African Journal of Biotechnology Vol. 10(76), pp. 17586-17592, 30 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1516
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Atorvastatin reverses the effects of interleukin-1β (IL-1β) on membrane potential in cultured rat aorta smooth muscle cells by activating big conductance Ca^{2+}-activated K^+ channels (BK) and scavenging hydrogen dioxide

Ying Yang[1#], Bo Yu[1]*, Yuan Gao[2#] and Guiping Wu[3],

[1]Department of Cardiology, First Affiliated Hospital, China Medical University, 110001, P. R. China.
[2]Department of Cardiology, First Affiliated Hospital, Xi'an JiaoTong University, 710049, P. R. China.
[3]Department of Cardiology, Second Affiliated Hospital, Shenyang Medical College, 110002, P. R. China.

Accepted 28 October, 2011

Statins are known to exert vasculopretective effects which are independent of their cholesterol lowering ability. The aims of this study were to investigate a possible effect of atorvastatin on Ca^{2+}-activated K^+ channels (BK) in cultured rat aorta smooth muscle cells (ASMCs) and to assess their contribution to interleukin-1β (IL-1β) induced changes of membrane potential and BK activity. The membrane potential was determined by the intensity of DiBAC4 (3) detected under confocal microscope. We found that atorvastatin hyperpolarized ASMCs in a concentration-dependent manner. 100 µmol atorvastatin activated BK channel directly which is determined by patch-clamp experiments. 12 h treatment of IL-1β resulted in decreased BK channel activity and depolarization of the cells, while atorvastatin or hydrogen dioxide (H_2O_2) scavenger catalase completely abolished the effects. There was no synergistic effect when catalase and atorvastatin were applied together. Furthermore, perfusion with atorvastatin resulted in a similar pattern of BK activation with hyperpolarization of ASMCs treated with IL-1β, which have significant differences statistically in comparison with saline group. Our results provide a potential important molecular mechanism of non-lipid-lowering effects of the atorvastatin by modulating BK channel.

Key words: Atorvastatin, interleukin-1β (IL-1β), hydrogen dioxide, smooth muscle cells, K^+ channels (BK) channel.

INTRODUCTION

The large conductance Ca^{2+}-activated K^+ (BK) channel is important in many electrically active cells. Its unique sensitivity to both intracellular calcium levels and membrane potential makes it a key regulator of intracellular calcium, a critical second messenger in cells. BK channel, abundantly expressed in vascular smooth muscle cells, plays a critical role in controlling membrane potential (Nelson and Brayden, 1993; Brenner et al., 2000). It has been shown that cardiovascular risk factors such as chronic cigarette smoking and aging down-regulates BK channel expression (Ye et al., 2004; Marijic et al., 2001). Therefore BK channels have been suggested as the therapeutic targets for treatment of cardiovascular diseases. It has been demonstrated that atorvastatin activates BK channels directly in human endothelial cells (Kuhlmann et al., 2004). So it is conceivable that atorvastatin activates BKca in vascular smooth muscle cells (VSMCs).

*Corresponding author. E-mail: ginoyy@hotmail.com. Tel: 86-24-83282602. Fax: 86-24-83282693.

#These authors contributed equally to this work.

The beneficial cardiovascular effects of statins are now well known to extend beyond their cholesterol-lowering activity (Lefer et al., 2001). One important action of the statins is their anti-inflammatory activity. Inflammation plays an important role in the development and progression of a variety of cardiovascular conditions, most notably coronary atherosclerosis and hypertension (Willerson and Ridker, 2004). Interleukin-1β (IL-1β) is one of the key mediators implicated in these processes. In our previous study, we found that long-term (12 to 48 h) treatment of IL-1β resulted in decreased BK channel activity and this process was mediated by hydrogen dioxide (H_2O_2) (Gao et al., 2010). So, we hypothesized that atorvastatin reverses the effects of IL-1β by activating BK channels and scavenging hydrogen dioxide.

In this study, we found that atorvastatin could act as a BK opener and effective therapeutic agents. Atorvastatin (dose-dependent) down-regulated membrane potential (MP) of aorta smooth muscle cells (ASMCs) by activating BK channels directly; it also reversed IL-1β effects on MP and the BK channel activity at least partialy by scavenging H_2O_2. These results may provide a novel molecular mechanism for non-cholesterol-lowering effect of statins, and for therapeutic strategies targeting BK channel in inflammation-related vascular diseases.

MATERIALS AND METHODS

Materials

IL-1β, Iberiotoxin (IBTX), 4-aminopyridine (4-AP), and catalase were purchased from Sigma. DiBAC4 (3) was from Invitrogen and atorvastatin was obtained from Parke Davis. Dulbecco's minimal essential medium (DMEM) was from GIBCO. Fetal bovine serum was obtained from HYCLONE. The external solution for patch-clamp experiments was composed of 40 mM K-Asp, 100 mM KCl, 1 mM $CaCl_2$ and 10 mM Hepes, at pH 7.2 to 7.4. The pipette solution contained 100 mM K-Asp, 40 mM KCl, 10 mM Hepes, and 2 mM EGTA, at pH 7.2 to 7.4. Incubation solution contained 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose.

Cell culture

Rat ASMCs were purchased from ATCC (NO:A-10) and were cultured with 5% CO_2 at 37°C in DMEM containing 10% fetal bovine serum, 100 IU/ml penicillin, and 100 mg/ml streptomycin sulfate. ASMCs between passages 4 to 8 were used for all the experiments. To measure the membrane currents, the cells from the stock culture were plated onto glass coverslips, and used for patch-clamp experiments 2 to 3 days after plating. The coverslips were transferred to a chamber (1 ml) mounted on the stage of an Olympus confocal microscope (FV1000S-IX81, Japan) for the experiments.

Measurement of membrane potential

We used DiBAC4 (3) to detect the membrane potential. DiBAC4 (3) are oxonol derivatives that are lipophilic and negatively charged with excitation maxima at approximately 490 nm. Hyperpolarization results in extrusion of the dye and then a decrease in cellular fluorescence, whereas depolarization causes enhanced fluorescence intensity (Epps et al., 1994). ASMCs were loaded with 1 μM DiBAC4 (3) for 30 min at room temperature in the dark. After loading, ASMCs were washed three times with HEPES-buffered Hank's solution. The fluorescence intensity was detected using an Olympus confocal microscope with the excitation and emission wavelength of 488 and 525 nm, respectively. Cells were preexposed to IL-1β and other chemicals for 12 h prior to loading the cells with DiBAC4 (3). Quantification of relative fluorescence intensity was performed using FluoView V6.1 software. Relative fluorescence intensity was determined by the subtraction of background value. Data were shown as the average of three independent experiments with five repeats for each sample.

Patch-clamp experiments

We used PC-10 puller (RWD Life Science, CN) to draw patch electrodes and the electrode resistance was 3 to 5 M Ω when filled with the pipette solution. The duration of patch clamp experiments was 10 min and no channel run-down was observed within 10 min. Using inside-out patch, we clamped the cell membrane potential at +50 mV (depolarization) that activates BK channels and studied BK channel activity with the same holding potential in the presence of 5 mM 4-AP. An Axopatch 700B amplifier (Axon Instruments, CA) was used for the recording. The signal was filtered at 1 kHz by digitada 1314 (Axon Ins.). Data were collected at sample rate of 5 kHz and were analyzed with software Axon patch 10.0. Channel activity was defined as NPo that was calculated from data samples of 120 s duration in the steady state as follows:

$$NPo = \frac{\sum t1 + 2\,t2 + ...iti}{T}$$

Where, ti is the fractional open time spent at each of the observed current levels, and T is total recording time.

Data analysis

Data were presented as means ± standard error of the mean (SEM). The changes of membrane potential and BK currents induced by atorvastatin and IL-1β vs. control were compared statistically by an analysis of variance (ANOVA) followed by Student-Newman-Keuls (S-N-K) post hoc test. To estimate the concentration-dependent effects of atorvastatin, the analysis of covariance and the Student t-test were used. The threshold of significance was $P < 0.05$ or $P < 0.01$.

RESULTS

BK channel mediated the effect of atorvastatin on MP

MP of ASMCs plays an important role in modulating vascular tone, so we first studied the effects of atorvastatin on MP. ASMCs were treated with 10, 25, 50, 100 or 150 μmol atorvastatin. The MP was determined by the intensity of DiBAC4 (3) detected under confocal microscope. As shown in Figure 1, treatment with atorvastatin caused hyperpolarization of ASMCs indicated by a decrease in fluorescence intensity. The application of

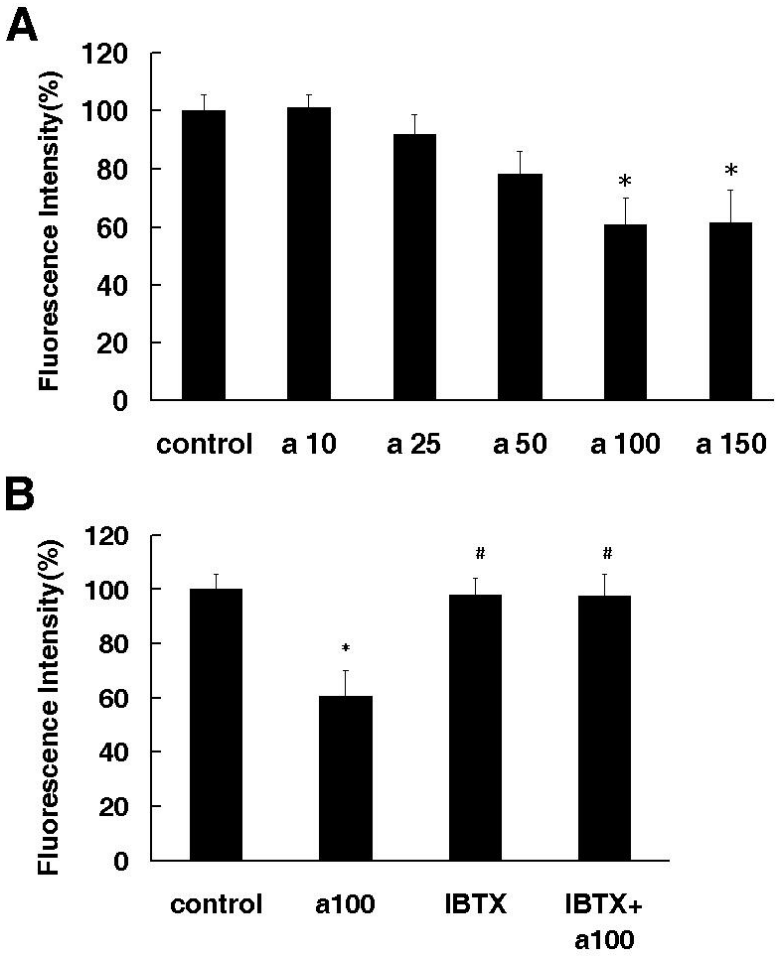

Figure 1. Atorvastatin-induced hyperpolarization in ASMCs. ASMCs were incubated with 50 ng/ml IL-1β for 12 h. The cells treated with saline were used as control. Quantification of relative fluorescence intensity was measured with DiBAC4 (3). (A) Atorvastatin (a; 10 to 150 μmol/L) hyperpolarized ASMCs in a concentration- dependent manner; (B) iberiotoxin (IBTX; 100 nmol/L) significantly inhibits 100 μmol/L atorvastatin induced membrane hyperpolarization. The fluorescence intensity of the control cells was considered as 100%. *P < 0.01, compared with control; # P < 0.01, compared with 100 μmol/L atorvastatin treament alone.

atorvastatin at a concentration of 100 μmol resulted in the strongest hyperpolarization of the MP (n = 15; P < 0.05). To test whether atorvastatin affects MP through modulating BK channel activity, BK channel inhibitor IBTX was applied to the cell bath. We found that IBTX could reverse atorvastatin-induced hyperpolarization. These data indicate that treatment with atorvastatin cause hyperpolarization of ASMCs and BK channel mediate the effect of atorvastatin on membrane potential.

Atorvastatin reversed IL-1β-induced depolarization in ASMCs

We studied the effect of IL-1β on MP in our laboratory previously and found that long time treatment of IL-1β suppressed BK channels via H_2O_2 resulting in depolarization of the MP. Since atorvastatin are well known to be an anti-inflammatory and reduce H_2O_2, we further tested whether atorvastatin could block the effect of IL-1β on MP and whether H_2O_2 mediated the effect of atorvastatin. ASMCs were treated with 50 ng/ml IL-1β for 12 h in the absence or presence of 40 μM catalase, a scavenger of ROS. 100 μmol atorvastatin was added to the cell bath alone or combined with catalase 5 min before the MP experiments started. Consistent with our previous results, 50 ng/ml IL-1β significantly depolarizarized ASMCs when applied for 12 h, which is almost compeletely abolished by H_2O_2 scavenger catalase. As shown in Figure 2, 100 μmol atorvastatin

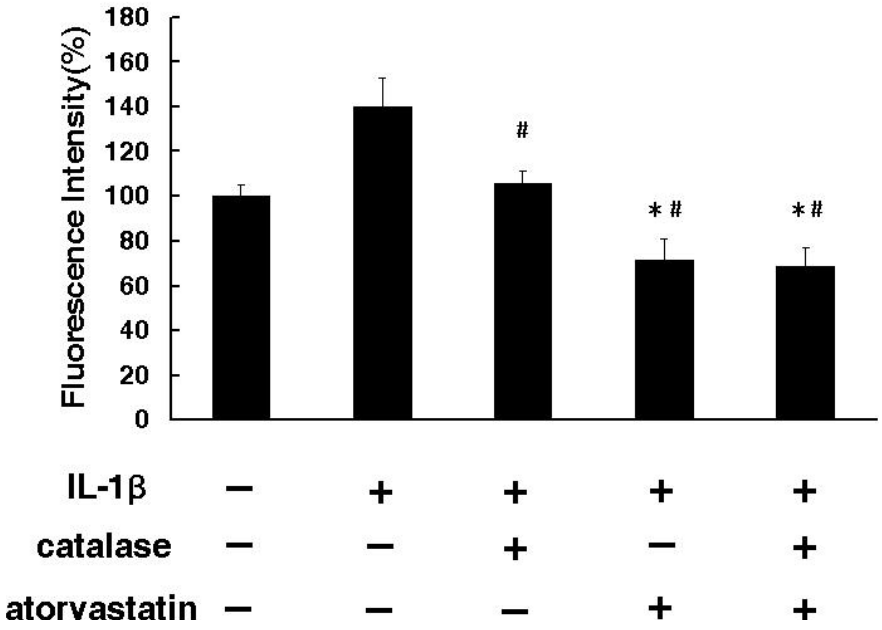

Figure 2. Atorvastatin reversed IL-1β-induced depolarization in ASMCs. ASMCs were treated with 50 ng/ml IL-1β for 12 h in the presence or absence of atorvastatin (a; 100 μmol/L), H_2O_2 scavenger catalase (cat; 200 U/ml), or together. The cells treated with saline were used as control. Membrane potential of ASMCs was measured with DiBAC4 (3) using confocal microscopy. Data were shown as quantification of the relative fluorescence intensity. The fluorescence intensity of control cells was considered as 100%. *P < 0.01, compared with control; # P < 0.01, compared with IL-1β treament alone.

could not only block the IL-1β induced depolarization, but also hyperpolarized the MP to some extent (n = 15; P < 0.05). Moreover, there was no synergistic effect when catalase and atorvastatin were applied together compared with atorvastatin alone, suggesting that atorvastatin may partially act in the same pathway with catalase. Taken together the previous finding that atorvastatin stimulates BK channels in ASMCs, these data suggest that atorvastatin affects MP at least partially through modulating BK channel-dependent K+ efflux and partially through reducing the H_2O_2 level.

Effect of atorvastatin on BK single-channel activity in ASMCs

BK was identified extensively in our laboratory previously. Using inside-out patch clamping, we measured single BK channel activity of ASMCs treated with 50 ng/ml IL-1β for 12 h in the present or absence of 40 μM catalase. We added 5μM 4-AP into the bath solution to inhibit the Kv channel activity and held the membrane potential at +50 mV. BK channel activity was defined by NPo as described previously in material and methods. As showed in Figure 3, BK channel activity was significantly suppressed during 12 h treatment of IL-1β while catalase completely abolished the effect (n = 10; P < 0.05). Next, we investigated whether atorvastatin directly affects BK channel activity and whether atorvastatin can reverse the effect of IL-1β. Atorvastatin was added to bath solution 10 min before the patch clamping. In the IL-1β - free bath solution, perfusion with atorvastatin resulted in BK channel activation and a significant increase of NPo (n = 10; P < 0.05).

Consistent with the previous MP experiments results, atorvastatin blocked the effects of IL-1β on BK channel activity and further increased NPo significantly compared with the control (saline group) (n = 10; P < 0.05). Still no synergistic effect was found when catalase and atorvastatin were applied together. These data demonstrated that atorvastatin reverse the IL-1β induced BK channel suppression by reducing H_2O_2, beside that, atorvastatin activates BK channel directly.

DISCUSSION

Ca^{2+} influx through voltage-gated Ca^{2+} channels is required for many essential pathways of vascular smooth muscle cells such as proliferation, migration, permeability and contractility (Bai et al., 2007). Because of this voltage dependence, membrane potential is the key determinant which, in turn, is largely controlled by potassium channels (Korovkina and England, 2002). BK channel, abundantly

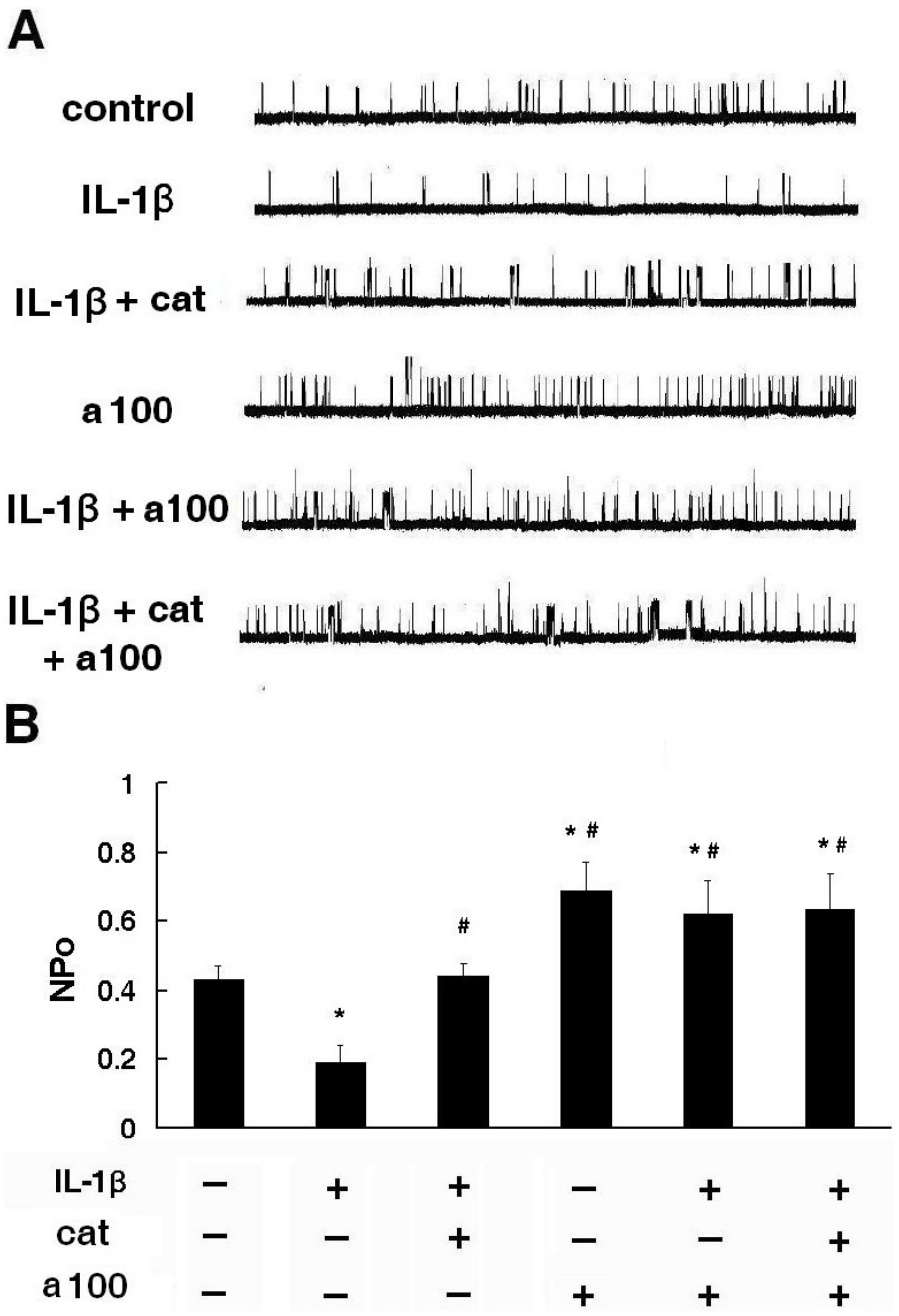

Figure 3. Effect of atorvastatin on BK single-channel activity in ASMCs. (A) Original recording of BKCa after 12 h treatment with IL-1β in the present or absence of atorvastatin (a; 100 μmol/L), H_2O_2 scavenger catalase (cat; 200U/ml), or they together. The cells treated with saline were used as control; (B) quantification of BK channel activity (NPo) shown in panel A. *P < 0.01 compared with saline-treated cells; # P < 0.05, compared with IL-1β treatment alone.

expressed in VSMCs, plays a critical role in controlling MP. A number of researches have showed that BK channel is involved in many physiological and pathological processes of VSMCs (Hui and Sachin, 2009).

It has been reported that cerivastatin activates endothelial BK channels and thereby modulates endothelial nitric oxide production and cell proliferation (Kuhlmann et al., 2004). In the field of electrophysiology, it is a well-established fact that activation of BK channels can influence essential endothelial functions by the modulation of calcium influx (Félétou, 2009). We present the first evidence for a functional role of atorvastatin in

the regulation of MP and BK channel. Our experiments suggest that a dose-dependent effect of atorvastatin on MP has the strongest hyperpolarization at a concentration of 100 μmol.This effect was abolished by preincubation with IBTX, the BK channel blocker. These data indicate that BK channel mediate the effect of atorvastatin-induced MP down regulation. In the patch-clamp experiments, we demonstrated for the first time that atorvastatin directly activate the BK channel in ASMCs defined by increasing NPo. Taken altogether, atorvastatin hyperpolarizes ASMCs by activating BK channel. The critical influence of BK channel in VSMCs suggests an involvement in cardiovascular diseases. It is reported that aldosterone-induced coronary dysfunction in mice involves the BK channels of VSMCs (Ambroisine et al., 2007). Chronic cigarette smoking and aging, well known as cardiovascular risker, correlated with a reduction of BK channel activity and expression (Toro, 2002). Hypercontractility and aborted sildenafil relaxations are always involved in the impaired BK channel function in erectile dysfunction model (Werner et al., 2008). Different calcium signals in smooth muscle target BK are channeled to modulate vascular function. These differential calcium signals represent multilayered opportunities for prevention and/or treatment of vascular dysfunctions.

Owing to its recognized role in mediating vascular functions, the BK channel is regarded as a therapeutic target for the treatment of cardiovascular diseases.The synthetic benzimidazolone NS-1619 has been shown to activate BK channels, but its functional effect also involves inhibition of Ca^{2+} currents and voltage-activated K^+ channels (Edwards et al., 1994) and its possibility of medical application is not clear. It has also been demonstrated that estrogen at high concentrations directly actives BK channel (De et al., 2006); but clinical safety of estrogen, especially at high concentrations, is still far from being satisfactory which is always subsequently with adverse reactions. Our present study showed that atorvastatin could act as a BK opener and an effective therapeutic agent. Because of its widely accepted safety and it is well tolerated, atorvastatin has the broad prospects in dealing with BK channel associated vascular dysfunction.

The statins of course are designed to reduce low-density lipoprotein (LDL), cholesterol levels and thus to exert an atherosclerotic preventing or delaying effect. Nevertheless, quite recently, the statins were found to exert direct cardiovascular effects which clearly are independent of their cholesterol lowering. A large body of studies has shown that one of the key non-lipid-lowering effects of the statins appears to attenuate the endothelial dysfunction (Reriani et al., 2010). The cytoprotective effects of statins are related to enhance endothelial NO (Ongini et al., 2004). Statins were shown to exert these salutary NO promoting effects by inhibitingthebiosynthesis of L-mevalonate, and of the protein Rho which is a potent nitric oxide synthase (NOS) inhibitor (Laufs et al., 1998). Now, it is well known that statins enhance the expression of NOS (Eto et al., 2002). Since the expression of Rho and even NOS need Ca^{2+} influx which is modulated by MP, it is conceivable that BK channel may take part in the process. Actually, it is reported that BK channel plays an important role in the signaling of statin-mediated endothelial NO production and proliferation (Kuhlmann et al., 2004). As to the effects of statins on VSMCs, although it is well known that a major vasculoprotective action of the statins is to promote basal release of endothelium-generated NO but this is not the only molecular effect of the statins. It is said that statins has the ability to scavenge oxygen-derived free radicals (Wagner et al., 2000) which is consistent with our finding. In our study, atorvastatin directly activated BK channel and reversed IL-1β effects on MP and the BK channel activity in ASMCs. Atorvastatin cannot synergize with catalase indicating atorvastatin partially affected BK channel in IL-1β treated ASMCs via H_2O_2. The use of cultured VSMCs excludes an influence of endothelium-generated NO and plasma lipid levels on our findings. Taken together with other findings, it is safe to say that statins exerts the non-lipid-lowering effects partially by modulating BK channel.

REFERENCES

Ambroisine ML, Favre J, Oliviero P, Rodriguez C, Gao J, Thuillez C, Samuel JL, Richard V, Delcayre C (2007). Aldosterone-Induced Coronary Dysfunction in Transgenic Mice Involves the Calcium-Activated Potassium (BKCa) Channels of Vascular Smooth Muscle Cells. Circulation, 116: 2435-2443.

Bai Y, Zhang M, Sanderson MJ (2007). Contractility and Ca2+ signaling of smooth muscle cells in different generations of mouse airways. Am. J. Respir. Cell. Mol. Biol. 58: 122-130.

Brenner R, Peréz GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW (2000). Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. Nature, 407: 870-876.

De WH, Allen M, Holmes C, Stobbart M, Lippiat JD, Callaghan R (2006). Modulation of the BK channel by estrogens: examination at single channel level. Mol. Membr. Biol. 23: 420-429.

Edwards G, Niederste-Hollenberg A, Schneider J, Noack T, Weston AH (1994). Ion channel modulation by NS 1619, the putative BKCa channel opener, in vascular smooth muscle. Br. J. Pharmacol. 113: 1538-1547.

Epps DE, Wolfe ML, Groppi V (1994). Characterization of the steady-state and dynamic fluorescence properties of the potential-sensitive dye bis-(1,3-dibutylbarbituric acid) trimethine oxonol (Dibac4(3)) in model systems and cells. Chem. Phys. Lipids, 69: 137-150.

Eto M, Kozai T, Cosentino F, Joch H, Lüscher TF (2002). Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. Circulation, 105: 1756-1759.

Félétou M (2009). Calcium-activated potassium channels and endothelial dysfunction: therapeutic options? Br J Pharmacol. 156: 545-562.

Gao Y, Yang Y, Guan Q, Pang X, Zhang H, Zeng D (2010). IL-1beta modulate the Ca (2+)-activated big-conductance K channels (BK) via reactive oxygen species in cultured rat aorta smooth muscle cells. Mol. Cell. Biochem. 338: 59-68.

Hui X, Sachin K (2009). BK channel function, venous tone and blood pressure regulation. FASEB J. 23: 1018-1021.

Korovkina VP, England SK (2002). Detection and implications of potassium channel alterations. Vascul. Pharmacol. 38: 3-12.

Kuhlmann CR, Gast C, Li F, Schafer M, Tillmanns H, Waldecker B,

Wiecha J (2004). Cerivastatin activates endothelial calcium activated potassium channels and thereby modulates endothelial nitric oxide production and cell proliferation. J. Am. Soc. Nephrol. 15: 868-875.
Lefer AM, Scalia R, Lefer DJ (2001). Vascular effects of HMG CoA-reductase inhibitors (statins) unrelated to cholesterol lowering: new concepts for cardiovascular disease. Cardiovasc. Res. 49: 281-287.
Laufs U, La Fata V, Plutzky J, Liao J K (1998) Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. Circulation, 97: 1129-1135.
Marijic J, Li Q, Song M, Nishimaru K, Stefani E, Toro L (2001). Decreased expression of voltage-and Ca(2+)-activated K(+) channels in coronary smooth muscle during aging. Circ. Res. 88: 210-216.
Nelson MT, Brayden JE (1993). Regulation of arterial tone by alcium-dependent K+ channels and ATP-sensitive K+ channels. Cardiovasc. Drugs Ther. 3:605-610.
Ongini E, Impagnatiello F, Bonazzi A, Guzzetta M, Govoni M, Monopoli A, Del Soldato P, Ignarro LJ (2004). Nitric oxide (NO)-releasing statin derivatives, a class of drugs showing enhanced antiproliferative and antiinflammatory properties. Proc. Natl. Acad. Sci. USA. 101: 8497-8502.
Reriani MK, Dunlay SM, Gupta B, West CP, Rihal CS, Lerman LO, Lerman A (2010). Effects of statins on coronary and peripheral endothelial function in humans: a systematic review and meta-analysis of randomized controlled trials. Eur. J. Cardiovasc. Prev. Rehabil. 18: 704-716.
Toro L, Marijic J, Nishimaru K, Tanaka Y, Song M, Stefani E (2002). Aging, ion channel expression, and vascular function. Vascul Pharmacol. 38: 73-80.
Wagner AH, Kohler T, Ru¨cscholoss U, Just I, Hecker M (2000). Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. Arterioscler. Thromb. Vasc. Biol. 20: 61-69.
Werner ME, Meredith AL, Aldrich RW, Nelson MT (2008). Hypercontractility and impaired sildenafil relaxations in the BKCa channel deletion model of erectile dysfunction. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295: R181-188.
Willerson JT, Ridker PM (2004). Inflammation as a cardiovascular risk factor. Circulation, 109: II2-10.
Ye H, Ma WL, Yang ML, Liu SY, Wang DX (2004). Effect of chronic cigarette smoking on large-conductance calcium-activated potassium channel and Kv1.5 expression in bronchial smooth muscle cells of rats. Sheng Li Xue Bao. 56: 573-578.