Biochimica et Biophysica Acta, 448 (1976) 633—637

BBA Report

BBA 71273

CHANGES DURING DEVELOPMENT IN TRANSPORT PROCESSES OF THE BLOOD-BRAIN BARRIER

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(Received August 24th, 1976)

Summary

The permeability of the blood-brain barrier to several classes of compounds was studied in rats between the ages of 15 days and 9 weeks. ^{14}C-labelled test substances were injected simultaneously with two reference isotopes, $^{3}H_2O$ and ^{113m}In-labelled EDTA, into the common carotid artery followed by decapitation 10 s later.

There was evidence that a monocarboxylic acid transport system in 15 to 23 day-old rats had a capacity at least six times greater than that present in adult animals. L-Lactate and acetate showed the highest permeability. At all ages there was a constant ratio between L-lactate and (—)D-3-hyroxybutyrate values. D-Glucose permeability increased with age, while that of several amino acids tested was the same in young and adult rats.

In recent years it has become well established that, in addition to glucose, the ketone-bodies acetoacetate and 3-hydroxybutyrate are quantitatively important as oxidizeable substrates for the brain. This is particularly so during infancy and early childhood [1,2] as well as in adulthood following prolonged starvation [3]. Both Persson et al .[1] and Krauss et al. [2] showed by arterio-venous difference measurements across the brains of young children that following relatively short periods of fasting, 6 to 12 h, the blood ketone body concentrations increased and there was a proportional net uptake of ketone-bodies by the brain. A further, interesting observation was that, although glucose also entered the brain, about 20 to 30% of it was returned to the blood as lactate [2]. The remainder

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of the glucose removed, together with the ketone-bodies taken up would account for the total oxygen consumed if combustion of these substrates were complete. Very similar findings have been obtained in studies on suckling rats [4,5]. The net efflux of lactate consistently observed to accompany a net influx of 3-hydroxybutyrate and acetoacetate suggested to us the possibility of a linked counter-transport system across the blood-brain barrier which, if it existed, might well be more effective in the young.

In adult rats it has been shown that glucose [6—8], lactate [9], 3-hydroxybutyrate and acetoacetate [10] are each transported between the blood and the brain by mediated diffusion processes that conform to simple Michaelis-Menten kinetics. One of the techniques used to study the properties of these "carriers" has been that described originally by Oldendorf [11]. Essentially, the clearance by the brain of a ^{14}C-labelled substance is determined relative to that of ^{3}HOH following a single, rapid injection of the isotopes into the common carotid artery. (Details of recent modifications are given in the legend to Fig. 1). One of the assumptions of the procedure, discussed previously [9,11], is that the injection is sufficiently rapid for the composition of the injected bolus to remain virtually unchanged by dilution with plasma.

To investigate possible changes in the transport processes during development we applied the technique to rats between the ages of 15 days and 9 weeks. (In animals younger than about 15 days we found the carotid artery too small to allow a satisfactory injection.) While this study was being completed Moore et al. [12] published their findings on developmental and dietary modulations of 3-hydroxybutyrate transport in rat brain. Although the method they used for measuring transport was indirect it provided evidence that the permeability of 3-hydroxybutyrate rose several-fold throughout suckling and then declined after weaning. The findings of the present report, using a more direct technique for transport measurement, both confirm and extend those of Moore et al. [12].

Results have been expressed as a brain uptake index, the definition of which is given in the legend to Fig. 1. Changes with age in the brain uptake index for L-lactate, D-lactate, (—)D-3-hydroxybutyrate and glucose are shown in Figs. 1a and b. There were several distinctive findings. The values for L-lactate were very high in the younger animals then, a few days after weaning, fell steadily to values 6 times less in mature adults. Very similar age changes were found for (—)D-3-hydroxybutyrate, although the brain uptake index values were always less than those for L-lactate. For glucose the brain uptake index was lower in the younger animals than in adults, a finding in agreement with observations by Moore et al. [13]. The transport of lactate showed some degree of stereo-selectivity [9,14]. At each age of animal tested the brain uptake index for D-lactate was about one-third the value for L-lactate (Fig. 1a).

There was some evidence from preliminary experiments in young animals that the transport of lactate and glucose showed saturation kinetics as in adults (Table I). Furthermore, it could be demonstrated that the addition of high concentrations of DL-3-hydroxybutyrate lowered the uptake of L-lactate (Table I). Similarly, a high concentration of L-lactate reduced the uptake of

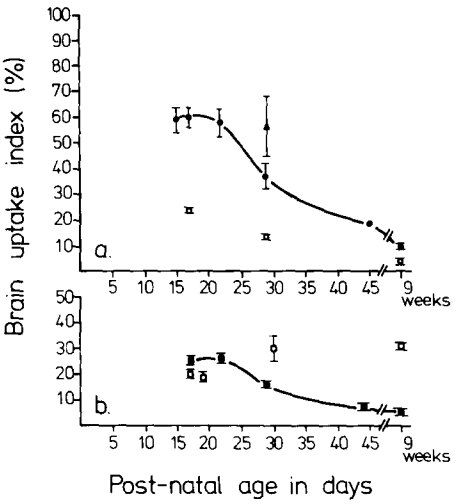

Fig. 1. The brain uptake index for several compounds in suckling and weaned rats. Values in Fig. 1a are for L-[U-^{14}C]lactate (●) and D-[U-^{14}C]lactate (○) and in Fig. 1b for (—)D-3-hydroxy[3-^{14}C]butyrate (■) and D-[2-^{14}C]glucose (□) in normal rats. The symbol, △, in Fig. 1a is for L-[U-^{14}C]lactate in rats injected subcutaneously at birth with 1 mg of cortisol acetate. The general procedure of the carotid artery injection, counting of radioactive brain samples and calculation of the brain uptake index was as described previously [15]. Rats were anaesthetized by intraperitoneal pentobarbital and the right common carotid artery exposed. In rats between the age of 15 to 35 days, a 0.1 ml bolus of Ringer solution buffered to pH 7.5 with 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethane sulphonate) containing between 0.13 to 0.25 μCi of ^{14}C-labelled test substance (specific radioactivities between 15 to 50 Ci/mol), 2 μCi of ^{3}HOH and about 50 μCi of [^{113m}In]EDTA, was injected rapidly using a 30-gauge needle. Rats were decapitated 10 s later and the right half of the brain rostral to the midbrain was removed for the determination of radioactivity content. The changes in procedure for the older animals were that the injection bolus was 0.2 ml and the needle used was of 27-gauge.

The ^{3}HOH served as the highly diffusable reference, whereas the [^{113m}In]EDTA, a non-diffusable complex, was used to correct for any of the bolus that remained in the blood compartment of the sample of brain taken for counting [15].

$$\text{The brain uptake index} \left[\frac{\text{tissue } ^{14}\text{C/tissue } ^{3}\text{H}}{\text{injectate } ^{14}\text{C/injectate } ^{3}\text{H}} \times 100 \right] - \left[\frac{\text{tissue } ^{113m}\text{In/tissue } ^{3}\text{H}}{\text{injectate } ^{113m}\text{In/injectate } ^{3}\text{H}} \times 100 \right]$$

Each value is the mean ± S.D. of the results from 3 animals.

TABLE I

COMPETITION OF THE UPTAKE OF ^{14}C-LABELLED LACTATE, 3-HYDROXYBUTYRATE AND GLUCOSE BY THE ADDITION OF UNLABELLED COMPOUNDS; AND THE EFFECT OF STARVATION ON LACTATE UPTAKE IN ADULT RATS

The general procedure was as described in Fig. 1 except that, where indicated an unlabelled compound was added to the injection bolus which contained a trace amount of a ^{14}C-labelled substance.

	Brain uptake index
18—22 day-old rats	
L-[U-^{14}C]Lactate 0.03 mM	59.6 ± 4.7 (3)
L-[U-^{14}C]Lactate + L-lactate 14 mM	34.5 ± 2.0 (3)
L-[U-^{14}C]Lactate + DL-3-hydroxybutyrate 10 mM	45.6, 43.6 (2)
(—)D-3-Hydroxy[3-^{14}C]butyrate 0.05 mM	26.1 ± 2.2 (3)
(—)D-3-Hydroxy[3-^{14}C]butyrate + L-lactate 14 mM	17.4, 18.0 (2)
D-[2-^{14}C]Glucose 0.05 mM	18.6 ± 2.2 (3)
D-[2-^{14}C]Glucose + glucose 10 mM	5.2 ± 0.3 (3)
Adult rats	
Fed, L-[U-^{14}C]lactate 0.03 mM	9.9 ± 1.4 (3)
Starved 4 days, L-[U-^{14}C]lactate 0.03 mM	14.6 ± 1.6 (4)

(−)D-3-hydroxybutyrate. These data are an indication that lactate and 3-hydroxybutyrate are transported by the same carrier process. This interpretation gained further support from the near constant ratio in the brain uptake index for lactate and 3-hydroxybutyrate over the whole age-course studied (Figs. 1a and 1b). Also, in starved adult rats, as compared with fed, we found a 1.5-fold increase in the uptake index for lactate (Table I) as has been found by others for 3-hydroxybutyrate [10]. Although the technique used here measures unidirectional uptake of the test substance from blood to brain, arterio-venous differences have shown that the net movement of the two compounds is in opposite directions across the blood-brain barrier [2,4] so that counter-transport is a possibility.

Because of the influence of hormones on normal development it was of interest to have the opportunity to test some 30 day-old rats treated at birth with cortisol. The uptake index for lactate remained high at an age when it was declining in control animals (Fig. 1a). This was suggestive of a modification of the usual changes in transport phenomena of the blood-brain barrier during development and might merit further investigation. The growth of these animals had been severely retarded giving a mean body weight of 33.5 ± 2.5 g (3) compared with 85 ± 14 g (8) for their litter-mate controls. Other changes in similarly treated rats have been described elsewhere [16,17]

Earlier studies by Oldendorf [9] using adult rats had indicated that a range of short chain monocarboxylic acids were transported by a common carrier system. This system was independent of the transport system for glucose and amino acids. In an attempt to gain more information about the specificity of the changes with age shown in Fig. 1 some other compounds were tested in rats of the younger age group.

Two rats of 17 days of age were injected with a tracer dose of [1-^{14}C]-acetate. The brain uptake index values were 68 and 73, which were very high compared with the mean adult value of 14 ± 1.9 [9].

Transport of amino acids across the blood-brain barrier in adult rats is mediated by carrier systems that have a selective affinity for either basic, neutral, or acidic amino acids [7,15]. Glycine, alanine and proline are exceptions and do not appear to be transported by a carrier mediated process. Values for the uptake index of lysine, valine and glycine in 19 to 23 day old rats are given in Table II.

TABLE II

BRAIN UPTAKE INDEX OF AMINO ACIDS IN YOUNG AND ADULT RATS

Young rats were injected with either L-[U-^{14}C]lysine, 0.012 mM; L-[U-^{14}C]valine, 0.012 mM; or [U-^{14}C]glycine, 0.06 mM; plus ^{3}HOH and [^{113m}In]EDTA as described in Fig. 1. Adult values are from reference [7] and are uncorrected for indium space.

	19—23 Day-old	Adult
Lysine	19.3 ± 2.5 (3)	16
Valine	21.6 (1)	21
Glycine	2.25 ± 0.52 (3)	2.5

Glycine uptake was extremely low (Table II), as it is in the adult [7,15]. This showed that the diffusion component for small molecular weight compounds across the blood-brain barrier was no greater than in adults. Ferguson and Woodbury [18] also found that the inulin space, which is high in the brains of rats at birth, had declined to the adult level at about 16 days of age. The brain uptake index values for both lysine and valine were the same in the young animals as in the adults (Table II). This evidence for the early maturation of the amino acid transport systems corroborates a recent report by Sershen and Lajtha [19] in which certain characteristics of the systems were shown to be present at birth.

Finally, we conclude that the data are sufficient to show that various transport systems previously described as part of the blood brain barrier in adult rats are also present in much younger animals. However, the capacities of the individual systems change with age. The most striking change is in the permeability of the blood-brain barrier to monocarboxylic acids, including lactate and 3-hydroxybutyrate. These latter two compounds may be part of a counter-transport system across brain capillary endothelial cell plasma membranes in vivo.

One of us (J.E.C.) wishes to acknowledge the support of the U.S.P.H.S. National Institute of Drug Abuse during the period in which this work was performed under an appointment as Visiting Professor in the Department of Neurology at University of California in Los Angeles. We thank Dr. Eain M. Cornford and Mrs. Stella Z. Oldedorf for their help, and Dr. A. Yuwiler for supplying the rats treated with cortisol. This research was also supported partly by the Veterans Administration.

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