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Enhanced nitric oxide generation from nitric oxide synthases as the cause of increased peroxynitrite formation during acute restraint stress: Effects on carotid responsiveness to angiotensinergic stimuli in type-1 diabetic rats

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ABSTRACT

Diabetes mellitus is associated with reactive oxygen and nitrogen species accumulation. Behavioral stress increases nitric oxide production, which may trigger a massive impact on vascular cells and accelerate cardiovascular complications under oxidative stress conditions such as Diabetes. For this study, type-1 Diabetes mellitus was induced in Wistar rats by intraperitoneal injection of streptozotocin. After 28 days, cumulative concentration-response curves for angiotensin II were obtained in endothelium-intact carotid rings from diabetic rats that underwent to acute restraint stress for 3 h. The contractile response evoked by angiotensin II was increased in carotid arteries from diabetic rats. Acute restraint stress did not alter angiotensin II-induced contraction in carotid arteries from normoglycaemic rats. However acute stress combined with Diabetes increased angiotensin II-induced contraction in carotid rings. Western blot experiments and the inhibition of nitric oxide synthases in functional assays showed that neuronal, endothelial and inducible nitric oxide synthase isoforms contribute to the increased formation of peroxynitrite and contractile hyperreactivity to angiotensin II in carotid rings from stressed diabetic rats. In summary, these findings suggest that the increased superoxide anion generation in carotid arteries from diabetic rats associated to the increased local nitric oxide synthases expression and activity induced by acute restrain stress were responsible for exacerbating the local formation of peroxynitrite and the contraction induced by angiotensin II.

1. Introduction

Diabetes mellitus is associated with accelerated cardiovascular complications, which are the major cause of morbidity and mortality among diabetic patients (Zheng et al., 2014). The pathophysiological mechanisms underlying type 1-diabetic cardiovascular events are not well understood (de Ferranti et al., 2014). The beginning and the progression of cardiovascular diseases have been attributed not only to hyperglycemia and inflammation but also to reactive oxygen and nitrogen species accumulation (Giacco and Brownlee, 2010; Fiorentino et al., 2013; Moreira et al., 2015). High glucose levels enhance superoxide anion generation, which leads to a pro-oxidant environment (Pandolfi and Filippis, 2007) and the subsequent induction of multiple mechanisms such as polyol pathway, cytochrome P450 monooxygenases and lipoxygenase activation, NADPH oxidase assemble, nuclear transcription factor κb activation, and nitric oxide synthases uncoupling (Valko et al., 2007; Giacco and Brownlee, 2010).

Nitric oxide is synthesized within endothelial cells during conversion of L-arginine to L-citrulline by a family of enzymes named nitric oxide synthases including the neuronal, the endothelial and the inducible isoforms (Sena, 2013). However, several studies have reported that the impaired nitric oxide synthesis and signaling are commonly observed in Diabetes (Williams et al.,

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1996; Pandolfi and Filippis, 2007).

Several studies have shown that behavioral stress increases nitric oxide production seemly to protect normotensive rats from stress-induced hypertension (Slezak et al., 2014). Recent findings report a sustained overproduction of nitric oxide via inducible nitric oxide synthase in some tissues such as rat brain (Olivenza et al., 2000) and aorta (Slezak et al., 2014).

Emotional reactions may trigger a massive impact on cell or organ function and pathophysiological mechanisms (Steptoe and Kivimäki, 2012). The body responds to many experiences by releasing chemical mediators that promote adaptation to the acute effects induced by stress mediators. Such adaptation contributes to the development of cardiovascular diseases (Kamarck et al., 1997; Kral et al., 1997; Sgoifo et al., 2001; McEwen, 2000).

The enhanced generation of nitric oxide does not suggest a higher nitric oxide bioavailability, especially under oxidative stress such as in Diabetes (Jay et al., 2006) or acute behavioral stress (Zafir and Banu, 2009; Puzserova et al., 2013). Therefore, acute behavioral stress associated with a redox imbalance in cells from diabetic rats might provide new insights on the mechanisms underlying the accelerated cardiovascular complications in Diabetes (Pandolfi and Filippis, 2007).

2. Materials and methods

The study was performed in adult male Wistar rats supplied by the Central Vivarium from the University of São Paulo (USP). Room temperature was regulated to 22 °C, relative humidity was kept at 60% and a 12 h light–12 h dark cycle was used. Animals had free access to food and water. The experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and approved by Ethics Committee on Animal Use (CEUA) from USP (Ribeirão Preto campus) in Brazil (protocol number: 13.1.441.53.2).

2.1. Type-1 Diabetes induction

Type-1 Diabetes was induced in eight-weeks-old male Wistar rats (350–400 g) by a single dose of streptozotocin (50 mg/kg) dissolved in citrate buffer (0.09 mol/l, pH 4.5) (Pernomian et al., 2012). Blood samples were taken from the tail vein prior to and 72 h (day 2) after streptozotocin injection to measure fasting glucose levels using an one-touch glucometer (Life Scan Inc., Milpitas, CA, USA) (Pernomian et al., 2013a). Diabetic rats presented a fasting glycaemia higher than 300 mg/dl (Pernomian et al., 2014) (Table 1).

2.2. Acute restraint stress

Acute stress intervention was performed by a single section of 3 h-lasting restraint stress model as previously described (Moreira et al., 2015). Serum corticosterone levels were determined as the standard protocol to validate acute stress. Normoglycaemic or diabetic rats (12-weeks-old) were exposed to acute restraint stress for 3 h (Kennett et al., 1985) in acrylic tubes (6.5 cm in diameter, adjustable in length of 20–30 cm) containing side holes to enable animal breath. Corticosterone levels were measured by radioimmunoassays in plasma samples collected immediately after acute stress for validating animal stress response (Table 2).

Table 1
Rat fasting glycaemia.

Rats	Glycaemia (mg/dl)	
	Day 0	Day 2
Non-treated (normoglycaemic)	94.5 ± 6.19	93.81 ± 8.17
Streptozotocin (STZ)-treated (diabetic)	97.3 ± 7.41	391.11 ± 10.931[a,b]

Data represent the mean ± S.E.M. (n 10). One-way ANOVA, Bonferroni post-hoc test.

[a] Significant difference ($P < 0.001$) from the non-treated group at the same day.
[b] Significant difference ($P < 0.001$) from the non-treated group at the same group at day 0.

Table 2
Rat corticosterone serum levels.

Rats	Corticosterone level (ng/ml)	
	Control (non-stressed)	Stressed
Normoglycaemic	27.51 ± 9.43	118.36 ± 16.15[a]
Diabetic	29.16 ± 6.78	74.81 ± 7.44[a,b,c]

Data represent the mean ± S.E.M. (n 7). One-way ANOVA, Bonferroni post-hoc test.

[a] Significant difference ($P < 0.001$) from the control normoglycaemic group.
[b] Significant difference ($P < 0.001$) from the control diabetic group.
[c] Significant difference ($P < 0.001$) from the stressed normoglycaemic group.

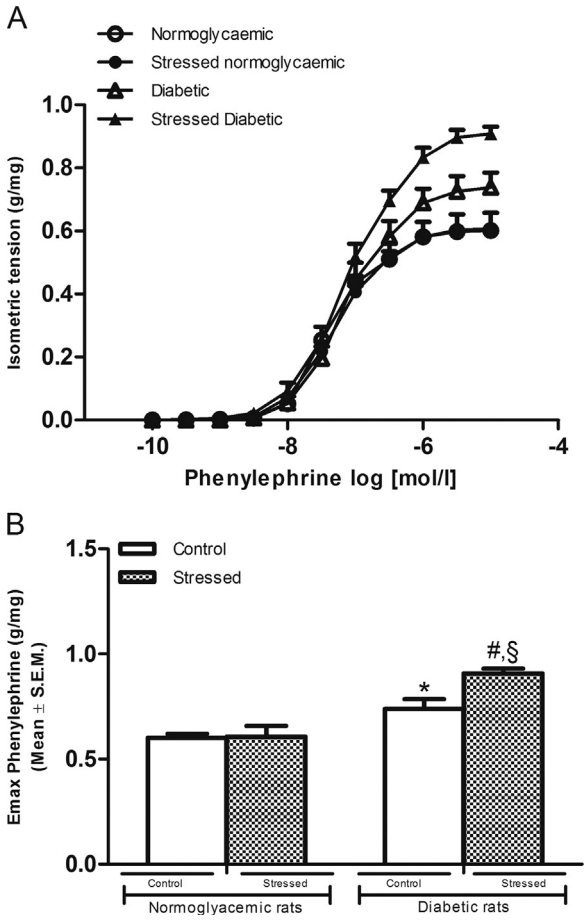

Fig. 1. Phenylephrine (Phe)-induced contraction in endothelium-intact carotid arteries from control (non-stressed) or stressed normoglycaemic or diabetic rats: (A) Phe concentration response curves in carotid arteries from control or stressed normoglycaemic or diabetic rats; (B) Maximum effect (Emax) values for Phe in carotid arteries from control or stressed normoglycaemic or diabetic rats. Significantly different ($P < 0.05$; n = 10; mean ± S.E.M.) from carotid arteries from control normoglycaemic (*), stressed normoglycaemic (#) or control diabetic (§) rats. One-way ANOVA, Bonferroni post-hoc test.