Pakistan J. Zool., pp 1-5, 2022. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20221112091131

Changes in Potassium Current and BK Channel Protein Expression in Coronary Artery Smooth Muscle Cells of Spontaneously Hypertensive Rats

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ABSTRACT

The objective of this study was to analyze the changes of potassium current (PC) and large conductance calcium activated potassium channel (BK) protein expression in coronary artery (CA) smooth muscle cells of spontaneously hypertensive rats. Eight healthy male spontaneously hypertensive rats (hypertension group) and 8 normal rats (Wistar group) were selected, and CA smooth muscle cells were isolated from the two groups. The PC of CA smooth muscle cells were recorded by patch clamp whole cell recording technique, and the BK currents and BK tail currents of CA smooth muscle cells were recorded and compared between the two groups. The BK α and BK β proteins expression in the two groups was detected by western blot. The average PC density of CA smooth muscle cells in hypertension group decreased to (68.72±5.14) % after the addition of 100nM IBTX, while that in Wistar group reduced to (33.21±1.76) % after the addition of 100nM IBTX. When the PC of coronary smooth muscle cells of hypertensive rats was + 120mV, + 110mV, + 100mV and + 90mV, the current density after 3mm4-AP inhibition was strikingly higher than that of rats in Wistar group. The tail current of BK channel was recorded by 4-AP inhibition, and the current density of hypertensive rats was dramatically higher than that of rats in Wistar group. There was no significant difference in BK channel BK α protein expression between the two groups. The expression level of BK β protein in hypertension group of rats was markedly higher than that in Wistar group. The BK channel current and protein of CA smooth muscle cells in spontaneously hypertensive rats were significantly higher than those in normal rats, suggesting that the diastolic function of smooth muscle cells was enhanced, which is of great significance to the protection of coronary circulation in hypertension.

Article Information
Received 12 November 2022
Revised 25 November 2022
Accepted 04 December 2022
Available online 13 March 2023
(early access)

Authors' Contribution
YF and ZY conducted the experiments in this study. CQ contributed to the design and interpretation of the current study and wrote the article.

Key words
Spontaneous hypertension, Coronary artery, Smooth muscle cell, Potassium current, BK channel

INTRODUCTION

Hypertension is a clinical synthesis characterized by increased blood pressure in the systemic circulation arteries (Liu and Xu, 2022). It is the most common cardiovascular disease in clinical practice. Patients with hypertension have no obvious symptoms in the early stage, but with the increase of blood pressure, dizziness, headache, and palpitation may occur, and most of the symptoms worsen after stress or fatigue. Severe cases can lead to angina pectoris, myocardial infarction and heart failure. Hypertension remains one of the leading causes of death from cardiovascular diseases (Humbert *et al*., 2017).

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0030-9923/2022/0001-0001 $ 9.00/0

Elevated blood pressure can change the hemodynamics of coronary arteries and increase the vascular smooth muscle tension. Vascular smooth muscle acts crucial role in maintaining vascular wall tension and regulating peripheral vascular resistance, thus achieving the purpose of changing the amount of blood perfusion in organs (Cooper, 2018). Potassium channels are widely expressed in the smooth muscle cell membrane of various blood vessels in human body, and are considered to be a kind of membrane channel protein with many important functions. Previous studies have confirmed that potassium channels can participate in the regulation of cell membrane resting potential by affecting vascular smooth muscle cells, and can regulate intracellular calcium concentration to participate in systolic and diastolic function of blood vessels (Chew *et al*., 2017). At least four different potassium channel types have been reported: Voltage-dependent potassium channel (Kv), ATP-sensitive potassium channel, large conductance calcium-activated potassium channel (BK), inward rectified potassium channel. BK channel is an important potassium channel with negative feedback regulation of cell tension in vascular smooth muscle cell membrane.

When depolarization occurs, enough calcium enters the cell and BK channels are activated to cause potassium outflow, which in turn causes vasodilation (Wang *et al*., 2017). Hypertension can increase the sensitivity of mesenteric artery smooth muscle cells to calcium ions. The molecular structure disorder of BK channel β1 subunit can cause vascular dysfunction, suggesting that BK channel has a crucial role in the development of hypertension (Ling *et al*., 2017). Therefore, this study analyzed the changes of potassium current (PC) and BK channel protein expression in coronary artery (CA) smooth muscle cells of spontaneously hypertensive rats to further elucidate the mechanism of hypertension.

MATERIALS AND METHODS

Experimental reagents and instruments

Heparin sodium, papain (Beijing Kairuiji Biotechnology Co., Ltd.); adenosine triphosphate disodium (Shanghai Hengfei Biotechnology Co., Ltd.); type II collagenase (Shanghai Chunshi Biotechnology Co., Ltd.); guanosine triphosphate (Suzhou Renuode Biotechnology Co., Ltd.); trypsinase inhibitor (Shanghai Shifeng Biotechnology Co., Ltd.); ineriotoxin (IBTX), elhylene diamine tetraacetic acid (EDTA) (Sigma Corporation, USA); Bkα, BKβ monoclonal antibodies (Shanghai Jingkang Biological Engineering Co., Ltd.); Fluorescein-labeled goat anti-mouse IgG (Wuhan Chundu Biotechnology Co., Ltd.) were used in this study.

Electric thermostatic water bath (Beijing Taize Jiaye Technology Development Co., Ltd.); inverted microscope (Beijing Jiayuan Xingye Technology Co., Ltd.); microelectrode puller (Hong Kong Youcheng Biotechnology Co., Ltd.); electrode manipulator (Sutter Instrument Company, USA); patch-clamp amplifier (Guangzhou Kezhilan Instrument Co., Ltd.); polishing straw (Beijing Lude Hengtai Trading Co., Ltd.); constant temperature oscillating water tank (Hangzhou Bori Technology Co., Ltd.); magnetic stirrer (Nanjing Shanben Biological Technology Co., Ltd.); low temperature refrigerator (Haier Company, Qingdao, China).

Experimental animal

Eight spontaneously hypertensive and eight normal control rats (Wistar-Kyoto), healthy males, aged 12 weeks (purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd.), and housed in the Animal Experimental Center of our hospital. All rats were fed at 22 to 24 ℃, with humidity of 50% to 60% in each day and night alternate for 12 h, with free diet and water intake. Rats were fed adaptively for one week and used in this study. The rats were injected intraperitoneally with heparin sodium and anesthetized by intraperitoneal injection of sodium pentobarbital 10 min later. The coronary arteries were isolated and the coronary smooth muscle cells were obtained by enzyme digestion. The main outward potassium flow of coronary smooth muscle cells was recorded in whole cell mode.

Detection methods

In voltage-clamp mode, the electrode was gradually depolarized from a clamping potential of -60mv to +150mV or +120mV, with a step of + 10mV, maintenance time of 80ms, stimulation frequency of 5KHz, the current map under various conditions of voltage can be recorded, including BK, Kv and other currents. The total PC was recorded, and then 100nM of highly selective BK channel blocker IBTX was added to the bath. After waiting for 5 to 10min, the recorded currents before and after IBTX addition were compared. The Kv blocker 3mM4-AP was added to the cell bath before recording, and the recorded current was also approximately BK current.

BK tail current measurement: In voltage-clamp mode, a condition pulse of 180ms was applied from the clamp potential of -60mV to +120m, followed by 180ms with a step of +10mV. BK tail currents were recorded from -60 to +90mV test pulse stimulation followed by a return to -60 mv clamp potential.

The expression of BK channel BK α and BK β proteins in the two groups was detected by western blot. Total protein of CA membrane was extracted from 5 rats in each group. The cells were lysed with RIPA lysate, and the amount of protein was checked by BCA kit. The solution volume of 40μg total protein was calculated as the loading volume, and SDS-PAGE was performed at the voltage of 40V to 60V for 4 to 5h. Electrophoresis was terminated when bromophenol blue just ran out, and then the protein was transferred to PVDF membrane, placed in blocking solution, and blocked at room temperature for 30min. Primary antibodies BKα and BKβ were added and incubated at 4℃ overnight, and the membrane was eluted three times by eluent. The membrane was exposed in the gel imaging system and developed and fixed by chemiluminescence.

Statistical method

All the data were analyzed using the SPSS21.0 software package. The data of this experiment were collected from three independent replicate experiments. Measurement data were represented by ($\bar{x}\pm s$), and t-test was used for pairwise comparison of data between two groups. $P<0.05$ was considered statistical difference.

RESULTS

As showed in Table I, there was no significant difference in the current density between the two groups before adding 100nM IBTX (P>0.05). The average PC density of CA smooth muscle cells in hypertension group of rats decreased to (68.72±5.14) % after the addition of 100nM IBTX, while that in Wistar group reduced to (33.21±1.76) % after the addition of 100nM IBTX, and the difference between the two groups was statistically significant (P<0.01).

As showed in Table II, when the PC of coronary smooth muscle cells of hypertensive rats was + 120mV, + 110mV, + 100mV and + 90mV, the current density after 3mm4-AP inhibition was strikingly higher than that of rats in Wistar group (P<0.01).

The tail current of BK channel was recorded by 4-AP inhibition, and the current density of hypertensive rats was dramatically higher than that of rats in Wistar group, and the difference was statistically significant (P<0.01). The results were shown in Table III.

There was no significant difference in BK channel BK α protein expression between the two groups (P > 0.05). The expression level of BK β protein in hypertension group of rats was markedly higher than that in Wistar group (P<0.01). The results were shown in Table IV.

DISCUSSION

Essential hypertension in humans and spontaneous hypertension in animals are both caused by increased total peripheral vascular resistance, which is one of the most important risk factors for cardiovascular diseases. Elevated blood pressure is more likely to cause CA diseases, which can lead to the decrease of coronary blood flow reserve capacity, atherosclerotic lesions and microvascular lesions, and the causes of which have become a hot topic of clinical scholars (Sokolović *et al*., 2019). In recent years, it has been shown that increased blood pressure can aggravate the damage of vascular intima and endothelial cells. Vascular smooth muscle cells participate in the formation of tunica media vasorum, and their contraction or relaxation can effectively regulate blood pressure. The increase of blood pressure can stimulate the proliferation and migration of vascular smooth muscle cells. The recombination and arrangement of smooth muscle cells in the tunica media

Table I. Current densities between hypertensive and Wistar rats before and after addition of 100nM IBTX ($\bar{x}\pm s$), pA/pF.

Groups	Cases	IBTX	+120mv	+110mv	+100mv	+90mv
Hypertension group	8	Before addition	213.07±27.03	160.56±18.49	120.13±11.81	84.33±10.06
	8	After addition	58.35±8.06*#	38.20±5.80*#	29.83±4.35*#	19.81±1.38*#
Wistar group	8	Before addition	204.71±18.42	150.29±16.15	126.40±14.57	85.43±10.26
	8	After addition	134.41±9.58*	96.84±10.04*	80.82±9.49*	66.31±4.29*

Notes: Compared with the same group before IBTX addition, *P<0.05; Compared with Wistar group after IBTX addition #P<0.05.

Table II. Current densities between hypertensive and Wistar rats after inhibition with 3mM 4-AP ($\bar{x}\pm s$), pA/Pf.

Groups	Cases	+120mv	+110mv	+100mv	+90mv
Hypertension group	8	110.44±10.42	88.10±7.16	66.17±5.61	50.37±6.11
Wistar group	8	54.54±6.01	40.34±2.81	31.30±2.09	21.28±1.82
t	-	13.144	17.563	16.475	12.906
P	-	<0.001	<0.001	<0.001	<0.001

Table III. Comparison of BK channel tail current density between hypertensive and Wistar rats ($\bar{x}\pm s$), pA/pF.

Groups	Cases	+50mv	+60mv	+70mv	+80mv	+90mv
Hypertension group	8	62.39±6.67	71.84±9.10	86.12±9.49	97.01±10.10	112.24±11.01
Wistar group	8	35.30±4.78	51.56±7.42	66.39±8.01	75.76±8.65	81.12±6.41
t		9.337	4.885	6.771	4.520	6.909
P		<0.001	<0.001	<0.001	0.001	<0.001

Table IV. Changes of BKα and BKβ protein expression in BK channel in hypertensive and Wistar rats ($\bar{x}$±*s*).

Groups	Cases	BKα	BKβ
Hypertension group	8	255.74±19.06	84.14±11.16
Wistar group	8	251.25±18.49	29.63±5.60
t	-	0.478	12.348
P	-	0.640	<0.001

vasorum leads to vascular restenosis, promotes the remodeling of CA structure, and ultimately leads to coronary atherosclerosis and many other cardiovascular diseases (Talwar *et al*., 2017; Cheng and Wang, 2017). In addition, due to the stimulatory effect of elevated blood pressure, the expression of scavenger receptor on the surface of smooth muscle cells can be increased, thereby accelerating the development of atherosclerosis and aggravating the degree of CA damage (Chung and Bailey, 2018). It can be concluded that CA smoothness leads to cell phagocytosis of a large number of foam cells with oxidized low-density lipoprotein, and the deposited lipids further aggravate the contraction, relaxation, migration and fibrosis of myocytes of phagocytic cells, which are related to cardiovascular diseases to a certain extent. Various ion channels on the surface of vascular smooth muscle cells play an important role in maintaining vascular wall tension and regulating peripheral vascular resistance by vascular smooth muscle cell. In recent years, studies have found that abnormal changes in the expression of ion channels on the surface of vascular smooth muscle cells are closely related to the pathogenesis of hypertension (Lee *et al*., 2018; Feng *et al*., 2009). As a large group of ion channels of the ion channel family, potassium channels play an important role in regulating peripheral vascular resistance. Among them, BK channel and Kv channel are the more important ion channels. However, smooth muscle cell channels and electrophysiology are different from cardiomyocytes. Potassium channels expressed by smooth muscle cells of different types and blood vessels have different structure and physiological characteristics, so as to ensure that they play a role according to physiological needs in different physiological states (Zhang *et al*., 2018). In order to understand the changes of the electrophysiological mechanism of CA contraction and relaxation in hypertensive patients, this study analyzed the changes of BK channel current and protein expression on the surface of coronary smooth muscle cells.

BK channels are widely expressed on mammalian nerve cells such as hippocampus and striatum, and smooth muscle cells. They are composed of the α subunit of the portal part and the β subunit of the auxiliary part, which can be regulated by voltage and calcium concentration, thus affecting the excitability of smooth muscle cells (Willis *et al*., 2017). Relevant data show that knockdown of BK channels can cause dysfunction of various organs and play an important role in cardiovascular diseases complicated by hypertension and diabetes (Gupta and Manchanda, 2017). BK channels play an important role in the feedback regulation of myogenicity and muscle tone of resistance vessels. BK current can be activated by depolarization of smooth muscle cell membrane and increase of intracellular Ca^{2+} concentration caused by outward vascular rectification, thereby increasing the outflow of intracellular K^+ and inducing the occurrence of cell membrane hyperpolarization (Brown *et al*., 2018). The results of the present study indicated that after addition of 100nM IBTX, the average current density of hypertension group decreased significantly than that of Wistar group ($P<0.01$). Moreover, after 3mm 4-AP inhibition, the current density of hypertension group was remarkably higher than that of Wistar group ($P<0.01$). Furthermore, the tail current density of hypertensive rats was significantly higher than that of wistar group ($P<0.01$). These results suggested that the sensitivity of hypertensive rats to IBTX and 4-AP was higher than that of Wistar rats, and the BK current of coronary smooth muscle cells in spontaneously hypertensive rats was significantly enhanced. The results of western blot showed that the expression of BKβ protein in hypertensive rats was significantly higher than that in wistar group ($P<0.01$), which again confirmed the up-regulation of BKβ protein in coronary smooth muscle cells of spontaneously hypertensive rats.

CONCLUSION

The BK channel current and protein of CA smooth muscle cells in spontaneously hypertensive rats were significantly higher than those in normal rats, suggesting that the diastolic function of smooth muscle cells was enhanced, which is of great significance to the protection of coronary circulation in hypertension.

ACKNOWLEDGEMENT

The research thanks the support from Ganzhou People's Hospital.

Funding

The study received no external funds.

IRB approval

Research experiments conducted in this article with animals or humans were approved by the Ethical Committee and responsible authorities of Ganzhou People's Hospital

following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Ethical statement

The study was approved and all patients provided written informed consent.

Statement of conflict of interest

The authors have declared no conflict of interest.

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