA	Neuronal marker Other compounds with <i>N</i> -acetyl groups contribute to the signal (NAAG)
Creatine/phosphocreatine	Cr	Source of phosphate to convert ADP to ATP
Choline	Cho	Sum of signals from all derivatives of choline including acetylcholine neurotransmitter, derivatives of membrane phosphatidylcholine and betaine
Myoinositol	mI	Glial marker, cell signalling molecule
Glutamate	Glu	Excitatory neurotransmitter
Glutamine	Gln	Product of the reaction between Glu and ammonia. Regulator of Glu and detoxification
Lactate	Lac	Final product of anaerobic glycolysis
γ -Aminobutyric acid	GABA	Neurotransmitter