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Research Report

# Reduced availability of brain amines during critical phases of postnatal development in a genetic mouse model of cognitive delay

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ABSTRACT

Serotonin (5-HT), dopamine (DA) and noradrenaline (NE) play important roles in brain postnatal maturation. Therefore, deficits in brain availability of biogenic amines during critical developmental phases might underlie neurodevelopmental disturbances associated with cognitive impairment. To test this hypothesis we evaluated brain availability of 5-HT, DA and NE, of their immediate precursors 5-hydroxytryptophan and 3,4-dihydroxy-L-phenylalanine, and of large neutral amino acids phenylalanine, tyrosine and tryptophan, in developing PahEnu2 mice, the genetic model of Phenylketonuria (PKU) a cause of severe cognitive delay. We found deficits of brain amine levels in PKU pups between day 14 and 35 of postnatal life, when pups of the healthy background showed developmental peak increases of amines and precursors. 5-HT deficits were most pronounced, were unrelated with brain availability of the aminoacid precursor tryptophan, but overlapped with peak brain phenylalanine concentrations and reduced availability of 5-HT direct precursor 5-hydroxytryptophan. These results identify a critical window of brain amine availability susceptible to disturbances in a genetic mouse model of pathological neurodevelopment and suggest a mechanism of interference with brain aminergic synthesis in PKU and non-PKU hyperphenylalaninemia.



## 1. Introduction

Biogenic amines, in addition to their well known function as neurotransmitters, have important roles in brain development (Herlenius and Lagercrantz, 2001). The availability of aminergic markers, such as tissue concentrations of amine precursors, receptors etc., in the developing brain follows a typical "phasic" evolution characterized by dramatic increases during specific age-windows followed by reduction to adult levels (Chugani et al., 1999; Goldman-Rakic and Brown, 1982). It has been suggested that neurotransmitter overproduction favors brain maturation in critical periods, i.e. in developmental stages during which condensing biochemical mechanisms are required for brain growth events. Moreover, several

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studies showed that, in developing animals, biogenic amines are implicated in the formation and maintenance of synapses (Okado et al., 2001; Whitaker-Azmitia, 2001).

These considerations suggest that deficits in biogenic amine availability during critical periods might be involved in neurodevelopmental disorders associated with cognitive delay.

Phenylketonuria (PKU; McKusick 2610600) is a genetic developmental disease that causes severe cognitive delay in humans. It is promoted by a deficiency of the phenylalanine hydroxylase enzyme, necessary to convert phenylalanine (PHE) in tyrosine, that causes elevated blood levels of PHE (Scriver and Kaufman, 2001). Although the pathogenic effects of hyperphenylalaninemia are still unknown, PKU is characterized by several neuropathological signs in structures whose maturation is directed by aminergic neurotransmitters (Bauman and Kemper, 1982; Williams et al., 1980). Moreover, hyperphenylalaninemia is associated with a decrease in brain availability of biogenic amines. Indeed, early studies reported reduced levels of dopamine (DA), norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) in *post mortem* brain tissue (McKean, 1972), and recent reports indicate reduced aminergic synthesis in the brain of adult PKU subjects on free PHE diet (Burlina et al., 2000) and in mild hyperphenylalaninemia with neurological signs (Bonafé et al., 2001).

In line with these human data, in recent studies we and others have reported reduced brain amine levels and metabolism in adult Pah$^{enu2}$ (ENU2) mice (Pascucci et al., 2002; Puglisi-Allegra et al., 2000; Joseph and Dyer, 2003), the murine model of PKU (McDonald et al., 1990), which are characterized by severe and generalized cognitive deficits (Cabib et al., 2003). Therefore, ENU2 mice represent a qualified model to study brain biochemical deficits in an animal model of neurodevelopmental disorder associated with cognitive delay. One further advantage of a PKU model is the known pathogenic determinant, i.e. excess in circulating PHE levels during postnatal development (Pietz, 1998). ENU2 mice, which resemble the human pathology as for

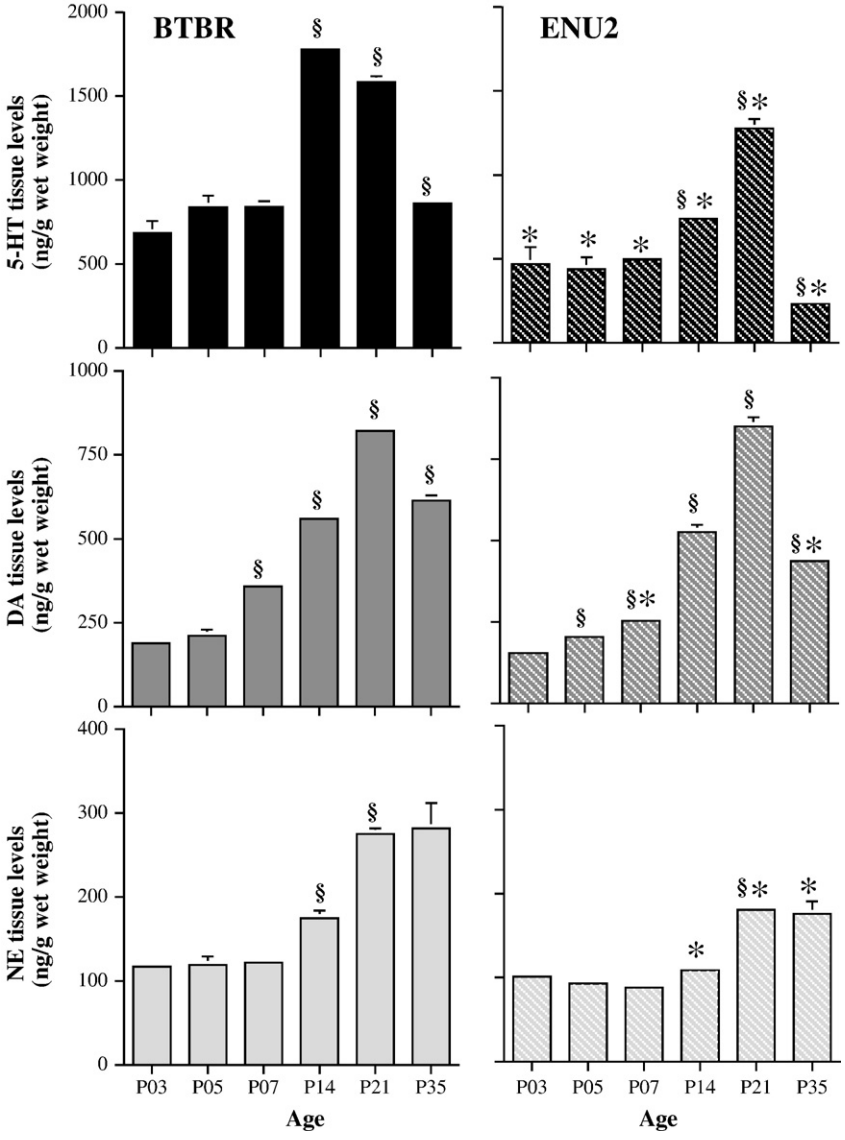


**Fig. 1 – Mean (+SE) brain concentrations of 5-HT (upper panel), DA (middle panel), and NE (lower panel) in BTBR and ENU2 mice at several postnatal days (P03–P35). *P<0.05 in comparison with BTBR mice. §P<0.05 in comparison with previous time point.**