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ORIGINAL ARTICLE

Chronic methionine load-induced hyperhomocysteinemia impairs the relaxation induced by bradykinin in the isolated rat carotid

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Abstract This study investigates the effects of chronic methionine intake on bradykinin (BK)-relaxation. Vascular reactivity experiments were performed on carotid rings from male Wistar rats. Treatment with methionine (0.1, 1 or 2 g kg^{-1} per day) for 8 and 16 weeks, but not for 2 and 4 weeks, reduced the relaxation induced by BK. Indomethacin, a non-selective cyclooxygenase (COX) inhibitor, and SQ29548, a selective thromboxane A_2 (TXA_2)/prostaglandin H_2 (PGH_2) receptor antagonist prevented the reduction in BK-relaxation observed in the carotid from methionine-treated rats. Conversely, AH6809, a selective prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) receptor antagonist did not alter BK-relaxation in the carotid from methionine-treated rats. The nitric oxide synthase (NOS) inhibitors L-NAME, L-NNA and 7-nitroindazole reduced the relaxation induced by BK in carotids from control and methionine-treated rats. In summary, we found that chronic methionine intake impairs the endothelium-dependent relaxation induced by BK and this effect is due to an increased production of endothelial vasoconstrictor prostanoids (possibly TXA_2) that counteracts the relaxant action displayed by the peptide.

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Introduction

Hyperhomocysteinemia is a known risk factor for the development of atherosclerosis and other vascular diseases. Homocysteine is a sulphur-containing amino acid that is derived from methionine, an essential amino acid found in abundance in proteins of animal origin, which is the only source of homocysteine in man (Perry 1999). Methionine taken orally is converted to homocysteine by demethylation (Selhub 1999), and it is well known that methionine loading can increase plasma homocysteine concentrations (Bonaventura et al. 2004). It has been suggested that elevated concentrations of homocysteine induce direct vascular dysfunction (Harker et al. 1976; Rodgers and Conn 1990; Weiss 2005). In this line, it has been described that elevations in plasma homocysteine levels are associated with increased contraction to vasoconstrictor agents such as phenylephrine (De Andrade et al. 2006) and angiotensin II (Bonaventura et al. 2004) and to a decreased vasorelaxation induced by acetylcholine (De Andrade et al. 2006) and histamine (Ungvari et al. 1999).

The mechanisms by which homocysteine alters the vessel functionality are most likely to involve direct injury of the vascular endothelium (Harker et al. 1976; Rodgers and Conn 1990; Weiss 2005). In this line, impairment of the endothelium-derived nitric oxide (NO) activity (Stuhlinger et al. 2001; Chen et al. 2002) and increased production of vasoconstrictor prostanoids (Wang et al. 1993; Signorello et al. 2002) are described to be associated with elevated plasma homocysteine levels.

Bradykinin (BK) is a potent agonist in numerous organs and acts through two BK receptor subtypes, B1 and B2 (Leeb-Lundberg et al. 2005), both of which are members of Family A (Horn et al. 2003) or rhodopsin family (Fredriksson et al. 2003) of G-protein-coupled receptors (GPCRs). In rats, BK induces relaxation of the femoral (Starr and West 1966), mesenteric (Wigg et al. 2001; Wimalasundera et al. 2003), coronary (Gonzalez et al. 2004), cerebral (Hernanz et al. 2004) and the carotid artery (Starr and West 1966; Tirapelli et al. 2006; Accorsi-Mendonca et al. 2004). In the latter, we recently showed that BK-induced relaxation involves the activation of endothelial B_2 receptors and the NO pathway (Tirapelli et al. 2007). Moreover, we found that endothelium-derived vasoconstrictor prostanoids (probably $PGF_{2\alpha}$, PGH_2 and TXA_2) counteract the vasorelaxant action displayed by BK. On the other hand, hyperhomocysteinemia enhances the synthesis of endothelial-derived vasoconstrictor prostanoids, such as TXA_2 (Ungvari et al. 1999; Graeber et al. 1982; Wang et al. 1993; Signorello et al. 2002) and $PGF_{2\alpha}$ (Davì et al. 2001).

Kinins may contribute to the regulation of the cardiovascular system in health and disease. Based on observations from experimental models of hypertension, hypertrophy, ischemia, remodeling and pre-conditioning one can assume that modulation of local kallikrein–kinin system pathways is instrumental for endogenous cardio- and vasculoprotective mechanisms (Scholkens 1996). Numerous observations obtained from clinical and experimental models of diabetes, hypertension, cardiac failure, ischemia, myocardial infarction and left ventricular hypertrophy, have suggested that the reduced activity of the local kallikrein–kinin system may be pivotal in the induction of cardiovascular-related diseases (Sharma and Sharma 2002). Thus, hyperhomocysteinemia-induced unbalance of local kallikrein–kinin system could contribute to the vascular disruption associated with hyperhomocysteinemia. Since hyperhomocysteinemia increases the production of vasoconstrictor prostanoids, which in turn are able to counteract the relaxation induced by BK in the rat carotid, we aimed to investigate whether methionine supplementation would affect the relaxation induced by this peptide.

Materials and methods

Male Wistar rats were housed under standard laboratory conditions (12-h light–dark cycle at 24°C) with free access to food and water. The housing conditions and experimental protocols were in accordance with the Ethical Animal Committee of the Campus of Ribeirão Preto (University of São Paulo).

Methionine treatment

Mild hyperhomocysteinemia was induced in male Wistar rats (3 weeks old, weighing 50–60 g) by daily administration of methionine (0.1, 1 and 2 g kg^{-1} per day body weight) in the drinking water for a period of 2, 4, 8 and 16 weeks, as previously described (Bonaventura et al. 2004). The doses administered were based on average daily fluid intake. The diet with L-methionine at 0.1, 1 and 2 g kg^{-1} per day was previously described to increase plasma total homocysteine levels in rats from 7 to 15 μmol l^{-1} (Bonaventura et al. 2004). The animals were weighed daily to allow the adjustment of methionine dosage.

Vascular reactivity studies

Male Wistar rats were anesthetized and killed by aortic exsanguination in accordance with standards and policies of the University of São Paulo's Animal Care and Use Committee.

The carotid artery was quickly removed, cleaned of adherent connective tissues and cut into 5-mm length rings. Two stainless-steel stirrups were passed through the lumen of each ring. One stirrup was connected to an isometric force transducer (Letica Scientific Instruments, Barcelona, Spain) to measure tension in the vessels. The rings were placed in 5 ml organ chambers containing Krebs solution, pH 7.4, gassed with 95%O_2/5%CO_2, and maintained at 37°C. The composition of Krebs solution was as follows (mmol l^{-1}): NaCl, 118.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 15.0; glucose, 5.5; $CaCl_2$, 2.5. The rings were stretched until a basal tension of 0.75 g, which was determined by length–tension relationship experiments, and then were allowed to equilibrate for 60 min with the bath fluid being changed every 15–20 min. In some rings, the endothelium was removed mechanically by gently rolling the lumen of the vessel on a thin wire. Endothelial integrity was assessed qualitatively by the degree of relaxation caused by acetylcholine (1 μmol l^{-1}) in the presence of contractile tone induced by phenylephrine (0.1 μmol l^{-1}). High homocysteine levels are described to induce endothelial dysfunction, and therefore impair the response to acetylcholine. Therefore, some of the tissues obtained from homocysteine-fed animals may have an impaired relaxation response to acetylcholine due to the effect of homocysteine and not due to mechanical damage. To deal with this issue, the rings were discarded if the relaxation caused by acetylcholine was less than 50%.

Effect of the treatment with methionine on BK- and acetylcholine-induced relaxation

Endothelium-intact rat carotid rings were pre-contracted with phenylephrine (0.1 μmol l^{-1}). After reaching a stable

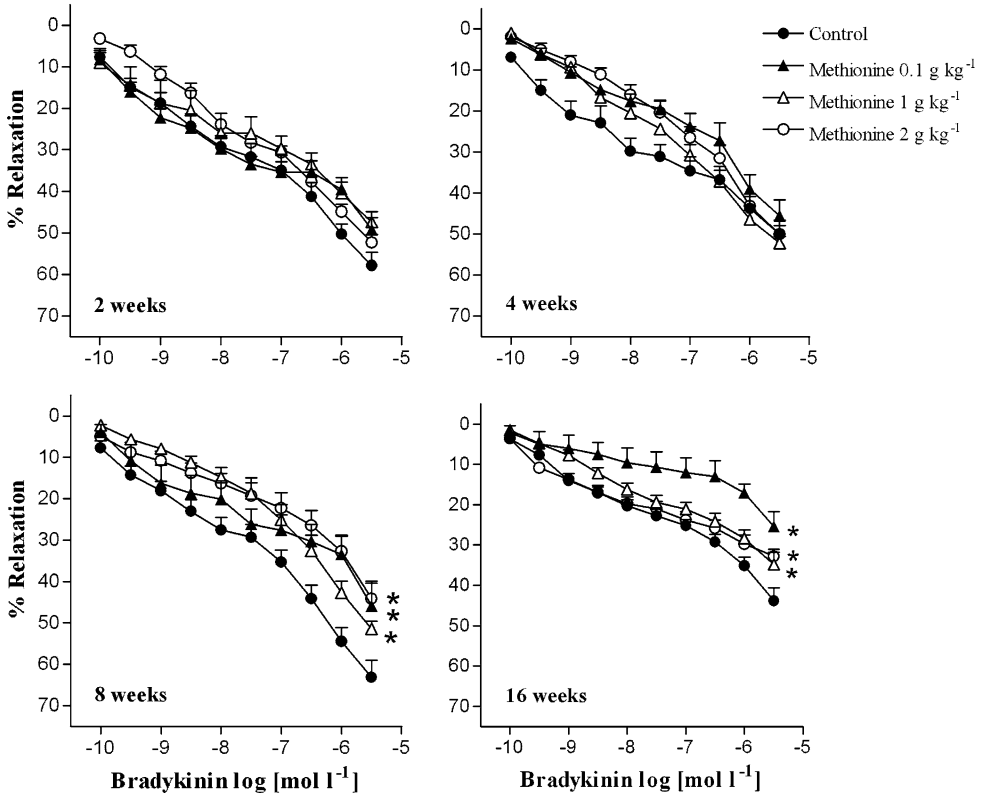

Fig. 1 Effect of treatment with methionine (0.1, 1 and 2 g kg^{-1} per day) on BK-induced relaxation. Relaxation induced by BK was determined in endothelium-intact carotid rings from 2, 4, 8 and 16 weeks methionine-treated rats and their respective age-matched controls. *Asterisks* Compared to respective control group ($P < 0.05$; ANOVA followed by Newman–Keuls)

and sustainable contraction, BK (0.1 nmol l^{-1}–3 μmol l^{-1}) or acetylcholine (10^{-10}–10^{-5} mol l^{-1}) was added cumulatively to the organ bath. The concentration–response curves for both agonists were performed in endothelium-intact carotid rings from rats treated with methionine (0.1, 1 and 2 g kg^{-1} per day) for 2, 4, 8 and 16 weeks and their respective age-matched controls. The vascular relaxation evoked by BK or acetylcholine was expressed as percentage change from the phenylephrine-contracted levels. To avoid a possible influence of the pre-contracting levels induced by phenylephrine on BK or acetylcholine-induced relaxation, the contraction was evoked with a dose of phenylephrine of 0.1 μmol l^{-1}, which was seen to contract with similar magnitude the tissues from control or methionine-treated rats.

Involvement of the endothelium on methionine-induced impairment of BK-mediated vasorelaxation

The following experiments designed to investigate the mechanisms underlying the effect of methionine treatment on BK-induced relaxation were performed in carotid rings from 8-week treated rats (2 g kg^{-1} per day) and their respective age-matched control animals. Concentration–response curves for BK were obtained in endothelium-intact or denuded carotid rings from control or methionine-treated rats. Steady tension was evoked by phenylephrine at 0.1 μmol l^{-1} for endothelium-intact rings and 0.03 μmol l^{-1} for endothelium-denuded rings to induce contractions of similar magnitude.

Involvement of prostanoids on methionine-induced impairment of BK-mediated vasorelaxation

Concentration–response curves for BK were obtained in endothelium-intact carotid rings from control or methionine-treated rats, in the presence of indomethacin (10 μmol l^{-1}), a non-selective cyclooxygenase inhibitor, SQ29548 (3 μmol l^{-1}), a selective thromboxane A_2 (TXA_2)/prostaglandin H_2 (PGH_2) receptor antagonist or AH6809 (10 μmol l^{-1}), a selective prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) receptor antagonist. The tissues were incubated with the inhibitors for 30 min.

Involvement of NO on methionine-induced impairment of BK-mediated vasorelaxation

Concentration–response curves for BK were obtained in endothelium-intact carotid rings from control or methionine-treated rats, in the presence of N^G-nitro-L-arginine-methyl-ester (L-NAME, 100 μmol l^{-1}), a non-selective NOS inhibitor, Nω-nitro-L-arginine (L-NNA, 100 μmol l^{-1}), a selective inhibitor of eNOS, 7-nitroindazole (7-NI, 100 μmol l^{-1}), a selective inhibitor of nNOS, and *N*-[3-

Table 1 Effect of methionine consumption on the E_{max} (% relaxation) values for BK in endothelium-intact carotid rings

Time (weeks)	Control	0.1 g kg^{-1} per day	1 g kg^{-1} per day	2 g kg^{-1} per day
2	57.9 ± 3.1 (11)	49.2 ± 2.9 (9)	47.4 ± 2.5 (7)	52.3 ± 2 (7)
4	50 ± 3.3 (10)	45.6 ± 4 (9)	52.3 ± 1.6 (7)	50 ± 2 (7)
8	59 ± 2 (10)	45.9 ± 5.6[b] (6)	51.5 ± 1.9[b] (6)	44.2 ± 4.2[b] (9)
16	43.8 ± 3.1[a] (15)	25.4 ± 3.7[b] (6)	34.8 ± 3[b] (9)	32.8 ± 1.7[b] (8)

Number between parentheses indicates the number of preparations. Values are mean ± SEM

[a] Compared to control 2, 4 and 8 weeks

[b] Compared to the respective age-matched control group (ANOVA followed by Newman–Keuls multiple comparison test, $P < 0.05$)

(aminomethyl)benzyl]acetaminide (1400 W, 10 nmol l^{-1}), a selective inhibitor of iNOS. The tissues were incubated with the inhibitors for 30 min.

Drugs

The following drugs were used: phenylephrine hydrochloride, acetylcholine hydrochloride, bradykinin, SQ29548, AH6809 (Sigma, St. Louis, MO, USA), L-NAME, L-NNA, 1400 W, 7-NI (Sigma/RBI, Natick, MA, USA), indomethacin (Calbiochem, USA). Indomethacin was dissolved in Tris buffer (pH 8.4) and 7-NI was dissolved in DMSO. The other drugs were dissolved in distilled water. The final bath concentration of DMSO did not exceed 0.5%, which was shown to have no effects per se on the basal tonus of the preparations or on the agonist-mediated relaxation.

Data analysis

Relaxant responses to BK and acetylcholine were expressed as "percent reversal" from phenylephrine precontracted levels. The E_{max} (maximal effect generated by the agonist) was calculated from the concentration–response curves for BK and acetylcholine. The pD_2 values for acetylcholine were calculated using a non-linear interactive fitting program (Graph Pad Prism 3.0; GraphPad Software Inc., San Diego, CA). Data are expressed as mean ± SEM. Differences between mean values have

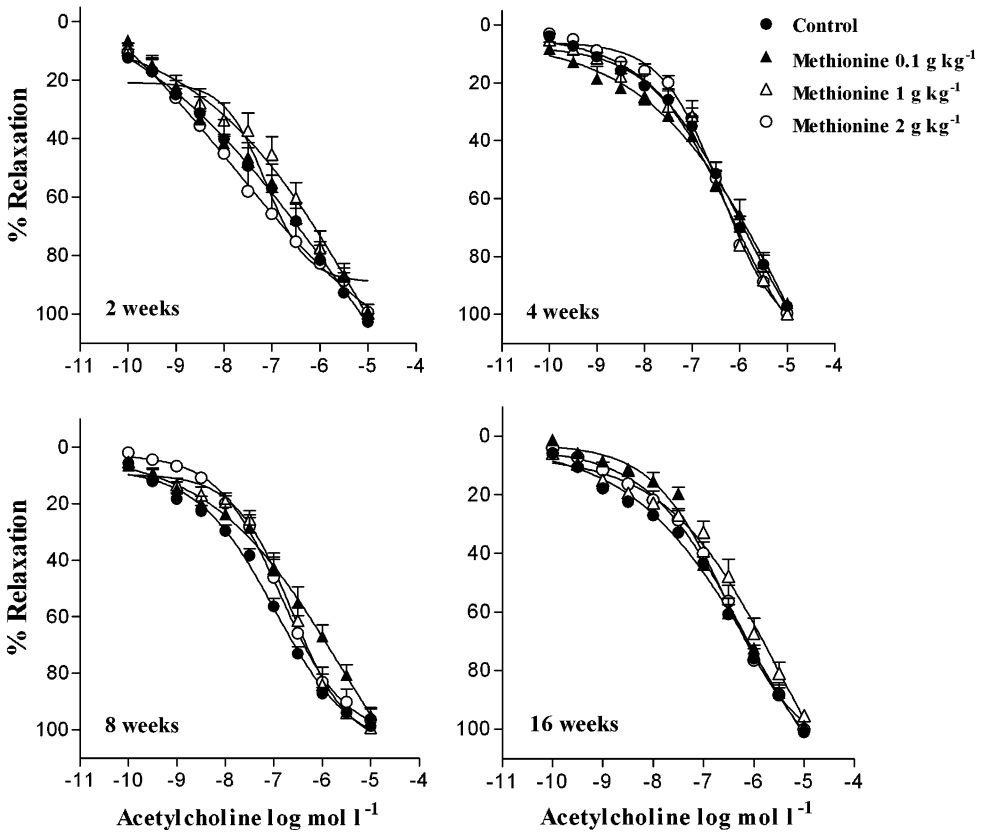

Fig. 2 Effect of treatment with methionine (0.1, 1 and 2 g kg^{-1} per day) on acetylcholine-induced relaxation. Relaxation induced by acetylcholine was determined in endothelium-intact carotid rings from 2, 4, 8 and 16 weeks methionine-treated rats and their respective age-matched controls

Table 2 Effect of methionine consumption on the E_{max} (% relaxation) and pD_2 values for acetylcholine in endothelium-intact carotid rings

Time (weeks)	Control	0.1 g kg^{-1} per day	1 g kg^{-1} per day	2 g kg^{-1} per day
E_{max}				
2	100.1 ± 3 (6)	98.3 ± 3.4 (8)	93.4 ± 3.1 (6)	96 ± 1.3 (5)
4	101 ± 1.3 (10)	96 ± 1 (4)	100 ± 1.7 (6)	101 ± 1.6 (8)
8	99 ± 0.5 (12)	98.5 ± 3.5 (5)	99.6 ± 0.7 (6)	96.5 ± 2 (9)
16	99.7 ± 1 (16)	98.6 ± 0.9 (6)	99.8 ± 1.8 (8)	98.2 ± 1.3 (6)
pD_2				
2	6.9 ± 0.22	7.4 ± 0.17	7.3 ± 0.18	7.5 ± 0.35
4	6.6 ± 0.1	6.8 ± 0.16	6.5 ± 0.11	6.5 ± 0.1
8	6.9 ± 0.11	7.1 ± 0.24	6.8 ± 0.1	7.2 ± 0.1
16	6.5 ± 0.1	6.5 ± 0.17	6.4 ± 0.14	6.7 ± 0.07

Number between parentheses indicates the number of preparations. Values are mean ± SEM

been assessed by Student's *t* test or one-way analysis of variance (ANOVA) followed by a Newman–Keuls post hoc test as indicated in the text and legends. The significance level considered in all tests was 0.05.

Results

Effect of methionine consumption on BK- and acetylcholine-induced relaxation

In endothelium-intact rat carotid rings, phenylephrine induced sustained contraction, which lasted at least 60 min without any predominant decrease in tension (data not shown). The addition of BK in concentrations ranging from 0.1 nmol l^{-1} to 3 µmol l^{-1} caused a concentration-dependent relaxation of endothelium-intact carotid rings. The maximum relaxant effect was obtained with 3 µmol l^{-1} (Fig. 1). We verified that repeated treatment by BK (10 µmol l^{-1}) produced no relaxation. Instead, at this concentration BK induced contraction in endothelium-intact or denuded carotids (data not shown). There was a decrease on the relaxation induced by BK in control tissues after 16 weeks when compared to 2, 4 and 8 weeks. Treatment with methionine (0.1, 1 and 2 g kg^{-1}) for 2 and 4 weeks did not alter the E_{max} for BK. Conversely, there was a reduction on BK-induced relaxation after treatment with the three different concentrations of methionine for 8 and 16 weeks (Fig. 1; Table 1). Surprisingly, the treatment with methionine did not affect the relaxation induced by acetylcholine (Fig. 2; Table 2).

Involvement of the endothelium on methionine-induced impairment of BK-mediated vasorelaxation

Removal of functional endothelium strongly reduced BK-induced relaxation in carotid rings from either control (E_{max}: 6.5 ± 0.7%, $n = 4$) or methionine-treated rats (E_{max}: 7.2 ± 3.1%, $n = 5$). Interestingly, no differences were observed between the groups after endothelial denudation (Student's *t* test) (Fig. 3).

Involvement of prostanoids on methionine-induced impairment of BK-mediated vasorelaxation

Figure 4 shows that pre-incubation with indomethacin increased BK-induced relaxation in endothelium-intact rings from control rats when compared to the relaxation obtained in the absence of the inhibitor. Interestingly, indomethacin prevented the reduction in BK-induced relaxation observed in the tissues from methionine-treated rats.

In endothelium-intact rings from control rats, BK-induced relaxation was not altered by SQ29548 or AH6809 when compared to the relaxation observed in the absence

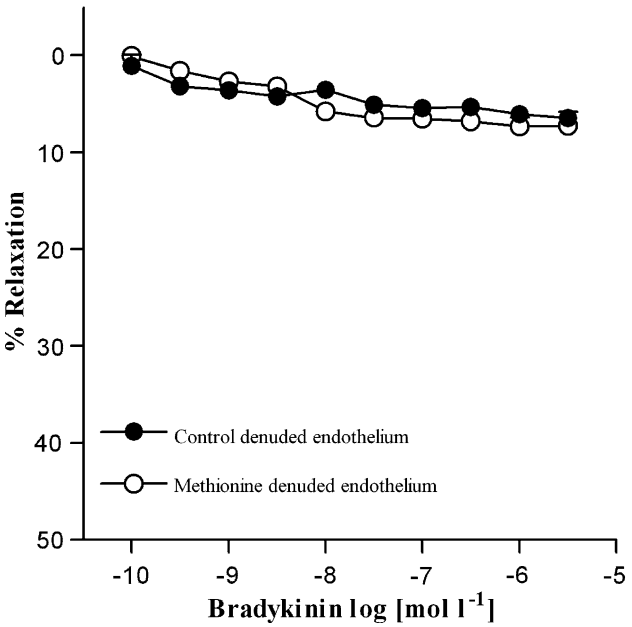

Fig. 3 Effect of treatment with methionine on BK-induced relaxation in endothelium-denuded carotid rings. Concentration–response curves for BK were determined in endothelium-denuded carotid rings from control or methionine-treated rats (8 weeks, 2 g kg^{-1})

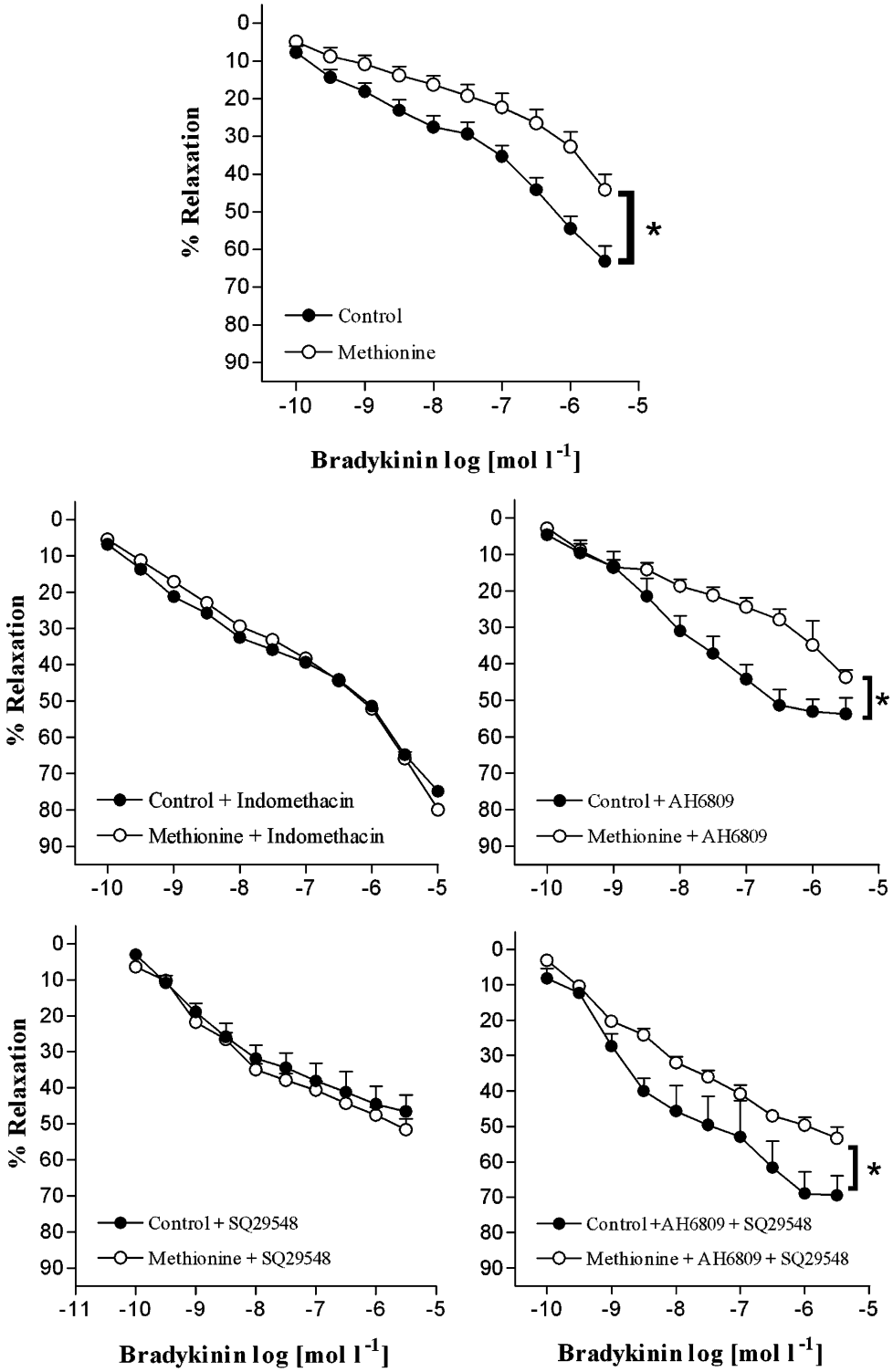

Fig. 4 Effect of indomethacin, SQ29548 and AH6809 on BK-induced relaxation of endothelium-intact carotid rings. Concentration–response curves for BK were determined in endothelium-intact carotid rings from control or methionine-treated rats (8 weeks, 2 g kg^{-1}). The curves were determined in the absence or after 30-min period of incubation with indomethacin (10 μmol l^{-1}), SQ29548 (3 μmol l^{-1}), AH6809 (10 μmol l^{-1}) or the combination of SQ29548 and AH6809. *Asterisks* Compared to the control group ($P < 0.05$; ANOVA followed by Newman–Keuls)

of the antagonists. SQ29548, but not AH6809, prevented the reduction in BK-induced relaxation observed in the tissues from methionine-treated rats. The combination of SQ29548 and AH6809 significantly enhanced BK-induced relaxation in control tissues. Moreover, when added simultaneously to the organ bath the antagonists prevented the decrease in relaxation induced by BK. The combination of SQ29548 and AH6809 showed no further effect than that observed with either SQ29548 or indomethacin alone in arteries from methionine-treated rats (Fig. 4; Table 3).

Table 3 Effect of methionine consumption (2 g kg^{-1}) for 8 weeks on the E_{max} (% relaxation) for BK in endothelium-intact carotid rings in the absence or presence of different inhibitors

Inhibitor	Control	Methionine
Absent	59 ± 2 (10)	44.2 ± 4.2[a] (9)
Indomethacin (10 μmol l^{-1})	74.8 ± 1.3[a] (6)	79.9 ± 3.1[a] (8)
AH6809 (10 μmol l^{-1})	53.7 ± 4.4 (5)	43.7 ± 1.9 (5)
SQ29548 (3 μmol l^{-1})	46.6 ± 4.6 (6)	51.6 ± 3.1 (5)
SQ29548 plus AH6809	69.5 ± 5.5[a] (5)	53.3 ± 3.1[b] (5)

Number between parentheses indicates the number of preparations. Values are mean ± SEM

[a] Compared to control group in the absence of the inhibitors

[b] Compared to respective control group in the presence of the inhibitor (ANOVA followed by Newman–Keuls multiple comparison test, $P < 0.05$)

Involvement of NO on methionine-induced impairment of BK-mediated vasorelaxation

In the presence of L-NAME and L-NNA the relaxation induced by BK in rings from control or methionine-treated rats was markedly reduced. 7-NI, a selective nNOS inhibitor significantly attenuated BK-induced relaxation in control or methionine-treated tissues. Conversely, 1400 W, a selective iNOS inhibitor did not alter the relaxation induced by BK. On the other hand, no differences in BK-induced relaxation were found between the tissues from control or methionine-treated rats in the presence of these inhibitors (Fig. 5; Table 4).

Discussion

Chronic methionine intake produced a time-dependent impairment on BK-induced relaxation in endothelium-intact carotids. On the other hand, after endothelial removal the impaired reactivity to BK was not observed, further supporting a role for the endothelium on the effects mediated by methionine consumption. The vascular endothelium is important in the regulation of the vascular tonus since it produces relaxing and contracting factors (Gris et al. 1991). In the rat carotid, the relaxation induced by BK is mediated by endothelial B_2 receptors. B_2 receptor is coupled to G-proteins of the Gi and Gq family (Liao and Homcy 1993). Both Gi and Gq can activate phosphoinositide-specific phospholipase C, which mobilizes intracellular calcium via the hydrolysis of phosphatidylinositol 4,5-bisphosphate (Taylor et al. 1991; Smrcka et al. 1991). This intracellular calcium signal is necessary for many of the vascular responses elicited by BK including the release of endothelial-derived nitric oxide (NO) (Loeb et al. 1988; Tirapelli et al. 2007). Thus, we can conclude that chronic methionine intake disrupts endothelial function, which leads to a reduced response to BK. Interestingly, the reduced reactivity to BK induced by methionine consumption is not the result of a non-specific impairment in the reactivity of the rat carotid, since the relaxant response of these arteries to acetylcholine was not affected. However, the result observed with acetylcholine does not rule out the possibility that methionine consumption affects the relaxation induced by other vasorelaxant agents. In fact, we have recently observed that α_{1D}-adrenoceptor-induced relaxation on rat carotid artery is impaired during early stages of hyperhomocysteinemia, further indicating that the effect of hyperhomocysteinemia on vascular reactivity is dependent on the agonist tested (De Andrade et al. 2006).

The lack of effect induced by methionine treatment on acetylcholine-induced relaxation described in the present investigation was also observed by Hanratty et al. (2001a, b) and Abularrage et al. (2007). These finding contrasts previous studies, which have reported that homocysteine impairs the endothelium-dependent relaxation induced by acetylcholine (Symons et al. 2002; De Andrade et al. 2006). Blood homocysteine level is a potential source of disparity between these results. Using the same experimental protocol for methionine treatment we previously described a maximum increase in blood homocysteine content of 15 μmol l^{-1} (Bonaventura et al. 2004). On the other hand, impairment of acetylcholine-induced relaxation is associated with higher levels of homocysteine as described by Abahji et al. (2007), Hanratty et al. (2001a), Symons et al. (2002), De Andrade et al. (2006), Shukla et al. (2006), who have detect plasma homocysteine levels of 30, 35, 58, 79, and 120 μmol l^{-1}, respectively.

An increased level of plasma homocysteine is a known risk factor for the development of atherosclerosis and other vascular diseases. The mechanisms by which homocysteine impairs the vessel wall are likely to be multifactorial. It has been suggested that elevated concentrations of homocysteine induce direct injury of vascular endothelium (Harker et al. 1976; Rodgers and Conn 1990; Weiss 2005). Blood vessels maintain a balanced state between vasodilatation and vasoconstriction through biochemical signaling by endothelial cells to smooth muscle cells (Rubanyi 1993). The disturbance of these cell functions is known to generally cause decreased relaxation and increased vasoconstriction (Luscher et al. 1993; Alexander 1995; Sellke et al. 1996). By using the same protocol for methionine feeding, we have previously described that administration of methionine in the drinking water enhanced angiotensin II-induced contraction in the rat carotid by a mechanism that involves increased production of vasoconstrictor prostanoids (Bonaventura et al. 2004). Increased levels of vasoconstrictor prostanoids associated with high levels of

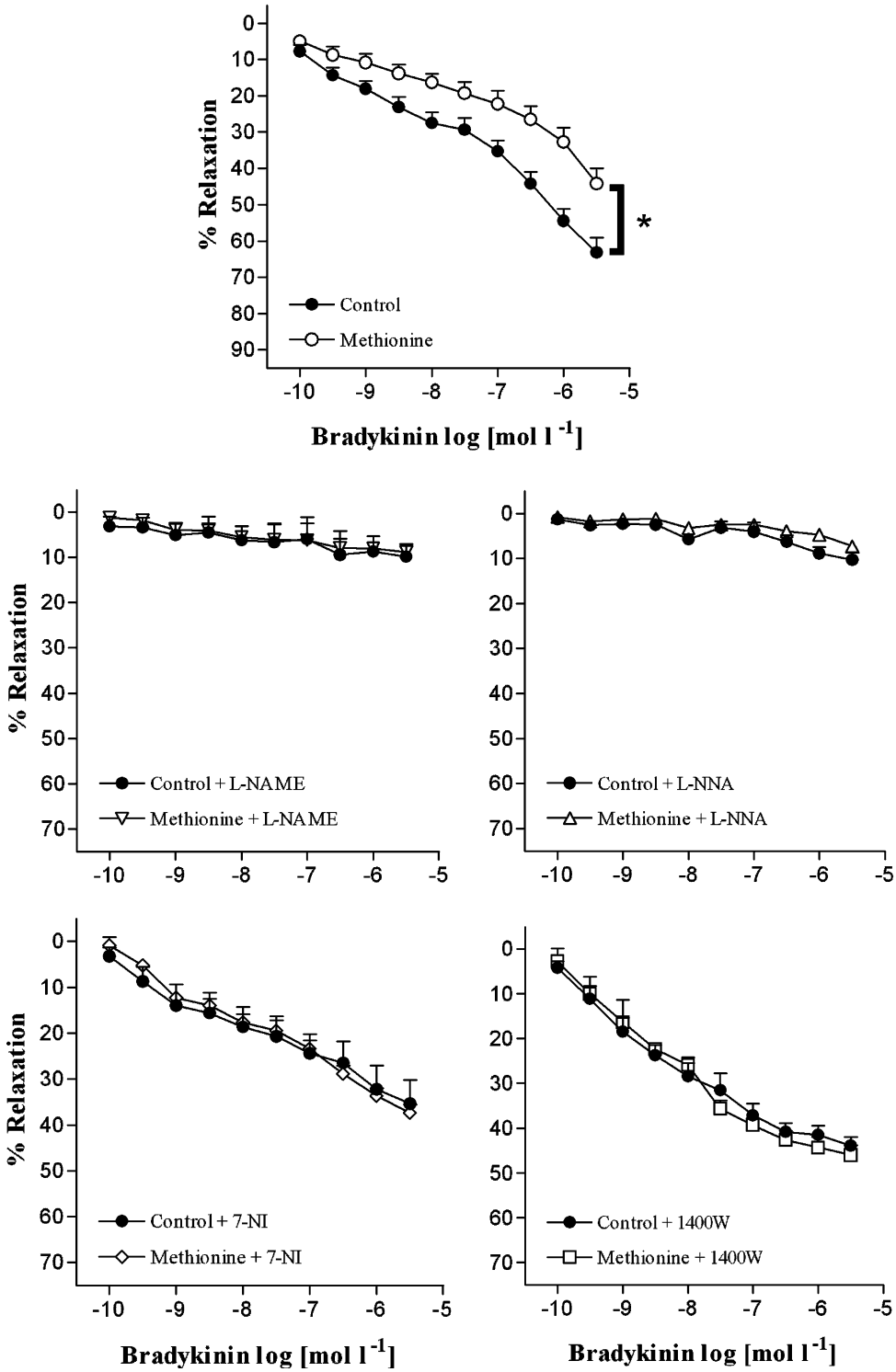

Fig. 5 Effect of L-NAME, L-NNA, 7-NI and 1400 W on BK-induced relaxation of endothelium-intact carotid rings. Concentration–response curves for BK were determined in endothelium-intact carotid rings from control or methionine-treated rats (8 weeks, 2 g kg^{-1}). The curves were determined in the absence or after 30-min incubation with L-NAME (100 μmol l^{-1}), L-NNA (100 μmol l^{-1}), 7-NI (100 μmol l^{-1}) or 1400 W (100 μmol l^{-1})

plasmatic homocysteine were also observed in other vascular tissues (Wang et al. 1993; Ungvari et al. 2000; Bagi et al. 2001). In the rat carotid, endothelium-derived vasoconstrictor prostanoids such as $PGF_{2\alpha}$, PGH_2 and TXA_2 counteract the vasorelaxant action displayed by BK (Tirapelli et al. 2007). In the present study, we found that chronic methionine consumption-induced impairment of BK-mediated relaxation was prevented by indomethacin and SQ29548. This observation supports the notion that methionine intake impairs BK-induced relaxation by a mechanism that involves increased production of endothelial vasoconstrictor prostanoids (probably TXA_2).

Table 4 Effect of methionine consumption (2 g kg^{-1}) for 8 weeks on the E_{max} (% relaxation) for BK in endothelium-intact carotid rings in the absence or presence of NOS inhibitors

Inhibitor	Control	Methionine
Absent	59 ± 2 (10)	44.2 ± 4.2[a] (9)
L-NAME (100 μmol l^{-1})	9.8 ± 2.7[a] (4)	8.9 ± 1.9[a] (5)
L-NNA (100 μmol l^{-1})	10.3 ± 1.6[a] (5)	7.3 ± 1[a] (5)
7-NI (100 μmol l^{-1})	35.4 ± 5.2[a] (8)	37.3 ± 1.7[a] (5)
1400 W (100 μmol l^{-1})	44.9 ± 1.9 (10)	46 ± 2.2 (5)

Number between parentheses indicates the number of preparations. Values are mean ± SEM

[a] Compared to control group in the absence of the inhibitors (ANOVA followed by Newman–Keuls multiple comparison test, $P < 0.05$)

In the rat carotid, the relaxation induced by BK involves the release of NO (Tirapelli et al. 2007). Incubation of carotid arteries from methionine-treated rats with L-NAME, L-NNA, 7-NI and 1400 W did not significantly modify the maximal relaxation induced by BK, when compared to control tissues. This data suggests that NO-mediated BK-relaxation was not attenuated by the treatment with methionine. Miyamoto et al. (1997) have identified a potentially important effect of NO on BK signaling pathways. They observed that NO can attenuate BK receptor ligand binding affinity and inhibit Gi and Gq proteins, that were necessary for relaxation induced by BK. Based on the fact that there was no difference between NO-mediated BK-relaxation in control and homocysteine rat carotid, we could suggest that the reduction in BK-relaxation in carotid rings from homocysteine group is not related to this important effect of NO on BK signaling pathways.

In various animal models and humans it has been shown that the stimulation of bradykinin B_2 receptors is implicated in powerful cardioprotective mechanisms (Spillmann et al. 2006). Decreased activity of this system may lead to cardiovascular diseases such as hypertension (Sharma and Thani 2004). Thus, pathophysiological conditions where the production of vascular prostanoids is increased, such as in diabetes (Akamine et al. 2006) and portal hypertension (Birney et al. 2003), may contribute to an altered response to BK, a fact that could decrease the cardioprotective effect displayed by this peptide. For this reason the present study showing that hyperhomocysteinemia impairs BK-induced relaxation, provides valuable information in the understanding of the vascular diseases associated with alterations on the vascular BK pathway.

Conclusion

Our findings show that chronic methionine consumption impairs the relaxant action displayed by BK in the isolated rat carotid and that this response is related to an increased production/release of vasoconstrictor prostanoids (possibly TXA_2) that acts counteracting the relaxant response induced by BK.

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