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Influence of acute arterial hypertension on blood–brain barrier permeability in streptozocin-induced diabetic rats

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Abstract

The effect of acute arterial hypertension on blood–brain barrier (BBB) permeability was studied in streptozocin-induced diabetic rats using Evans blue as a barrier tracer. Four groups of rats were studied: Group 1, normotensive normoglycemia; Group II, normotensive + diabetes mellitus; Group III, arterial hypertension + diabetes mellitus; Group IV, arterial-hypertension + normoglycemia. During adrenaline-induced acute arterial hypertension the mean arterial blood pressure increased in both non-diabetic and diabetic animals. Changes in BBB permeability were observed in 52% of the non-diabetic rats, and in 72% of the diabetic rats after adrenaline-induced acute arterial hypertension. Mean levels of Evans blue in the whole brain were found to be 0.63 ± 0.1 mg% in non-diabetic and 0.90 ± 0.2 mg% in diabetic rats. The difference between the non-diabetic and the diabetic rats was found to be statistically significant ($P < 0.01$). From these results it was suggested that the extravasation of Evans blue albumin is more pronounced in the brains of diabetic rats in comparison with non-diabetic rats after adrenaline-induced acute hypertension, which is indicative of changes in BBB permeability due to diabetes mellitus.

Keywords: Diabetes mellitus; Blood–brain barrier; Acute arterial hypertension; Evans blue; Adrenaline

The most characteristic vascular pathology of long-term diabetes mellitis (DM) is the increased permeability of the microvessels [18]. DM produces an increase in the permeability of the peripheral vascular beds [7]. Clinical observations suggest indirectly that the BBB might share in this abnormality [9]. Under normal conditions, the BBB minimizes the entry of molecules into brain. This restriction is accomplished by tight junctions between adjacent endothelial cells, and by a slight pinocytotic activity [31. The relationship between DM and the development of the BBB dysfunction has not been definitively established yet [4]. Experimental studies have yielded contradictory findings [4,10,14]. On the other hand, it has been shown in many studies that acute arterial hypertension can increase the BBB permeability to protein in human beings and experimental animals [12,15]. Acute severe increases in arterial blood pressure produce an increase in cerebral blood flow, passive dilatation of cerebral blood vessels, and disruption of the BBB [12]. Arterial hypertension is a common problem in patients with either type I or type II DM [2,5].

DM appears to be a contributing factor in the pathogenesis of many cerebrovascular events, including stroke [1,8]. Many studies have shown degeneration of the endothelium in cerebral arterioles during DM [6,9,14]. The aim of the present study was to examine the permeability of BBB under control conditions and during acute increases in arterial blood pressure in streptozocin induced-diabetic rats.

The experiments were carried out on adult male Wistar rats. They were anesthetised with diethyl ether. A femoral artery was cannulated for recording of mean arterial blood pressure (MABP). MABP was recorded by connecting the arterial catheter to a stain gauge transducer (Ugo-Basil). Another catheter was inserted in a femoral vein for drug injection. Evans blue which binds to serum albumin was used as a BBB tracer and was given intravenously at a dose of 4 ml of a 2% solution in saline per kg body weight [16].

DM was induced with intravenously administered streptozocin (65 mg/kg body weight), and blood glucose

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Table 1
Mean arterial blood pressure (MABP) and changes in blood brain barrier (BBB) permeability during experimental groups

Exp. groups[a]	n	MABP (mmHg)		Changes in BBB permeability			
	n	Initial	Maximal	0	1+	2+	3+
Group I	10	98 ± 13	–	10	–	–	–
Group II	8	116 ± 14	–	4	4	–	–
Group III	14	110 ± 11	176 ± 12*	4	5	3	2
Group IV	17	96 ± 10	170 ± 16*	8	5	4	–

n, number of animals.
[a] See text.
* In comparison to initial value $P < 0.01$.

levels were measured three times after the streptozocin injection. The diabetic and non-diabetic rats received standard laboratory rat diet. The body weight of every rat was recorded four times during the experiments. The rats were divided into four groups: Group I, normotensive normoglycemia ($n = 10$); Group II, normotensive + DM ($n = 8$) (approximately 60 days after the induction of DM); Group III, arterial hypertension + DM ($n = 14$); Group IV, arterial hypertension + normoglycemia ($n = 17$). The first and the second groups served as controls and received Evans blue only. In Groups III and IV, after recording the initial blood pressure, Evans blue was injected intravenously; 5 min later adrenaline (40 μg/kg) was injected rapidly. At the end of experiments, i.e. approximately 20 min after Evans blue or drug injection under diethyl ether anesthesia, all the rats were killed by perfusion through the heart with saline solution to avoid artificial staining of the brain during removal. Then the brains were removed and examined for Evans blue albumin extravasation and the extent and intensity of the staining. Staining of each hemisphere and the coronal sections by Evans blue was graded as follows: grade 0, no staining; grade 1+, just noticeable staining; grade 2+, moderate blue staining; and grade 3+, dark blue staining [13,19]. A quantitative estimation with a spectrophotometer using homogenized brain to release the dye was performed as described previously [16]. Briefly, the brain was removed and divided at the midline. Each half cerebrum and cerebellum was placed in tared tubes which were immediately reweighed. They were homogenized with 5 ml of phosphate-buffered saline containing a 5 ml% solution of 1 N NaOH. The homogenized brain was centrifuged (10 000 rev./min for 10 min) and spectrophotometric analysis at 620 nm was performed to measure the amount of the dye [16]. Data are expressed as means ± SD, and statistical analysis was performed by Student's t-test. Adrenaline, streptozocin and Evans blue were obtained from Sigma.

Three days after streptozocin injection, plasma glucose was 360 ± 23 mg/100 ml versus 118 ± 12 mg/100 ml in controls, and after 60 ± 5 days it was 418 ± 36 mg/100 ml ($P < 0.001$). The experimental animals lost 25–50 g of their body weight during their diabetic periods.

The degree of BBB breakdown and mean arterial blood pressure before and after drug administration are presented in Table 1. In all rats a single rapid intravenous administration of adrenaline resulted in an immediate increase in MABP. The initial MABP was 96 ± 10 mmHg in non-diabetic animals (Group IV) and 110 ± 11 mmHg in diabetic rats (Group III). These pressures rapidly increased to 170 ± 16 mmHg in non-diabetic and 176 ± 12 mmHg in diabetic animals after the adrenaline injections. MABP increased similarly in diabetic rats and normoglycemic rats.

No Evans blue albumin extravasation was seen in the brains from non-diabetic normotensive rats except in the pineal body, pituitary gland and choroid plexus, i.e regions in which capillaries are known to be leaky. Evans blue dye concentration was found to be 0.28 ± 0.05 mg% whole brain in this group (Group I). In the second group, no macroscopically evident Evans blue albumin complex was observed in the brain in 4 out of the 8 rats. Minimal extravasation of Evans blue albumin was observed in four rats (grade 1+). Passage of Evans blue albumin through the BBB was spotty in macroscopic examination, particularly in the septal area, hypothalamus and the area around the ventricle. No Evans blue albumin extravasation was observed in the cerebral cortex. The mean value for Evans blue dye was found to be 0.40 ± 0.1 whole brain in this group (Group II). This difference between normotensive diabetic and normotensive non-diabetic animals was found to be significant ($P < 0.05$)

In non-diabetic rats, after adrenaline injection, Evans blue albumin extravasation was observed predominantly in the frontal and occipital regions of the cortex which

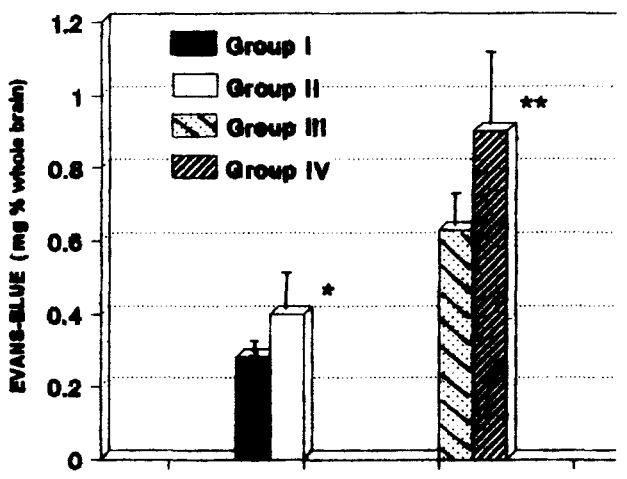

Fig. 1. Evans blue (mg% whole brain) content in the experimental groups. Group I, normotensive normoglycemia; Group II, normotensive + DM; Group III, arterial hypertension + DM; Group IV, arterial hypertension + normoglycemia. *$P < 0.05$ Group I versus Group II; **$P < 0.01$ Group IV versus Group III. Means ± SD.

were the most common area for BBB dysfunction in acute arterial hypertension (Group IV). In normotensive diabetic rats, after adrenaline injection (Group III), BBB leakage pattern was similar to that in non-diabetic rats but the extravasation of Evans blue was most pronounced in the brains of diabetic rats in comparison with non-diabetic ones. The mean value for Evans blue dye was found to be 0.63 ± 0.2 mg% whole brain in non-diabetic and 0.90 ± 0.2 mg% whole brain in diabetic animals after adrenaline induced acute arterial hypertension (Fig. 1). This difference between non-diabetic and diabetic animals was found to be significant ($P < 0.01$).

The major finding of the present study is that DM appears to alter the susceptibility of the BBB to disruption during acute arterial hypertension. Although adrenaline-induced blood pressure elevations were similar in amplitude in non-diabetic and diabetic animals, the extravasation of Evans blue albumin increased significantly in diabetic rats. These results suggest that diabetes aggravates the permeability of the BBB in arterial hypertension. However, it has previously been reported that the permeability of the BBB remained unaltered in diabetic rats during acute arterial hypertension [11]. These conflicting results may be related to the age of rat at the time of induction of DM; strain of rats; and severity and duration of DM. The detailed mechanisms of the vulnerability of BBB in DM during acute arterial hypertension is not known, but the results may lead to the following conclusions. There is evidence of thickening of basement lamina around cerebral capillaries in both human DM and experimental DM in the rat [6,17]. Disturbances in the structure and function of microvessels in many tissues are also recognized as occurring in both human DM and in experimental animal models [4]. In rats, 60 ± 5 days corresponds approximately to 6 years of human life which is the approximate time required for the development of diabetic microangiopathy. Several studies have also indicated that 80% of insulin-dependent DM develop macroproteinuria over the subsequent 6–14 years [21]. Parving and Rossing have allowed that both hypertensive and diabetic patients had transcapillary albumin ascape rates that were approximately 30% higher than the controls [17]. Therefore, microangiopathy may be one of the mechanisms underlying the BBB permeability changes.

There is ample study related to the effect of DM on the permeability of the BBB [4,6,11,14]. However, the relationship between DM and the development of the BBB dysfunction has still not been definitely established [4]. Experimental studies have yielded contradictory findings. Stauber et al. [20] reported that the permeability of the BBB to albumin was increased in 2 weeks after the induction of DM. Lorenzi et al. [10] have also demonstrated that the leakage of ^{3}H-labelled inulin into three different areas of the brain is significantly increased in streptozocin diabetic rats, but they could not show any leakage of horseradish peroxidase from blood to brain after 4 weeks of DM. Mayhan [11] examined the permeability of the BBB 3.5 months after the induction of DM and found that the number of microvascular leaky sites was greater in diabetic rats compared to non-diabetic rats. Finally, studies by Bradbury et al. [4] showed that the permeability of the BBB to sucrose was not altered at 3 weeks, 6, 7 months and 13, 14 months after the induction of DM in rats. In the present study, we examined the permeability of the BBB 60 ± 5 days after induction of DM; our findings suggest that the permeability of the BBB may be increased during DM.

That diabetic rats are more prone to develop permeability disturbances than non-diabetic rats during acute arterial hypertension may be of clinical interest. It has long been known that patients with DM have an increased risk of mortality from arterial hypertension. Arterial hypertension may have serious effects on the diabetic organism [5]. However, the neurological consequences of these barrier disturbances are at present uncertain.

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