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Short communication

Enhanced endothelium-independent vasodilator response to calcitonin gene-related peptide in hypertensive rats

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Abstract

In isolated superior mesenteric arteries, vasodilation induced by calcitonin gene-related peptide (CGRP) was significantly larger in 12-week-old spontaneously hypertensive rats (SHR) than Wistar–Kyoto rats (WKY). In WKY and 6-week-old SHR, most of the vasodilator response to CGRP was abolished by N^{ω}-nitro-L-arginine and indomethacin. In contrast, the inhibitors caused no significant change in the response in 12-week-old SHR. Vasodilations induced by acetylcholine and isoproterenol were smaller in 12-week-old SHR than WKY, and that induced by sodium nitroprusside was comparable in both tissues. These results suggest that endothelium-independent vasodilator activity of CGRP is enhanced in hypertensive SHR, which overwhelms the decreased endothelium-dependent effects.

Keywords: CGRP (calcitonin gene-related peptide); Spontaneously hypertensive rat (SHR); Mesenteric artery

1. Introduction

Calcitonin gene-related peptide (CGRP) is a neuropeptide which is widely distributed in the perivascular nerves and elicits potent vasodilation through specific receptors (Bell and McDermott, 1996). The role of endothelium in mediating the vasodilator activity of CGRP varies in different blood vessels. In most vessels, the relaxant effect of CGRP is not affected by removal of the endothelium (Bell and McDermott, 1996). Conversely, the removal of endothelium abolishes CGRP's vasodilator effects in several vessels, such as rat thoracic aorta (Brain et al., 1985; Grace et al., 1987) and renal artery (Elhawary and Pang, 1995). In rat thoracic aorta, CGRP is likely to cause endothelium-dependent vasodilation via the release of nitric oxide (NO) (Gray and Marshall, 1992). It has also been suggested that endothelium-dependent vasodilation is mediated, at least in part, by the release of prostacyclin in rat renal artery (Villarreal et al., 1988).

We previously reported that CGRP is released from capsaicin-sensitive sensory nerves by electrical nerve stimulation and causes vasodilation in perfused mesenteric vascular bed of rats (Kawasaki et al., 1988; Fujimori et al., 1989). These findings suggest that CGRP acts as a vasodilator neurotransmitter and may participate in the cardiovascular regulation. In spontaneously hypertensive rats (SHR), which are used as a model of human essential hypertension, the vasodilator response to exogenously applied CGRP in perfused mesenteric vascular bed is greater than in normotensive control, Wistar–Kyoto rats (WKY) (Kawasaki et al., 1990). In contrast, decreased response to endothelium-dependent vasodilators such as acetylcholine has been detected consistently in hypertensive animals (Shirasaki et al., 1988; Dohi et al., 1990). The present study was therefore designed to examine the effects of hypertension on endothelium-dependent and -independent vasodilator responses to CGRP in isolated mesenteric arteries of SHR and WKY.

2. Materials and methods

Male SHR and WKY (6 and 12 weeks of age) were purchased from Charles River Japan (Kanagawa, Japan). Before the experiments, systolic blood pressure was mea-

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sured with a tail-cuff sphygmomanometer (Riken Kaihatsu PS-100, Kanagawa, Japan).

The rats were anesthetized with pentobarbital (50 mg/kg, i.p.). The superior mesenteric arteries were excised and placed in Krebs solution of the following composition (mM): NaCl 113, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 1.8, $MgSO_4$ 1.2, and glucose 5.5. After removal of connective tissues, arterial ring segments (4 mm long) were mounted using a stainless steel rod and tungsten wire in isolated tissue baths containing 30 ml Krebs solution. The tungsten wire was anchored to a plastic gate, and the steel rod was connected to a transducer (Nihon Kohden TB-612T, Tokyo, Japan) to measure the isometric contraction. The resting tension was adjusted to 1.0 g. The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at 37°C. After equilibration for 2 h, the response to 50 mM K^+ was measured repeatedly at intervals of 30 min until steady responses were obtained (usually three or four times). Vascular relaxation was studied in preparations constricted with 3 μM methoxamine, an α_1-adrenoceptor agonist. The amplitude of contractions induced by 3 μM methoxamine was not significantly difference between SHR and WKY; 572 ± 32 and 600 ± 29 mg at 12 weeks old ($n = 14$) and 565 ± 36 and 501 ± 29 mg at 6 weeks old ($n = 6$), respectively. In some experiments, N^{ω}-nitro-L-arginine (L-NNA) and indomethacin were added to the tissue baths 30 min before the application of methoxamine and were then present throughout the experiment. Preincubation of tissues with the drugs caused a slight contraction, which was usually less than 10 mg. When the contraction induced by 3 μM methoxamine reached a plateau, cumulative addition of vasodilators was performed. The responses to the vasodilators were expressed as the percentage of the maximum relaxation induced by 0.1 mM papaverine. The amplitude of relaxations induced by 0.1 mM papaverine was not significantly different between SHR and WKY; 581 ± 42 and 625 ± 22 mg at 12 weeks old ($n = 14$) and 569 ± 58 and 519 ± 36 mg at 6 weeks old ($n = 6$), respectively.

Data are presented as mean ± S.E.M. Statistical evaluation was performed by unpaired Student's *t*-test. A P level less than 0.05 was considered statistically significant.

The following drugs were used: human α-CGRP (Peptide Institute, Osaka, Japan); L-NNA, indomethacin, isoproterenol, methoxamine, sodium nitroprusside (Sigma, St. Louis, MO, USA); acetylcholine chloride, papaverine (Wako Pure Chemicals, Osaka, Japan). High K^+ solution was prepared by replacement of NaCl with an equimolar amount of KCl.

3. Results

Systolic blood pressure in 12-week-old SHR was significantly higher than that in age-matched WKY (185 ± 6 and 133 ± 4 mmHg, respectively; $n = 6$). In contrast, there was no difference in systolic blood pressure between 6-week-old SHR and WKY (128 ± 6 and 121 ± 4 mmHg,

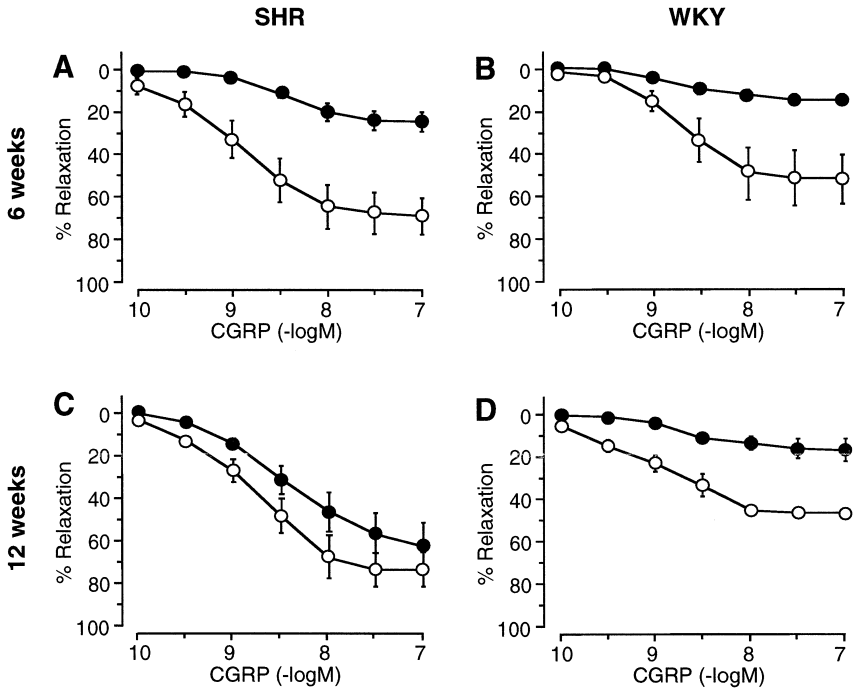

Fig. 1. Vasodilator activity of CGRP in isolated superior mesenteric arteries of 6- and 12-week-old SHR and WKY. Graphs show concentration–response relationship for CGRP-induced relaxation in preparations isolated from 6-week-old SHR (A) and WKY (B), and 12-week-old SHR (C) and WKY (D) in the absence (○) and presence (●) of N^{ω}-nitro-L-arginine (0.1 mM) and indomethacin (10 μM). Relaxation is expressed as the percentage of the maximum relaxation induced by 0.1 mM papaverine. Values represent mean ± S.E.M. for six experiments.

respectively; $n = 6$). These results indicate that 6-week-old SHR were normotensive, while 12-week-old SHR had developed a hypertensive state.

As shown in Fig. 1, CGRP caused dose-dependent vasodilation in all preparations from 6- and 12-week-old SHR and WKY. The maximum vasodilator response to CGRP was significantly larger in 12-week-old SHR than WKY ($73.5 \pm 7.6\%$ in SHR vs. $47.1 \pm 2.9\%$ in WKY), whereas there was no difference between 6-week-old SHR and WKY ($68.6 \pm 8.9\%$ and $51.5 \pm 11.8\%$, respectively). Mean EC_{50} values for CGRP-induced relaxation in 6- and 12-week-old SHR (1.21 and 1.83 nM) were not significantly different from those in 6- and 12-week-old WKY (1.70 and 1.06 nM), respectively.

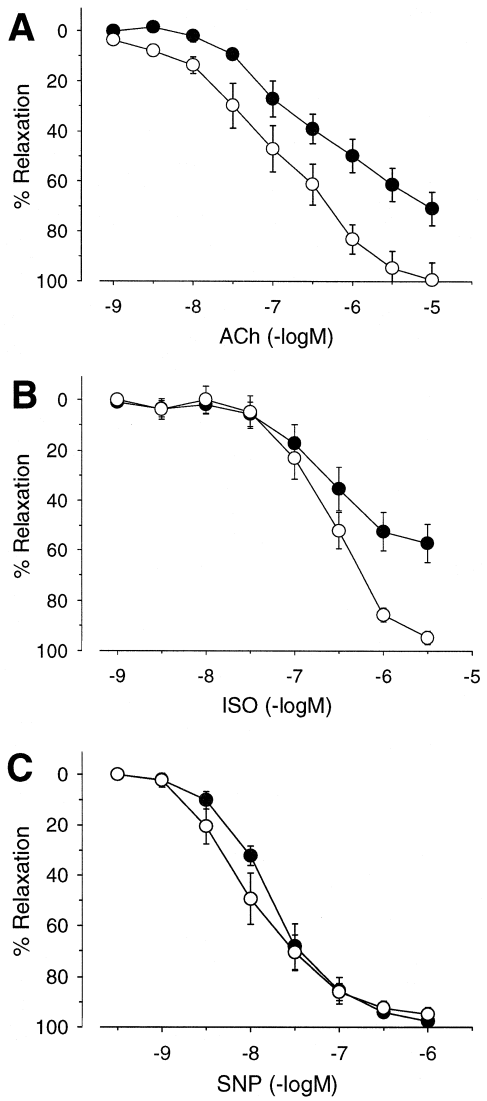

Fig. 2. Vasodilator activity of acetylcholine (ACh), isoproterenol (ISO) and sodium nitroprusside (SNP) in isolated superior mesenteric arteries of 12-week-old WKY and SHR. Graphs show concentration–response relationship for ACh (A)-, ISO (B)-, and SNP (C)-induced relaxation in preparations isolated from WKY (○) and SHR (●). Relaxation is expressed as the percentage of the maximum relaxation induced by 0.1 mM papaverine. Values represent mean ± S.E.M. for four to eight experiments.

The concentration–response relationship for CGRP-induced relaxation was also investigated in the presence of both L-NNA (0.1 mM), an NO synthase inhibitor, and indomethacin (10 μM), a cyclooxygenase inhibitor. As shown in Fig. 1, in 6- and 12-week-old WKY and 6-week-old SHR, the maximum vasodilator responses to CGRP were profoundly suppressed by the inhibitors. In contrast, combined treatment with the inhibitors caused no significant change in the vasodilator response to CGRP in the preparations isolated from 12-week-old SHR (Fig. 1C).

Fig. 2 illustrates the maximum vasodilator responses induced by acetylcholine, isoproterenol and sodium nitroprusside in 12-week-old SHR and WKY. The responses to acetylcholine and isoproterenol were significantly smaller in SHR than WKY, while there was no difference in the response to sodium nitroprusside between SHR and WKY. Mean EC_{50} values for vasodilation induced by acetylcholine, isoproterenol and sodium nitroprusside were not significantly different between SHR and WKY ($-\log EC_{50} = 6.51 \pm 0.14$ and 6.73 ± 0.28 for acetylcholine, 6.55 ± 0.14 and 6.84 ± 0.09 for isoproterenol, and 7.65 ± 0.16 and 8.10 ± 0.15 for sodium nitroprusside, respectively). The vasodilator activity of acetylcholine was abolished in the presence of both L-NNA (0.1 mM) and indomethacin (10 μM) (data not shown).

4. Discussion

The present study demonstrated that CGRP produced vasodilation via mainly endothelium-dependent mechanism in the superior mesenteric arteries of normotensive WKY and 6-week-old normotensive SHR, whereas in 12-week-old hypertensive SHR a large part of the vasodilator response to CGRP was endothelium-independent. This result indicates that endothelium-dependent vasodilator activity of CGRP is decreased in hypertensive animals. It has been reported that the endothelium-dependent vasodilator response to acetylcholine is reduced in the thoracic aorta and femoral artery of SHR as compared with age-matched WKY (Konishi and Su, 1983). A similar result was also observed in the superior mesenteric artery; acetylcholine-induced endothelium-dependent relaxation in 12-week-old SHR was about a half that in WKY. It is likely, therefore, that the impaired endothelium-dependent vasodilator response to CGRP in SHR at the hypertensive stage is due primarily to altered endothelial function, i.e., the decreased production and/or release of NO and prostacyclin. Nevertheless, the mesenteric artery of 12-week-old SHR exhibited obviously greater responsiveness to CGRP as compared with that of age-matched WKY, suggesting that the endothelium-independent action of CGRP is enhanced in SHR at the hypertensive stage. This enhanced endothelium-independent vasodilation in SHR is likely to

be specific for CGRP, because the responses to isoproterenol and sodium nitroprusside were not increased in SHR.

A number of studies have shown differences from normotensive animals in responsiveness to various vasoconstrictor and vasodilator agents in vascular tissues isolated from hypertensive animals. In particular, the decreased responsiveness of vascular smooth muscle to β-adrenoceptor agonists has been described in a variety of hypertensive animals including SHR (Cohen and Berkowitz, 1976; Asano et al., 1982; the present study). β-Adrenoceptor-mediated vasodilation involves increased cellular cyclic AMP through the activation of adenylate cyclase (Hardman, 1981). Furthermore, Masuzawa et al. (1989) have shown that vasodilator responses to adenosine A_2, histamine H_2 and dopamine D_1 receptor agonists, all of which act by activating adenylate cyclase, are significantly attenuated in the femoral and renal arteries of SHR. Since the vasodilator activities of both forskolin, an activator of adenylate cyclase, and dibutyryl cyclic AMP are not different between SHR and WKY (Asano et al., 1988), the events distal to cyclic AMP production are unlikely to be responsible for the decreased responsiveness to cyclic AMP-increasing agents in SHR. It is suggested, therefore, that a reduced coupling of the receptors to adenylate cyclase is mainly involved in the decreased responsiveness to these agents in SHR. In contrast to these agents, CGRP-induced vasodilation was enhanced in the superior mesenteric artery of SHR as compared with WKY, although CGRP elicits vasodilation by activating adenylate cyclase (Shoji et al., 1987). This discrepancy may be explained by dynamic changes in CGRP-containing nerves during the development of hypertension. CGRP is a vasodilator neurotransmitter released from capsaicin-sensitive sensory nerves (Kawasaki et al., 1988; Fujimori et al., 1989). In SHR at the hypertensive stage, CGRP-containing vasodilator innervation of the mesenteric vascular bed is decreased compared with that in age-matched WKY (Kawasaki et al., 1990). It is speculated, therefore, that the enhanced vasodilator response to CGRP in SHR at the hypertensive stage might result from upregulation of CGRP receptors on smooth muscle cells, which is induced by the decreased release of CGRP from CGRP-containing nerves in SHR. Increased number of CGRP receptors might overwhelm the impaired receptor-adenylate cyclase system in vascular smooth muscle cells of SHR. Further experiments such as a receptor binding assay will be needed to clarify this.

In conclusion, the present results suggest that in the superior mesenteric artery of aged SHR with established hypertension, endothelium-independent vasodilator activity of CGRP is enhanced, which overwhelms the decreased endothelium-dependent action. It has been reported that intravenous injection of CGRP induces a significant hypotensive effect in patients with essential hypertension (Tang et al., 1989). The effective vasodilator activity of CGRP in the hypertensive state suggests the therapeutic usefulness of the application of CGRP in essential hypertension.

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