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Enalapril treatment corrects the reduced response to bradykinin in diabetes increasing the B2 protein expression

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ABSTRACT

Considering the growing importance of the interaction between components of kallikrein–kinin and renin–angiotensin systems in physiological and pathological processes, particularly in diabetes mellitus, the aim of the present study was to investigate the effect of enalapril on the reduced response of bradykinin and on the interaction between angiotensin-(1–7) (Ang-(1–7)) and bradykinin (BK), important components of these systems, in an insulin-resistance model of diabetes. For the above purpose, the response of mesenteric arterioles of anesthetized neonatal streptozotocin-induced (n-STZ) diabetic and control rats was evaluated using intravital microscopy. In n-STZ diabetic rats, enalapril treatment restored the reduced response to BK but not the potentiation of BK by Ang-(1–7) present in non-diabetic rats. The restorative effect of enalapril was observed at a dose that did not correct the altered parameters induced by diabetes such as hyperglycemia, glicosuria, insulin resistance but did reduce the high blood pressure levels of n-SZT diabetic rats. There was no difference in mRNA and protein expressions of B1 and B2 kinin receptor subtypes between n-STZ diabetic and control rats. Enalapril treatment increased the B2 kinin receptor expression. From our data, we conclude that in diabetes enalapril corrects the impaired BK response probably by increasing the expression of B2 receptors. The lack of potentiation of BK by Ang-(1–7) is not corrected by this agent.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [15,52]. Insulin resistance of skeletal muscle glucose transport represents a major defect in the normal maintenance of euglycemia [57]. Both the chronic hyperglycemia and the insulin-resistance state in diabetes have been often associated with hypertension and with the development of cardiovascular disease [24,52].

Activation of the renin–angiotensin system (RAS) appears to play an important pathogenic role in diabetes mellitus, contributing not only to beta-cell dysfunction but also to potentiation of the other pathogenic pathways such as

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glucotoxicity, lipotoxicity, and advanced glycation [11]. For this reason, the effects of the drugs of this group on insulin resistance have been highlighted [5,56].

Angiotensin converting enzyme (ACE) inhibitors represent one of the most widely used antihypertensive interventions, and it is important to consider their effects not only on blood pressure but also on relevant metabolic parameters [28,33]. The physiological actions of ACE inhibitors are mediated via reduction in the conversion of angiotensin I to angiotensin II, leading to a reduction in the circulating and local levels of this potent vasoconstrictor and growth factor angiotensin II. In addition, increase in the circulating level of bradykinin (BK), which is a powerful vasodilator, and a modulator of actions of several hormones such as insulin [16,54,10,40,41] also contribute to the effects of ACE inhibitors. Moreover, in diabetes, ACE inhibitors improve endothelial function [42] and the whole-body insulin sensitivity [12] as well as decrease free fatty acid concentrations [51].

The inhibition of RAS using ACE inhibitors consistently and significantly reduces the incidence of type 2 diabetes mellitus. The mechanism underlying this protective effect appears to be complex and various mechanisms may be involved [50,2]. A direct effect of the inhibition of angiotensin II and/or the enhancement of BK on various steps of insulin cascade signaling as well as an increase in glucose transporter GLUT 4 after RAS inhibition has been described [49]. The role of Ang-(1–7), whose levels are enhanced after RAS inhibition, has not been investigated in diabetes mellitus. However, recently it has been suggested that Ang-(1–7) is involved in the beneficial effects of ACE inhibitors, in which increased levels of BK may be implicated [18,50].

In diabetes mellitus, it has been demonstrated that BK can induce translocation of glucose transporter GLUT4, with increase in insulin sensitivity and decrease in plasma glucose and free fatty acids [13]. Therefore, the reduced response to BK observed in diabetes [19,20,21,22,32] might contribute to the pathogenesis of diabetes mellitus.

The enhancement of the biological effect of BK by the interaction between Ang-(1–7) and BK has been intensively demonstrated. Most of the interaction between both the peptides has been reported to occur in blood vessels and endothelial cells seem to be involved in the response [1,8,34,45,47,48]. Potentiation of BK by Ang-(1–7) has been observed in conscious normotensive and hypertensive rats in terms of the BK hypotensive effect in the whole animal [35] or of the vasodilating action of BK in mesenteric microvessels *in situ* [34,45]. In mesenteric resistance vessels of alloxan-diabetic rats (a model of type 1 diabetes) and of n-STZ diabetic rats (a model of type 2 diabetes), Ang-(1–7) induced vasodilatory effect but did not potentiate BK-induced vasodilation, contrasting the observation made in case of non-diabetic rats. The potentiating effect of Ang-(1–7) on BK-induced vasodilation can be restored by chronic but not by acute insulin treatment in both models. In type 1 model, membrane hyperpolarization but not nitric oxide (NO) was demonstrated to be involved in the restoring effect of insulin [44] whereas in type 2 model NO, prostanoids, and membrane hyperpolarization were proposed to be involved [46].

Diabetes mellitus is a rather complex human disease. Several pathogenic processes are involved in its development. Recently, a classification system has included evidences to demonstrate that it is an etiologically and clinically heterogeneous group of disorders that share hyperglycemia in common [52]. In a type 1 diabetes model, enalapril, though capable of restoring BK-reduced response, did not recover the potentiation of BK by Ang-(1–7). Therefore, it is important that diabetes-induced alterations are demonstrated and investigated in different experimental models of the disease.

Considering all these points, the aim of the present study was to evaluate the effect of ACE inhibition on the reduced response to BK and the lack of potentiation of BK-induced vasodilation by Ang-(1–7) in a model different from the one we used previously [18,44]. We chose the neonatal streptozotocin-induced diabetes because it seems to better simulate the human diabetes [4], among other reasons. The study of the interaction between BK and Ang-(1–7) was based on the growing importance of the interaction between components of the kallikrein–kinin system and RAS in diabetes mellitus and the important role of ACE inhibitors in the progression and prevention of diabetes.

2. Material and methods

2.1. General

Diabetes mellitus was induced in male newborn (2-day-old) Wistar rats with an injection of STZ (150 mg/kg, i.p.) dissolved in citrate buffer. Control rats were sham-injected with the same volume of citrate buffer [23]. The STZ-injected and the corresponding control rats were housed according to institutional guidelines (room temperature 22 ± 0.5 °C, 12 h light/dark cycle, 60% humidity, standard rat chow and water *ad libitum*). Twelve weeks after STZ injection, diabetes was confirmed through determination of blood glucose levels by oxidase glucose method using Diastix Reagents strips (Bayer Diagnostics) and determination of urine glucose by God-finder Method (CELM Brazil). The 24-h urinary volume, food and water intake and changes of body weight were monitored. The Animal Ethics Committee of the Institute of Biomedical Sciences of University of São Paulo, Brazil approved the protocol for these experiments (Protocol no. 007, p. 3, book 2), which was in accordance with the guidelines established by the Brazilian College of Animal Experimentation.

2.2. Insulin tolerance test

Insulin tolerance test (itt) was performed according to the method described by Bonora et al. [7], and the values were used to calculate the glucose disappearance constant (K_{itt}).

2.3. Measurement of systolic blood pressure

In unanaesthetized rats, systolic blood pressure levels were determined using the indirect tail-cuff method (PowerLab 4/S pneumatic transducer; AD Instruments, Milford, MA, USA). After a training period of 3 days, the rats were placed in a box that was pre-warmed to 40 °C for 5 min, and three consecutive measurements of blood pressure were recorded and averaged.

Care was taken in selecting the appropriate cuff size for each animal.

2.4. Enalapril treatment

Twelve weeks after STZ injection, 14 n-STZ diabetic rats were subdivided into two groups: untreated and treated with enalapril (10 mg/kg/21 days; by gavage). All the experiments with enalapril-treated rats were performed 12–15 h after the last enalapril dose. The duration of treatment was chosen based on previous studies conducted in our laboratory [18].

2.5. Intravital microscopy

The rats were anesthetized with a subcutaneous injection of chloral hydrate (450–500 mg/kg). The mesentery was exteriorized and arranged for microscopic observation according to Zweifach [58], with slight modifications [19]. Second-order arterioles were distinguished on morphological grounds [6]. Following the application of vasoactive drugs (1.5 pmol of BK and 100 pmol of Ang-(1–7)), changes in vessel diameter were measured. The doses of the vasodilator agents were selected based on the findings of a previous study conducted in our laboratory and were the lowest doses that induce maximum vasodilation [43]. Drugs were removed by flushing with warmed Ringer-Locke solution, after which the initial vessel diameter was restored. For each animal, at least three different microscopic fields were used for diameter estimation.

2.6. Experimental protocols

2.6.1. Effect of BK and Ang-(1–7) alone

The effects of BK (1.5 pmol) and Ang-(1–7) (100 pmol) were tested in control, n-STZ diabetic, and chronically enalapril-treated n-STZ diabetic rats. The doses of BK and Ang-(1–7) were chosen in non-diabetic rats in preliminary experiments.

2.6.2. Effect of Ang-(1–7) on BK-induced vasodilation

The dose of 1.5 pmol of BK was applied, and after an interval of 3 min, Ang-(1–7) (100 pmol) was added to the preparation 30 s before a second application of BK (1.5 pmol). This protocol was tested in control, diabetic, and diabetic rats treated with enalapril.

2.6.3. Effect of HOE-140 on bradykinin-induced vasodilation in diabetic rats treated with enalapril

To interpret the role of B2 BK receptors in the correction of the reduced response of diabetic rats by enalapril, we chose a dose of BK in previous experiments. The dose of 0.15 pmol BK was chosen in previous experiments in control non-diabetic and diabetic rats, and BK at such a dose did not alter the basal diameter in these rats but it did induce a vasodilation in diabetic rats treated with enalapril. HOE-140, B2 BK receptor antagonist was used. The dose and the time of exposure to this antagonist were selected in previous experiments. The following criteria were followed: no alteration of the basal diameter and block in the response to BK in control non-diabetic and diabetic rats. The dose of 100 pmol was chosen and was in accordance with previous studies from our laboratory [18,43].

2.6.4. Immunohistochemical detection of kinin receptors

Immunohistochemistry was realized according to Argañaraz et al., [3]. Mesenteric arterioles (<300 μm in diameter) were fixed in formalin solution (5%, for 24 h) and then submerged in paraffin and transversal sectioning was done. The slices were incubated overnight with primary polyclonal anti-B1 (1:25) and anti-B2 (1:50) receptors (Santa Cruz Biotechnology, CA) in blocking buffer. Experimental protocols on control tissues, in which the primary antibody was replaced by blocking buffer, were also carried out. Antibody anti-B1 and anti-B2 were detected using a biotinylated rabbit anti-goat IgG (Vector, Burlingame, CA; BA-2000) with a dilution of 1:1000. The complex was then incubated with ABC Kit (Elite PK-6102; Vector) and visualized using 3,3′-diaminobenzidine (1 mg/ml in 1% H_2O_2). The sections were then dehydrated and coverslipped. The intensity of brown staining, which is an estimation of the immunoreactivity for B1 and B2 receptor antibodies, was evaluated on a Axioskop Zeiss microscope using a 32× objective at an aperture of 0.40 and was analyzed by light microscopy using bright-field illumination. Immunoreactivity for B1 and B2 receptor antibodies were determined by a computer system (KS-300® Software, ZEISS), which was calibrated to detect the brown color (generated by 3,3′-diaminobenzidine).

2.6.5. Detection of kinin receptors using reverse transcriptase-polymerase chain reaction (RT-PCR)

Mesenteries were dissected, frozen in liquid nitrogen, and stored at −70 °C. Total cellular RNA was isolated from the mesenteric vascular bed using TRizol Reagent (Invitrogen Co., CA, USA), according to the manufacturer's instructions. In order to avoid genomic contamination, RNA samples were treated with DNAse I Amplification Grade. Total RNA (2 μg) was used for first-strand cDNA synthesis (reverse transcriptase—RT) using SuperScript II. RNaseOUT was also added to protect the RNA during this process. To find the ideal conditions for the PCR reaction, a curve of the number of cycles and a curve of temperature of annealing were constructed for each pair of genes studied. Values localized in the linear phase of the curves were chosen. PCR amplifications were carried out on a portion of the cDNA using TaqDNA polymerase and specific oligonucleotide for B1 (forward—GCA TCC CCA CAT TCC TTC TA; reverse—AAG AAG TGG TAA GGG CAC CA) and B2 (forward—CTT GGG TGA GCT CAG TGT CA; reverse—TAG GGG CAG ACA TTT GAA GG) kinin receptors. GAPDH was used as an internal control for the co-amplification (forward—GGTGCTGAGTATGTCGTG; reverse—TTCAGCTCT GGGATGACC). The conditions for PCR were as follows: initial denaturation at 94 °C for 5 min, followed by 38 (B1 receptor), 30 (B2 receptor), or 26 (GAPDH) cycles at 94 °C for 30 seg; annealing temperature of 62 °C for 30 seg, and 72 °C for 30 seg. The final extension step occurred at 72 °C for 10 min. Three pooled RNA aliquots were routinely sham reverse-transcribed (i.e., reverse transcriptase omitted) to ensure the absence of DNA contaminants. PCR products were resolved on 1.6% agarose gel containing 0.5 μg/ml ethidium bromide. The gel was subjected to ultraviolet light and photographed. The band intensities were measured using a software package (Kodak, USA) and the signals were expressed in relation to the intensity of the GAPDH amplicon in each co-amplified sample.

All primers and enzymes utilized in this protocol were purchased from Invitrogen Co. (CA, USA).

2.7. Drugs and reagents

Chloral hydrate and STZ were purchased from Sigma Chemical CO., St. Louis, MO, USA; Ang-(1–7), BK and Ang-(1–7) from Bachem-CA, USA; enalapril from Merck Sharp & Dohme, Campinas, SP, Brazil.

2.8. Statistical analysis

The results are expressed as means ± S.E.M.; the data were analyzed by one-way ANOVA followed by Bartlett's test for homogeneity of variances and by Tukey–Kramer multiple comparisons test when appropriate. Values of $p < 0.05$ were considered significant. The number of rats (n) used in each group is referred to in the text or is presented inside the bars in the figures.

3. Results

3.1. General characteristics of the animals

After 12 weeks of STZ injection, the gain in body weight was lower (Table 1) and blood glucose concentrations were higher in n-STZ diabetic (229.57 ± 36.94 mg/dl; $n = 8$) than in control rats (control 89.8 ± 1.9 mg/dl; $n = 8$). Urine glucose concentrations, food and water intake, and 24-h urine volume were significantly higher in the n-STZ diabetic group. K_{itt} values in n-STZ diabetic rats were lower than those in control rats, indicating insulin resistance. None of these parameters were significantly altered by treatment with 10 mg/day of enalapril for 21 days (Table 1). Blood pressure levels were significantly higher in the diabetic group and enalapril significantly lowered the blood pressure (control group: 119.0 ± 2.7 mmHg, $n = 8$; diabetic group: 135.2 ± 4.1 mmHg, $n = 14^{*}$; diabetic + enalapril group 119.2 ± 2.4 mmHg, $n = 8$; $^{*}p < 0.05$ compared with control and diabetic + enalapril-treated rats).

3.2. Response of mesenteric microvessels in situ

Under resting conditions, there was no difference in the initial diameters of comparable types of arterioles in n-STZ diabetic rats (21.4 ± 0.8 μm, $n = 10$), and their respective controls (18.2 ± 0.5 μm, $n = 13$), or in the enalapril-treated n-STZ diabetic rats (22.2 ± 0.5 μm, $n = 8$). The responses of arterioles to Ang-(1–7) were not altered in n-STZ diabetic rats. Reduced responses to BK were observed in these rats. The vasodilation elicited by BK was significantly potentiated by Ang-(1–7) in control but not in n-STZ diabetic rats. Although enalapril was capable of restoring the reduced BK vasodilation observed in n-STZ diabetic rats, it did not restore the potentiating effect of Ang-(1–7) on BK vasodilation (Fig. 1). The restored responses to BK observed in enalapril-treated diabetic rats were almost completely blocked by HOE-140, a B2 BK receptor antagonist (before 6.0 ± 0.4% and after 1.0 ± 0.1%, $n = 6$, $^{*}p < 0.05$).

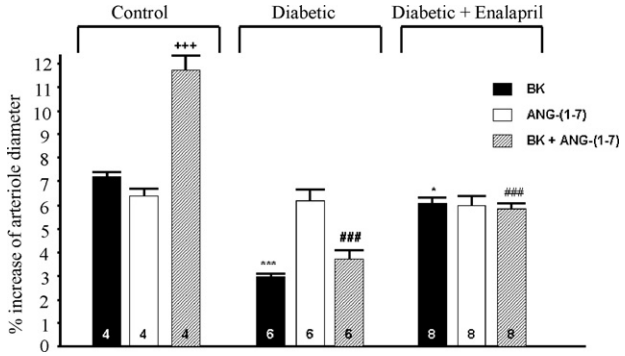

Fig. 1 – Bar graphs showing the increase in arteriolar diameter (in %) induced by topical application of bradykinin (BK), angiotensin-(1–7) [Ang-(1–7)], and BK + Ang(1–7) in control, untreated, and enalapril-treated n-STZ diabetic rats. The results are means ± S.E.M. The number of rats per group is shown inside the bars. $^{+++}p < 0.001$ versus BK; $^{***}p < 0.001$ versus BK in control rats; $^{###}p < 0.001$ versus BK + Ang-(1–7) in control rats, $^{*}p < 0.001$ versus BK in diabetic rats.

3.3. Immunohistochemical detection of kinin receptors

There was no difference in the protein expression of B1 and B2 kinin receptors between n-STZ diabetic and control rats (Fig. 2). Enalapril treatment increased B2 protein expression without interfering with B1 kinin receptor expression in mesenteric arterioles of n-STZ diabetic (Fig. 2).

Table 1 – General characteristics of the animals

Group	Urine glucose (mg/dl)	Urine volume (ml/24 h)	Water intake (ml/24 h)	Food intake (g/24 h)	Body weight (g)	K_{itt} (%min)
Control	<25	2.5 ± 0.7 $n = 5$	14.2 ± 3.2 $n = 5$	9.8 ± 1.6 $n = 5$	266.7 ± 7.5 $n = 7$	4.34 ± 0.3 $n = 5$
Diabetic	>1000	23.7 ± 8.6* $n = 5$	83.4 ± 15.6** $n = 5$	28.8 ± 3.0* $n = 5$	190.1 ± 6.5** $n = 8$	0.76 ± 0.47*** $n = 7$
Diabetic + enalapril[a]	>1000	30.2 ± 10.9* $n = 5$	85.33 ± 23.2* $n = 6$	27.9 ± 6.00* $n = 8$	189.1 ± 15.7** $n = 8$	1.23 ± 0.45*** $n = 6$

The results are presented as means ± S.E.M.
* $p < 0.05$ in comparison with control.
** $p < 0.01$ in comparison with control.
*** $p < 0.001$ in comparison with control.
[a] n-STZ diabetic rat treated with enalapril (10 mg/kg/day/v.o.). n = Number of animals.

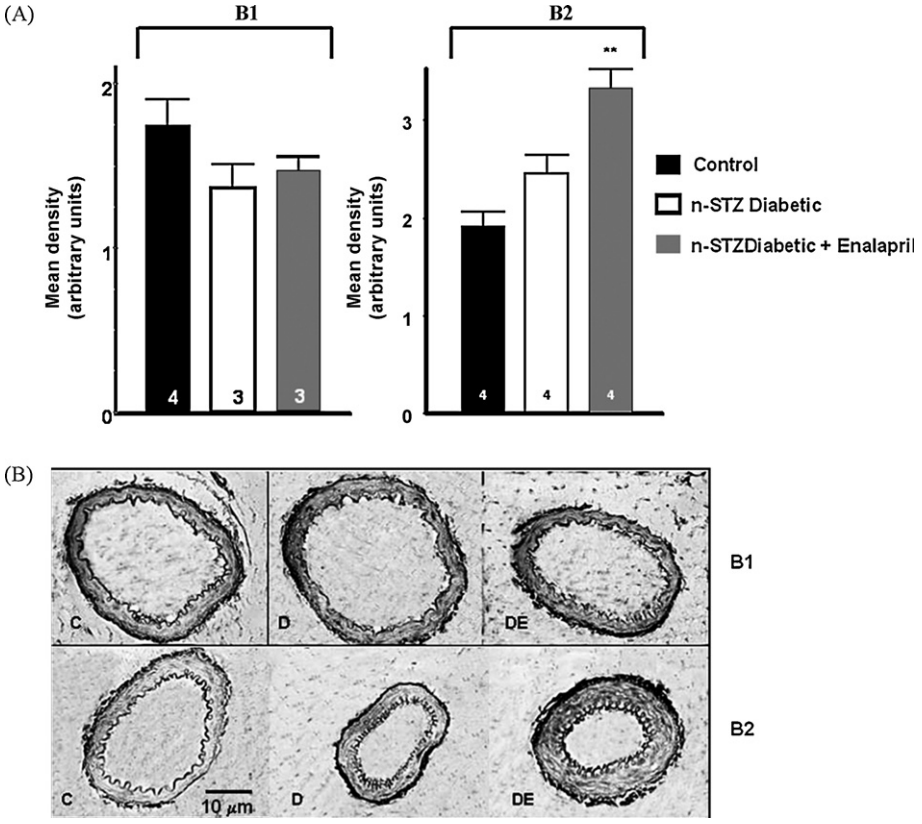

Fig. 2 – Immunohistochemical analysis (A) and representative images staining (B) of B1 and B2 kinin receptors in the mesenteric arterioles of control (■), n-STZ diabetic (□) and enalapril-treated n-STZ diabetic rats (▩). C1 and C2 = control, D1 and D2 = n-STZ diabetic, DE 1 and DE 2 enalapril-treated n-STZ diabetic rats. (A) The bar graphs show the mean density for B1 and B2 kinin receptors. Values are expressed as means ± S.E.M. $^{**}p < 0.001$ versus control. (B) Magnification, 950×

3.4. *Kinin receptors mRNA expression*

There was no difference in mRNA of B1 and B2 kinin receptors between n-STZ diabetic and control rats (Fig. 3). Enalapril treatment did not alter the expression for mRNA levels of kinin receptors (Fig. 3).

4. Discussion

In the present study, we demonstrated that enalapril treatment corrected the reduced response to BK observed in n-STZ diabetic rats. The lack of potentiation of BK by Ang-(1–7), observed in these rats, was not corrected by enalapril treatment. Increased expression of B2 BK receptors might be involved in the restorative effect of enalapril.

Characteristics of human diabetes can be found in animals in which diabetes is experimentally induced. The n-STZ model can be considered as a suitable animal model of type 2 diabetes mellitus [25,27,30,55]. In this model, various stages of type 2 diabetes such as impaired glucose tolerance as well as mild, moderate, and severe hyperglycemia can be observed. The n-STZ diabetic rats can exhibit slightly lowered plasma insulin levels, slightly elevated plasma glucose levels, and lowered pancreatic insulin content [4].

In the present study, rats that received STZ in the neonatal period (2 days of age) presented characteristics similar to human diabetes such as polyuria, urine glucose, polydipsia, polyphagia, and lower body weight gain (Table 1). These characteristics are also found in models of type 1 diabetes such as the alloxan model. However, alloxan-induced diabetes compared with neonatal STZ-induced differs in toxicity mechanism, the age of induction of experimental diabetes, and the time needed to achieve the full development of metabolic alterations. Alloxan administered to adult rats causes a complete ablation of insulin production, resulting in severe diabetes after 3 days of insulin production. In the neonatal STZ diabetes model, the drug is administered to 2-day-old rats. Similar to alloxan, STZ destroys B pancreatic cells, but part of the B cells can be regenerated depending on the age at which it was administered. Consequently, they did not show complete ablation of insulin production and gradually developed diabetes, moreover, diabetes was completely established when they became adults [4]; therefore, in spite of similar characteristics of both models, such as insulin resistance, impaired weight gain, large increase in urine volume, we chose n-STZ model because it better simulates human diabetes, i.e., the diabetes and its complications develop gradually and becomes more aggravated in the adult life. In addition, the n-STZ diabetic rats exhibited glucose

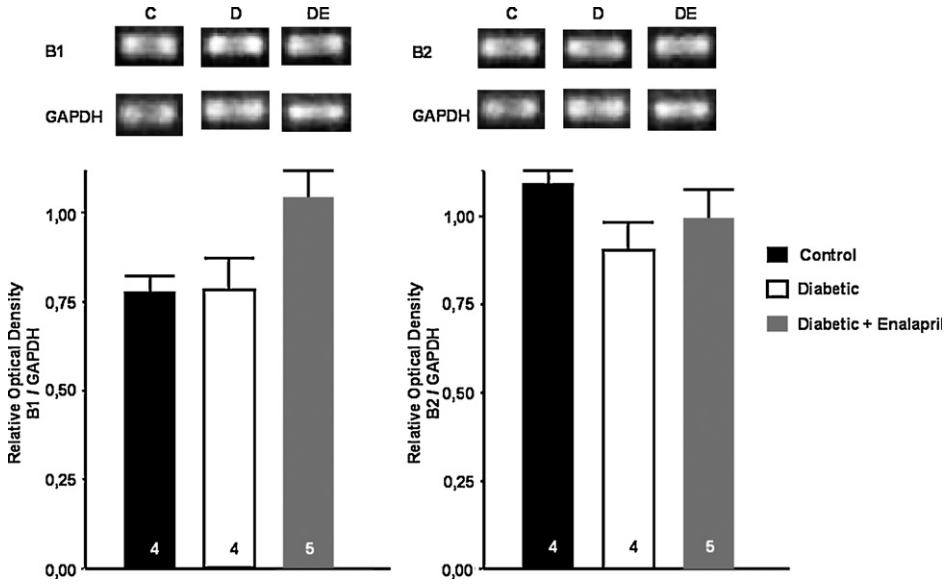

Fig. 3 – (Top) Representative RT-PCR products of 2 μg total RNA extracted from mesenteries of control (C), n-STZ diabetic (D), and enalapril-treated n-STZ diabetic (DE) rats. (Bottom) Bar graphs show the relative optical density values for B1 and B2 kinin receptors. The corresponding RT-PCR products are normalized values for GAPDH, used as internal control. Values are expressed as means ± S.E.M.

intolerance, insulin resistance (lower K_{itt} values) (Fig. 1) and changes in arterial blood pressure, characteristics that can be found in diabetic patients as well.

The fact that no difference was found in the initial arteriolar diameters between n-STZ diabetic and control rats suggests that diabetes mellitus had no effect on the microvascular caliber under basal conditions. Enalapril did not modify the initial arteriolar diameters as well, indicating that the alterations we observed were not due to an effect of the treatments on the basal vascular tonus.

Despite the fact that enalapril treatment was not capable of restoring most of the parameters altered by diabetes, it significantly lowered the blood pressure levels. This finding indicates that the treatment was effective in inhibiting ACE.

Similar to the finding in type 1 diabetes mellitus [44], we demonstrated that the BK response was reduced and the response to Ang-(1–7) was unaltered in arterioles of n-STZ diabetic rats. Enalapril treatment restored the decreased response without correction of the altered metabolic parameters. The fact that the restoration effect of enalapril treatment (Fig. 2) occurred without correction of all the metabolic parameters and that it was observed after chronic treatment might indicate that the effect of the ACE inhibitor is time-dependent and independent of the full recovery of the metabolic alterations.

Several factors can contribute to the restorative effect of enalapril treatment on the reduction such as ACE-BK B2 receptors ''cross-talk'' that goes beyond blocking hydrolysis. Also, it has been shown in CHO cells transfected with B2 receptors and ACE as well as in endothelial cells that ACE inhibitors potentiate BK effects by increasing the number of cell-surface B2 receptors, blocking B2 receptor desensitization and decreasing B2 receptor internalization [39,17,37]. All these alterations might be involved in the potentiation of BK action by ACE inhibitors and could promote more efficient signal transduction in response to activation of BK B2 receptors.

An increase in the number of B2 receptors could well explain our data in n-STZ diabetic rats. An interference of type 2 diabetes with gene expression of these receptors could explain the altered response to BK and consequently enalapril, interfering with it, would correct the alterations observed. We could exclude this hypothesis since no alteration in mRNA for BK B2 receptors could be found. However, although enalapril treatment did not alter the expression mRNA of B2 kinin receptors, B2 protein expression was increased after the treatment of n-STZ diabetic rats. Therefore, the restorative effect of enalapril might be at least partially explained by an increased number of B2 receptors. The fact that HOE-140, a B2 BK receptor antagonist, almost abolished the vasodilation induced by BK in diabetic rats treated with enalapril supports this hypothesis.

Besides the effects of ACE inhibitors upon B2 receptors, these agents also have some effect on B1 receptors. They induce B1 receptor upregulation [38] and can act as B1 receptor agonists, stimulating NO release at nanomolar concentrations [29]. This mechanism can explain the restorative effect of enalapril on the vasodilation induced by BK. However, since we could not find an alteration of either mRNA or protein B1 receptor, this hypothesis seems not plausible.

Ang-(1–7) potentiates several BK effects, including its vasodilatory effect on isolated dog coronary arteries [8], the vasoconstrictor action of BK on rabbit jugular vein [26], and the hypotensive effect of BK in normotensive [45] and hypertensive [35] rats.

In a previous study, we demonstrated that in *in vivo in situ* mesenteric microvessel preparations of normotensive rats, Ang-(1–7) increases the BK-induced vasodilation through release of NO and cyclooxygenase (COX) products [43]. Also

the potentiation of BK by Ang-(1–7) *in vivo* was demonstrated to be dose-dependent and independent of ACE blockade [35]. However, in a model of type 1 diabetes mellitus, though Ang-(1–7) response was not altered, this agent was not capable of potentiating BK vasodilation in mesenteric arterioles *in vivo* [44]. Similarly, although enalapril restored the decreased response to BK in arterioles of type 2 diabetic rats, the potentiating effect of Ang-(1–7) on BK vasodilation was not restored after treatment. This might indicate that restoration of BK-reduced response is not sufficient to overcome the potentiating effect of Ang-(1–7). Lack of potentiation was also demonstrated in human subjects treated with ACE inhibitors [14].

The lack of potentiation cannot be explained by our data; however, we may speculate that the increased Ang-(1–7) by many folds [9,31,47] with ACE inhibition would hinder any further effect of exogenous administration of the peptide [36].

Another possibility for explaining the absence of potentiation after enalapril treatment could be that, at high concentrations, interaction of Ang-(1–7) with AT1 receptors would induce vasoconstriction. The vasoconstricting effect would oppose the vasodilating effect of BK, reducing the potentiating effect of Ang-(1–7). This is a plausible hypothesis since it has been demonstrated that at high levels, Ang-(1–7) stimulates AT1 receptors [53].

In summary, we demonstrated that, similar to that observed in a type 1 model of diabetes, Ang-(1–7) did not potentiate BK vasodilation in a type 2 model of diabetes mellitus as it does in non-diabetic rats. In n-STZ diabetic rats, enalapril restoring effect on BK response might involve, besides the well-known effect reducing the degradation of BK, the increase in BK B2 protein expression in mesenteric arterioles. Our data allow us to speculate that restoration of the reduced response to BK is not sufficient to recover the potentiation of BK by Ang-(1–7) which is absent in diabetes.

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