Cation permeability of the blood-brain barrier in streptozotocin-diabetic rats

J. Jakobsen, G. M. Knudsen and M. Juhler

Department of Neurology, University Clinic, Rigshospitalet, Copenhagen, Denmark

Summary. Decreased sodium permeability across the blood-brain barrier occurs in streptozotocin-treated rats after 2 weeks of diabetes. To establish whether this is a phenomenon specific for cations, the blood-brain barrier permeability for sodium, potassium and calcium was studied with an arterial integral uptake technique. Experiments were performed in control rats and, after two weeks after diabetes induction, in untreated streptozotocin-diabetic rats and in insulin-treated streptozotocin rats. In untreated diabetes, the neocortical blood-brain barrier permeability for sodium decreased by 35% (5.2 ± 1.7 vs $3.4 \pm 1.1 \cdot 10^{-5} \cdot \text{cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$) and potassium permeability by 39% (19.8 ± 5.7 vs $12.1 \pm 3.9 \cdot 10^{-5} \cdot \text{cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$), whereas no differences in calcium permeability occurred. Insulin treatment was associated with an increase in

the blood-brain barrier permeability to sodium ($4.8 \pm 1.0 \cdot 10^{-5} \cdot \text{cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$) as compared to untreated diabetes ($3.4 \pm 1.1 \cdot 10^{-5} \cdot \text{cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$). It is concluded that the observed changes in sodium and potassium permeability cannot be caused by electrostatic membrane changes. More specific abnormalities of the transport of sodium and potassium across the blood-brain barrier are likely to occur; disturbances in the sodium-potassium-pump activity could account for such alterations.

Key words: Experimental streptozotocin-diabetes, blood-brain barrier permeability, cations, arterial integral uptake technique.

Recently we have shown that blood-brain barrier (BBB) permeability to sodium is decreased in rats with a few weeks duration of streptozotocin diabetes. The abnormality of the permeability seems rather specific, as the passage of chloride and sucrose across the barrier is unchanged [1].

Abnormalities of cation permeability in various tissues have been described in rats with streptozotocin-diabetes. In peripheral nerve voltage clamp, in vitro studies have shown a shift in electrical membrane characteristics compatible with a decrease in leakiness of sodium [2]. In the isolated and perfused heart the uptake of calcium ions is decreased [3].

The present study examined BBB cation permeability (sodium, potassium and calcium) in streptozotocin-diabetic rats with 1–2 weeks duration of diabetes. To evaluate whether changes in cation permeability might be due to a toxic effect of streptozotocin and not to the metabolic disturbance, an insulin treatment control experiment was also performed.

Materials and methods

Diabetes induction

Age- and weight-matched male Wistar rats were randomly assigned to three groups: (a) non-treated diabetes; (b) insulin-treated diabetes; (c) controls. For induction of diabetes, streptozotocin (Zanosar)

was injected into a tail vein at a dose of 85 mg/kg. Only rats with a blood glucose value of more than 15 mmol/l (measured with a glucose oxidase method) at the following day and at the day of the tracer experiment were included. In the insulin study, rats were kept diabetic for three days before insulin treatment for a period of 7–11 days was begun. Daily injections of long-acting and modified (pH 5.5) Ultralente (Novo, Copenhagen, Denmark) were given at 16.00 hours according to blood glucose determinations at a time when glucose levels are at their nadirs in night-eaters [4]. The individual mean daily dosage of insulin was 6.8 IU (4.8–9.4).

Tracer technique

The BBB permeability of sodium, potassium and calcium was studied with an integral uptake technique as earlier described [1, 5]. A tracer mixture containing 100 μCi $^{42}\text{K}^+$ (Risø, Roskilde, Denmark), 8 μCi $^{45}\text{Ca}^{++}$ and 10 μCi $^{22}\text{Na}^+$ (Amersham, Buckinghamshire, UK) was diluted in 1 ml saline. For determination of the cerebral vascular space 50 μCi of $^{113\text{m}}\text{In}$ (Amersham) in 1 ml of rat plasma was injected 3 min before decapitation. $^{113\text{m}}\text{In}$ binds to plasma transferrin, which does not leave the intravascular space.

Animal preparation

The rats were anaesthetized with halothane and tracheotomized. Blood gases were measured in each experiment with conventional microelectrodes (Radiometer, Copenhagen, Denmark), and respiration was maintained at normocapnia (CO_2 tension: 36–44 mm Hg). Catheters were inserted into both femoral arteries and veins, and animals were heparinized and relaxed with suxametonium. Arterial blood pressure was measured continuously; animals with a systolic blood pressure below 60 mm Hg were not included in the study.

Table 1. Body weight at start and end of the experiment and blood glucose, plasma sodium, plasma potassium and plasma calcium on the day of the experiment in control rats ($n=9$), streptozotocin-diabetic rats ($n=9$) and insulin-treated diabetic rats ($n=11$). Values are means and ranges

	Body weight (g)		Blood glucose (mmol/l)	Plasma sodium (mmol/l)	Plasma potassium (mmol/l)	Plasma calcium (mmol/l)
	At start	At end				
Controls	—	386 (300–539)	5.3 (3.8–8.4)	139 (130–146)	5.8 (4.7–7.7)	2.6 (2.2–3.2)
Diabetic rats	361 (296–441)	303 (258–336)	18.7 (15.5–25.0)	138 (129–146)	5.3 (3.7–6.5)	2.5 (2.1–2.8)
Insulin-treated diabetic rats	396 (345–445)	412 (372–449)	2.2 (1.0–5.3)	139 (129–143)	5.4 (3.3–7.8)	2.4 (2.2–2.7)

Table 2. Neocortical blood-brain barrier permeability of sodium, potassium and calcium in controls, streptozotocin-diabetic rats and insulin-treated rats

	Sodium	Potassium	Calcium
Controls	5.2 ± 1.7	19.8 ± 5.7^b	5.9 ± 3.7
Diabetic rats	3.4 ± 1.1^a	12.1 ± 3.9	6.6 ± 3.5
Insulin-treated diabetic rats	4.8 ± 1.0	14.4 ± 4.7	4.9 ± 3.3

^a Level of significance ($p < 0.05$) as compared with the insulin-treated group; ^b level of significance ($p < 0.01$) as compared with the two diabetic groups. A model I ANOVA test showed statistically significant differences of sodium and potassium permeability between the three groups, whereas no change of calcium permeability occurred. Permeability-surface area product (PS) values are given as mean $\pm$ SD, units being $10^{-5} \cdot \text{cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$

Body temperature was held constant at $37.0 \pm 0.5^\circ\text{C}$ by means of a thermostat-controlled heat lamp.

After an intravenous bolus injection of 0.2 ml, the tracer solution was constantly infused at a rate of 0.053 ml/min. Arterial plasma tracer integral concentration (C_p) was obtained by continuous sampling of arterial blood at a constant rate (0.053 ml/min). The circulation time was 15 min. Immediately before decapitation, a final blood sample was taken for determination of the blood tracer concentration (C_b). After removal of the brain, samples (10–130 mg) were taken from mid-frontal, mid-parietal, and mid-occipital cortices, cerebellum, and midbrain.

Tissue preparation

Each tissue samples was weighed and the tracer content of tissues and blood samples (25 μl of plasma from the arterial integral blood sample, and 25 μl of the final blood sample) were counted by gamma-spectrophotometry and corrected for decay and spillover. Tissues were dissolved in 1 ml of Soluene 350^R (Packard, Downers Grove, Ill, USA), decolorized with hydrogen peroxide (35%) and neutralized with hydrochloric acid before addition of the scintillation fluid (Picofluor, Packard). After two weeks of decay of $^{42}\text{K}^+$ ($T_{0.5}=12.4\text{h}$) and of $^{113\text{m}}\text{In}$ ($T_{0.5}=1.5\text{h}$), betaemission was counted and corrected for spillover and quenching.

Statistical analysis

The PS (the permeability-surface area product) for each of the ions is calculated by use of a modified version of the arterial integral uptake technique [5, 6]. The amount of tracer taken up by the brain following the intravenous injection is determined by the equation

$$\frac{dC_b}{dt} = \text{PS} \cdot (C_p - C_b/V_e) \quad (1)$$

C_p =arterial tracer plasma concentration (cpm/ml); C_b =tracer brain concentration (cpm/mg); PS=permeability surface area product; V_e =tracer distribution volume in the brain. C_b/V_e is negligible as compared to C_p because of the relatively short circulation time and because of the maintained tracer plasma levels obtained by the constant tracer infusion. The integration of Eq. (1) when the amount of intravascular tracer is deducted leads to the following expression:

$$\text{PS} = \frac{C_b(T) - C_i(T)}{\int_0^T C_p(t) dt} \quad (2)$$

C_i =intravascular tracer content; T=experimental time (15 min). The method requires that tracer permeation across the BBB is independent of the cerebral blood flow (CBF) within physiologic limits.

For substances such as sodium, potassium and calcium with a low extraction, CBF plays essentially no role.

A model I ANOVA was applied to test the statistical significance of differences between any of the three groups. In case of a significant difference at the 5% level, comparisons between subsets of groups were subsequently made by a priori F-tests using the same level of significance. The most powerful design of the study allowed the following comparisons: controls vs the two diabetic groups and untreated diabetic rats vs insulin-treated diabetic rats. To ensure minimum likelihood of type I errors, differences between controls and untreated diabetic rats could not be included unless other test options were disregarded.

Results

Diabetic rats lost 16% of body weight, whereas the insulin-treated diabetic rats had a small weight gain. In the insulin-treated group, blood glucose levels at start of treatment were 18.4 (15.2–24.2) mmol/l, and during the last 3 days prior to the tracer experiment the values were 5.4 (1.4–19.6) mmol/l compared to 5.3 mmol/l in the control group and 18.7 mmol/l in the untreated group. Table 1 shows body weight, blood glucose levels and plasma levels of sodium, potassium and calcium at the time of the tracer experiment. Plasma levels of cations did not differ significantly between the groups (Table 1).

Table 2 shows BBB permeability of three cations in the neocortex and Table 3 shows the permeability in various brain regions.

BBB permeability to sodium ($^{22}\text{Na}^+$) in neocortical tissues differed significantly between the three groups ($2p=0.022$). Untreated diabetic rats had a lower BBB permeability of sodium than insulin-treated rats ($2p=0.013$). Although the significance of the difference

Table 3. Blood-brain barrier permeability of sodium, potassium and calcium of frontal, parietal and occipital cortex as well as of midbrain and cerebellum in control rats, streptozotocin-diabetic rats and insulin-treated streptozotocin-diabetic rats

	Cortex			Midbrain	Cerebellum
	Frontal	Parietal	Occipital		
Sodium					
Controls (<i>n</i> =9)	4.6 ± 1.3	4.7 ± 1.4	6.4 ± 2.7	3.1 ± 1.7	4.8 ± 1.7
Diabetic rats (<i>n</i> =8)	2.1 ± 0.9	3.3 ± 0.7	4.9 ± 2.2	2.7 ± 0.8	4.7 ± 1.2
Insulin-treated diabetic rats (<i>n</i> =10)	4.2 ± 1.4	4.1 ± 1.1	6.2 ± 0.8	3.7 ± 1.3	4.5 ± 0.7
Potassium					
Controls (<i>n</i> =9)	17.8 ± 6.3	18.4 ± 5.4	23.1 ± 7.2	9.6 ± 3.4	15.5 ± 2.0
Diabetic rats (<i>n</i> =9)	8.0 ± 3.4	11.1 ± 4.2	16.3 ± 5.8	7.9 ± 2.7	12.3 ± 3.2
Insulin-treated diabetic rats (<i>n</i> =11)	11.9 ± 4.3	12.7 ± 3.9	18.6 ± 6.3	9.6 ± 2.0	14.0 ± 2.4
Calcium					
Controls (<i>n</i> =9)	6.0 ± 3.5	5.9 ± 4.5	6.0 ± 3.6	3.9 ± 2.1	3.6 ± 1.3
Diabetic rats (<i>n</i> =9)	6.8 ± 4.1	5.4 ± 2.7	7.7 ± 4.4	4.4 ± 1.6	4.8 ± 2.8
Insulin-treated diabetic rats (<i>n</i> =10)	4.9 ± 2.0	4.1 ± 1.8	5.7 ± 2.4	4.8 ± 1.8	3.1 ± 0.8

PS values are given as mean ± SD, units being $10^{-5} \cdot \text{cm}^3 \cdot \text{g}^{-1} \cdot \text{s}^{-1}$

Fig. 1. Blood-brain barrier permeability of sodium ($^{22}\text{Na}^+$), potassium ($^{42}\text{K}^+$) and calcium ($^{45}\text{Ca}^{++}$) in frontal cortices in controls (C), in untreated diabetic rats (D), and in insulin-treated diabetic rats (D+I). Values are individual permeability surface area products, the unit being $10^{-5} \cdot \text{cm}^3 \cdot \text{g}^{-1} \cdot \text{s}^{-1}$. The height of the columns indicates mean values for each group

between controls and untreated diabetic rats could not be tested directly, it appears that sodium permeability was maximal in controls.

BBB permeability to potassium ($^{42}\text{K}^+$) in the neocortex differed significantly between the three groups ($2p=0.008$). Taken together, the two diabetic groups had a lower potassium permeability than the control group ($2p=0.003$). Insulin treatment was not associated with an increase in permeability as compared to untreated diabetes.

In contrast, the BBB permeability to calcium ($^{45}\text{Ca}^{++}$) did not differ between any of the three groups according to the model I ANOVA test.

In the frontal (Fig. 1), parietal and occipital neocortices, this pattern of abnormalities of cation permeability was found in all regions. In the midbrain and in the cerebellum, however, permeabilities for each of the three cations appeared similar between the groups.

Discussion

The findings of the present study on BBB function in early streptozotocin-diabetes are a decrease in PS for potassium in cerebral cortices as well as a confirmation of the previously described observation of a decrease

in PS for sodium [1]. To obtain a *true* PS, in the sense that only unidirectional flux into the brain is measured, no significant transport of the tracer from brain to blood should occur. Since the brain distribution volume for sodium is very large and the extraction low, the lowered PS values found in untreated diabetes can not be explained by an increase in backflux. The conclusion that the transport of sodium and potassium are decreased in untreated diabetes is thus valid.

PS for calcium is unchanged, indicating that other factors than electrical charge are decisive for the observed abnormalities. Insulin treatment was associated with an increased BBB permeability of sodium, making it unlikely that the abnormality is induced by a toxic effect of streptozotocin. Potassium permeability was not reversed by insulin treatment. This discrepancy between the two cations could be explained either by biological variation or by different transport mechanisms for sodium and potassium uptake.

In studies of streptozotocin-diabetic rats an increased inulin space in specific brain regions (the periaqueductal grey and in parts of the hypothalamus) has been described in rats with 4 weeks, but not with 2 weeks, duration of untreated diabetes [7]. This is in agreement with our previous finding of unchanged BBB permeability for sucrose and chlorine [1] after 2 weeks' duration of diabetes. The changes found for sodium and potassium permeability, therefore, seem rather specific.

The endothelial cells of the brain microvessels constitute the BBB, which regulate the exchange of solutes between blood and the brain extracellular fluids. It contains mechanisms for facilitated and active specific transport of substances. The concentrations of many ions are kept remarkably constant in the cerebral extracellular fluid even in case of considerable changes in the blood concentrations [8, 9]. Ions cross the BBB very slowly with an extraction of approximately 1–2% after a single capillary passage. The sodium exchange presumably takes place by means of at least two specific Na^+ -transport systems on the luminal side whereas an energy-dependent carrier is located only to the abluminal side of the brain endothelial cell [10, 11]. Potassium is actively transported from brain to blood by Na^+/K^+ -ATPase of brain microvessels and a high sodium-potassium-pump activity is present in brain endothelial cells [12].

The decreased K^+ and Na^+ passage into the brain in experimental diabetes either could be caused by raised blood osmolarity, by metabolic effects of a raised blood glucose level, or by insulin deficiency. In the untreated diabetic rats of the present study, plasma osmolarity presumably was raised by about 2% (estimated $\text{osmolarity} = 2 \cdot ([\text{Na}^+] + [\text{K}^+] + [\text{glucose}])$). It seems less likely that a permanent state of plasma hyperosmolarity of 2% can induce changes in the PS-products in the range of 35 to 39%. However, the possibility should be tested in future studies.

The observed decrease in BBB permeability for sodium and potassium in untreated diabetes could be due to alterations of the Na^+/K^+ -pump activity or to other changes of the brain endothelial membrane properties. Experimental diabetes seems to initiate a defect in Na^+/K^+ -pump activity in peripheral nerves [13, 14] and in skeletal and heart muscle [15] of streptozotocin-diabetic as well as in spontaneously diabetic rats. In rat brains, an initial decrease followed by an increase in Na,K -ATPase activity which can be corrected by insulin administration has, in fact, been found in alloxan-diabetes [16].

In the present study, we have confirmed our previous observation that the non-cortical brain structures do not show any changes in BBB permeability, suggesting an interesting difference between the metabolically active cortical regions and less active deep brain regions. The intimate contact to the fourth ventricle and to brain regions without a BBB could, however, result in artificially high PS values in the midbrain and obscure differences between control rats and diabetic rats.

It has been suggested that the vulnerability of brain capillaries due to the dependence of oxidative metabolism for active ions exchange can cause brain oedema in several metabolic diseases, such as in diabetes [12]. Our findings of decreased passage of Na^+ and K^+ across the BBB in untreated diabetes may be involved in the development of brain oedema which possibly occurs during the treatment of diabetic ketoacidosis [17]. If the passage of Na^+ into the brain is normalized or raised by sudden administration of large amounts of insulin, as in the treatment of diabetic coma, it is possible that brain oedema occurs. The alterations of the cation permeability of the BBB in experimental diabetes might, therefore, have clinical implications.

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Dr. Gitte Moos Knudsen
Department of Neurology N2082
Rigshospitalet
9, Blegdamsvej
DK-2100 Copenhagen Ø
Denmark