Relaxant Effects of Calcitonin Gene-related Peptide on Isolated Small Renal Arteries in Stroke-Prone Spontaneously Hypertensive Rats

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Abstract

The relaxant effects of calcitonin gene-related peptide (CGRP) on the 3rd branches of renal arteries obtained from stroke-prone spontaneously hypertensive rats (SHRSP), and Wistar-Kyoto rats (WKY) were investigated in vitro. CGRP elicited concentration-dependent relaxation, and the relaxant response was not affected by the mechanical removal of endothelium in either SHRSP or WKY. The CGRP-induced relaxant response was markedly greater in SHRSP than in WKY, whereas there was no significant difference in acetylcholine-induced relaxation, which was endothelium-dependent, between the two groups. Additionally, significantly enhanced reactivity to CGRP was also shown in spontaneously hypertensive rats compared to WKY; however, this reactivity was less than that observed in SHRSP. There were also no significant differences between WKY and SHRSP in the relaxation induced by forskolin, dibutyryl cyclic AMP, and 3-isobutyl-1-methylxanthine (IBMX). CGRP-induced relaxation was significantly potentiated in similar manner by the pretreatment with IBMX in both WKY and SHRSP. Incubation with glibenclamide (10^-6 M) had no effect on CGRP-induced relaxation in either group, the WKY or the SHRSP. These results suggest that CGRP produces endothelium-independent relaxation in the small renal arteries in the rat, and that the increased CGRP-induced relaxant response found in SHRSP may not be associated with the altered vasodilation mediated by cyclic AMP, or with functional changes in ATP-sensitive potassium channels.

Keywords: Calcitonin gene-related peptide, Stroke-prone spontaneously hypertensive rats, Renal artery, Endothelium, Vascular relaxation

Introduction

Calcitonin gene-related peptide (CGRP) (Amara et al., 1982; Rosenfeld et al., 1983) has potent vasodilatory effects (Brain et al., 1985; Haass et al., 1985; Marshall et al., 1986a; 1986b). CGRP-like immunoreactivity and specific binding sites for CGRP have been demonstrated in blood vessels as well as in some regions of the heart (Gibbins et al., 1985; Saito et al., 1986; Sigrist et al., 1986; Uddman et al., 1986; Del Bianco et al., 1991; Franco-Cereceda, 1991). These evidences have suggested the possibility that nerves containing CGRP function as
a regulator of systemic or local circulations. It is well documented that hypertensive animal models exhibit impaired vascular responses to various relaxant agents when compared to normotensive controls. Thus, an altered vascular response to CGRP would be expected in hypertensive animals. Kawasaki et al. (1990a; 1991; 1992) have demonstrated that CGRP-containing vasodilator innervation in mesenteric vascular beds was greatly decreased, and that vasodilatory response to exogenously applied CGRP was increased in adult spontaneously hypertensive rats (SHR). Nishimura et al. (1992) have also shown enhanced CGRP-induced relaxation in basilar arteries from stroke-prone spontaneously hypertensive rats (SHRSP). However, little is known about the vascular response to CGRP in the renal arteries of hypertensive animals.

This study was designed to investigate whether the response to CGRP was altered in small renal arteries obtained from SHRSP. We also examined the relaxant effects of cyclic AMP-related drugs, and the influences of 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, and glibenclamide, an ATP-sensitive potassium channel (K<sub>ATP</sub>-channel) antagonist, on CGRP-induced relaxation. In addition, we compared the reactivity of small renal arteries to CGRP in SHR with that in WKY and SHRSP.

Materials and Methods

Animals

Male SHRSP and SHR at 6-7 months of age, and age-matched Wistar-Kyoto rats (WKY) were used in this study. All rats were given food and water ad libitum. They were housed in the Experimental Animal Center at Kinki University School of Medicine at constant temperature of 23°C, under a 12-hr light/dark cycle. Systolic blood pressure was measured by the standard tail cuff method; it was 255.3±3.2 (n=26), 206.2±9.6 (n=5) and 143.8±1.9 (n=26) mmHg for the SHRSP, SHR, and WKY respectively. Body weight was 324.2±8.3 g (n=26), 352.2±10.0 g (n=5), 371.8±7.5 g (n=26) for the SHRSP, SHR, and WKY respectively.

Experimental protocol

Under sodium pentobarbital anesthesia (40 mg/kg, i.p.), the rats were exsanguinated from the abdominal aorta. The kidneys were isolated, quickly removed, and placed in physiological salt solution (PSS; 120 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, and 10 mM glucose, pH 7.3-7.4). Using a dissecting microscope, we carefully dissected the 3rd branches of the renal artery (outside diameter 0.15-0.2 mm), from within the kidneys, cleared them of renal and connective tissue, and cut them into rings approximately 1 mm long. The endothelium was removed by gently rubbing the intimal surface with a stainless steel rod. Successful removal of endothelium was confirmed by the absence of small renal arteries to CGRP in SHR with that in WKY and SHRSP.
CGRP and SHRSP Small Renal Arteries

The resting tension was adjusted to 200 mg, which was found to be the optimal tension in preliminary experiments.

After at least 90 min equilibration, the preparations were precontracted with noradrenaline (NA, $6 \times 10^{-7} \text{M}$) to induce submaximal contraction (80% of the maximum). When the NA-induced contraction reached a plateau, the drugs were added in a cumulative manner to induce a relaxant response. The relaxation response to each concentration was expressed as a percentage of the maximal relaxation generated by the addition of $10^{-4} \text{M}$ papaverine at the end of each series of experiments. When tested, IBMX ($3 \times 10^{-6} \text{M}$) or glibenclamide ($10^{-6} \text{M}$) was added 20 min before the experiment was initiated.

**Chemicals**

The following chemicals were used: rat synthetic CGRP (Peptide Institute Inc.); acetylcholine chloride (ACh, Daiichi); norepinephrine bitartrate, glibenclamide, dibutryl cyclic AMP sodium salt and IBMX (Sigma); forskolin (Calbiochem); and papaverine hydrochloride (Dainippon).

CGRP was dissolved in distilled water to form a $10^{-4} \text{M}$ stock solution and was stored at $-40^\circ \text{C}$. Forskolin and IBMX were dissolved in 50% ethanol and dilutions were prepared in distilled water. Other chemicals were all dissolved in distilled water immediately before the experiments. The concentrations expressed below are all final concentrations in the organ bath.

**Statistical analyses**

Results are expressed as the mean±S.E.M. for n separate experiments. Significant differences were assessed by either a two-tailed unpaired Student's t-test or one way analysis of variance, followed by the Newman-Keuls' test (Bruning et al., 1977). P values of less than 0.05 were considered significant.

**Results**

**Relaxant effects of CGRP and ACh**

The cumulative addition of CGRP ($10^{-11} \text{M} - 10^{-7} \text{M}$) produced concentration-dependent relaxation in the small renal arteries of both SHRSP and WKY that had been pre-contracted at submaximal contraction with NA. These relaxant effects of CGRP were not affected by mechanical removal of the endothelium. CGRP-induced relaxation was significantly greater in both the endothelium-intact and rubbed arteries obtained from SHRSP than in those obtained from WKY (Figs. 1A and 2A). ACh relaxed endothelium-intact arteries in a concentration-dependent manner, and this effect was abolished by removal of the endothelium. However, there was no significant difference between the two groups for this response (Figs. 1B and 2B).

The effects of CGRP on SHR small renal arteries were also investigated and compared with the effects on these arteries from SHRSP and WKY. As shown in Figure 3, CGRP-induced relaxation in endothelium-intact preparations was significantly greater in SHR than in WKY; nevertheless, it was smaller than that in SHRSP. Removal of the endothelium did not
change the reactivity of SHR small renal arteries to CGRP (data not shown).

Relaxant effects of cyclic AMP–related drugs

The relaxant effects of forskolin (an adenylate cyclase activator), dibutyryl cyclic AMP (an analogue of cyclic AMP), and IBMX were examined in endothelium–intact preparations from SHRSP and WKY (Fig. 4). There were no significant differences between the two groups in the relaxation induced by these three drugs.

Effects of IBMX on CGRP–induced relaxation

The relaxant response to CGRP was tested in endothelium–intact preparations after pretreatment with IBMX ($3 \times 10^{-6}$ M) for 20 min. CGRP–induced relaxation was significantly potentiated by the pretreatment with IBMX in both WKY and SHRSP in similar manner (Fig. 5). The magnitude of NA–induced contraction was not significantly altered by the incubation
Fig. 2. Relaxant responses of small renal arteries to CGRP (A) and ACh (B). Small renal arteries from WKY, with and without endothelium, are denoted by ◊ and △, respectively; arterial preparations from SHRSP, with and without endothelium, are denoted by ● and ▲, respectively. All preparations were precontracted with $6 \times 10^{-7}$ M NA. Relaxation induced by CGRP and ACh is expressed as a percentage of the maximal relaxation induced by $10^{-4}$ M papaverine. Each point is the mean of 5 experiments. Vertical lines represent the S.E.M. **$p<0.01$, compared with respective preparations from WKY (Newman-Keuls' test).

Fig. 3. Relaxant effects of CGRP on small renal arteries from SHR. WKY, SHR, and SHRSP are denoted by ○, □, and ●, respectively. Arterial preparations were precontracted with $6 \times 10^{-7}$ M NA. The relaxant effects of CGRP are expressed as a percentage of the maximal relaxation induced by $10^{-4}$ M papaverine. Each point is the mean of 5 experiments. Vertical lines represent the S.E.M. *$p<0.05$, **$p<0.01$, compared with WKY, *$p<0.05$, **$p<0.01$, compared with SHRSP (Newmen-Keuls' test).
Fig. 4. Relaxant effects of forskolin (A), dibutyryl cyclic AMP (B), and IBMX (C) on small renal arteries from WKY (○) and SHRSP (●). All arteries were precontracted with $6 \times 10^{-7}$ M NA. The relaxation is expressed as a percentage of the maximal relaxation induced by $10^{-4}$ M papaverine. Each point is the mean of 5 experiments. Vertical lines represent the S.E.M.

Fig. 5. Influence of IBMX on CGRP-induced relaxation of small renal arteries from WKY and SHRSP. Arteries from WKY, with and without IBMX pretreatment, are denoted by □ and ○; preparations from SHRSP, with and without IBMX pretreatment, are denoted by ■ and ●. IBMX ($3 \times 10^{-6}$ M) was added 20 min before the initiation of the experiment. Contraction was induced by $6 \times 10^{-7}$ M NA, and the relaxation was expressed as a percentage of the maximal relaxation induced by $10^{-4}$ M papaverine. Each point is the mean of 6 experiments. Vertical lines represent the S.E.M. *$p<0.05$, **$p<0.01$, compared with respective control; ***$p<0.01$, compared with WKY control (Newmen-Keuls' test).
Fig. 6. Influence of glibenclamide on CGRP-induced relaxation of small renal arteries from WKY and SHRSP. Arteries from WKY, with and without glibenclamide pretreatment, are denoted by □ and ○; preparations from SHRSP, with and without glibenclamide pretreatment, are denoted by ■ and ●. Glibenclamide (10⁻⁶ M) was added 20 min before the initiation of the experiment. Contraction was induced by 6×10⁻⁷ M NA, and the relaxation was expressed as a percentage of the maximal relaxation induced by 10⁻⁴ M papaverine. Each point is the mean of 5 experiments. Vertical lines represent the S.E.M.

with this concentration of IBMX, although the onset of contraction became a little slow.

Effects of glibenclamide on CGRP-induced relaxation

After the arteries were incubated with 10⁻⁶ M glibenclamide for 20 min, the effect of CGRP was tested in both endothelium-intact and rubbed preparations from SHRSP and WKY. However, the glibenclamide pretreatment had no effect on the CGRP-induced relaxation (Fig. 6). NA-induced contraction was not affected by the pretreatment of glibenclamide.

Discussion

This study demonstrated that CGRP induced potent relaxation in the 3rd branches of rat renal arteries via an endothelium-independent mechanism. It has been reported that CGRP did not require intact endothelium to produce relaxation of rat basilar (Nishimura et al., 1992), gastric, splenic, and hepatic arteries (Bratveit et al., 1991). However, it has been shown that CGRP produced vasodilation of the rat aorta (Brain et al., 1985; Kubota et al., 1985; Grace et al., 1987) and superior mesenteric artery (Bratveit et al., 1991) in an endothelium-dependent manner. Prieto et al. (1991) found that CGRP-induced relaxation of rat proximal epicardial coronary arteries was almost abolished by the removal of endothelium, whereas such relaxation in the distal intramyocardial arteries was not affected. Thus, CGRP-induced relaxation
in the rat vasculature appears to show heterogeneous endothelium dependency.

An enhanced relaxant response to CGRP was observed in small renal arteries from SHR and SHRSP, compared to the response for WKY. Further, the enhancement of relaxation was greater in SHRSP than in SHR. It seemed likely that this enhancement might be related to the different levels of elevated blood pressure in SHR and SHRSP. Whether these alterations in hypertensive animal models are hereditary or are a secondary effect of hypertension remains to be elucidated. It has been reported that CGRP-induced relaxation of mesenteric vascular beds was not increased in 8-week-old SHR (Kawasaki et al., 1990a); this would suggest that the enhanced relaxation response to CGRP might be a secondary effect due to hypertension. On the other hand, in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, no such alteration was observed (Kawasaki et al., 1990b), indicating that this change might be hereditary.

There is much evidence that the action of CGRP is mediated through the generation of cyclic AMP in endothelial and vascular smooth muscle cells (Edvinsson et al., 1985; Kubota et al., 1985; Hirata et al., 1988; Crossman et al., 1990; Edwards et al., 1991). In the present study on small renal arteries from WKY and SHRSP, we found that the relaxant response to CGRP was significantly potentiated by IBMX, a phosphodiesterase inhibitor, in similar manner. Thus, it is indicated that the relaxation induced by CGRP in these arteries are mediated by the elevation of cyclic AMP, although cyclic AMP level was not actually measured. Furthermore, there were no significant differences between WKY and SHRSP in relaxation responses to forskolin, dibutyryl cyclic AMP and IBMX. These results suggest that the increased CGRP-induced relaxation observed in SHRSP small renal arteries is unlikely to be due to the altered vasodilation mediated by cyclic AMP.

A recent study (Nelson, 1990) has demonstrated that CGRP relaxed rabbit mesenteric arteries by activating K<sub>ATP</sub>-channels. Glibenclamide, a blocker of K<sub>ATP</sub>-channels, was shown to have markedly attenuated the relaxation induced by CGRP, and single potassium channel activity was increased by CGRP. Furthermore, potassium channel activity was reportedly increased in carotid arteries from SHRSP (Miyata et al., 1990). Therefore, it is necessary to identify whether the increased reactivity to CGRP of SHRSP small renal arteries was mediated by enhanced K<sub>ATP</sub>-channel involvement. However, we found that pretreatment with glibenclamide had no influence on the effects of CGRP on these arteries in either group. Other investigators have also found that CGRP-induced relaxation did not involve the activation of K<sub>ATP</sub>-channels (Prieto et al., 1991; Abdelrahman et al., 1992; Kageyama et al., 1993). Thus, it seems reasonable to postulate that the activation of K<sub>ATP</sub>-channels by CGRP, if it does occur, plays a minor role in CGRP-induced relaxant effects in rat small renal arteries.

CGRP has been shown to be involved in non-adrenergic, non-cholinergic vasodilatory responses in the mesenteric resistance vessels of the rat (Kawasaki et al., 1988). Further, marked decrease in the release of CGRP-like immunoreactive substance evoked by perivascular stimulation, and significant enhancement of relaxation in response to exogenously applied CGRP have been shown in the mesenteric vascular beds of aged SHR (Kawasaki et al., 1991). There has been no direct evidence so far reported for the release of CGRP from perivascular nerves in rat small renal arteries. However, radioimmunoassay has found that renal artery of
rat contained abundant CGRP (Mulderry et al., 1985), and immunohistochemical studies also revealed that CGRP immunoreactive nerve fibers were mainly observed surrounding blood vessels in the cortex and outer medulla of rat kidney (Geppetti et al., 1989). Thus, it might be reasonable to speculate that if the release of CGRP from perivascular nerves was impaired in the renal vasculature of SHRSP, this might cause up-regulation of CGRP receptors in vascular smooth muscles, and hence enhance reactivity to exogenous CGRP.

In conclusion, this study demonstrated that CGRP-induced relaxation in rat small renal arteries was endothelium-independent. Increased reactivity to CGRP was shown in SHR and SHRSP; this seemed to be due to the up-regulation of CGRP receptors in vascular smooth muscle cells. However, further investigation is needed to elucidate the pathophysiological role played by CGRP in the regulation of renal circulation and in the pathogenesis and maintenance of hypertension in hypertensive animals.

References


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