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ORIGINAL ARTICLE

IMPAIRMENT OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR RESPONSES IN SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract To examine the effect of chronic hypertension on endothelium-derived hyperpolarizing factor (EDHF) responses, the hearts of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were isolated and perfused using Langendorff system with constant perfusion pressure. Bradykinin increased coronary flow (CF) dose-dependently and this was not affected by N^G-nitro-L-arginine methyl ester or indomethacin, indicating that bradykinin's effect on CF was not mediated by nitric oxide or prostacyclin but by EDHF. Bradykinin-induced CF increase was smaller in SHR than in WKY. Tetrabutylammonium (a non-specific K_{Ca} channel blocker) abolished bradykinin-induced CF increase in both rats. 1-Ethyl-2-benzimedazolinone (1-EBIO, an agonist of intermediate conductance K_{Ca} channel)-induced increase in CF was smaller in SHR than in WKY. 1,3-Dihydro-1-[2-hydroxy-5-(trifluoromethyl) phenyl]-5-(trifluoromethyl) -2H-benzimidazol-2-one (NS1619, an agonist of large conductance K_{Ca} channel)-induced increase in CF did not differ between SHR and WKY. In early stage of hypertension, there was no significant difference between SHR and WKY in bradykinin- and 1-EBIO-induced increases in CF. In conclusion, EDHF response in coronary microcirculation is impaired in SHR due to dysfunction of intermediate-conductance calcium-activated potassium channels.

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Key words: Endothelium-derived hyperpolarizing factor (EDHF); Coronary microcirculation; Calcium-activated potassium channel; Hypertension.

原　著

高血圧自然発症ラットの内皮依存性過分極因子反応における カルシウム活性化カリウムチャネルの障害

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抄録　高血圧自然発症ラット（SHR）と正常血圧ラット（WKY）のランゲンドルフ灌流心を用いて，慢性高血圧の内皮依存性過分極因子反応におよぼす影響を検討した．ブラジキニンは用量依存性に冠血流量を増加し，その増加は N^G-nitro-L-arginine methyl ester とインドメサシンの影響を受けなかった．ブラジキニンによる冠血流量の増加は，SHR が WKY に比して小であった．Tetrabutylammonium（非特異的 Ca 活性化 K チャネル阻害薬）はブラジキニンによる冠血流量の増加を両群で阻害した．1-EBIO（Intermediate conductance Ca 活性化 K チャネルアゴニスト）による冠血流量の増加は，SHR が WKY に比して小であった．NS1619（Large conductance Ca 活性化 K チャネルアゴニスト）による冠血流量の増加は，両群で差はなかった．高血圧早期では，ブラジキニンと 1-EBIO による冠血流量の増加は両群で差はなかった．以上から，SHR の冠動脈微小循環における内皮依存性過分極因子反応は intermediate conductance Ca 活性化 K チャネルの機能異常を介して障害されていることが示唆された．

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キーワード：内皮依存性過分極因子；冠微小循環；カルシウム活性化カリウムチャネル；高血圧．

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1. Introduction

Vascular endothelium produces and releases various factors that modulate vascular tone. Endothelium-derived hyperpolarizing factor (EDHF) hyperpolarizes vascular smooth muscle cells and dilates microvessels[1, 2]. It has been suggested that a cytochrome P-450 metabolite can act as EDHF in the rat coronary and guinea-pig carotid arteries[3–5]. On the other hand, potassium ion was reported to act as EDHF in the rat hepatic artery[6]. In addition, recent studies demonstrated that hydrogen peroxide acts as EDHF in mice mesenteric and human coronary arteries[7, 8]. And other studies demonstrated that myoendothelial gap junctions mediate EDHF responses in the rat hepatic and mesenteric arteries[9, 10]. Although several factors are regarded as EDHF, the nature has been unknown. The action of these EDHFs is characterized as 1) hyperpolarization of the vascular smooth muscle cell, 2) mediation through calcium-activated potassium (K_{Ca}) channels, and 3) independence of nitric oxide (NO) and prostacyclin.

Hypertension is one of the most important risk factor of coronary heart disease. Impairment of endothelium-dependent vasodilation has been demonstrated in several models of experimental hypertension[11, 12]. Recent studies suggested that EDHF responses in the rat coronary artery are reduced during chronic hypertension[13], but its mechanism is still unclear. Several studies have suggested that vascular K_{Ca} channel function is impaired during chronic hypertension in the rat mesenteric and pulmonary arteries[14, 15]. On the other hand, K_{Ca} channels play an important role in the regulation of myogenic tone of the carotid, femoral and mesenteric arteries in spontaneously hypertensive rat (SHR)[16]. Although K_{Ca} channels are involved in EDHF responses, the effect of chronic hypertension on K_{Ca} channel in coronary circulation is not known. In this study, we examined the influence of chronic hypertension in EDHF response and K_{Ca} channel function in coronary circulation.

2. Methods

2.1. Experimental protocols

This study protocol was approved by the Animal Welfare Committee at the Hirosaki University School of Medicine. The experiments were performed in 27 male SHR aging 23 to 26 weeks as a hypertension model and 23 male Wistar-Kyoto rats (WKY) aging 23 to 26 weeks as a control model. Rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and heparinized (2000 IU, i.p.). The heart was rapidly excised and promptly bathed in 4℃ saline. The ascending aorta was cannulated for perfusion of the coronary arteries. The heart was perfused using a Langendorff system with Krebs-Henseleit solution containing (in mM) NaCl 118, KCl 4.7, $CaCl_2$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, and D(+)-glucose 11.5. Coronary perfusion pressure was maintained at 80 mmHg. Heart rate was kept constant (330 beats/min) by atrial pacing during the experiment. A thin fluid-filled high-density polyethylene balloon connected to a 1 mm caliber polyethylene tube was inserted and positioned in the left ventricle via the left atrium to monitor isovolumetric left ventricular pressure (LVP) and its first derivative (dp/dt). Balloon volume was adjusted to set the left ventricular diastolic pressure below 10 mmHg at the beginning of the experiment[17]. Once a stable LVP level was attained, no further volume change was made. Aortic pressure and coronary flow were continuously monitored. After the instrumentation, at least 20 minutes were allowed for stabilization of the monitored variables. All drugs were dissolved in the perfusate and were infused into the coronary arteries through the aortic perfusion cannula.

2.2. Role of EDHF in bradykinin-induced increase in coronary flow

Bradykinin (1-100 nM) was infused into the coronary arteries and the peak changes in coronary flow were observed. N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and indomethacin (INDO, 10 μM) were then administered to the perfusate and bradykinin-induced increases in coronary flow were reexamined. To study the involvement of K_{Ca} channel in EDHF responses, the change in coronary flow in response to bradykinin (1-100 nM) was examined in the presence and absence of tetrabutylammonium (TBA, a non-specific K_{Ca} channel blocker, 30 μM) under the treatment with L-NAME and INDO.

2.3. Role of K_{Ca} channels in EDHF responses

After the treatment with L-NAME (100 μM) and INDO (10 μM), 1-Ethyl-2-benzimedazolinone (1-EBIO, an agonist of intermediate conductance K_{Ca} (IK_{Ca}) channel, 3-300 nM) and 1,3-Dihydro-1-[2-hydroxy-5-(trifluoromethyl) phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619, an agonist of large conductance K_{Ca} (BK_{Ca}) channel, 1-100 nM) were administered. The vascular dilatory responsiveness was verified by examining the change in coronary flow in response to sodium nitroprusside (SNP, 0.1-1 μM) under treatment with L-NAME and INDO. To verify the direct influence of TBA for vascular smooth muscle, SNP-induced change in coronary flow was reexamined after administration of TBA (30 μM). To determine the influence of the genetic factors to bradykinin- and 1-EBIO-induced vasodilation, the changes in coronary flow by bradykinin (1-100 nM) and 1-EBIO (3-300 nM) were also examined in WKY and SHR at the age of 8-10 weeks.

2.4. Drug

Drugs and chemicals were obtained from Sigma Chemical (St. Louis, MO). INDO was dissolved in Na_2CO_3 (0.1 M). 1-EBIO and NS1619 were dissolved in dimethyl sulfoxide (DMSO, 0.03 M). The final concentration of Na_2CO_3 and DMSO was 0.01% and 0.1%, respectively, and had no effect on hemodynamic parameters. Other drugs were dissolved in distilled water. pH of the solution was maintained at 7.4 by bubbling with a mixture of 95% oxygen and 5% carbon dioxide. The perfusate was maintained at 37℃ by a circulating water bath with an external heat exchanger. L-NAME and INDO, and TBA were perfused for 20 minutes and their influences to the effects of the vasodilators used were examined. In the preliminary study, we confirmed that coronary flow responses to the vasodilators used were reproducible in the absence of inhibitors.

2.5. Measurements

Blood pressure and heart rate were measured by tail-cuff manometry (Model UR-5000, Ueda, Tokyo, Japan) in the conscious state before the experiment at the temperature of 37℃. An electromagnetic flow probe (FF-O45F, Nihon Kohden, Tokyo, Japan) was attached to the middle portion of the tube connected to the aorta, and coronary flow was continuously measured with a flowmeter (Model MFV-3100, Nihon Kohden, Tokyo, Japan). Coronary perfusion pressure was measured with a pressure transducer connected to a side arm of the aortic perfusion cannula. All data including LVP and dP/dt were recorded continuously on a polygraph system (Model RMP-6008M, Nihon Kohden, Tokyo, Japan). At the end of the experiment, the left ventricle was separated from the right ventricular free wall, the atria and the great vessels, and its wet weight was measured.

2.6. Statistics

All data are shown as mean±SEM. Blood pressure and baseline hemodynamic parameters were compared between SHR and WKY with an unpaired t-test. The changes in coronary flow were quantified by computing percent changes from the baseline. The changes in coronary flow

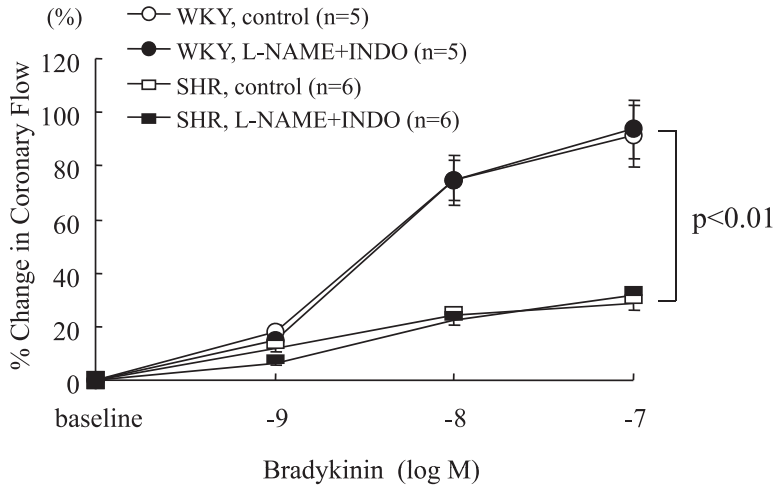

Figure 1 Graph showing the effect of bradykinin (1-100 nM) on coronary flow and the influence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and indomethacin (INDO, 10 μM) treatment to bradykinin-induced increase in coronary flow in Wistar-Kyoto rats (WKY) (n=5) and spontaneously hypertensive rat (SHR) (n=6). All data are shown as mean±SEM.

in response to various drugs between SHR and WKY, and those in response to bradykinin and SNP before and after TBA were analyzed with two-way ANOVA. Significance was accepted at $p<0.05$.

3. Results

3.1. Body weight and heart weight

The body weight of SHR (338±3 g) did not differ from that of WKY (342±5 g) (p=NS). The heart wet weight of SHR (1.52±0.13 g) was greater than that of WKY (1.18±0.12 g) ($p<0.05$). The ratio of whole heart weight to body weight, and the ratio of left and right ventricular weight to body weight in SHR were greater than those in WKY (both $p<0.05$).

3.2. Blood pressure and heart rate in the conscious state

The systolic, diastolic, and mean blood pressures measured in the conscious state before experiments were all higher in SHR than in WKY [207±2, 128±3, and 154±3 mmHg, respectively, in SHR and 139±3, 72±5, and 95 ±4 mmHg, respectively, in WKY (all $p<0.01$)]. The heart rate in SHR (434±8 beats/min) was greater than that in WKY (303±12 beats/min) ($p<0.01$).

3.3. Hemodynamic parameters

At the baseline, coronary flow was 10.0±0.3 ml/min in SHR (n=27) and 11.1±0.5 ml/min in WKY (n=23) (p=NS). Coronary resistance in SHR and WKY were 7.9±0.3 and 7.2±0.4 mmHg/ml/min, respectively (p=NS).

3.4. Bradykinin-induced vasodilation in WKY and SHR

The changes in coronary flow in response to bradykinin were examined in the presence and absence of L-NAME and INDO (fig. 1). Bradykinin increased coronary flow in a dose-dependent manner in both rats. The bradykinin-induced increase in coronary flow in SHR (n=6) was smaller by 65% than that in WKY (n=5) ($p<0.01$). L-NAME and INDO did not affect the bradykinin-induced increase in coronary flow in both rats.

3.5. The effect of TBA on bradykinin-induced increase in coronary flow

Under the treatment of L-NAME and INDO, the change in coronary flow in response to

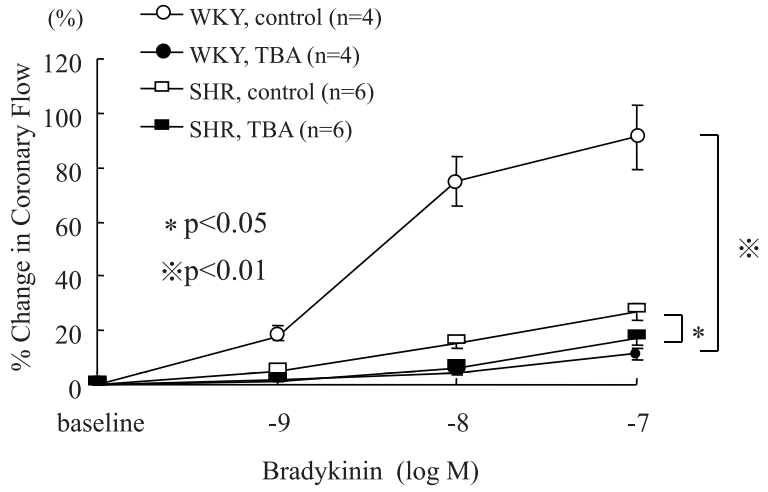

Figure 2 Graph showing the effect of tetrabutylammonium (TBA, 30 μM) on bradykinin-induced increase in coronary flow in Wistar-Kyoto rats (WKY) (n=4) and spontaneously hypertensive rat (SHR) (n=6). All data are shown as mean±SEM.

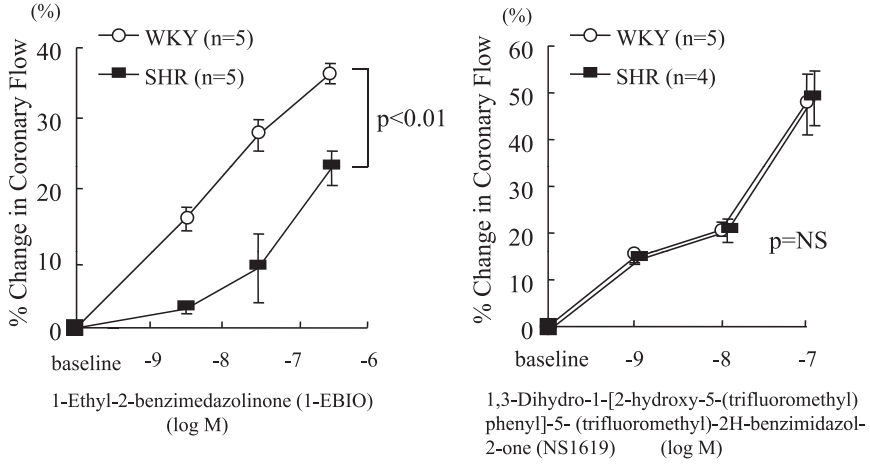

Figure 3 Left graph showing the effect of 1-Ethyl-2-benzimedazolinone (1-EBIO, 3-300 nM) and right graph showing the effect of 1,3-Dihydro-1-[2-hydroxy-5-(trifluoromethyl) phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619, 1-100 nM) on coronary flow in Wistar-Kyoto rats (WKY) (n=5 and n=5, respectively) and spontaneously hypertensive rat (SHR) (n=5 and n=4, respectively). All data are shown as mean±SEM.

bradykinin was examined in the presence and absence of TBA (fig. 2). TBA did not affect either LVP or dp/dt, but strongly attenuated the bradykinin-induced increase in coronary flow in WKY (n=4) by 90% ($p<0.01$). After TBA, bradykinin-induced increase in coronary flow in SHR did not differ from that in WKY.

3.6. 1-EBIO and NS1619-induced vasodilation in WKY and SHR

In the presence of L-NAME and INDO, the changes in coronary flow in response to 1-EBIO and NS1619 were examined (fig. 3). 1-EBIO and NS1619 increased coronary flow in a dose-dependent manner. 1-EBIO-induced increase in coronary flow in SHR (n=5) was smaller by 54% than that in WKY (n=5) ($p<0.01$). On the other hand, NS1619-induced increase in coronary flow did not differ between WKY (n=5) and SHR (n=4) (p=NS).

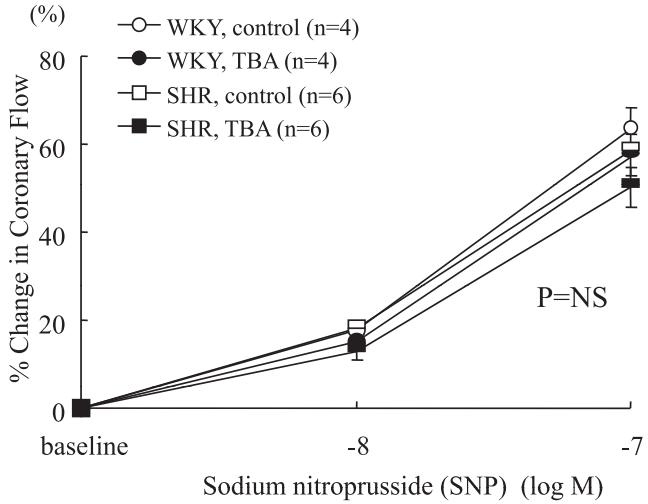

Figure 4 Graph showing the effect of sodium nitroprusside (SNP, 0.1-1 μM) on coronary flow in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and indomethacin (INDO, 10 μM) before and after administration of tetrabutylammonium (TBA, 30 μM) in Wistar-Kyoto rats (WKY) (n=4) and spontaneously hypertensive rat (SHR) (n=6). All data are shown as mean±SEM.

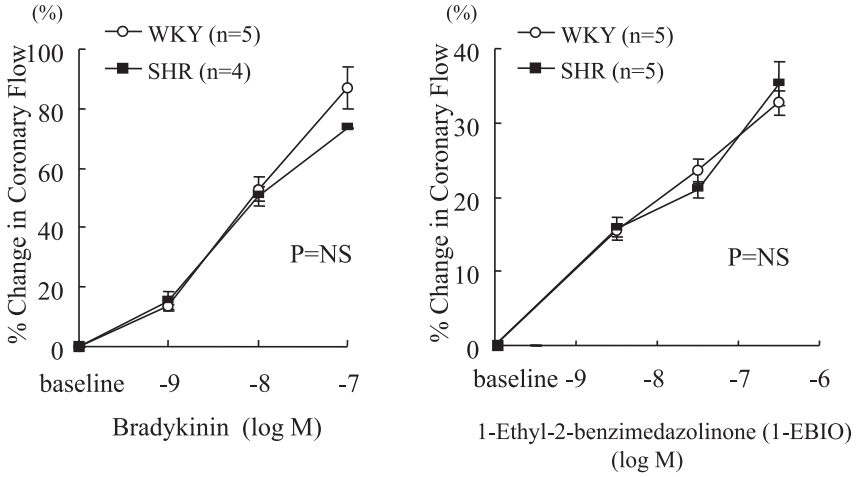

Figure 5 Graphs showing the effect of bradykinin (left panel) and 1-Ethyl-2-benzimedazolinone (1-EBIO) (right panel) on coronary flow in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and indomethacin (INDO, 10 μM) in Wistar-Kyoto rats (WKY) (n=5 and n=5, respectively) and spontaneously hypertensive rat (SHR) (n=4 and n=5, respectively) in early stage of hypertension (at the age of 8-10 weeks). All data are shown as mean±SEM.

3.7. SNP-induced vasodilation in WKY and SHR

Under the treatment with L-NAME and INDO, the changes in coronary flow in response to SNP were examined in the presence and absence of TBA (fig. 4). SNP increased coronary flow in a dose-dependent manner. This increase in coronary flow was not different between WKY (n=4) and SHR (n=6). TBA did not affect the SNP-induced increase in coronary flow in both rats.

3.8. Influence of the genetic factors

The effects of bradykinin and 1-EBIO on coronary flow in WKY and SHR in early stage of hypertension were examined with the use of rats at the age of 8-10 weeks. Systolic blood pressure level was 180±10 mmHg in SHR and 110±10 mmHg in WKY ($p<0.01$). Bradykinin showed comparable increase in coronary flow in SHR and WKY (fig. 5). 1-EBIO-induced

increases in coronary flow in SHR and WKY were also comparable.

4. Discussion

The major findings of the present study were as follows. 1) Bradykinin-induced increase in coronary flow was smaller in SHR than in WKY. 2) Bradykinin-induced increase in coronary flow was not attenuated by L-NAME and INDO. 3) TBA strongly attenuated bradykinin-induced increase in coronary flow in WKY. 4) 1-EBIO-induced increase in coronary flow was smaller in SHR than in WKY, but NS1619-induced one was not different between them. These findings indicate that bradykinin-induced dilation of coronary microvessel is mediated via an EDHF response, and is impaired in the animal with hypertension. This impairment of EDHF responses may be explained by IK_{Ca} channel dysfunction.

4.1. The role of EDHF-mediated dilation in bradykinin-induced dilation

Response of vessels to bradykinin, which shows a kinin B2-receptor mediated vasodilation[18], is mainly dependent on NO and slightly dependent on EDHF and prostaglandins in the rat aorta[19]. On the other hand, this response is mainly dependent on EDHF and independent or slightly dependent on NO and prostaglandins in the human coronary and rat mesenteric arteries[19, 20]. In our study, L-NAME and INDO did not affect bradykinin-induced increase in coronary flow in both rats, indicating that the contribution of NO and prostanoids to bradykinin-induced dilation may be absent or minimum in the rat coronary microcirculation. EDHF is appeared to have a major role in bradykinin-induced dilation of small diameter vessels and microvessels as compared with NO and prostanoids as shown in previous reports[21].

4.2. Impairment of bradykinin-induced dilation in SHR and the role of K_{Ca} channels in it

Bradykinin-induced increase in coronary flow in SHR was significantly smaller than that in WKY. SNP-induced increase in coronary flow in SHR, however, did not differ from that in WKY. These results suggest that attenuation of bradykinin-induced increase in coronary flow is caused by a specific mechanism, not by a nonspecific impairment of vasodilatory responsiveness of vessels in SHR. Since bradykinin-induced dilation is reported to be mediated by K_{Ca} channels[22], we examined the vascular K_{Ca} channel function in SHR and WKY. TBA strongly attenuated bradykinin-induced increase in coronary flow, indicating that bradykinin-induced increase in coronary flow is mediated by K_{Ca} channels in our preparation. Since SNP-induced increase in coronary flow was not affected by TBA, the present dose of TBA selectively inhibited vasodilation via K_{Ca} channels. After inhibiting K_{Ca} channels by TBA, bradykinin-induced increase in coronary flow was strongly attenuated, indicating that K_{Ca} channels have a major role in vasodilatory effect of bradykinin in the rat coronary microcirculation. Since bradykinin-induced increases in coronary flow in SHR and in WKY were comparable after inhibiting K_{Ca} channel function by TBA, attenuation of bradykinin-induced dilation in SHR may be explained by dysfunction of K_{Ca} channel in SHR. Presently, three types of K_{Ca} channel are known; BK_{Ca}, IK_{Ca} and small conductance K_{Ca} channels, according to the difference in single channel conductance. The functions of IK_{Ca} and BK_{Ca} channels in bradykinin-induced change in coronary flow in SHR and WKY were examined while observing the effects of 1-EBIO, an IK_{Ca} channel opener[23], and NS1619, a BK_{Ca} channel opener[24]. As shown in figure 3, 1-EBIO-induced increase in coronary flow in SHR was smaller than that in WKY, whereas NS1619-induced one was not. These results suggest that IK_{Ca} but not BK_{Ca} channel-mediated coronary dilation is impaired in SHR.

It is possible that vascular K_{Ca} channels in the present hypertension model are impaired genetically and not by the influence of hypertension. This contention was challenged by examining bradykinin- and 1-EBIO-induced increases in coronary flow in WKY and SHR at early stage of hypertension. The increases in coronary flow by these vasodilators in SHR were similar to those in WKY, indicating that K_{Ca} including IK_{Ca} channel function may be not impaired genetically in this hypertension model. Impairment of IK_{Ca} channel function in SHR appeared to be caused by the long-term exposure of microvessels to hypertension. Although the precise mechanism that results in the IK_{Ca} channel dysfunction is unclear, it is possible that IK_{Ca} channel density or function in the coronary artery is reduced mainly after long-term exposure to hypertension.

4.3. Limitations

To confirm that vasodilator response is mediated by EDHF, it seems to be necessary to measure the membrane potential directly with the use of a microelectrode technique[25]. The present experiments were performed with the use of isolated, Langendorff-perfused hearts, and therefore we could not measure the membrane potential of the coronary vessels directly. On the other hand, several studies considered that vasodilator responses to bradykinin or acethylcholine after inhibition of NO and prostacycline with NO synthase inhibitor and cyclooxygenase inhibitor are mediated by EDHF[26]. We examined the vasodilator effect of bradykinin after treatments with L-NAME and INDO.

It is established by the studies on an isolated vessel that apamin- and charybdotoxin-sensitive K_{Ca} channels mediate EDHF response[27]. We used TBA, a nonselective K_{Ca} channel blocker, and did not use selective blockers such as apamin and charybdotoxin. These selective blockers seem to show cardiotoxicity, and we considered that these blockers were not suitable for the study on a Langendorff-perfused heart. Further studies on the coronary microvessels isolated from the hearts of SHR and WKY would be required.

4.4. Implications

EDHF is released from endothelial cells to regulate blood flow, especially in smaller resistance arteries[28]. In addition, K_{Ca} channels play a crucial role in the coronary microvascular autoregulation, and coronary microvascular dilation to EDHF[29]. Since these play a crucial role in the regulation of coronary blood flow, the dysfunction of K_{Ca} channels in hypertension would result in an inadequate oxygen supply metabolic demand of the heart is increased. Hypertension is one of the most common diseases in the cardiovascular system. In addition to the dysfunction of K_{Ca} channels, hypertension per se increases myocardial oxygen demand through hemodynamic loading to the heart and left ventricular hypertrophy. Also hypertension is one of the most important risk factors that promote coronary artery disease. Thus, it is conceivable that chronic hypertension seems to produce severe myocardial ischemia through the impairment of metabolic regulation in patients, especially in those with significant coronary artery disease. A therapeutic strategy for improving K_{Ca} channel dysfunction would be necessary in patients with hypertension.

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