Impaired β-Adrenergic Hyperpolarization in Arteries From Prehypertensive Spontaneously Hypertensive Rats

Kenichi Goto, Koji Fujii, Isao Abe

Abstract—Stimulation of β-adrenoceptors leads to vascular smooth muscle hyperpolarization, presumably through the β-adrenoceptors/Gs protein/adenylate cyclase/ATP-sensitive K⁺-channels (KATP) signaling cascade, which may play an important role in the sympathetic control of membrane potential. β-Adrenoceptor–mediated hyperpolarization has been shown to be impaired in the established stage of experimental hypertension. The present study tested the hypothesis that β-adrenergic hyperpolarization may be defective before the development of hypertension in some forms of genetic hypertension. We evaluated β-adrenoceptor–mediated hyperpolarization using microelectrodes in mesenteric resistance arteries from 5-week-old, prehypertensive, spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto rats (WKY). Isoproterenol-induced hyperpolarization was significantly smaller in SHR than in WKY (10⁻⁷ mol/L: −4.6±0.6 mV versus −7.8±0.8 mV, P<0.01; 10⁻⁶ mol/L: −7.8±0.5 mV versus −9.8±0.6 mV, P<0.05; n=9). Furthermore, hyperpolarization to cholaria toxin, a direct activator of Gs protein, was also impaired in SHR. On the other hand, hyperpolarization to forskolin, an adenylate cyclase activator, and to levcromakalim, a KATP opener, was comparable between groups. These findings suggest that β-adrenoceptor–mediated hyperpolarization is defective in SHR before the development of hypertension, presumably because of an abnormality at the Gs protein site. Considering the importance of membrane potential in the control of vascular tone, altered β-adrenergic control of membrane potential might play a role in the development of hypertension in SHR. (Hypertension. 2001;37[part 2]:609-613.)

Key Words: receptors, adrenergic ■ membrane potentials ■ hyperpolarization ■ hypertension, genetic ■ rats, spontaneously hypertensive

Stimulation of vascular β-adrenoceptors leads to smooth muscle hyperpolarization and relaxation, presumably through the β-adrenoceptors/Gs protein/adenylate cyclase/cAMP signaling cascade.¹ ² We recently demonstrated that isoproterenol-induced hyperpolarization is mediated by the opening of ATP-sensitive K⁺ channels (KATP) in rat mesenteric resistance arteries,³ which parallels the case of the canine saphenous veins,⁴ and that this β-adrenergic hyperpolarization plays an important role in the sympathetic control of membrane potential by opposing α-adrenoceptor–mediated depolarization.⁴ In view of the importance of membrane potential as a determinant of smooth muscle tone,⁵ β-adrenoceptor–mediated hyperpolarization may play an important role in the control of vascular tone.

β-Adrenoceptor–mediated relaxation is impaired in arteries from hypertensive rats,⁶ ⁷ and in some models, such an abnormality has been shown to precede the development of hypertension.⁶ ⁷ On the other hand, information is limited regarding β-adrenoceptor–mediated hyperpolarization in hypertension. To the best of our knowledge, the only previous report on this topic, made by Stekiel et al.,¹ demonstrated that in situ hyperpolarization to isoproterenol is impaired in arterioles from reduced renal mass hypertensive rats. It is unclear, however, whether such an abnormality occurs merely as a result of high blood pressure or rather could play a causal role in the development of hypertension, particularly in the case of genetic hypertension.

The present study, using a conventional microelectrode technique, tested the hypothesis that β-adrenoceptor–mediated arterial hyperpolarization may be defective before the development of hypertension in spontaneously hypertensive rats (SHR), a genetic model of hypertension, and subsequently attempted to elucidate the underlying mechanisms of the impairment.

Methods

Preparation of Arteries

Five-week-old SHR/Izm and age-matched Wistar-Kyoto rats (WKY)/Izm (Disease Model Cooperative Research Association, Kyoto, Japan) were used in the present study. The rats had free access to tap water and were fed a normal rat chow. The study protocol was approved by the Animal Experimentation Ethics Committee of Kyushu University. Rats were anesthetized with ether and exsanguinated. The third or fourth branch of the mesenteric arteries (external diameter, 100 to 150 μm) was excised and bathed in cold Krebs’ solution of the following composition (in mmol/L):

- NaCl, 122.0
- KCl, 4.7
- CaCl₂, 1.25
- MgSO₄, 1.2
- NaHCO₃, 24.7
- NaH₂PO₄, 1.2
- glucose, 11.0

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After an equilibration of $t$ minutes, the membrane potentials of SHR were larger (P<0.05) than those of WKY. (Figure 1). Isoproterenol-induced hyperpolarization was impaired in resistance arteries compared between the 2 strains. These findings suggest that $\beta$-adrenoceptor–mediated hyperpolarization is defective in SHR even before the development of hypertension, and this abnormality may be due to a defect at the level of the Gs protein. The present study demonstrated that (1) isoproterenol-induced hyperpolarization was impaired in resistance arteries from prehypertensive SHR compared with those from age-matched WKY; (2) cholera toxin–induced hyperpolarization was also attenuated in prehypertensive SHR; and (3) hyperpolarization to forskolin and that to levromakalim were comparable between strains. These findings suggest that $\beta$-adrenoceptor–mediated hyperpolarization is defective in SHR even before the development of hypertension, and this abnormality may be due to a defect at the level of the Gs protein.

**Statistical Analysis**

Data are expressed as mean±SEM; n refers to the number of animals examined. The concentration-response curves of hyperpolarization were analyzed by 2-way ANOVA followed by Scheffé’s test for multiple comparisons. The concentrations of agonists causing half-maximal responses (EC$_{50}$ value) were calculated with a nonlinear regression analysis. The EC$_{50}$ values were expressed as the negative logarithm of the molar concentration (pD$_{2}$ values). Other variables were analyzed by 2-way ANOVA followed by Scheffé’s test for multiple comparisons or unpaired Student’s $t$ test. A level of $P<0.05$ was considered statistically significant.

**Results**

Body weight was similar between 5-week-old SHR and age-matched WKY (97.8±4.0 versus 102.8±2.0 g, $P=NS$; n=10 in each group). Systolic blood pressure did not differ between 5-week-old SHR and WKY (137.5±4.3 versus 138.1±2.3 mm Hg, $P=NS$; n=10 in each group). Resting membrane potential of the mesenteric resistance arteries was $-62.3±0.9$ mV for SHR and $-61.7±0.5$ mV for age-matched WKY ($P=NS$; n=10 in each group).

Representative tracings and a summary of the data of isoproterenol-induced hyperpolarization in the mesenteric resistance arteries are shown in Figure 1. Isoproterenol hyperpolarized the membrane in a concentration-dependent manner in both strains. However, the amplitude of isoproterenol-induced hyperpolarization was significantly smaller in 5-week-old SHR than in age-matched WKY ($P<0.05$ by 2-way ANOVA; n=9 in each group), although pD$_{2}$ values did not differ between the 2 strains (pD$_{2}$ values: SHR, 7.5±0.2; WKY, 8.0±0.2; $P=NS$; maximal hyperpolarization: SHR, $-8.2±0.5$; WKY, $-10.1±0.6$ mV; $P<0.05$) (Figure 1). Hyperpolarization to choler toxin, a direct activator of Gs protein, was also significantly smaller in SHR than in WKY ($10^{-6}$ g/mL: $-2.1±0.9$ versus $-4.5±0.6$ mV; $P<0.05$; n=6 in each) (Figure 2). On the other hand, hyperpolarization to forskolin, a direct activator of adenylate cyclase, was comparable between the 2 groups (Figure 3). Hyperpolarization to levromakalim, a direct activator of KATP, also did not differ between the 2 groups (Figure 4).

**Discussion**

The following drugs were used: isoproterenol hydrochloride, forskolin, cholera toxin (all from Sigma Chemical Co), and levromakalim (kindly provided by Smith-Kline Beecham Pharmaceuticals). Forskolin was dissolved in 9.95% dimethyl sulfoxide. Levromakalim was dissolved in ethanol. The other drugs were dissolved in distilled water. All drugs were further diluted $\times1000$ in the Krebs’ solution to give the final chamber concentrations.

**Impaired Isoproterenol-Induced Hyperpolarization in Prehypertensive SHR**

Stekiel et al showed that isoproterenol-induced hyperpolarization in situ was markedly reduced in cremaster muscle arterioles from reduced renal mass hypertensive rats, suggesting that the $\beta$-adrenoceptor–mediated hyperpolarization may be impaired at the established stage of hypertension.
The present findings show that the β-adrenoceptor–mediated arterial hyperpolarization is defective in prehypertensive SHR, indicating that the impairment of β-adrenergic hyperpolarization may not merely occur as a consequence of hypertension but could play a causal role in the development of genetic hypertension. This parallels the case of β-adrenoceptor–mediated arterial relaxation in SHR, which has been shown to be impaired not only at the established stage of hypertension but also at the prehypertensive stage. Fujimoto et al. and Cheng et al. showed that β-agonist–induced relaxation is impaired in conduit arteries, such as femoral arteries, superior mesenteric arteries, and aortae, from prehypertensive SHR. Our study, however, is distinct from these previous studies in that we compared electrical responses, (ie, isoproterenol-induced hyperpolarization) and that we focused on mesenteric resistance arteries, which are thought to play a pivotal role in determining total peripheral resistance in rats.

**Underlying Mechanisms of the Impaired β-Adrenoceptor–Mediated Hyperpolarization in Prehypertensive SHR**

As mentioned previously, isoproterenol-induced hyperpolarization in the rat mesenteric arteries is likely to be achieved...
through the following signaling cascade: β-adrenoceptors/Gs/adenylate cyclase/KATP.1–3 Although endothelium-derived nitric oxide has been suggested to account in part for β-adrenoceptor-mediated relaxation in certain blood vessels,15 isoproterenol appears to act directly on smooth muscle cells to elicit hyperpolarization in the rat mesenteric arteries.2,4 In the present study, hyperpolarization to forskolin, a direct activator of adenylate cyclase,13 and to levcromakalim, a direct opener of KATP,14 did not differ between prehypertensive SHR and age-matched WKY, suggesting that the main defect responsible for the impaired β-adrenoceptor-mediated hyperpolarization in prehypertensive SHR may lie upstream of adenylate cyclase.

Recently, increased activity of G-protein–coupled receptor kinase, which phosphorylates and downregulates G-protein–linked receptors, has been documented in lymphocytes from hypertensive subjects.16 However, the present findings that hyperpolarization to cholora toxin, a direct activator ofGs, as well as β-agonist–induced responses, was reduced in prehypertensive SHR may favor the defect at the Gs protein site rather than the alteration of β-adrenoceptors per se. The β-adrenoceptor density has also been reported to be unchanged in the mesenteric vascular bed of SHR.11 Stekiel et al17 suggested that the impaired isoproterenol-induced hyperpolarization in arterioles from reduced renal mass hypertensive rats may be due to a defect at the Gs protein–adenylate cyclase coupling step, because hyperpolarization to cholora toxin was markedly impaired whereas that to forskolin was preserved. It has also been suggested that the reduced function of Gs protein may be responsible mainly for the impaired β-agonist–induced relaxation in femoral, superior mesenteric, renal, and carotid arteries from 13-week-old SHR compared with age-matched WKY.9,10 because relaxation to norepinephrine and cholora toxin, but not to forskolin, was attenuated. The present findings are largely in agreement with the findings in these studies9,10 and demonstrates for the first time the possibility that function of Gs protein, involved in β-adrenergic hyperpolarization, may be defective in resistance arteries before the development of hypertension in genetically hypertensive rats.

Gs protein levels of vascular smooth muscle cells of SHR have been reported to be unchanged both before17,18 and after the development of hypertension.19,20 Tissue levels of inhibitory guanine nucleotide binding protein, which inhibits the Gs protein function, have been reported to be either increased17,18 or unchanged21 in SHR. The precise mechanism of impaired function of Gs protein in prehypertensive SHR is unclear from the present findings alone and remains to be determined by further investigation.

Pathophysiological Implications

β-Adrenergic hyperpolarization may contribute to the sympathetic control of membrane potential by opposing α-adrenergic depolarization in the rat mesenteric resistance arteries.2,4 The resting membrane potentials in this study are rather negative, and the magnitude of the hyperpolarizations that occur in response to β-agonists is relatively small. However, if the membrane potentials were measured in situ, the resting membrane potentials would be much less negative because of neuronal and hormonal influences1 and possibly the influence of a higher pressure in the vessel. As a result, the hyperpolarizations that occur in vivo should have a much greater effect on vessel tone, because the less negative membrane potential occurring in vivo should be in the range at which voltage-gated Ca2+ channels are activated. Therefore, any hyperpolarization that occurs should have a substantial effect on vessel tone, presumably a greater relaxation in the normotensive animals and less relaxation in the SHR.

Notably, several recent studies have demonstrated that activation of KATP is involved in β-agonist–induced vaso-dilatation.22–24 Defective β-adrenoceptor–mediated hyperpolarization might therefore permit enhanced contractile responses to sympathetic nerve stimulation in vascular smooth muscle, possibly leading to an increase in total peripheral resistance.

Impairment of β-adrenergic responses has been documented not only in patients with hypertension25 but also in certain population with higher prevalence of essential hypertension.19 Gs protein function may also be reduced in hypertensive subjects.26 Furthermore, genetic variants of the β2-adrenoceptor gene have been suggested to be associated with high blood pressure.28,29 These findings imply possible involvement of the altered β-adrenergic system in the pathogenesis of essential hypertension.

In conclusion, β-adrenoceptor–mediated hyperpolarization is impaired in the mesenteric resistance arteries from prehypertensive SHR presumably as a result of a defect at the level of Gs protein. It remains to be determined whether the reduced β-adrenergic hyperpolarization leads to an increase in peripheral resistance, thereby contributing to the development of hypertension.

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References


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