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Effects of a selective bradykinin B_1 receptor antagonist on increased plasma extravasation in streptozotocin-induced diabetic rats: Distinct vasculopathic profile of major key organs

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Abstract

Diffuse vasculopathy is a common feature of the morbidity and increased mortality associated with insulino-dependent type 1 diabetes. Increased vascular permeability leading to plasma extravasation occurs in surrounding tissues following endothelial dysfunction. Such micro- and macro-vascular complications develop over time and lead to oedema, hypertension, cardiomyopathy, renal failure (nephropathy) and other complications (neuropathy, retinopathy). In the present investigation, we studied the effect of a selective bradykinin B_1 receptor antagonist, R-954, on the enhanced vascular permeability in streptozotocin (STZ)-induced diabetic Wistar rats compared with age-matched controls. Plasma extravasation was determined using Evans blue dye in selected target tissues (left and right heart atria, ventricles, lung, abdominal and thoracic aortas, liver, spleen, renal cortex and medulla), at 1 and 4 weeks following STZ administration. The vascular permeability was significantly increased in the aortas, cortex, medulla, and spleen in 1-week STZ rats and remained elevated at 4 weeks of diabetes. Both atria showed an increased vascular permeability only after 4-week STZ-administration. R-954 (2 mg/kg, bolus, s.c.), given 2 h prior to Evans blue dye, to 1- and 4-week diabetic rats significantly inhibited (by 48–100%) plasma leakage in most tested tissues affected by diabetes with no effect in healthy rats. These results showed that the inducible bradykinin B_1 receptor subtype participates in the modulation of the vascular permeability in diabetic rats and suggest that selective bradykinin B_1 receptor antagonism could have a beneficial role in reducing diabetic vascular complications.

Keywords: Type 1 diabetes; Vascular permeability; Plasma extravasation; Bradykinin B_1 receptor

1. Introduction

Chronic hyperglycemia occurring in uncontrolled diabetes leads to significant long-term damage, and foreseeable failure of various organs. Endothelial dysfunction is linked to altered micro- and macro-vascular permeability leading to several diabetic complications such as diffuse vasculopathy (that accelerates atherosclerosis leading to arterial hypertension), retinal micro-angiopathy, as well as serious conditions of coronary and renal failure (Christrieb, 1973; Porta et al., 1987; Plante et al., 1996).

Chemically induced type 1 diabetes is associated with significant increases in vascular permeability in many organs of streptozotocin (STZ)-diabetic rats (Hulthén et al., 1995; Chakir and Plante, 1996) and mice (Simard et al., 2002) models. The mechanisms responsible for the control of vascular permeability are complex and involve a number of pro-inflammatory mediators such as the kinins, which are released by circulating blood cells and the endothelium.

The kinins are involved in many biological effects such as cardiovascular homeostasis, inflammation and nociception (Marceau et al., 1998). The action of kinins reflect many key features of inflammation including an increase in

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blood flow and tissue oedema, as well as the release of other mediators such as nitric oxide (NO), prostanoids and cytokines (Couture et al., 2001). The kinins exert their biological effects through two G-protein-coupled receptors designated B_1 and B_2. The B_2 receptor subtype, which mediates many of the physiological effects of kinins, is constitutively expressed and is believed to be involved in the acute phase of inflammation. On the other hand, the bradykinin B_1 receptor subtype, usually absent or of little impact in normal tissues, is highly induced and over-expressed during tissue injury and following the administration of agents such as bacterial endotoxins and cytokines (Marceau et al., 1998; Couture et al., 2001).

Experimental evidence suggests that under diabetic conditions, the bradykinin B_1 receptor is up-regulated. Current evidence indicates that insulin-dependent diabetes mellitus is triggered by an autoimmune response associated with over-production of cytokines, including interleukin-1β and tumor necrosis factor-α, that leads to the destruction of pancreatic islet β-cells (Hussain et al., 1996; Rabinovitch and Suarez-Pinzon, 1998; Rabinovitch, 1998). These cytokines induce and over-express the bradykinin B_1 receptor by mechanisms involving the mitogen-activated protein kinase and the transcriptional nuclear factor κB (Larrivee et al., 1998; Ni et al., 1998; Schanstra et al., 1998; Zhou et al., 1998; Sardi et al., 1998; Campos et al., 1999). In addition, hyperglycemia and the resulting oxidative stress observed alongside diabetes can activate the transcriptional nuclear factor κB (Yerneni et al., 1999), which is known to induce the bradykinin B_1 receptor (Marceau et al., 1998). Therefore, both the overproduction of cytokines and hyperglycemia could trigger the expression of the bradykinin B1 receptor through the transcriptional nuclear factor κB in diabetes. Other mechanisms that are involved in the induction of bradykinin B_1 receptor in diabetes include the long-term exposure of the bradykinin B_1 receptor to its endogenous agonist desArg^{9}bradykinin, which results in increased receptor expression (Faussner et al., 1999) and the cross up-regulation by the bradykinin B_2 receptor activation (via autocrine production of cytokines and activation of nuclear factor κB) and/or bradykinin B_2 receptor sensitization (Phagoo et al., 1999).

Previous findings documented the expression profile of the bradykinin B_1 receptor in animal models of type 1 diabetes and the implication of such receptor subtype in diabetic complications (Koyama et al., 2000; Simard et al., 2002; Mage et al., 2002; Vianna et al., 2003; Abdouh et al., 2003; Ongali et al., 2004). Selective bradykinin B_1 receptor antagonists were shown to attenuate renal abnormalities (including increased diuresis and excretion of proteins, nitrite and kallikrein; Zuccollo et al., 1996, 1999), and to decrease vascular permeability changes in the liver, pancreas, duodenum, ileum, spleen, heart, kidney and stomach (Simard et al., 2002) in STZ-diabetic mice. Studies conducted in our laboratory also demonstrated the involvement of the bradykinin B_1 receptor in mediating thermal hyperalgesia in STZ-mice (Gabra and Sirois, 2002, 2003a,b; Gabra et al., 2005) and in models of type 1 and type 2 diabetes in rats (data not shown): selective bradykinin B_1 receptor antagonists blocked neuronal alteration that could have been triggered, in part, by dysfunction of the peripheral micro-circulation in the nerve endings.

The present study aimed at evaluating the effect of the selective bradykinin B_1 receptor antagonist, R-954, (Neugebauer et al., 2002) on established plasma extravasation in seven key organs of STZ-induced type 1 diabetic rats, some of which can lead to severe complications such as cardiomyopathy, vasculopathy and nephropathy.

2. Materials and methods

2.1. Animals

Male Wistar rats (7–8 weeks of age; mean body weight 290±7 g; Charles River, St-Constant, QC, Canada) were used. The rats were housed four by cage with free access to food (normal rat chow diet #5075; Charles River, St-Constant, QC, Canada) and tap water. They were maintained under conditions of standard lighting (alternating 12-h light/dark cycle), temperature (22±0.5 °C) and humidity (60±10%) for at least 1 week before the experiments. All experiments were carried out in accordance with the recommendations of the IACUC (Institutional Animal care and Use Committee) and were approved by the local Animal Care Committee of University of Sherbrooke.

2.2. Induction of type 1 diabetes

Rats were given a single intraperitoneal dose (65 mg/kg) of STZ (Chakir and Plante, 1996). Diabetes was confirmed by measuring the venous circulating plasma concentrations of glucose, 4 days post-STZ injection. Blood samples were obtained from the rat-tail vein and the glucose concentration was determined with an automatic analyzer (Glucometer Elite XL, Bayer Incorporation, Toronto, ON, Canada) using glucose oxidase/potassium ferricyanide reagents strips. Diabetic rats used in our study had a blood glucose level higher than 30 mmol/l, while the normal value in control healthy rats injected with saline ranges from 5 to 7 mmol/l.

2.3. Physiological markers of diabetes

Circulating arterial blood samples were collected in heparin-lithium vacutainers from euthanized control versus STZ-diabetic rats at time 0, 1 and 4 weeks post-saline or STZ injection. Plasma concentrations of glucose (mmol/l), cholesterol (mmol/l) and triglycerides (mmol/l) were measured using a Vitros 950 from Beckman Coulter Inc (Hialeah, FL, USA) through UV/visible colorimetric assays, whereas insulin (ng/ml) was measured with a commercially available rat insulin kit (S-1238; ELIS7536) from Peninsula Labo-

ratories Inc. (San Carlos, CA, USA). The consumption of drinking water (ml/day) and food (g/day) was also determined.

2.4. Measurement of capillary permeability using the extravasation of Evans blue dye

Non-anaesthetized control and diabetic rats were injected with the Evans blue dye (20 mg/kg) through the tail vein. The dye was injected 10 min before the rats were sacrificed by decapitation. Thereafter, the chest cavity was opened, and the rats were perfused, via a catheter implanted though the right ventricle up to the pulmonary artery, with 15 ml of heparinized saline (4 U/ml) under constant peristaltic flow (10 ml/min). Both heart atria and ventricles, lung, thoracic and abdominal aortas, kidney cortex and medulla, liver and spleen were harvested, dissected, weighed and divided in two equal sections. A first portion was desiccated at 60 °C for 24 h prior to weighing the dry tissue and a second portion was immersed in formamide (4 ml/g wet tissue weigh) at 24 °C for 24 h for the extraction of the Evans blue dye. The absorbance of Evans blue dye extracted in formamide was then measured by spectrophotometry at 620 nm using a plate reader (SPECTRAmax® GEMINI XS, Molecular Devices, Sunnydale, CA, USA). The concentration of the Evans blue dye was then calculated from a standard curve and expressed as μg of Evans blue per g of dry tissue.

2.5. Drugs

Streptozotocin (Pharmacia & Upjohn Inc., Mississauga, ON, Canada) was dissolved in saline and administered by intraperitoneal injection. Evans Blue dye (Sigma, St Louis, MO, USA) was dissolved in saline and administered by intravenous injection. The selective bradykinin B_1 receptor antagonist R-954 (Ac-Orn-[Oic^2, α-Me Phe^5, D-β Nal^7, Ile^8]desArg^{9}bradykinin) (bradykinin B_1 receptor Ki=2.4 nmol/l; bradykinin B_2 receptor Ki>10 μmol/l; Neugebauer et al., 2002) was obtained from IPS Pharma Inc. (Sherbrooke, QC, Canada), dissolved in saline and administered by subcutaneous (s.c.) injection.

2.6. Experimental protocol

Rats were divided into four groups each of 6–8 animals: (i) control group, injected with saline; (ii) control group treated with R-954 (2 mg/kg; bolus, s.c.); (iii) STZ-diabetic group treated with saline; (iv) STZ-diabetic group treated with R-954. One and four weeks after the induction of diabetes, the rats were given an acute subcutaneous injection of saline or R-954, 2 h before the administration of Evans blue dye which was allowed to circulate for an additional 10 min. Pharmacodynamics studies (unpublished data) showed that the present dose and route of administration of the bradykinin B_1 receptor antagonist R-954 provided optimal coverage (maximum blockade of the receptor) over a period of 2–4 h following its injection in rats.

2.7. Statistical analysis

Data are expressed as the mean values±S.E.M. The percentage of inhibition associated with the effects of R-954 was calculated as follows:

$$(\%\text{inhibition}) = \frac{(\text{STZ value} - \text{control value}) - (\text{STZ/R954 value} - \text{control value})}{(\text{STZ value} - \text{control value})} \times 100$$

Analysis of variance (ANOVA) followed by the "Student–Newman–Keuls Multiple Comparisons Test" were performed to assess significance. $P<0.05$ was considered significant.

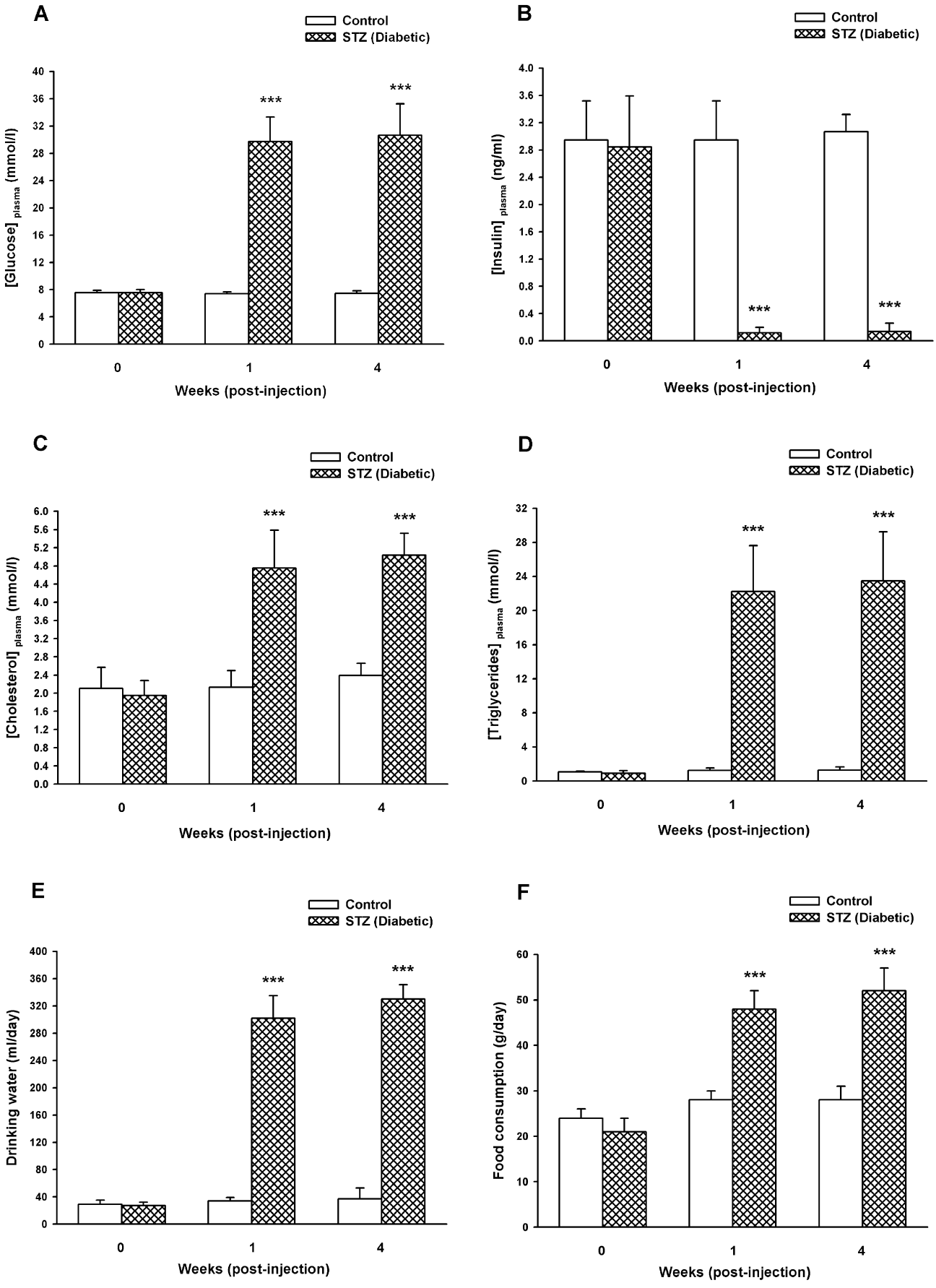
3. Results

3.1. Physiological markers of diabetes

Single treatment of wistar rats with STZ (65 mg/kg, bolus i.p.) produced a marked hyperglycemia (Fig. 1A), hypoinsulinemia (Fig. 1B), hypercholesterolemia (Fig. 1C), hypertriglyceridemia (Fig. 1D), polydipsia (Fig. 1E) and polyphagia (Fig. 1F). The mean plasma glucose concentration, measured 1 week post-STZ administration, was reported at 29.75±3.60 mmol/l and was stable (3.5-fold elevation) for up to 4 weeks (30.66±4.60 mmol/l) in diabetic rats compared to 7.57±0.37 mmol/l in normal healthy rats along the same time. Insulin secretion was almost abolished (96% decrease) after 1 and up to 4 weeks post-STZ injection, from 2.94±0.88 to 0.12±0.08 ng/ml. Both cholesterol and triglycerides increased from 1.95±0.32 to 4.75±0.84 (2.5-fold) and from 0.93±0.29 to 22.22±5.41 mmol/l (24-fold), respectively after 1 week from STZ administration, and remained steadily elevated (2.6- and 25-fold) at 4 weeks post-treatment. Diabetic rats drank and ate much more (7.5- and 8.6-fold, and 1.4- and 1.8-fold), 1 and 4 weeks post-STZ, respectively, than age-matched control Wistar rats.

3.2. Alteration of vascular permeability in STZ-diabetic rats

One week following the injection of STZ, the capillary permeability to albumin-bound Evans blue dye increased in the thoracic aorta, abdominal aorta, renal cortex, renal medulla and spleen of diabetic rats ($70 \approx 67 \gg 26 \approx 31 > 18\%$, respectively) compared to age-matched non-diabetic controls (Fig. 2A–C). The mean values of [Evans blue] in μg/g dry tissue were 97.94±9.86 vs. 29.33±3.26 (thoracic aorta), 111.36±14.23 vs. 33.41±5.82 (abdominal aorta), 266.17±23.35 vs. 197.02±18.21 (renal cortex), 527.42±49.37 vs. 366.73±18.75 (renal medulla) and


A
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C
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E
Control
STZ (Diabetic)
Drinking water (ml/day)
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Control
STZ (Diabetic)
Food consumption (g/day)
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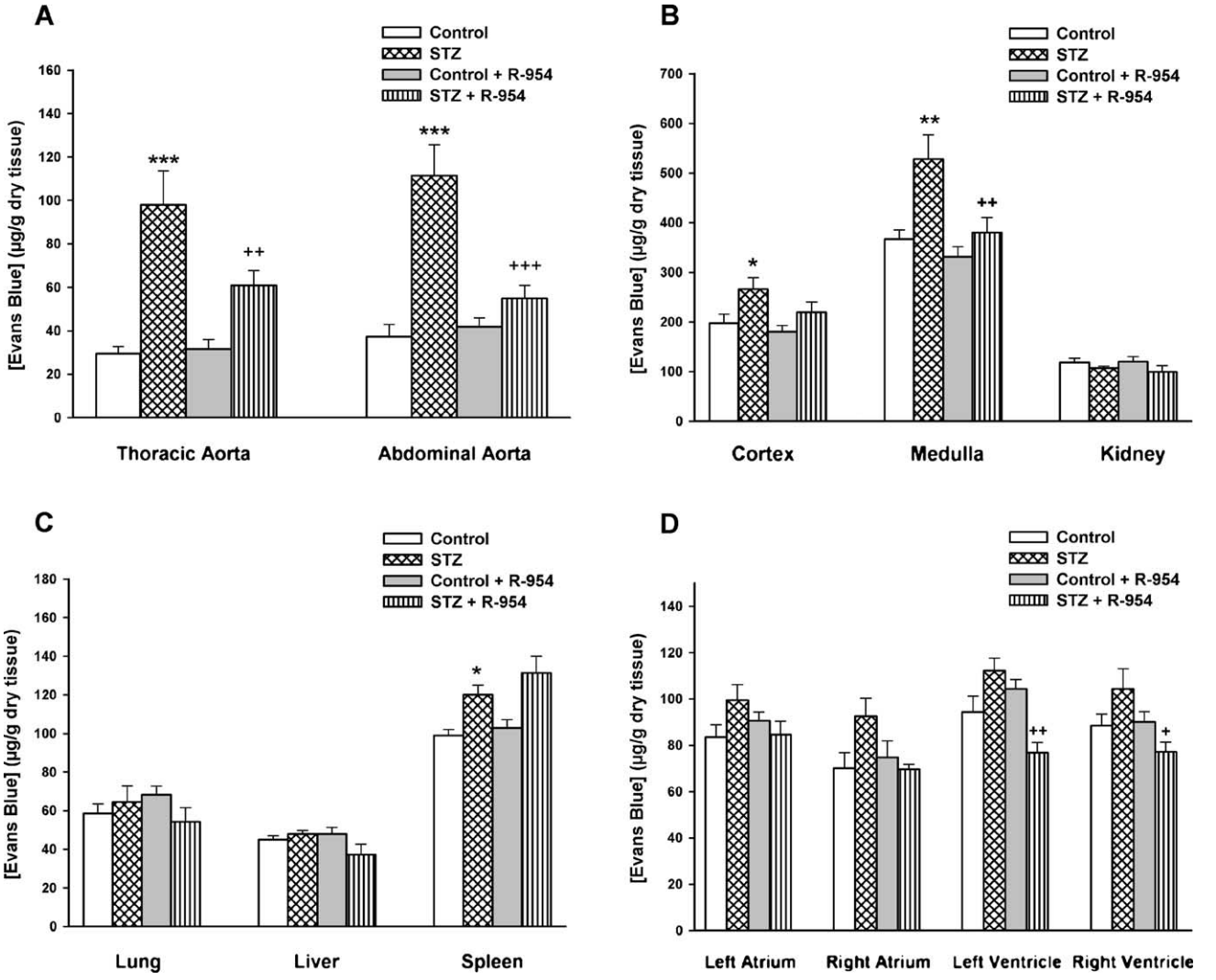

Fig. 2. Effects of the selective bradykinin B_1 receptor antagonist R-954 on altered vascular permeability in 1-week STZ-induced diabetic rats. Type 1 diabetes was induced in male Wistar rats using STZ (65 mg/kg, bolus i.p.). One week following the induction of diabetes, animals were injected with saline or R-954 (2 mg/kg, s.c.) 2 h before the injection of Evans blue dye (20 mg/kg). Non-diabetic control rats injected with saline or R-954 received the same amount of Evans blue dye. After 10 min of Evans blue circulation, capillary permeability was assessed in thoracic and abdominal aortas (A), renal cortex, medulla and kidney (B), lung, liver and spleen (C), both heart atria and ventricles (D), by quantifying the extravasation of albumin-bound Evans blue. Data are expressed as mean [Evans blue] (µg/g dry tissue)±S.E.M. ($n=6–8$). *, ** and *** significantly different from the control non-diabetic group at $P<0.05$, $P<0.01$ and $P<0.001$, respectively; $^{+}$, $^{++}$ and $^{+++}$ significantly different from the STZ-diabetic group at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

120±4.93 vs. 98.94±2.95 (spleen), for the 1-week diabetic rats vs. age-matched controls ($P<0.001$; Fig. 2A, B and C). Non-significant increases in plasma extravasation were observed in the four cardiac compartments compared with healthy control rats ($P>0.05$; Fig. 2D).

Four weeks after the induction of diabetes, the increase in the vascular permeability in the thoracic aorta was less marked but still elevated (up 59%) compared to 1 week (70%) (Fig. 3A). The other tissues were similarly affected at 4 weeks post-STZ compared to 1 week of diabetes with elevation values of $74 \gg 33 \approx 40 > 21\%$ vs. controls, respectively in the abdominal aorta (Fig. 3A), renal cortex and medulla (Fig. 3B), and spleen (Fig. 3C). The mean values of [Evans blue] in µg/g dry tissue were as follows: 173.72±34.45 vs. 45.22±5.45 (abdominal aorta), 265.05±25.12 vs. 178.38±15.53 (renal cortex), 610.68±55.76 vs. 365.28±23.46 (renal medulla) and 123.69±3.70 vs. 97.14±3.54 (spleen) for the 4-week diabetic rats vs. age-matched controls ($P<0.001$). Only after 4 weeks of STZ injection, the left and right atria also showed 27% and

Fig. 1. Physiologica markers of diabetes. Type 1 diabetes was induced in male Wistar rats using STZ (65 mg/kg, bolus i.p.). At 1 and 4 weeks post-STZ injection, circulating plasma concentrations of glucose (A), insulin (B), cholesterol (C), triglycerides (D), water consumption (E) and food consumption (F) were measured in age-matched control-saline compared to STZ-diabetic rats. Data are expressed as mean values±S.E.M. ($n=8–10$). *** significantly different from the control non-diabetic group at $P<0.001$.

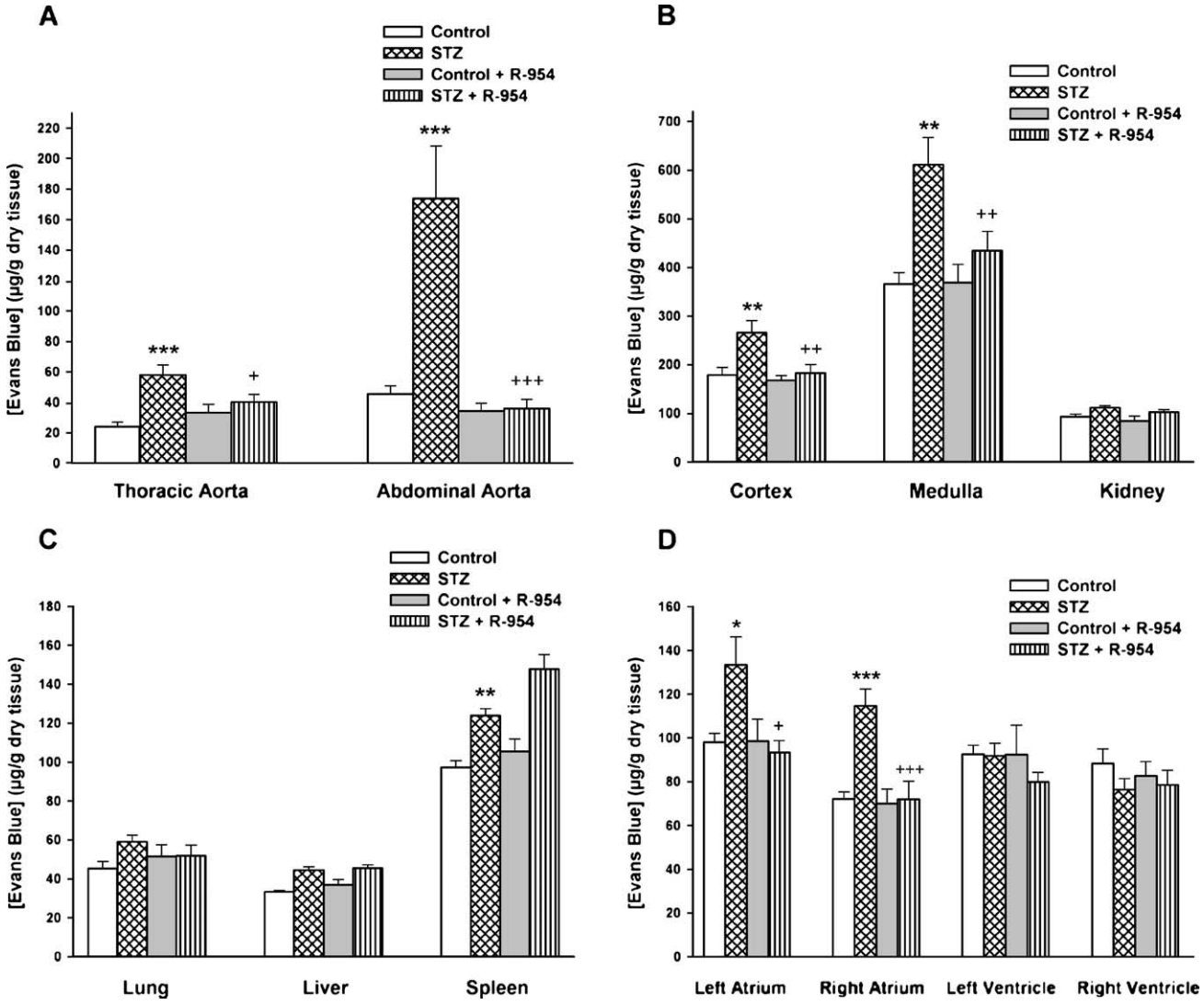

Fig. 3. Effects of the selective bradykinin B_1 receptor antagonist R-954 on altered vascular permeability in 4-week STZ-induced diabetic rats. Type 1 diabetes was induced in male Wistar rats using STZ (65 mg/kg, bolus i.p.). Four weeks following the induction of diabetes, animals were injected with saline or R-954 (2 mg/kg, s.c.) 2 h before the injection of Evans blue dye (20 mg/kg). Non-diabetic control rats injected with saline or R-954 received the same amount of Evans blue dye. After 10 min of Evans blue circulation, capillary permeability was assessed in thoracic and abdominal aortas (A), renal cortex, medulla and kidney (B), lung, liver and spleen (C), both heart atria and ventricles (D), by quantifying the extravasation of albumin-bound Evans blue. Data are expressed as mean [Evans blue] (µg/g dry tissue)±S.E.M. ($n=6–8$). **, *** significantly different from the control non-diabetic group at $P<0.01$ and $P<0.001$, respectively; $^{+}$, $^{++}$ and $^{+++}$ significantly different from the STZ-diabetic group at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

37% increase vascular permeability, respectively (Fig. 3D) compared to 1-week diabetic or 4-week non-diabetic control healthy rats. The mean values of [Evans blue] in µg/g dry tissue were 133.30±12.78 vs. 97.91±4.22 (left atrium) and 114.59±7.69 vs. 72.09±3.11 (right atrium).

No significant changes were observed in both heart ventricles after 4 weeks post-STZ as well as in the lung, liver and kidney taken as a whole, at 1 and 4 weeks after the induction of diabetes.

3.3. Effect of the bradykinin B_1 receptor antagonist, R-954 on altered vascular permeability in STZ-diabetic rats

Following the acute administration of the selective bradykinin B_1 receptor antagonist R-954 (2 mg/kg; s.c.; bolus 2 h pre-injection of Evans blue) to 1-week STZ-diabetic rats, the elevated vascular permeability in the thoracic and abdominal aortas (Fig. 2A) and in the renal medulla (Fig. 2B) was significantly decreased by 58%, 86% and 95%, respectively, towards baseline values in non-diabetic age-matched controls. The mean values of [Evans blue] in µg/g dry tissue were 60.93±6.82 vs. 97.94±15.59 (thoracic aorta), 54.83±6.02 vs. 111.36±14.23 (abdominal aorta) and 379.75±30.05 vs. 527.42±49.37 (renal medulla) in the 1-week diabetic rats treated with R-954 vs. age-matched untreated diabetic rats ($P<0.001$). The R-954-induced decrease in plasma extravasation in the renal cortex was non-significant (Fig. 2B). The bradykinin B_1 receptor antagonist did not reverse the elevated vascular permeability in the spleen (Fig. 2C). On the other hand, treatment of 1-

week diabetic rats with R-954 decreased, but non-significantly ($P>0.05$), the vascular permeability in the left and right atrium and significantly ($P<0.001$) in the left and right ventricle (Fig. 2D) versus age-matched untreated diabetic rats, even though there were no significant increases in plasma extravasation in these 1-week diabetic rats compared to controls.

In the 4-week STZ rats, R-954 significantly reduced the elevated vascular permeability in the thoracic aorta (Fig. 3A) and renal medulla (Fig. 3B) by 48% and 76%, respectively. The mean values of [Evans blue] in μg/g dry tissue were 40.07±5.11 vs. 57.79±6.66 (thoracic aorta) and 433.43±39.73 vs. 610.68±55.76 (renal medulla) in the 4-week diabetic rats treated with the antagonist versus age-matched untreated diabetic rats ($P<0.001$). The same dose of the antagonist totally normalized the permeability in the abdominal aorta (Fig. 3A), renal cortex (Fig. 3B) and in the left and right atria (Fig. 3D).

Noticeably, the treatment with R-954 had no effect on the vascular permeability of all tested organs in age-matched non-diabetic control rats ($P>0.05$).

4. Discussion

The present study showed that STZ-induced type 1 diabetes is associated with a marked increase in the capillary permeability to albumin-bound Evans blue in several rat tissues such as the thoracic and abdominal aortas, the renal cortex and medulla as well as the spleen. This elevated vascular permeability was detected as early as 1 week following the induction of diabetes and hyperglycemia, and remained over 4 weeks in most tissues. Studies conducted in our laboratory also showed that plasma extravasation was increased in the skin and retina of STZ-treated rats (Lawson et al., 2005). The present findings are in agreement and further extend the observations recorded in previous studies carried out in STZ-induced diabetic rats (Chakir and Plante, 1996) and in murine (Simard et al., 2002) models of diabetes which showed that type 1 diabetes is associated with increases in plasma extravasation in many organs.

The regulation of vascular permeability, under both normal and pathophysiological conditions such as diabetes, is linked to the release and the actions of several agents (endothelins, natriuretic peptides, nitric oxide, eicosanoids, prostanoids, growth factors, free radicals or reactive oxygen species) that affect micro-vascular homeostasis (Bassirat and Khalil, 2000; Wilkinson-Berka, 2004). Their gene expression, production, release, degradation and receptor density are also altered in diabetes (Haluzik and Nedvidkova, 2000; Khan and Chakrabarti, 2003). In addition, the diabetic state is accompanied by vascular hypertrophy where tissue remodeling is related to an increase of the extracellular matrix, the formation of advanced glycation end products (AGEs) and the over-production of collagen IV (Williamson et al., 1988; Brownlee et al., 1988; Rumble et al., 1997). However, the precise mechanisms responsible for these increases are not fully established.

The regulation and potential roles of kinins and the bradykinin system have been evoked in diabetes (Margolius, 1989). Diabetes influences renal and vascular responses to bradykinin. The excretion of bradykinin is increased in severely hyperglycemic STZ-rats (Tschope et al., 1996), concomitant to the chronic activation of the inducible bradykinin B_1 receptor subtype (Mage et al., 2002). This up-regulation is likely to be amplified by the accumulation of desArg^{9}bradykinin, the metabolite resulting from the partial degradation of bradykinin at the site of inflammation (Marceau et al., 1998), which is the natural selective agonist of the bradykinin B_1 receptor subtype.

Retinopathy, painful neuropathy and nephropathy are the major complications associated with long-term diabetes. The desArg^{9}bradykinin binding sites were found to be induced in the retina of STZ-rats for up to 21 days following STZ administration (Abdouh et al., 2003) and the exogenous administration of desArg^{9}bradykinin evoked relaxation of the retinal vessels (observed via an increase in diameter). In addition, a significant increase in the level of the bradykinin B_1 receptor mRNA expression in the spinal cord and brain of STZ-diabetic rats (2 and 7 days following the injection of STZ) and of its specific binding sites (2, 7 and 21 days following STZ injection) was reported by Ongali et al. (2004). Indeed, bradykinin B_1 receptor antagonists attenuate diabetic hyperalgesia in murine and rat models that reflect painful diabetic neuropathy in patients (Gabra and Sirois, 2002; 2003a,b; Gabra et al., 2005). Moreover, STZ-diabetic rats, with moderate hyperglycemia, showed increased renal and urinary excretion of active kallikrein and kinin, in conjunction with reduced renal vascular resistance, increased glomerular filtration rate and increased renal plasma flow (Jaffa et al., 1995). The expression of the bradykinin B_1 receptor was reported to be sustained in the renal glomeruli of STZ-rats over 21 days (Christopher and Jaffa, 2002). Finally, a bradykinin B_1 receptor antagonist was found to normalize impaired renal functions in STZ-mice (Zuccollo et al., 1996, 1999).

Here, we studied other diabetic-related complications, investigating the variations in vascular permeability in major blood vessels (aorta), the four heart compartments, the renal cortex and medulla, and other target organs (lung, liver, spleen).

Two major arterial blood vessels are affected alongside diabetes since vascular permeability is increased. Albumin permeability was also increased in the aorta smooth muscle cells of STZ-diabetic rat (Tilton et al., 1992) and the thoracic aorta of diabetic guinea pig (Schlosser and Verlangieri, 1988). The development and presence of vasculopathy have been further suggested in the thoracic aorta of 1-week-old STZ-mice in which the vasodilatory action of endothelium-derived prostacyclin (prostaglandin I_2) on vascular smooth muscles was enhanced (Shen et al., 2003). Thus, an increase

in vascular permeability may constitute the first step toward vasculopathy associated with structural (smooth muscle cell (SMC) hypertrophy, matrix collagen deposition and dysfunction in proteoglycan metabolism) and functional (SMC hyper-reactivity and disturbances of the vaso vasorum microcirculation) abnormalities, characteristics of large artery rigidity (El-Taouil et al., 2003).

Moreover, the diabetic heart develops a condition known as cardiomyopathy with myocardiac hypertrophy as well as intersitial and perivascular fibrosis (Factor et al., 1980). In the present set of experiments, vascular permeability increased in both atria 4 weeks post-STZ, as previously reported by Sitniewska and Wisniewska (2001) in the same model. We did not observe vascular permeability changes in the ventricles of STZ-rats but other reports showed depressed rates of contraction and relaxation (Fein et al., 1984) as well as diastolic dysfunction of the left ventricle of diabetic rats (Mihm et al., 2001). Since young diabetic patients present diastolic dysfunction with a reduction in early diastolic filling and an increase in atrial filling (Schannwell et al., 2002), this effect could be attributed to the changes we observed over atrial permeability. However, the exact mechanisms responsible for the progressive diabetic heart failure remain unknown.

Finally, diabetic nephropathy, that leads to renal failure and dialysis, constitutes one of the most, if not the most severe complications of diabetes. It is often associated with hypertension and characterized by glomerulosclerosis, thickening of the glomeruli basement membrane and expansion of the mesangium due to accumulation of the extracellular matrix (Candido et al., 2002). Our observations that both the cortex and medulla of STZ-rats presented an increase in vascular permeability tend to support these findings.

Major organs such as the lung and the liver were not affected by 1–4 weeks of hyperglycemia (diabetes) in this study. However, in another study conducted with STZ-diabetic rats, the vascular permeability of the upper bronchus was increased over 6 weeks of diabetes (Chakir and Plante, 1996). In a similar study with a murine model of STZ-induced diabetes, the liver plasma extravasation was significantly increased after 3 weeks of diabetes (Simard et al., 2002).

The increase in plasma extravasation in organs affected by vasculopathy, cardiomyopathy and nephropathy was consistently attenuated by treatment with a selective bradykinin B_1 receptor antagonist strongly suggesting that the bradykinin system plays a prominent role in microvascular homeostasis under inflammatory conditions observed in a rat model of type 1 diabetes. Only the microvascular leakage observed in the spleen was not reversed by antagonism of the inducible B_1 receptor subtype of the bradykinin system, suggesting that other mechanisms and/or mediators might also be involved in diabetic conditions including the constitutively expressed bradykinin B_2 receptor (Watanabe et al., 1999), endothelins (Chakir and Plante, 1996; Cukiernik et al., 2004), nitric oxide (Takeda et al., 2001; Cukiernik et al., 2004), prostaglandins (Rudberg et al., 1993) or sorbitol pathway metabolism (Ido et al., 1996) which may even interact with the bradykinin system.

In conclusion, the bradykinin B_1 receptor subtype is induced alongside diabetes and likely plays a strategic role in key organs associated with diabetic complications. We demonstrated that a selective bradykinin B_1 receptor antagonist can significantly reduce, even in some cases reverse, the increased vascular permeability in diabetic rats. These results suggest a novel approach in the treatment of diabetic complications where increased vascular permeability is observed with induction of the bradykinin B_1 receptor subtype.

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