

Robert Surtees · Nenad Blau

The neurochemistry of phenylketonuria

Abstract The mechanisms by which deficiency of hepatic phenylalanine hydroxylase causes central nervous system disease are reviewed. The neurological disease appears to be secondary to increased concentrations of phenylalanine and a decrease in the concentrations of other large neutral amino acids, especially methionine and tyrosine, within the central nervous system. This causes a deficiency of the neurotransmitter dopamine, reduced protein synthesis and demyelination. Similar mechanisms appear to be operating when blood phenylalanine concentrations are in the range expected for early continuously treated phenylketonuria.

Conclusion The severe brain disease found in phenylketonuria is caused by a raised blood phenylalanine content which increases the brain free phenylalanine and decreases the concentration of other large neutral amino acids. Brain protein synthesis is decreased, myelin turnover is increased and there are abnormalities in amine neurotransmitter systems.

Key words Phenylalanine hydroxylase deficiency · Hyperphenylalaninaemia · Neurotransmitters · Demyelination · Central nervous system

Abbreviations *BH₄* tetrahydrobiopterin · *GTP* guanosine triphosphate · *GFRP* GTPCH feed-back regulatory protein · *GTPCH* GTP cyclohydrolase I · *HPA* hyperphenylalaninaemia · *PAH* phenylalanine 4-hydroxylase · *Phe* phenylalanine · *PKU* phenylketonuria · *PTPS* 6-pyruvoyltetrahydropterin synthase · *SR* sepiapterin reductase

Introduction

Phenylketonuria (PKU) is caused by deficiency of the hepatic enzyme phenylalanine 4-hydroxylase (PAH). The biochemical consequences of this are the accumulation of phenylalanine (Phe) and its metabolites in the blood and a relative deficiency of tyrosine that becomes an essential amino acid. However, the main impact of the disease is upon the brain that normally does not

contain PAH. Untreated, the early manifestations of PKU are microcephaly, severe mental retardation and epilepsy and, in the second or third decade, there is the emergence or progression of a motor disorder. The neuropathological correlates of this are microcephaly with white matter gliosis and delayed myelination in the younger patients and focal or diffuse demyelination in the older patients.

Severe neurological disability in PKU can largely be prevented by a strict reduced Phe diet started in early

R. Surtees (✉)
Institute of Child Health (UCLMS), 30, Guilford Street,
London WC1N 1EH, UK
e-mail: r.surtees@ich.ucl.ac.uk
Tel.: +44-20-7837-7618; Fax: +44-20-7833-7618

N. Blau
University Children's Hospital,
Steinwiesstrasse 75, 8032 Zurich,
Switzerland

infancy. Despite such treatment, patients with PKU are of lower intelligence [38], may have neuropsychological [9] or neurological [41] abnormalities and have cerebral white matter abnormalities [6, 7, 40]. There is also the risk of late motor and cognitive decline in adults who have relaxed their diet [26, 39].

Current understanding of the biochemical pathogenesis of the neurological consequences of PKU is that these are secondary to the accumulation of Phe in the blood. This will be discussed in four sections: (1) the effect on the free amino acids of the brain; (2) the effect upon dopamine and other neurotransmitters; (3) the effect upon protein synthesis in the brain; and (4) the effect upon myelin turnover and synthesis. The accumulation of potentially toxic metabolites of Phe in the blood does not appear to be of a degree sufficient to cause brain injury.

Free amino acids in the brain

McKean [27] showed that the brains of patients with PKU contained an increased concentration of free Phe whilst the content of the amino acids tyrosine and tryptophan were decreased. In earlier work he had shown that rats made acutely hyperphenylalaninaemic had a raised brain Phe concentration and deficiencies of the other large neutral amino acids valine, leucine, isoleucine, threonine, histidine, tryptophan, methionine and tyrosine [28]. This has since been confirmed by many investigators using a variety of animal models that cause acute and chronic hyperphenylalaninaemia (HPA) across all ages. Because the large neutral amino acids share a common transporter across the blood-brain barrier, the simplest explanation is when blood Phe concentrations are raised competition at the transporter causes a decreased uptake of the other large neutral amino acids across the blood-brain barrier; thereby resulting in deficiency of these within the central nervous system. Furthermore, the large neutral amino acid carrier has a greater affinity for Phe than the other amino acids, across all species, and this is more marked in humans than rodents [34]. Thus humans may be selectively more vulnerable to HPA. The hypothesis of competition across the blood-brain barrier has been tested in a rat model of chronic HPA where the uptakes of the large neutral amino acids were shown to be impaired by HPA [2]. Further support for this mechanism has been obtained from experiments that show that supplementation of leucine, isoleucine, valine, threonine, tryptophan, tyrosine and methionine in animal models of HPA restores the brain concentrations of these large neutral amino acids [3] and reduces brain Phe concentration [1].

Such a mechanism is also likely to account for the findings in brains from human patients with PKU. The kinetics of the human large neutral amino acid carrier at the blood-brain barrier are very similar to that in rodents and methionine uptake into the brain is

depressed in patients with PKU [33]. Recently, new radioactive tracer and magnetic resonance spectroscopic techniques have been employed for direct measurements in humans, although these currently show high variability [20, 30–32]. Subtraction magnetic resonance spectroscopy has shown increased brain Phe concentrations in patients with PKU comparable to that found by direct measurement [30, 31]; furthermore, comparison of plasma and brain concentrations suggests a saturable carrier for Phe across the blood-brain barrier with kinetics similar to the human large neutral amino acid carrier [31]. Assessment of blood-brain barrier permeability to Phe and leucine using a double indicator method at different plasma Phe concentrations in normal volunteers and patients has demonstrated a reduction in the net uptake of large neutral amino acids in PKU [20].

In summary, experimental evidence from both human and animal studies suggests that the primary effect of HPA upon the brain is both to raise Phe concentrations and to reduce the concentrations of other large neutral amino acids; an effect of competition for transport across the blood-brain barrier.

Dopamine

The biosynthesis of the neurotransmitter monoamines dopamine, noradrenaline and serotonin is dependent upon the availability of the precursor amino acids tyrosine and tryptophan within the brain and the presence of a normal Phe concentration [15]. The excess of Phe and the relative lack of tyrosine and tryptophan in the brain in HPA would be expected to result in deficiencies of these amines. In humans with PKU, a reduction in the production of the metabolites of these was shown in urine [8] and CSF [4, 23]. In these studies a reduction in plasma Phe caused an increase in the amine neurotransmitter metabolites. These neurotransmitters are normally concerned with executive functions of the brain and the initiation of movement. However, measurement of the physiological effect of their deficiency in PKU has been difficult. An increase in the variability of visual reaction time has been shown and this effect was normalised by a reduction in plasma Phe [22]. Indirect evidence also suggests that even in treated PKU, dopamine deficiency causes defects in executive functions of the brain. The dorsolateral prefrontal cortex of mammals receives a large dopamine projection with a very high dopamine turnover. In primates, this brain region subserves specific executive functions that are impaired when dopamine levels are reduced [9]. Infants and children with early treated PKU and moderate HPA are also impaired on these tasks when compared to age-matched controls and the degree of impairment is directly related to the degree of HPA [9]. Rat pups made chronically hyperphenylalaninaemic with α -methylphenylalanine (a PAH inhibitor) and Phe show impaired performance on tasks dependant on frontal lobes and

have significantly reduced levels of homovanillic acid (an acidic metabolite of dopamine) in their frontal cortex [10].

The role of tetrahydrobiopterin

Tetrahydrobiopterin (BH₄) is the essential cofactor of Phe, tyrosine, and tryptophan mono-oxygenases. BH₄ is synthesised from guanosine triphosphate (GTP) in three enzymatic steps involving the enzymes GTP cyclohydrolase I (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS), and sepiapterin reductase (SR); very little is known about its cellular regulation. However, it has been shown that patients with classical PKU excrete increased amounts of pterins in urine [21]. At serum Phe concentrations of 43 to 1004 $\mu\text{mol/l}$, a good correlation was also found with serum biopterin and neopterin levels [29]. Following oral loading with Phe (100 mg/kg), serum and red blood cell biopterin concentrations increased in patients with classical PKU. The kinetics of the biopterin response to simple Phe loading was characterised by its early increase in serum, followed by an increase in erythrocytes. Similar information, consistent with biopterin transfer from serum into erythrocytes, was obtained by performing a combined Phe and BH₄ loading test [35]. As an explanation, it has recently been shown that increased Phe stimulates BH₄ production by activating GTPCH via the GTP cyclohydrolase stimulating protein (GFRP) [29] (Fig. 1). Thus, BH₄ controls its own biosynthesis by a feed-back regulatory circuit through the action of the GFRP. The regulatory protein mediated end-product feed-back inhibition of GTPCH by BH₄ is reversed by Phe [12]. In patients with classical PKU, Phe also acts as a competitive inhibitor of the brain tyrosine and tryptophan mono-oxygenases (Fig. 1). Thus, although the bioavailability of BH₄ at high Phe levels in these patients is probably increased, its inhibitory effect on mono-oxygenases

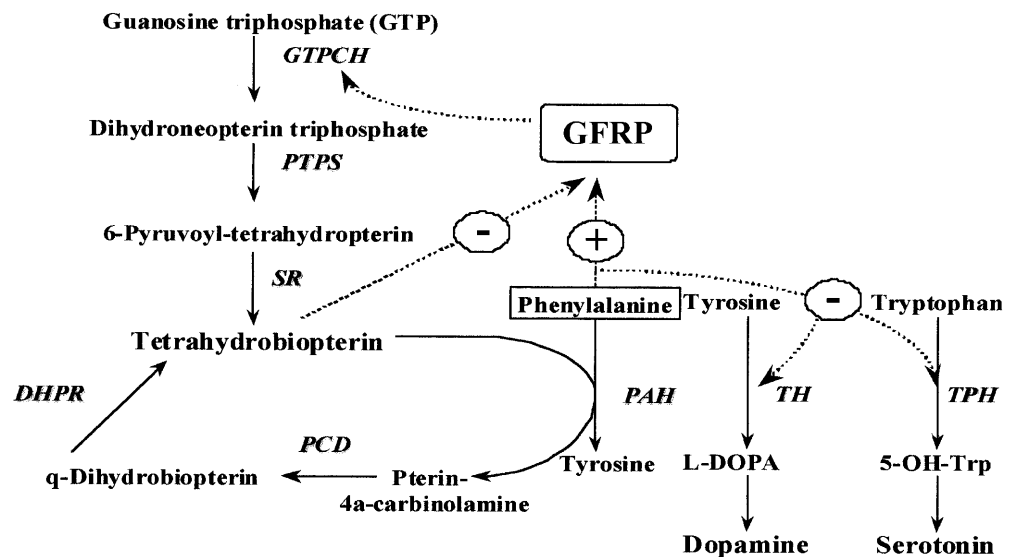
is more dominant. This results in a diminished production of catecholamines and serotonin. Brain Phe concentrations of $>40 \mu\text{mol/l}$ were shown to inhibit rat tyrosine mono-oxygenase even in the presence of BH₄.

Protein synthesis

The effect of HPA on protein synthesis in the brain has been studied exclusively in animal models. HPA causes a reduction in brain weight, cell number and deoxyribonucleic acid in the immature rat with no effect on the kidney [18]. This organ-specific effect has been thought to be secondary to "cellular undernutrition" because of competing amino acids. Acute and chronic HPA reduces the incorporation of amino acids into protein in the brain [3]. This effect on protein synthesis is reflected by a reduction in the number of polyribosomes and a corresponding rise in the number of monoribosomes [3], and can be partially reversed by supplementation with other large neutral amino acids [3, 19]. The effect on protein synthesis seems to be due in part to competition between Phe and other large neutral amino acids for transport across the blood-brain barrier. Such a view is supported by the finding that, despite a reduction in the concentration of free amino acids, there is actually a rise in the equivalent aminoacyl-transfer ribonucleic acids (tRNA) with the exception of methionyl-tRNA and especially the initiator species tRNA^{met} [19]. Here deficiency of the initiator methionyl-tRNA is believed to inhibit protein synthesis and cause the rise in the other aminoacyl-tRNA species. HPA also prevents the normal phosphorylation of the ribosomal protein S₆ which influences the initiation of protein synthesis; such an effect is reversed by supplementation with the other large neutral amino acids [36].

More recently, in rats made hyperphenylalaninaemic by carotid perfusion of Phe, an inverse relationship

Fig. 1 Effect of Phe on tetrahydrobiopterin and biogenic amines pathways. (*DHPR* dihydropteridine reductase, *PCD* pterin-4a-carbinolamine dehydratase, *TH* tyrosine-3-mono-oxygenase, *TPH* tryptophan-5'-mono-oxygenase)



between cerebral protein synthesis and carotid perfusate Phe concentration has been shown [34]. This relationship holds for Phe concentrations in the range seen in early treated PKU.

Myelination

The demonstration that there are abnormalities in myelination in patients with PKU led to the suggestion that disordered myelination and demyelination may be the primary process causing the brain disease [37]. Although a reduced amount of myelin is consistently found, it was thought to have a normal gross composition [37]. However, some human studies suggested a selective reduction in the sulphatide content [37]. Animal studies too have shown that in experimental HPA there is a reduced amount of myelin with a grossly normal composition [17], but earlier studies suggested a selective reduction in sulphatide [5]. The latter investigators also showed that the decreased synthesis of sulphatide was due to inhibition of the sulphate activating enzyme.

In a series of studies of experimental HPA in young rats, Hommes and colleagues [13, 14, 16] have shown that there is an increased turnover of the "fast component" of the central nervous system myelin induced by HPA and this is associated with inhibition of the sulphate activating system. The sulphate activating system consists of two enzymes which sequentially convert inorganic sulphate to adenosine phosphosulphate (ATP-sulphurylase) and then phosphoadenosine phosphosulphate (APS-kinase). Phosphoadenosine phosphosulphate is the sulphate donor in a variety of reactions which produce the sulphatides of myelin amongst other sulphated products. In experiments with partially purified sulphate activating system enzymes it was shown that the brain (but not the liver) has a Phe-sensitive ATP-sulphurylase [25]. Furthermore, the development of the Phe-sensitive ATP-sulphurylase paralleled the rate of myelination and was distributed exclusively in the white matter of the brain [24]. These experiments led to the hypothesis that inhibition of oligodendroglial ATP-sulphurylase by Phe causes decreased availability of cerebroside sulphate that cannot then protect myelin basic protein effectively from proteolytic degradation [13].

More recent studies in a genetically modified mouse model with PAH deficiency (Pah^{enu2}, previously Pah^{HPH-5}) have shown hypomyelination and gliosis of the white matter which appears to be caused by the transformation of oligodendrocytes to a non-myelinating phenotype [11]. Furthermore, wild-type oligodendrocytes cultured in the presence of elevated Phe also show such a transformation [11]. The abnormalities in myelination that complicate PKU thus appear to be a combination of increased myelin turnover and decreased myelin production; both appearing secondary to an increase in brain Phe.

Conclusions

The neurochemical studies discussed above suggest that the severe brain disease found in PKU is caused by a raised blood Phe content which increases the brain free Phe and decreases the concentration of other large neutral amino acids. As a consequence, brain protein synthesis is decreased, myelin turnover is increased and there are abnormalities in amine neurotransmitter systems. Similar mechanisms appear to operate when blood Phe concentrations are in the ranges seen in early treated PKU. However, the relative contributions of these to the development of the central nervous system disease are not understood. Despite this, there is abundant evidence that supplementation with other large neutral amino acids can reverse the neurochemical changes.

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