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Mechanical stretch increases L-type calcium channel stability in cardiomyocytes through a polycystin-1/AKT-dependent mechanism

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ABSTRACT

The L-type calcium channel (LTCC) is an important determinant of cardiac contractility. Therefore, changes in LTCC activity or protein levels could be expected to affect cardiac function. Several studies describing LTCC regulation are available, but only a few examine LTCC protein stability. Polycystin-1 (PC1) is a mechanosensor that regulates heart contractility and is involved in mechanical stretch-induced cardiac hypertrophy. PC1 was originally described as an unconventional Gi/o protein-coupled receptor in renal cells. We recently reported that PC1 regulates LTCC stability in cardiomyocytes under stress; however, the mechanism underlying this effect remains unknown. Here, we use cultured neonatal rat ventricular myocytes and hypo-osmotic stress (HS) to model mechanical stretch. The model shows that the Cavβ2 subunit is necessary for LTCC stabilization in cardiomyocytes during mechanical stretch, acting through an AKT-dependent mechanism. Our data also shows that AKT activation depends on the G protein-coupled receptor activity of PC1, specifically its G protein-binding domain, and the associated Gβγ subunit of a heterotrimeric Gi/o protein. In fact, over-expression of the human PC1 C-terminal mutant lacking the G protein-binding domain blunted the AKT activation-induced increase in Cav1.2 protein in cardiomyocytes. These findings provide novel evidence that PC1 is involved in the regulation of cardiac LTCCs through a Giβγ-AKT-Cavβ2 pathway, suggesting a new mechanism for regulation of cardiac function.

1. Introduction

Every heartbeat is produced by an action potential that causes the opening of the L-type calcium channel (LTCC). The ensuing calcium current triggers a massive release of calcium from the sarcoplasmic reticulum via ryanodine receptors, inducing contraction [1]. In addition to this critical role in excitation-contraction coupling, calcium controls several other aspects of cardiac function such as action potential duration, mitochondrial metabolism and transcriptional gene expression that regulates normal and pathologic cell growth [1,2].

LTCCs are members of the high-voltage calcium channel family, localized at the plasma membrane. In the heart, LTCCs are heteromeric complexes of three subunits named Cav1.2, Cavβ and Cavα2δ. The Cav1.2 subunit is a transmembrane protein that harbors both the channel pore and voltage sensor [3–5]. The Cavβ subunit is intracellular and binds to a cytoplasmic loop of the Cav1.2 subunit. Cavα2δ is a heterodimer bound together by disulfide bridges [3,6,7]. The Cavβ2 and Cavα2δ subunits are involved in trafficking, anchorage and regulation of the gating properties of the LTCC complex [4,5,8].

After transcription and synthesis, LTCC subunits are assembled into a complex and transported by vesicles to the plasma membrane to exert their function [5,8]. Cav1.2 protein density in the plasma membrane is a crucial determinant for calcium influx into cardiomyocytes, but regulation of Cav1.2 levels is not fully understood. When not directed to the plasma membrane, Cav1.2 is degraded by the proteasome [9,10]. In neonatal cardiomyocytes, IGF-1 induces AKT-dependent Cavβ2 phosphorylation, inhibiting its degradation and inducing its translocation to the plasma membrane. The resulting increase in LTCC protein density enhances calcium entry and contractility [11].

Polycystin-1 (PC1) is a large mechanosensing protein with 11 transmembrane domains, a large extracellular N-terminus and a relatively short intracellular C-terminus that contains a G-protein binding domain (Gi/o), a coiled-coil domain, and phosphorylation sites [12–17]. PC1 is expressed in many cells, including cardiomyocytes

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[18–20]. PC1 modulates calcium channel proteins such as polycystin-2 and stromal interaction molecule 1 (STIM1), and therefore intracellular Ca^{2+} levels [20,21], as well as other signaling pathways such as calcineurin/NFAT, Wnt, STAT6, mTOR and AKT [14,18,21–23]. In addition, the C-terminal tail of PC1 binds and activates a heterotrimeric Gi/o protein to further activate the PI3K/AKT pathway in renal epithelial cell lines [22,24].

It has been recently shown that cardiomyocyte-specific PC1 knockout mice exhibit decreased heart contractility and low LTCC protein levels. Moreover, these mice fail to develop hypertrophy after pressure overload by transverse aortic constriction [19]. In addition, cultured neonatal rat ventricular myocytes subjected to cyclic mechanical stretch or stretch induced by hypo-osmotic stress (HS) show increased LTCC content attributable to Cav1.2 stabilization through a PC1-dependent mechanism [19]. However, the mechanism by which PC1 regulates LTCC protein levels in cardiomyocytes is unknown.

We investigated the mechanism whereby PC1 regulates LTCC content in neonatal rat ventricular myocytes (NRVM) submitted to HS as a mechanical stretch model. We found that PC1 induced an increase in Cav1.2 content through a Gi/o(βγ)-dependent AKT pathway.

2. Materials and methods

2.1. Animals

All experiments adhered to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication, 8th Edition, 2011) and was approved by the Institutional Ethics Review Committees of the Universidad de Chile. We used Sprague-Dawley rat pups on postnatal day 1 − 3 to isolate neonatal rat ventricular myocytes (NRVMs).

2.2. NRVM culture, transfections and stimuli

NRVM cultures were developed as described previously [19]. Briefly, NRVMs were obtained by digestion with pancreatin (1 μg/mL, Sigma-Aldrich), and cells were pre-plated to separate myocytes from fibroblasts. NRVMs were cultured for 24 h in Dulbecco's modified Eagle's medium (DMEM, Cyclone)/Medium 199 (M199, HyClone), 3:1, containing 5% fetal bovine serum (FBS, HyClone), 10% donor equine serum (HyClone), 100 mmol/L bromodeoxyuridine (Sigma-Aldrich) and penicillin-streptomycin solution (100 U/mL − 100 μg/mL, HyClone).

To generate PC1 knockdown NRVMs, cells were transfected with a siRNA specific to PC1 (siPC1, 120 nmol/L, Sigma-Aldrich), as described previously [19]. To obtain voltage-dependent calcium channel β2 subunit knockdown NRVMs, we used a siRNA from Qiagen (siCavβ2, 120 nmol/L). To over-express the human full-length, membrane-anchored PC1 C-terminus (FLM-PC1) or the mutated G protein-binding domain-deficient PC1 C-terminus (CTM-PC1), NRVMs were transduced with the corresponding adenovirus at a multiplicity of infection of 20 plaque-forming units per cell, 36 h after plating. FLM-PC1 and CTM-PC1 were a gift from Thomas Weimbs, corresponding to Addgene plasmids #41567 and #41573, respectively. Empty vector (cytomegalovirus (CMV)) was used as a control for transduction. For adenovirus-mediated overexpression of the Giβγ inhibitor (βARkct) cells were transduced at a multiplicity of infection of 300,000 plaque-forming units per cell. LacZ adenovirus was used as a control for transduction. Experiments were performed 16 h after protein knockdown or adenoviral transduction. To model mechanical stretch, NRVMs were exposed to 50% hypo-osmotic solution for 2 h, as described previously [19,25]. AKT inhibitor VIII (10 μmol/L), pertussis toxin (1.0 μg/mL) and G-protein βγ binding peptide (mSIRK, 10 μmol/L) were obtained from Calbiochem and used 20 min before and during stimulation.

2.3. Protein extraction and Western blot analysis

Proteins were obtained from NRVMs with cold T-PER buffer (Thermo Scientific) in the presence of a protease and phosphatase inhibitor cocktail (Roche Diagnostics). Proteins were separated by SDS-PAGE (8% gels), transferred to PVDF (Millipore Corp) membrane and immunoblotted. The primary antibodies used were: anti-Cav1.2, anti-GRK2 and anti-PC1 (Santa Cruz Biotechnology), anti-AKT-pSer473, anti-AKT-pThr308 and anti-AKT total (Cell Signaling Technology), anti-Cavβ2 (Abcam) and anti-GAPDH (Sigma-Aldrich Corp., St Louis, MO). After incubation with the appropriate secondary antibody, the antigen-antibody reaction was detected by ECL (Amersham Biosciences), and blots were quantified using Image Lab software. Results were normalized to GADPH.

2.4. RNA isolation and qRT-PCR

NRVMs were homogenized in the presence of TRIzol (Bio-Rad). Chloroform was added 1:1 *v*/v and samples centrifuged at 10,000 x g for 10 min at 4 °C. The aqueous phase was obtained and RNA was precipitated in the presence of isopropanol. RNA was reconstituted in DNase/RNase-free ddH_2O. 150 ng RNA from each sample were collected for RT using the iScript cDNA synthesis kit (Bio-Rad). cDNA was diluted 10-fold with ddH_2O and used for quantitative PCR analysis (Roche). Primers used for RT-PCR were as described previously [19]. A ΔCt method was used to calculate relative transcript abundance.

2.5. Measurement of intracellular calcium

Intracellular calcium (Ca^{2+}) signals were assessed optically using the Ca^{2+} probe Fluo-4 as described [26]. In brief, the cells were loaded with 2 μmol/L of Fluo-4 AM (Invitrogen) for 25 min at 37 °C in Tyrode solution, containing (in mmol/L): NaCl 137, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 10, and HEPES 25 adjusted to pH 7.4. Fluorescence was measured in an AxioCam MRm (Carl Zeiss) coupled to a Carl Zeiss inverted microscope Axio Vert.A1 equipped for epifluorescence (magnification 63 ×). To determine NRVM intracellular Ca^{2+} concentration ($[Ca^{2+}]i$), we used an excitation wavelength of 485 nm and an emission wavelength of 530 nm. We measured the maximum (Fmax) and the minimum fluorescence (Fmin) of Fluo-4 and we use the following equation to calculate the resting calcium concentration: $[Ca^{2+}]i = K_d[(F - F_{min}) / (F_{max} - F)]$, where *K*d is the dissociation constant (345 nmol/L for Fluo-4), [27].

Fluo-4 signals in response to HS were analyzed using the imageJ software and the data are shown as ΔF/F0, where F0 represents the resting fluorescence. The LTCC channel inhibitor, Nifedipine (10 μmol/L, Sigma-Aldrich Corp., St Louis, MO) or the TRPP3 and PC2 inhibitor, Amiloride (100 μmol/L, Sigma-Aldrich Corp., St Louis, MO), were incubated 20 min before the Ca^{2+} measurement.

2.6. Statistical analysis

Data are shown as mean ± SEM of the indicated number (n) of independent experiments. Data were analyzed using Student's unpaired *t*-test or ANOVA followed by Tukey's test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. The Cavβ2 LTCC subunit is necessary to stabilize Cav1.2 during mechanical stretch induced by hypo-osmotic stress (HS)

To investigate the signaling pathway involved in PC1-induced Cav1.2 subunit stabilization, we used a HS-dependent mechanical stretch model in neonatal rat ventricular myocytes (NRVM) knockdown for PC1, siPC1 (Fig. 1A–B). Our results confirmed that HS produced a

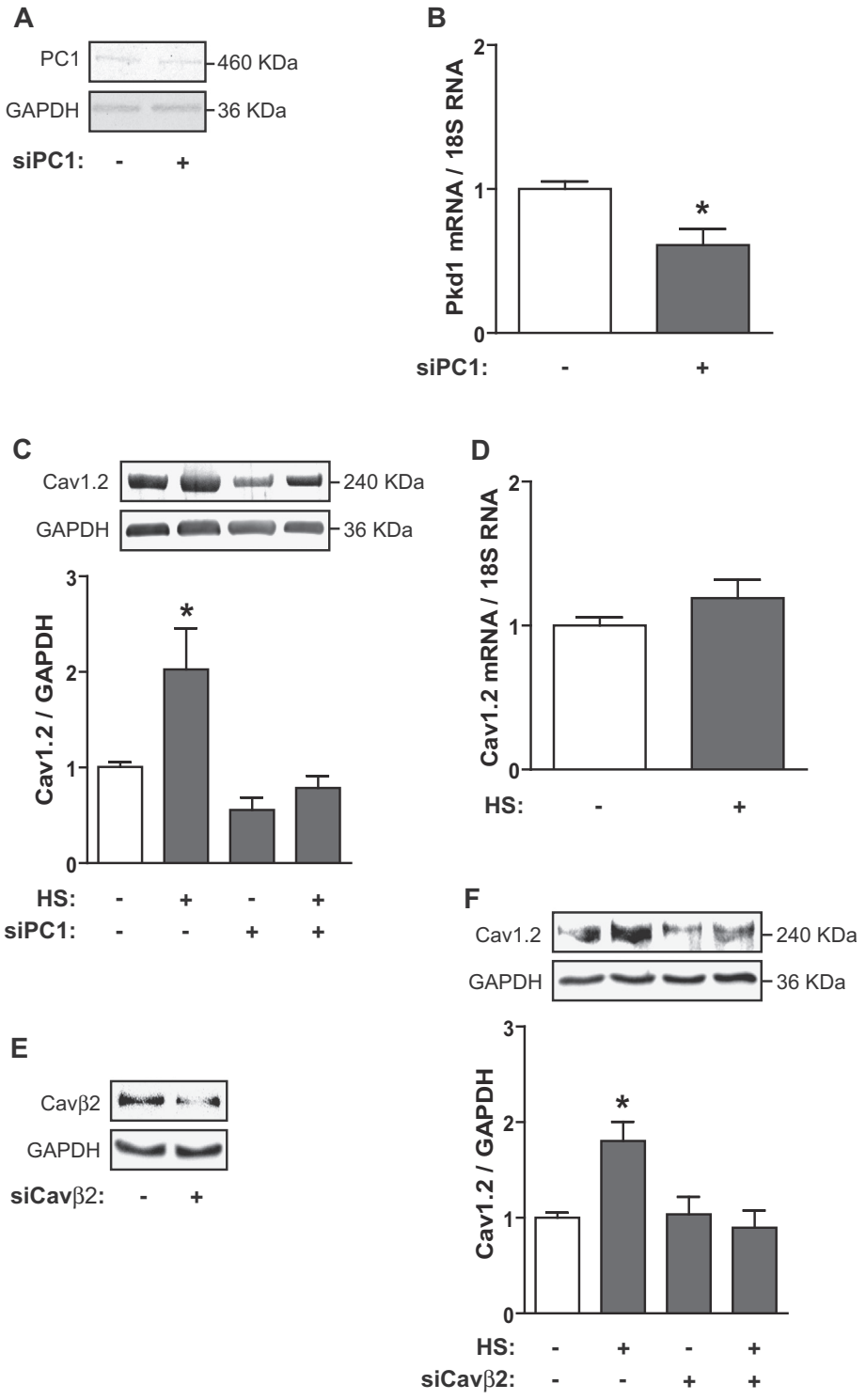

Fig. 1. Hypo-osmotic stress-induced mechanical stretch increases Cav1.2 protein levels through a Cavβ2-dependent mechanism. Cultured cardiomyocytes were exposed to hypo-osmotic stress (HS) for 2 h. Representative Western blot of PC1 (A) and Pkd1 mRNA levels (B), transfected with specific siRNA for PC1 (siPC1) are depicted. (C) Representative Western blots and average results obtained from densitometric analysis of Cav1.2 and GAPDH in cardiomyocytes siPC1. (D) Cav1.2 mRNA levels determined by qRT-PCR are expressed relative to 18S RNA. (E) Western blot showing Cavβ2 protein levels in controls and in cardiomyocytes transfected with specific siRNA for Cavβ2 (siCavβ2). (F) Representative Western blots and average results for Cav1.2 normalized according to GAPDH content in cardiomyocytes transfected with siCavβ2 and stimulated with HS. Values are shown as mean ± SEM ($n = 5 - 9$). *$p < 0.05$ vs. control.

two-fold increase in Cav1.2 protein levels in NRVM after 2 h of treatment. As shown previously, this increase was inhibited in siPC1 cells (Fig. 1C) despite unaltered Cav1.2 mRNA levels (Fig. 1D).

Since the Cavβ2 LTCC subunit is necessary to stabilize and transport the Cav1.2 subunit to the sarcolemma [11], we assessed whether Cavβ2 was also necessary to stabilize Cav1.2 in our model. For this purpose, we knockdown Cavβ2 using a specific targeted siRNA, siCavβ2 (Fig. 1E). We found that the HS-dependent increase in Cav1.2 was blunted in siCavβ2-treated NRVMs (Fig. 1F). Together, these data confirm that HS increases LTCC protein levels through a non-transcription-dependent mechanism that also involves Cavβ2.

3.2. Calcium increase induced by hypo-osmotic stress depends on LTCC and polycystin-1 in NRVMs

NRVMs exposed to hypo-osmotic stress (HS) show a fast and transitory increase in intracellular $[Ca^{2+}]i$ which is dependent on the external Ca^{2+} and at least in part, on LTCC [28]. To investigate whether the decrease of LTCC in siPC1 cells modify basal $[Ca^{2+}]i$ or the transient increase in $[Ca^{2+}]i$ in response to HS stress, we measured basal

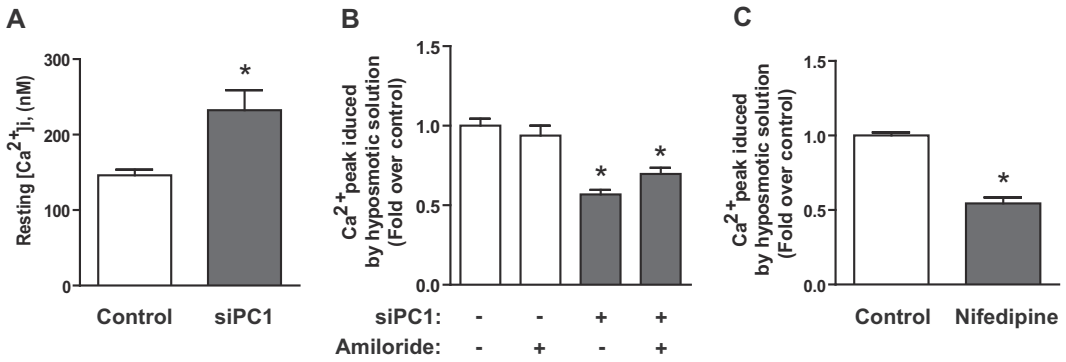

Fig. 2. Increase in cytoplasmic Ca^{2+} by hypo-osmotic stress depends of LTCC protein content. (A) Resting calcium concentration ($[Ca^{2+}]i$) in control and siPC1 NRVMs (B) Average Ca^{2+} signal during HS in control and siPC1 cells with or without amiloride (100 μmol/L) (C) Average Ca^{2+} signal during HS in control cells with or without nifedipine (10 μmol/L). Bars represent the mean ± SEM of 55–80 cells from 4 different experiments in each case. *$p < 0.05$ vs. control.

$[Ca^{2+}]i$ and during HS stimulus in NRVMs control and knockdown to PC1 (siPC1). We found that basal $[Ca^{2+}]i$ was higher in siPC1 cells than controls (Fig. 2A), although in both cases values are within the $[Ca^{2+}]i$ concentration range reported in NRVM [29,30]. The increase in $[Ca^{2+}]i$ in response to HS stimulus in siPC1 NRVMs, however, was significantly lower than the increased observed in control cells (Fig. 2B). This decreased $[Ca^{2+}]i$ response to HS was similar to the response observed in cell incubated with the LTCC inhibitor, nifedipine (10 μmol/L, Fig. 2C). To investigate if Polycystin-2 plays a role in the increase in $[Ca^{2+}]i$ in response to HS we used the inhibitor amiloride (100 μmol/L). As shown in Fig. 2B amiloride did not modify the $[Ca^{2+}]i$ increase in response to HS stimulus in control or siPC1 NRVMs, suggesting that Polycystin-2 is not involved in this response. As reported before [28], we found no increase in $[Ca^{2+}]i$ in response to HS in the absence of extracellular Ca^{2+} (not shown) indicating that Ca^{2+} influx from the extracellular space is necessary for the Ca^{2+} response. Together, these data suggest that the decrease in LTCC content in siPC1 cells determines the magnitude of the calcium response to HS stimulus.

3.3. Mechanical stretch-dependent increase in Cav1.2 protein level depends on AKT activation by polycystin-1

The Cavβ2 subunit is phosphorylated by AKT to bind and stabilize Cav1.2 [11]. It has been previously shown that PC1 induces AKT activation in epithelial renal cells during shear stress [14,22]. To investigate whether AKT is activated during mechanical stretch, NRVMs were stimulated with HS, and AKT phosphorylation on Ser473 (AKT-pSer473) was measured. Our results showed an increase in AKT-pSer473 levels after HS stimulation. Moreover, HS-induced AKT phosphorylation was prevented in NRVM transfected with a specific siRNA for PC1 (siPC1), (Fig. 3A). These data suggest that the mechanical stretch induced by HS triggers AKT activation through a PC1-dependent mechanism. To assess whether PC1-induced AKT activation is involved in the elevation of Cav1.2 protein levels, NRVMs were treated with an AKT inhibitor (AKTi, Fig. 3B). Our results showed that pretreatment of NRVMs with AKTi blunted the HS-induced increase in Cav1.2 protein content (Fig. 3C). These data suggest that PC1-dependent AKT activation is necessary to stabilize LTCC proteins through mechanical stretch.

3.4. Giβγ activates AKT and stabilizes LTCC during mechanical stretch

Various signaling pathways activate AKT in NRVMs. G-protein coupled receptors (GPCRs) induce several transduction pathways mediated by heterotrimeric G protein activation. In particular, Gi/o-coupled GPCRs activate PI3K and AKT through their Gβγ subunits [31,32]. Previous reports indicate that PC1 acts as an unconventional heterotrimeric G protein-coupled receptor [14,16]. Moreover, PC1 constitutively activates Gi/o-type G proteins and modulates Ca^{2+} and K^{+} channels via the Gβγ subunits in neuronal cells cultures [24]. To clarify the mechanism by which HS activates AKT and stabilizes LTCC, we used pertussis toxin (PTX) as a Gi inhibitor and measured AKT activation after the HS stimulus. Our results showed that NRVMs stimulated with HS in the presence of PTX failed to activate AKT (Fig. 4A), suggesting the involvement of a Gi/o protein in AKT activation.

In addition, we over-expressed a Giβγ inhibitor (βARkct) in NRVMs (Fig. 4B). βARkct inhibited both the activation of AKT and the increase in Cav1.2 protein levels after the HS stimulus (Fig. 4C–D). On the other hand, activation of Gβγ with the G-protein βγ binding peptide (mSIRK) activated AKT and increased Cav1.2 protein levels (Fig. 4E–F).

Taken together, these results suggest that a heterotrimeric G protein, specifically a Giβγ, is involved in the activation of AKT and the stabilization of LTCC during mechanical stretch. Therefore, a Giβγ associated with PC1 is likely involved in HS-induced LTCC stabilization.

3.5. Polycystin-1 acts as a G protein-coupled receptor to activate AKT and stabilize LTCC

The binding of the heterotrimeric G protein to PC1 depends on 74 amino acids contained in the G protein-binding domain of the C-terminus of PC1 [16]. To investigate whether the G protein-binding domain of PC1 is involved in LTCC stabilization, we over-expressed the human PC1 C-terminal tail (full-length, membrane-anchored PC1 tail construct, FLM-PC1, Fig. 5A) and the same construct without the G protein-binding domain (CTM-PC1, Fig. 5A), as described previously [33]. Empty CMV promoter construct was used as a control. The over-expression of FLM-PC1 (~75 KDa) and CTM-PC1 (~55 KDa) in NRVMs is shown in Fig. 5B. As described, we also observed a peptide with a molecular weight similar to FLM when CTM-PC1 was expressed [34].

After 24 h of FLM-PC1 over-expression, NRVMs showed AKT activation (Fig. 5C, D and E) and increased Cav1.2 protein content (Fig. 5F). These effects were not observed in CTM-PC1-transduced cells. Moreover, cells FLM-PC1 stimulated with HS activated AKT (Fig. 5G) and increased Cav1.2 protein content (Fig. 5H) similar to HS alone, however that was prevented in cells over expressing CTM-PC1.

These data suggest that the G protein-binding domain of PC1 is necessary for AKT phosphorylation and Cav1.2 stabilization.

4. Discussion

Our study describes a new signaling pathway by which PC1, through its GPCR activity and the Gβγ subunit of a heterotrimeric Gi/o protein, activates AKT and stabilizes LTCC in cardiomyocytes during mechanical stretch.

Heart contractility depends on Ca^{2+} influx to cardiomyocytes through L-type calcium channel (LTCC) during membrane depolarization [5]. Changes in LTCC activity or protein levels alter heart function

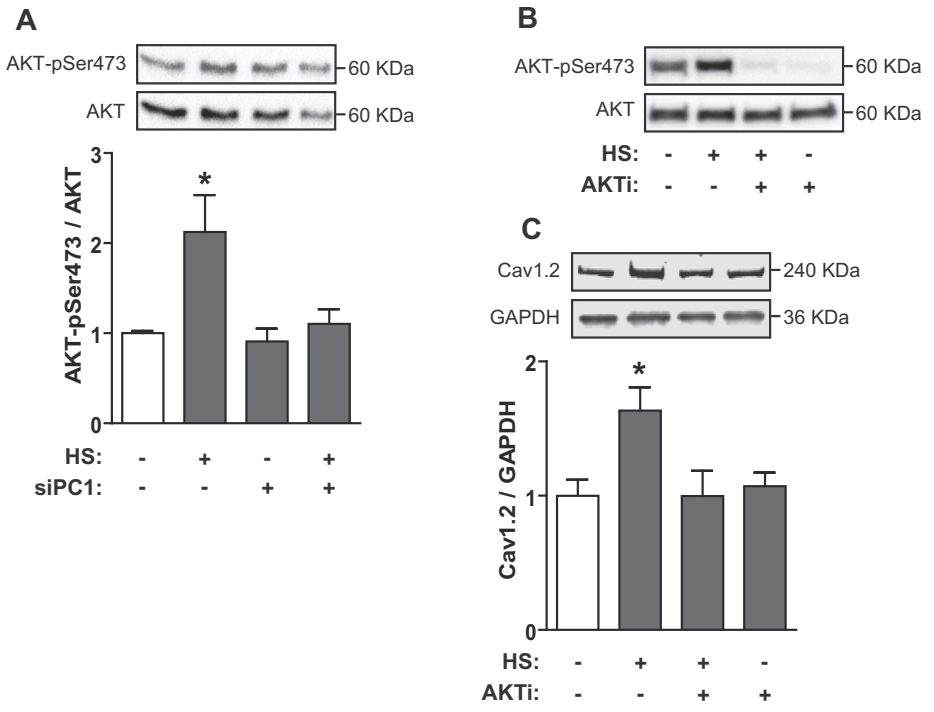

Fig. 3. Hypo-osmotic stress-induced mechanical stretch activates AKT through a polycystin 1-dependent mechanism and increases Cav1.2 protein levels. Representative Western blots of AKT phosphorylation on Ser473 (AKT-pSer473) and total AKT in (A) cardiomyocytes pretreated with PC1 siRNA (siPC1) and stimulated with HS (bar graph shows the AKT-pSer473/AKT ratio) and (B) cardiomyocytes pretreated with AKT inhibitor (AKTi, 10 μmol/L). (C) Cav1.2 and GAPDH protein levels determined by Western blot in cardiomyocytes pretreated with AKTi. Bar graph shows the Cav1.2/GAPDH ratio. Values are shown as mean ± SEM (n = 5–9). *p < 0.05 vs. control.

and may elicit various pathologies, such as cardiac hypertrophy [6,35,36].

Mechanical stretch is one of the most important stimuli for regulation of heart function [37–39]. The heart is under continual physiological mechanical stretch that modulates various signaling pathways within cardiac cells, converting mechanical stimuli to intracellular biochemical responses and shaping their function [38]. Sustained mechanical stretch induces a pathological response, i.e. pressure overload-induced cardiomyocyte hypertrophy [40]. However, relatively little is known about the mechanosensors implicated and how they trigger intracellular signaling pathways.

Recently, we described that the mechanosensor PC1 is crucial for maintaining normal cardiac function and is involved in the development of cardiac hypertrophy induced by mechanical stretch [19].

A critical determinant of calcium influx is Cav1.2 protein density at the plasma membrane. However, regulation of Cav1.2 abundance is not fully understood. Most studies are focused on the regulation of LTCC activity, but only few describe its degradation and/or stabilization. Previous reports indicate that Cav1.2 is degraded by the proteasome and that the binding of Cavβ2 to Cav1.2 in the endoplasmic reticulum prevents Cav1.2 ubiquitination and degradation [9]. In a previous work, we showed that PC1 regulates LTCC protein levels through protein stabilization, under conditions of mechanical stretch [19]. Here, we confirm that PC1 regulates LTCC stabilization and the magnitude of the Ca^{2+} response during mechanical stretch.

Although the basal cytoplasmic Ca^{2+} in siPC1 cardiomyocytes was higher than controls, the level was within the reported range of cytoplasmic calcium in NRVM [29,30,41]. In cardiomyocytes, cytoplasmic Ca^{2+} is buffered by several Ca^{2+} binding proteins and also by the activities of the different cellular Ca^{2+} transport systems, therefore changes in LTCC should not produce major changes in intracellular Ca^{2+} under basal conditions. The increase in Ca^{2+} concentration in response to HS, however, was greatly diminished in siPC1 cells compared to controls and was not affected by amiloride, a polycystin-2 channel inhibitor. These results suggest that Ca^{2+} influx through LTCC is involved in the response to HS and support a role for PC1 in cell signaling since reduction of this protein causes a decreased response to stress.

The present study describes the mechanism by which PC1 stabilizes LTCC in NRVMs and shows that Cavβ2 is necessary to stabilize LTCC during HS-induced mechanical stretch. Our findings also indicate that AKT activation is crucial for LTCC stabilization, consistent with previous findings in cardiomyocytes stimulated with IGF-1 [11].

Our data indicate that PC1 is the protein responsible for AKT activation in response to mechanical stretch, corroborating the idea that PC1 regulates this kinase [14,22].

GPCRs are important cell surface receptors that regulate physiological cardiac function [32,42,43]. GPCR dysfunction contributes to cardiac hypertrophy, failure and arrhythmias [31,44]. One-third of the drugs used in cardiology have GPCRs as targets, demonstrating the importance of these receptors in heart pathologies [42]. Various mechanosensors have been identified as GPCRs [45].

PC1 is a transmembrane protein with a large extracellular N-terminus, 11 transmembrane domains and a short intracellular C-terminus [18]. PC1 binds, through its G protein-binding domain in the C-terminus, multiple classes of Gα subunits, especially Gα12 and Gαi/o [14,16]. Moreover, PC1 is a constitutive activator of Gi/o that modulates Ca^{2+} and K^{+} channels via a Gβγ subunit through a heterologous expression system [24]. PC1 is also an inhibitor of N-type Ca^{2+} channels [46], and shows anti-apoptotic activity via activation of the Gβγ-PI3K-AKT pathway [22]. This report provides the first description of PC1 regulation of these signaling pathways in cardiomyocytes.

Our results show that blocking the G-protein binding domain of PC1 totally prevented AKT activation and LTCC stabilization. Therefore, our data is consistent with a model in which PC1 acts as a GPCR in NRVMs and, through this activity, regulates steady state LTCC levels in the heart under stress.

Interestingly, according to our results, the N-terminal domain does not seem to be necessary. Furthermore, the transmembrane domain is not always involved in the mode of action of mechanosensors. Some proteins act as mechanosensors through linkage with cytoskeleton proteins, intermediate filaments, or microtubules (the tethered model of mechanosensing). On the other hand, other proteins expose cryptic regions when subjected to mechanical stress (Protein unfolding model). Moreover, PC1 interacts with several other membrane and cytoplasmic proteins and thus, mechanical stress could be sensed by different

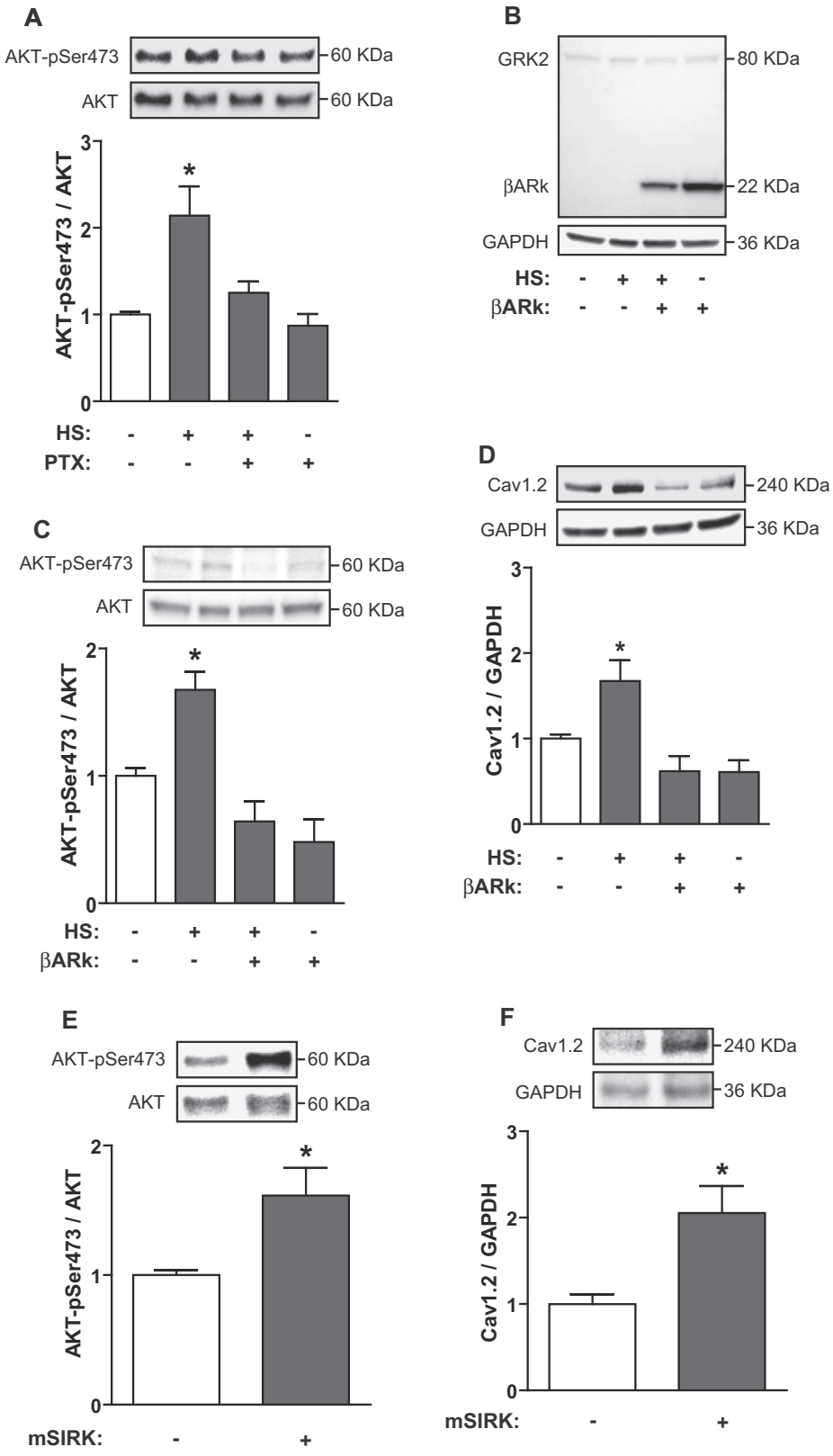

Fig. 4. Activation of AKT by hypo-osmotic stress and increase in Cav1.2 depends on a Gi protein. (A) Representative Western blots of AKT phosphorylation on Ser473 (AKT-pSer473) and total AKT in cardiomyocytes pretreated with pertussis toxin (PTX, 1.0 μg/mL) and exposed to HS for 2 h. Bar graphs show the AKT-pSer473/AKT ratio calculated by densitometric analysis. (B) Western blots showing the overexpression of the C-terminus of GRK2 (βARk), a Giβγ inhibitor. LacZ adenovirus was used as a control. Representative Western blots and average results for: (C) AKT-pSer473 and total AKT and (D) Cav1.2 and GAPDH protein levels. Representative Western blots and average results for (E) phospho-AKT (AKT-pSer473) and total AKT and (F) Cav1.2 and GAPDH in cardiomyocytes pretreated with a G-protein βγ binding peptide (mSIRK, 10 μmol/L). Values are shown as mean ± SEM (n = 4–9). *p < 0.05 vs. control.

mechanism presently unexplored [20].

Taken together, our results uncover a previously-unrecognized route for LTCC stabilization through PC1 in cardiomyocytes. This finding opens the prospect of therapeutic manipulation. Understanding how LTCC protein levels are regulated by PC1 could also help to explain mechanisms of physiological calcium regulation in cardiomyocytes and dysregulation in cardiac diseases. Future studies will be necessary to define other pathways modulated by PC1 in the cardiovascular system.

Transparency document

The Transparency document associated with this article can be

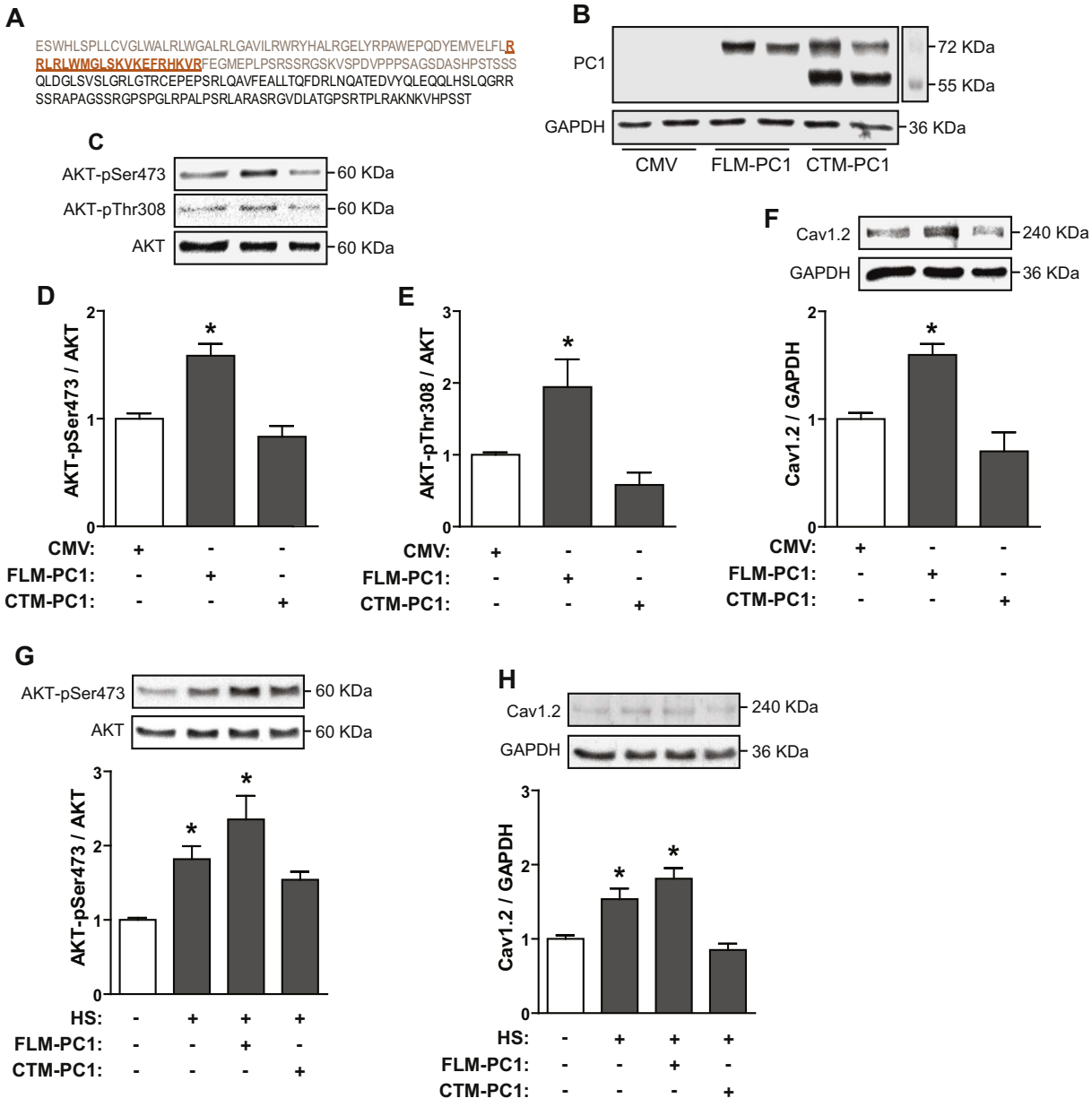

Fig. 5. The G-protein domain of polycystin-1 is crucial for activating AKT and modulates Cav1.2 content in cardiomyocytes. (A) Full-length sequence of the human membrane-anchored PC1 C-terminal tail (FLM-PC1). Amino acids deleted in the mutated PC1 C-terminal tail (CTM-PC1) are shown in orange. The G protein-binding domain is underlined. (B) PC1 immunoreactivity in cardiomyocytes transfected with control empty vector (CMV), FLM-PC1 or CTM-PC1. (C) Representative Western blots and average results in bar graphs for AKT-pSer473 (D) and AKT-pThr308 (E), respect the total AKT and, (F) Cav1.2 and GAPDH. Western blots and levels of AKT-pSer473 (G) and Cav1.2 (H), in cardiomyocytes over expressing FLM-PC1 and CTM-PC1 stimulated with HS. Values are shown as mean ± SEM (n = 4–5). *$p < 0.05$ vs. control.

found, in online version.

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Conflict of interest

The authors declare no competing financial interest.

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