The balloon catheter induces an increase in contralateral carotid artery reactivity to angiotensin II and phenylephrine

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1   The effects of balloon injury on the reactivity of ipsilateral and contralateral carotid arteries were compared to those observed in arteries from intact animals (control arteries).

2   Carotid arteries were obtained from Wistar rats 2, 4, 7, 15, 30 or 45 days after injury and mounted in an isolated organ bath. Reactivity to angiotensin II (Ang II), phenylephrine (Phe) and bradykinin (BK) was studied. Curves were constructed in the absence or presence of endothelium or after incubation with $10\,\mu$M indomethacin, $500\,\mu$M valeryl salicylate or $0.1\,\mu$M celecoxib.

3   Phe, Ang II and BK maximum effects (Emax) were decreased in ipsilateral arteries when compared to control arteries. No differences were observed among pD2 or Hill coefficient.

4   Emax to Phe (4 and 7 days) and to Ang II (15 and 30 days) increased in the contralateral artery. In addition, Phe or Ang II reactivity was not significantly different in aorta rings from control or carotid-injured animals.

5   The increased responsiveness of contralateral artery was not due to changes in carotid blood flow or resting membrane potential. The endothelium-dependent inhibitory component is not present in the contraction of contralateral arteries and it is not related to superoxide anion production.

6   Indomethacin decreased contralateral artery responsiveness to Phe and Ang II. Valeryl salicylate reduced the Ang II response in contralateral and control arteries. Celecoxib decreased the Phe Emax of contralateral artery.

7   In conclusion, decreased endothelium-derived factors and increased prostanoids appear to be responsible for the increased reactivity of contralateral arteries after injury.
British Journal of Pharmacology (2004) **142**, 79–88. doi:10.1038/sj.bjp.0705732

Introduction

Balloon catheter angioplasty is a widely accepted procedure for treatment of peripheral and coronary obstructive disease to restore blood flow. Despite its widespread application, there is still uncertainly regarding the effects of the procedure on smooth muscle cells and arterial responsiveness, as the technique may also cause extensive endothelial injury (Schwartz *et al.*, 1995).

In vitro and *in vivo* studies have confirmed the involvement of the renin—angiotensin system in the growth of the neointimal layer after balloon injury. Ang II stimulates DNA synthesis in the media and neointima after angioplasty (Hutchinson *et al.*, 1999). Powell *et al.* (1989) and others (Hanson *et al.*, 1991; Farhy *et al.*, 1992; Rakugi *et al.*, 1994; Fernandez-Alfonso *et al.*, 1997) reported that angiotensin-converting enzyme inhibitors or AT_1 antagonist are able to prevent balloon-induced neointima formation in rats. The Ang

II-induced DNA synthesis observed in the media and the neointima after angioplasty is partly mediated by α1-adrenoceptor activation (Bruijns *et al.*, 1998).

Conversely, the renin–angiotensin system also influences the noradrenergic system. Ang II facilitates sympathetic transmission, inhibits norepinephrine reuptake (Starke, 1977) and releases catecholamine from the adrenal medulla (Feldberg & Lewis, 1964). Additionally, the renin–angiotensin system modulates the kallikrein–kinin system, a regulatory system involved in a multitude of physiologic and pathologic conditions. In addition, stimulation of Ang 1–7 or AT_2 receptor has been shown to activate tissue kallikrein, increasing formation (Tschöpe *et al.*, 2002).

There are reports that vascular injury can cause changes in tissues of normal appearance located at a distance from the injury site. Renal artery manipulation was reported to cause a centripetal spread of endothelial cell replication along the periureteric collateral artery (Hollenberg & Odori, 1987). Reidy (1990) observed that focal endothelial cell loss and formation of platelet thrombi in rat aorta caused a widespread

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increase in smooth muscle cell replication at distant aortic sites. In patients with acute myocardial infarction, the coronary vasodilator response is significantly reduced in areas of the myocardium that are not directly supplied by the impaired artery (Uren *et al.*, 1994).

Immunohistochemical examination of the contralateral artery, 1 day after balloon catheter-induced endothelial injury, revealed a substantial increase in the density of protein gene product 9.5 (PGP) – substance P (SP) – and calcitonin-gene-related peptide (CGRP)-containing nerves innervating the carotid artery and vasa vasorum compared to arteries from intact animals, which was no longer observed twenty-eight days after surgery (Milner *et al.*, 1997).

In the present study, we examined the effect of balloon catheter-induced injury on the responsiveness of ipsilateral artery and distant vessel (contralateral carotid artery) to Ang II, Phe and BK at days 2, 4, 7, 15, 30 and 45 after surgery and compared the responsiveness to those of carotid arteries from intact animals (control arteries).

Methods

Surgery

All experimental procedures conformed to International Guidelines and Ethical Animal Committee of the Ribeirão Preto Campus, University of São Paulo, Brazil.

Adult male Wistar rats (3.5–4 months) were divided into three groups: control, sham-operated and animals that underwent unilateral balloon catheter injury. Care was taken for all the animals to be of the same age on the day of the experiment.

Balloon angioplasty was carried out as previously described (Clowes *et al.*, 1983). In brief, general anaesthesia was installed using the short-lasting anaesthetic tribromoethanol (2.5 mg kg^{-1}, i.p.). The left common carotid artery (ipsilateral to injury) was exposed and the external carotid artery was ligated for access of the 2F Fogarty balloon catheter (Baxter, the Netherlands), which was passed three times along the common carotid artery and distended saline. The catheter was removed, the external carotid artery was ligated, and the wound was closed. Sham-operated rats underwent anaesthesia, the left common carotid artery was exposed and the external carotid artery was ligated without performing catheterisation.

Vascular studies

At various time points after injury (2, 4, 7, 15, 30 and 45 days), rats were anaesthetised with tribromoethanol i.p. (2.5 mg kg^{-1}). and killed. The carotid or aorta arteries were removed and immediately placed in Krebs–Henseleit solution, which was composed of the following (mmol l^{-1}): NaCl, 118.4; KCl, 4.7; CaCl$_2$, 2.5; KH$_2$PO$_4$,1.2; MgSO$_4\cdot$7H$_2$O, 1.2; NaHCO$_3$, 25; C$_6$H$_{12}$O$_6$, 11.6; pH 7.4, at 37°C. Carotid artery rings (4 mm) or aorta rings (6 mm) were mounted in organ chambers bubbled with a carbogenic mixture (95% O$_2$ and 5% CO$_2$) and connected to force transducers (Letica, Spain).

Preliminary experiments showed that a 1 g resting tension was at the optimal plateau of the length–tension relationship for 90 mM KCl. Cumulative concentration–response curves were obtained using 0.1 nM–10 μM Phe or 0.01 nM–1 μM Ang II. Response to 90 mM KCl was also recorded. Concentration–

relaxation curves to 0.1 nM–10 μM BK were performed in arteries previously contracted using 0.1 μM Phe. In some preparations, the endothelium was mechanically removed or the rings were incubated with 10 μM indomethacin for 45 min, 500 μM valeryl salicylate for 1 h (Bhattacharyya *et al.*, 1995) or 0.1 μM celecoxib for 1 h (Penning *et al.*, 1997).

Results were expressed as gram (g) of tension (Phe or Ang II curves) or % relaxation (BK curves) and were represented as mean ± s.e.m. of 6–10 animals. Pharmacological parameters, that is, the agonist potency (pD$_2$), maximum effect (Emax) and the Hill coefficient were derived from the concentration–effect curves (Kenakin, 1996).

Carotid blood flow

Animals were anaesthetised with 7 mg kg^{-1} ketamine + 0.4 mg kg^{-1} xylazine, i.p. (suggested anaesthetic protocol for flow measurements – Guideline for use of anaesthetics from Transonic Systems Inc., U.S.A.) and the common carotid arteries were exposed. A noninvasive transit-time flow probe (model 1.5RB; Transonic Systems, Inc., Ithaca, NY, U.S.A.) was placed around the carotid arteries. The flow probe was connected to a flow meter (model T-206; Transonic system, U.S.A.) and carotid blood flow was recorded using a computerised acquisition system (Dataq, U.S.A.). The system provided actual volume flow measurements with a resolution of 0.05 ml.min^{-1}. The carotid was minimally manipulated to avoid vasospasm.

Resting membrane potential (RMP)

Isolated carotids were placed in a 2 ml perfusion chamber with Krebs–Henseleit solution gassed with 95% CO$_2$/5% O$_2$ at a rate of 3 ml.min^{-1}. Micropipettes from borosilicate glass capillaries were prepared using an horizontal puller (Narishige, Model PN3) filled with 2 M KCl (tip resistance 20–40 MΩ and tip potential <6 mV), (1B120F-6, World Precision Instruments, U.S.A.). The microelectrodes were mounted in Ag/AgCl half-cells on a Leitz micromanipulator and connected to an electrometer (Intra 767, World Precision Instruments, U.S.A.). Impalements were made in smooth muscle cells. Electrical signals were continuously monitored on an oscilloscope (54645-A, Hewlett Packard, U.S.A.) and recorded on a potentiometric chart recorder (2210, LKB, Sweden).

Successful implantation of the electrode was demonstrated by a sharp voltage drop upon entry into the cell, a stable 3 mV potential for at least 1 min after impalement, a sharp return to zero upon exiting the cell and a minimal change (<10%) in microelectrode resistance following impalement. Isolated arteries were initially equilibrated for 1 h.

Superoxide analyses

Production of superoxide anion was measured by the coelenterazine-enhanced chemiluminescence technique, as previously described by Laurindo *et al.* (2002), with a final coelenterazine concentration of 10 μM. Rats were anaesthetised, killed and the carotid arteries were removed and cut into 0.5 mm segments which were incubated in Krebs-HEPES bicarbonate buffer and bubbled with 95%O$_2$/5% CO$_2$ at 37°C for 30 min.

Artery rings were exposed to potassium phosphate buffer containing 0.1 mM *N*-methyl-L-arginine (L-NMMA) and 0.1 mM diethylenetriamine pentaacetic acid (DTPA) during the experiments. After 2 min equilibration, photon emission was continuously recorded for 4 min in a Biolumat LB9505 (Berthold, Germany). The specific chemiluminescence was expressed as c.p.m. minus background. mg^{-1} dry weight min^{-1}.

Drugs

Phe (Sigma, U.S.A.), Ang II (Sigma, U.S.A.), BK (Sigma, U.S.A.), ketamine (União Química, Brazil), xylazine (Calier Laboratory, Brazil), coelenterazine (ProLume, U.S.A.), L-NMMA (Calbiochem, U.S.A.), DTPA (Sigma, U.S.A.), indomethacin (Calbiochem, U.S.A.) valeryl salicilate (Cayman Chemical, U.S.A.) and celecoxib (Pfizer, U.S.A.).

Statistical analyses

pD_2 ($-\log EC_{50}$), Emax and Hill coefficient were determined from concentration–response curves using the Prism software (version 2.0,. GraphPad Software Inc., U.S.A.). Parameters were analysed using one-way ANOVA and Bonferroni's post-test or unpaired 't' test, $P < 0.05$, using the Prism software.

Results

Initially, the arterial reactivity of the arteries from intact animals was compared to that of sham operated animals (2, 4, 7, 15, 30 and 45 days after sham -surgery). Phe, Ang II and BK evoked similar concentration-dependent responses in carotid arteries from intact or sham-operated rats. No differences were observed in the pD_2 and Emax values for Phe, Ang II or BK between ipsilateral and contralateral arteries from sham animals or between those and the arteries from intact animals (data not shown). Thus, thereafter, arteries from intact rats were used as control arteries to avoid unnecessary animal suffering.

In arteries from injured rats, Phe and Ang II evoked concentration-dependent contractions in ipsilateral and con-tralateral arteries removed 2, 4, 7, 15, 30 and 45 days after balloon catheter surgery. The injury significantly reduced the maximum response to Phe and Ang II in ipsilateral arteries when compared to control arteries from intact animals (Table 1). The pD_2 values for Phe and Ang II were not

different for the control and ipsilateral (injured) arteries (Table 2).

In contrast, an increase in the Emax to Ang II and Phe was observed in contralateral when compared to control arteries. The Emax to Phe was increased in contralateral arteries at days 4 and 7 after injury (Figure 1a and Table 1). The Emax to Ang II was increased in contra-lateral arteries at days 15 and 30 after injury (Figure 2a and Table 1). There were no differences in Emax to Phe and Ang II between contralateral arteries and control arteries on the other days studied (Figure 1b and 2b, respectively).

BK induced significant relaxation (52%) in control carotid arteries precontracted with Phe ($EC_{50} = 0.1 \mu M$). BK-induced relaxation was decreased in ipsilateral vessels on days 2, 4 and 7 after removal of the endothelium by balloon injury. Relaxation was re-established at days 15, 30 and 45 after injury (Table 1). The maximal relaxation induced by BK in contralateral vessel was similar to that observed in control arteries (Table 1).

There were no differences in the pD_2 values for Ang II, Phe and BK among control, ipsilateral and contralateral arteries (Table 2). The analysis of the concentration–response curves showed that Hill coefficients were not significantly different from unity (data not shown).

Vasoconstrictions of ipsilateral or contralateral arteries in response to a high potassium concentration (90 mM) were not different from those observed in control arteries (Figure 3).

To investigate whether this increased contraction effect was selective for the contralateral carotid or if it also occurred in other vessels, we studied the contractions evoked by Phe or Ang II in aorta rings from carotid injured rats at the times when increased Emax was observed in the contralateral arteries.

The maximal responses to Phe in the aorta artery at 4 and 7 days after the lesion to the carotid artery (Figure 4a) were similar to that of the aorta artery of the control animal (2.15 ± 0.20; 2.30 ± 0.18 and 1.87 ± 0.21 g, respectively, not significant). Similarly, the maximum force developed in response to Ang II in control aorta arteries (1.07 ± 0.10 g) was not different from that of the aorta, artery of carotid-injured animals 15 days or 30 days after injury (1.18 ± 0.13, 1.15 ± 0.07 g, respectively) (Figure 4b). There were no significant differences in the pD_2 values of Phe and Ang II in isolated aorta as shown by the curves from control or injured animals (data not shown).

To establish the role of blood flow in the contractile effect, we measured the blood flow in carotid arteries. In control

Table 1 Emax values for agonists in rat carotid artery after endothelial injury

Days after injury	Phe (g)		Ang II (g)		BK (% relaxation)	
	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral
Control	0.28 ± 0.02		0.28 ± 0.01		52.12 ± 6.34	
2	0.22 ± 0.03	0.17 ± 0.02^a	0.24 ± 0.02	0.24 ± 0.03	49.16 ± 4.31	19.19 ± 3.25^a
4	0.37 ± 0.03^a	0.16 ± 0.04^a	0.26 ± 0.03	0.16 ± 0.01^a	57.74 ± 6.86	21.79 ± 3.48^a
7	0.35 ± 0.03^a	0.14 ± 0.02^a	0.24 ± 0.02	0.13 ± 0.02^a	52.50 ± 5.96	27.31 ± 8.99^a
15	0.32 ± 0.02	0.20 ± 0.02^a	0.35 ± 0.04^a	0.16 ± 0.01^a	50.95 ± 6.89	44.27 ± 6.10
30	0.27 ± 0.03	0.17 ± 0.03^a	0.36 ± 0.03^a	0.12 ± 0.02^a	53.88 ± 4.69	55.43 ± 0.70
45	0.26 ± 0.03	0.16 ± 0.03^a	0.29 ± 0.04	0.10 ± 0.03^a	55.13 ± 6.03	50.40 ± 9.51

Anova and Bonferroni's post test, $P < 0.05$.
[a]vs control group.

Table 2 pD$_2$ values ($-$log EC$_{50}$) for agonists in rat carotid artery after endothelial injury

Days after injury	Phe		Ang II		BK	
	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral
Control	8.64 $\pm$ 0.20		9.12 $\pm$ 0.13		6.61 $\pm$ 0.50	
2	8.72 $\pm$ 0.30	8.13 $\pm$ 0.19	8.76 $\pm$ 0.16	9.08 $\pm$ 0.18	6.45 $\pm$ 0.14	ND
4	8.26 $\pm$ 0.10	8.50 $\pm$ 0.53	9.45 $\pm$ 0.25	8.92 $\pm$ 0.12	6.23 $\pm$ 0.33	6.97 $\pm$ 0.18
7	8.09 $\pm$ 0.19	7.83 $\pm$ 0.40	9.03 $\pm$ 0.33	8.74 $\pm$ 0.25	6.08 $\pm$ 0.17	6.85 $\pm$ 0.20
15	8.33 $\pm$ 0.19	8.20 $\pm$ 0.14	8.66 $\pm$ 0.12	9.10 $\pm$ 0.27	6.45 $\pm$ 0.18	7.41 $\pm$ 0.13
30	8.39 $\pm$ 0.27	7.89 $\pm$ 0.32	8.91 $\pm$ 0.16	8.32 $\pm$ 0.17	6.06 $\pm$ 0.12	6.16 $\pm$ 0.41
45	8.12 $\pm$ 0.17	8.11 $\pm$ 0.37	8.88 $\pm$ 0.13	8.63 $\pm$ 0.19	6.21 $\pm$ 0.28	6.73 $\pm$ 0.13

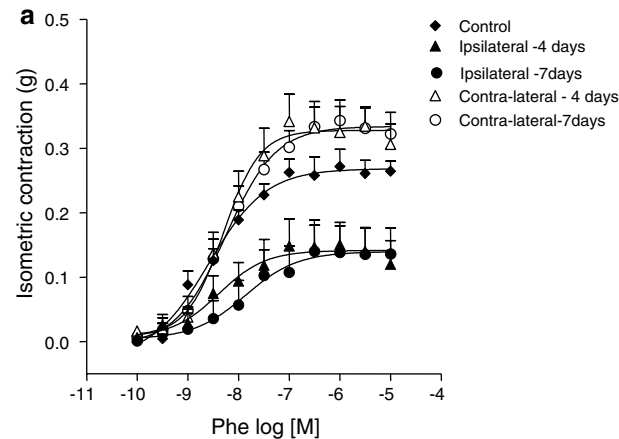ANOVA: not significant. ND: not determined.
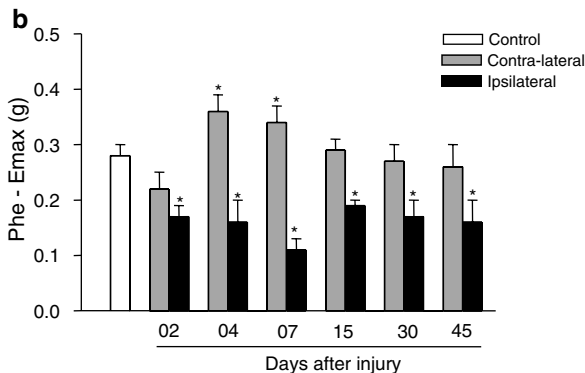
Figure 1 (a) Phe concentration–effect curves from control carotid arteries and contralateral and ipsilateral arteries 4 and 7 days after the injury; (b) Emax values (g) for Phe obtained in control, contralateral and ipsilateral carotid arteries (2, 4, 7, 15, 30 and 45 days after the injury). One-way ANOVA followed by Bonferroni's post test, $P < 0.05$, *significantly different from control group.
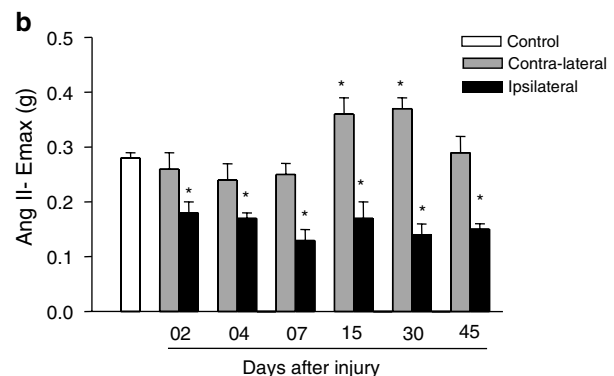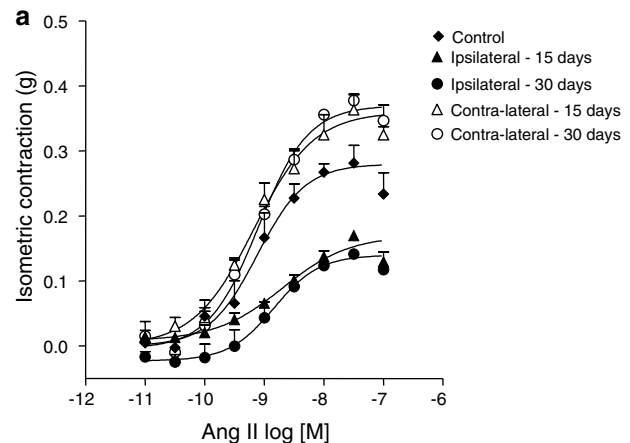

Figure 2 (a) Ang II concentration–effect curves from control carotid arteries and contralateral and ipsilateral carotid arteries taken 15 and 30 days after the injury; (b) Ang II Emax values (g) from control, contralateral and ipsilateral arteries (2, 4, 7, 15, 30 and 45 days after the injury). One-way ANOVA followed by Bonferroni's post test, $P < 0.05$, *significantly different from control group.

animals, carotid blood flow was 5.5 ± 0.9 ml min^{-1}. The blood flow values for contralateral arteries were similar to the values from control animals; however, blood flow was decreased in the ipsilateral arteries of injured rats (Figure 5).

The electrophysiological study showed that the resting membrane potentials (RMP) of the contralateral artery and the control animal artery did not differ statistically from each other. In contrast, RMP were significantly lower in ipsilateral arteries 2, 4 and 7 days after surgery than in intact artery; furthermore, at 15 days after injury the resting potential membrane of the arteries returned to normal (Figure 6).

To evaluate the role of the endothelium in increased contraction in the contralateral artery, the arteries were denuded

mechanically before the experiments. The absence of endothelium significantly increased the contractions caused by Phe or Ang II in vessels from the control group. However, in the contralateral arteries, the absence of endothelium did not affect the Phe or Ang II response (Figures 7a and 7b), suggesting that endothelium-derived factors are decreased in contralateral arteries 4, 7, 15 and 30 days after injury.

Superoxide production was measured to demonstrate the reduction of the endothelium-derived factors in the contralateral artery. Figure 8 demonstrates the chemiluminescence of coelenterazine in the control and contralateral carotid rings under basal conditions. The chemiluminescence activity of the
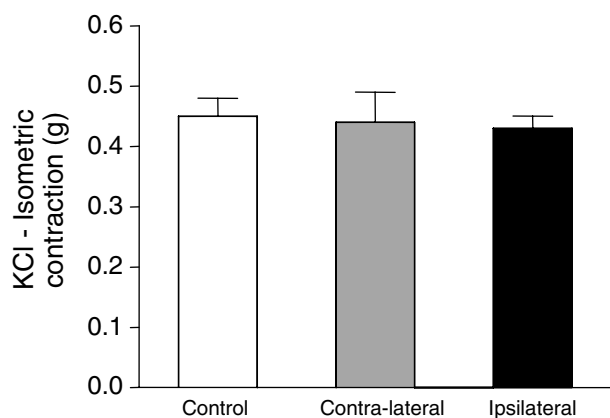
Figure 3 KCL-induced contraction (90 mM) in isolated carotid from control or injured animals killed 15 days after the injury. One-way ANOVA (not significant).
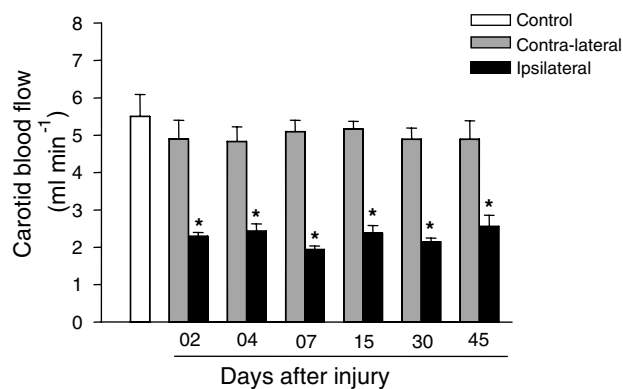

Figure 5 Carotid blood flow (ml min^{-1}) in control, contralateral or ipsilateral arteries (2, 4, 7, 15, 30 and 45 days after the injury). One-way ANOVA followed by Bonferroni's post test, $P < 0.05$, * significantly different from control group.
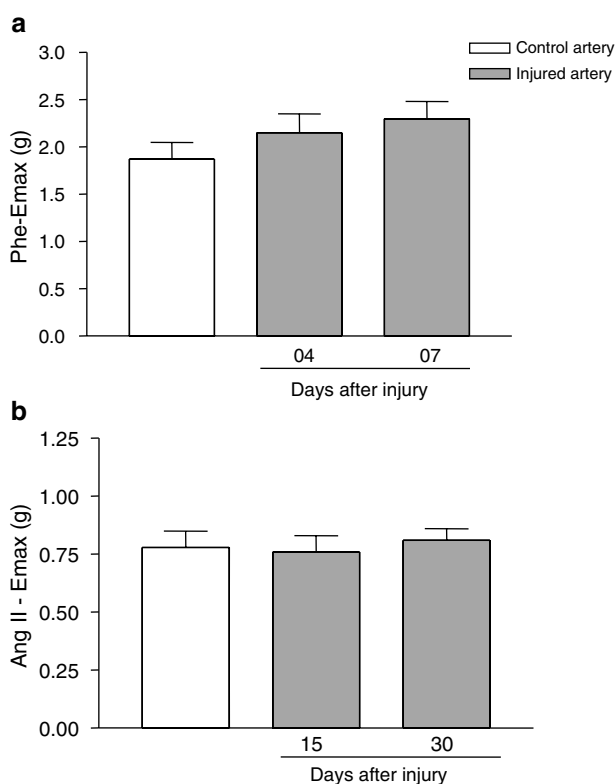

Figure 4 (a) Phe Emax (g) in aorta rings from control or operated rats 4 and 7 days after the injury in carotid artery. One-way ANOVA: not significant. (b) Ang II Emax (g) in aorta rings from control or operated rats 15 and 30 days after the injury in carotid artery. One-way ANOVA: not significant.

Figure 6 Resting potential membrane (mV) of control, contralateral or ipsilateral arteries (2, 4, 7, 15, 30 and 45 days after the injury). One-way ANOVA followed by Bonferroni's post test, $P < 0.05$, * significantly different from control group.

Indomethacin did not significantly alter the Phe responses in the control carotid artery. In contrast, it depressed maximal contraction to adrenergic agonist in contralateral arteries (4 and 7 days after injury) (Figure 9a) and it reduced the Ang II-induced contraction in control and contralateral carotid arteries (Figure 9b).

The selective cyclooxygenase 1 inhibitor, valeryl salicylate, did not affect the Phe Emax in control or contralateral artery 4 days after the injury, while celecoxib (a selective cyclooxygenase 2 inhibitor) reduced the adrenergic response in the contralateral artery without affecting the control artery response (Figure 10a).

Valeryl salicylate decreased the Ang II Emax in control or contralateral artery 15 days after the injury, but celecoxib did not alter the peptide maximum response in control or contralateral arteries (Figure 10b).

Discussion and conclusions

This study compared the effects of balloon catheter-induced injury on the vascular reactivity of the ipsilateral and contralateral carotid arteries with the vascular reactivity of control arteries from intact animals.

control or contralateral arteries was not significantly different at 4, 7, 15 or 30 days after injury.

To evaluate the possible role played by prostanoids in the Ang II or Phe effect in the contralateral artery indomethacin (a nonselective cyclooxygenase inhibitor), valeryl salicylate (selective cyclooxygenase 1 inhibitor) and celecoxib (selective cyclooxygenase 2 inhibitor) were employed. The baseline tone in isolated arteries was increased in the presence of indomethacin and valeryl salicylate.
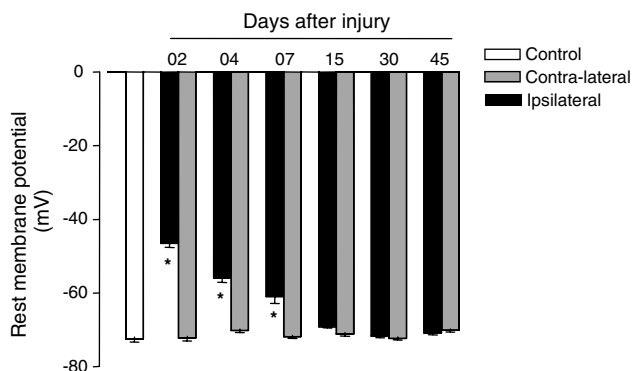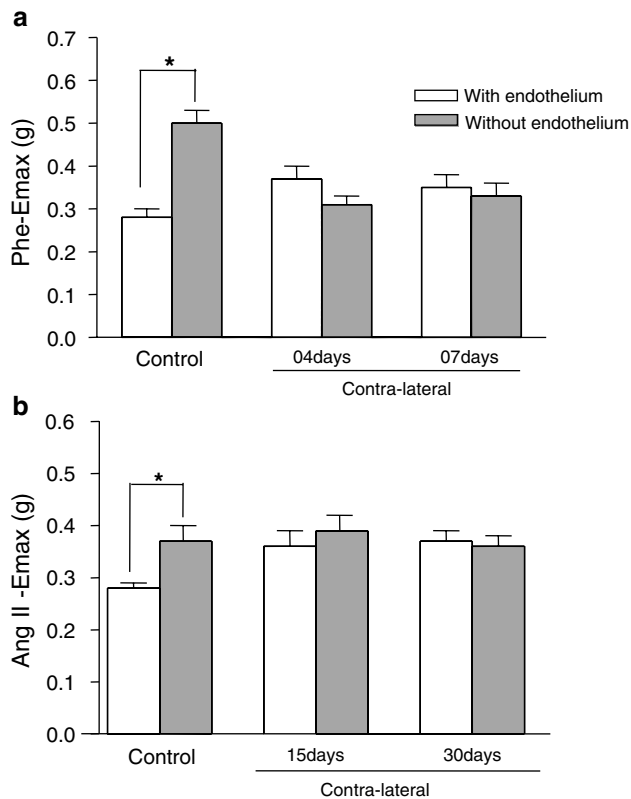
Figure 7 (a) Phe Emax (g) of control or contralateral (4 and 7 days after the injury) arteries with or without endothelium; (b): Ang II Emax (g) of control or contralateral (15 and 30 days after the injury) arteries with or without endothelium. Unpaired '*t*' test, $P<0.05$, *significantly different from respective group with endothelium.
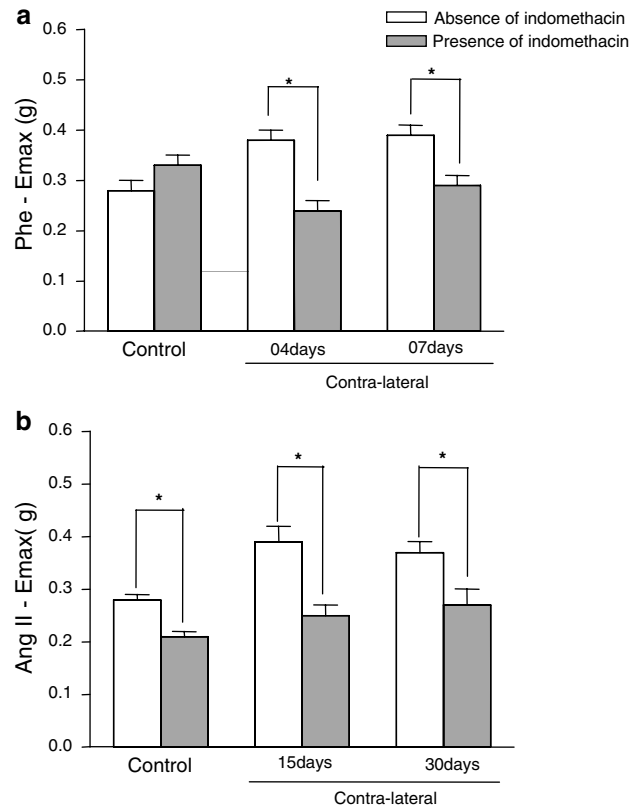

Figure 9 Effect of indomethacin ($10\,\mu$M) on Emax of Phe (a) or Ang II (b) in control or contralateral arteries. Unpaired '*t*' test, $P<0.05$, *significantly different from respective group in the absence of inhibitor.
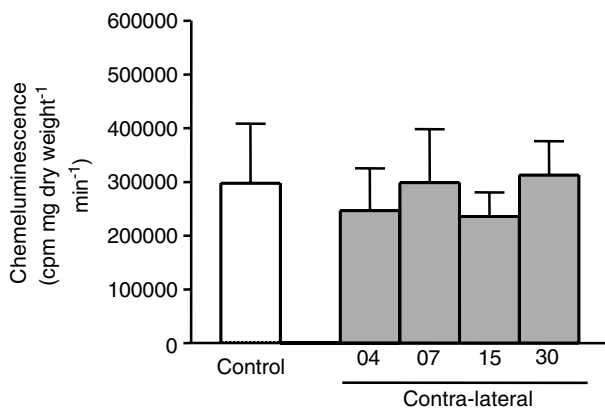

Figure 8 Coelenterazine chemiluminescence's response (cpm mg.dry weight^{-1} min^{-1}) in control or contralateral carotid arteries 4, 7, 15 or 30 days after the injury. One-way ANOVA: not significant.

The vascular responses (Emax, pD_2 and Hill coefficient values) to the three agonists used (Ang II, Phe and BK) in the ipsilateral and contralateral arteries of sham-operated rats were not significantly different from those of arteries from intact animals. These findings indicate that surgical procedures, such as the dissection of the vagus nerve and ligation of the external carotid artery, did not affect the vascular reactivity. Thus, vessels isolated from intact animals were used as controls to compare to injured animals.

The pD_2 values for Ang II, Phe or BK were similar in control, ipsilateral and contralateral arteries, suggesting that balloon catheter surgery does not affect agonist potency, as previously described by Lemay *et al.* (2000). However, the endothelium injury-induced balloon caused an immediate increase in the endothelin-1 contractile potency and it could be related to an increase in affinity for the endothelin-1 receptor subtype mediating vasoconstriction, an increase in agonist bio-availability at this smooth muscle cell receptor or the loss of some type of physiological antagonism, an intrinsic mechanism which normally exists to selectively oppose the contractile actions of this peptide (Douglas *et al.*, 1994). Hill coefficients from all curves were similar to unity, suggesting a homogeneous response and the positive cooperative phenomenon does not affect the response (Kenakin, 1996).

Data demonstrate that balloon catheter injury decreased the maximum contractions evoked by Ang II or Phe in the ipsilateral artery. These results are in agreement with those of Major *et al.* (1985), Joly *et al.* (1992) and Antonnacio *et al.* (1994), who all demonstrated a reduction in the Emax value for several agonists in injured arteries.

The decreased Ang II-induced contraction in the ipsilateral artery may be the consequence of changes in the activities of AT_2 receptors. Activation of AT_2 has been associated with vasodilator effects (Tschöpe *et al.*, 2002) and AT_2 receptors re-expresssion was reported to occur 48–72 h after injury (Hutchinson *et al.*, 1999). This effect may be the consequence of an increased production of inducible nitric oxide in the
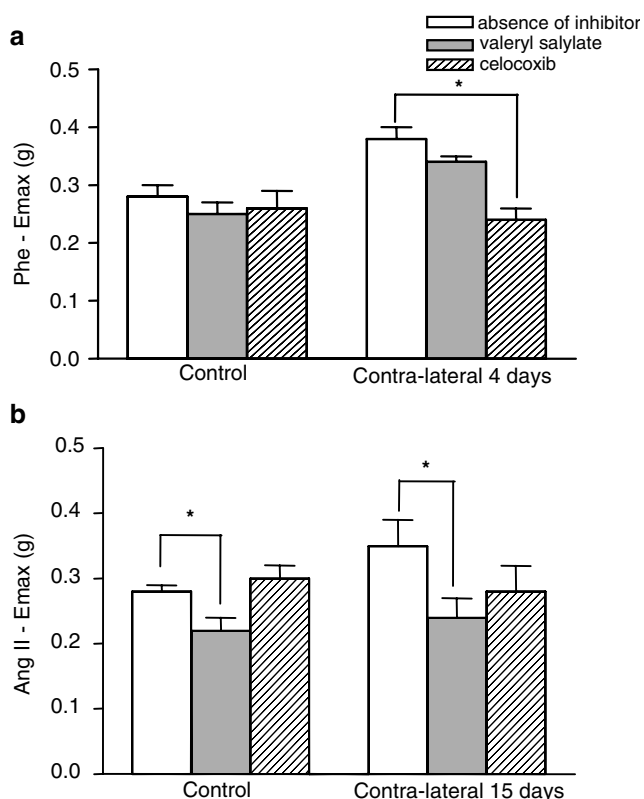
Figure 10 Effect of valeryl salicylate (500 μM) or celecoxib (0.1 μM) on Emax of Phe(a) or Ang II (b) in control or contralateral arteries. Unpaired '*t*' test, $P < 0.05$, *significantly different from respective group in the absence of inhibitor.

ipsilateral vessel wall, as suggested by several studies (Major *et al.*, 1985; Joly *et al.*, 1992; Douglas *et al.*, 1994; Hansson *et al.*, 1994).

The decreased response to Phe could also be related to the desensitisation to exogenous ligands (Candipan *et al.*, 1994), to a decrease in α1-adrenoceptors from day 3 up to 10 weeks after injury (Bruijns *et al.*, 1998) or increased production of inducible nitric oxide in injured vessel (Major *et al.*, 1985; Joly *et al.*, 1992; Douglas *et al.*, 1994; Hansson *et al.*, 1994; Yan & Hansson, 1998).

The vascular hyporeactivity to Phe and Ang II described above was not selective for these agonists. The BK-induced relaxation was reduced during the early phases of injury (2, 4 and 7 days), possibly due to the absence of endothelium (Clowes *et al.*, 1983). After 15 days, BK-induced relaxation returned to control levels. Similar results were reported for carbachol-induced relaxations in the rat carotid (Major *et al.*, 1985) or for BK relaxation of the pig coronary, after angioplasty (Thollon *et al.*, 2002). At 15 days after balloon catheterisation, the injured vessel develops a neointima layer, in which the smooth muscle cells are primarily of the synthetic rather than the contractile phenotype (Campbell *et al.*, 1988) with a different shape and organisation of smooth muscle cells (Majesky *et al.*, 1992). These cells could induce vasodilatation, explaining the recovery of relaxation obtained.

The balloon injury did not interfere with the intrinsic contractility of muscle cells since the maximal responses to a high potassium concentration were not influenced by balloon

catheter, as previously described by Douglas *et al.* (1994) and Lemay *et al.* (2000).

We also demonstrate that balloon injury promoted an increase in the responsiveness of contralateral arteries, which was agonist-dependent and occurred at different postinjury times. These results could indicate the existence of a compensatory mechanism in the contralateral artery that was activated after the balloon catheter procedure. Several examples of vascular changes in normal-appearing tissues, which take place at a distance from the injury site, have been reported (Segal & Duling, 1986; Hollenberg & Odori, 1987; Reidy, 1990; Milner *et al.*, 1997). Compensatory changes may also occur in the contralateral sympathetic neurons of the superior cervical ganglion and in their terminals in the pineal gland following unilateral ganglionectomy (Dornay *et al.*, 1995).

Evidence exists to demonstrate that balloon catheter dilatation may induce transient changes in the innervations of the contralateral artery. At 1 day after the surgery, there was a substantial increase in the density of PGP, SP and CGRP-containing nerves innervating the vasa vasorum and vessel wall in the contralateral artery, which were no longer observed 28 days after surgery (Milner *et al.*, 1997).

The increased response of the contralateral artery to Ang II and Phe may be related to an increase in receptor density after surgery. However, balloon injury did not alter the α1-adrenoceptor binding in contralateral arteries (Bruijns *et al.*, 1998). There are no data concerning the density of Ang II receptors in contralateral arteries after balloon injury and this possibility cannot be excluded.

We hypothesised that if the increased maximal effect observed in the contralateral artery was related to a humoral phenomenon other vessels should be similarly affected. Phe and Ang II Emax values in aorta rings from rats with injured carotid arteries were not different from those in rings from control intact arteries, suggesting that humoral factors are not responsible for the increased reactivity of the contralateral artery and that this effect is vascular-bed dependent.

Hemodynamic stimuli such as altered flow or circumferential stress can induce arterial remodelling and changes in the characteristics of vessels (Reidy, 1990; Pasterkamp *et al.*, 2000). The results of the current study suggest that the increased response of the contralateral artery is not related to blood flow. The decreased blood flow in the ipsilateral artery of the injured animals may be due to the ligation of the external carotid during the surgery and did not change the vessel response, since the ipsilateral arteries from sham animals demonstrated the same reduction (data nor shown).

The RMP may influence vascular reactivity. Accorsi-Mendonça *et al.* (2002) showed a correlation between Ang II potency and RMP in nonvascular muscle cells. Furthermore, endothelial hyperpolarisation increases the electrochemical driving force for Ca^{2+} influx into the endothelium and, thus, indirectly augments Ca^{2+}-dependent formation of vasodilating factors (Luckhoff & Busse, 1990). In addition, the neointimal proliferation may be caused by the depolarised state of the smooth muscle cells (Dalle-Lucca *et al.*, 2000).

Our data showed that injured carotids were significantly more depolarised than control muscle cells until 7 days after lesion; after 15 days, the RMP from vascular muscle cells are similar to control values, although Köhler *et al.* (2001) have suggested that the neointima cells have a reduced capacity to

hyperpolarise and dilate because of the decreased expression of K_{Ca} channels. Our findings also demonstrate that there is no difference in RMP between control and contralateral carotids, suggesting that the Phe and Ang II increased contraction in the contralateral artery after injury is not caused by alterations in membrane potential.

To further understand the role of the endothelium in the increased contraction of the contralateral vessels, we examined the response to Phe and Ang II in arteries without intact endothelium. After *ex vivo* endothelium denudation, the effect of both agonists in control arteries is significantly increased. However, the endothelium-dependent inhibitory component of the response to Phe or Ang II is not present in the contraction of contralateral arteries, suggesting that endothelium-derived factors were decreased in contralateral arteries after the injury.

Endothelium-derived factors may be decreased due to the existence of strong oxidants such as the superoxide radical, which may change reactivity following its reaction with NO, thus reducing the bio-availability of NO (Hanafy *et al.*, 2001; Lum & Roebuck, 2001). Vascular production of reactive oxygen species is reported to be increased 2 min after balloon injury (Azevedo *et al.*, 2000). In smooth muscle cells, both superoxide and peroxide disrupt the sarcoplasmic reticulum Ca^{2+}-ATPase and inactivate plasma membrane Ca^{2+} pumps leading to both short- and long-term effects on smooth muscle Ca^{2+} handling (Wolin *et al.*, 2002). Thus, we evaluated superoxide production to analyse the participation of oxidative stress in contralateral artery reactivity. Our results showed that there was no difference in superoxide production between control or contralateral arteries, despite the difference in the arteries 7 and 30 days after balloon placement. These data suggest that superoxide does not participate in the increased reactivity observed in the contralateral artery.

The vascular responsiveness can be affected by prostaglandins that are molecules that can affect several physiological and pathological functions. The prostaglandin generation is enhanced in patients with atherosclerosis and after balloon angioplasty (Braden *et al.*, 1991; Belton *et al.*, 2000).

Prostaglandins are synthesized from arachidonic acid by the cyclooxygenase (COX) enzyme, of which there are two isoforms, COX_1 and COX_2 (Hla & Neilson, 1992). COX_1 is constitutively expressed in most tissues and is largely responsible for TXA_2 formation. COX_2 is normally undetectable or is expressed in low amounts (Masferrer *et al.*, 1994) and it is induced in the vascular smooth muscle and inflammatory cells of human atherosclerotic plaque (Schonbeck *et al.*, 1999; Belton *et al.*, 2000). Prostanoids are also related to the adrenergic response in cultured vascular rabbit smooth muscle cells (Nebigil & Malik, 1992), adrenergic-induced constriction of endothelium-denuded rat abdominal aorta (Asbún-Bojalil *et al.*, 2002) and carotid artery (Furuhashi *et al.*, 2000).

Normal, uninjured vessels showed COX_1, but no COX_2 expression. At 14 days after balloon injury, both COX_1 and COX_2 were expressed in the neointima. Balloon angioplasty resulted in a marked increase in prostaglandin excretion (Pritchard *et al.*, 1994; Connolly *et al.*, 2002).

Then, we analysed the prostaglandin role in increase responsiveness of contralateral artery. Indomethacin, a nonselective inhibitor, induced a decrease in Phe effect of contralateral artery without affect the response of control artery, suggesting that prostanoids are involved in the increased effect to Phe observed in the contralateral artery. Our data demonstrated that the COX_2 isoform is involved in the prostaglandin production during adrenergic stimulation in contralateral artery 4 days after injury. The balloon catheter is an inflammatory response (Ross, 1999) and it is probable that the COX_2 isoform is present in injured tissue.

The COX1-dedrived prostanoids are involved in Ang II effect in control artery since indomethacin and valeryl salicylate reduced the peptide Emax. The endothelium injury did not modify the involvement of prostanoids in the Ang II contraction since the response to the peptide in contralateral arteries was also decreased by indomethacin and valeryl salicylate.

These data suggest that some form of cellular communication generated by the focal mechanical injury might be responsible for vascular remodelling in other uninjured vessels. Vascular compensation and remodelling are poorly understood complex processes. To date, no other report exists that explains the altered reactivity seen in the contralateral artery, and therefore further studies are required to examine which of the above mechanisms is responsible for this phenomenon. Our results indicate that important information concerning possible compensatory mechanisms may be derived from the study of effects of balloon injury on the contralateral vessels. Although the basic molecular mechanisms involved in the response remain to be fully elucidated, these responses provide potential targets through which it may be possible to influence remodelling after angioplasty.

In conclusion, we report that after unilateral balloon catheter dilatation of the carotid artery there is an increase in Phe and Ang II, but not BK reactivity, in the contralateral artery when compared to the effect on control arteries. This phenomenon was not observed in aorta rings after the angioplasty in the carotid artery, suggesting that it is a vascular bed-specific effect, which may involve increased prostanoids or decreased endothelium-derived factors in this mediation.

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