The effects of anti-hypertensive drugs and the mechanism of hypertension in vascular smooth muscle cell-specific ATP2B1 knockout mice

Yuki Okuyama1, Nobuhiro Hirawa2, Megumi Fujita1, Akira Fujiwara2, Yosuke Ebara1, Keisuke Yatsu1, Koichiro Sumida1, Minako Kagimoto1, Mari Katsumata2, Yusuke Kobayashi1, Sanae Saka2, Satoshi Umemura1 and Kouichi Tamura1

ATP2B1 is a gene associated with hypertension. We reported previously that mice lacking ATP2B1 in vascular smooth muscle cells (VSMC ATP2B1 KO mice) exhibited high blood pressure and increased intracellular calcium concentration. The present study was designed to investigate whether lack of the ATP2B1 gene causes a higher response to calcium channel blockers (CCBs) than to other types of anti-hypertensive drugs. Both VSMC ATP2B1 KO and control mice were administered anti-hypertensive drugs while monitoring blood pressure shifts. We also examined the association of nitric oxide synthase (NOS) activity in those mice to investigate whether another mechanism of hypertension existed. VSMC ATP2B1 KO mice had a higher response to CCBs for blood pressure-lowering effects than other anti-hypertensive drugs. These results mean increased intracellular calcium concentration in VSMCs due to lack of ATP2B1 gene causes a higher response to CCBs for blood pressure-lowering effects than other anti-hypertensive drugs. These results mean increased intracellular calcium concentration in VSMCs due to lack of ATP2B1 gene causes phenotypic changes in VSMCKO mice.7,8 We considered this upregulation as compensation for the lack of ATP2B1 gene.9 We previously reported increased blood pressure, intracellular calcium concentration and vascular contractility in these mice.7,8

INTRODUCTION

Many studies have proven that ATP2B1, which encodes plasma membrane calcium ATPase (PMCA) 1, is one of the candidate genes for hypertension. PMCAs are ATP-driven, calmodulin-dependent pumps that contribute to the regulation of intracellular calcium levels by extruding calcium ions from the cells.1 In the Millennium Genome Project in Japan,2 we demonstrated that single-nucleotide polymorphisms (SNPs) located upstream of the ATP2B1 gene were significantly associated with hypertension. These SNPs have been replicated in individuals of European descent,3 Korean descent4 and worldwide.5 We showed that SNPs in the ATP2B1 gene caused phenotypic changes in human tissue. ATP2B1 mRNA expression in human umbilical artery smooth muscle cells was significantly lower in those with a risk allele for hypertension than in those having no risk allele.6 The results of the study suggested that the low expression of ATP2B1 was associated with elevated blood pressure. Therefore, we generated a conditional knockout mouse model of ATP2B1 in vascular smooth muscle cells (VSMCs), and we reported increased blood pressure, intracellular calcium concentration and vascular contractility in these mice.7,8

On the other hand, ATP2B4 mRNA expression was upregulated in VSMCs of ATP2B1 KO mice.7 We considered this upregulation as compensation for the lack of ATP2B1. Mice overexpressing human PMCA4 in arterial SMCs exhibited high blood pressure, and PMCA4 overexpression was associated with the regulation of arterial contractility through neuronal nitric oxide synthase (nNOS) activity.9 We previously reported that systemically heterozygous ATP2B1-null mice exhibited enhanced vasoconstriction and elevated blood pressure, and the phenotype was associated with impaired endothelial NOS (eNOS) activity.10 However, NOS activity in VSMC-specific ATP2B1 knockout mice remains unknown.

In considering the treatment strategy for the hypertension caused by the lack of the ATP2B1 gene, which results in decreased extrusion of...
calcium ions from VSMCs, we hypothesize that calcium channel blockers (CCBs) are specifically effective compared with other anti-hypertensive drugs. CCBs are the most frequently prescribed anti-hypertensive agents in Japan because of their established blood pressure-lowering efficacy and their ability to maintain blood pressure control.\textsuperscript{11} Although there are few reports on the association between anti-hypertensive drug response and ATP2B1 gene expression, it is important that efficacious treatment for this type of hypertension is discussed. The present study was undertaken to investigate whether lack of the ATP2B1 gene would cause a higher response to CCBs than other types of anti-hypertensive drugs. We compared the efficacies of several anti-hypertensive drugs on blood pressure, examined the NOS expression to investigate whether another mechanism of hypertension existed, and demonstrated the importance of calcium channel blockade.

METHODS

Animal care

Animals were housed under a 12-h light–dark cycle at a temperature of 25 °C. Mice were fed a normal-salt diet (0.3% NaCl) with free access to drinking water. The experiments were conducted under the guidelines for animal experiments set by the Animal Experiment Committee of Yokohama City University School of Medicine.

Mice

ATP2B1\textsuperscript{loxP/loxP} mice were generated as previously described.\textsuperscript{7} To target inactivation of the ATP2B1 gene to VSMCs, ATP2B1\textsuperscript{loxPfloxed} mice were intercrossed with transgenic mice expressing Cre recombinase under control of the mouse transgelin (smooth muscle protein 22-) promoter (B6.Cg-Tg(Taggln-Cre)1Her/J, stock No.017491, Jackson Laboratory, Sacramento, CA, USA).\textsuperscript{12} We generated ATP2B1\textsuperscript{floxed/SM22Cre} (VSMC ATP2B1 KO) mice, and ATP2B1\textsuperscript{loxP/loxP} littermates were used as control mice.

Blood pressure measurement by tail-cuff method and radio telemetric method

Systolic blood pressure was measured by the tail-cuff method (BP-monitor MK-2000; Muromachi Kikai Co., Tokyo, Japan). Furthermore, direct blood pressure measurement was performed by a radio telemetric method in which a blood pressure transducer (PA-C10; Data Sciences International, Primetech Syste, Cupertino, CA, USA), and blood pressure and heart rate were monitored by simultaneously implanted osmotic pump (Alzet Osmotic Pumps; Durect Corp., Tokyo, Japan) was inserted into the left carotid artery at the age of 7 days. Direct blood pressure was recorded every 3 or 5 min by the Dataquest ART Gold Acquisition software (Data Sciences International, Primetech Corp., Tokyo, Japan), and the data were calculated as the average value per hour.

Effects of anti-hypertensive drugs on blood pressure

To survey the mechanism of hypertension in VSMC ATP2B1 KO mice, the L-type CCBs nicardipine (1 mg kg\textsuperscript{-1}), and amlopidine (5 mg kg\textsuperscript{-1}), the angiotensin II receptor blocker (ARB), candesartan (10 mg kg\textsuperscript{-1}), the \(\alpha\)-antagonist prazosin (1 mg kg\textsuperscript{-1}), or the NOS inhibitor No-nitro-L-arginine methyl ester (L-NAME, 30 mg kg\textsuperscript{-1}) were administered intraperitoneally to 14- to 18-week-old mice. All administrations were done at 3:00 pm. The injection volume was 0.3 ml. Saline was injected as a vehicle, and we confirmed that each administration of these drugs had blood pressure-lowering effects. The drugs were washed out by resting three days between each injection. Blood pressure and heart rate shifts were evaluated by telemetry measurements. Next, amlopidine (5 mg kg\textsuperscript{-1} per day) or candesartan (0.5 mg kg\textsuperscript{-1} per day)\textsuperscript{15} was administered for 2 weeks through a subcutaneously implanted osmotic pump (Alzet Osmotic Pumps; Durect Corp., Cupertino, CA, USA), and blood pressure and heart rate were monitored by tail-cuff or telemetric method. As there are several reports that candesartan could decrease blood pressure sufficiently in very low doses (0.1–1 mg kg\textsuperscript{-1} day) when mice were administered candesartan by osmotic pumps,\textsuperscript{15–17} we decided to reduce the doses of candesartan compared with the single injection for the long-term administration study.

Vascular smooth muscle cell culture

The aorta was dissected out from the aortic arch to the abdominal aorta from 8-week-old mice. VSMCs were prepared by the explant method and cultured in 10% fetal bovine serum–Dulbecco’s modified Eagle’s Medium as described previously.\textsuperscript{18} After the desired incubation period, cells were rinsed with phosphate-buffered saline and then lysed and sonicated.

Real-time quantitative reverse transcription-PCR analysis

Total RNA was extracted from aorta and cultured VSMCs with ISOGEN (Nippon Gene, Tokyo, Japan), and cDNA was synthesized using the SuperScript III First Strand System (Thermo Fisher Scientific, Waltham, MA, USA). Real-time quantitative reverse transcription-PCR (qRT-PCR) was performed by incubating the reverse transcription product with TaqMan PCR Master Mix and a designed TaqMan probe (Thermo Fisher Scientific, Waltham, MA, USA). RNA quantities were expressed relative to the 18S mRNA control.

Western blot analysis

Western blot analysis was performed as described previously.\textsuperscript{19} Briefly, VSMCs extracts were used for electrophoresis, and membranes (Thermo Fisher Scientific, Waltham, MA, USA) were incubated with anti-Ca1.2 (Alomone Labs, Jerusalem, Israel), anti-nNOS antibody (Cell Signaling Technology, Danvers, MA, USA), or anti-\(\beta\)-actin antibody (Abcam, Cambridge, MA, USA) and subjected to enhanced chemiluminescence (EMD Millipore, Darmstadt, Germany). The images were analyzed quantitatively using a FUJI LAS3000 Image Analyzer (FUJI Film, Tokyo, Japan) to determine each protein level.

Evaluation of nitric oxide production by measuring nitrate/nitrite

Measurement of combined urinary nitrite and nitrate (NOx) excretion is widely used as a marker of nitric oxide (NO) production. Urine samples were collected for 24 h with metabolic cages and were deproteinized by centrifugation with an Amicon Ultra-0.5 filter (EMD Millipore, Darmstadt, Germany). We colorimetrically measured urinary NOx excretion applying the Griess reaction (Griess Reagent Kit; Dojindo, Kumamoto, Japan).

Statistical analysis

For statistical analysis of differences among groups, an unpaired Student’s t-test or analysis of variance (ANOVA) followed by the Bonferroni correction was used. All quantitative data are expressed as the mean ± s.e.m. Values of P less than 0.05 were considered significant.

RESULTS

Baseline characteristics of VSMC ATP2B1 KO mice and control mice

As we reported previously,\textsuperscript{7} the blood pressure of VSMC ATP2B1 KO mice was higher than that of control mice, but heart rate and body weight were not significantly different. Serum and urinary electrolytes, including calcium and phosphate, were not different either (Supplementary Table). Systolic blood pressure was higher in the dark period (as the active phase for mice) than in the light period in both control and knockout mice (Supplementary Figure).

Single injection of anti-hypertensive drugs

Figure 1 shows the delta systolic blood pressure (SBP) by a single injection of vasodilators. Blood pressure-lowering effects were significantly greater in VSMC ATP2B1 KO mice when nicardipine, which has a short half-life, was injected (Figure 1a, \(P<0.05\)). On the other hand, no significant change was seen when mice were injected with an ARB or \(\alpha\)-adrenoceptor blocker (Figures 1b and c). We administered amlopidine to mice as a long-acting L-type CCB\textsuperscript{19} to...
confirm the greater effects of a CCB on blood pressure in KO mice, but unexpectedly, SBP shifts were not significantly different (Figure 1d). Figure 1e shows the delta SBP for every 6 h after nicardipine injection, and greater anti-hypertensive effects were seen in VSMC ATP2B1 KO mice until 12 h after administration (1–6 h; \( P < 0.05 \), 7–12 h; \( P < 0.01 \)).

**Long-term administration of anti-hypertensive drugs**

Although the short half-life L-type CCB response could be evaluated by a single injection, we considered that long-term injection was needed for an amlodipine response because the drug has a long half-life. As shown in Figures 2a and b, SBP was significantly decreased by a 2-week-treatment of amlodipine in VSMC ATP2B1 KO mice as measured by the tail-cuff method (\( \Delta \text{SBP} = -0.7 \pm 3.8 \) vs. \( -12.7 \pm 4.0 \) mm Hg \( P < 0.05 \)). On the other hand, the difference was not seen when mice were administered candesartan (\( \Delta \text{SBP} = -14.6 \pm 2.8 \) vs. \( -15.9 \pm 3.3 \) mm Hg, Figures 2c and d), which also has a long half-life. We also examined both short- and long-term effects of amlodipine by the radio telemetric method. Although there was no significant difference between the two groups on day 2 or 3 as an early phase, CCB demonstrated greater anti-hypertensive effects after 1-week and 2-week treatments in VSMC ATP2B1 KO mice (Figure 3a). By observing the changes in ambulatory blood pressure monitoring for 24 h at 1 week, the blood pressure-lowering effects of

---

**Figure 1** Changes in SBP produced by a single injection of anti-hypertensive drugs. (a) Delta SBP in response to the administration of nicardipine (1 mg kg\(^{-1}\), i.p., \( n = 7 \) for each group). (b) Delta SBP in response to the administration of candesartan (10 mg kg\(^{-1}\), i.p., \( n = 8 \) for each group). (c) Delta SBP in response to the administration of prazosin (1 mg kg\(^{-1}\), i.p., \( n = 6 \) for each group). (d) Delta SBP in response to the administration of amlodipine (5 mg kg\(^{-1}\), i.p., \( n = 8 \) for each group). (e) Delta SBP in response to the administration of nicardipine every 6 h. The data are means ± s.e.m. of each group. *\( P < 0.05 \) vs. the control group. **\( P < 0.01 \) vs. the control group. Each arrow indicates the time of drug injection. A horizontal line shows the time after (or before) injection and each injection was at ~15:00. C, control mice; K, VSMC ATP2B1 KO mice.
Figure 2. SBP shifts produced by long-term administration of amlodipine and candesartan measured by the tail-cuff method. (a) SBP and (b) Delta SBP from the baseline in mice administered amlodipine for 2 weeks (5 mg kg\(^{-1}\) per day, s.c., \(n=9\) for each group). (c) SBP and (d) Delta SBP from the baseline in mice administered candesartan for 2 weeks (0.5 mg kg\(^{-1}\) per day, s.c., \(n=7\) for each group). The data are means ± s.e.m. of group. * \(P<0.05\) vs. the control group. # \(P<0.05\) vs. own baseline.

Figure 3. SBP shifts produced by amlodipine administration on days 2, 3, 7, and 14 were examined by radio telemetric measurement. (a) Changes in delta SBP of mice treated with amlodipine (5 mg kg\(^{-1}\) per day, s.c., \(n=6-8\)). Circadian patterns of SBP of (b) Control and (c) VSMC ATP2B1 KO mice treated with amlodipine for 1 week, measured by the radio telemetric method. Basal SBPs were also measured (\(n=6-8\)). 12-h light (8:00 AM to 20:00 PM)/dark (20:00 PM to 8:00 AM) cycle are shown. Values plotted are hourly means. Data are means ± s.e.m. of group. * \(P<0.05\) vs. the control group.
amlodipine in VSMC ATP2B1 KO mice were especially seen in the dark period, and the effects were diminished throughout the day in control mice (Figures 3b and c).

Expression of L-type calcium channels
On the basis of the above results, we examined mRNA and protein expression of the α1C subunit of the L-type calcium channel (Cav1.2) in VSMCs to investigate the mechanism of augmented effects of calcium channel blockers in KO mice. The expression of CACNA1C mRNA was upregulated (3.3-fold $P<0.001$, Figure 4a) in VSMCs of KO mice. Cav1.2 protein expression was also increased in KO VSMCs (2.7-fold $P<0.05$, Figures 4b and c).

NO production of VSMC and urinary excretion of NO
We examined nNOS protein expression in VSMCs and NOx production in urine to investigate whether KO mice had impaired NOS activity. Neural NOS protein expression was not different between VSMCs of control and KO mice (Figures 5a and b). Urinary excretion of NOx was not significantly different either (Figure 5c). Delta SBP was not changed significantly between control and knockout mice when L-NAME was administered (Figure 5d).

DISCUSSION
In the present study, we examined the efficacy of anti-hypertensive drugs to investigate the specificity of drug response and the mechanism of hypertensive phenomenon by deletion of the ATP2B1 gene in VSMCs. To the best of our knowledge, this is the first report to demonstrate the association between the ATP2B1 gene and CCB response. In response to a single administration of nicardipine, which is categorized as a dihydropyridine CCB with a short half-life, the blood pressure-lowering effects in KO mice were greater than in control mice. However, the anti-hypertensive effects of candesartan and prazosin were not different between ATP2B1 knockout mice and the genetic control. Candesartan is categorized as an ARB, and no difference in the anti-hypertensive effect of an ARB administration suggests that high blood pressure was not caused by renin-angiotensin system stimulation. In the same way, prazosin, which primarily blocks vasoconstriction induced by $\alpha_1$-adrenergic receptors, decreased blood pressure equally between the two groups, indicating that elevated blood pressure in VSMC ATP2B1 KO mice may not be caused by sympathetic activation. These results suggest that the elevated intracellular calcium concentration of KO VSMCs contributes to high blood pressure and that blocking calcium entry through L-type calcium channels is effective, even with a single injection.

A single injection of amlodipine was thought to not sufficiently increase the blood concentration of the drug due to its long half-life, thereby causing no significant difference to be observed between the two groups. Therefore, we tried long-term administration. Two weeks of continuous amlodipine treatment significantly reduced blood pressure in VSMC ATP2B1 KO mice compared with control mice. The difference was seen after 1 week, but not at day 2 or 3 as an early phase measurement. One previous study demonstrated that repeated administration of amlodipine in humans resulted in steady state plasma drug concentration being reached after seven days. In comparison to the human data, our results indicated that amlodipine in VSMC ATP2B1 KO mice exhibited high blood pressure-lowering effects due to abnormalities in calcium handling after reaching a steady state. In other studies, mice treated with a high salt diet or angiotensin II received 5–6 mg kg$^{-1}$ of amlodipine for several weeks, and amlodipine did not decrease blood pressure. Their results support our results that the systolic blood pressure of control mice treated with amlodipine for 2 weeks returned to baseline levels. Control mice may be fully compensated by other calcium transporters, whereas KO mice would be able to respond to CCBs permanently due to the lack of the
ATP2B1 gene and subsequent abnormal expression of other calcium transporters (e.g., NCX17 and Cav1.2). We also administered candesartan for 2 weeks because it has a long half-life, similar to amlodipine, and confirmed that the blood pressure-lowering effects were similar between the two groups.

Both CACNA1C gene and Cav1.2 protein expression were significantly increased in KO VSMCs. Cav1.2 channels are multi-subunit protein complexes composed of the three subunits α, α2δ, and β25 and all calcium channel blockers bind to the α1C subunit.26 There are considerable data that augmented Ca2+ in flux through Cav1.2 channels contributes to the augmented peripheral resistance and contractile responses of the vascular smooth muscle in hypertension.27 In previous studies, Cav1.2 expression was reported to be upregulated in SHR28 and increased by high blood pressure29 or angiotensin II infusion30 in mice. These reports demonstrated that elevated Cav1.2 protein expression means functional activation of the channel. As several studies reported that increased calcium entry through Cav1.2 showed high response to CCBs in both human and animal models,29,33 upregulation of Cav1.2 in VSMC KO mice may have promoted a high CCB response.

As reviewed by Harraz and Altier,32 the L-type calcium channel is activated by depolarization in response to action potentials or sub-threshold stimuli. Pasic et al.29 suggested that high intravascular blood pressure-induced activation of Cav1.2 protein involved membrane depolarization. Thus, the elevated intracellular calcium concentration due to lack of the ATP2B1 gene in VSMCs may promote an increase in membrane potentials and Cav1.2 expression. Korb et al.33 reported that Cav1.2 and PMCA4b bind promiscuously to several PDZ domains. Thus, the association between Cav1.2 and PMCA1 may be recognized. Further study is needed to clarify the fundamental mechanism of the association between L-type calcium channels and ATP2B1.

On the other hand, systemic heterozygous ATP2B1-null mice exhibited impaired eNOS activity.10 Activity of NOS was unclear in VSMC ATP2B1 KO mice until we examined it in this study. It is well known that NO can influence contractility of peripheral blood vessels, and it is becoming more apparent that vascular NO production is not just mediated by eNOS but also via nNOS activity.34 Associations between nNOS and ATP2B4 are reported by several researchers.9,35 We speculated that nNOS was the most likely target if associations were seen between NOS and VSMC ATP2B1 KO mice because ATP2B4 mRNA expression was upregulated in those mice.7 However, nNOS protein expression was not changed in knockout mice compared with control mice. Furthermore, we administered L-NAME as a non-selective NOS inhibitor and checked urinary NOx excretion, which could be taken as an index of NO production,36 but no significant difference was seen in these experiments. These results indicated that NO production and all NOS isoform activity in VSMC ATP2B1 KO mice were not impaired, and upregulation of ATP2B4 in KO VSMCs did not influence the high blood pressure observed in these mice. Therefore, we considered that the increased intracellular calcium concentration was the main cause of the hypertensive phenomena in VSMC ATP2B1 KO mice.

There is a limitation in this study. We chose an L-type calcium channel blocker rather than T-type or N-type in the present study because extracellular Ca2+ influx is mainly mediated by the opening of L-type Ca2+ channels.37 Further studies are needed to investigate whether other calcium channels, such as T-type or N-type, contribute to the phenotype of increased intracellular calcium concentration in VSMCs of KO mice.
In conclusion, we found that ATP2B1 VSMC-specific knockout mice had a high susceptibility to CCBs. The results mean that the effects of a lack of ATP2B1 on blood pressure are mainly due to increased intracellular calcium concentration and subsequent activation of L-type calcium channels. Our study indicates that low ATP2B1 gene in humans may induce higher efficacy of CCBs. It is of great importance that we investigate the response to CCBs in humans to promote tailored medicine. As the genomic data of CCBs-sensitive patients among hypertensive people more easily and of candidate genes for hypertension are obtained in the future, genetic variants in novel pathways in ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. Hypertension 2012; 59: 854–860.

6. Tabara Y, Kohara K, Yita H, Hirawa N, Katsuya T, Okubo T, Hiura Y, Tajima A, Morisaki T, Miyata T, Nakayama T, Takahashi N, Nakura J, Kawamoto R, Takahashi N, Hata A, Soma M, Imai Y, Kubo K, Okuma L, Tomohiro A, Iwai N, Ogiura T, Iwae I, Tokunaga K, Johnson A, Caufield M, Murnue P, Global Blood Pressure Genetics. Emunera S, Ushina M, Hikita M, T. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project. Hypertension 2010; 56: 671–675.

7. Kobayashi Y, Hirawa N, Tabara Y, Muraoka H, Fujita M, Miyazaki N, Fujikawa A, Ichikawa Y, Yamamoto Y, Ichihara N, Saka S, Sakai H, Yoshida S, Saku K, Taya K, Yasuda G, Kohara K, Yita H, Takayama K, Goshima Y, Ichikawa H, Ushina M, Hikita M, T. Mice lacking hypertension candidate gene ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. Hypertension 2012; 59: 854–860.

8. Hirawa N, Fujisawa A, Emunera S, ATP2B1 gene to blood pressure: from associations to pathophysiology. Curr Opin Nephrol Hypertens 2013; 22: 177–184.

9. Gros P, Athane T, You XM, Van Wert R, Kalair W, Mungrue IN, Williams AJ, Bhat RH, Jin DL, Hicks AE, Murgue IN, Husain M. Plasma membrane calcium ATPase expression in arterial smooth muscle increases vasomotor responsiveness and blood pressure. Circ Res; 2003; 93: 614–621.

10. Fujikawa A, Hirawa N, Fujita M, Kobayashi Y, Ogiura T, Iwai N, Takahashi N, Hata A, Soma M, Imai Y, Kubo K, Okuma L, Tomohiro A, Iwai N, Ogiura T, Iwae I, Tokunaga K, Johnson A, Caufield M, Murnue P, Global Blood Pressure Genetics. Emunera S, Ushina M, Hikita M, T. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project. Hypertension 2010; 56: 671–675.

11. Kobayashi Y, Hirawa N, Tabara Y, Muraoka H, Fujita M, Miyazaki N, Fujikawa A, Ichikawa Y, Yamamoto Y, Ichihara N, Saka S, Sakai H, Yoshida S, Saku K, Taya K, Yasuda G, Kohara K, Yita H, Takayama K, Goshima Y, Ichikawa H, Ushina M, Hikita M, T. Mice lacking hypertension candidate gene ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. Hypertension 2012; 59: 854–860.

12. Hirawa N, Fujisawa A, Emunera S, ATP2B1 gene to blood pressure: from associations to pathophysiology. Curr Opin Nephrol Hypertens 2013; 22: 177–184.

13. Gros P, Athane T, You XM, Van Wert R, Kalair W, Mungrue IN, Williams AJ, Bhat RH, Jin DL, Hicks AE, Murgue IN, Husain M. Plasma membrane calcium ATPase expression in arterial smooth muscle increases vasomotor responsiveness and blood pressure. Circ Res; 2003; 93: 614–621.

14. Fujikawa A, Hirawa N, Fujita M, Kobayashi Y, Ogiura T, Iwai N, Takahashi N, Hata A, Soma M, Imai Y, Kubo K, Okuma L, Tomohiro A, Iwai N, Ogiura T, Iwae I, Tokunaga K, Johnson A, Caufield M, Murnue P, Global Blood Pressure Genetics. Emunera S, Ushina M, Hikita M, T. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project. Hypertension 2010; 56: 671–675.

15. Kobayashi Y, Hirawa N, Tabara Y, Muraoka H, Fujita M, Miyazaki N, Fujikawa A, Ichikawa Y, Yamamoto Y, Ichihara N, Saka S, Sakai H, Yoshida S, Saku K, Taya K, Yasuda G, Kohara K, Yita H, Takayama K, Goshima Y, Ichikawa H, Ushina M, Hikita M, T. Mice lacking hypertension candidate gene ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. Hypertension 2012; 59: 854–860.

16. Hirawa N, Fujisawa A, Emunera S, ATP2B1 gene to blood pressure: from associations to pathophysiology. Curr Opin Nephrol Hypertens 2013; 22: 177–184.

17. Gros P, Athane T, You XM, Van Wert R, Kalair W, Mungrue IN, Williams AJ, Bhat RH, Jin DL, Hicks AE, Murgue IN, Husain M. Plasma membrane calcium ATPase expression in arterial smooth muscle increases vasomotor responsiveness and blood pressure. Circ Res; 2003; 93: 614–621.

18. Fujikawa A, Hirawa N, Fujita M, Kobayashi Y, Ogiura T, Iwai N, Takahashi N, Hata A, Soma M, Imai Y, Kubo K, Okuma L, Tomohiro A, Iwai N, Ogiura T, Iwae I, Tokunaga K, Johnson A, Caufield M, Murnue P, Global Blood Pressure Genetics. Emunera S, Ushina M, Hikita M, T. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project. Hypertension 2010; 56: 671–675.

19. Kobayashi Y, Hirawa N, Tabara Y, Muraoka H, Fujita M, Miyazaki N, Fujikawa A, Ichikawa Y, Yamamoto Y, Ichihara N, Saka S, Sakai H, Yoshida S, Saku K, Taya K, Yasuda G, Kohara K, Yita H, Takayama K, Goshima Y, Ichikawa H, Ushina M, Hikita M, T. Mice lacking hypertension candidate gene ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. Hypertension 2012; 59: 854–860.

20. Hirawa N, Fujisawa A, Emunera S, ATP2B1 gene to blood pressure: from associations to pathophysiology. Curr Opin Nephrol Hypertens 2013; 22: 177–184.
19 Leenen FH, Fourney A, Notman G, Tansier J. Persistence of anti-hypertensive effect after ‘missed doses’ of calcium antagonist with long (amlodipine) vs. short (diltiazem) elimination half-life. Br J Clin Pharmacol 1996; 41: 83–88.

20 Curran MP, Robinson DM, Keating GM. Intravenous nicardipine: its use in the short-term treatment of hypertension and various other indications. Drugs 2006; 66: 1755–1782.

21 Duka I, Gavras I, Johns C, Handy DE, Gavras H. Role of the postsynaptic alpha(2)-adrenergic receptor subtypes in catecholamine-induced vasoconstriction. Gen Pharmacol 2000; 34: 101–106.

22 Faulkner JK, McGibney D, Chasseaud LF, Perry JL, Taylor IW. The pharmacokinetics of amlodipine in healthy volunteers after single intravenous and oral doses and after 14 repeated oral doses given once daily. Br J Clin Pharmacol 1986; 22: 21–25.

23 Chen X, Rateri DL, Howatt DA, Balakrishnan A, Moorleghen JJ, Morris AJ, Charnigo R, Cassis LA, Daugherty A. Amlodipine reduces AngII-induced aortic aneurysms and atherosclerosis in hypercholesterolemic mice. PLoS ONE 2013; 8: e81743.

24 Devarajan S, Yahiro E, Uehara Y, Habe S, Nishiyama A, Miura S, Saku K, Urata H. Depressor effect of chymase inhibitor in mice with high salt-induced moderate hypertension. Am J Physiol Heart Circ Physiol 2015; 309: H1987–H1996.

25 Hofmann F, Flockerzi V, Kahl S, Wegener JW. L-type CaV1.2 calcium channels: from in vitro findings to in vivo function. Physiol Rev 2014; 94: 303–326.

26 Abernethy DR, Schwartz JB. Calcium-antagonist drugs. N Engl J Med 1999; 341: 1447–1457.

27 Chen Y, Zhang H, Zhang Y, Lu N, Zhang L, Shi L. Exercise intensity-dependent reverse and adverse remodeling of voltage-gated Ca(2+) channels in mesenteric arteries from spontaneously hypertensive rats. Hypertens Res 2015; 38: 656–665.

28 Pratt PF, Bonnet S, Ludwig LM, Bonnet P, Rusch NJ. Upregulation of L-type Ca2+ channels in mesenteric and skeletal arteries of SHR. Hypertension 2002; 40: 214–219.

29 Pesic A, Madden JA, Pesic M, Rusch NJ. High blood pressure upregulates arterial L-type Ca2+ channels: is membrane depolarization the signal? Circ Res 2004; 94: e27–104.

30 Wang WZ, Pang L, Palade P. Angiotensin II causes endothelial-dependent increase in expression of Ca(V)1.2 protein in cultured arteries. Eur J Pharmacol 2008; 599: 117–120.

31 Hutri-Kahonen N, Kahonen M, Wu X, Sand J, Nordback I, Taurio J, Porti I. Control of vascular tone in isolated mesenteric arterial segments from hypertensive patients. Br J Pharmacol 1999; 127: 1735–1743.

32 Harraz OF, Altier C. STIM1-mediated bidirectional regulation of Ca(2+) entry through voltage-gated calcium channels (VGCC) and calcium-release activated channels (CRAC). Front Cell Neurosci 2014; 8: 43.

33 Korb D, Trg PY, Milenkovic VM, Reichhart N, Strauss O, Ritter O, Fischer T, Benz PM, Schuh K. Identification of PDZ domain containing proteins interacting with 1.2 and PMCA4b. JGRN Cell Biol 2013; 2013: 1–16.

34 Zhao Y, Vanhoutte PM, Leung SW. Vascular nitric oxide: Beyond eNOS. J Pharmacol Sci 2015; 129: 83–94.

35 Schuh K, Quaschning T, Knauer S, Hu K, Kocak S, Roethlein N, Neyes L. Regulation of vascular tone in animals overexpressing the sarcoplasmic calcium pump. J Biol Chem 2003; 278: 41246–41252.

36 Bank N, Ayedjian HS. Role of EDRF (nitric oxide) in diabetic renal hyperfiltration. Kidney Int 1993; 43: 1306–1312.

37 Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, Morgan KG. Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. Pharmacol Rev 2016; 68: 476–532.