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Total stenosis triggers compensatory responsiveness of carotid and basilar arteries to endothelin-1 and phenylephrine

Claudia Roberta de Andrade [a], Carlos Renato Tirapelli [b], Fernando M. de A. Corrêa [a], Leandra N.Z. Ramalho [c], Ana Maria de Oliveira [d,*]

[a] *Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, Brazil*
[b] *Department of Psychiatric Nursing and Human Sciences, College of Nursing of Ribeirão Preto, USP, Ribeirão Preto, SP, Brazil*
[c] *Department of Pathology, School of Medicine of Ribeirão Preto, USP, Ribeirão Preto, SP, Brazil*
[d] *Department of Physics and Chemistry, Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, USP, Avenue do Café s/n°, 14040-903 Ribeirão Preto, SP, Brazil*

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Abstract

The aim of this study was to investigate whether total stenosis of the common carotid artery (ipsilateral) would affect the vascular responsiveness of the contralateral carotid as well as the basilar artery from guinea pigs. With this purpose, the carotid artery was occluded with a silk thread at a position close to its origin. Vascular reactivity experiments using standard muscle bath were performed 7, 15, 30, and 90 days after carotid occlusion. Stenosis induced a progressive reduction in the contraction induced by endothelin-1, phenylephrine and KCl in the ipsilateral carotid, when compared to their respective age-matched SHAM groups. Endothelial removal or incubation of endothelium-intact ipsilateral carotids with L-NAME, a nitric oxide synthase inhibitor, did not alter the response to endothelin-1 or phenylephrine, when compared to the endothelium-intact ipsilateral carotid in the absence of the inhibitor. Interestingly, an increased contractile response to endothelin-1, phenylephrine and KCl was observed in the contralateral carotid. Indomethacin, a non-selective cyclooxygenase inhibitor, prevented the increased contraction to endothelin-1 in the contralateral carotid. Stenosis also induced an increase in the contractile response to endothelin-1 in the basilar artery while the contractile response to phenylephrine and KCl were reduced. Indomethacin, but not L-NAME, prevented the increased contraction to endothelin-1 in the basilar artery. Morphometric analysis showed no differences in the medial area (wall thickness) of carotid or basilar arteries from the stenosis group when compared to their respective age-matched SHAM groups. The present study confirms the importance of adaptation to stenosis on the vascular reactivity of the stenosed artery to different vasoconstrictor agents. Moreover, our results show that stenosis induces alterations of the vascular reactivity on arteries distant from the site of injury. The increased response to endothelin-1 in the contralateral carotid artery and basilar artery seems to involve the release of vasoconstrictor prostanoids from endothelial origin.

Keywords: Total stenosis; Contralateral carotid artery; Collateral basilar artery; Endothelin-1; Phenylephrine; KCl; Vascular reactivity

1. Introduction

Vascular diseases remain the most common cause of death in industrialized nations [1]. Those are chronic diseases that progress slowly with age and can affect the reactivity of blood vessels [2,3]. Arterial stenosis results in impairment of vascular reactivity, which is particularly important in the development of vascular diseases [4,5]. In this line, it has been described that stenosis increases the vasoconstrictive response of the dog carotid artery to noradrenaline *in vitro* [6]. Stenosis was also described to increase the vasoconstrictor response to noradrenaline *in vivo* [7]. Moreover, total stenosis affects the vascular reactivity of rat carotid artery to KCl, phenylephrine, and noradrenaline [8]. Furthermore, the effects of stenosis on the vascular contraction induced by KCl were reported to be both, tissue- and age-dependent [9].

In this way, a close relationship between stenosis and alterations in the function of arterial vessels has been established [10,11]. The alterations induced by stenosis in blood vessels include the increased release of endothelin-1 in some vascular beds [12–14], which in turn affects the responsiveness of vascular smooth muscle to exogenous endothelin-1. Additionally,

* Corresponding author. Tel.: +55 16 3602 4165; fax: +55 16 3602 4880.
E-mail address: amolive@usp.br (A.M. de Oliveira).

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Viswanathan et al. [15] reported an up-regulation of endothelin-1 receptors in the rat carotid artery after stenosis induced by balloon catheter. Milner et al. [16] reported that the injury of carotid arteries induced by balloon catheter triggers compensatory mechanisms in arteries located far from the site of injury. Such effects were also observed by Accorsi-Mendonça et al. [17], who have showed that these compensatory mechanisms alter the vascular reactivity of rat carotid arteries to phenylephrine and angiotensin II, after balloon injury.

Although it has been described that stenosis alters the vascular responsiveness of arteries that are located far from the site of injury, the mechanisms involved in such response are not fully understood. Based on the aforementioned observations, the aim of this study was to investigate whether total stenosis of the common carotid artery (ipsilateral) would affect the vascular responsiveness of the contralateral carotid as well as of the basilar artery from guinea pigs. With this purpose, concentration–response curves for endothelin-1, phenylephrine and KCl were performed on ipsilateral (stenosed) or contralateral carotid arteries and basilar arteries. Additionally, it was verified whether there is a relationship between the period of stenosis and the alterations in the vascular reactivity to these vasoactive agents.

2. Materials and methods

2.1. Total stenosis induction

Male guinea pigs were housed under standard laboratory conditions with free access to food and water. Housing conditions and experimental protocols are in accordance with the Ethical Animal Committee from the University of São Paulo, *Campus* of Ribeirão Preto.

Male guinea pigs (aged 3–6 months) were randomly divided into two groups: SHAM-operated group and a group that was submitted to unilateral total stenosis of the left common carotid arteries.

Animals from SHAM and stenosis groups were anesthetized with dihydrotiazine chloride (Rompum®; 0.2 ml kg^{-1}, i.m.; Bayer®, Germany). In animals from the SHAM group, the left common carotid artery was separated from the vagus nerve. In the total stenosis group, the carotid artery was occluded with a silk thread at a position close to its origin. Carotid occlusion was performed 7, 15, 30, and 90 days before the animals reached the age of 6 months, when it was assumed that they had reached adulthood (data obtained from the animal facility from the *Campus* of Ribeirão Preto, University of São Paulo). The scheme outlined in Fig. 1 represents the design of the experimental protocol.

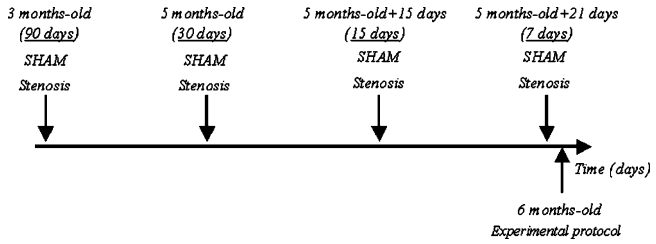

Fig. 1. Schematic diagram representing the design of the experimental protocol. Carotid occlusion was performed 7, 15, 30, and 90 days before the animals reached 6 months of age.

2.2. Vessel rings preparation

The guinea pigs were anaesthetized and sacrificed by aortic exsanguination. The carotid arteries were quickly removed, cleaned of adherent connective tissues and cut into rings. Contralateral and ipsilateral carotids were removed. After isolation of the carotid rings, the skull was opened and the brain was carefully removed. The brain stem was isolated to allow dissection of the basilar artery. The basilar artery was quickly removed, cleaned of adherent connective tissues and one ring was dissected. Two stainless-steel stirrups were passed through the lumen of each ring (carotid or basilar). One stirrup was connected to an isometric force transducer (Letica Scientific Instruments, Barcelona, Spain) to measure vessel tension. The rings were placed in a 10-mL organ chamber containing Krebs solution (pH 7.4). The composition of the Krebs solution was as follows (in mmol L^{-1}): NaCl, 118.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11.6; and $CaCl_2$, 1.9. The solution was continuously gassed with carbogen (95% O_2 + 5% CO_2) at 37 °C. The rings were initially stretched until a basal tension of 0.8 g (carotid arteries) and 0.3 g (basilar artery), which were determined by length–tension relationship exper-

Table 1
Tissues dry weight (mg)

	Period after stenosis			
	7 days	15 days	30 days	90 days
Carotid				
SHAM contralateral	0.57 ± 0.02	0.57 ± 0.03	0.58 ± 0.03	0.57 ± 0.02
SHAM	0.54 ± 0.03	0.54 ± 0.05	0.54 ± 0.03	0.57 ± 0.03
Stenosis contralateral	0.55 ± 0.03	0.57 ± 0.03	0.54 ± 0.03	0.57 ± 0.02
Stenosis	0.58 ± 0.03	0.57 ± 0.03	0.57 ± 0.02	0.54 ± 0.03
Basilar				
SHAM	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Stenosis	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.13 ± 0.01[a]

Values are means ± S.E.M. of 7 tissues.
[a] Compared to the respective age-matched SHAM group ($p < 0.05$, ANOVA followed by Dunnett's multiple comparison test).