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Amino Acids

Introduction: Vulnerability of the CNS to metabolic and drug-related insults (Chapter 1)

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The CNS is particularly vulnerable to metabolic and drug-related insults occurring along development. At the gestational period, the blood-brain barrier (BBB) provides protection against many insults affecting the mothers, or drugs taken by the mothers. Indeed, the BBB, as a barrier that excludes proteins, many charged or large molecules, and other substances present in the blood, is formed very early during development, probably, when the vessels first penetrate into the brain parenchyma and choroid epithelial cells differentiate.

Still, it has been suggested that the BBB of young animals is more permeable to small molecules than that in adults, either reflecting an immaturity of the tight junctions between the cerebral endothelial cells (Saunders and Møllgård, 1984; Møllgård and Saunders, 1986), or a greater metabolic demand by a rapidly growing tissue (Baños et al., 1978). Indeed, it has been reported that immature animals show a larger number of large pores in cerebral vessels and choroid plexus than that shown by more developed animals (Dziegielewska et al., 1979). In agreement, J. W. Olney first demonstrated that glutamate is toxic when administered subcutaneously to young animals (Olney, 1969), although it has been argued that the transport of excitotoxic amino acids to the brain by the BBB is low, and that the neurotoxicity of systemically administered glutamate is mainly found on cells of the arcuate nucleus, which is a circumventricular region outside the BBB (see Lefauconnier, 1992).

In the present chapter, Leon Navarro et al. (2005) present a paper investigating the effect of glutamate given by the drinking water to pregnant rats on metabotropic glutamate receptors assayed in the brain of mothers and pups, finding that the treatment produced a down-regulation of mGlu receptors and Gq/11 and phospholipase Cβ1 proteins in mothers, but not foetuses brains, supporting the idea that glutamate excitotoxicity does not primarily relate to BBB, but to increased endogenous viability. Glutamate is ubiquitously synthesized and released at high concentrations. In the extracellular space, glutamate is modulated by a powerful transport system to glia and nerve terminals, which, however, is very vulnerable to metabolic impairments, elevating extracellular glutamate to excitotoxic levels.

There are environmentally and synthetically produced amino acids that can penetrate the BBB over-stimulating glutamate receptors in the CNS. Kusama-Eguchi et al. (2005) show that 3-N-oxyalyl-L-2,3-diamino propanoic acid (L-β-ODAP; also known as β-N-oxalyl-amino-L-alanine [BOAA]), contained in *Lathyrus Satirus* grass pea, can accumulate in the spinal cord following parenteral administration, producing paraparesis of the hind legs in neonatal rats, associated to atrophy of the ventral root of the lumbar cord and degeneration of motor neurons. The mechanism by which L-β-ODAP is taken up and accumulated in the spinal cord has to be further investigated, because the incidence of the effect produced by L-β-ODAP is low. In humans, lathyrism was first described by the Greek physician Hippocrates and the Roman physician Galen, and was a major epidemic in Spain as recently as the 1940s (Spencer and Schaumburg, 1983). Lathyrism in humans is a slowly progressing irreversible spastic parapesis, disproportionately affecting young males. When >400 grams of *L. sativus* is consumed per day for several days ($\sim$15–50 mg/kg/day), lathyrism can occur in just a few weeks (Cohn and Streifler, 1981). Pathologically, human lathyrism is thought to be attributable to selective loss of cortical Betz neurons specific to the leg region (Filiminoff, 1926), and in some ways the symptom-

atology resembles amyotrophic lateral sclerosis (Calne et al., 1986). BOAA has also been associated with degeneration of anterior horm cells in the spinal cord (Pearson and Nunn, 1981).

Propofol is a substituted phenol used for intravenous anaesthesia, producing, however, several side effects, including hallucination, sexual dis-inhibition and euphoria by, until now, unknown mechanisms. The hedonic and rewarding quality of these effects has suggested an action on mesolimbic dopamine circuitry, but seizures have also been reported, indicating an action on glutamatergic systems. These possibilities were investigated with *in vivo* microdialysis by Grasshoff et al. (2005), showing that propofol locally administered by a microdialysis probe decreases dopamine levels in ventral striatum, without affecting glutamate levels. Whether the decrease of dopamine levels relates to the side effects produced by propofol in humans remains to be investigated. Nevertheless, the methodological approach used by Grasshoff et al. (2005) is a competent method for dissecting in detail the action of a particular drug on specific neurocircuitry systems.

Perinatal asphyxia is still a serious clinical problem despite advances in perinatal and obstetric care, probably because of a lack of a suitable model for investigating the mechanisms by which short- and long-term consequences of perinatal asphyxia are produced. At the Karolinska Institutet, Stockholm, Sweden, a model for investigating perinatal asphyxia in the rat has been developed (Bjelke et al., 1991; Herrera-Marschitz et al., 1993), showing that dopamine (Chen et al., 1997a, b, c; Kohlhauser et al., 1999), as well as amino acid (Chen et al., 1997a; Kohlhauser et al., 1999; Engidawork et al., 2001) neurocircuitries are vulnerable to perinatal asphyxia. In the present chapter, Klawitter et al. (2005) show studies combining an *in vivo* and *in vitro* approach. After inducing perinatal asphyxia *in vivo*, surviving pups were used to prepare organotypic cultures, as shown by Morales et al. (2003), demonstrating that cultures prepared from asphyctic pups showed a decreased number of neurons labelled with an antibody against the NR1 subunit of the N-Methyl-D-aspartate (NMDA) receptor, and a decreased number of secondary to higher level branching of dopamine neurons labelled with an antibody against tyrosine hydroxylase.

The treatment of Parkinson's patients with the amino acid L-DOPA is well established, being an efficient treatment for rigidity and hypokinesia. L-DOPA is less effective against tremor, and does not reverse the course of the disease. Furthermore, the effectiveness of L-DOPA gradually declines by, until now, unknown mechanisms. It has been suggested that L-DOPA's declining efficiency is due to impairment of L-DOPA transport and reduced biodisponibility in the targeted brain areas. The motor dyskinesia attending long-term L-DOPA has been attributed to the overexpression of striatal (i.e., basal ganglia) dopamine D_3 receptors, consequent to increased action of L-DOPA-induced production of brain-derived neurotrophic factor (BDNF) (Bezard et al., 2003; Bordet et al., 2000).

L-DOPA is transported into the brain by the L- transport system (Oldendorf, 1971), which is sodium independent and modulated by the availability of L-amino acids. The transport of L-DOPA competes with amino acids and proteins in the food. Furthermore, L-DOPA can be decarboxylated into dopamine and further de-aminated in the endothelial cells, before reaching the targeted areas. Thus, only a low percentage of systemically administered L-DOPA is transported into the brain. Indeed, as demonstrated by Zetterström et al. (1986), the amount of dopamine synthesized from a single clinically-relevant dose of L-DOPA reaches a tenth of the dopamine levels found in the striatal extracellular compartment of an intact brain, but that amount is enough to stimulate dopamine receptors rendered supersensitive following the lack of endogenous dopamine, and to revert the motor deficits.

In the targeted brain areas, L-DOPA can be taken up by the remaining dopamine nerve terminals, and further released as dopamine, or intracellularly metabolised by MAO dependent and/or independent mechanisms. It has been suggested that intracellular oxidation of L-DOPA or dopamine accelerates the progression of the disease (Fahn, 1997; Kostrzewa et al., 2000, 2002). In this chapter, however, Kostrzewa et al. (2005) discuss the idea that L-DOPA is actually neuroprotective, because the L-DOPA treatment reduces striatal tissue content of reactive oxygen species, and also induces BDNF release, which in turn enhances the expression of D3 receptors (Guillin et al., 2001), and perhaps neuritogenesis.

Thus, in this chapter several aspects related to the vulnerability of the CNS to metabolic and drug-related insults are discussed, enlightening the role of the BBB, CNS maturity for amino acid-induced excitotoxicity, and drug selectivity on particular neurocircuitries of the brain.

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