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Review

Barriers in the developing brain and *Neurotoxicology*

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ABSTRACT

The brain develops and grows within a well-controlled internal environment that is provided by cellular exchange mechanisms in the interfaces between blood, cerebrospinal fluid and brain. These are generally referred to by the term "brain barriers": blood–brain barrier across the cerebral endothelial cells and blood–CSF barrier across the choroid plexus epithelial cells. An essential component of barrier mechanisms is the presence of tight junctions between the endothelial and epithelial cells of these interfaces. This review outlines historical evidence for the presence of effective barrier mechanisms in the embryo and newborn and provides an up to date description of recent morphological, biochemical and molecular data for the functional effectiveness of these barriers. Intercellular tight junctions between cerebral endothelial cells and between choroid plexus epithelial cells are functionally effective as soon as they differentiate. Many of the influx and efflux mechanisms are not only present from early in development, but the genes for some are expressed at much higher levels in the embryo than in the adult and there is physiological evidence that these transport systems are functionally more active in the developing brain. This substantial body of evidence supporting the concept of well developed barrier mechanisms in the developing brain is contrasted with the widespread belief amongst neurotoxicologists that "the" blood–brain barrier is immature or even absent in the embryo and newborn. A proper understanding of the functional capacity of the barrier mechanisms to restrict the entry of harmful substances or administered therapeutics into the developing brain is critical. This knowledge would assist the clinical management of pregnant mothers and newborn infants and development of protocols for evaluation of risks of drugs used in pregnancy and the neonatal period prior to their introduction into clinical practice.

Contents

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1. Introduction

In pregnancy and following birth, the developing brain is vulnerable to the effects of exposure to drugs and xenobiotics via the mother (Landrigan and Goldman, 2011). It is therefore essential to have available accurate information about both protective mechanisms and potential vulnerabilities in the developing brain. This will ensure that clinical advice to patients is based on reliable evidence and relevant agencies are able to formulate regulations that are in accord with the real hazards rather than assumed ones.

In the adult, the brain is protected from many drugs and xenobiotics by a series of mechanisms described by the term "blood–brain barrier". In the embryo and fetus an important degree of protection is provided by the placenta, with potential additional protection provided by blood–brain barrier mechanisms in the developing brain. However, there is a widespread belief, particularly amongst neurotoxicologists, going back nearly 100 years that "the" blood–brain barrier is "immature" or even absent in the embryo, fetus and newborn. As it will be discussed below, this belief is promulgated in many reviews and textbooks (e.g. Costa et al., 2010; Ahmed et al., 2005) particularly in the journals *Neurotoxicology* (Table 1) and *Environmental Health Perspectives* (Table 2) as well as in official government sources such as the CDC in the US (ATSDR, 2011; see Tables 3 and 4). This belief is not supported by most evidence going back almost as far as when the original concept of the blood–brain barrier was suggested more than 100 years ago (Lewandowsky, 1900).

The permeability of the brain barrier interfaces during development has been inferred from measurements of the concentrations of marker compounds in the brain or CSF in relation to their concentrations in plasma (i.e. CSF/plasma and brain/plasma concentration ratios). However, these ratios only measure apparent permeability, not the actual permeability of the barrier interfaces. This is because the brain or CSF concentration of a compound is a function not only of its rate of influx, but also its rate of efflux and any developmental changes in the volume of the compartment that it is distributing in. Changes in any of these factors directly affect concentration ratios and thus the apparent permeability (Johansson et al., 2008). Unfortunately, apparent permeability changes have often been confused in the literature as only reflecting changes in influx and thus interpreted as changes in

Table 1
Statements of blood–brain barrier incompleteness or immaturity in papers published in Neurotoxicology, with comments regarding errors of cited evidence.

Authors (year)	Quotation and comments
Zeng et al. (2011)	"*This status cannot be excluded, because the blood–brain barrier in embryonal and neonatal rats is quite immature and porous, and is not completed until PND24* (Chang et al., 2009)". Chang et al. (2009) cites Kniesel et al. (1996) which was an EM freeze fracture study without permeability data.
Costa et al. (2010)	"*. . . incomplete development of the blood–brain barrier* (Mayer, 2000)" Mayer cites 3 references (Al-Sarraf et al., 1997; Habgood et al., 1998; Muramatsu et al., 1997) none of which contains evidence for incomplete blood–brain development.
Bal-Price et al. (2010)	"*Since even after the birth BBB is not entirely differentiated (until about 6 months after birth).*" No evidence cited.
Maia et al. (2009)	"*. . . the developing CNS goes through a critical period for the formation of the basic circuitry of the nervous system, while the adult CNS already has a developed blood–brain barrier to give much more protection* (Costa et al., 2004; Rodier, 1995)." Costa et al. (2004) cite Rodier (1995) who cites Adinolfi (1985), see text.
Boucher et al. (2009)	"*. . . weaker efficacy of the blood–brain barrier,*" No evidence cited.
Myers et al. (2009)	"*The blood brain barrier does not completely form until after birth* (Rodier, 2004)." Rodier (2004) cites Adinolfi (1985), see text.
Soldin and Aschner (2007)	"*. . . an incompletely formed blood–brain barrier, . . .*" No evidence cited.
Ahmed et al. (2005)	"*. . . immaturity of the blood–brain barrier* (Risau and Wolburg, 1990)." Risau and Wolburg (1990) injected intracardially excessive volumes of horseradish peroxidase solution (see Saunders, 1992)
Thiruchelvam et al. (2002)	"*The development of the BBB proceeds from late gestation and continues through the PN period and may be a time of increased permeability. Thus, exposure to an environmental toxicant during this period could be associated with increased uptake and also disruption of the normal development and maturation of this crucial barrier* (Kniesel et al., 1996; Rodier, 1994, 1995; Saunders et al., 1991). *An incomplete or immature BBB in the developing central nervous system could result in greater uptake of neurotoxic compounds into the central nervous system.*" Kniesel et al. (1996) was a freeze fracture study without permeability data. Rodier (1995) cited Adinolfi (1985). Saunders et al. (1991) not Saunders and Møllgård as cited, provided evidence of a well functioning blood–brain barrier in the embryo and fetus.