Effect of Cholecalciferol Treatment on the Relaxant Responses of Spontaneously Hypertensive Rat Arteries to Acetylcholine

Antonio C.R. Borges, Teresa Feres, L.M. Vianna, Therezinha B. Paiva

Abstract—We studied the effect of oral cholecalciferol treatment on the endothelium-dependent vascular relaxation and hyperpolarization induced by acetylcholine (ACh), which is impaired in spontaneously hypertensive rats (SHR). Adult female SHR and normotensive Wistar-Kyoto rat (WKY) controls received 125 µg of cholecalciferol per kilogram body weight per day for 6 weeks. The responses to ACh of the isolated mesenteric vascular bed and mesenteric artery rings were measured, as well as the smooth muscle cell membrane potential. After cholecalciferol treatment, the systolic blood pressure and basal perfusion pressure of the mesenteric vascular bed of the SHR fell to control levels. The relaxant and hyperpolarizing effects of ACh, which are reduced in SHR, were also brought to control levels after cholecalciferol treatment. These effects of ACh were inhibited by Nω-nitro-L-arginine in SHR and by apamin in WKY. After cholecalciferol treatment, SHR hyperpolarizing responses showed the same inhibition pattern as those of WKY. This indicates that, after cholecalciferol treatment, SHR vascular mesenteric preparation responses to ACh are mediated by endothelium-derived hyperpolarizing factor, which induces activation of Ca2+-dependent K+ channels, as in WKY. In untreated SHR, the ACh-mediated response is entirely due to ACh acting via the release of nitric oxide. (Hypertension. 1999;34[part 2]:897-901.)

Key Words: rats, inbred SHR ■ arteries ■ membranes ■ potassium ■ acetylcholine ■ cholecalciferol

Many reports in the literature have demonstrated that the vascular responsiveness to various stimuli is altered in spontaneously hypertensive rats (SHR).1,2 The major observations are that some blood vessels from SHR are hyperactive to constrictor stimuli,3,4 whereas endothelium-dependent vasodilator responsiveness is impaired.5–8

Acetylcholine (ACh) induces endothelium-dependent vasorelaxation in precontracted arteries through release of nitric oxide (NO), prostacyclin, and endothelium-dependent hyperpolarizing factor (EDHF).9–17 EDHF is thought to act by opening different K1 channels in arteries in various species and vascular beds.12,17–19 Hyperpolarization is an effective mechanism for relaxing vascular smooth muscle, since it decreases the open-state probability of L-type voltage-dependent Ca2+ channels, thereby reducing the level of intracellular Ca2+, leading to vasorelaxation. Different K+ channels have been implied in this mechanism, depending on the type of artery and animal species considered.20 In the case of the rat mesenteric artery, evidence in the literature suggests that apamin-sensitive Ca2+-dependent K+ channels may be the main ion channels mediating the endothelium-dependent hyperpolarizing response to ACh.21,22 These channels were found to be impaired in mesenteric vessels23 as well as in other visceral24 smooth muscles of the SHR. This could be responsible for the reduced endothelium-dependent hyperpolarizing and relaxation responses to ACh in SHR mesenteric arteries, since the role of NO in the response was well preserved, and the participation of prostacyclin in the relaxation appeared to be insignificant.11,13,14,18,25,26

Oral administration of cholecalciferol was shown to normalize the blood pressure4,27 as well as the functioning of Ca2+-dependent K+ channels in SHR visceral24 and vascular smooth muscles without increasing the serum calcium concentration.4 We have now investigated the effect of that treatment on the impaired endothelium-dependent relaxation and hyperpolarizing responses to ACh in the isolated mesenteric vascular bed and mesenteric arterial rings of SHR.

Methods

Animals

Experiments were performed with female Okamoto and Aoki SHR and their Wistar-Kyoto rat (WKY) normotensive controls derived from an original colony supplied by the National Institutes of Health, Bethesda, Md. Normotensive Wistar rats (NWR) from the Wistar Institute, Philadelphia, Pa, inbred at Escola Paulista de Medicina, Sao Paulo, Brazil, were also used. The rats were aged 20 to 30 weeks and weighed 200 to 220 g. They were fed a standard diet (Labina rat chow, Purina), containing 6600 IU vitamin D3 per kilogram. After a basal period of 10 days, the treated groups received, by oral gavage,
a daily supplement of 125 μg (500 IU) cholecalciferol per kilogram body weight (Sigma Chemical Co), dissolved in 0.3 mL of coconut oil. The duration of treatment was 6 weeks, and the control groups received only the vehicle. Systolic blood pressure was measured twice weekly from the tail of prewarmed unanesthetized rats by a plethysmographic method (LE 5650/6, Letica Scientific Instruments). An average of 3 readings was recorded for each animal. After 6 weeks of treatment, some animals were decapitated to remove the mesenteric vascular bed, which was dissected away from the intestine for perfusion pressure measurements. Others were decapitated to remove the superior mesenteric arteries, which were cleaned of adherent connective tissue and cut into rings (3 to 4 mm in length) for tension and electrophysiological measurements. Care was taken to ensure that the endothelial layer was not damaged during the processing of tissue preparation. All procedures complied with the norms of the Ethic Committee for Research of the São Paulo Hospital/Federal University of São Paulo.

Mechanical Responses
Mesenteric vascular bed preparations were set up as previously described2,28 and perfused at a constant flow of 4.0 mL/min, with the use of a peristaltic pump (model 2115, LKB-Produkter AB), with Krebs’ solution of the following composition (in mmol/L): NaCl 137, NaHCO3 5.9, KHCO3 5.9, CaCl2 2.3, MgCl2 1.2, and glucose 11.8. The solution was bubbled with a 5% CO2 /95% O2 gas mixture and maintained at pH 7.4 and 37°C. Indomethacin (10 μmol/L). Micropipettes (Borosilicate glass capillaries 1B120F-6, World Precision Instruments [WPI]) were made by means of a vertical puller (Pal-100, WPI) and filled with 2 mol/L KCl (tip resistance 20 to 40 MΩ and tip potential <6 mV). The microelectrodes were mounted in Ag/AgCl half cells on a micromanipulator (Leitz, Leica) and connected to an electrometer (Intra 767, WPI). The impalpitations were made in the smooth muscle cells from the adventitial side in rings with intact endothelium. The electrical signals were continuously monitored on an oscilloscope (54645A, Hewlett Packard) and recorded in a potentiometric chart recorder (2210, LKB-Produkter AB). The successful implantation of the electrode was evidenced by a sharp drop in voltage on entry into a cell, a stable potential (±3 mV) for at least 1 minute after impalement, a sharp return to zero on exit, and minimal change (<10%) in microelectrode resistance after impalement.

Measurements of membrane potential of mesenteric rings were obtained in Krebs’ solution before and after stimulation of the vessels with ACh (10 nmol/L to 10 μmol/L), ACh (1 μmol/L) in the presence of a NO synthesis inhibitor, Nω-nitro-L-arginine (L-NNA) (30 μmol/L for 20 minutes, Sigma Chemical), or ACh (1 μmol/L) in the presence of the K+ channel blocker apamin (100 nmol/L, for 10 minutes, Sigma Chemical). We have previously observed that L-NNA (30 μmol/L) had no effect on the resting membrane potential of NWR or SHR rings, whereas apamin (100 nmol/L) induced a significant depolarization in rings from NWR but did not affect the resting membrane potential of SHR preparations.23

Statistical Analysis
All data are expressed as mean±SEM, with the number of animals in parentheses. Statistical analysis was performed by 1-way ANOVA followed by the Newman-Keuls test in the case of pairwise comparisons between groups. When the data consisted of repeated observations at successive time points, ANOVA for repeated measurements was applied to determine differences between groups. Where >1 implantation was made on the same mesenteric ring from the same rat, the measurements were averaged and considered as n=1. Differences were considered significant at P<0.05.

Results
Blood Pressure and Body Weight
The systolic blood pressure of SHR aged 20 weeks was significantly higher than that of WKY (Table). The oral supplementation with 125 μg cholecalciferol per kilogram of body weight per day caused, from the second week on, a significant reduction of the systolic blood pressure of SHR (Table). WKY did not present a significant change in blood pressure.
pressure during the 6 weeks of cholecalciferol treatment (Table). These results are in agreement with the previous findings of Vianna et al and Borges et al. No significant differences in body weight and serum calcium concentration were observed between treated and nontreated animals (Table).

We did not measure 1,25 vitamin D₃ content in these animals, but previous work has shown that SHR are unable to sustain appropriate circulating levels of 1,25 vitamin D₃ even when receiving very high doses of that vitamin.

**Measurements of Mechanical Response**

The Table shows that the resting perfusion pressure of the mesenteric vascular bed was higher in SHR than in WKY or in NWR (44.4±2.7 mm Hg; n=16). Cholecalciferol treatment significantly reduced the resting perfusion pressure of SHR but not of WKY or NWR (not shown) mesenteric vascular beds.

Concentration-response curves for the vasorelaxation induced by ACh on mesenteric vascular beds showed similar ED₅₀ values for NWR (ED₅₀=2.9±0.06×10⁻¹¹ mol), WKY (ED₅₀=3.6±0.05×10⁻¹¹ mol), or SHR (ED₅₀=3.4±0.05×10⁻¹¹ mol) preparations, but the maximum responses to ACh were significantly decreased in SHR compared with NWR or WKY (Figure 1). The cholecalciferol treatment clearly improved the maximum responses of the SHR preparations, bringing them to levels similar to those of the NWR and WKY preparations, which themselves were not affected by treatment with the vitamin (Figure 1).

In the mesenteric arterial ring preparations, ACh also induced concentration-dependent relaxation, with similar ED₅₀ values for NWR (ED₅₀=5.8±0.05×10⁻⁸ mol/L), WKY (ED₅₀=6.5±0.1×10⁻⁸ mol/L), and SHR (ED₅₀=4.4±0.2×10⁻⁸ mol/L) preparations; maximum relaxation was also impaired in SHR compared with NWR and WKY rings (Figure 2). In addition, similar to what was observed in the mesenteric vascular bed, the maximum responses of SHR mesenteric rings to ACh were significantly increased after cholecalciferol treatment (Figure 2).

**Measurements of Membrane Potential**

The resting membrane potential of smooth muscle cells was significantly less negative in SHR (−38.4±1.2 mV) than in WKY (−47.6±1.8 mV) (Figure 3) or NWR (−46.6±1.2 mV; n=6; not shown) arterial rings. However, in rings from cholecalciferol-treated SHR, the membrane potentials were significantly more negative than those of arteries from untreated SHR, being comparable to those measured in preparations from normotensive rats (Figure 3).

The membrane hyperpolarization induced by ACh in smooth muscle cells of endothelium-intact mesenteric arterial
artery and animal species considered. In the case of the rat role of NO in the response was well preserved. In SHR mesenteric arteries, in which the polarization has been shown to account for the impaired as well as in SHR. Deficient endothelium-dependent hyperpolarization to ACh in SHR mesenteric arteries, in which the hyperpolarizing effect induced by ACh, rings with endothelium were pretreated with the NO synthesis inhibitor L-NNA (30 μmol/L for 20 minutes) or with a toxin selective for the small-conductance Ca²⁺-dependent K⁺ channels, apamin (100 nmol/L for 10 minutes); the results are shown in Figure 3. Whereas preincubation with L-NNA had no effect on the hyperpolarization induced by ACh in mesenteric arterial rings from WKY or cholecalciferol-treated SHR, it significantly reduced the responses to this agonist in rings from untreated SHR. On the other hand, preincubation with apamin significantly reduced the hyperpolarizing effect of ACh in WKY and treated SHR rings but not in untreated SHR.

**Discussion**

Vascular endothelium plays an important role in the regulation of arterial tone by producing dilator mediators such as NO, prostacyclin, and EDHF. Several reports have shown that endothelial function is impaired in hypertensive patients as well as in SHR. Deficient endothelium-dependent hyperpolarization has been shown to account for the impaired relaxation to ACh in SHR mesenteric arteries, in which the role of NO in the response was well preserved.

The mechanism of ACh-induced endothelium-dependent vasorelaxation is complex, and different K⁺ channels have been implied in this mechanism, depending on the type of artery and animal species considered. In the case of the rat mesenteric artery, evidence in the literature suggests that apamin-sensitive Ca²⁺-dependent K⁺ channels may be the main ion channels involved in this mechanism.

The present study shows that the relaxations induced by ACh in norepinephrine-precontracted vessels are impaired in the SHR mesenteric vascular bed and in mesenteric rings even in the presence of indomethacin and that cholecalciferol treatment brings these responses to levels similar to those of normotensive rats. In addition, it was also demonstrated that ACh, in the presence of indomethacin, caused a concentration-dependent hyperpolarization that was markedly reduced in mesenteric arteries from SHR, and this was also reversed by cholecalciferol treatment.

Several hypotheses may be proposed to explain these results, including release of depolarizing substances by ACh; impaired synthesis, release, or diffusion of EDHF; and reduced responsiveness of the smooth muscle to hyperpolarizing agents.

Fuji et al showed that the hyperpolarizing response to ACh in the rat mesenteric artery was not affected by indomethacin, thereby excluding the possibility of release of cyclooxygenase products capable of producing depolarization. In contrast, Chen and Cheung demonstrated that the hyperpolarization by ACh in mesenteric arteries from normotensive rats is mainly due to opening of apamin-sensitive K⁺ channels.

Since we have already demonstrated a reduced response of smooth muscle to hyperpolarizing agents in SHR mesenteric arteries without endothelium, the decreased hyperpolarizing response to ACh could result from a reduced responsiveness of smooth muscle to hyperpolarizing agents. This could be attributed to impaired activity of K⁺ channels, as was observed for the responses to α₁-adrenergic agonists, rather than to impaired synthesis, release, or diffusion of EDHF.

In addition, our results showed that ACh-induced hyperpolarization of mesenteric rings from SHR was abolished by the inhibitor of the NO synthesis, L-NNA, whereas other groups (WKY and treated SHR) showed L-NNA resistance. In contrast, pretreatment with the K⁺ channel inhibitor apamin had no effect on the ACh-induced hyperpolarization in SHR mesenteric rings. In agreement with our results, Kahonen et al, Wu et al, and Onaka et al showed that the relaxing responses to ACh in SHR mesenteric vascular preparations are due only to NO, being completely inhibited by L-NNA.

Since previous studies already reported an interaction between NO and EDHF systems, it is likely that the reduced hyperpolarization induced by ACh in untreated SHR mesenteric arteries, due to the impairment of K⁺ channels, may upregulate the production of NO.

After cholecalciferol treatment, the relaxation and hyperpolarization induced by ACh in SHR mesenteric arteries, as well as in rings from normotensive rats, were augmented, and apamin reduced this effect, suggesting that it may be due to the restoration of functioning or synthesis of new apamin-sensitive K⁺ channels.

In agreement with our findings, a link between antihypertensive treatments and recovery of K⁺ channels in SHR arterial smooth muscle was also reported with cholecalciferol, hydralazine, and ramipril.
In conclusion, our findings show a beneficial effect of antihypertensive treatment with cholecalciferol on the hyperpolarization induced by ACh in mesenteric arteries from SHR.

References

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