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Diabetes confers a vasoprotective role to the neurocompensatory response elicited by carotid balloon injury: Consequences on contralateral carotid tone and blood flow

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ABSTRACT

The purpose from this study was to investigate the consequences of sensory neurocompensation to carotid balloon injury in diabetic rats on angiotensin II-induced contraction and basal blood flow in contralateral carotid. Concentration-response curves for angiotensin II and blood flow were obtained in contralateral carotid from non-treated or capsaicin-treated streptozotocin-induced diabetic rats that underwent carotid balloon injury. Diabetes increased angiotensin II-induced contraction and impaired the blood flow in non-operated rat carotid. In diabetic rats, balloon injury led to neointima formation, which reduced the blood flow in ipsilateral carotid. Carotid balloon injury in diabetic rats reduced angiotensin II-induced contraction and restored the blood flow in contralateral carotid when compared to diabetic non-operated rat carotid. Capsaicin inhibited the effects evoked by carotid balloon injury on diabetic rat contralateral carotid. Endothelium removal, PEG-catalase (hydrogen peroxide scavenger) or L-NPA (neuronal nitric oxide synthase, *n*NOS, inhibitor) increased angiotensin II-induced contraction in contralateral carotid from diabetic operated rats to the levels observed in diabetic non-operated rat carotid. Our findings suggest that carotid balloon injury in diabetic rats elicits a neurocompensation that attenuates the diabetic hyperreactivity to angiotensin II in contralateral carotid by a sensory nerves-dependent mechanism mediated by hydrogen peroxide derived from endothelial *n*NOS. This sensory mechanism also restored the blood flow in this vessel, compensating the impaired blood flow in diabetic rat ipsilateral carotid. Thus, our major conclusions are that Diabetes confers a vasoprotective significance to the neurocompensation to carotid balloon injury in preventing further damage at carotid cerebral irrigation after angioplasty in diabetic subjects.

1. Introduction

Type I-Diabetes is an important risk factor for carotid atherosclerosis, which can impair cerebral irrigation and lead to stroke (Gül et al., 2010; Polak et al., 2011). Balloon angioplasty has been effectively used to restore blood flow in atheromatous carotid from diabetic patients (Brown, 2001). However, its beneficial outcomes have been limited by post-angioplasty complications (Pernomian et al., 2011), which has been widely studied trough balloon catheter injury model in rat carotid. In this model, the introduction of balloon catheter into carotid leads to endothelial denudation and medial disruption, so that the injured artery is called ipsilateral carotid (Clowes et al., 1983).

Until 1997, researchers often used contralateral (non-injured) carotid as the control parameter for ipsilateral carotid, since none morphological change had been described for contralateral artery (Antonaccio et al., 1994). But in 1997, Milner et al. observed that balloon injury reduces the expression of sensory nerves in ipsilateral carotid, which triggers a neurocompensatory response that increases these nerves expression in contralateral carotid. Thereafter, our laboratory showed that the neurocompensatory response to balloon injury increases angiotensin II-induced contraction in contralateral carotid without changing its blood flow (Accorsi-Mendonça et al., 2004). The contractile hyperreactivity to angiotensin II in contralateral carotid is important to compensate the hyporesponsiveness of ipsilateral carotid to angiotensin II, while the normal blood flow in contralateral artery compensates

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the impaired blood flow in the restenotic ipsilateral artery (Accorsi-Mendonça et al., 2004), which avoids further damages in carotid cerebral irrigation.

Although these findings point a pathophysiological significance to compensatory response elicited by balloon injury in carotid cerebral irrigation, they represent a phenomenon that occurs only in healthy conditions. Usually, clinical carotid angioplasty is performed in disease conditions characterized by vascular dysfunction, such as Diabetes (Brown, 2001). Furthermore, type I-Diabetes induces the loss of sensory perivascular innervation (Cellek et al., 2005; Sima, 2006), which mediates the neurocompensatory response to balloon injury (Milner et al., 1997). Based on these, we hypothesized that type I-Diabetes would abrogate the neurocompensatory response to carotid balloon injury. Considering that type I-Diabetes promotes a higher increase in angiotensin II-induced contraction in rat carotid (Pernomian et al., 2012) than that one induced by neurocompensatory response to balloon injury (Accorsi-Mendonça et al., 2004), we also hypothesized that the abrogation of this neuronal mechanism would keep the diabetic hyperreactivity to angiotensin II in contralateral carotid, which could be enough to impair the local blood flow. According to this hypothesis, the impaired blood flow in contralateral carotid, added to the reduced ipsilateral carotid flow, could lead to further damage in carotid cerebral irrigation after angioplasty during type I-Diabetes. Thus, we aimed to investigate the consequences of balloon injury on angiotensin II-induced contraction in contralateral carotid from type I-diabetic rat, as well as the influence of the reactivity changes on blood flow in this vessel. Since oxidative stress induced by uncoupled nitric oxide synthases (NOS) is a marker from type I-Diabetes (Szabó, 2009), we also investigated the role of reactive oxygen species and NOS in these reactivity changes.

2. Materials and methods

All the experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals. A prior approval was granted by the Animal Ethics Committee of the Faculty of Medicine from Ribeirão Preto (FMRP) from the University of São Paulo (USP) in Brazil (approval reference number: 007/2009). The animals used in this study were kept under a 12 h light:12 h darkness cycle (light from 06:00 to 18:00 h) and fed with regular chow and water *ad libitum*.

2.1. Type I-Diabetes induction

8-weeks-old male Wistar rats (350–400 g) underwent to a single intraperitoneal injection of streptozotocin (STZ, 55 mg/kg) dissolved in citrate buffer (0.09 mol/l, pH 4.5) (day 0) (Pernomian et al., 2012). A positive control group was composed by age-matched normoglycaemic rats that underwent to citrate buffer injection. Fasting glucose levels were determined from rat tail blood samples prior to and 48 h (day 2) after STZ-injection, using a one-touch glucometer (LifeScan Inc., Milpitas, CA, USA). Diabetic rats presented a glycaemia higher than 300 mg/dl (Pernomian et al., 2012) (Table 1).

2.2. Balloon catheter injury

Four weeks after STZ- or vehicle-injection (day 28) (Yousif et al., 2006), balloon injury was performed in the left carotid artery. Rats were anaesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg), by intraperitoneal injection. The depth of the anaesthesia was assessed by the loss of the motor reflexes and by the immobility after pinching the rat tail tip. The left common carotid was exposed for access of a 2F Fogarty balloon catheter, which was distended and passed three times through the carotid. The catheter was removed, the external carotid was ligated and the wound was closed. In anaesthetized sham operated rats, the left common carotid was exposed and the external carotid artery was ligated without performing catheterisation (Accorsi-Mendonça et al., 2004; Pernomian et al., 2011).

Table 1
Fasting blood glucose levels from intact no treated, vehicle- or STZ-treated rats.

Groups	Glucose level (mg/dl)	
	Day 0	Day 2
No treated (normoglycaemic)	79.3 ± 3.62	77.4 ± 1.24
Vehicle-treated	75.4 ± 2.95	80.7 ± 3.41
STZ-treated (diabetic)	81.1 ± 1.68	364.7 ± 7.43[a,b]

Data represent the mean ± S.E.M. ($n=11$).

[a] Significant difference ($P<0.001$) from the no treated group at the same day.

[b] Significant difference ($P<0.001$) from the no treated group at the same group at day 0.

To investigate if the compensatory response evoked by carotid balloon injury is a sensory mechanism, some newborn rats (two-day old) underwent to sensory chemodenervation by capsaicin treatment (50 mg/kg, subcutaneous injection into the dorsal region of the neck, under ice anaesthesia) (Newson et al., 2005) before Diabetes induction.

2.3. Experimental groups

Fifteen days after balloon injury (Accorsi-Mendonça et al., 2004), the fasting glucose was measured and the experiments were performed. For the *in vitro* assays, the rats were sacrificed by abdominal aortic exsanguination and the common carotid arteries were isolated. The experimental groups used in all protocols included: (1) the control carotid from normoglycaemic intact (non-operated) rat; (2) the ipsi- (left) and (3) contralateral (right) carotid arteries from normoglycaemic operated rat; (4) the control carotid from diabetic intact rat; and (5) the ipsi- and (6) contralateral carotid arteries from diabetic operated rat. Some protocols also required a few more experimental groups. For instance, the validation of STZ-induced Diabetes by measuring fasting glucose levels required blood samples from citrate buffer-treated (intact) rats. In turn, the validation of the consequences of balloon injury on carotid responsiveness required the carotid from normoglycaemic sham-operated rat in the *in vitro* arterial reactivity studies. Furthermore, in order to assess the role of sensory nerves on the functional consequences triggered by the neurocompensatory response to carotid balloon injury, the following additional groups were included in the functional assays (*i.e.*, the *in vitro* arterial reactivity studies and the *in vivo* carotid blood flow and blood pressure measurement): (1) the control carotid from capsaicin-treated normoglycaemic intact rat; (2) the ipsi- and (3) contralateral carotid arteries from capsaicin-treated normoglycaemic operated rat; (4) the control carotid from capsaicin-treated diabetic intact rat; and (5) the ipsi- and (6) contralateral carotid arteries from capsaicin-treated diabetic operated rat.

2.4. Histological analysis of carotid rings

Carotid arteries were fixed *in situ* with formalin (10%) for 24 h, and then embedded in paraffin. The 3 μm-cut sections of the paraffin-included carotid rings were stained with hematoxylin and eosin (HE) to morphological and morphometrical analysis, by using an optic microscopy coupled to a digital camera (Coolpix 4500, Roper Scientific, Japan). The images were edited in the Adobe Photoshop