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Inhibitory effect of a novel bradykinin B_1 receptor antagonist, R-954, on enhanced vascular permeability in type 1 diabetic mice

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Abstract: The morbidity and mortality associated with type 1 diabetes are essentially related to the micro- and macrovascular complications that develop over time and lead to several diabetic complications, including hypertension, atherosclerosis, and retinopathy, as well as coronary and renal failure. Normally absent in physiological conditions, the bradykinin B_1 receptor (BKB_1-R) was recently found to be overexpressed in pathological conditions, including type 1 diabetes. In the present study, we evaluated the effect of the new BKB_1-R antagonist, R-954 (Ac-Orn-[Oic^2, α-MePhe^5, D-βNal^7, Ile^8]desArg^9-bradykinin, on the increase in vascular permeability in streptozotocin (STZ) - diabetic mice. The capillary permeability to albumin was measured by quantifying the extravasation of albumin-bound Evans blue dye in selected target tissues (liver, pancreas, duodenum, ileum, spleen, heart, kidney, stomach, skin, muscle, and thyroid gland). Acute single administration of R-954 (300 μg/kg, i.v.) to type 1 diabetic mice 4 weeks after STZ significantly inhibited the enhanced vascular permeability in most tissues. These data provide further experimental evidence for the implication of BKB_1-R in the enhanced vascular permeability associated with type 1 diabetes.

Key words: vascular permeability, type 1 diabetes, bradykinin B_1 receptor.

Résumé : La morbidité et la mortalité associées au diabète de type 1 sont essentiellement liées aux complications micro et macrovasculaires qui se développent avec le temps et mènent à de nombreuses complications diabétiques comprenant l'hypertension, l'athérosclérose, la rétinopathie ainsi que l'insuffisance coronaire et rénale. Normalement absent en conditions physiologiques, le récepteur B_1 de la bradykinine a été récemment rapporté être surexprimé dans des conditions pathologiques incluant le diabète de type 1. Dans la présente étude, nous avons évalué l'effet du nouvel antagoniste du récepteur B_1 de la bradykinine, le R-954 (Ac-Orn-[Oic^2, α-MePhe^5, D-βNal^7, Ile^8]desArg^9-bradykinine, sur l'augmentation de la perméabilité vasculaire dans un modèle de diabète de type 1 chez la souris traitée à la streptozotocine. La perméabilité capillaire à l'albumine a été mesurée en quantifiant le colorant bleu d'Evans lié à l'albumine dans des différents tissus cibles (foie, pancréas, duodénum, iléum, rate, cœur, rein, estomac, peau, muscle et glande thyroïde). L'administration simple du R-954 (300 μg/kg, i.v.) aux souris diabétiques de type 1, 4 semaines après le traitement avec la STZ, a significativement inhibé l'augmentation de la perméabilité vasculaire au niveau de la plupart des tissus. Les données obtenues dans cette étude supportent l'implication des récepteurs B_1 des kinines dans l'augmentation de perméabilité vasculaire liée au diabète de type 1.

Mots clés : perméabilité vasculaire, diabète de type 1, récepteur B_1 de la bradykinine.

Introduction

The chronic hyperglycemia of type 1 diabetes mellitus is associated with significant long-term damage and failure of various organs. The dysfunction of the vascular endothelium and the altered micro- and macro-vascular permeability lead to many diabetic complications, such as arterial hypertension, retinal microangiopathy, and accelerated atherosclerosis, as well as coronary and renal failure (Steil 1999).

Homeostasis of the vascular endothelium has important functions in various pathophysiological conditions, including type 1 diabetes. Endothelial cells play a significant role in controlling the passage of macromolecules across the intima of large vessels, potentially leading to the deposition of macromolecular material, including lipoproteins (Ross 1986). An intact endothelium also selectively modulates the transfer of albumin, fluid, and small solutes from the vascular to the interstitial fluid compartment of different capillary

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networks (Sirois et al. 1990). Such an action may produce local tissue oedema and alteration in the traffic of substrates and waste products between the vascular and cellular volumes, thereby potentially leading to target-organ damage and development of morbid conditions (Aukland and Reed 1993).

Kinins are important mediators known to be implicated in many biological effects, including cardiovascular homeostasis, inflammation, and nociception (Marceau et al. 1998). They promote many features of inflammation, including an increase in blood flow and tissue oedema, as well as the release of other mediators such as nitric oxide (NO), prostanoids, and cytokines. Two types of receptors, namely, B_1 and B_2, mediate the biological effects of kinins. The B_2 receptor, which mediates many of the physiological effects of kinins, is constitutively expressed and is reported to be responsible for the acute phase of inflammation. On the other hand, the bradykinin (BK) B_1 receptor (BKB_1-R), usually absent in normal tissues, is highly induced and overexpressed during tissue injury and following the administration of agents like bacterial endotoxins and cytokines. The BKB_1-R does not desensitize after repeated binding and participates in the chronic phase of the inflammation (Couture et al. 2001).

Current experimental evidence suggests that type 1 diabetes upregulates BKB_1-R as a consequence of the overproduction of cytokines, in addition to hyperglycemia and oxidative stress (Rabinovitch 1998; Yerneni et al. 1999). Our study aimed to evaluate the effect of the novel and selective BKB_1-R antagonist R-954 (Neugebauer et al. 2002) on the already stabilized increased vascular permeability in STZ-diabetic mice.

Materials and methods

Animals

Male CD-1 mice weighing 25–30 g (Charles River Breeding Laboratory, St-Constant, Que.) were used. The mice were housed four to a cage with free access to food and water. They were maintained under conditions of standard lighting (12 h light : 12 h dark), temperature (22 ± 0.5°C), and humidity (60 ± 10%). All experiments were carried out in accordance with the ethical recommendations and guidelines of the Canadian Council on Animal Care.

Drugs

STZ (Pharmacia & Upjohn Inc., Mississauga, Ont.) was dissolved in saline (pH 4.5) and administered i.p. to mice. Evans blue dye (EB; Sigma, St. Louis, Mo.) and R-954 (Ac-Orn-[Oic^2, α-MePhe^5, D-βNal^7, Ile^8]desArg^9-BK; Institute of Pharmacology of Sherbrooke, School of Medicine, University of Sherbrooke, Que.) were dissolved in saline and administered i.v. to mice. Standard abbreviations for amino acids and peptides follow the recommendations of the IUPAC Commission on Nomenclature in Organic Chemistry and IUPAC-IUB Commission on Biochemical Nomenclature (1975) and the IUPAC–IUB Joint Commission on Biochemical Nomenclature (1984). Additional abbreviations are as follows: Oic, (2*S*,3a*S*,7a*S*)-octahydro-1H-indole-2-carboxylic acid (3a and 7a refer to the joint carbon atoms between the cyclohexyl and pyrrolidine rings; C-3a follows C-3, while C-7a follows C-7 in the numbering of the octahydroindole ring); α-MePhe, α-methylphenylalanine; D-βNal, β-(2-naphthyl)-D-alanine.

Induction of type 1 diabetes

Male CD-1 mice received a single high dose of streptozotocin (200 mg/kg, i.p.) (McEvoy et al. 1984). The induction of type 1 diabetes was confirmed by measuring the blood glucose level 96 h after streptozotocin administration (Katovich et al. 1995; Chakir and Plante 1996). Blood was withdrawn from the retro-orbital sinus with a 50-μL heparinized capillary tube. Blood glucose levels were determined with an automatic analyzer (Glucometer Elite XL, Bayer Inc., Toronto, Ont.) using glucose oxidase – potassium ferricyanide reagents strips. The type 1 diabetic animals used in our study had blood glucose levels higher than 20 mmol/L, while the normal value is from 5 to 8 mmol/L (Chakir and Plante 1996; Plante et al. 1996).

Measurement of capillary permeability using the extravasation of EB dye

The vascular permeability to albumin was evaluated following i.v. injection of EB dye by measuring the levels of albumin-bound EB in selected tissues. Unanaesthetized mice were given caudal venous injections of EB dye (20 mg/kg, in a final volume of 100 μL). The dye was allowed to circulate for 10 min, and thereafter the mice were killed by cervical dislocation and were exsanguinated (Béliveau et al. 2002). The liver, pancreas, duodenum, ileum, spleen, heart, kidney, stomach, skin, muscle, and thyroid were harvested, dissected, and weighed, and a portion of each was immersed in formamide (4 mL/g wet weight at 24°C for 24 h). The remaining portion was desiccated at 60°C for 24 h. The concentration of EB dye extracted in formamide from selected tissues was determined spectrophotometrically at 620 nm using Titertek Multiscan®MC (Titertek, Instruments Inc., Huntsville, Ala.) against a standard curve and expressed as micrograms of EB per gram of dry tissue.

Experimental protocol

Mice were divided into four groups, each made up of 4–6 animals: (*i*) control group treated with saline; (*ii*) group treated with R-954 (300 μg/kg, i.v.); (*iii*) group treated once with STZ (200 mg/kg, i.p.); (*iv*) group treated with STZ and R-954. Four weeks after the induction of type 1 diabetes, the mice were given an acute i.v. injection of saline or R-954 together with the EB dye, and the vascular permeability test was performed 10 min later.

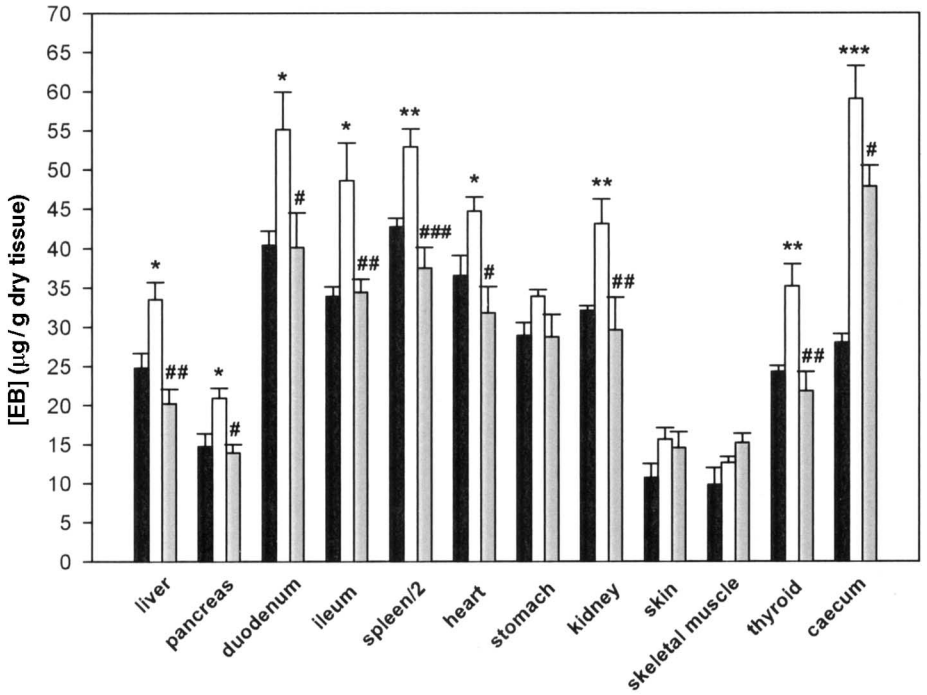
Statistical analysis

Data are expressed as mean [EB] (μg/g dry tissue) ± SE, and analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparisons test were performed. $P < 0.05$ was considered significant.

Results

STZ-induced insulin-dependent diabetes mellitus (IDDM) was associated with marked alterations in vascular permeability 4 weeks post STZ injection in mice. As shown in Fig. 1, the capillary permeability to albumin-bound EB was increased by 35% in liver, 42% in pancreas, 36% in duodenum,

Fig. 1. Acute effect of the selective BKB_1-R antagonist R-954 on vascular permeability changes associated with STZ-induced type 1 diabetes in mice. Type 1 diabetes was induced in CD-1 mice using STZ (one dose of 200 mg/kg, bolus i.p.). Four weeks following the induction of diabetes, animals were injected with saline (STZ; open bars) or R-954 (300 μg/kg, bolus i.v.; grey bars), together with EB dye (20 mg/mL). Control nondiabetic mice (saline; black bars) received equal amounts of EB dye. Capillary permeability was assessed in selected tissues collected 10 min later by quantifying the extravasation of albumin-bound EB. Data are expressed as mean [EB] (μg/g dry tissue) ± SE (n = 4–6). [EB] values in tissues from nondiabetic animals (saline or R-954 treated) were statistically indistinguishable. *, $P < 0.05$ vs. saline; **, $P < 0.01$ vs. saline; ***, $P < 0.001$ vs. saline; #, $P < 0.05$ vs. STZ; ##, $P < 0.01$ vs. STZ; ###, $P < 0.001$ vs. STZ.



43% in ileum, 24% in spleen, 17% in stomach, 22% in heart, 34% in kidney, 46% in skin, 28% in skeletal muscle, 46% in thyroid gland, and 110% in caecum ($P < 0.001$).

Acute administration of the novel selective BKB_1-R antagonist R-954 (300 μg/kg, i.v.) to STZ-diabetic mice at the same period of time abolished the elevated vascular permeability in most tissues, bringing them back to normal control levels, except in the caecum where the plasma extravasation was reduced by only 35% ($P < 0.001$; Fig. 1). However, no significant changes in capillary permeability were observed in the skin or skeletal muscle. It is noteworthy that treatment of control mice with R-954 had no effect on vascular permeability. The mean values of [EB] (μg/g dry tissue) for the control non-diabetic mice treated with R-954 versus those given saline, respectively, were as follows: liver, 22.4 ± 1.7 vs. 24.8 ± 1.9; pancreas, 15.1 ± 1.6 vs. 14.7 ± 1.7; duodenum, 39.2 ± 2.9 vs. 40.4 ± 1.8; ileum, 32.5 ± 1.9 vs. 33.9 ± 1.2; spleen, 38.5 ± 2.7 vs. 42.7 ± 1.1; heart, 33.2 ± 2.0 vs. 36.5 ± 2.6; stomach, 29.5 ± 2.1 vs. 28.9 ± 2.2; kidney, 33.2 ± 1.9 vs. 32.1 ± 0.6; thyroid, 22.8 ± 2.3 vs. 24.3 ± 1.6; and caecum, 30.1 ± 1.9 vs. 28.0 ± 1.1.

Discussion

In the current study, we demonstrated for the first time that STZ-induced IDDM caused a marked increase in capillary permeability to albumin-bound EB in a number of selected mice tissues. It has been demonstrated that a high concentration of glucose significantly increases transcellular transport by macromolecules in bovine aortic endothelial cells in culture (Yamashita et al. 1995). Several mediators have been proposed to be involved in the control and development of the vascular permeability in various rat models. However, the exact mechanisms responsible for its increase remain unknown. A limited number of clinical studies showed that treatment with indomethacin, a cyclooxygenase inhibitor, reduced microalbuminuria in type 1 diabetic patients (Rudberg et al. 1993). Furthermore, Chakir and Plante (1996) reported that the aldose-reductase and NO pathways play a selective and dominant role in endothelial permeability dysfunction, since the aldose reductase inhibitor sorbinil, which tackles polyol production in IDDM, and aminoguanidine, which modulates NO production, normalize capillary permeability in the kidney and duodenum of rats.

Recent studies showed that type 1 diabetes is associated with an upregulation of BKB_1-R, induced by the overproduction of cytokines, in addition to hyperglycemia and oxidative stress (Rabinovitch 1998; Yerneni et al. 1999). In addition, in vivo studies reported that BKB_1-R intervenes in the pathogenesis of STZ-induced IDDM in mice, since the BKB_1-R antagonist [Leu^8]desArg^9-BK normalized glycemia and renal functions (Zuccollo et al. 1996, 1999). When administered with STZ, this selective antagonist reversed elevated blood glucose levels and prevented renal abnormalities including increased urine volume and increased excretion of protein, nitrite, and kallikrein.

In the present set of experiments, we showed that a novel selective BKB_1-R antagonist (R-954) administered 4 weeks after the onset of IDDM abolished the enhanced vascular permeability observed in type 1 diabetic mice back to the normal nondiabetic levels observed in nondiabetic murine tissues.

Therefore, enhanced vascular permeability in IDDM can be explained by the BK-mediated pathway. First, it is known that BKB_1-R, which is selectively activated by BKB_1-R agonists, is normally absent or exhibits little activity under normal physiological conditions (Couture et al. 2001), whereas such selective agonists are effective upon overexpression of the BKB_1-R in pathological conditions, as alongside IDDM. The BKB_1-R was found to be induced on bovine aortic endothelial cells (D'Orléans-Juste et al. 1996), as well as on the portal vein of type 1 diabetic rats (Chakir 1996). In addition, it is expressed on vascular smooth muscle cells (SMC) and seems to relax arterial preparations such as the rabbit coeliac artery (Ritter et al. 1989).

Secondly, in inflammatory conditions such as type 1 diabetes chronic activation of the inducible BKB_1-R is likely to be amplified by the accumulation of $desArg^9$-BK (DBK), the metabolite resulting from the degradation of BK, at the site of inflammation (Marceau et al. 1998). This can be attributed in part to the upregulation of carboxypeptidase M (kininase I, the enzyme responsible for the metabolism of BK to DBK), increasing the endogenous level of DBK, a BKB_1-R agonist, as observed in pigs aorta infused with lipopolysaccharide (Schremmer-Danninger et al. 1998).

Thus, DBK may directly increase the plasma extravasation of albumin by acting on endothelial BKB_1-R, inducing endothelial contraction and hence increasing intracellular spaces. Alternatively, DBK binding to BKB_1-R on precapillary endothelial cells has been reported to activate phospholipase C and NO production (Regoli 1984), the later of which will lead to the dilatation of precapillary vessels. Such an effect will increase blood flow in capillary beds and thus increase the plasmatic extravasation of proteins. On postcapillary SMCs, the binding of DBK to BKB_1-R will lead to phospholipase C activation, inositol triphosphate production, and Ca^{2+} release, causing the contraction of the SMC. The postcapillary vessel constriction will result in the formation of fenestrations in the wall of microvessels, enabling extravasation of blood constituents. Taken together, the vasodilatation of precapillary arterioles and the constriction of postcapillary venules result in the increase of hydrostatic pressure in the capillaries, leading to plasma extravasation (D'Orléans-Juste et al. 1996). In conclusion, the BKB_1-R, when overexpressed in pathological conditions, is likely to play a strategic role in diseases with an immune component such as IDDM. We demonstrated that the new BKB_1-R antagonist R-954, being highly resistant to enzymatic degradation by kininases, exercises competitive inhibition to its inducible receptor, leading to attenuation of the increased vascular permeability in type 1 diabetic mice. These results suggest a novel approach in the treatment of IDDM complications using the BKB_1-R antagonists.

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