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Hypercholesterolemia alters vascular functions and gene expression of potassium channels in rat aortic smooth muscle cells[1]

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KEY WORDS hypercholesterolemia; thoracic aorta; potassium channels; gene expression regulation

ABSTRACT

AIM: To investigate the effects of hypercholesterolemia on functions of rat aorta and on gene expression of inward rectifier and ATP-sensitive potassium channels in aortic smooth muscle. **METHODS**: Rats were treated with high-cholesterol emulsion ig for 2 weeks; the aortic rings with and without endothelium were prepared to examine the aortic contractile and relaxation responses; RT-PCR was used to observe the gene expression of inward rectifier and ATP-sensitive potassium channels. **RESULTS**: Hypercholesterolemia damaged the endothelium-dependent vascular functions severely, but did not affect the endothelium-independent vascular functions; Kir6.2 mRNA expression was upregulated ($P < 0.05$) and Kir3.1 mRNA expression was downregulated markedly ($P < 0.05$) in hypercholesterolemic smooth muscle. **CONCLUSION**: Hypercholesterolemia altered the vascular functions and regulated gene expression levels of specific inward rectifier and ATP-sensitive potassium channel subtypes.

INTRODUCTION

Hypercholesterolemia is recognized as one of major independent risk factors for initiating the complex process of atherosclerosis. Former studies demonstrated that endothelium-dependent relaxation was attenuated in the aorta of hypercholesterolemic rabbits, and the effect of cholesterol (particularly oxidized LDL cholesterol) to induce endothelial vasodilator dysfunction occurs rapidly[1]. Inward rectifier and ATP-sensitive potassium channels are two important types of K^+ channels expressed in vascular smooth muscle[2], they have functions related to the control resting membrane potential and play critical role under pathologic conditions such as ischemia and/or hypoxia. Little is known about regulation of potassium channels expression in hypercholesterolemic vascular smooth muscle. In the present study we investigate the inward rectifier subtypes: Kir2.1 (strong inward rectifier K^+ channel) and Kir3.1 (G-protein coupled K^+ channel) and ATP-sensitive potassium channel (K_{ATP}) subunits mRNA expression alteration in the hypercholesterolemic rat aorta.

MATERIALS AND METHODS

Animal and tissue preparation Wistar ♂ rats weighing (290 ± 15) g (Animal Center of Chinese Academy of Medical Sciences) were treated with cholesterol-rich and fat-rich emulsion ig 10 mL·kg·d^{-1} for 2 weeks. The 500 mL emulsion contained following composition: (cholesterol 50 g, pig bile salt 10 g, methylthiouracil 5 g, pig fatty oil 100 g, Tween 80 100 mL, 1,2-propanediol 100 mL, added water to 500 mL). Plasma samples for measuring plasma cholesterol and triglyceride levels were drawn from the orbital venous plexus at the end of the administration period. Total plasma cholesterol and triglyceride concentrations were determined by spectrophotometric assay kit (Zhongsheng, China). Then rats were killed by cervical dislocation and exsanguinated. The thoracic aorta were quickly excised and rinsed in freshly prepared, ice-cold Krebs' buffer (mmol·L^{-1} composition: NaCl 118.3; KCl 4.7; $CaCl_2$ 1.8; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; edetic acid 0.026; glucose 11.1; pH 7.40) aerated with 95 % O_2/5 % CO_2. In some arteries the endothelium was mechanically removed by gently rubbing the intimal surface

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of the vessels with the closed tip of a pair of forceps.

Organ bath experiments Aortic rings were mounted on stainless steel wires connected to force transducers and placed in individual organ chambers for isometric tension recording as described previously[3]. Presence and absence of endothelium responses were confirmed by the ability of the preparation precontracted with 0.1 mmol·L^{-1} phenylephrine to relax in response to acetylcholine (ACh) and sodium nitroprusside (SNP) respectively. After reaching a stable contraction plateau, rings with endothelium were relaxed with cumulative concentration of ACh (0.001 – 100 μmol·L^{-1}); rings without endothelium were relaxed with cumulative concentration of SNP (0.001 – 1 μmol·L^{-1}).

RT-PCR studies Total RNA of thoracic aorta was extracted by using TRIzol (Life Technologies) according to the manufacture's instruction. Total RNA was reverse-transcribed into first-strand complementary DNA using random hexamer primers (Promega). cDNA samples were amplified in a PCR reaction carried out in a total volume of 50 μL containing 12.5 pmol specific primers; each 2.5 mmol·L^{-1} dNTP 4 μL; Taq DNA polymerase (MBI) 2 U; 10 × PCR buffer 5 μL. The expression of the housekeeping gene, GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA, was considered as an internal control. The following specific primer sequences were used: 1) Kir6.1: Forward: 5′-GAGTGAACTGTCGCACCAGA-3′; Reverse: 5′-CGATCACCAGAACTCAGCAA-3′; 209 bp. 2) Kir6.2: Forward: 5′-TCCAACAGCCCGCTCTAC-3′; Reverse: 5′-GATGGGGACAAAACGCTG-3′; 167 bp. 3) SUR2A/2B: Forward: 5′-ACACGCTCCGCTCCAGGCTG-3′; Reverse: 5′-GCCAGGCAGAACAGCTGTCT-3′; 2A: 407 bp; 2B: 231 bp fragment, respectively. 4) GAPDH: Forward: 5′-GACGCAAAAGAAGATGCGGCT-3′; Reverse: 5′-ATGGATGACCCGGTTCAGGGGTG-3′; 540 bp. PCR conditions were: 28 cycles of 94 ℃ for 60 s, 57 ℃ for 30 s, and then 72 ℃ for 60 s. PCR reaction in the presence of SUR2A/2B primer was performed as follows[4]: 30 cycles of 94 ℃ for 45 s, 55 ℃ for 60 s, and then 72 ℃ for 2 min. PCR products were separated by 1.7 % agarose gel electrophoresis. Gel were visualized and photographed under UV light, and analyzed by computerized densitometric scanning of the images using Bio-Rad GS-700 Imaging Densitometer. The intensities of ethidium Bromide fluorescence signals were determined from the areas under the curve for each peak and the data were plotted on graphs.

Drugs Phenylephrine (Phe) and acetylcholine (ACh) were purchased from Sigma. All other chemicals were of AR grade.

Statistics All data were expressed as $\bar{x} \pm s$ and analyzed with t test.

RESULTS

Plasma cholesterol and triglyceride levels and maximum responses to phenylephrine (Phe) In treated group plasma cholesterol level was increased [(3.5 ± 1.4) mmol·L^{-1} *vs* (1.8 ± 0.3) mmol·L^{-1}, $P < 0.05$, $n = 15$]. Plasma triglyceride level, however, was not elevated [(1.1 ± 0.3) mmol·L^{-1} *vs* (0.9 ± 0.2) mmol·L^{-1}, $P > 0.05$, $n = 15$]. The maximal responses to Phe 100 μmol·L^{-1} of control and hypercholesterolemic groups were (0.7 ± 0.2) and (1.0 ± 0.3) g, respectively ($P < 0.05$, $n = 10$).

Endothelium-dependent and -independent relaxation responses In the aortas with endothelium obtained from the hypercholesterolemic rats, ACh (0.001 to 100 μmol·L^{-1}) evoked concentration-dependent relaxations to the precontraction by Phe (100 μmol·L^{-1}) were attenuated, compared to those noted in the control aortae. The EC_{50} values of control and hypercholesterolemic groups were (0.16 ± 0.09) μmol·L^{-1} *vs* (14 ± 6) μmol·L^{-1}, respectively ($P < 0.01$, $n = 10$). In the endothelium-free rings of aortae from the hypercholesterolemic group, the relaxation to SNP (0.001 to 1 μmol·L^{-1}) precontracted by Phe (100 μmol·L^{-1}) was not different from that obtained in the control group. The EC_{50} values of control and hypercholesterolemic groups were (2.9 ± 0.4) nmol·L^{-1} *vs* (3.9 ± 0.8) nmol·L^{-1}, respectively ($P > 0.05$, $n = 10$).

RT-PCR studies mRNA expressions of Kir2.1, Kir3.1, Kir6.1, Kir6.2, SUR2B, and GAPDH were detected in aortic vascular smooth muscles from control and hypercholesterolemic rats. Ratios of the corresponding intensity areas (Kir2.1/GAPDH, Kir3.1/GAPDH, Kir6.1/GAPDH, Kir6.2/GAPDH, and SUR2B/GAPDH, GAPDH served as internal controls, Fig 1 and Fig 2) were calculated for comparisons by imaging densitometer (Bio-Rad, GS-700). Kir6.2 mRNA expression was increased (0.47 ± 0.11 *vs* 0.17 ± 0.04, $P < 0.05$) and Kir3.1 mRNA expression was reduced (0.49 ± 0.09 *vs* 1.00 ± 0.21, $P < 0.05$) markedly in the hypercholesterolemic rats compared to those in control group. However, Kir2.1, Kir6.1, and SUR2B mRNA expression levels had no alteration between control and

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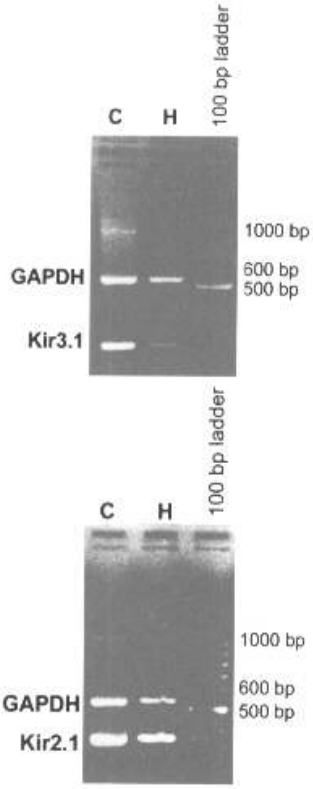

Fig 1. Gel electrophoresis in 1.7 % agarose gel of the RT-PCR fragments of Kir3.1, Kir2.1 channel mRNA and GAPDH mRNA served as an internal standard in aortic smooth muscle. C: Control; H: hypercholesterolemic rats. The PCR fragments length: Kir2.1 (373 bp); Kir3.1 (221 bp); GAPDH (540 bp).

hypercholesterolemic groups (Fig 3).

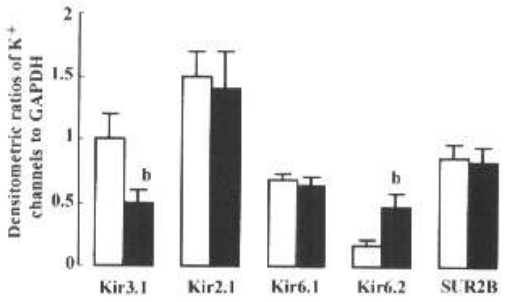

Fig 3. Comparison of expression levels of Kir3.1, Kir2.1, Kir6.1, Kir6.2, and SUR2B in the control (open columns) and hypercholesterolemic (hatched columns) rat aortic smooth muscle. Data were obtained from the densitometric ratios of K_{ATP} subunits/GAPDH mRNA expression levels. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

DISCUSSION

Our study demonstrated that hypercholesterolemia damaged the vascular endothelium-dependent functions severely, however, the vascular endothelium-independent functions were not affected. The results showed accordance with the observations of O'Rourke *et al*[5] and were similar to the results from other animal models[6,7] confirming that our hypercholesterolemic rat model was successful. In the present study we showed that mRNA

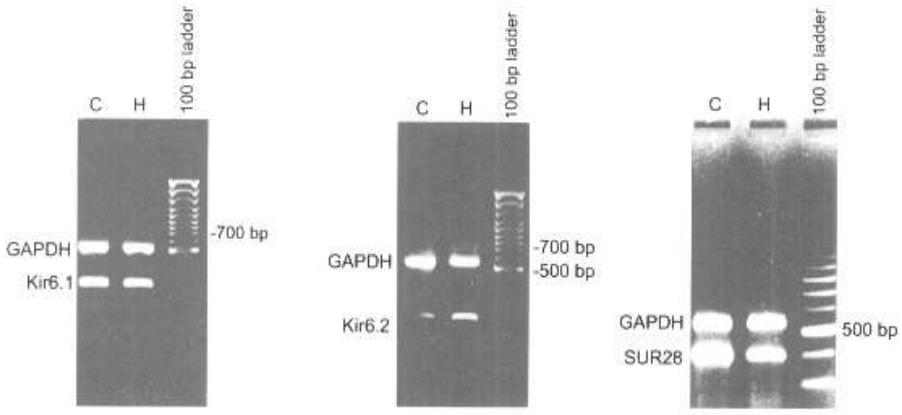

Fig 2. Gel electrophoresis in 1.7 % agarose gel of the RT-PCR fragments of K_{ATP} channel subunits mRNA and GAPDH mRNA served as an internal standard in the aortic smooth muscle cells. C: control; H: hypercholesterolemic rats. The PCR fragments length: Kir6.1 (247 bp); Kir6.2 (167 bp); SUR2B (231 bp); GAPDH (540 bp).

expression levels of Kir6.2 were enhanced and of Kir3.1 (GIRK-1) were downregulated in hypercholesterolemic rat vascular smooth muscle. However, Kir6.1, Kir2.1 and SUR2B mRNA expression levels were not changed. It suggested that hypercholesterolemia may alter some specific inward rectifier K^+ channels subtypes mRNA expressions.

Kir3.1 is the most important and widely expressed member in Kir3.0 subfamily (G-protein coupled potassium channels, GIRK). It is accepted that other members in Kir3.0 subfamily function combine with Kir3.1; in cardiac myocytes heteromultimer of Kir3.1 and Kir3.4 form the muscarinic K^+ channel (K_{ACh})[8]. Our study showed that hypercholesterolemia downregulated the Kir3.1 mRNA expression in vascular smooth muscle. Although the vascular endothelium-independent responses were not altered in 2-week hypercholesterolemic rats, the gene expression of Kir3.1 was reduced markedly in vascular smooth muscle.

Recent molecular studies have shown that functional K_{ATP} channels are formed by the combination of sulfonylurea receptor (SUR) and inward rectifier K^+ channel member of Kir6.0 subfamily[9,10]. SUR are responsible for the regulatory functions of K_{ATP}, which contain the sulfonylurea-binding site. Kir6.0 members serve as a pore-forming subunit allowing the K^+ to pass through[11]. Our lab has investigated that Kir6.1, Kir6.2, and SUR2B are expressed in rat aorta and Kir6.1 and Kir6.2 are widely expressed in several other tissues. It was interesting to note that hypercholesterolemia only affected the expression of Kir6.2 subunit in K_{ATP} channels. We have postulated that hypercholesterolemia may affect the balance of Kir6.1 and Kir6.2 subunits of K_{ATP} channels in vascular smooth muscles and/or reduce Kir3.1 induced elevation of Kir6.2 mRNA expression to protect vascular smooth muscle from pathologic damages.

Recently Kim *et al*[12] found that the endothelium-independent relaxation by α_2-adrenoceptor antagonists was abolished by increased external K^+ and was reduced by tetraethylammonium 0.01 mol/L (a Ca^{2+}-dependent K^+ channel blocker), but was not inhibited by glibenclamide 0.01 mmol/L (ATP-sensitive K^+ channel blocker). It is concluded that the endothelium-independent relaxation may be caused by the opening of potassium channels in the vascular smooth muscle. Our research indicates that some specific inward rectifier K^+ channel subtypes may be involved in the endothelium-dependent relaxation. Further studies should determine the physiologic functional changes of Kir and K_{ATP} channels in hypercholesterolemia.

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高胆固醇血症改变大鼠血管功能及动脉平滑肌细胞钾通道基因表达[1]

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关键词　高胆固醇血症；胸主动脉；钾通道；基因表达调控

目的：研究高胆固醇血症对大鼠主动脉功能及主动脉平滑肌上内向整流和 ATP 敏感钾通道基因表达的影响．**方法**：大鼠灌胃高胆固醇乳剂两周，制备带内皮和去内皮的主动脉血管环检测收缩和舒张反应，应用 RT-PCR 观察基因表达的变化．**结果**：高胆固醇血症明显损伤血管内皮功能，但不影响血管内皮非依赖性舒张反应；在高胆固醇血症血管平滑肌中 Kir6.2mRNA 表达明显升高（$P < 0.05$），而 Kir3.1 mRNA 表达却显著降低（$P < 0.05$）．**结论**：高胆固醇血症可改变血管功能，并影响某些内向整流和 ATP 敏感钾通道亚型的基因表达．

（责任编辑　朱倩蓉）

关于申报评选 2001 年第五届 中国药理学会 Servier 青年药理学工作者奖的通知

中国药理学会经与法国 Servier 研究院商定，第五届中国药理学会 Servier 青年药理工作者奖将于 2001 年 4 月评出．现将有关事宜通知如下：

一．获奖候选人条件：

1．2000 年底以前入会的中国药理学会会员；

2．年龄在 37 岁（1964 年 5 月 1 日以后出生）以下；

3．在国内从事药理学研究并取得优秀成绩，不包括在国外做过的工作；

4．在国内工作至少 2 年以上，（硕士、博士生在读学位前有 2 年以上工作经历也可以）

5．报奖者的两名推荐专家必须是中国药理学会会员；

6．提交近三年发表与未发表的研究内容写成的论文参加评选．

二．报名及评选程序：

符合上述条件的青年药理学工作者接到通知后，将报奖材料统一送到学会办公室，由学会评奖委员会评选．报名时需报以下材料：（1）按要求填写申报表（申报表可在中国药理学会网页及《中国药理学会通讯》第 18 卷第 1 期上查找或向学会办公室索取）；（2）论文一篇，全文用中文和英文撰写（用 16kB5 纸打印）并附软盘（Word 格式或文本文件）．（3）两位药理学会专家（教授或相当职称者）的推荐书，材料不符合标准及弄虚作假者取消评审资格．报奖者请于 2001 年 4 月 10 前（以邮戳为准），将材料报送中国药理学会办公室．学会将评选出 12 名候选人送法方专家评审．12 名候选人经终审落选的 4 位候选人给予中国药理学会青年药理学工作者奖和发给一定的奖金．荣获 Servier 奖的获奖者将免费参加 7 月在法国里昂召开的法中药理学术会议和欧洲药理学术会议．

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