European Journal of Pharmacology 723 (2014) 397–404

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Impairment of α_1-adrenoceptor-mediated calcium influx in contralateral carotids following balloon injury: Beneficial effect of superoxide anions

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ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form
20 October 2013
Accepted 27 October 2013
Available online 8 November 2013

Keywords:
Balloon injury
Contralateral carotid
Compensatory mechanism
Calcium influx
Superoxide anion

ABSTRACT

There are many evidences indicating a compensatory mechanism in contralateral carotids following balloon injury. Previously it was observed α_1-adrenoceptor-mediated hyper-reactivity and impairment of calcium influx in contralateral carotids 4 days after injury. At a later stage, α_1-adrenoceptor-mediated contraction is similar to the control and we hypothesized that downstream signaling was normal. In the present study, we aimed to evaluate α_1-adrenoceptor-mediated calcium influx in contralateral carotids 15 days after balloon injury. Concentration–response curves for $CaCl_2$ in presence of the α_1-adrenoceptor agonist (phenylephrine), measurement of the intracellular calcium transient and the levels of reactive oxygen species using fluorescent dyes were performed in control and contralateral carotids. Phenylephrine-induced intracellular calcium mobilization in contralateral carotids was not altered, while phenylephrine-induced calcium influx was reduced in the contralateral artery. Nitric oxide synthase inhibitors, L-NAME or L-NNA, restored this response, but nitrite and nitrate levels were decreased in contralateral carotids. Additionally, a rise in oxygen free radicals was observed in contralateral carotids. Furthermore, Tiron, a superoxide anion scavenger, restored α_1-adrenoceptor-mediated calcium influx in contralateral carotids to the control level. Similar results were observed with the selective potassium channels blockers 4-aminopyridine and charybdotoxin. In conclusion, data showed that balloon catheter injury resulted in increased superoxide anions levels, activation of potassium channels (K_v and BK_{Ca}), inhibition of calcium channels (Ca_v) and preservation of α_1-adrenoceptor-mediated contraction at a later stage after injury.

1. Introduction

The procedure of balloon catheter angioplasty is a widely accepted technique, frequently used to restore blood flow in peripheral and coronary obstructive disease, but it may cause extensive endothelial injury and restenosis (Schwartz and De Blois, 1995; Schwartz and Henry, 2002). Restenosis is a complex vascular wound healing process that occurs in almost all patients after this procedure (Serruys et al., 1988). These changes reduce the vascular reactivity of injured and remodeled vessels to contracting agents (Joly et al., 1992; Lippolis et al., 2003) and also impair blood flow in this vessel (Accorsi-Mendonça et al., 2004; Pernomian et al., 2013).

Interestingly, balloon catheter injury induces specific changes in the contralateral rat carotid artery (Milner et al., 1997; Bruijins et al., 1998; Accorsi-Mendonça et al., 2004). Our group previously observed hyper-reactivity to both phenylephrine and angiotensin II occurring between 4–7 days and 15–30 days following balloon injury, respectively, thereafter returning to control levels (Accorsi-Mendonça et al., 2004). Studies also showed that hyper-reactivity to both contracting agents in contralateral carotids did not change blood flow in this vessel (Accorsi-Mendonça et al., 2004), suggesting a compensatory mechanism.

Additionally, we recently demonstrate that despite increased α_1-adrenoceptor-mediated contraction, calcium influx was reduced in contralateral carotids 4 days after balloon injury (Pereira et al., 2010). On later stages after injury, α_1-adrenoceptor-mediated contraction is similar to the control (Accorsi-Mendonça et al., 2004) and we hypothesized that calcium signaling is not altered in contralateral carotids. Calcium signaling plays a crucial role in many physiological events, as in the regulation of vascular tone and blood flow (Hidalgo and Donoso, 2008; Berridge, 2008; Knot and Nelson, 1998). Although a rise in intracellular calcium is not essential to trigger contraction (Kim et al., 2008), calcium levels modulate vascular processes and we aimed to evaluate α_1-adrenoceptor-mediated calcium signals in contralateral carotids 15 days following balloon injury.

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http://dx.doi.org/10.1016/j.ejphar.2013.10.066

2. Methods

2.1. Drugs

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich. KCl, $CaCl_2$ and others salts were purchased from Synth (São Paulo, Brazil), Ketamine (União Química, Brazil), Xylazine (Calier Laboratory, Brazil) and Isoflurane (Forane, ABBOTT). Tiron (4,5-Dihydroxy-1,3-benzenedisulfonic acid disodium salt monohydrate), L-NAME (*N*-nitro-L-arginine methyl ester), L-NNA (*N*-nitro-L-arginine), 1400W, TEA (tetraethylammonium chloride), 4-AP (4-aminopyridine), apamin (American Peptide Company), charybdotoxin and verapamil were dissolved in distilled water. Indomethacin was dissolved in Tris buffer (pH 8.4). Glibenclamide was prepared as stock solution in ethanol, while 7-NI (7-nitroindazole), DHE (dihydroethidium) and Fluo 3-AM were prepared in DMSO and stored at −20 °C.

2.2. Surgery

All procedures employed in this study conformed to the International Guidelines and Ethical Animal Committee of the Ribeirão Preto Campus, University of São Paulo, Brazil (process 06.1.1063.53.4). Adult male Wistar rats (80 days) underwent unilateral balloon catheter injury. The surgery was carried out as previously described (Clowes et al., 1983; Accorsi-Mendonça et al., 2004). In brief, general anaesthesia was installed with Ketamine and Xylazine (2.5 mg/kg, intraperitoneally). The left common carotid artery was exposed and the 2F Fogarty balloon catheter (Baxter, the Netherlands) was distended and passed three times along the external carotid. The catheter was removed, the external carotid artery was ligated, and the wound was closed. Since vascular reactivity was not altered in sham animals, intact rats were used as control in all experimental protocols (Accorsi-Mendonça et al., 2004).

2.3. Vascular studies

Fifteen days after surgery, rats were anaesthetized with Isoflurane and killed by aortic exsanguination. Contralateral (right) carotids were removed and immediately placed in Krebs solution (NaCl 118.4; KCl 4.7; $CaCl_2$ 1.9; KH_2PO_4 1.2; $MgSO_4$. $7H_2O$ 1.2; $NaHCO_3$ 25; $C_6H_{12}O_6$ 11.6, in mM), pH 7.4, at 37°C, bubbled with 95% O_2 and 5% CO_2. The same procedure was employed with the right carotid from intact animals. Carotid rings (4 mm) were connected to an isometric force transducer (Letica Scientific Instruments, Barcelona, Spain) to measure tension in the vessels, as previously described (Pereira et al., 2010). Concentration–response curves to phenylephrine (10^{-10}–10^{-5} M) and calcium mobilization studies were performed in presence and absence of functional endothelium.

In order to perform calcium signals studies, Krebs solution was changed to a solution without calcium, and phenylephrine (10^{-7} M) was added. The observed contractile response corresponded to intracellular calcium mobilization. Successive phenylephrine (10^{-7} M) stimulations were performed in the presence of EGTA (1 mM), to deplete intracellular Ca^{2+} stores. Preparations were rinsed in Ca^{2+}-free solution (without EGTA) containing phenylephrine (10^{-7} M or 10^{-5} M). Cumulative concentration–response curves for $CaCl_2$ (0.05–2.5 mM) (Tirapelli et al., 2006; Tostes et al., 1995) were obtained in the absence and presence of polyethylene glycol-catalase (PEG-catalase, a membrane permeable superoxide dismutase mimetic, 250 U/ml), tiron (a specific membrane-permeable superoxide scavenger, 10^{-3} M), indomethacin (non-selective cyclooxygenase inhibitor, 10^{-5} M), TEA (non-selective K^+ channels blocker, 10^{-2} M), 4-AP (, voltage-activated potassium channel specific blocker, K_v, 10^{-3} M), glibenclamide (ATP sensitive potassium channel specific blocker, K_{ATP}, 3×10^{-6} M), apamin (small-conductance calcium-activated potassium channel specific blocker, SK_{Ca} 10^{-6} M), charybdotoxin (mainly large and intermediate-conductance calcium-activated potassium channel blocker, BK_{Ca} and IK_{Ca}, 10^{-7} M), L-NAME (nonselective inhibitor of nitric oxide synthase, NOS, 10^{-4} M), 7-NI (neuronal NOS inhibitor, 10^{-4} M), 1400W (induced NOS inhibitor, 10^{-7} M), L-NNA (endothelial NOS inhibitor, 10^{-4} M) or Verapamil (voltage-activated calcium channel blocker, Ca_v, 10^{-6} M).

2.4. Detection of reactive oxygen species in carotid rings

DHE was used to detect superoxide production in isolated carotid arteries as described earlier (Miller et al., 1998). Cells are permeable to DHE, which in the presence of superoxide is oxidized to fluorescent ethidium bromide. Ethidium bromide is trapped by intercalation into DNA, and the number of fluorescent nuclei indicates the relative level of superoxide production. Carotid arteries were previously incubated with tiron (10^{-3} M) for 30 minutes in Krebs solution. Then, it was cryosectioned and put on glass slides covered with poly-L-lysine. DHE (10^{-6} M in phosphate buffer solution) was then added for 20 min followed by washing (phosphate buffer solution). Frozen sections of arteries were then visualized through a water immersion objective (63 ×) and photographed using a confocal scanning laser microscope (Leica TCS SP2). Images were analyzed using the Image J software. The intensities of ethidium bromide fluorescence were measured in the endothelium and media slice regions.

2.5. Detection of intracellular calcium transient in carotid rings

Calcium levels were detected as previously described (Lunard et al., 2006; Oliveira et al., 2009; Pereira et al., 2010). After isolation and removal of carotids, they were placed in Hanks solution (NaCl 145.0; KCl 5.0; $MgSO_4$; $7H_2O$ 1.0; NaH_2PO_4 0.5; $CaCl_2$ 1.6; Glucose 10.0; Hepes 10.0 in mM), pH 7.4, at 25 °C. Rings were placed in a glass coverslip covered with poly-L-lysine (50% Hanks solution). Then, the tissue was loaded with Fluo-3 AM (10^{-5} M, Sigma Probes) for 30 min at room temperature. After washing, coverslips were placed on a chamber (1.0 ml in volume), which was put on a confocal microscope (Leica TCS SP5) and observed through a water immersion objective (63 ×). Fluo-3 AM was excited with the 488 nm line of an argon ion laser, and the emitted fluorescence was measured at 510 nm. Time course software was used to capture the images before and after phenylephrine (10^{-7} M) addition. By applying the computer software of the Leica Mycrosystem LAS AF Lite, the intensities of the intracellular fluorescence were measured in the media slice.

2.6. Nitrite and nitrate measurement

Nitrite and nitrate levels were measured from total carotid arteries homogenates as previously described (Lizarte et al., 2009). Arteries were prepared in Tris buffer (pH 7.4). Following a centrifugation at 4000*g* for 10 min, supernatants were assayed for nitrite and nitrate, using a Nitric Oxide Analyser (Sievers, Model 280i). Data were collected for endothelium-intact rat carotids from control and contralateral arteries.

2.7. Statistical analysis

The maximum effect generated by the agonist (E_{max}, expressed as g of tension) and the pD_2 ($-\log EC_{50}$, EC_{50} is the agonist concentration that leads to 50% of the E_{max}) were obtained from concentration–response curves through nonlinear regression