Published in final edited form as:
*Hypertension*. 2013 January ; 61(1): 137–142. doi:10.1161/HYPERTENSIONAHA.112.197863.

# The β3 Subunit Contributes to Vascular Calcium Channel Upregulation and Hypertension in Angiotensin II-infused C57BL/6 Mice

**Sujay V. Kharade**[*], **Swapnil K. Sonkusare**[*], **Anup K. Srivastava**, **Keshari M. Thakali**, **Terry W. Fletcher**, **Sung W. Rhee**, and **Nancy J. Rusch**
Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205 USA

## Abstract

Voltage-gated L-type $Ca^{2+}$ ($Ca_v1.2$) channels in vascular smooth muscle cells (VSMCs) are a predominant $Ca^{2+}$ influx pathway that mediates arterial tone. Channel biogenesis is accomplished when the pore-forming $\alpha_{1C}$ subunit co-assembles with regulatory $Ca_v\beta$ subunits intracellularly, and the multi-protein $Ca_v1.2$ complex translocates to the plasma membrane to form functional $Ca^{2+}$ channels. We hypothesized that the main $Ca_v\beta$ isoform in VSMCs, $Ca_v\beta3$, is required for the upregulation of arterial $Ca_v1.2$ channels during the development of hypertension, an event associated with abnormal $Ca^{2+}$-dependent tone. $Ca_v1.2$ channel expression and function was compared between 2nd order mesenteric arteries (MA) of C57BL/6 wild-type (WT) and $Ca_v\beta3^{-/-}$ mice infused with saline (control) or angiotensin II (Ang II) for 2 weeks to induce hypertension. The MA of Ang II-infused WT mice showed increased $Ca_v1.2$ channel expression and accentuated $Ca^{2+}$-mediated contractions compared to saline-infused WT mice. In contrast, $Ca_v1.2$ channels failed to upregulate in MA of Ang II-infused $Ca_v\beta3^{-/-}$ mice and $Ca^{2+}$-dependent reactivity was normal in these arteries. Basal systolic blood pressure (SBP) was not significantly different between WT and $Ca_v\beta3^{-/-}$ mice (98±2 mm Hg and 102±3 mm Hg, respectively), but the $Ca_v\beta3^{-/-}$ mice showed a blunted pressor response to Ang II infusion. Two weeks after the start of Ang II administration, the SBP of $Ca_v\beta3^{-/-}$ mice averaged 149±4 mm Hg compared to 180±5 mm Hg in WT mice. Thus, the $Ca_v\beta3$ subunit is a critical regulatory protein required to upregulate arterial $Ca_v1.2$ channels and fully develop angiotensin II –dependent hypertension in C57BL/6 mice.

### Keywords

Calcium channel; $Ca_v1.2$; β3 subunit; hypertension; mesenteric arteries

## Introduction

During the development of hypertension, the L-type $Ca^{2+}$ ($Ca_v1.2$) channel encoded by the $Ca_v1.2$ gene is upregulated in vascular smooth muscle cells (VSMCs).[1-3] An over-

Corresponding Author: Nancy J. Rusch, Ph.D., Professor and Chair, Department of Pharmacology & Toxicology, University of Arkansas for Medical Sciences (UAMS), 4301 West Markham Street, Mail Slot 611, Little Rock, AR 72205, (501) 686-5510 phone; (501) 686-5521 fax, nrusch@uams.edu.
[*]Shared first authorship

**Conflict of interest/disclosures**: None.

abundance of vascular $Ca_v1.2$ channels has been documented in rats with genetic, renal and salt-dependent forms of hypertension [for review, see 4]. At least one study suggests that even a short-term elevation of intravascular pressure increases expression of arterial $Ca_v1.2$ channels *in vivo*, implicating the upregulation of $Ca_v1.2$ channels as an early event in the pathogenesis of hypertension.[1] The increased expression of $Ca_v1.2$ channels enhances voltage-dependent $Ca^{2+}$ influx resulting in abnormal vasoconstriction, which can be reversed by pharmacological antagonists of the $Ca_v1.2$ channel.[1,2,5-7] Correspondingly, an increased $Ca_v1.2$ channel –mediated arterial tone in hypertensive animals and humans with essential hypertension may partly explain their sensitivity to the antihypertensive effect of clinical $Ca^{2+}$ channel antagonists that block $Ca_v1.2$ channels. The same drugs only mildly lower blood pressure in normotensive subjects.[8-11]

The mechanisms that mediate the upregulation of arterial $Ca_v1.2$ channels during hypertension are unclear, but appear to primarily rely on post-transcriptional events since the increased expression of the $Ca_v1.2$ pore-forming $\alpha_{1C}$ subunit has not been linked to a corresponding increase of $\alpha_{1C}$ transcript.[2,3] In the present study, we hypothesized that the regulatory β3 subunit ($Ca_v\beta3$) of the $Ca_v1.2$ channel, which acts post-transcriptionally to promote surface expression of the $\alpha_{1C}$ pore, may increase $Ca_v1.2$ channel abundance during hypertension. It is presumed that in VSMCs, similar to other cell types, the $\alpha_{1C}$ subunit co-assembles with smaller β and $\alpha_2\delta$ subunits to form functional $Ca^{2+}$ channels.[12-15] Four gene families (β1, β2, β3, β4) encode $Ca_v\beta$ subunits in a tissue-specific manner. $Ca_v\beta3$ is the principal subtype in VSMCs,[16,17] where it is presumed to bind to $\alpha_{1C}$ in the endoplasmic reticulum (ER) to enhance its trafficking to the plasma membrane (PM). At the cell surface, the multi-protein $Ca_v1.2$ channel assumes its critical function of mediating voltage-dependent $Ca^{2+}$ influx during membrane depolarization.

The precise domains that permit interaction between $\alpha_{1C}$ and $Ca_v\beta3$ in the ER and the sequence of events that promote $Ca_v1.2$ expression on the surface of VSMCs remain elusive. However, Murakami *et al*[16,17] reported that the aortae of $Ca_v\beta3^{-/-}$ mice express fewer $Ca_v1.2$ channels compared to wild-type (WT) mice, implying that $Ca_v\beta3$ contributes to the basal expression of $Ca_v1.2$ channels in VSMCs *in vivo*. Unexpectedly, despite the loss of arterial $Ca_v1.2$ channels, the $Ca_v\beta3^{-/-}$ mice exhibited normal systolic blood pressure (SBP).[16,17] This seeming dichotomy may indicate that blood pressure was sustained by compensatory mechanisms and/or indicate that $Ca^{2+}$ influx through $Ca_v1.2$ channels contributes only minimally to basal blood pressure in normotensive subjects.[8-11]

Here, we considered the hypothesis that the regulatory $Ca_v\beta3$ subunit promotes vascular $Ca_v1.2$ channel expression in hypertension, a disease in which this channel is pathogenically over-expressed. Our studies utilized $Ca_v\beta3^{-/-}$ mice to explore if deletion of the $Ca_v\beta3$ gene attenuates the upregulation of arterial $Ca_v1.2$ channels and the development of hypertension. The findings demonstrate that although $Ca_v\beta3^{-/-}$ mice show normal resting SBP, they fail to fully develop angiotensin II (Ang II)-dependent hypertension and this loss of function is associated with an inability to upregulate arterial $Ca_v1.2$ channels. Thus, $Ca_v\beta3$ may be a previously unrecognized but critical protein that contributes to the vascular $Ca_v1.2$ channel abnormalities that elevate blood pressure.

## Methods

### Blood pressure measurement

Procedures using animals were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. SBP was recorded using tail-cuff plethysmography before and after implanting subcutaneous osmotic minipumps containing either saline or Ang II (infused at 2 ng/g/min).

### Vessel perfusion assays

Second order mesenteric arteries (MA) were isolated from saline-infused (SAL) and Ang II-infused hypertensive (AHT) mice, and used to perform vessel perfusion assays.

### Patch clamp studies

The VSMCs were enzymatically isolated from 2nd order MA branches and whole-cell $Ca^{2+}$ channel currents were recorded using $Ba^{2+}$ (10 mmol/L) as a charge carrier with standard pulse protocols, solutions and a patch-clamp station described previously.[1]

### Quantitative real-time PCR

Whole tissue RNA was isolated from 2nd order branches of MA pooled from two mice. Then qRT-PCR was performed using primers specific for $\alpha_{1C}$, $Ca_v\beta3$ and GAPDH.

### Western blotting

Protein lysates were prepared by pooling MA from two mice.[1] Specific polyclonal antibodies directed against epitopes specific to $\alpha_{1C}$ and $Ca_v\beta3$ were used as probes.

### Statistics

Data represent mean ± standard error of the mean for the number (n) of animals indicated in parentheses. Student's *t*-test was used to compare two data sets and one-way ANOVA with Bonferroni's post-hoc test was used for multiple group comparisons. $P \leq 0.05$ was considered significant.

## Results

### $Ca_v1.2$ channel $\alpha_{1C}$ and β3 subunits upregulate in MA of AHT mice

Initially we determined whether vascular $Ca_v1.2$ channels upregulate in Ang II-infused C57BL/6 mice, since previous studies demonstrating $Ca_v1.2$ upregulation were performed only in hypertensive rat models. Figure S1 shows that 14 days of subcutaneous Ang II infusion (2 ng/g/min) resulted in SBP values of 106±2, 155±5 and 172±8 mm Hg at 0, 7 and 14 days, respectively (n=10 each). The SBP values in SAL mice did not significantly differ from a basal value of 104±3 mm Hg. Subsequent studies were performed using mice infused with saline or Ang II for two weeks.

Next, we determined if the MA of AHT mice showed an increased expression of the $\alpha_{1C}$ and $Ca_v\beta3$ subunits that co-assemble to form functional $Ca_v1.2$ channels. Adjacent lanes on Western blot (WB) were loaded with MA lysate from SAL or AHT mice. The $\alpha_{1C}$ protein was upregulated 2.4±0.4 fold in the MA of AHT mice compared to SAL mice (Fig. 1A,B). The abundance of the $Ca_v\beta3$ subunit also increased during the development of hypertension. The immunodensity of the $Ca_v\beta3$ band was 2.8±0.6 fold higher in MA lysate from AHT mice compared to SAL mice (Fig. 1A,C). Interestingly, qRT-PCR experiments using total RNA isolated from MA of SAL and AHT mice revealed that the transcript levels of both $\alpha_{1C}$ ($\Delta C_T$ values SAL 24.2±0.3, AHT 24.1±0.3) and $Ca_v\beta3$ ($\Delta C_T$ values SAL 25.1±0.5, AHT 25.1±0.6) were similar between the two isolates (Fig. 1D). The $C_T$ values were normalized to GAPDH, which was used as an amplification standard.

### Increased $Ca_v1.2$ current and vascular reactivity in AHT mice

We confirmed that the over-abundance of $Ca_v1.2$ channels in MA of AHT mice was associated with enhanced channel function. Patch-clamp studies revealed increased $Ca_v1.2$ channel -mediated current in mesenteric VSMCs of AHT compared to SAL animals (Fig. 2A, top traces), which were blocked by nifedipine (Fig. S2). Current-voltage relationships

suggested a 1.9±0.2 fold increase in peak current density in the VSMCs of AHT mice (-10.7±1.0 pA/pF) compared to SAL mice (-5.6±0.7 pA/pF) (Fig. 2A, n=23 each). Voltage-sensitive activation and inactivation were not different between groups (Fig. S3), revealing no abnormalities of $Ca_v1.2$ channel properties in VSMCs of AHT mice. The MA from AHT mice also exhibited enhanced reactivity to the $Ca_v1.2$ channel opener, FPL 64176 (Fig. 2B) resulting in a 5.1 -fold lower $EC_{50}$ value of 224±37 nmol/L compared to 1144±331 nmol/L in arteries of SAL mice (Fig. 2C).

### $Ca_v\beta3^{-/-}$ mice show normal resting SBP

After linking an overabundance of arterial $Ca_v1.2$ channels in AHT mice to increased $Ca_v\beta3$ expression for the first time, we directly tested if $Ca_v\beta3$ subunits are required for $Ca_v1.2$ channel expression by employing WT and $Ca_v\beta3^{-/-}$ mice. We observed no difference in $\alpha_{1C}$ expression between MA from WT and $Ca_v\beta3^{-/-}$ mice (Fig. 3A-B, n=12 each). Resting SBP levels also were similar between WT mice (98±2 mm Hg) and $Ca_v\beta3^{-/-}$ mice (102±3 mm Hg) (Fig. S4A, n=10). The resting heart rate of 555±8 beats/min in WT mice (n=10) and 563±10 beats/min in $Ca_v\beta3^{-/-}$ mice (n=11) also was not different (Fig. S4B).

### $Ca_v\beta3^{-/-}$ mice fail to fully develop hypertension

Subsequently, we explored if the $Ca_v\beta3$ subunit contributes to the development of Ang II-dependent hypertension, which we postulated may partly rely on upregulation of $Ca_v1.2$ channels. WT and $Ca_v\beta3^{-/-}$ mice were infused with saline (Sal) or Ang II for two weeks, and SBP was recorded for 3 days before infusion (day 0) and on days 4, 7, 10 and 14 after infusion. As expected, WT and $Ca_v\beta3^{-/-}$ mice infused with saline maintained normal SBP that averaged 104±2 mm Hg and 102±5 mm Hg on day 14, respectively (Fig. 4, n=7 each). The WT mice infused with Ang II showed steadily rising SBP reaching an average value of 180±5 mm Hg on day 14 (Fig. 4, n=7). In contrast, the SBP response to Ang II infusion was significantly blunted in $Ca_v\beta3^{-/-}$ mice, averaging only 149±4 mm Hg on day 14 (Fig. 4, n=7).

### MA of $Ca_v\beta3^{-/-}$ mice fail to upregulate $Ca_v1.2$ channels

Next, we compared the expression level of the $Ca_v1.2$ channel $\alpha_{1C}$ subunit between MA of WT and $Ca_v\beta3^{-/-}$ mice infused with saline or Ang II. As expected, Ang II infusion was associated with an upregulation of the $\alpha_{1C}$ and $Ca_v\beta3$ subunits in MA of WT mice (Fig. 5A, lanes 1 and 3), which correlated to a 1.90±0.1 fold increase in $Ca_v1.2$ $\alpha_{1C}$ (Fig. 5B, n=6) and a 2.65±0.7 fold increase in $Ca_v\beta3$ immunosignal. In contrast, $\alpha_{1C}$ failed to upregulate in MA of Ang II –infused $Ca_v\beta3^{-/-}$ mice (Fig. 5A, lanes 2 and 4), showing only a 2.3% average increase compared to Sal-infused $Ca_v\beta3^{-/-}$ animals (Fig. 5B, n=6).

### MA of $Ca_v\beta3^{-/-}$ mice fail to show abnormal $Ca_v1.2$ channel–mediated reactivity

Finally, we explored whether the failure of $Ca_v1.2$ channels to upregulate in MA of Ang II-infused $Ca_v\beta3^{-/-}$ mice also attenuated the heightened $Ca^{2+}$-dependent reactivity observed in MA of Ang II-infused WT mice (Figs. 2B-C). To test this hypothesis, an ~$EC_{50}$ of FPL 64176 (0.3 μmol/L) was used to activate $Ca_v1.2$ channels in isolated MA. The diameter response to FPL 64176 in MA from Ang II-infused $Ca_v\beta3^{-/-}$ of 34±11 μm was markedly less compared to a 72±12 μm reduction in MA from Ang II-infused WT mice (Fig. 5C-E; n=7).

## Discussion

Our findings demonstrate that the MA of $Ca_v\beta3^{-/-}$ mice infused with Ang II fail to upregulate $Ca_v1.2$ channels or show enhanced $Ca^{2+}$-dependent reactivity, which are

abnormalities observed in MA of Ang II-infused WT mice. Correspondingly, $Ca_v\beta3^{-/-}$ mice fail to fully develop Ang II-dependent hypertension compared to WT mice. Collectively our findings draw attention to the $Ca_v\beta3$ subunit in VSMCs as a critical protein necessary to upregulate $Ca_v1.2$ channels and elevate blood pressure in Ang II-dependent hypertension. The residual rise in SBP in response to Ang II infusion in $Ca_v\beta3^{-/-}$ mice infers that factors in addition to the upregulation of $Ca_v1.2$ channels contribute to the elevated blood pressure in this form of hypertension, reflecting its complex pathogenesis as reviewed by Hall.[18]

Many studies during the past decade have shown that hypertension is associated with an elevated $Ca^{2+}$ influx through arterial $Ca_v1.2$ channels.[14] At least three rat models of hypertension show an increased density of functional $Ca_v1.2$ channels in the VSMCs of the aorta and in vascular beds involved in blood pressure regulation.[1-3,5,6] In the renal circulation of aortic-banded rats, an increased $Ca_v1.2$ expression occurs by 48 hours after blood pressure elevation.[1] Thus, an upregulation of $Ca_v1.2$ channels, the opening of which may be driven by the depolarized membrane potential of VSMCs exposed to high intravascular pressure, appears to contribute to the elevated vascular tone of hypertension.[1,2] An increased $Ca^{2+}$-dependent reactivity, and an enhanced vasodilator sensitivity to nifedipine, also has been reported in MA segments from hypertensive patients.[7] These findings, combined with the observation that the blood pressure lowering effect of calcium channel blockers is exaggerated in hypertensive patients,[8,9] raise the possibility that $Ca_v1.2$ channel abnormalities extend to essential hypertension in humans.

Here, we extend earlier findings in rats to the Ang II –infused hypertensive mouse by confirming that MA from AHT mice show increased $Ca_v1.2$ expression and $Ca^{2+}$-dependent reactivity compared to arteries from SAL mice with normal blood pressure. Similar to earlier studies in rats,[1-3] we failed to detect a major increase of $\alpha_{1C}$ transcript in arteries of AHT mice that could account for the marked over-abundance of the $\alpha_{1C}$ protein. Thus, the upregulation of arterial $Ca_v1.2$ channels in AHT mice appears to rely on post-transcriptional mechanisms that could include increases in translational efficiency and/or channel biogenesis, trafficking or surface stability.

In this regard, $Ca_v\beta$ subunits are known to increase the surface density of $Ca^{2+}$ channels, although most studies have been performed in heterologous expression systems[19,20] or nonvascular cell types.[21,22] The $Ca_v\beta$ subunits co-assemble with pore-forming α-subunits during channel biogenesis to promote the trafficking of the $Ca^{2+}$ channel complex from the ER to the PM.[14,19] Although $Ca_v\beta$ are postulated to mask an ER retention signal in α-subunits as a mechanism to target $Ca^{2+}$ channels to the cell surface, no specific motif has been identified. Altier *et al.*[23] has alternatively demonstrated in HEK293 cells that the interaction between $Ca_v\beta$ and $\alpha_{1C}$ prevents ubiquitination of the $\alpha_{1C}$ subunit by ubiquitin ligase, thereby rescuing $Ca_v1.2$ channels from the ER-associated protein degradation (ERAD) complex that marks the channel for proteasomal degradation. Waithe *et al.*[24] reported a similar interaction between $Ca_v\beta$ and $Ca_v2.2$ channels in neurons and concluded that $Ca_v\beta$ increases $Ca_v2.2$ surface density by conferring protection from proteosomal degradation. Finally, by co-expressing Nedd4 ubiquitin ligases and $Ca_v1.2$ channels in tsA-201 cells, Rougier *et al.*[25] showed that Nedd4-1 ubiquitin ligase directly interferes with the $Ca_v\beta$-mediated delivery of $Ca_v1.2$ channels to the PM.

The precise physiological role of $Ca_v\beta3$ in VSMCs is unknown. Murakami *et al.*[16,17] reported that $\alpha_{1C}$ expression and $Ca_v1.2$ -mediated $Ca^{2+}$ current were reduced in aortae from $Ca_v\beta3^{-/-}$ mice, although SBP was normal. Here, we also observed comparable basal SBP between WT and $Ca_v\beta3^{-/-}$ mice, and additionally, the MA of these mice exhibited similar basal $Ca_v1.2$ channel expression. However, MA of $Ca_v\beta3^{-/-}$ mice failed to upregulate $Ca_v1.2$ channels or show accentuated $Ca^{2+}$-dependent reactivity in response to Ang II

infusion, which were abnormalities consistently observed in arteries of hypertensive WT mice. The absence of $Ca_v1.2$ channel upregulation in MA of Ang II –infused $Ca_v\beta3^{-/-}$ mice was associated with a blunted blood pressure response to Ang II. Thus, our findings suggest an association between the $Ca_v\beta3$ subunit, $Ca_v1.2$ upregulation and hypertension. Notably, the deletion of $Ca_v\beta3$ was not limited to VSMCs in the $Ca_v\beta3^{-/-}$ mice of our study, and thus we cannot state with certainty that the failure of $Ca_v\beta3^{-/-}$ mice to fully develop Ang II –dependent hypertension relied on $Ca_v\beta3$ deletion in VSMCs rather than nonvascular cell types. Although the $Ca_v\beta3^{-/-}$ mouse has no gross phenotype, the $Ca_v\beta3$ subunit also is extensively expressed in the brain and may negatively regulate NMDA receptors and potentially affect other neuronal functions.[26] For this reason, the design of new $Ca_v\beta3^{-/-}$ mice showing conditional and SMC-specific $Ca_v\beta3$ gene deletion will be required to directly test the hypothesis that arterial $Ca_v\beta3$ subunits contribute to experimental hypertension.

Notably, Fan *et al.*[22] designed mutant $Ca_v\beta$ subunits that interact with $\alpha_{1C}$ intracellularly, but lack the motifs required to target the $Ca_v1.2$ channel complex to the PM. These β subunit "decoys" represent potential therapeutics to reduce $Ca_v1.2$ surface expression in cardiovascular pathologies characterized by an overabundance of $Ca_v1.2$ channels.[22,27,28] It may be advantageous to ameliorate anomalous $Ca^{2+}$ influx by preventing the upregulation of $Ca_v1.2$ channels rather than using pharmacological blockers to reduce the activity of over-expressed channels. Our findings provide proof-of-principle for this concept by showing that $Ca_v\beta3$ deletion can normalize $Ca_v1.2$ channel expression in VSMCs of hypertensive mice in which $Ca_v1.2$ over-expression is a contributing defect.

## Perspectives

To our knowledge, the present study provides the first evidence to implicate a distinct $Ca^{2+}$ channel β subunit in the development of hypertension. It has been proposed that small molecule inhibitors of $Ca_v\beta$ subunits represent future therapies to lower $Ca^{2+}$ channel abundance in conditions associated with excessive $Ca^{2+}$ influx including hypertension. Our findings support the concept that strategies to knockdown or inhibit the $Ca_v\beta3$ subunit in VSMCs and thereby reduce $Ca_v1.2$ channel expression in the arterial circulation may represent novel therapeutic approaches for lowering blood pressure in hypertensive subjects.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We are grateful to Dr. Hee-Sup Shin of the Korea Institute of Science and Technology for designing and sharing the $Ca_v\beta3^{-/-}$ mice. We also thank Dr. William Guido from Virginia Commonwealth University for directly providing the mice from which on-site colonies were generated.

**Sources of Funding**: This work was supported by R01 HL064806-12 from the National Institutes of Health (NJR, SWR) and predoctoral grant PRE 092250224 from the South Central Affiliate of the American Heart Association (SVK).

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## Novelty and Significance

**What is New?**

- A β3 protein increases the number of voltage-gated $Ca^{2+}$ channels in arteries during hypertension.
- Mice in which the β3 protein is deleted fail to fully develop hypertension.

**What Is Relevant?**

- The abnormal contraction of small arteries during hypertension is partly caused by the presence of too many $Ca^{2+}$ channels.
- Here, we report that a β3 protein is required for $Ca^{2+}$ channels to increase in arteries during hypertension and lack of this protein reduces the level of blood pressure elevation.

## Summary

The β3 subunit is a critical protein in arteries that contributes to the upregulation of voltage-gated $Ca^{2+}$ channels and the development of hypertension.

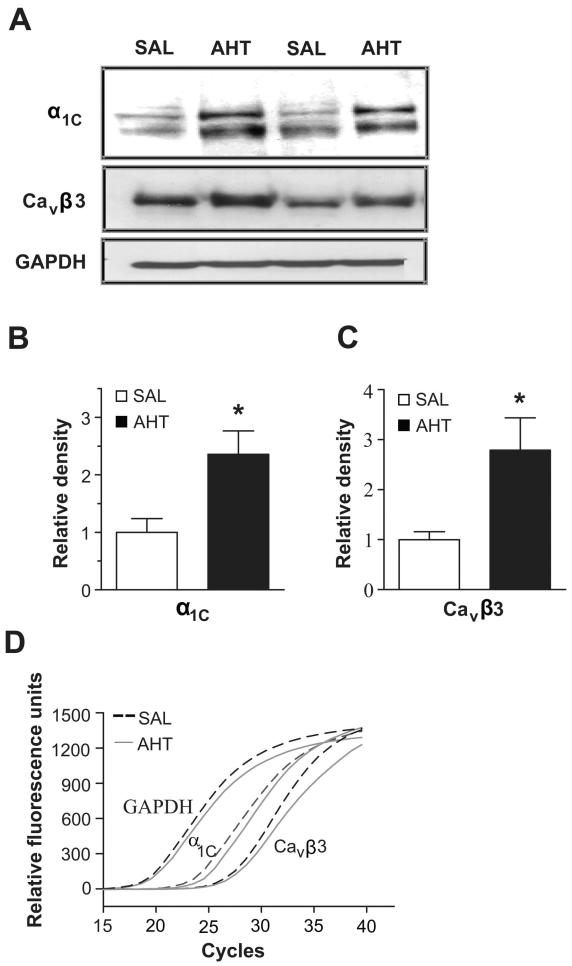


**Figure 1.**

**A.** WB showing adjacent lanes loaded with 40 μg of MA protein lysate pooled from either two SAL or AHT mice and probed with anti- $\alpha_{1C}$ and $Ca_v\beta3$. GAPDH was a loading control. **B, C.** Densitometric analyses of $\alpha_{1C}$ and $Ca_v\beta3$ immunoreactivity (n=6; * = $p<0.05$). **D.** qRT-PCR amplification corresponding to $\alpha_{1C}$, $Ca_v\beta3$ and GAPDH transcripts averaged from 4 MA samples from SAL and AHT mice.

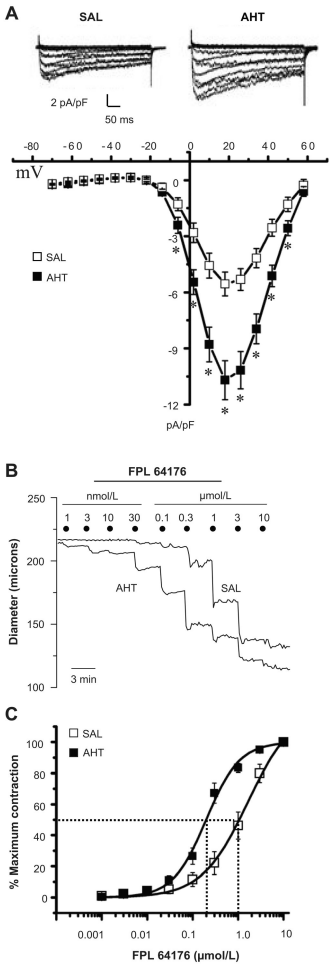


**Figure 2.**

**A.** $Ca_V1.2$ channel current elicited by 8 mV steps from -70 mV to +58 mV in mesenteric VSMCs of SAL and AHT mice (upper panel) and averaged I-V curves (n=23 each). **B.** On-line diameter responses of MA from SAL and AHT mice to increasing concentrations of FPL 64176 (1 nmol/L to 10 μmol/L, half-log units). **C.** Concentration-response relationships to FPL 64176 reveal a lower $EC_{50}$ value for MA from AHT mice compared to SAL mice (n=7 each). * = $p<0.05$.

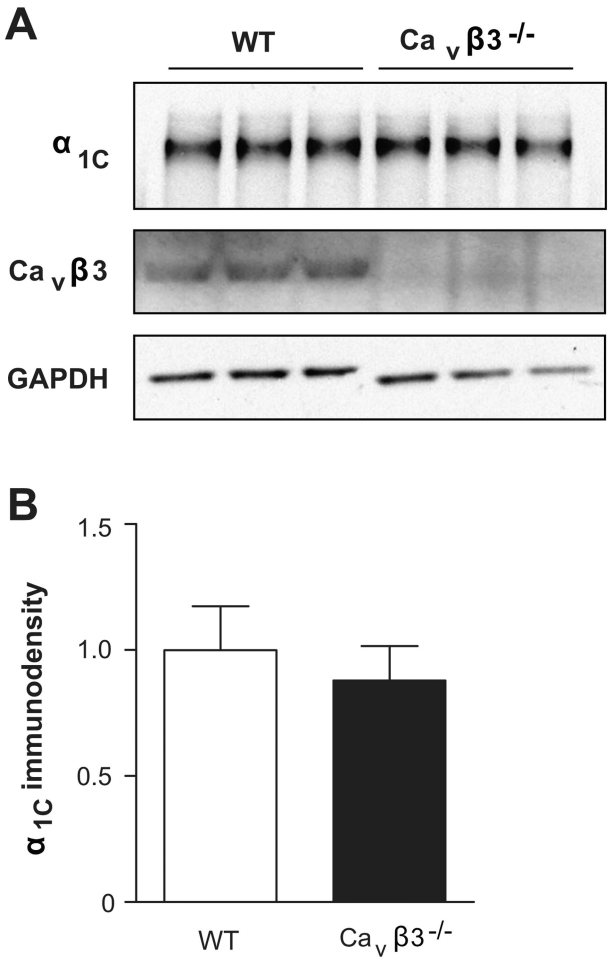


**Figure 3.**

**A.** WB showing adjacent lanes loaded with 40 μg of MA protein lysate each pooled from two WT or $Ca_v\beta3^{-/-}$ mice and probed with anti-$\alpha_{1C}$ or $Ca_v\beta3$. GAPDH was a loading control. **B.** Average densitometric values for $\alpha_{1C}$ (n=12).

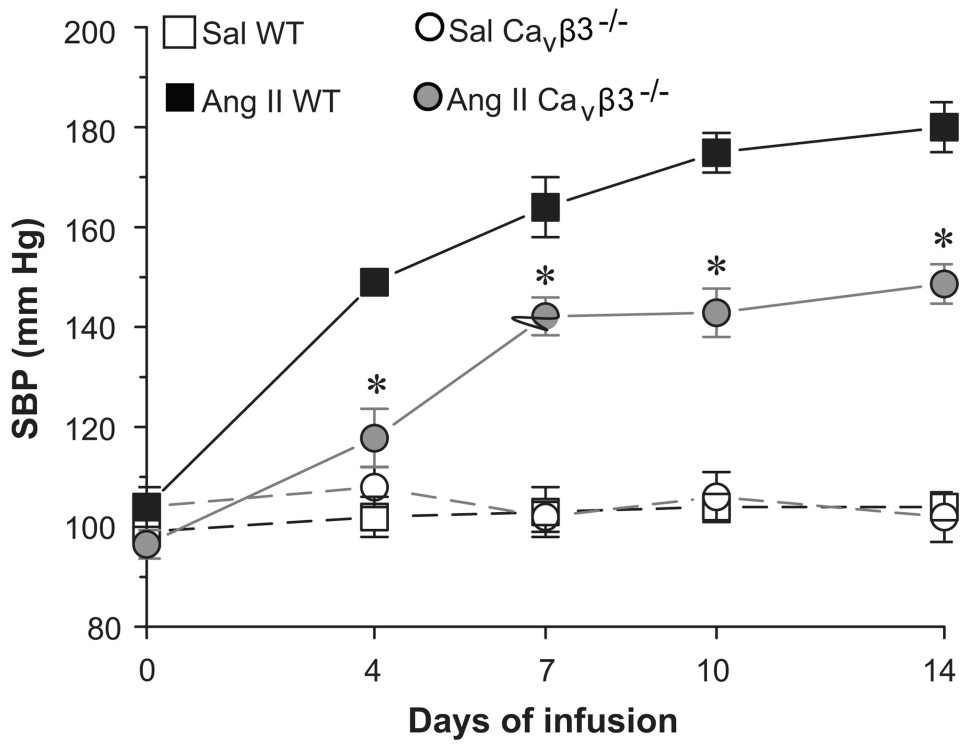


**Figure 4.**

The SBP response to two weeks of saline (Sal) or Ang II infusion in WT and $Ca_v\beta3^{-/-}$ mice. Sal-infused WT and $Ca_v\beta3^{-/-}$ mice showed normal SBP during the infusion period. SBP progressively increased in Ang II-infused WT mice and reached 180±5 mm Hg at 14 days. $Ca_v\beta3^{-/-}$ mice showed a blunted blood pressure response to Ang II infusion and SBP at 14 days averaged only 149±4 mm Hg (n=7 each). * = $p<0.05$, significant difference between Ang II-infused WT and $Ca_v\beta3^{-/-}$.

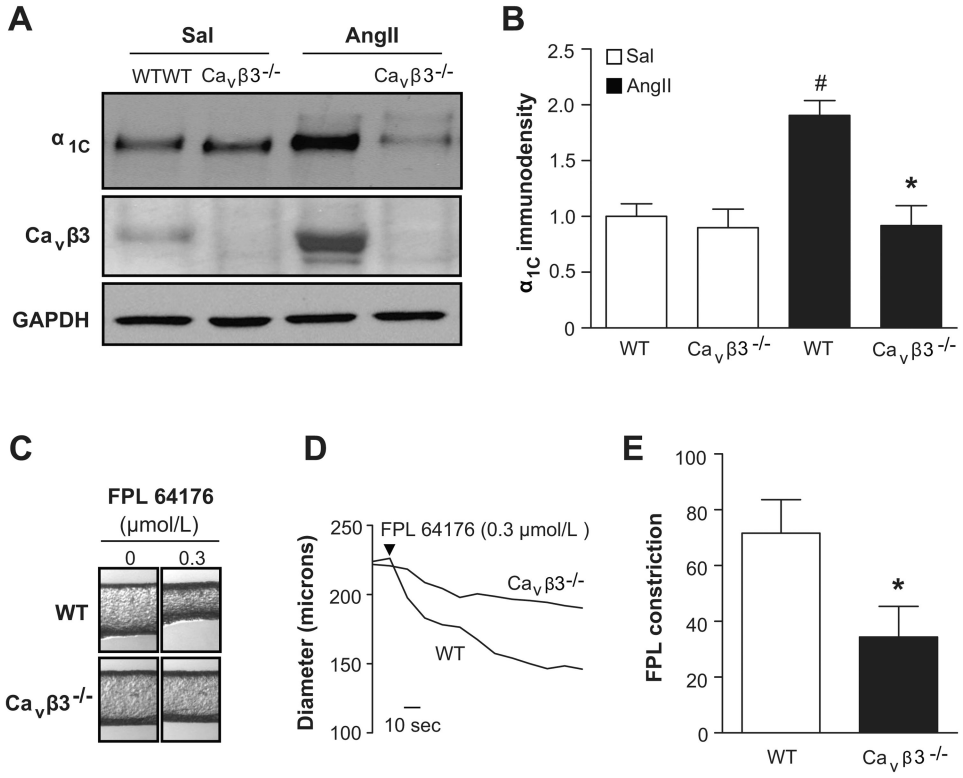


**Figure 5.**

**A.** WB probed with antibodies directed against $\alpha_{1C}$ and $Ca_v\beta3$. Each lane was loaded with 40 μg of protein lysate pooled from the MA of two WT or $Ca_v\beta3^{-/-}$ mice infused with either saline (Sal) or Ang II for two weeks. GAPDH was a loading control. **B.** Densitometric analyses revealed that only MA of WT mice upregulated $\alpha_{1C}$ in response to Ang II (n=6). **C, D.** On-line picture and diameter recordings, respectively, of MA from Ang II-infused WT or $Ca_v\beta3^{-/-}$ mice. The contraction to FPL 64176 (0.3 μmol/L) was blunted in arteries of $Ca_v\beta3^{-/-}$ mice. **E.** Average diameter responses to FPL 64176 in MA from Ang II-infused WT and $Ca_v\beta3^{-/-}$ mice (n=7 each). $^{*,\#}$ = $p<0.05$, # indicates significant difference between Sal-infused mice and Ang II-infused WT mice. * indicates value is significantly different than Ang II -infused WT mice.