Journal of the American Society of Hypertension 4(3) (2010) 128–134

Research Article

Influence of calcium-dependent potassium channel blockade and nitric oxide inhibition on norepinephrine-induced contractions in two forms of genetic hypertension

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Manuscript received October 7, 2009 and accepted February 11, 2010

Abstract

The activation of Ca^{2+}-dependent K^+ channels (BK_{Ca}) leads to the attenuation of vascular contraction. Our study aimed to evaluate BK_{Ca} influence on norepinephrine (NE)-induced femoral artery contraction in two forms of genetic hypertension. NE dose-response curves were studied before and after BK_{Ca} blockade or after combined blockade of BK_{Ca} and NO synthase (NOS) in femoral arteries with intact endothelium from normotensive Wistar (WIS), hypertensive hereditary hypertriglyceridemic (HTG), or spontaneously hypertensive rats (SHR). NE-induced contractions of femoral arteries were augmented in both hypertensive strains compared with Wistar rats, but acetylcholine-induced relaxation was impaired in HTG only. The increase of basal vascular tone of isolated arteries after BK_{Ca} blockade was similar in all rat strains, but subsequent NOS inhibition increased basal vascular tone more in vessels from both hypertensive rat strains. NOS inhibition increased sensitivity to NE in all strains, but BK_{Ca} blockade in SHR only. Neither treatment enhanced maximal NE-induced contraction. NO-dependent attenuation of NE-induced contractions was greater in SHR than HTG or Wistar vessels, whereas large conductance Ca^{2+}-dependent K^+ channels may play a greater role in modulating vascular contraction in the severe form of hypertension. J Am Soc Hypertens 2010;4(3):128–134.

Keywords: Femoral artery; SHR; HTG; norepinephrine; BK_{Ca}; tetraethylammonium; L-NNA.

Introduction

Genetic forms of experimental hypertension, such as spontaneously hypertensive rats (SHR), are characterized by enhanced sympathetic tone and augmented vascular contractile response to norepinephrine (NE).[1] There are multiple factors influencing pressor action of sympathetic nervous system, which range from the modulation of sympathetic of activity by central nervous mechanisms up to the modification sympathetic effects by intracellular signaling. NE, which is the main mediator of sympathetic nervous system, causes vascular smooth muscle contraction by elevation of cytosolic free Ca^{2+} (Ca_i^{2+}), which is raised through Ca^{2+} mobilization from the calcium stores in sarcoplasmic reticulum followed by Ca^{2+} influx mainly through L-type voltage-dependent Ca^{2+} channels (L-VDCC). Both Ca^{2+} release and Ca^{2+} influx are regulated by numerous mechanisms.[2–8] The prominent role of Ca^{2+} influx through L-VDCC in the control of vascular tone and blood pressure can be demonstrated both under the in vivo and in vitro conditions as we did in conscious SHR and their isolated femoral arteries.[9]

Large conductance Ca^{2+}-dependent K^+ channels (BK_{Ca}) represent an important feedback tool by which increased cytosolic Ca^{2+} concentration can limit further Ca^{2+} influx into vascular smooth muscle cells through their membrane hyperpolarization.[10,11] Their major role in blood pressure control or pathogenesis of hypertension has already been outlined by Furspan and Bohr.[12,13] Jones[14,15] was the first

The experimental work was partially supported by the research grants of Internal Grant Agency of the Academy of Sciences of the Czech Republic (IAA500110902).

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1933-1711/$ – see front matter
doi:10.1016/j.jash.2010.02.006

who reported the enhanced Ca^{2+}-activated K^+ efflux from the aorta of rats with various forms of experimental hypertension. Recently, Cox and Rusch[16] suggested that the upregulation of BK_{Ca} in vascular smooth muscle of hypertensive animals compensates for the downregulation of voltage-dependent K^+ channels.

At the level of peripheral circulation, the vasoconstrictor effects of NE are usually well balanced by vasodilator action of nitric oxide (NO) or other vasodilators (eg, PGI_2, dopamine), which lower Ca^{2+} influx through L-VDCC. In hypertensive rats, this balance of vasoactive systems is altered in favor of vasoconstrictor systems.[17]

Endothelium-derived hyperpolarizing factor might compensate for the loss of NO and preserve the endothelium-dependent relaxation in the mesenteric arteries of genetically hypertensive rats[18] or in rats in which hypertension is induced by a high-salt diet.[19] Recent studies suggest that endothelium-derived hyperpolarizing factor causes endothelial hyperpolarization generated by the activation of endothelial small and intermediate conductance Ca^{2+}-dependent K^+ channels that spreads passively to vascular smooth muscle cell via myoendothelial gap junctions.[20,21] Membrane hyperpolarization can also be achieved by enhanced activity of BK_{Ca} located directly in vascular smooth muscle cells. As far as NO vasodilator action is concerned, it is generally known that NO stimulates cGMP formation. The activated protein kinase G can close L-VDCC either by membrane hyperpolarization caused by the stimulation of Ca^{2+}-dependent K^+ channels[21] or by a direct effect on L-VDCC.[22]

Prague hereditary hypertriglyceridemic rats (HTG), which were selected from Wistar rats,[23] represent another model of genetic hypertension characterized by moderate blood pressure elevation resulting from enhanced sympathetic vasoconstriction.[24,25] This inbred strain with pronounced abnormalities of lipid and carbohydrate metabolism is also considered as interesting nonobese model of insulin resistance.[25–27]

The aim of the present study was to evaluate the role of BK_{Ca} and NO in the control of basal vascular tone and NE-induced contractile response of femoral arteries with preserved endothelium that were isolated from normotensive Wistar rats (WIS), hereditary hypertriglyceridemic rats with moderate hypertension or spontaneously hypertensive rats. Using Mulvany-Halpern myograph, we have investigated the influence of BK_{Ca} blockade on isolated femoral arteries both in the presence and in the absence of NO synthesis.

Methods

Experiments were carried out in 16–18-week old male normotensive Wistar, HTG, and SHR rats (Table 1), which were housed under standard laboratory conditions (temperature 23 ± 1°C, 12-hour light-dark cycle, standard pelleted diet for laboratory rats, tap water ad libitum). All procedures and experimental protocols, which were approved by the Ethical Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic, conform to European Convention on Animal Protection and Guidelines on Research Animal Use.

Animals were anesthetized with ether and blood pressure was measured directly by the puncture of carotid artery. Isolated and cleaned femoral arteries with intact endothelium were cut into 2-mm long segments and placed in a Mulvany-Halpern isometric myograph (Model 510A, DMT, Denmark). The myograph chamber was filled with modified Krebs-Henseleit solution (119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.18 KH_2PO_4, 0.03 mM EDTA, 2.5 mM $CaCl_2$, 200 mg/L ascorbic acid, 2 g/L glucose, 37°C) and bubbled with 95% O_2 and 5% CO_2. The inner arterial diameter was set to be 90% of the diameter predicted for the pressure of 100 mm Hg in wire myograph. After 30 minutes' stabilization, the vessels achieved their basal tone. To provide maximal depolarization-induced contraction, vessels were incubated with a depolarizing solution (124 mM K^+, without Na^+). Further contractions were induced by cumulative doses of NE (10^{-9} to 10^{-5} mol/L). Acetylcholine-induced relaxation (10^{-6} mol/L) of NE-precontracted vessels was measured. One segment with preserved endothelium was studied in each rat (n = 9-12). After repeated washing and stabilization of basal tone, NE dose-response curves were again determined in the same artery segment in the presence of Ca^{2+}-dependent K^+ channel blocker tetraethylammonium (TEA, 10^{-3} mol/L).[28] Final dose-response curves were measured in the presence of TEA and NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NNA, 10^{-4} mol/L). Using additional arterial segments (n = 8, two arterial rings were isolated from four animals in each strain), we have determined NE dose-response curves in the presence of 100 μM L-NNA only. All chemicals were obtained from Sigma (Heidelberg, Germany).

Changes of basal tone (Δ basal tone, mN/mm) were calculated after the addition of TEA as well as after combined addition of TEA and L-NNA. Pharmacodynamic characteristics — EC50 (half-maximal effective concentration, log mol/L) and E_{max} (maximal contraction, mN/mm) — were calculated for each NE dose-response curve. The sum of Δ basal tone and E_{max} represents a maximal contraction, which can be achieved at highest NE concentrations.

Data are presented as mean ± SEM. Statistical analysis was performed with one-way analysis of variance and post-hoc least significant difference test.

Results

Wistar rats had slightly greater body weight than rats of both hypertensive strains: HTG and SHR. There was a moderate blood pressure elevation in HTG rats, whereas SHR were severely hypertensive (Table 1). The inner diameter of studied femoral arteries was significantly