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Hypertension. Author manuscript; available in PMC 2013 October 01.

Published in final edited form as:
Hypertension. 2012 October ; 60(4): 1006–1015. doi:10.1161/HYPERTENSIONAHA.112.199661.

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TRANSCRIPTIONAL UPREGULATION OF $\alpha_2\delta$-1 ELEVATES ARTERIAL SMOOTH MUSCLE CELL CA$_V$1.2 CHANNEL SURFACE EXPRESSION AND CEREBROVASCULAR CONSTRICTION IN GENETIC HYPERTENSION

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Abstract

A hallmark of hypertension is an increase in arterial myocyte voltage-dependent Ca^{2+} (Ca$_V$1.2) currents that induces pathological vasoconstriction. Ca$_V$1.2 channels are heteromeric complexes comprising a pore forming Ca$_V$1.2α_1 with auxiliary $\alpha_2\delta$ and β subunits. Molecular mechanisms that elevate Ca$_V$1.2 currents during hypertension and the potential contribution of Ca$_V$1.2 auxiliary subunits are unclear. Here, we investigated the pathological significance of $\alpha_2\delta$ subunits in vasoconstriction associated with hypertension.

Age-dependent development of hypertension in spontaneously hypertensive rats (SHR) was associated with an unequal elevation in $\alpha_2\delta$-1 and Ca$_V$1.2α_1 mRNA and protein in cerebral artery myocytes, with $\alpha_2\delta$-1 increasing more than Ca$_V$1.2α_1. Other $\alpha_2\delta$ isoforms did not emerge in hypertension. Myocytes and arteries of hypertensive SHR displayed higher surface-localized $\alpha_2\delta$-1 and Ca$_V$1.2α_1 proteins, surface $\alpha_2\delta$-1 to Ca$_V$1.2α_1 ratio ($\alpha_2\delta$-1:Ca$_V$1.2α_1), Ca$_V$1.2 current-density and non-inactivating current, and pressure- and - depolarization-induced vasoconstriction than those of Wistar-Kyoto controls. Pregabalin, an $\alpha_2\delta$-1 ligand, did not alter $\alpha_2\delta$-1 or Ca$_V$1.2α_1 total protein, but normalized $\alpha_2\delta$-1 and Ca$_V$1.2α_1 surface expression, surface $\alpha_2\delta$-1:Ca$_V$1.2α_1, Ca$_V$1.2 current-density and inactivation, and vasoconstriction in myocytes and arteries of hypertensive rats to control levels.

Genetic hypertension is associated with an elevation in $\alpha_2\delta$-1 expression that promotes surface trafficking of Ca$_V$1.2 channels in cerebral artery myocytes. This leads to an increase in Ca$_V$1.2 current-density and a reduction in current inactivation that induces vasoconstriction. Data also suggest that $\alpha_2\delta$-1 targeting is a novel strategy that may be used to reverse pathological Ca$_V$1.2 channel trafficking to induce cerebrovascular dilation in hypertension.

Keywords

Calcium channels; Genetic Hypertension; Vasodilation; Vasoconstriction

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Conflict of Interest/Disclosures

None.

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Introduction

Hypertension is associated with an elevation in arterial contractility that increases systemic blood pressure and restricts organ blood flow, leading to end-organ damage.[1] Hypertension is also a major predictor for a variety of cerebral diseases, including stroke, Alzheimer's disease, and dementia. One characteristic pathological alteration that occurs in hypertension is an elevation in vascular smooth muscle cell (myocyte) voltage-dependent Ca^{2+} influx.[2, 3] Voltage-dependent L-type Ca^{2+} ($Ca_V1.2$) channels are the primary Ca^{2+} entry pathway in arterial myocytes and are essential for contractility regulation by a wide variety of stimuli, including intravascular pressure, membrane potential, and vasoconstrictors.[4–8] A hypertension-associated elevation in $Ca_V1.2$ currents leads to an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and vasoconstriction.[9–11] However, molecular mechanisms that elevate arterial myocyte $Ca_V1.2$ currents in hypertension, leading to vasoconstriction, are unclear.

$Ca_V1.2$ channels are heteromeric complexes comprising a pore forming α_1 with auxiliary $\alpha_2\delta$ and β subunits.[12] Four $\alpha_2\delta$ (1 through 4) subunit isoforms have been identified that are each encoded by different genes.[13, 14] $\alpha_2\delta$ subunits undergo post-translational cleavage into a highly glycosylated extracellular α_2 and a smaller δ subunit, which are subsequently coupled by a disulfide bond to form a single functional protein.[14, 15] $\alpha_2\delta$ subunits are membrane-bound by the bilayer-spanning δ subunit. Recently, $\alpha_2\delta$-1 was identified as being critical for functional trafficking of $Ca_V1.2\alpha_1$ subunits to the plasma membrane (surface) in arterial myocytes.[16] To date, no studies have investigated pathological or disease-associated molecular changes in $Ca_V1.2$ auxiliary subunits, including $\alpha_2\delta$ subunits, in myocytes of resistance-size arteries. In addition, it is unclear whether the subunit composition of arterial myocyte surface $Ca_V1.2$ channels is altered in disease. Given that arterial myocyte $Ca_V1.2$ currents are elevated during hypertension, leading to vasoconstriction, we determined the subunit composition of $Ca_V1.2$ channels and investigated the involvement of $\alpha_2\delta$ subunits in this pathological alteration.[9–11] Elucidating molecular mechanisms governing $\alpha_2\delta$ subunit regulation of $Ca_V1.2$ channels in hypertension could lead to the development of novel approaches to treat cardiovascular diseases.

Here, we used a genetic model of hypertension, the spontaneously hypertensive rat (SHR), to investigate the pathological significance of arterial myocyte $\alpha_2\delta$ subunits in hypertension. We show that that during hypertension, an elevation in $\alpha_2\delta$-1 expression increases plasma membrane $Ca_V1.2$ currents in arterial myocytes, leading to vasoconstriction. We also identify $\alpha_2\delta$-1 as a novel therapeutic target to induce cerebrovascular dilation in hypertension.

Methods

Cell isolation and tissue preparation

All animal protocols used were reviewed and approved by the Animal Care and Use Committee at the University of Tennessee Health Science Center. Male 6 or 12 week old SHR and Wistar Kyoto (WKY) rats were euthanized by intraperitoneal injection of sodium pentobarbital (150 mg/Kg body weight, Vortech Pharmaceuticals, Dearborn, MI). Middle cerebral, posterior cerebral, and cerebellar arteries (~100–200 μm diameter) were studied. Myocytes were enzymatically dissociated from dissected cerebral arteries, as previously described.[4]

Blood pressure measurements

Diastolic and systolic blood pressures were measured in conscious rats using a tail cuff sphygmomanometer (Kent Scientific, Torrington, Conn).

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RT-PCR

RT-PCR was performed on myocytes individually collected under a microscope using an enlarged patch-clamp pipette to prevent contamination from other arterial wall cell types, as previously described.[4]

Quantitative real-time PCR

Total RNA was isolated from cerebral arteries using Trizol (Invitrogen, Grand Island, NY). cDNA was transcribed using Affinity Script Multiple temperature reverse transcriptase (Stratagene, Clara, CA). Gene specific primers and probes were designed using the Universal Probe Library (UPL). Sequences of primers and probes used and PCR reaction efficiencies are given in Table S1.

Protein analysis and biochemistry

Proteins were separated on SDS-PAGE gels and analyzed by Western blotting. Blots were cut at the 75 kDa marker to allow simultaneous probing of the upper section for $\alpha_2\delta$-1 and lower section for actin. The upper portion of the blot was then re-probed for $\mathrm{Ca_V1.2\alpha_1}$. Protein band intensities were determined using Quantity One (BioRad, Hercules, CA) software. For quantification, protein band intensities were first normalized to actin and then to appropriate control samples.

Artery surface biotinylation

To determine the distribution of $\alpha_2\delta$-1 and $\mathrm{Ca_V1.2\alpha_1}$ subunit proteins between surface and intracellular compartments, artery surface biotinylation was used, as previously described.[16]

Patch-clamp electrophysiology

Whole cell $\mathrm{Ca_V1.2}$ currents were recorded in isolated myocytes using the whole cell patch-clamp configuration, as previously described.[16]

Pressurized artery myography

Endothelium-denuded artery diameter was measured over a range of intravascular pressures (20–100 mmHg) in the presence and absence of nimodipine (1 μmol/L) using edge-detection myography, as previously described.[17] Diameter responses to elevating extracellular K^+ from 6 to between 20 and 60 mmol/L at 10 mmHg in the presence of pinacidil (10 μmol/L), a K_{ATP} channel opener, were also recorded. Arteries treated with pregabalin for 24 h were also maintained in pregabalin throughout these experiments to inhibit $\mathrm{Ca_V1.2}$ subunit membrane re-insertion.

Statistical analysis

Summary data are presented as mean ± SEM. Significance was determined using paired or unpaired t-tests with Welsh correction, or ANOVA followed by Student-Newman Keuls for multiple groups. $P<0.05$ was considered significant. Power analysis was carried out where $P>0.05$ to verify that sample size was sufficient to give a value of >0.8.

An expanded Methods section is available as Supplemental Documentation.

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Results

Age-dependent development of genetic hypertension is associated with an elevation in arterial myocyte $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ subunit expression

The pathological involvement of arterial myocyte $Ca_V1.2$ subunits was studied using a rat genetic model of hypertension. At 6 weeks of age, WKY and SHR rat diastolic, systolic, and mean arterial blood pressures were similar (Fig. S1). In contrast, at 12 weeks of age, diastolic, systolic, and mean arterial pressures were ~63, 65, and 72 mmHg higher in SHR than WKY rats, respectively (Fig S1).

Four different $\alpha_2\delta$ isoforms have been described, with $\alpha_2\delta$-1 the only isoform expressed in normotensive Sprague-Dawley (SD) rat cerebral artery myocytes.[14, 16] We tested the hypothesis that hypertension is associated with a shift in $\alpha_2\delta$ isoform expression in myocytes of resistance-size arteries. RT-PCR detected only $\alpha_2\delta$-1 in pure cerebral artery myocytes from 12 week old WKY and hypertensive SHR rats (Fig. 1A). In contrast, the same primers amplified transcript for all $\alpha_2\delta$ isoforms in WKY and SHR whole brain (Fig. 1A).

Quantitative PCR was performed to compare $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ message levels in 6 and 12 week old WKY and SHR cerebral arteries. Eight different reference genes were screened to identify those with similar mRNA levels in cerebral arteries of WKY and SHR (Table S1). Rps5 mRNA levels were similar in WKY and SHR arteries and thus, Rps5 was used as the reference gene for these experiments (Table S2). Quantitative PCR indicated that mean $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ mRNA levels were similar in 6 week old WKY and SHR arteries (Fig. 1B). In contrast, $\alpha_2\delta$-1 and $Ca_V1.2$ mRNAs were ~2.1- and 1.5-fold higher, respectively, in 12 week old SHR than WKY arteries (Fig. 1B). Age-dependent development of hypertension was also associated with a larger increase in $\alpha_2\delta$-1 than $Ca_V1.2\alpha_1$ mRNA (Fig. 1B). These data indicate that hypertension is associated with an elevation in $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ subunit mRNA, but not with the appearance of other $\alpha_2\delta$ isoforms, in arterial myocytes.

Next, we investigated whether age-dependent development of genetic hypertension is associated with upregulation of $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins in cerebral arteries. $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ protein levels were similar in 6 week old WKY and SHR arteries (Fig. 1C, D). Aging between 6 and 12 weeks did not alter $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ protein in WKY rat arteries, but increased these proteins ~2.1- and 1.4-fold in SHR arteries (Fig. S2). At 12 weeks of age, $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins were ~2.5- and 1.7-fold higher in SHR than age-matched WKY arteries (Fig. 1C, D). In agreement with message levels, age-dependent development of hypertension also increased $\alpha_2\delta$-1 more than $Ca_V1.2\alpha_1$ protein (Fig. 1C, D, S2).

In summary, these data indicate that genetic hypertension is associated with transcriptional upregulation of both $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ in cerebral artery myocytes. $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins are elevated more than their respective mRNAs (Fig. 1B–D, S2), suggesting that hypertension-associated changes in post-translational events also contribute to increased $Ca_V1.2$ channel subunit expression during hypertension. Furthermore, during hypertension there is a larger increase in mRNA and protein for $\alpha_2\delta$-1 than for $Ca_V1.2\alpha_1$.

Hypertension is associated with an elevation in surface $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins in arteries

$\alpha_2\delta$-1 induces membrane trafficking of $Ca_V1.2\alpha_1$ subunits in SD rat arterial myocytes.[16] Therefore, we tested the hypothesis that an increase in $\alpha_2\delta$-1 contributes to elevated surface $Ca_V1.2$ expression in hypertension. Surface (plasma membrane) and intracellular $\alpha_2\delta$-1 and

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$Ca_V1.2\alpha_1$ proteins were measured in age-matched WKY and hypertensive SHR cerebral arteries using biotinylation. Surface-localized $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins were ~2.6- and 2-fold higher, respectively, in SHR than WKY rat arteries (Fig. 2A, B). A larger percentage of total $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ was located at the plasma membrane in SHR than WKY arteries (Fig. 2A, C). In WKY arteries, more of the total amount of $\alpha_2\delta$-1 (~85 %) than $Ca_V1.2\alpha_1$ (~77%) was located at the surface. In contrast, in SHR arteries the percentage of total $\alpha_2\delta$-1 (~93 %) and $Ca_V1.2\alpha_1$ (~92 %) located at the surface were similar (Fig. 2A, C). These data indicate that during hypertension, an elevation in $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ total protein translates to an increase in surface expression of these subunits in arterial myocytes. Furthermore, hypertension is associated with an alteration in the distribution of $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins between intracellular and surface compartments.

Pregabalin reduces surface trafficking of $Ca_V1.2$ channel subunits more effectively in hypertensive than normotensive rat arteries

Pregabalin, an $\alpha_2\delta$-1/2 ligand, reduces surface trafficking of $Ca_V1.2$, 2.1, and 2.2 channels in neurons and arterial myocytes.[14, 16, 18–20] Next, we studied pregabalin regulation of $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ subunit surface expression and subunit cellular distribution in WKY and SHR cerebral arteries. For these experiments, arteries were incubated for 24 h with or without pregabalin. Pregabalin (24 h) did not alter total protein of $\alpha_2\delta$-1 (% control: WKY, 115 ± 9; SHR, 118 ± 20) or $Ca_V1.2\alpha_1$ (% control: WKY, 116 ± 10; SHR, 109 ± 14) (Fig. 3A, WKY n= 4–5, SHR n= 5, $P>0.05$ for each). In contrast, pregabalin reduced surface $\alpha_2\delta$-1 and $Ca_V1.2$ and increased intracellular levels of these proteins in both WKY and SHR arteries (Fig. 3A, B, C, S3). Pregabalin reduced plasma membrane $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ ~3.1 and 1.9-fold more, respectively, in hypertensive SHR than WKY control arteries (Fig. 3C). To evaluate pregabalin regulation of $\alpha_2\delta$-1 and $Ca_V1.2$ cellular distribution, surface:intracellular protein ratios were calculated. Consistent with data shown in figure 2C, a larger proportion of $\alpha_2\delta$-1 and $Ca_V1.2$ subunits were present at the plasma membrane in SHR than WKY arteries (Fig. 3D). Pregabalin induced a larger reduction in surface:intracellular $\alpha_2\delta$-1 and $Ca_V1.2$ in SHR than in WKY arteries (Fig. 3D).

Hypertension was associated with a larger increase in surface $\alpha_2\delta$-1 than $Ca_V1.2\alpha_1$ protein in arteries (Fig. 2A, B). We calculated the band intensity ratio of surface $\alpha_2\delta$-1 to $Ca_V1.2\alpha_1$ and regulation by pregabalin. While this methodology cannot determine subunit stoichiometry, total protein loaded in each lane is identical, allowing comparison of this ratio in SHR and WKY arteries from the same blot. The mean surface $\alpha_2\delta$-1:$Ca_V1.2\alpha_1$ band intensity ratio was ~1.38 in SHR arteries and ~1.06 in WKY arteries, or ~1.3-fold higher in SHR (Fig. 3E). Pregabalin reduced the surface $\alpha_2\delta$-1 to $Ca_V1.2\alpha_1$ band intensity ratio to ~0.91 in SHR rat arteries, but did not change the ratio in WKY rat arteries (Fig. 3E).

Collectively, these data indicate that pregabalin blocks surface expression of $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ subunits more effectively in hypertensive than normotensive rat arteries. During hypertension, surface $\alpha_2\delta$-1 protein is elevated more so than $Ca_V1.2\alpha_1$ protein, leading to an increase in the ratio of plasma membrane $\alpha_2\delta$-1 to $Ca_V1.2\alpha_1$ subunits. Pregabalin reverses this elevation in surface $\alpha_2\delta$-1 to $Ca_V1.2\alpha_1$ subunits. These data also indicate that $\alpha_2\delta$-1 is essential for upregulation of surface $Ca_V1.2$ channels in arterial myocytes during genetic hypertension.

$\alpha_2\delta$-1 targeting reverses hypertension-associated modifications in $Ca_V1.2$ current density and inactivation in arterial myocytes

To investigate the functional impact of elevated $\alpha_2\delta$-1 expression and effects of $\alpha_2\delta$-1 targeting, $Ca_V1.2$ currents were measured in age-matched WKY and hypertensive SHR cerebral artery myocytes. Mean peak $Ca_V1.2$ current density (Ba^{2+} as charge carrier) was

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~5.3 pA/pF in hypertensive SHR compared with ~2.4 pA/pF in WKY cells, or ~2.2-fold larger (Fig. 4A, B and Table 1). Pregabalin (24 h) reduced peak $Ca_V1.2$ current density in SHR cells to ~2.2 pA/pF, or by ~59 %, and to ~1.6 pA/pF in WKY cells, or by ~32 % (Fig. 4A, B and Table 1). Pregabalin reduced peak $Ca_V1.2$ current density in SHR myocytes to the current density of untreated WKY cells (Fig 4A, B and Table 1). The relationship between cell capacitance and peak $Ca_V1.2$ current was investigated (Fig. 4C). When data were fit with a linear function, the slope was −5.41 for SHR and −2.40 for WKY cells, or 2.3-fold higher (Fig. 4C). Pregabalin reduced slopes by ~58 and 25 % in SHR and WKY cells, respectively (Fig. 4C). Slopes were similar for untreated WKY and pregabalin-treated SHR cells (Fig. 4C, $P>0.05$). Mean cell capacitance for WKY (16.3 ± 0.8 pF) and SHR (SHR 17.1 ± 1.3 pF) cells were similar and were not altered by pregabalin (WKY, 18.8 ± 1 pF; SHR 15.8 ± 0.8 pF; $P>0.05$ when comparing all), indicating that current density and slope increased due to changes in $Ca_V1.2$ channels (Fig. 4B, C).

The voltage-dependence of half-maximal $Ca_V1.2$ current activation ($V_{1/2act}$) and slope (k) were similar in untreated control and pregabalin-treated WKY and SHR arterial myocytes (Fig. 4D and Table 1). The voltage-dependence of half-maximal inactivation ($V_{1/2inact}$) and k were also similar in untreated control and pregabalin-treated WKY and SHR cells (Fig. 4E and Table 1). In contrast, untreated SHR cells displayed a non-inactivating $Ca_V1.2$ current that was ~2-fold larger than in WKY cells (Fig. 4A, E). Pregabalin (24 h) reduced the non-inactivating current in SHR cells such that it was similar to WKY cells (Fig. 4A, E). $Ca_V1.2$ current inactivation rates (τ) were similar in control and pregabalin-treated WKY and SHR cells (Fig. S4).

In addition to acting as an inhibitor of $\alpha_2\delta$-1-induced $Ca_V1.2$ channel trafficking, pregabalin is a weak $Ca_V1.2$ channel pore blocker that does not directly alter $Ca_V1.2$ current voltage-dependence in normotensive SD rat arterial myocytes.[16] To determine whether the reduction in $Ca_V1.2$ current amplitude in pregabalin-treated WKY and SHR rat myocytes was due to $Ca_V1.2$ pore block, we measured $Ca_V1.2$ current regulation in untreated cells by acute bath application of pregabalin. Acute pregabalin reduced $Ca_V1.2$ currents in WKY cells by ~12 % (Fig. 4F). In contrast, pregabalin reduced $Ca_V1.2$ currents in SHR myocytes by ~23 %, or ~1.9-fold more than in WKY cells (Fig. 4F). Acute pregabalin-induced $Ca_V1.2$ current inhibition was significantly smaller than that induced by 24 h pregabalin treatment in both WKY (~32 % inhibition) and SHR (~59 % inhibition) cells (Figs. 4B, C, F). When combined with the biochemical data illustrated in Figure 3, these data indicate that acute and chronic pregabalin inhibit $Ca_V1.2$ currents through distinct mechanisms in arterial myocytes.

Collectively, data indicate that genetic hypertension is associated with an elevation in $\alpha_2\delta$-1 expression that stimulates surface expression of $Ca_V1.2\alpha_1$ subunits, leading to a $Ca_V1.2$ current elevation and an increase in non-inactivating current. $\alpha_2\delta$-1 targeting reduces the hypertension-associated $\alpha_2\delta$-1-induced elevation in $Ca_V1.2\alpha_1$ surface expression, leading to a reduction in $Ca_V1.2$ current density. $\alpha_2\delta$-1 targeting also restores $Ca_V1.2$ current inactivation.

$\alpha_2\delta$-1 targeting reverses elevated pressure- and depolarization-induced vasoconstriction in hypertension

The functional significance of hypertension-associated alterations in $\alpha_2\delta$-1 signaling was studied by measuring arterial contractility. Diameter regulation by intravascular pressure (20–100 mmHg) was measured in WKY and SHR cerebral arteries that had been incubated for 24 h with or without pregabalin. SHR arteries developed more myogenic tone than WKY arteries over the entire pressure range (Fig. 5A, B). Pregabalin reduced myogenic tone in WKY and SHR arteries, decreased tone more in SHR than in WKY arteries (e.g., % reduction in myogenic tone at 60 mmHg: SHR, ~41 %; WKY, ~28 %), and reduced tone in

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SHR arteries to levels in untreated WKY arteries (Fig. 5A, B). Nimodipine (1 μmol/L), a voltage-dependent Ca^{2+} channel blocker, fully dilated control and pregabalin-treated WKY and SHR arteries at all pressures (20–100 mmHg) studied, indicating that myogenic tone occurred due to $Ca_V1.2$ channel activation (Fig. 5B). Passive arterial diameters were similar for WKY (249 ± 11 μm) and hypertensive SHR (242 ± 9μm) cerebral arteries (values given at 60 mmHg, n=10 for each, P>0.05).

Elevating extracellular K^+ induces depolarization, activation of voltage-gated Ca^{2+} channels, Ca^{2+} influx and vasoconstriction.[4] As an alternative approach to investigate the functional impact of $\alpha_2\delta$-1 targeting, we studied K^+-induced vasoconstriction in WKY and SHR arteries. Increasing extracellular K^+ from 6 to 20, 40, or 60 mmol/L induced graded vasoconstriction that was larger in SHR than WKY cerebral arteries (Fig. 6A, B). Pregabalin reduced K^+-induced vasoconstriction more in SHR than WKY arteries (Fig. 6A, B). For example, pregabalin reduced the mean 60 mmol/L K^+-induced constriction by ~54 % in SHR and ~37 % in WKY (Fig. 6B). These data indicate that $\alpha_2\delta$-1 targeting reduces pressure- and depolarization-induced vasoconstriction more effectively in hypertensive SHR than in control WKY rat arteries.

Discussion

To date, no studies have investigated involvement of $Ca_V1.2$ channel auxiliary subunits in the pathological elevation of arterial myocyte $Ca_V1.2$ currents and vasoconstriction in hypertension. Here, we demonstrate for the first time that genetic hypertension is associated with transcriptional and post-translational upregulation of $\alpha_2\delta$-1 subunits in myocytes of resistance-size arteries. The additional $\alpha_2\delta$-1 subunits increase surface trafficking of $Ca_V1.2\alpha_1$ subunits, which are also elevated in hypertension. The consequent increase in surface $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins elevates $Ca_V1.2$ current density and generates a non-inactivating current, leading to vasoconstriction. We also demonstrate that $\alpha_2\delta$-1 targeting normalizes myocyte $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ surface expression, re-establishes $Ca_V1.2$ current density and inactivation, and reduces hypertensive rat artery contractility to levels in controls. These data indicate that $\alpha_2\delta$-1 elevates $Ca_V1.2$ currents and $Ca_V1.2$-dependent vasoconstriction during hypertension and demonstrate that $\alpha_2\delta$-1 targeting is a viable therapeutic strategy to reverse these pathological alterations and induce cerebrovascular dilation.

Our data indicate that the development of genetic hypertension is associated with a transcriptional and post-translational increase in $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ in arterial myocytes. In contrast, other $\alpha_2\delta$ isoforms did not emerge during hypertension, an alteration that could have contributed to pathological $Ca_V1.2$ current modifications. Previous studies have described that $Ca_V1.2\alpha_1$ mRNA and protein is higher in mesenteric arteries and aorta of hypertensive SHR than WKY rat controls.[21, 22] In contrast, angiotensin II- and hypoxia-induced hypertension did not alter $Ca_V1.2\alpha_1$ mRNA, but elevated $Ca_V1.2\alpha_1$ protein in cultured mesenteric arteries and neonatal piglet pulmonary arteries.[23, 24] These findings lead to the proposal that hypertension may not be associated with an increase in $Ca_V1.2\alpha_1$ message, but post-translational upregulation of $Ca_V1.2\alpha_1$ protein.[21–24] Here, we used both age-dependent development of hypertension in SHR and comparison to WKY rat controls to investigate relative changes in $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ mRNA and protein. Our data indicate that the increase in $\alpha_2\delta$-1 (~2.1-fold) and $Ca_V1.2\alpha_1$ (~1.5-fold) mRNA cannot fully account for the elevation in $\alpha_2\delta$-1 (~2.5-fold) and $Ca_V1.2\alpha_1$ (~1.7-fold) proteins during hypertension. These data indicate that both transcriptional and post-translational mechanisms elevate $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins in cerebral artery myocytes during hypertension.

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Using a novel application of biotinylation, we recently determined the surface to intracellular distribution of arterial $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins in normotensive rats.[16] Essentially all (>95 %) $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins locate to the surface in cerebral artery myocytes of normotensive SD rats.[16] Here, a smaller percentage of total $\alpha_2\delta$-1 (~85%) and $Ca_V1.2\alpha_1$ (~77 %) was located in the plasma membrane in WKY rat arteries. Explanations for slight differences in $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ distribution between SD and WKY rats include the different rat strains and animal age (7 weeks in ref. [16] vs 12 weeks here). To determine the cellular distribution of $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ subunit proteins in SHR and WKY cerebral arteries, we compared the percentage of total protein expressed at the surface and the surface:intracellular protein ratio in both SHR and WKY cerebral arteries. Both of these analysis methods indicate that a higher proportion of $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ is located at the plasma membrane in hypertensive rat arteries than in controls. The net result of both the transcription and translational increase in $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ protein and higher relative surface expression elevates plasma membrane levels of these proteins. Our data also indicate that there is a fractional shift in surface $\alpha_2\delta$-1:$Ca_V1.2\alpha_1$ during hypertension, a change that occurs due to a larger elevation in surface $\alpha_2\delta$-1 than $Ca_V1.2\alpha_1$. These results provide evidence that an elevation in $\alpha_2\delta$-1 to $Ca_V1.2\alpha_1$ subunit ratio can modify native $Ca_V1.2$ current properties and that there may not be rigid $\alpha_2\delta$-1:$Ca_V1.2\alpha_1$ subunit stoichiometry in arterial myocytes. Also possible is that in normotension, a proportion of arterial myocyte $Ca_V1.2$ channel complexes may not contain $\alpha_2\delta$-1 subunits. During hypertension, the higher elevation in surface $\alpha_2\delta$-1 than $Ca_V1.2\alpha_1$ may increase the proportion of channels that contain $\alpha_2\delta$-1. Future studies should be designed to further investigate native $Ca_V1.2$ channel stoichiometry in arterial myocytes and changes that occur in cardiovascular disease. Collectively, these results indicate that $\alpha_2\delta$-1 increases surface expression and functionality of $Ca_V1.2\alpha_1$ subunits in arterial myocytes during hypertension.

Pregabalin is a gabapentinoid drug used to treat neuropathic pain, fibromyalgia, and epileptic seizures.[25, 26] Of all four $\alpha_2\delta$ isoforms, only $\alpha_2\delta$-1 and -2 contain complete metal ion adhesion site and RRR motifs which are required for gabapentanoid drug binding.[14, 20] Gabapanetin reduced $\alpha_2\delta$ subunit recycling from Rab-11-positive recycling endosomes.[27] Pregabalin also reduced surface expression of both $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins in cerebral artery myocytes of normotensive SD rats.[16] Here, pregabalin did not alter total $\alpha_2\delta$-1 or $Ca_V1.2\alpha_1$ protein. Rather, pregabalin reduced surface $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ more in hypertensive arteries than in control rat arteries, essentially normalizing surface levels of these proteins to those in WKY. Pregabalin also reduced the $\alpha_2\delta$-1 to $Ca_V1.2\alpha_1$ subunit ratio in hypertensive rat arteries to that in WKY controls. Given that pregabalin normalized elevated $Ca_V1.2$ current density and the proportion of non-inactivating current to those in WKY cells, our data indicate that upregulated $\alpha_2\delta$-1 functionality contributes to the increase in $Ca_V1.2$ currents in arterial myocytes in hypertension.

Voltage-dependent Ca^{2+} currents are elevated in myocytes from vasculature including renal, cerebral and mesenteric arteries, when studying a variety of different hypertension models such as SHR, angiotensin II-induced, aortic banding, stroke-prone SHR, hypoxia-induced pulmonary hypertension, and Osborne-Mendel rats on a high fat diet.[2, 3, 7, 9, 21, 23, 24, 28–30] $Ca_V1.2$ current density measured here is consistent with that previously reported in cerebral artery myocytes when using WKY and SHR rat models.[2, 30] Our data indicate that $Ca_V1.2$ current density was ~2.2-fold larger in hypertensive than control rat arterial myocytes. In contrast, $Ca_V1.2$ current $V_{1/2act}$ and $V_{1/2inact}$ were similar in WKY and SHR cells, consistent with previous reports.[2, 9, 28, 30] A non-inactivating $Ca_V1.2$ current in myocytes of hypertensive rats was double that in controls, a modification that would significantly increase Ca^{2+} influx at steady-state membrane potentials, thereby stimulating vasoconstriction. Pregabalin (24 h) reduced elevated $Ca_V1.2$ current density and non-inactivating current to levels in controls, suggesting that these pathological modifications

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occurred due to an increase in $\alpha_2\delta$-1 surface expression. Our data are consistent with pregabalin acting as both a weak $Ca_V1.2$ channel pore blocker and an effective chronic inhibitor of $\alpha_2\delta$-1 surface expression in hypertensive rat arterial myocytes. In our previous study, acute pregabalin reduced $Ca_V1.2$ currents by ~33 % in SD rat cerebral artery myocytes.[16] Here, the same acute pregabalin concentration reduced $Ca_V1.2$ currents by ~12 % in WKY rat myocytes. Our previous study used 7 week old SD rats, whereas here acute pregabalin effects were measured in 12 week old WKY rat myocytes. Our data indicate that $Ca_V1.2$ channel properties in 12 week old WKY myocytes are not identical to those in 7 week old SD rat myocytes, including the percentage of $Ca_V1.2\alpha_1$ protein that is located at the surface. Data here indicate that acute pregabalin is a more effective inhibitor of $Ca_V1.2$ currents in SHR than WKY myocytes. This may be due to the higher number of surface $\alpha_2\delta$-1 subunits and the higher $\alpha_2\delta$-1:$Ca_V1.2$ ratio in SHR myocytes. Acute gabapentin also inhibited voltage-dependent Ca^{2+} currents in pyramidal neocortical cells, but did not alter currents generated by recombinant $Ca_V2.1$ channels or endogenous Ca^{2+} channels in dorsal root ganglia neurons.[31, 32] In a model of neuropathic pain in which $\alpha_2\delta$-1 is upregulated, chronic pregabalin inhibited $\alpha_2\delta$-1 trafficking to pre-synaptic terminals, thereby inhibiting Ca^{2+} channel function.[19] Our data indicate that chronic pregabalin inhibits $\alpha_2\delta$-1-induced trafficking of $Ca_V1.2\alpha_1$ channel subunits, thereby reducing $Ca_V1.2$ currents in arterial myocytes during hypertension.

Intravascular pressure and depolarization both stimulated a larger vasoconstriction in arteries of hypertensive rats than in controls. Consistent with our findings, pressure-induced Ca^{2+} influx and associated vasoconstriction were larger in arteries from animal models with both genetic- and induced-hypertension.[7, 21, 23, 24] We show that nimodipine abolished myogenic tone at all pressures, indicating that $Ca_V1.2$ channel activity was essential to generate tone in hypertensive and control rat arteries. Chronic pregabalin (24 h) was a more effective vasodilator of hypertensive than control rat arteries, effectively reversing the pathological vasoconstriction. Pregabalin is also a weak $Ca_V1.2$ channel pore blocker, which induces a small vasodilation.[16] Thus, pregabalin-induced $Ca_V1.2$ pore block may also have contributed to vasodilation in both WKY and SHR arteries. Although unlikely, pregabalin could have caused vasodilation through additional mechanisms, including through inducing membrane hyperpolarization. Our data are inconsistent with this possibility as pregabalin similarly reduced surface $Ca_V1.2$ subunits, $Ca_V1.2$ currents, myogenic tone and K^+-induced constriction and inhibition of pressure- and depolarization-induced vasoconstriction were equivalent. Thus, data demonstrate that pregabalin dilates hypertensive rat arteries primarily by reducing surface expression of $Ca_V1.2$ subunits in myocytes.

Hypertension is associated with increased risk for cerebral diseases, including stroke, Alzheimer's disease, and dementia. Cerebral blood flow is reduced in hypertensive humans and 12 week old SHR rats, when compared to normotensive controls.[33, 34] Voltage-dependent Ca^{2+} channel blockers have been used for over two decades to treat hypertension.[35] However, Ca^{2+} channel blockers inhibit $Ca_V1.2$ channels in multiple cell types *in vivo* and induce multiple side effects, including sweating, edema, and nausea.[35, 36] Therefore, the development of alternative approaches to target $Ca_V1.2$ channels in arterial myocytes could provide significant benefits over current inhibitors. Here, we used pregabalin, as an *in vitro* tool to test the concept that $\alpha_2\delta$-1 targeting induces vasodilation in cerebral arteries of hypertensive animals. Data here provide a foundation for future studies aimed at developing novel approaches to target $\alpha_2\delta$-1 in arterial myocytes. All data in our study were obtained by studying cerebral arteries that regulate brain regional blood flow but do not control systemic blood pressure. Clinical pregabalin does not appear to modify systemic blood pressure in normotensive humans at doses used to treat neuropathic pain, fibromyalgia, and epileptic seizures.[26] There are several explanations for this observation. First, there are a large number of distinct mechanisms that control cerebral and systemic

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artery contractility. To date, no studies have examined the molecular identity or physiological functions of $\alpha_2\delta$ subunits in systemic artery myocytes that regulate diastolic and systolic blood pressure. $\alpha_2\delta$-1 may not be the principal $\alpha_2\delta$ isoform, or $\alpha_2\delta$ subunits may not regulate $Ca_V1.2$ channel activity in systemic artery myocytes. Pregabalin is an $\alpha_2\delta$-1/2 ligand. If $\alpha_2\delta$-1 or $\alpha_2\delta$-2 are not expressed or do not regulate $Ca_V1.2$ channels in systemic artery myocytes, pregabalin should not induce systemic vasodilation or alter blood pressure. Second, clinical doses of pregabalin that are used to treat neuropathic pain, fibromyalgia, and epileptic seizures may be insufficient to induce vasodilation *in vivo*. Gabapentin, a lower affinity pregabalin analogue, enters cells through system-L neutral amino acid transporters.[32] Arterial myocytes may not uptake pregabalin as effectively as neurons. *In vivo*, intracellular myocyte pregabalin concentrations may be less than those obtained *in vitro* that alter $Ca_V1.2$ function. Third, many *in vivo* mechanisms, including those mediated by baroreceptors or the renin-angiotensin sytem, may compensate for pregabalin-induced systemic vasodilation, leading to no net change in blood pressure. Fourth, our data indicate that pregabalin is more effective at inhibiting $Ca_V1.2\alpha_1$ subunit trafficking in cerebral artery myocytes of hypertensive than normotensive rats. *In vivo*, pregabalin may be a more effective vasodilator in hypertensive subjects and have a smaller effect in normotensive subjects in which clinical systemic blood pressure measurements have been obtained. Our study provides the first evidence that arterial myocyte $\alpha_2\delta$-1 functionality is upregulated in hypertension and that $\alpha_2\delta$-1 targeting is a novel approach for reducing pathological vasoconstriction in hypertension. Data also indicate that $\alpha_2\delta$-1 targeting can modify cerebral artery contractility, setting the stage for future studies to use a variety of other $\alpha_2\delta$-1 targeting strategies, including RNA interference and genetic models, to investigate physiological and pathological involvement of $\alpha_2\delta$ subunits in arteries of other vascular beds and *in vivo*.

In summary, we identify for the first time that a hypertension-associated increase in $\alpha_2\delta$-1 elevates $Ca_V1.2\alpha_1$ surface expression in arterial myocytes leading to pressure- and depolarization-induced vasoconstriction. Our data also indicate that $\alpha_2\delta$-1 targeting is a novel approach to reverse elevated $Ca_V1.2$ channel surface expression in arterial myocytes and vasoconstriction in hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Marie Dennis Leo for critical reading of the manuscript.

Funding Sources

This work was supported by NIH grants to J.H.J.

Non-standard Abbreviations and Acronyms

None

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Perspectives

A hallmark of hypertension is an increase in voltage-dependent $Ca_V1.2$ currents in arterial myocytes that induces vasoconstriction.[1–3] Molecular mechanisms that elevate arterial myocyte $Ca_V1.2$ currents in hypertension and the significance of auxiliary subunits in this pathological alteration are unclear. We show that the development of genetic hypertension is associated with a transcriptional and post-translational upregulation of $\alpha_2\delta$-1 subunits in cerebral artery myocytes. This increase in $\alpha_2\delta$-1 subunits elevates $Ca_V1.2$ channel surface expression, $Ca_V1.2$ current density, and vasoconstriction. $\alpha_2\delta$-1 targeting using pregabalin, an $\alpha_2\delta$-1 ligand, reduced $\alpha_2\delta$-1 and $Ca_V1.2$ surface expression and $Ca_V1.2$ current density in myocytes. Pregabalin also dilated cerebral arteries of hypertensive rats. Our study provides the first evidence that $\alpha_2\delta$-1 subunits are upregulated in cerebral artery myocytes during hypertension and contribute to the pathological elevation in myocyte $Ca_V1.2$ currents and vasoconstriction. We also identify $\alpha_2\delta$-1 as a potential novel therapeutic target for inducing cerebrovascular dilation in hypertension.

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Novelty and Significance

1) What is new?

- We demonstrate for the first time that genetic hypertension is associated with a transcriptional and post-translational upregulation of $\alpha_2\delta$-1 subunits in myocytes of resistance-size cerebral arteries that increase $Ca_V1.2\alpha_1$ subunit surface trafficking, thereby elevating $Ca_V1.2$ current density and arterial contractility.
- Pharmacological targeting of $\alpha_2\delta$-1 inhibits the pathological increase in $Ca_V1.2$ current density and cerebral artery contractility during hypertension. This study identifies $\alpha_2\delta$-1 as a novel therapeutic target for inducing cerebrovascular vasodilation in hypertension.

2) What Is Relevant?

- Upregulation of $\alpha_2\delta$-1 subunits is essential for the elevation in $Ca_V1.2$ current density and cerebrovascular tone in genetic hypertension.
- Pharmacological targeting of $\alpha_2\delta$-1 can reverse the pathological elevation in surface $Ca_V1.2$ channels, $Ca_V1.2$ current density and vasoconstriction in cerebral artery myocytes.

3) Summary

- Upregulation of $\alpha_2\delta$-1 subunits during genetic hypertension increases $Ca_V1.2$ channel surface expression and $Ca_V1.2$ current density, leading to vasoconstriction. $\alpha_2\delta$-1 targeting reverses this pathological increase in $Ca_V1.2$ channel surface expression, $Ca_V1.2$ current density and contractility in cerebral arteries.

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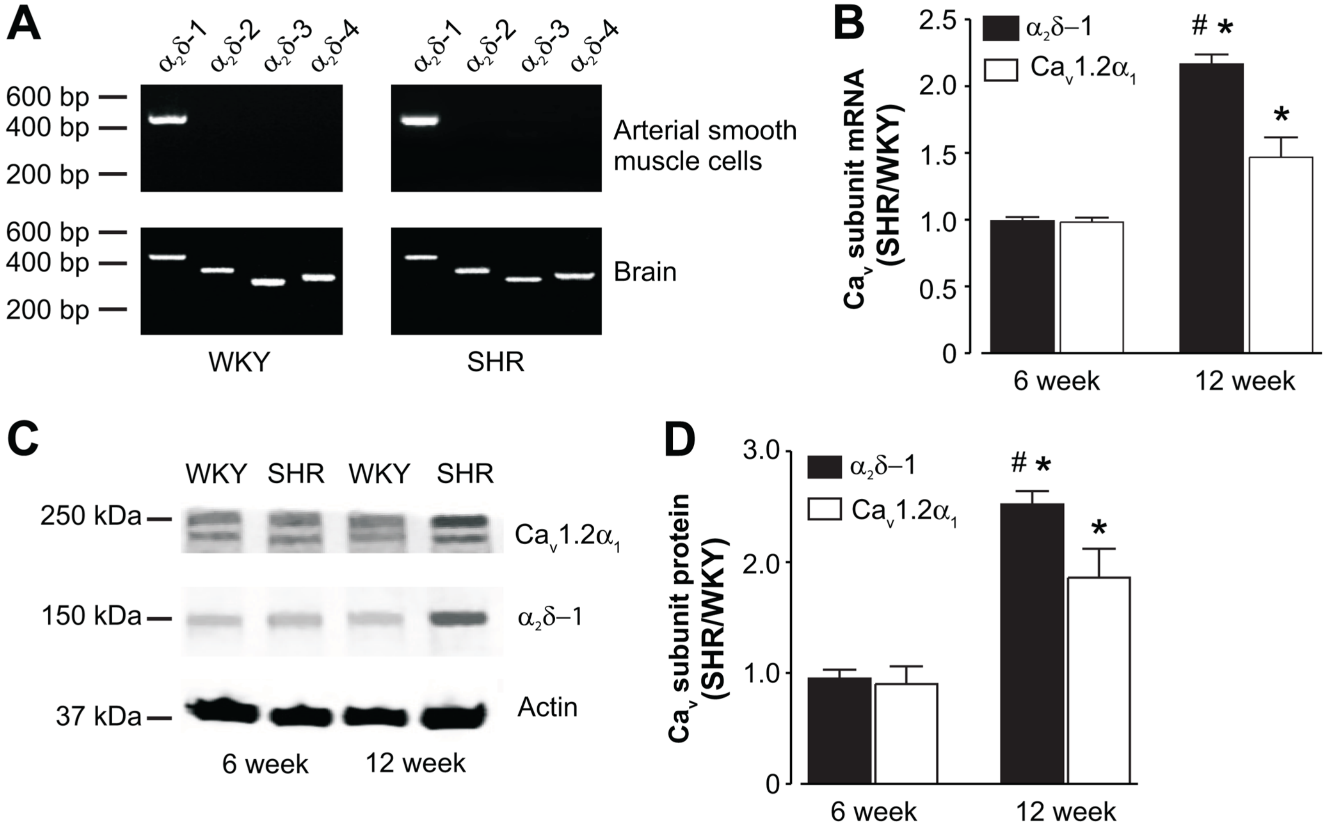

Figure 1.

$\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ subunit mRNA and protein are elevated in hypertension. A, Representative gel (of 3 experiments) illustrating RT-PCR amplification of transcripts for $\alpha_2\delta$-1 through -4 in isolated arterial myocytes and whole brain of 12 week old WKY and SHR rats. B, Mean quantitative PCR data for $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ mRNA in 6 (n=8 for each) and 12 (n=6 for each) week old SHR rat arteries normalized to Rps5 and then to age-matched WKY controls. C, Exemplar Western blot illustrating $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ protein from 6 and 12 week old WKY and SHR rat whole arterial lysate. Blots were physically cut at 75 kDa to allow probing for actin and $\alpha_2\delta$-1/$Ca_V1.2\alpha_1$. D, Mean data illustrating that $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins are elevated in hypertension. * indicates $P<0.05$ when compared with the respective mRNA/protein at 6 weeks, # indicates $P<0.05$ when compared with $Ca_V1.2\alpha_1$ mRNA/protein at 12 weeks (n=8 for each protein at each age).

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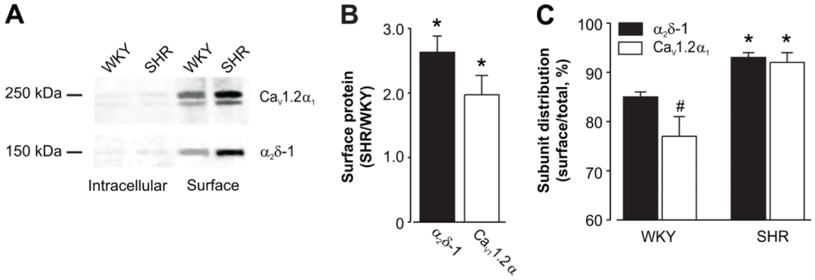

Figure 2.

Arterial surface $\alpha_2\delta$-1 and Ca$_V$1.2α_1 subunits are elevated in hypertension. A, Representative Western blot illustrating increased surface expression of both $\alpha_2\delta$-1 and Ca$_V$1.2α_1 proteins in 12 week old WKY and SHR arteries. Blot was physically cut at 75 kDa to allow probing for $\alpha_2\delta$-1 and Ca$_V$1.2α_1. B, Mean data illustrate that surface levels of $\alpha_2\delta$-1 and Ca$_V$1.2α_1 subunits are higher in arteries during hypertension. C, Mean data illustrating the percentage of total (surface + intracellular) $\alpha_2\delta$-1 and Ca$_V$1.2α_1 proteins located at the surface * indicates $P<0.05$ versus same protein in age-matched WKY rat arteries, # indicates $P<0.05$ when compared with WKY $\alpha_2\delta$-1 (n=5 – 9 each for WKY and SHR).

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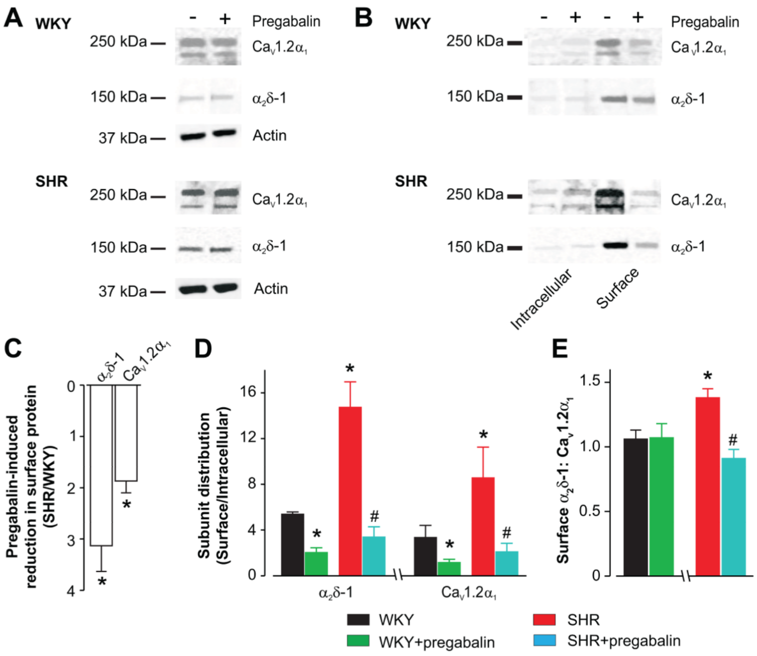

Figure 3.

Pregabalin reduces surface expression of $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ channel proteins more effectively in arteries of hypertensive rats than in controls. A, Representative Western blot illustrating that pregabalin does not change total (whole arterial) $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins in WKY and SHR arteries. B, Representative Western blots illustrating pregabalin (24 h)-induced changes in surface and intracellular $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins. Blots were cut at 75 kDa to allow probing for $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$. C, Pregabalin reduced surface $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ proteins more in SHR than WKY arteries. D, Mean data illustrating $\alpha_2\delta$-1 and $Ca_V1.2\alpha_1$ subunit distribution in WKY and SHR arteries and regulation by pregabalin. E, Surface $\alpha_2\delta$-1 to $Ca_V1.2\alpha_1$ and modulation by pregabalin. Pregabalin concentration in all figures was 100 μmol/L. * indicates $P<0.05$ compared with untreated WKY and # indicates $P<0.05$ versus untreated SHR rat arteries (n=4–5 each for untreated and pregabalin-treated WKY and SHR).

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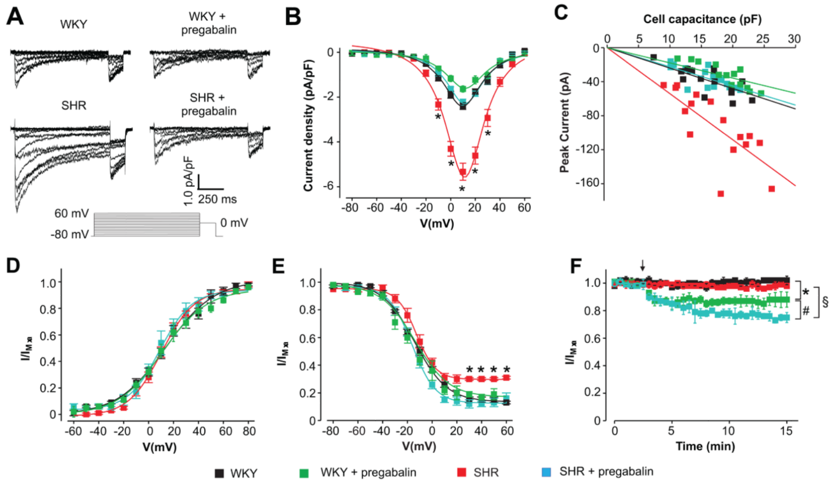

Figure 4.

Pregabalin reverses elevated Ca$_V$1.2 currents in hypertensive rat arterial smooth muscle cells. A, Representative Ca$_V$1.2 current density recordings from control and pregabalin-treated WKY and SHR arterial smooth muscle cells (10 mmol/L Ba^{2+} as charge carrier). B, Mean current density-voltage relationships of WKY (n=17), pregabalin-treated WKY (n=13), SHR (n=16) and pregabalin-treated SHR (n=18) cells. C, Scatter plot with linear fit for peak Ca$_V$1.2 current versus cell capacitance in WKY (n=17), pregabalin-treated WKY (n=13), SHR (n=16) and pregabalin-treated SHR (n=18) cells. WKY: slope=−2.40, r=−0.76, p=3.3×10-4. WKY+pregabalin: slope=−1.79, r=−0.90, p=7.5×10-4. SHR: slope=−5.41, r=−0.77, p=3.5×10-4. SHR+pregabalin: slope=−2.25, r=−0.72, p=1.1×10-4. D, Voltage-dependent Ca$_V$1.2 current activation in WKY (n=13), pregabalin-treated WKY (n=9), SHR (n=12) and pregabalin-treated SHR (n=6) cells. E, Voltage-dependent current inactivation of WKY (n=17), pregabalin-treated WKY (n=13), SHR (n=16) and pregabalin-treated SHR (n=18) cells. *indicates significance from WKY at indicated potentials ($P<0.05$). F, Graph illustrating the time course of Ca$_V$1.2 currents (at +20 mV) and inhibition by acute pregabalin (100 μmol/L). WKY (control n=8, pregabalin n=9), SHR (control n=14, pregabalin n=6) cells. The arrow (not applicable for controls) indicates where pregabalin was added. Pregabalin concentration in all figures was 100 μmol/L. * indicates $P<0.05$ when compared to untreated WKY, # indicates $P<0.05$ when compared to pregabalin-treated WKY and § indicates $P<0.05$ when compared to untreated SHR.

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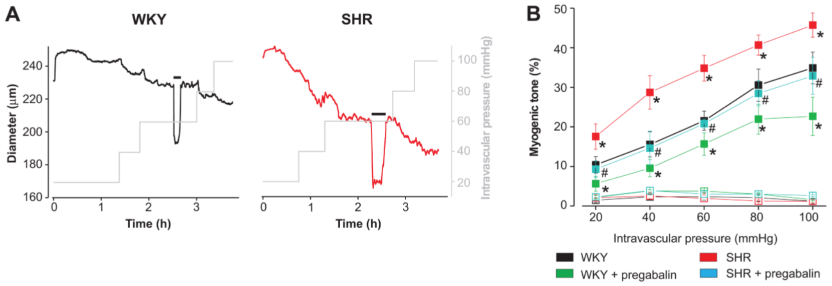

Figure 5.

Pregabalin reverses elevated pressure-induced vasoconstriction in hypertension. A, Representative traces illustrating steady-state myogenic tone in response to increasing intravascular pressure in a WKY (black) and SHR (red) artery. Horizontal black bars indicate an increase in bath K^+ from 6 to 60 mmol/L. B Pregabalin (24 h, 100 µmol/L) reduced pressure (20 – 100 mmHg)-induced myogenic tone (filled symbols) more so in arteries from hypertensive rats than in controls. Myogenic tone was abolished by nimodipine (1 µmol/L, empty symbols). Mean data (n: WKY, 6–10; WKY + pregabalin, 6–9; SHR, 6–10; SHR+pregabalin, 6–7). * indicates P<0.05 when compared with untreated WKY and # indicates P<0.05 for SHR+pregabalin when compared with untreated SHR.

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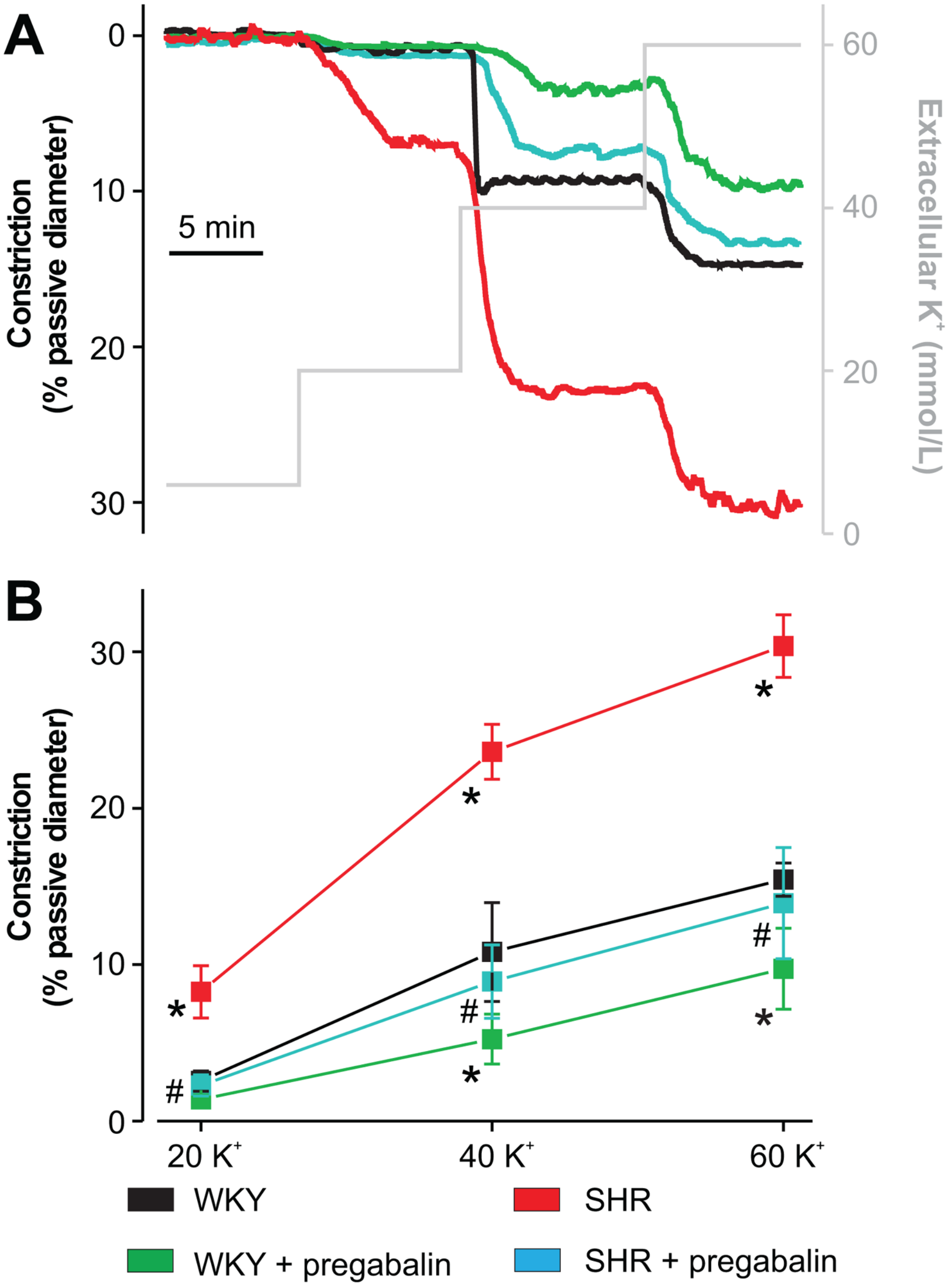

Figure 6.

Pregabalin reverses elevated depolarization-induced vasoconstriction in hypertension. A, Representative traces illustrating diameter responses to increasing extracellular K^+. B, Pregabalin (24 h, 100 μmol/L) reduced depolarization (20, 40, and 60 mmol/L K^+)-induced vasoconstriction more so in arteries from hypertensive rats than controls. Mean data (n: WKY, 6; WKY + pregabalin, 6; SHR, 6; SHR + pregabalin, 6). * indicates $P<0.05$ when compared with untreated WKY, and # indicates $P<0.05$ for SHR + pregabalin when compared with untreated SHR.

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Table 1

Properties of arterial myocyte $Ca_V1.2$ currents. Numbers in parentheses indicate experimental number.

	WKY	**WKY + pregabalin**	**SHR**	**SHR + pregabalin**
IV relationship				
Peak current density (pA/pF)	2.40±0.15 (17)	1.65±0.13 (13) *	5.33±0.38 (16) $^{*,\#}$	2.23±0.14 (16)
Peak voltage (mV)	10.25±0.74 (17)	12.31±1.45 (13)	11.09±1.03 (16)	11.26±0.89 (18)
Voltage-dependent activation				
$V_{1/2act}$ (mV)	9.24±1.88 (12)	10.70±3.72 (9)	11.62±1.80 (12)	11.18±3.29 (5)
Slope	13.09±1.55 (12)	14.56±1.66 (9)	12.74±2.84 (12)	10.51±1.09 (5)
Voltage-dependent inactivation				
$V_{1/2inact}$ (mV)	−14.23±1.57 (8)	−17.20±1.28 (13)	−11.33±1.90 (16)	−14.71±1.15 (11)
Slope	6.44±0.76 (8)	8.58±1.16 (13)	7.86±0.70 (16)	8.09±0.69 (11)

*indicates P<0.05 compared to WKY and

$^\#$ indicates P<0.05 versus SHR + pregabalin.