TITLE: PERMEABILITY AND VASCULARITY OF THE DEVELOPING BRAIN: CEREBELLUM VS CEREBRAL CORTEX

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INTRODUCTION: The stability of the internal environment of the brain, made possible by the blood-brain barrier (BBB), is critical for the normal functioning of the central nervous system (CNS). Various characteristics of the BBB in adult organisms have been extensively investigated; however, considerably less information is available on immature barrier systems. Because of potentially deleterious effects of pharmacological agents on the developing brain, it is important to evaluate factors which affect the rate and extent to which drugs gain access to nervous tissue at various stages of development. In the present ontogenetic study the short-term uptake of mannitol by the cerebellum (CER) and cerebral cortex (CC) was compared to that of inulin. The data provide insight on the time course of development of BBB permeability in different regions of the CNS.

METHODS: To evaluate permeability, the uptake by brain of ^{3}H-mannitol (or ^{3}H-inulin) was investigated in Sprague-Dawley rats 0.5, 1, 2, 3 and > 5 weeks of postnatal age. Each animal was etherized and nephrectomized (to prevent excretion of isotope), injected with a tracer (0.5 μc/.01 ml saline/g), and killed 1 hour later by exsanguination. To evaluate vascularity, the steady-state distribution of ^{51}Cr-tagged erythrocytes (RBC's) in various tissues was analyzed; ^{51}Cr-RBC's were injected into the inferior vena cava (3 μc/g) and allowed to mix with the circulating blood for 10 minutes. Overall, 103 animals were used in the investigation: mannitol experiments (n=40); inulin (n=35); ^{51}Cr-RBC's (n=28). Radioisotope distribution in all tissues was expressed as a space (%) = 100 x (dpm/g wet tissue ÷ dpm/g plasma H_2O [or g RBC's]). Several non-neural tissues were also analyzed.

RESULTS: As the CNS undergoes maturation, the greatest decrease in the 1 hour radiomannitol space in the CER takes place during the 2nd postnatal week (Table 1) whereas the greatest reduction in the CC occurs earlier (0.5-1 week), $p < 0.05$, multiple range test. At 3 weeks the permeability of the BBB in the CER to mannitol is comparable to that in the CC.

TABLE 1: One Hour ^{3}H-Mannitol Spaces in the CNS

Tissue	0.5	1	2	3	>5 wks
Cerebral cortex	6.4	5.5	5.0	4.6	3.9
Cerebellum	9.5	8.8	5.5	4.5	3.8

(Each value is a mean for 8 rats. In all tables standard errors are generally < 6% of means.)

Interpretation of tracer distribution is facilitated by the availability of data for vascularity. The residual erythrocyte-^{51}Cr space, which provides an estimate of the degree of patency of the vasculature in the developing brain, is greater in the CER than in the CC at each age studied (Table 2), $p < 0.05$, paired t-test. Moreover, the more-or-less progressive increase in this parameter with age indicates that the density of patent vessels in both CER and CC increases in postnatal life; thus it seems likely that a hemodynamic factor can be ruled out as the explanation for the continual decline (with advancing age) in the uptake of mannitol and inulin.

TABLE 2: ^{51}Cr-RBC Spaces in Brain Tissue

Tissue	1	2	3	>5 wks
Cerebral cortex	0.17	0.20	0.55	0.47
Cerebellum	0.39	0.34	0.60	0.55

n = 7 for each mean value

In Table 3 are values for the ratio of the 1 hour radioisotope spaces (mannitol:inulin), corrected for residual blood, in the CER as well as the CC of rats of various ages. (These values are calculated from hematocrit data and the distribution spaces for ^{51}Cr-RBC's, ^{3}H-mannitol and ^{3}H-inulin.) An analysis of the ratio values provides information on the relative restriction to the diffusion of tracers out of the cerebral capillaries into brain tissue. In both CER and CC the ratios tend to increase with age.

TABLE 3: Ratio of ^{3}H-Mannitol to ^{3}H-Inulin Space

Tissue	0.5	1	2	3	>5 wks
Cerebral cortex	2.5	2.7	2.8	8	5
Cerebellum	2.9	2.8	3.1	10	5

The BBB is relatively leaky in the CER and CC of the neonatal rat; however, permeability is a relative phenomenon, and there is a barrier effect in the brain even in the youngest animals studied; for example, during the 1st postnatal week the magnitude of the space in the CER into which mannitol penetrates is only a 1/7, 1/4 and 1/3 of that into which this tracer permeates in the liver, lungs and heart, respectively.

TABLE 4: One Hour ^{3}H-Mannitol Spaces Outside CNS

Tissue	0.5	1	2	3	> 5 wks
Heart	24.6	24.6	25.4	22.8	20.1
Liver	66.3	67.8	68.2	66.6	67.6
Lungs	36.0	32.7	35.2	36.0	38.2

n = 8 for each mean value

DISCUSSION: The uptake of mannitol by the postnatal rat brain represents a convenient model for studying the penetration of hydrophilic agents across barrier systems in the immature mammalian CNS. The present study provides evidence for regional differences in the temporal development of the blood-brain barrier; thus, during development the cerebellum (compared to the cerebral cortex) may remain vulnerable to potentially harmful agents in the blood for a longer period of time as the CNS undergoes maturation. However, the time course of vascularization in the cerebellum runs more-or-less parallel to that in the cerebral cortex. Analysis of the uptake ratios for the tracers indicates that there is a continual tightening of the blood-brain barrier, even during the relatively late period 2-3 weeks after birth. A comparison of tracer penetration into non-neural and neural tissues indicates that even in the immature brain there is a limited barrier effect; the functional significance of this limited barrier effect, as well as the time course of development of the blood-brain barrier in other regions of the CNS, are problems that deserve additional investigation.

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