Developmental changes in blood–brain barrier potassium permeability in the rat: relation to brain growth

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1. The potassium permeability of the blood–brain barrier (BBB) was determined in anaesthetized rats aged between 21 days gestation and adult using $^{86}Rb^+$ as a marker for potassium.

2. The brain influx rate constant for $^{86}Rb^+$ was high in fetal cortex at 21 days gestation ($42{\cdot}5 \pm 4{\cdot}3$ μl g^{-1} min^{-1}) but had decreased markedly by just after birth ($12{\cdot}2 \pm 0{\cdot}6$ μl g^{-1} min^{-1}). There was a further, gradual, postnatal decline to $7{\cdot}0 \pm 0{\cdot}3$ μl g^{-1} min^{-1} by 50 days after birth.

3. Developmental changes in passive BBB permeability were examined over the same age range using ^{14}[C]urea. These studies showed similar developmental changes in influx rate to those found for $^{86}Rb^+$. Specifically, a marked perinatal decline followed by a more gradual postnatal fall. Thus, the changes in potassium permeability probably reflect a decrease in the BBB paracellular leak during development.

4. The changes in BBB permeability coincide with changes in the rate of brain growth and the associated rate of brain potassium accumulation. As the potassium permeability properties of the adult BBB would provide insufficient potassium influx to meet the requirement associated with fetal brain growth, it is suggested that need for potassium may be the reason for the greater BBB permeability early in development.

In adult mammals, the blood–brain and blood–cerebrospinal fluid (CSF) barriers, situated at the cerebral capillaries and the choroid plexuses, form an impediment to the movement of solutes from blood to brain. These barriers are formed, respectively, by the capillary endothelial cells and the plexus epithelial cells. As well as acting as passive permeability barriers, both tissues have numerous transport systems (Bradbury, 1979). Such systems are necessary to circumvent the barriers to move nutrients like glucose into the brain, to remove waste products from the brain and to provide a stable brain microenvironment.

During brain development, compounds are required not only to enable function but also to support growth. It appears that this need is reflected by changes in some of the transport systems at the blood–brain barrier (BBB). For example, the transport of β-hydroxybutyrate (Cremer, Braun & Oldendorf, 1976; Moore, Lione, Sugden & Regen, 1976), lactate (Cremer *et al.* 1976; Cornford, Braun & Oldendorf, 1982) and some amino acids (Banos, Daniel & Pratt, 1978; Cornford *et al.* 1982) is enhanced in young animals. The extent to which there are also changes in passive permeability has been the subject of much controversy (Risau & Wolburg 1991; Saunders, Dziegielewska & Mollgard, 1991), though most studies have shown a decline in BBB permeability to small polar compounds with age (Johanson 1989; Tuor, Simone & Bascaramurty, 1992). Another study has also shown a marked increase in the transendothelial resistance of pial microvessels just prior to birth in the rat, indicating a tightening of the BBB (Butt, Jones & Abbott, 1990). There is, however, a scarcity of quantitative measurements of BBB permeability in the fetus.

Potassium, the major cation present in the brain, is essential for growth. The mechanism by which it crosses the adult BBB is uncertain, but it has a high permeability relative to sodium (Bradbury, 1979 (Table 8.1)) and, during hyperosmotic stress, BBB potassium permeability is markedly increased even though there is little change in BBB passive permeability (Cserr, DePasquale & Patlak, 1987). This suggests that there are specific endothelial potassium transporters and channels (Keep, Xiang & Betz, 1993*b*). The purpose of this study was to measure developmental changes in BBB potassium permeability (using rubidium as a marker for potassium) in the rat in relation to changes in brain growth. Whether these changes reflect alterations in passive permeability or movement via specific endothelial transporters and channels was examined by also measuring developmental changes in urea, a passive permeability marker. The results serve to extend our knowledge of BBB permeability back into the

rat fetus, to confirm that there is a marked change in BBB permeability around birth in the rat, and to suggest that there is a link between BBB permeability and the potassium requirement for brain growth.

An abstract of some of this work has been published (Keep, Xiang, Beer & Betz, 1993*a*).

METHODS

Brain uptake experiments

Rationale. The isotope $^{86}Rb^+$ was used to examine developmental changes in brain potassium uptake as it has a longer half-life than $^{42}K^+$. Cserr *et al.* (1987) found no differences in brain $^{42}K^+$ and $^{86}Rb^+$ uptake during control and hyperosmotic conditions in the rat. Developmental changes in $^{86}Rb^+$ uptake were compared to those for [^{14}C]urea, a compound that probably crosses the adult BBB by diffusion rather than saturable transport (Fenstermacher & Rapoport, 1984).

Analysis. Brain influx rate constants (K_1) for $^{86}Rb^+$ and [^{14}C]urea were measured using multiple-time point graphical analysis, the theory of which has been published previously (Patlak, Blasberg & Fenstermacher, 1983). Under conditions of unidirectional uptake, the concentration of tracer in the brain (C_{br}) is related to the concentration of tracer in the plasma (C_p) by the equation:

$$C_{br} = K_1 \int C_p dt + V_1 C_t, \quad (1)$$

where V_1 equals the sum of the brain vascular and rapidly filling spaces for the tracer, and C_t is the terminal plasma tracer concentration. Thus, a plot of C_{br}/C_t (the isotope space) against $(\int C_p dt)/C_t$ (the stretch time) will have a slope of K_1 and a y-intercept of V_1.

In large animals, brain exposure to isotope (the integral of the plasma curve $\int C_p dt$), would be determined for each individual animal by either taking serial blood samples or by using a continuous arterial withdrawal. The small size of the fetal and neonatal rats, however, make this an impossibility. Instead, for all ages, we used the terminal samples from individual animals killed after different circulation times to determine the overall plasma curve. Care was taken that each animal received the same dose of isotope per gram body weight. The integral of the plasma curve was then calculated by trapezoidal analysis using the mean terminal plasma isotope concentration at each time point.

A similar graphical analysis approach was used to calculate the influx rate constants for uptake into CSF and red blood cells. The concentration of isotope in the CSF (C_{csf}) and red blood cells (C_{rbc}) is used in the graphical analysis instead of C_{br}. For CSF, cisterna magna CSF samples were used and the influx rate constant reflects entry into that sample which may differ from entry into pure ventricular CSF.

Experimental method. Sprague–Dawley rats were used at 21 days gestation, and at 2, 10, 21 and 50 days after birth. The fetal rats had an average crown–rump length of 38 mm, the postnatal rats had mean weights of 7, 20, 47 and 190 g respectively. The animals were anaesthetized with sodium pentobarbitone (30–50 mg kg^{-1} I.P.) and body temperature was maintained by a heating blanket set at 37 °C in young animals or regulated to 37 °C by a rectal probe in adults.

For brain uptake experiments, postnatal animals received an intraperitoneal injection of either 0·25–1·25 μCi g^{-1} of $^{86}Rb^+$ or 0·15–0·4 μCi g^{-1} of [^{14}C]urea, the dose (per gram body weight) decreasing with age. The volume of the injectate was 30–50 μl for neonates (just 2-day-olds) and 100 μl in older rats. At six to ten time intervals, up to 20 min, pairs of animals were decapitated, a terminal (neck) blood sample was taken, and the forebrain was removed and divided into the cerebral hemispheres (hereafter called the cortex) and the remainder (hereafter called the basal ganglia). Whole blood was sampled and the plasma separated by centrifugation within 90 s. In 10- and 50-day [^{14}C]urea experiments, a CSF sample was also taken from the cisterna magna just prior to decapitation to measure CSF influx.

For fetal experiments, the dam was anaesthetized as quoted previously, a femoral vein cannulated for isotope injection and a femoral artery cannulated for monitoring blood pressure and sampling maternal blood. Individual isotopes were given intravenously to the dam either as a single bolus ([^{14}C]urea; 200 μCi animal^{-1}) or as a bolus followed by a constant infusion ($^{86}Rb^+$; 70 μCi bolus, infusion at 7 μCi min^{-1}). After the injection, consecutive fetuses were exteriorized from the uterus at intervals up to 20 min, the umbilical vessels cut and the fetus decapitated. The colour of the fetal skin and umbilical vessels was used as a gauge of the physiological state of the fetus. Fetal blood and brain samples were processed as the samples from postnatal animals.

Tissue, plasma and whole blood samples were dissolved either in protosol or methyl-benzethonium hydroxide prior to addition of scintillation cocktail. Whole blood samples were decolourized with hydrogen peroxide prior to the addition of the fluor. Dual-label counting was performed on a Beckman LS 3801 Liquid Scintillation Counter (Fullerton, CA, USA).

As $^{86}Rb^+$ is accumulated by red blood cells, accurate determination of the brain uptake of this isotope requires a correction for erythrocyte uptake. Brain red blood cell volumes were determined with ^{51}Cr-labelled red blood cells using a method described previously (Lo, Ennis, Goldstein, McNeely & Betz, 1987). Red blood cells (RBC) from donor rats of the appropriate age were incubated with ^{51}Cr (300 μCi (ml RBC)$^{-1}$) for 30 min and then washed three times and kept on ice until use.

A femoral vein was catheterized under pentobarbitone anaesthesia (30–50 mg kg^{-1} I.P.). The animals were then injected I.V. with the ^{51}Cr-labelled RBC (approximately 1 μl of cells diluted with saline per gram body weight). After 3 min, animals were decapitated, a terminal whole blood sample taken, the haematocrit measured and the brain removed. Samples were then processed and counted as described above. The brain RBC volume was calculated as $(C_{br}Hct)/C_{bl}$, where C_{br} and C_{bl} are the isotope concentrations in the brain and whole blood, respectively, and Hct is the systemic haematocrit. Because of the difficulty in cannulating the femoral vein in fetuses and neonates, these experiments were limited to animals aged 10 days and above.

Brain growth, potassium and sodium

Rats at 17, 19 and 21 days gestation (mean crown–rump lengths of 15, 23 and 36 mm respectively), at 2, 5, 10 and 30 days after birth and at adulthood were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} I.P.) and then decapitated. The brains were quickly removed and weighed. The brains were dried to constant weight at 100 °C to determine water content and dry

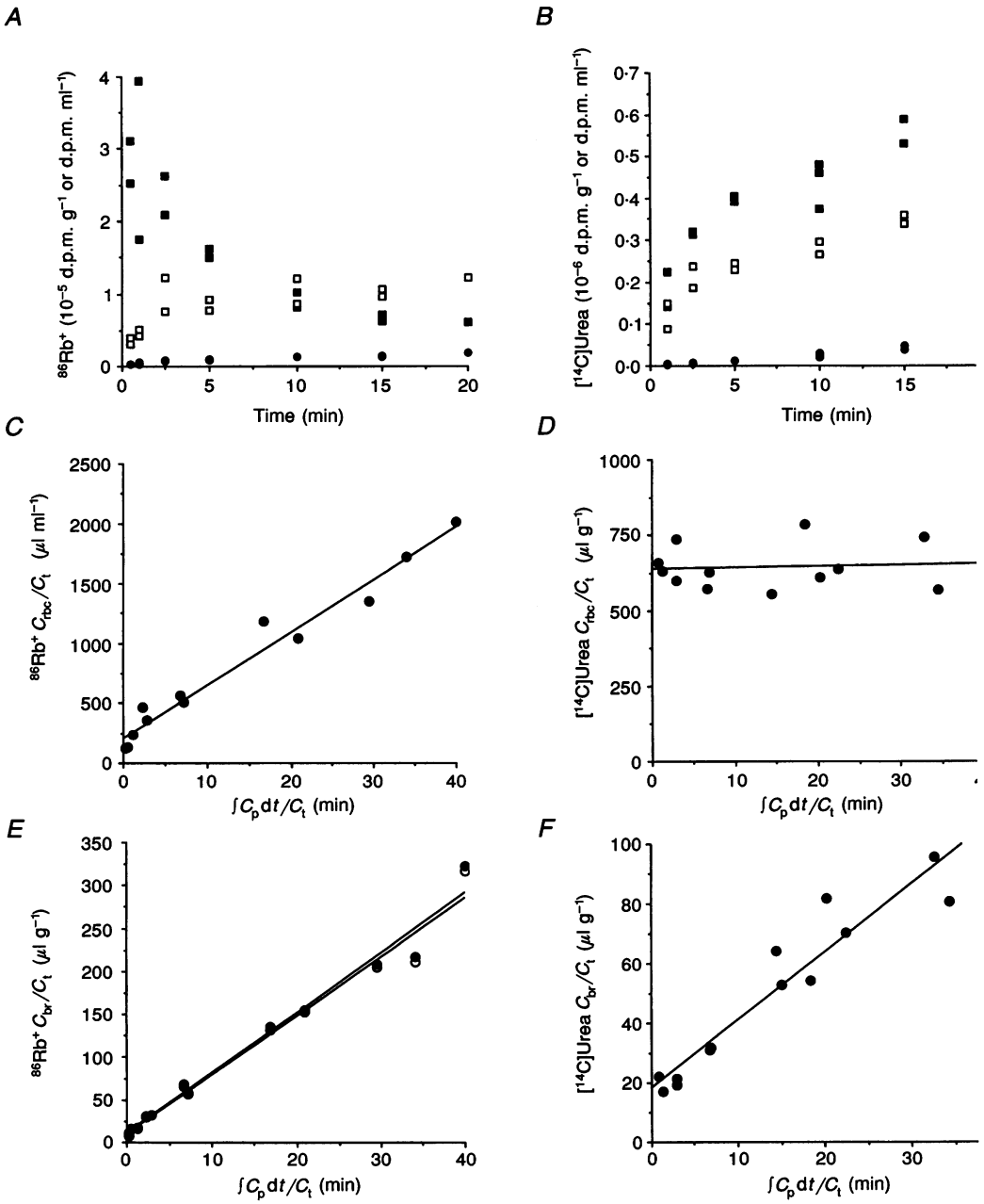

Figure 1. Analysis of rubidium and urea uptake in 50-day-old rats

Results from $^{86}Rb^+$ (*A*, *C* and *E*) and [^{14}C]urea (*B*, *D* and *F*) uptake experiments. *A* and *B*, plasma (■), red blood cell (□) and cortex (●) $^{86}Rb^+$ and [^{14}C]urea concentrations in samples taken from individual animals sampled after different circulation times. *C* and *D*, graphical analysis of the uptake of $^{86}Rb^+$ and [^{14}C]urea into red blood cells. For $^{86}Rb^+$, the plot of C_{rbc}/C_t against $\int C_p dt/C_t$ was linear and the slope equals the rate constant for red blood cell uptake (44 $\mu l\ g^{-1}\ min^{-1}$, $y = 201 + 44{\cdot}4x$). Unlike $^{86}Rb^+$, [^{14}C]urea did not steadily accumulate in the red blood cells, but there was a rapid equilibration between the red blood cells and plasma ($y = 638 \pm 0{\cdot}87x$). *E* and *F*, graphical analysis of the uptake of $^{86}Rb^+$ and [^{14}C]urea into cortex. The rubidium graphical analysis is shown with (●, $y = 10{\cdot}8 + 7{\cdot}02x$) or without (○, $y = 10{\cdot}2 + 6{\cdot}88x$) a correction for red blood cell uptake. For urea uptake in the cortex $y = 18{\cdot}2 + 4{\cdot}55x$. The influx rate constants are equal to the slopes of the regression lines.

weight. They were then ashed at 400 °C, redissolved in deionized water and the K^+ and Na^+ content determined on a IL943TM Automatic Flame Photometer (Allied Instrumentation Laboratory, Lexington, MA, USA) with CsCl as an internal standard.

Brain growth rate was estimated as the increase in brain weight per day divided by the mean brain weight for the period studied. A similar calculation was used to estimate the rate of potassium and sodium accumulation per gram of brain.

Statistical analysis

Influx rate constants were calculated by linear regression analysis. Comparison between influx rate constants were by analysis of covariance. Differences were considered significant at the $P < 0{\cdot}05$ level, two tailed. Where necessary, a Newman–Keuls correction was used for multiple comparisons (Zar, 1984). All data are presented as means ± S.E.M.

Materials

[^{14}C]Urea, $^{86}Rb^+$, and Protosol were obtained from Du Pont. All other chemicals were from Sigma.

RESULTS

Application and limitation of methodology

An example of plasma, red blood cell and brain $^{86}Rb^+$ measurements from experiments on postnatal (50 days old) animals is given in Fig. 1*A*. Intraperitoneal injections resulted in a peak in plasma [$^{86}Rb^+$] around 1 min, followed by an exponential decline. Despite the decline in plasma [$^{86}Rb^+$], both brain and red blood cell [$^{86}Rb^+$] increased with time. Graphical analysis of red blood cell uptake showed a linear relation between the $^{86}Rb^+$ space (C_{rbc}/C_t) and stretch time ($\int C_p dt/C_t$, Fig. 1*C*) indicating unidirectional uptake with an influx rate constant (K_1) of about 44 μl (ml red blood cells)$^{-1}$ min^{-1}. Uptake into cortex was also unidirectional during the time of this experiment (Fig. 1*E*). The cortical K_1, corrected for red blood cell uptake (with a measured red blood cell volume of 3·3 ± 0·6 μl g^{-1}), was 6·88 ± 0·29 μl g^{-1} min^{-1}. If no correction was used, a value of 7·02 ± 0·29 μl g^{-1} min^{-1}

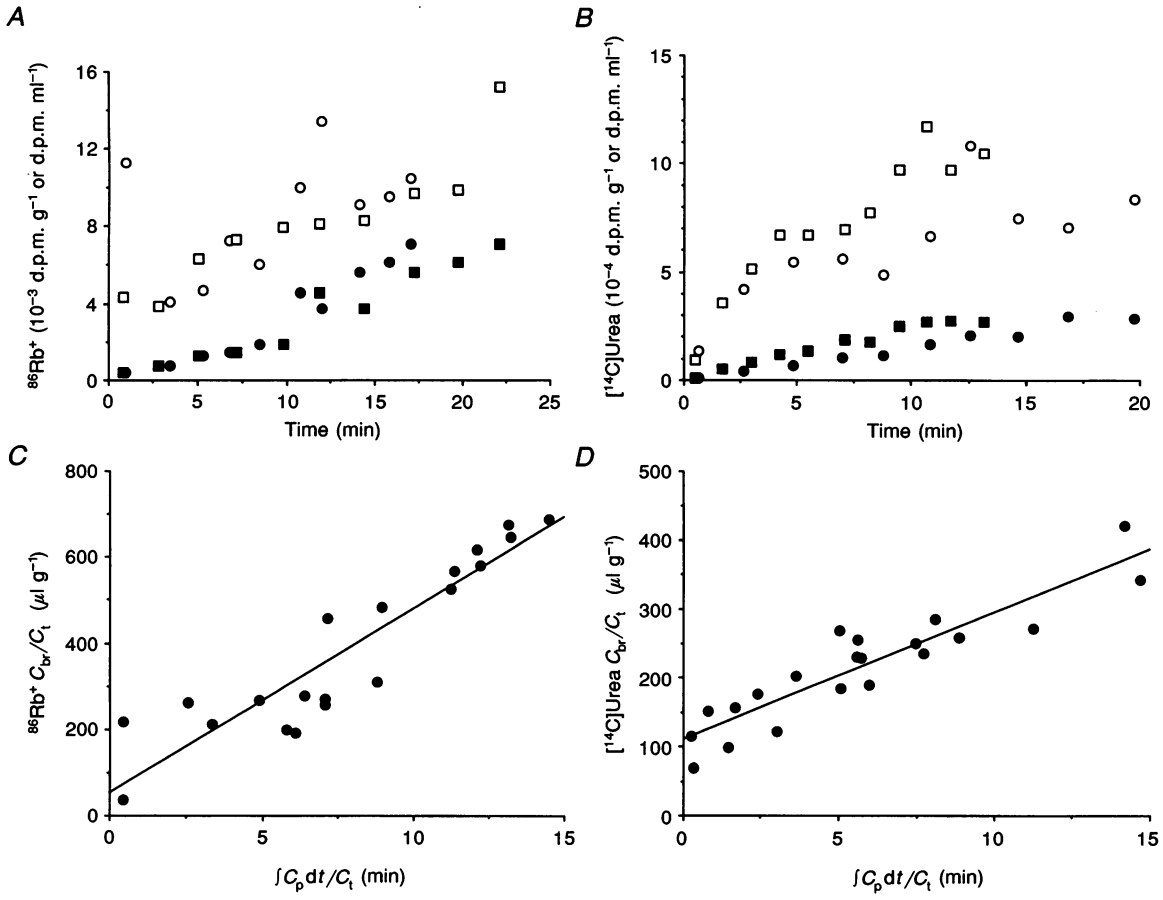

Figure 2. Analysis of rubidinium and urea uptake in fetal rats

Results from $^{86}Rb^+$ (*A* and *C*) and [^{14}C]urea (*B* and *D*) uptake experiments on 21-day-gestation fetuses. *A* and *B*, plasma (open symbols) and cortex (filled symbols) $^{86}Rb^+$ and [^{14}C]urea concentrations in samples taken from individual animals sampled after different circulation times. For each isotope, experiments on two different dams are shown (indicated by squares and circles). *C* and *D*, graphical analysis of the uptake of $^{86}Rb^+$ and [^{14}C]urea into cortex. The influx rate constants are equal to the slopes of the regression lines (*C*, $y = 53{\cdot}7 + 42{\cdot}5x$; *D*, $y = 110{\cdot}7 + 18{\cdot}4x$).

was obtained, i.e. an error of 2·0%. As vascularity increases (Bär, 1980; Keep & Jones, 1990) and permeability decreases (see below) with age, the error in K_1 due to a lack of a red blood cell correction will be even smaller in younger animals. In 20- and 10-day-old rats, where brain red blood cell volumes could be measured, the error had decreased to 1·7 and 1·2% respectively. The results on $^{86}Rb^+$ in the rest of the paper are presented without a red blood cell correction to facilitate comparisons with fetal and neonatal rats where such corrections were not feasible.

Two $^{86}Rb^+$ experiments using 21-day-gestation dams are shown in Fig. 2. Despite the high dose of $^{86}Rb^+$ given to the dams (approximately triple the dose per gram given to the 50-day-old animals shown in Fig. 1), the fetal plasma $^{86}Rb^+$ concentrations are approximately one to two orders of magnitude less than those found in the experiment on the 50-day-old animals. Presumably, this reflects the placental barrier and a high rate of tissue uptake by the fetus. Initial experiments showed that a single bolus injection of $^{86}Rb^+$ into the dam resulted in only a transient appearance of $^{86}Rb^+$ in the fetal blood. The experiments shown, therefore, are the result of bolus injections followed by a slow infusion. This results in an initial peak in plasma $^{86}Rb^+$ followed by a gradual rise. The cortical [$^{86}Rb^+$] of serially sampled fetuses gradually increases with time (Fig. 2*A*). Graphical analysis shows unidirectional uptake with a K_1 of 42·5 ± 4·3 μl g^{-1} min^{-1} (Fig. 2*C*).

In contrast to $^{86}Rb^+$, intraperitoneal injections of [^{14}C]urea into postnatal rats resulted in a gradual increase in plasma isotope concentration to a plateau level after about 5 min (e.g. Fig. 1*B*). Although there was some variation in plasma isotope concentration between animals sampled at the same circulation time, this variation was generally small.

With urea, there was significant red blood cell uptake (Fig. 1*D*). Expressed as a percentage of the plasma [^{14}C]urea concentration, however, the red blood cell concentration did not vary with circulation time and the effect of the presence of red blood cells in the cerebral circulation was to alter the y-intercept of the graphical analysis and not the transfer constant (K_1) determined from the slope. The amount of isotope present in the cerebral red blood cells is also relatively small compared to the total amount present in the brain. For example, in the experiments shown in Fig. 1*B* on 50-day-old rats, after 20 min of isotope circulation, the total cortical isotope concentration was approximately $4{\cdot}5 \times 10^4$ d.p.m. g^{-1} of which only 1×10^3 d.p.m. g^{-1} (i.e. 2%) would be due to the [^{14}C]urea present in the red blood cells (red blood cell volume equals 3·3 μl g^{-1}). The uptake of urea into the brain appeared unidirectional (i.e. the graphical analysis was linear; Fig. 1*F*), with a K_1 of 4·5 ± 0·5 μl g^{-1} min^{-1}.

Fetal urea experiments, with a single bolus injection into the dam, resulted in fetal plasma and brain isotope concentrations that increased with circulation time (Fig. 2*B*). The graphical analysis appeared linear (Fig. 2*D*) indicating unidirectional uptake with a K_1 of 18·4 ± 1·9 μl g^{-1} min^{-1}. However, there was a large y-intercept and the volume of distribution reached at the longest circulation times was approximately 40% of that for urea in the adult brain. Both of these facts suggest there was some efflux which may have led to an underestimate of the true K_1.

Brain uptake rates during development

The influx rate constant for $^{86}Rb^+$ showed a very marked (~68%) decline between 21 days gestation and 2 days after birth. ($P < 0{\cdot}001$, Fig. 3*A* and Table 1). There was a further, significant ($P < 0{\cdot}001$) decline between 2 and 50 days after birth. This decline, however, was more gradual than the perinatal change. At all ages the cortical K_1 was significantly ($P < 0{\cdot}05$) higher than that found in the basal ganglia.

Qualitatively, the influx rate constants for the passive permeability marker, [^{14}C]urea, showed a similar developmental pattern to that found for $^{86}Rb^+$ (Fig. 3*B* and Table 1). There was an abrupt reduction in K_1 ($P < 0{\cdot}001$) between 21 days gestation and 2 days after birth followed by a further postnatal decline between 2 and 50 days after birth.

Table 1. Developmental changes in the influx rate constants (K_1) for $^{86}Rb^+$ and [^{14}C]urea

	$^{86}Rb^+$ K_1 (μl g^{-1} min^{-1})			[^{14}C]Urea K_1 (μl g^{-1} min^{-1})	
Age	Cortex	Basal ganglia	Red blood cell	Cortex	Basal ganglia
21 days gestation	42·5 ± 4·3	29·5 ± 3·8	—	18·4 ± 1·9	14·5 ± 1·5
2 days	12·2 ± 0·6	10·0 ± 0·7	55·7 ± 3·9	8·1 ± 0·5	5·9 ± 0·4
10 days	10·7 ± 0·7	8·4 ± 0·6	48·8 ± 9·7	6·8 ± 0·5	5·9 ± 0·3
21 days	9·3 ± 0·7	6·0 ± 0·7	55·5 ± 6·4	6·6 ± 0·3	6·1 ± 0·3
50 days	7·0 ± 0·3	4·3 ± 0·2	44·4 ± 2·4	4·5 ± 0·4	3·8 ± 0·4

Values were determined from the slope of graphical analyses and are given as means ± S.E.M.; $n = 10$–30. No correction for red blood cell uptake has been used in the $^{86}Rb^+$ brain uptake calculations. See text for statistical comparisons.

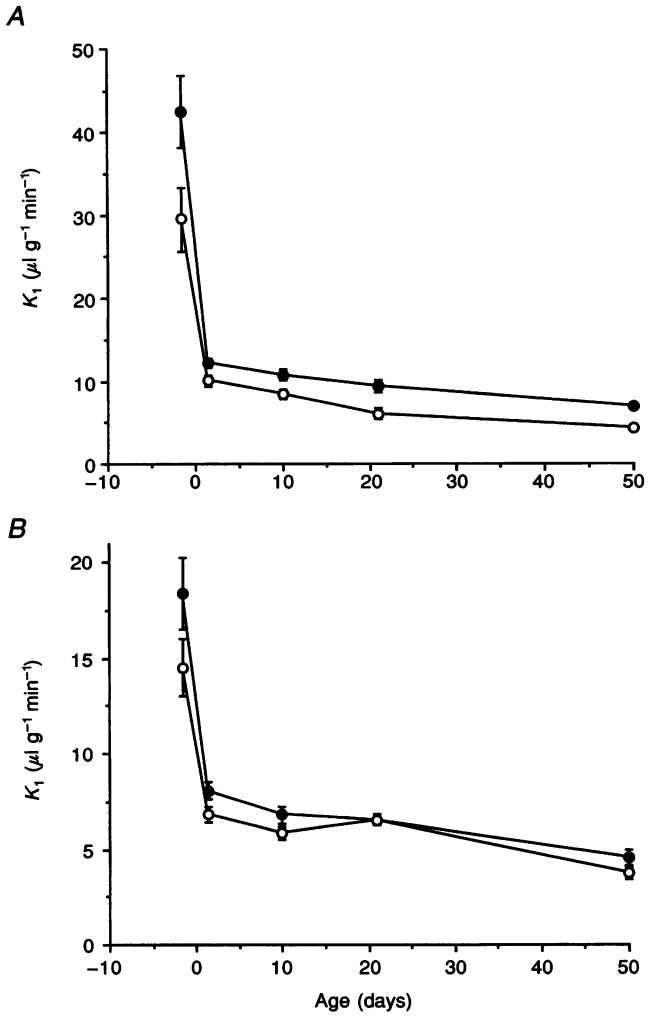

Figure 3. Brain influx rate constants for rubidium and urea during development

Developmental changes in the influx rate constants (K_1) for $^{86}Rb^+$ (*A*) and [^{14}C]urea (*B*) in the rat cortex (●) and basal ganglia (○). All values were calculated by graphical analysis and are shown as means ± S.E.M.; $n = 10$–30.

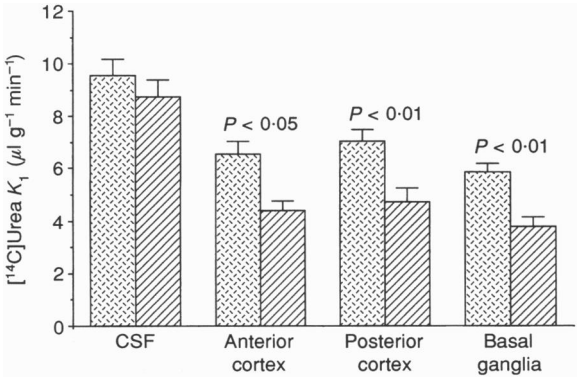

Figure 4. Regional [^{14}C]urea influx rate constants during development

Influx rate constants (K_1) for [^{14}C]urea in different brain regions and CSF in 10 (▨)- and 50 (▨)-day-old rats. All values were calculated by graphical analysis and are shown as means ± S.E.M.; $n = 8$–26. Statistical differences are by analysis of covariance.

Except for the prenatal experiments (see previous section), the y-intercepts from the [^{14}C]urea experiments were close to expected cerebral vascular volumes ([^{14}C]urea, 6–19 μl g^{-1}). The y-intercepts for $^{86}Rb^{+}$ were generally higher than vascular volume (8–46 μl g^{-1}). The origin of this rapidly filling space is unknown.

The developmental changes in brain influx rate constants might reflect changes in blood–brain or blood–CSF barrier permeability. This latter possibility was investigated in [^{14}C]urea experiments in 10- and 50-day-old rats. At these ages, cisternal CSF was sampled and the brain was divided into anterior and posterior cortex, as well as basal ganglia. The results (Fig. 4) show that there was no significant decline in CSF urea K_1 between the two ages, but that all three brain regions, including the anterior cortex where CSF influence is probably least (Smith & Rapoport, 1986), showed a similar, significant ($P < 0{\cdot}05$ or $P < 0{\cdot}01$), percentage decline between 10 and 50 days (34–37%).

In contrast to the postnatal decline in rubidium K_1 found in both cortex and basal ganglia (43 and 57% between 2 and 50 days for the respective brain regions), there was no significant change in the red blood cell influx rate constant for this ion (Table 1).

Brain growth, potassium and sodium

Figure 5*A* shows the changes that occur in brain weight and brain total potassium and sodium during the developmental period studied. These data were used to calculate the fractional increase in brain weight per day and the increase in brain potassium or sodium per gram wet

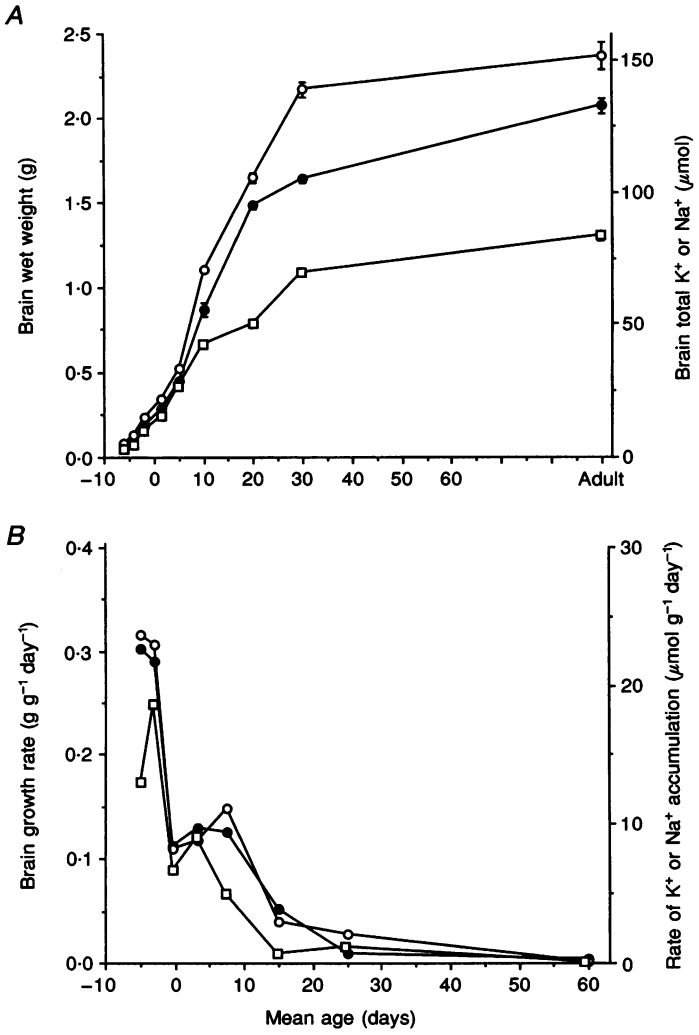

Figure 5. Brain growth and ion content changes in development

A, changes in brain weight (●) and in brain total potassium (○) and sodium (□) contents during development in the rat. All values are means ± S.E.M., $n = 5$–24. *B*, rates of brain growth (●) and potassium (○) and sodium (□) accumulation during rat development. Graph derived from data presented in Fig. 5*A*.

weight per day (Fig. 5*B*). Qualitatively, the developmental changes in these parameters are similar to those found in the influx rate constants for rubidium and urea (Fig. 3). There is a steep decline between the late gestation fetus and the neonate, followed by a further, more gradual, postnatal reduction.

DISCUSSION

Methodological considerations

Measurements of brain influx rate constants (K_1) in small mammalian fetuses and neonates are hindered by technical problems in directly determining the brain exposure to isotope and the brain blood volume correction. In this paper, we have used a composite plasma curve and graphical analysis to circumvent these problems. This method does not account for red blood cell uptake, as found with $^{86}Rb^+$, but the error involved in measuring K_1 is small ($< 2\%$).

The method gave 50-day-old cortical K_1 values that are similar to those reported in adult rats using individual blood curves (Takasato, Rapoport & Smith, 1984; Cserr *et al.* 1987). The small, gradual, postnatal decline in influx rate constant is similar to that found in a number of studies of small hydrophilic compounds (Johanson, 1989) including $^{42}K^+$ (Katzman & Liederman, 1953) and [^{14}C]urea (Parandoosh & Johanson, 1982). A postnatal increase in the transendothelial resistance of pial microvessels, however, was not found by Butt *et al.* (1990). This may reflect some vessel trauma in their studies due to increased difficulty in removing the arachnoid membrane with age. This suggestion is supported by the finding that an H_2 histamine antagonist can increase the resistance of postnatal pial vessels (Butt & Jones, 1992).

In this study, we have extended isotopic influx measurements of $^{86}Rb^+$ and [^{14}C]urea back into the rat fetus. The physiological state of the fetal rat was only gauged by visual inspection of the fetuses and umbilical vessels prior to sampling and there is the possibility that progressive sampling of fetuses might affect the blood supply and BBB function of the remaining fetuses. However, inspection of the brain and blood data presented in Figs 1 and 2 show no obvious discontinuity as more fetuses are sampled. Also, Butt *et al.* (1990) have measured the transendothelial resistance of pial microvessels in fetal and neonatal rats. They found a marked (4-fold) increase in resistance just prior to birth indicating a tightening of the BBB. The results presented in this paper confirm that study using a different technique and using fetuses sampled periodically from the uterus rather than exteriorized for prolonged periods of time.

Developmental changes in brain uptake

Mechanism

The results presented indicate that during development there is a marked perinatal decrease in influx rate constant for both of the solutes examined. This is followed by a smaller, gradual, postnatal decrease. The postnatal changes in brain influx rate constants appear to reflect changes at the BBB since for [^{14}C]urea there was no decline in the CSF influx rate constant between 10 and 50 days, but there was a decrease for each of the brain regions examined. Even with $^{22}Na^+$, where CSF entry is a large proportion of total brain entry, a large increase in the CSF influx rate constant between 14 and 35 days is not reflected in the brain (Smith, Woodbury & Johanson, 1982). The perinatal change in brain influx rate constant also appears to be due to a change in blood–brain rather than blood–CSF barrier permeability as Butt *et al.* (1990) showed a marked increase in the transendothelial resistance in pial microvessels at a similar time (although there could be a simultaneous change in the blood–CSF barrier).

With compounds of low permeability, such as rubidium and urea, influx rate constants approximate the permeability surface (*PS*) products for the tissue, as blood flow does not limit uptake (Fenstermacher, Blasberg & Patlak, 1981). Between 10 and 20 days after birth, there is a marked (3-fold) increase in brain vascularization in the rat (Bär 1980; Keep & Jones 1990). Thus, the postnatal decrease in K_1 (*PS*) actually reflects a greater decline in the permeability (*P*) of the BBB. For example, between 2 and 50 days the *PS* for $^{86}Rb^+$ declines from 12·2 to 7·0 μl g^{-1} min^{-1} (43% decrease), but the surface area (*S*) per gram increases from 4·8 to 11·2 mm^2 mm^{-3}, indicating a decline in permeability from $4{\cdot}2 \times 10^{-6}$ to $1{\cdot}0 \times 10^{-6}$ cm s^{-1} (76% decrease).

In the adult brain, the two compounds studied probably have different entry mechanisms. Urea appears to cross the BBB by diffusion rather than saturable transport (Fenstermacher & Rapoport, 1984) whereas rubidium and potassium probably cross the BBB by specific transporters and channels (Keep *et al.* 1993*b*). However, developmentally $^{86}Rb^+$ and [^{14}C]urea show qualitatively similar changes in influx rate constant suggesting a common mechanism for these changes, an alteration in the paracellular pathway. Structural changes in the tight junctions that link the BBB endothelial cells and limit movement through the paracellular pathway have been described during development (Stewart & Hayakawa, 1987; Schulze & Firth, 1992).

Implications

The increased permeability of the BBB in the fetal rat may explain the poor regulation of brain interstitial fluid [K^+] and [Ca^{2+}] at that age (Jones & Keep, 1987, 1988). Whether the improvement in regulation around birth also coincides with a maturation in active ion transport mechanisms is unknown.

The developmental changes in BBB permeability may not only affect the movement of solute from blood to brain but also bulk water flow. In the adult, interstitial fluid formation is probably driven by the asymmetrical

distribution of Na^+–K^+-ATPase at the cerebral endothelial cells (Betz, Firth & Goldstein, 1980), as the low hydraulic conductivity of the BBB (Fenstermacher & Johnson, 1966) is such that capillary hydrostatic pressure is probably an insufficient driving force. However, a leakier BBB in the fetus may result in greater flow due to hydrostatic pressure. Estimates of brain fluid secretion rate, from measurements of CSF pressure and resistance to drainage, show a dip in secretion around birth (Jones, Deane & Bucknall, 1987). This may indicate a hiatus between hydrostatically driven fluid formation in the fetus and a postnatal increase in Na^+–K^+-ATPase-driven CSF and brain interstitial fluid secretion. Evans, Reynolds, Reynolds, Saunders & Segal (1974) also found that CSF secretion rate (in $\mu l\ g^{-1}\ min^{-1}$) was high in 60-day-gestation lambs, a time when the BBB permeability is increased.

As well as increasing the supply of nutrients to the brain, increased BBB permeability may also have the effect of allowing entry of drugs and toxins into the fetal brain. It should be noted, however, that only small polar solutes were examined in these experiments and the BBB permeability to larger compounds remains to be elucidated. The observed changes could reflect either large focal or small general changes in permeability.

Potassium, BBB permeability and brain growth

Developmental changes in BBB permeability might be related to factors internal to the brain, such as growth rate or developmental stage, or external factors such as birth. Determining the importance of these different factors requires information on BBB permeability from different species, information that is, unfortunately, scarce. Evans *et al.* (1974), however, measured a brain sucrose space after 90 min circulation in the fetal lamb. They found a marked increase in the space, presumably reflecting an increased K_1, in fetuses prior to 70 days gestation (term is approximately 145 days) but not at birth. At 50 days gestation, the sucrose space is approximately four times that of a week-old lamb. At that age the fetal lamb brain is growing at about 8% per day (calculated from data in Richardson, Herbert & Terlecki, 1976), similar to the growth rate in the neonatal rat that also shows a 2- to 4-fold increase in permeability to urea. The similarity of these results suggests that the changes in BBB permeability are linked to brain growth rate.

The possible temporal link between changes in brain growth rate and changes in BBB passive permeability suggests that the permeability of proliferating blood vessels is greater. This is the case in vessels that proliferate in certain tumors (e.g. Brem, Zagzag, Tsanaclis, Gately, Elkouby & Brien, 1990). Whether the permeability is altered to increase nutrient supply to the brain is uncertain. However, an element necessary for brain growth is potassium, the major cation in the brain, and it is possible that developmental changes in BBB permeability may reflect the need for this ion. The adult BBB has a potassium unidirectional influx of about 50 $\mu mol\ g^{-1}\ day^{-1}$ (assuming a plasma K^+ concentration of 4·7 mM measured in the brain growth experiments and a K_1 of 7 $\mu l\ g^{-1}\ min^{-1}$). As the capillary surface area in the 21-day-gestation fetus is about one-third that of the adult (Keep & Jones, 1990), a potassium influx of about 17 $\mu mol\ g^{-1}\ day^{-1}$ would be expected at this age if there were no alterations in BBB function. However, in the fetal rat the net accumulation of potassium is about 23 $\mu mol\ g^{-1}\ day^{-1}$ suggesting that a BBB with the permeability of the adult could not provide the potassium needed for growth, even assuming that there was no potassium efflux from the fetal brain.

In the 21-day-gestation fetus, the potassium concentration of brain interstitial fluid is about 85% of that in plasma (Jones & Keep, 1987). If, at that age, the unidirectional influx and efflux of potassium are determined by diffusion and thus potassium concentration, influx would only exceed efflux by about 15%. Thus, to accumulate 23 $\mu mol\ g^{-1}\ day^{-1}$, the BBB influx would have to be about 150 $\mu mol\ g^{-1}\ day^{-1}$, or 3 times the adult influx rate constant (and 9 times the adult permeability assuming the surface area is one-third of the adult). Using $^{86}Rb^+$ as a marker for potassium we found a 6-fold increase in influx rate constant between adult and fetus (an 18-fold increase in permeability).

If changes in influx rate constants are linked to the rate of brain growth and the requirement for potassium, it should be possible to estimate when developmental changes will occur in the influx rate constants at the human BBB. Available evidence extending back to the 10-week fetus (Rao & Sarma, 1976) would suggest a growth rate at that age of about 4% per day. This rate occurs at about postnatal day 20 in the rat and fetal day 100 in the sheep (calculated from Richardson *et al.* 1976). In both species, the BBB influx rate constants are close to adult by that stage (above and Evans *et al.* 1974) suggesting that the human BBB would only show substantial increases in influx rate constants to small polar molecules in the embryo (< 10 weeks).

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