Channels 5:3, 280-286; May/June 2011; © 2011 Landes Bioscience

L-type calcium channel β subunit modulates angiotensin II responses in cardiomyocytes

Tamara Hermosilla,[1] Cristian Moreno,[1] Mircea Itfinca,[2] Christophe Altier,[3] Ricardo Armisén,[1] Andrés Stutzin,[1] Gerald W. Zamponi[2] and Diego Varela[1,*]

[1]Centro de Estudios Moleculares de la Célula; Instituto de Ciencias Biomédicas; Universidad de Chile; Santiago, Chile; [2]Department of Physiology and Pharmacology; Hotchkiss Brain Institute; [3]Department of Physiology and Pharmacology; Snyder Institute; University of Calgary; Calgary, AB Canada

Key words: β subunits, $Ca_V1.2$, angiotensin II, neonatal cardiomyocytes, L-type calcium current, arachidonic acid

Abbreviations: AngII, angiotensin II; $Ca_V\beta$, calcium channel β subunit; $Ca_V\alpha_2\delta_1$, calcium channel $\alpha_2\delta_1$ subunit; R_{250}, residual current after a 250 ms pulse; AT1, angiotenisn receptor type 1; G_qPCR, G_q-protein coupled-receptors; VDCC, voltage-dependent Ca^{2+}-channels; PLC, phospholipase C; IP_3, inositol triphosphate; DAG, diacylglycerol; PKC, protein kinase C; PIP_2, phosphoinositol-4,5-bisphosphate; AA, arachidonic acid; DAGL, DAG lipase

Angiotensin II regulation of L-type calcium currents in cardiac muscle is controversial and the underlying signaling events are not completely understood. Moreover, the possible role of auxiliary subunit composition of the channels in Angiotensin II modulation of L-type calcium channels has not yet been explored. In this work we study the role of $Ca_V\beta$ subunits and the intracellular signaling responsible for L-type calcium current modulation by Angiotensin II. In cardiomyocytes, Angiotensin II exposure induces rapid inhibition of L-type current with a magnitude that is correlated with the rate of current inactivation. Semi-quantitative PCR of cardiomyocytes at different days of culture reveals changes in the $Ca_V\beta$ subunits expression pattern that are correlated with the rate of current inactivation and with Angiotensin II effect. Overexpression of individual β subunits in heterologous systems reveals that the magnitude of Angiotensin II inhibition is dependent on the $Ca_V\beta$ subunit isoform, with $Ca_V\beta_{1b}$ containing channels being more strongly regulated. $Ca_V\beta_{2a}$ containing channels were insensitive to modulation and this effect was partially due to the N-terminal palmitoylation sites of this subunit. Moreover, PLC or diacylglycerol lipase inhibition prevents the Angiotensin II effect on L-type calcium channels, while PKC inhibition with chelerythrine does not, suggesting a role of arachidonic acid in this process. Finally, we show that in intact cardiomyocytes the magnitude of calcium transients on spontaneous beating cells is modulated by Angiotensin II in a $Ca_V\beta$ subunit-dependent manner. These data demonstrate that $Ca_V\beta$ subunits alter the magnitude of inhibition of L-type current by Angiotensin II.

Introduction

Angiotensin II (AngII) is a well known hormone that plays a crucial role in physiology and physiopathology by regulating the function of many cell types.[1] In particular, the acute effect of AngII on L-type calcium currents has been shown to be dependent on the cell type: while in smooth muscle is accepted that AngII induce activation of L-type calcium current;[2-4] in neurons and kidney the opposing effect (inhibition of L-type calcium current) is observed.[5-7] On the other hand, the effect of this hormone in cardiac muscle is still controversial as AngII was reported to induce an increase[8,9] or a decrease[10,11] of L-type calcium current in heart cells. Although the reason for this disagreement is not known, it is proposed that the experimental approach is relevant.[12,13]

The Angiotensin type I receptor (AT1) is responsible for most of the classical actions associated with AngII, such as the increase in peripheral vascular resistance or sodium and water uptake. This receptor belongs to the super-family of seven transmembrane helix G_q-protein coupled receptor (G_qPCR) and its activation is linked to phospholipase C (PLC) activation and to the production of inositol triphosphate (IP_3) and diacylglycerol (DAG), which in turn activates the classical and novel isoforms of protein kinase C (PKC). In parallel to PKC activation, PLC activation induces a decrease in phosphoinositol 4,5-bisphosphate (PIP_2) membrane levels and DAG production induces an increase in arachidonic acid (AA) levels trough the action of DAG lipase (DAGL). Interestingly, while the role of PKC in the AngII-dependent modulation of L-type calcium channel is controversial,[10,13-15] recent reports show that either PIP_2 depletion[16] or DAGL-dependent AA production[17] are able to modulate L-type calcium channels.

In the heart, L-type Ca^{2+}-currents are carried by a multi-subunit membrane complex that includes $Ca_V1.2$ as the pore-forming subunit that co-assembles with the auxiliary $Ca_V\alpha_2\delta_1$ and $Ca_V\beta$ subunits.[18-20] To date, four genes that encode the $Ca_V\beta_{1-4}$ isoforms

*Correspondence to: Diego Varela; Email: dvarela@bitmed.med.uchile.cl
Submitted: 03/24/11; Revised: 04/06/11; Accepted: 04/14/11
DOI: 10.4161/chan.5.3.15833

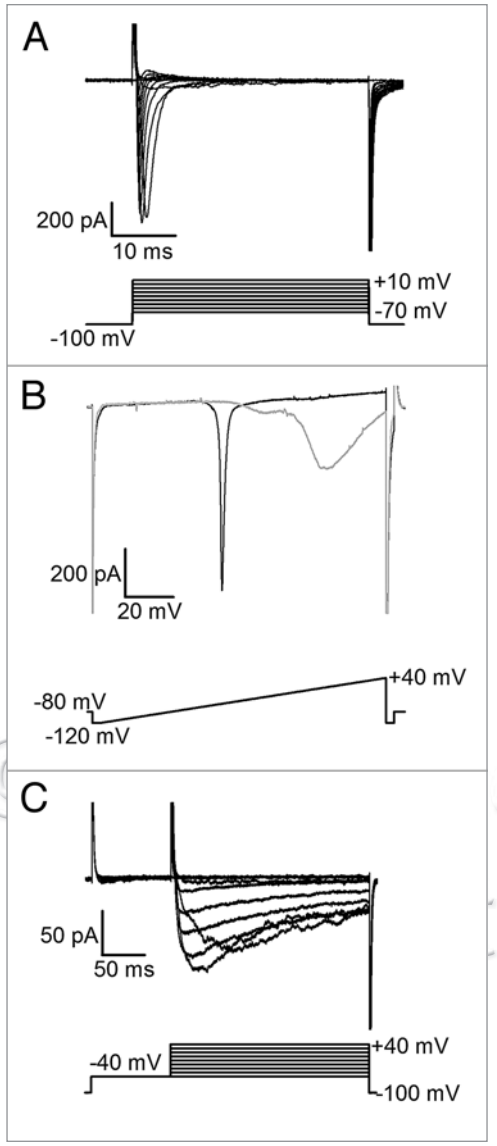

Figure 1. Na^+ and L-type currents in cardiomyocytes. (A) Representative nystatin-perforated whole-cell voltage-dependent Na^+ current traces activated by the voltage protocol shown in the bottom. (B) Current-Voltage relation of whole-cell currents with 100 mM Na^+ external solution (black trace) and 20 mM Ba^{2+} (gray trace), using a 180 ms duration voltage ramp from -120 to 40 mV. (C) Nystatin-perforated whole-cell L-type barium current traces activated by the voltage protocol shown in the bottom. All data were obtained from the same cardiomyocyte.

have been identified and shown to differentially alter channel behavior, including open probability, gating kinetics activation and inactivation over L-type Ca^{2+}-current, either in heterologous expression systems[21] or in cardiomyocytes.[22] While these $Ca_V\beta$ subunit effects have been extensively studied, less is known about their ability to fine tune the modulation of L-type calcium channels by neurotransmitters and hormones.

Here we show that the magnitude of AngII inhibition of L-type calcium current in neonatal rat cardiomyocytes correlate with the rate of inactivation of the currents and is dependent on DAGL activity, moreover, we demonstrate that this correlation is due to a $Ca_V\beta$ subunit dependence of AngII action. Finally, we show that calcium transients in spontaneous beating cells are modulated by AngII in a $Ca_V\beta$ subunit manner. In conclusion, we postulate that the effect of AngII over L-type calcium current is significantly dependent on $Ca_V\beta$ subunits and arachidonic acid production.

Results

Macroscopic barium currents were recorded from dispersed beating cardiomyocytes using the nystatin perforated patch-clamp method to ensure minimal disruption of the cytosolic environment. Only cells that were beating at the onset of experiments were chosen, and to verify proper voltage-control, voltage-dependent Na^+ currents were measured before each experiment (see Methods and **Fig. 1**).

We found that L-type currents with fast inactivation kinetics are more sensitive to AngII, while barium currents with slow inactivation are barely inhibited by AngII (**Fig. 2A and B**). This observation was confirmed after plotting the residual current after a 250 ms pulse to 10 mV (R_{250}, a measure of current inactivation) against the percentage of current inhibition after AngII (100 nM) exposure. **Figure 2C** shows that these two variables correlate ($R > 0.8$) suggesting that the percentage of current inhibition by AngII is linked to the inactivation rate of the L-type current.

It has been reported that calcium currents in neonatal cardiomyocytes change throughout the time of culture,[23] therefore we examined whether changes in R_{250}, and consequently AngII sensitivity could be attributable to different days in culture. We found that both parameters are correlated with the time in culture of the cardiomyocytes after isolation (**Fig. 3A and B**). As the speed of inactivation (and therefore R_{250}) is correlated with the $Ca_V\beta$ subunit expressed, semi-quantitative PCR was performed to detect changes in $Ca_V\beta$ subunit expression. As seen in **Figure 3C–E** there is a decrease in $Ca_V\beta_{1b}$ expression after cell isolation while $Ca_V\beta_{2a}$ increase its expression and $Ca_V\beta_3$ remain constant.

The latter results could implicate that changes in the magnitude of AngII inhibition is in fact due to changes in $Ca_V\beta$ subunits. To address this point directly, the cardiac form of $Ca_V1.2$ ($+Ca_V\alpha_2\delta_1$) was overexpressed in a stable cell line that overexpresses the AT1 receptor (HEK-AT1) with different $Ca_V\beta$ subunits. As seen in **Figure 4**, when cells overexpressing $Ca_V\beta_{1b}$ were exposed to AngII (100 nM) a fast current inhibition (~70%) was observed (**Figs. 4A and B and 5C**). Cells expressing $Ca_V\beta_3$ showed a lower degree of current inhibition (~50%) (**Figs. 4E, F and 5C**); however, in $Ca_V\beta_{2a}$ expressing cells, almost no inhibition was observed (**Figs. 4C and D and 5C**), demonstrating that $Ca_V\beta$ subunits are potent regulators of AngII mediated effects on L-type currents.

$Ca_V\beta_{2a}$ differs from other $Ca_V\beta$ subunits by the existence of two cysteine residues near the N-terminus which form palmitoylation sites,[24] and many of the unique properties of this subunit are dependent on these residues.[24] Elimination of the putative palmitoylation sites via site-directed mutagenesis[25] ($\beta_{2aC(3,4)S}$) resulted in a significant enhancement of the AngII mediated modulation, albeit not to the levels observed with other $Ca_V\beta$ subtypes

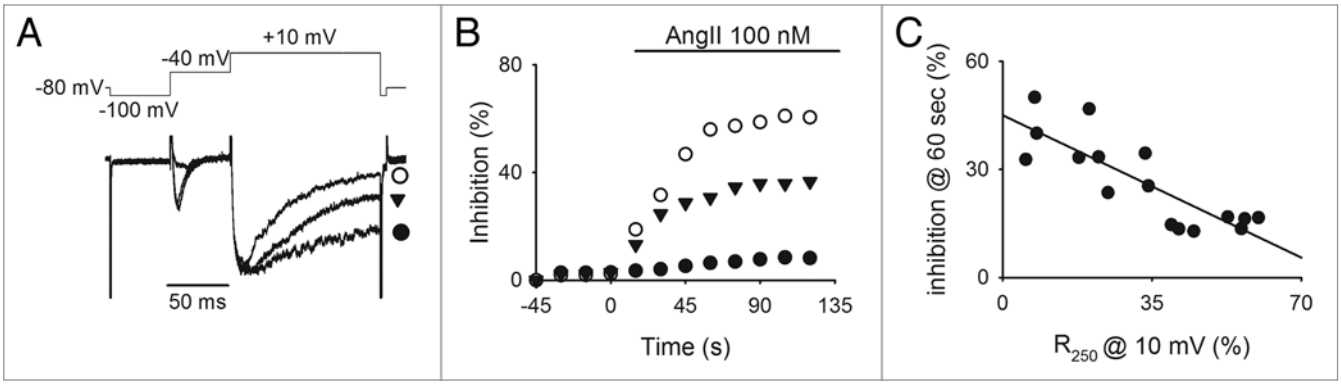

Figure 2. The magnitude L-type Ba^{2+}-currents inhibition depends on the inactivation kinetics of the channels. (A) Representative L-type currents, normalized to I_{max}, from 3 different cardiomyocytes, recorded using the voltage protocol shown in the upper part (B) Time course of L-type Ba^{2+}-current inhibition induced by AngII (100 nM) obtained from the cardiomyocytes shown in (A). (C) Plot of the remaining current after a pulse of 250 ms to 10 mV (R_{250}) versus AngII inhibition obtained at 60 seconds for individual cardiomyocytes. The regression line corresponds to a linear fit of the data (R = 0.84, n = 16).

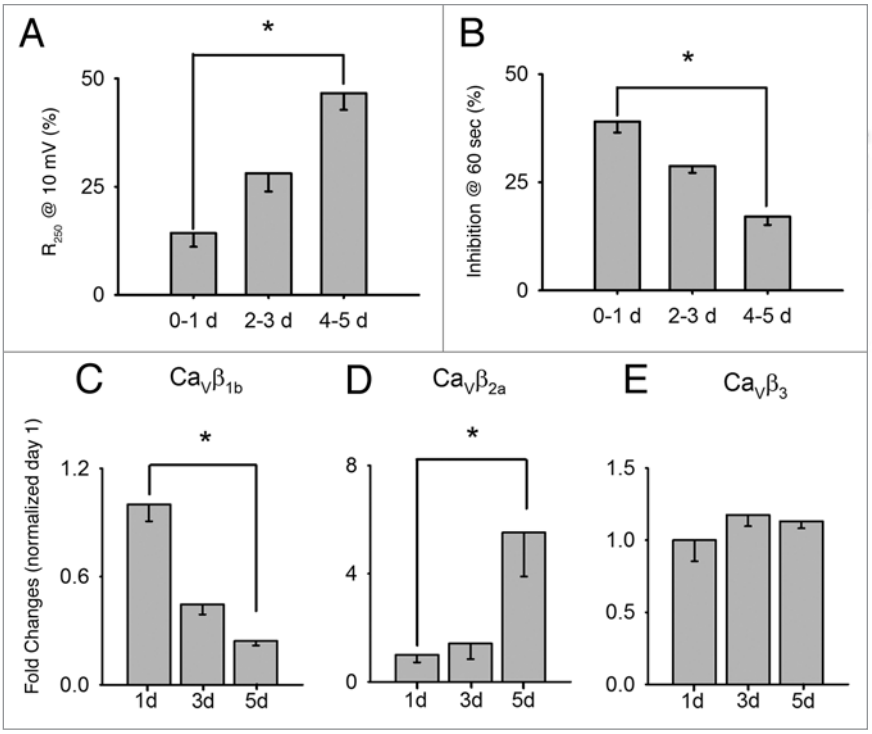

Figure 3. Changes in $Ca_V\beta$ subunits expression pattern in cultured cardiomyocytes (A) R_{250} of L-type barium current (mean ± SEM) in cardiomyocytes from different days in culture (n > 4). (B) Percent inhibition by AngII (mean ± SEM) in cardiomyocytes from different days in culture (n > 4). $Ca_V\beta$ subunits mRNA levels relative to GADPH mRNA in cardiomyocytes cultures at different days for $Ca_V\beta_{1b}$ (C), $Ca_V\beta_{2a}$ (D) or $Ca_V\beta_3$ (E). n = 4. *p < 0.01, compared with day 1.

(**Fig. 5A–C**). This indicates that the palmitoyl groups of $Ca_V\beta_{2a}$ contribute to the insensitivity of $Ca_V\beta_{2a}$ containing L-type channels to AngII.

Next, we decided to explore the intracellular signaling pathways responsible for the effect of AngII on L-type currents. In order to focus on cardiomyocytes exhibiting a fast rate of inactivation and therefore, with higher L-type current inhibition by AngII, only cardiomyocytes with less of 48 hours (0–1 days) in culture were used (**Fig. 3B**). As shown in **Figure 6**, PLC inhibition with the generic inhibitor U73122 prevented the effect of AngII on L-type currents. However, in cardiomyocytes treated with the PKC inhibitor chelerythrine, the AngII-dependent L-type current inhibition was unaffected. Interestingly, inhibition of DAGL was enough to prevent the effect of AngII, suggesting a role for AA in L-type calcium channel inhibition (**Fig. 6**).

As calcium influx via L-type Ca^{2+}-current is critical for Ca^{2+}-induced Ca^{2+}-release,[26] an inhibition of L-type Ca^{2+}-currents by AngII should be reflected in a decrease in the magnitude of Ca^{2+}-transients. Consequently, we explored whether $Ca_V\beta$ subunits could modulate AngII responses on Ca^{2+}-transients in spontaneous beating cardiomyocytes. All these experiments were done in cardiomyocytes with less of 48 hours in culture. In control cardiomyocytes, AngII produced a strong inhibition of the magnitude of Ca^{2+}-transients as measured with Fura-2 (**Fig. 7A, B and G**). A similar response was observed in cardiomyocytes overexpressing $Ca_V\beta_{1b}$ (**Fig. 7C, D and G**) whereas $Ca_V\beta_{2a}$ overexpressing cardiomyocytes showed only little reduction in the magnitude of calcium transient after exposure to AngII (**Fig. 7E–G**).

Discussion

In this report, we show that L-type calcium channels $Ca_V\beta$ subunits are critical modulators of L-type current responses to AngII. Using primary cultures of neonatal rat cardiomyocytes we have demonstrated that AngII inhibition of L-type currents correlates

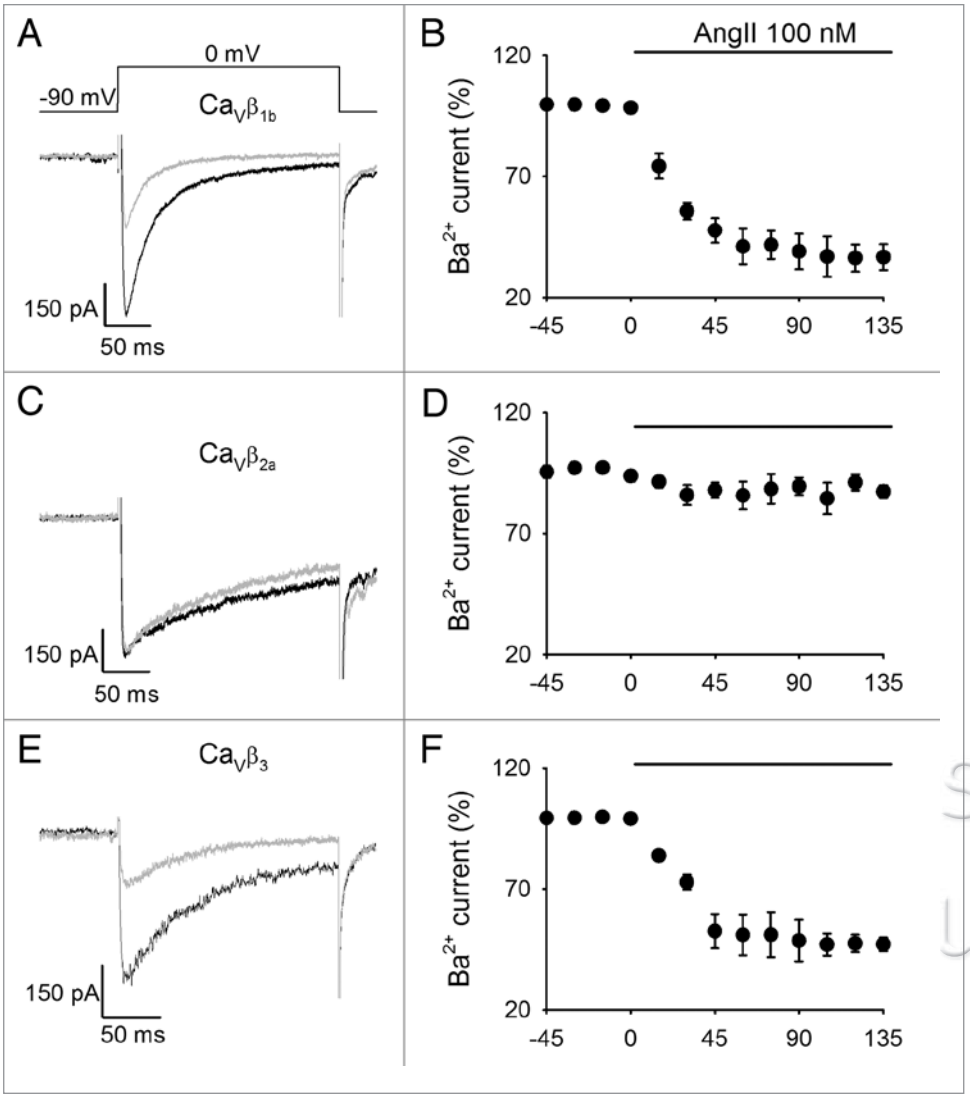

Figure 4. L-type Ba^{2+}-current inhibition by AngII is β subunit dependent. Representative barium currents before (black line) and 60 seconds after (gray line) exposure to AngII (100 nM). The currents were recorded from a HEK-AT1 cell line overexpressing the cardiac form of $Ca_V1.2$ ($+Ca_V\alpha_2\delta_1$) and $Ca_V\beta_{1b}$ (A) or $Ca_V\beta_{2b}$ (C) or $Ca_V\beta_3$ (E). Time course of the percentage of remaining current (mean ± SEM) of L-type Ba^{2+}-current in a HEK-AT1 cell line overexpressing the cardiac form of $Ca_V1.2$ ($+Ca_V\alpha_2\delta_1$) and $Ca_V\beta_{1b}$ (B) or $Ca_V\beta_{2a}$ (D) or $Ca_V\beta_3$ (F).

with the inactivation speed of the current. At a first glance, this observation could imply that inhibition may be dependent on the inactivation state of $Ca_V1.2$ channels, however, the experimental protocol was designed to avoid this possibility with short (50 ms) depolarizing pulses and long inter-pulses intervals (15 seconds), channels are expected to be in a closed state during AngII application. As every record shown here was performed with external Ba^{2+}, no Ca^{2+} dependent inactivation is observed, and therefore, it is more likely that the correlation with the rate of inactivation may be an epiphenomenon of a different underlying molecular event, such as channel subunit composition.

Indeed, cardiomyocytes express more than one isoform of $Ca_V\beta$ subunits, the main component of the L-type calcium channel multi-protein complex that determines the time course of inactivation.[27] As we show here, the expression pattern of $Ca_V\beta$ subunits changes throughout the time that the cardiomyocytes remain in culture, therefore overexpression of individual $Ca_V\beta$ subunits in cells that overexpress the AngII receptor, AT1, and the cardiac form of $Ca_V1.2$ ($+Ca_V\alpha_2\delta_1$) was chosen to demonstrate the impact of these subunits in AngII modulation. As suggested from cardiomyocytes data, the magnitude of inhibition is correlated with the type of $Ca_V\beta$ subunit that is expressed. Hence, a more likely explanation for the observed effects is that cells showing a lower macroscopic rate of inactivation simply express more $Ca_V\beta_{2a}$ to give rise to a greater proportion of AngII insensitive channels in a given cell.

In contrast with previous results showing that the $Ca_V\beta$ subunit dependence of PKA modulation of $Ca_V1.2$ is due to alterations in channel open probability by a particular $Ca_V\beta$ subunit,[28] our $Ca_V\beta$ subunit dependency is reminiscent of results showing that $Ca_V\beta$ subunit subtype regulates the modulation of N-type Ca^{2+}-current by G_q-coupled receptors[29] and of the inhibition of $Ca_V1.3$ channels by AA.[30] In line with these results, we show that DAGL activity is necessary for AngII-mediated L-type calcium channel inhibition, suggesting that AA production is necessary for this effect and indicating that ancillary calcium channel subunits can serve to fine-tune second messenger modulation of several VDCCs.

In mouse heart,[31] changes in $Ca_V\beta$ subunits during development has been described; along these lines, changes in these subunits has been observed not only between different chambers of the heart but also between young and old rats.[32] We show here that the magnitude of L-type current inhibition by AngII is dependent on the $Ca_V\beta$ subunit isoform, and therefore is expected that AngII may differentially modulate L-type calcium channels during these stages. The impact of this differential regulation is yet to be studied.

On the other hand, increases in $Ca_V\beta_{2a}$ subunit expression[33] as well as increases in AngII levels[34] and changes in ion channels expression[35] are associated with cardiac hypertrophy. In fact, the solely overexpression of $Ca_V\beta_{2a}$ subunits is sufficient for inducing a hypertrophic response.[36] Our results demonstrating a lack of AngII dependent modulation of L-type currents and calcium transients when $Ca_V\beta_{2a}$ is expressed suggest that the

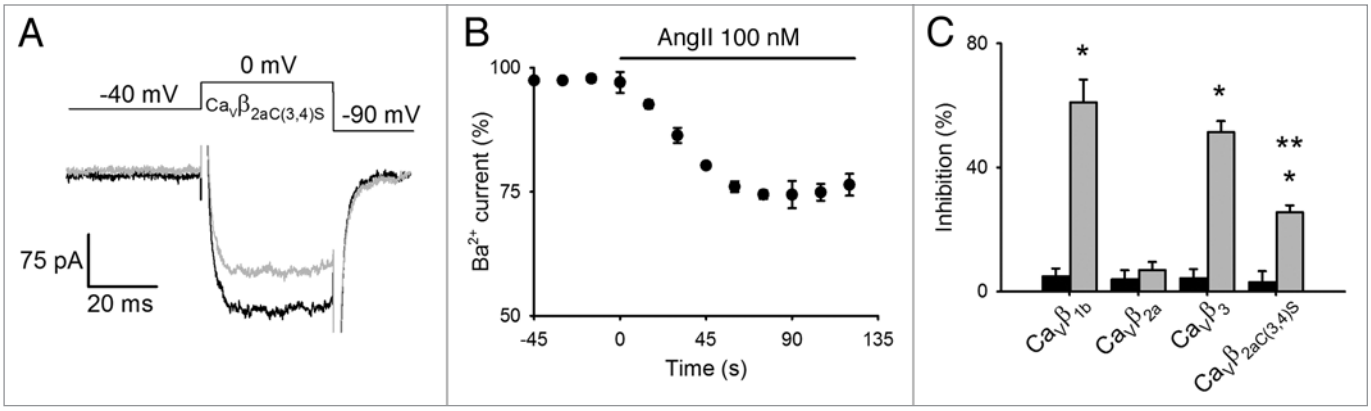

Figure 5. Palmitoyl groups of $Ca_V\beta_{2a}$ are partially responsible for the lack of response after AngII exposure. Representative currents before (black line) and 60 seconds after (gray line) exposure to AngII (100 nM) to a HEK-AT1 cell line overexpressing the cardiac form of $Ca_V1.2$ ($+Ca_V\alpha_2\delta_1$) and $Ca_V\beta_{2a(C3,4S)}$ (A). Time course of the percentage of current (mean ± SEM) of L-type barium current in the HEK-AT1 cell line overexpressing the cardiac form of $Ca_V1.2$ ($+Ca_V\alpha_2\delta_1$) and $Ca_V\beta_{2a(C3,4S)}$ (B). (C) Summary bar graph for percent inhibition by AngII for different $Ca_V\beta$ subunits. n > 6. *p < 0.01 compared with parental HEK cell lines. **p < 0.01 compared with $Ca_V\beta_{2a}$.

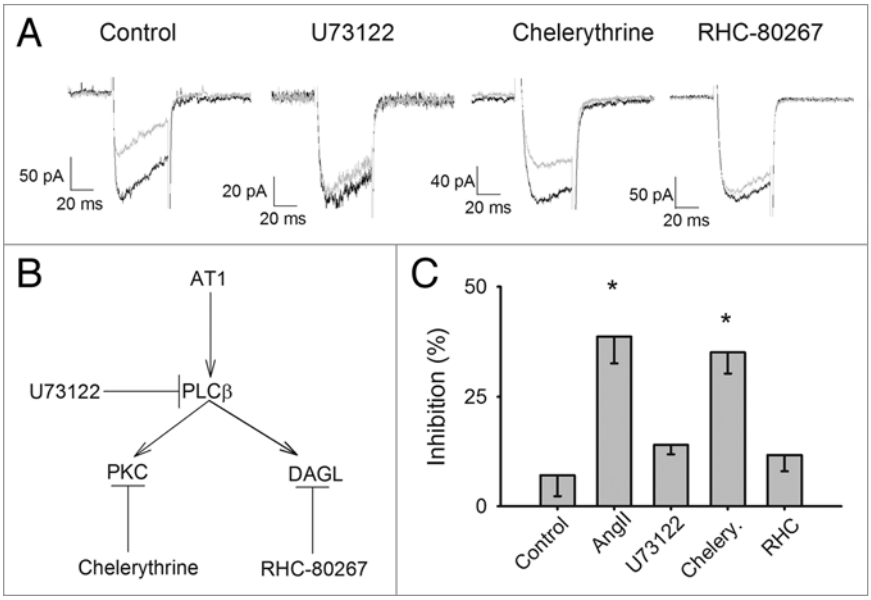

Figure 6. DAGL inhibition prevents the AngII effects on L-type calcium channels. (A) Representative cardiomyocyte barium currents before (black line) and 60 seconds after (gray line) exposure to AngII (100 nM) and treated with different drugs. (B) Pharmacological strategy used to establish the enzymes involved in the effect of AngII on L-type calcium channel. (C) Summary bar graph for percent inhibition by AngII for cardiomyocytes treated with different drugs. n > 6. *p < 0.01 compared with cardiomyocytes control.

AngII-dependent calcium homeostasis in a hyperthrophic heart is different compared with a healthy heart, and could reflect a novel mechanism that may contribute to the understanding of the deleterious effects of AngII.

Materials and Methods

Constructs. cDNA encoding the angiotensin receptor 1, AT1, was obtained from the University of Missouri-Rolla cDNA resource center. cDNAs for calcium channel subunits were kindly provided by Dr. Snutch. The palmitoylation deficient $Ca_V\beta_{2a}$ was described previously in reference 25.

Cardiomyocyte isolation. Rats were bred in the Animal Breeding Facility from the Facultad Ciencias Químicas y Farmacéuticas, Universidad de Chile (Santiago, Chile). All studies were done with the approval of our Institutional Bioethics Committee. Cardiac myocytes were prepared from hearts of 1- to 3-day-old Sprague Dawley rats as described previously.[37] Briefly, cardiomyocytes were dissociated in a solution of collagenase (0.2 mg/ml) and pancreatin (1.2 mg/ml). The collected cells were plated in nitrocellulose-coated glass cover slips and cultured in DMEM containing 10% Horse Serum, 5% FBS, 2 mmolar/L L-glutamine and 50 U/ml penicillin-streptomycin (Sigma).

Transfection. Tissue culture and transfection of tsA-201 cells was described previously in detail.[38] Briefly, cells were grown at 37°C (5% CO_2) in DMEM (+5% FBS, 50 U/ml penicillin-streptomycin) and plated on glass cover slips. Transfection solutions for individual culture dishes contained a mixture of cDNA expression vectors (2 μg for each L-type calcium channel subunit and 0.2 μg of a pEGFP marker vector (Clontech)) and were transfected into cells by the calcium phosphate method. Cells were transferred to 30°C 24 h after transfection, and recordings were conducted 2–3 days later. Cardiomyocytes were transfected the same day of isolation with the same protocol described above with the exception that the cells were kept at 37°C and experiments were performed 48 hr after transfection.

Electrophysiology. Prior to recordings, cells were transferred into an external bath solution of 100 mM sodium (mM):

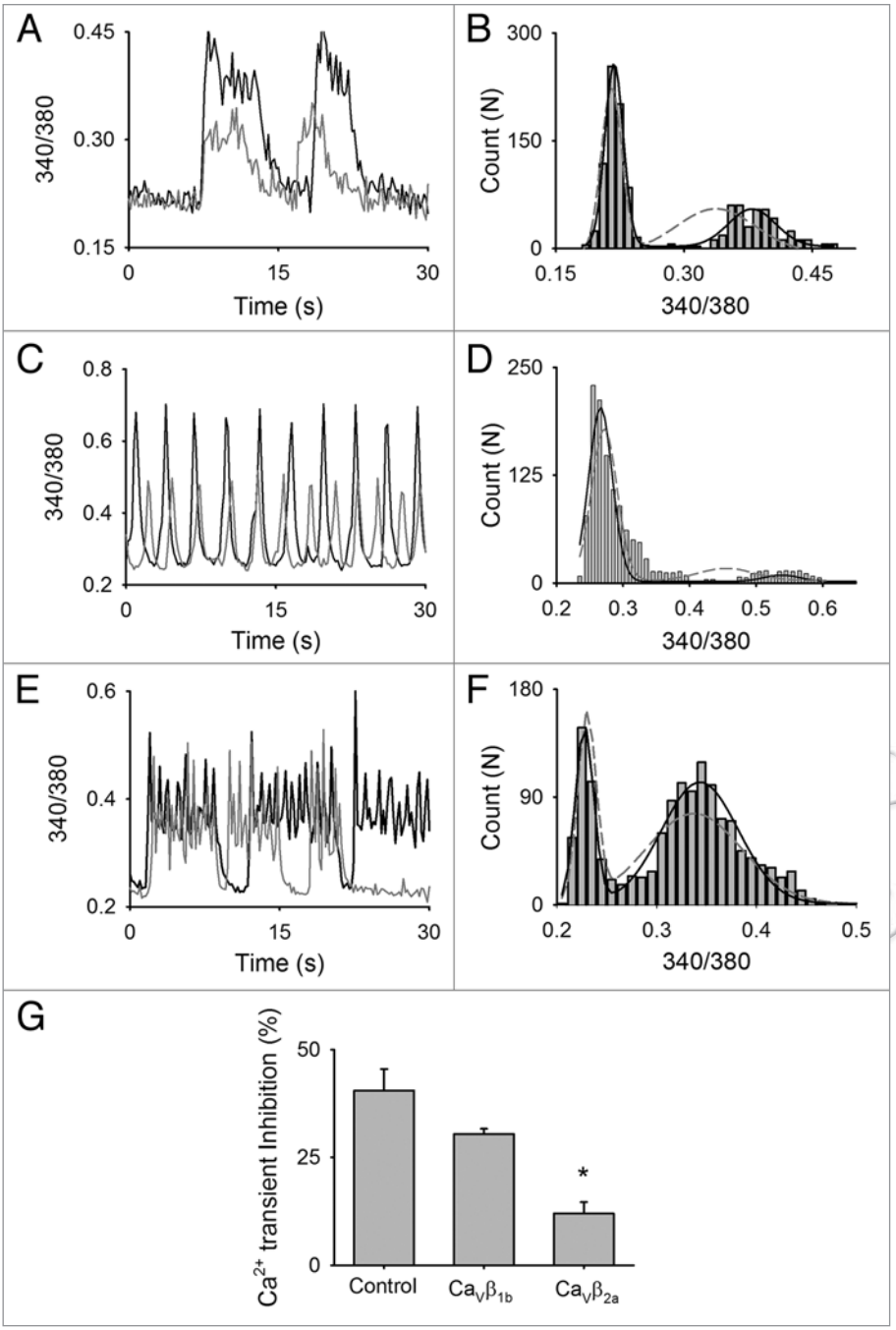

Figure 7. Calcium transients in rat cardiomyocytes decrease upon AngII treatment. Changes of cytosolic [Ca^{2+}] in spontaneous beating control (A); $Ca_V\beta_{1b}$-transfected (C) or $Ca_V\beta_{2a}$-transfected (E) cardiomyocytes loaded with Fura-2 (Ratio 340/380). To determine the magnitude of calcium transient, the fitting of two Gaussians to an all-points histogram (5 minutes duration) was used (B, D and F). The black line represents the best fitting before AngII, the gray line after AngII (G) Summary bar graph showing the percentage of inhibition of calcium transient amplitude after AngII (100 nM) exposure. n > 6, *p < 0.01.

100 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 95 sorbitol and 10 Hepes, pH 7.4, adjusted with Tris. Borosilicate glass pipettes were pulled and polished to 2–4-MΩ resistance and filled with internal solution contained (mM): 108 CsCl, 4 $MgCl_2$, 2 $CaCl_2$ 10 EGTA and 10 HEPES (pH 7.2 adjusted with CsOH). Data were acquired at room temperature using an Axopatch 200B amplifier and pClamp 8 software (Axon Instruments), low pass-filtered at 1 kHz and digitized at 10 kHz. Series resistance was compensated to 85%, leak currents were negligible. In all experiments the perforated-patch configuration was obtained by supplementing the pipette internal solution with Nystatin[39] to a final concentration of 800 μg/ml. Ramp protocols (-120 mV to +40 mV, 0.8 mV/ms every 15 sec) were used to monitor the gradual increase of the sodium current. After a stable current was achieved (≈10 min, access resistance (R_a) of 15 ± 8 MΩ) bath solution was replaced with a solution contained (mM): 20 $BaCl_2$, 1 $MgCl_2$, 10 HEPES, 40 TEA-Cl, 10 glucose and 65 CsCl (pH 7.2 adjusted with TEA-OH) changes in liquid junction potential were calculated,[40] and voltages corrected for.

Semi-quantitative PCR. Real-time semi-quantitative PCR was performed using a Strategene Mx300P thermal cycler (Stratagene). Briefly, cDNAs amplified out of total RNA from cardiomyocytes in culture at different days. PCR amplification of the GADPH RNA was used as internal control. PCR reactions were done with Brilliant SYBR Green according to the manufacturer's directions, primers used for qPCR were:

$Ca_V\beta_{1b}$ S: ATG GTC CAG AAG AGC GGC ATG TCC; AS: TTG ATG TGC AGG AAG TCC TTG GG

$Ca_V\beta_{2a}$ S: ATG CAG TGC TGC GGG CTG GTA C; AS: TCC GAA CTG CAA ATG CAA CAG G

$Ca_V\beta_3$ S: ATG TAT GAC GAC TCC TAC GTG CC; AS: TTG ACT CCA GAG CCC TGG ACT GG.

To confirm amplification specificity, PCR products were subjected to a melting curve program. Relative RNA amount was calculated with the ΔΔCt method and normalized with the amount of GADPH for each sample, measured in triplicates.

Calcium imaging. Plated cardiomyocytes were mounted in a perfusion chamber on the stage of an inverted microscope (Olympus IX-81, UPLFLN 40XO 40 x/1.3 oil-immersion objective). Cells were incubated with Fura-2 AM (Molecular Probes; 1 μM) and then superfused for 10–20 min with a solution contained (mM): 100 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 90 sorbitol, 5 glucose and 10 Hepes, pH 7.4, adjusted with Tris. AngII was applied by local perfusion system. Fura-2 was alternately excited

at 340 and 400 nm, and the fluorescence filtered at 510 nm was collected and recorded at 5 Hz using a CCD-based imaging system (Olympus DSU) running CellR software (Olympus). For every experiment, signals were recorded and the background intensity was subtracted, using a same-size region of interest outside the cells. Results are expressed as the ratio between the 340 nm and 400 nm (R340/400) signals.[41]

Reagents. All reagents were of analytical grade and were purchased from Sigma (USA) and Merck (Germany).

Statistical analysis. All results are presented as means ± SEM. Statistical analysis of the data was performed using Statgraphics Plus 5.0 (Statistical Graphics Corp.). Statistically significant differences between means were assessed with Student's t tests or a one-way ANOVA (Dunnett's method) and considered significant at $p < 0.05$.

Acknowledgments

This work was supported by Fondecyt 11080019 to D.V. and FONDAP-Fondecyt 15010006 to Andrés Stutzin, Chile, and by a Canadian Institutes of Health Research grant to Gerald W. Zamponi. Gerald W. Zamponi is a Scientist of the AHFMR and a Canada Research Chair.

References

1. Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol Rev 2000; 52:639-72.
2. Macrez-Lepretre N, Morel JL, Mironneau J. Angiotensin II-mediated activation of L-type calcium channels involves phosphatidylinositol hydrolysis-independent activation of protein kinase C in rat portal vein myocytes. J Pharmacol Exp Ther 1996; 278:468-75.
3. Quignard JF, Mironneau J, Carricaburu V, Fournier B, Babich A, Nurnberg B, et al. Phosphoinositide-3-kinase gamma mediates angiotensin II-induced stimulation of L-type calcium channels in vascular myocytes. J Biol Chem 2001; 276:32545-51.
4. Seki T, Yokoshiki H, Sunagawa M, Nakamura M, Sperelakis N. Angiotensin II stimulation of Ca^{2+}-channel current in vascular smooth muscle cells is inhibited by lavendustin-A and LY-294002. Pflugers Arch 1999; 437:317-23.
5. Guenther E, Hewig B, Zrenner E, Kohler K. Angiotensin II-induced inhibition and facilitation of calcium current subtypes in rat retinal ganglion cells. Neurosci Lett 1997; 231:71-4.
6. Ikegami H, Endoh T, Suzuki T. Angiotensin II-induced inhibition of calcium currents in hamster submandibular ganglion neurons. Neurosci Res 2001; 41:227-32.
7. Maturana AD, Casal AJ, Demaurex N, Vallotton MB, Capponi AM, Rossier MF. Angiotensin II negatively modulates L-type calcium channels through a pertussis toxin-sensitive G protein in adrenal glomerulosa cells. J Biol Chem 1999; 274:19943-8.
8. Allen IS, Cohen NM, Dhallan RS, Gaa ST, Lederer WJ, Rogers TB. Angiotensin II increases spontaneous contractile frequency and stimulates calcium current in cultured neonatal rat heart myocytes: insights into the underlying biochemical mechanisms. Circ Res 1988; 62:524-34.
9. De Mello WC, Monterrubio J. Intracellular and extracellular angiotensin II enhance the L-type calcium current in the failing heart. Hypertension 2004; 44:360-4.
10. Bkaily G, Sculptoreanu A, Wang S, Nader M, Hazzouri KM, Jacques D, et al. Angiotensin II-induced increase of T-type Ca^{2+} current and decrease of L-type Ca^{2+} current in heart cells. Peptides 2005; 26:1410-7.
11. Habuchi Y, Lu LL, Morikawa J, Yoshimura M. Angiotensin II inhibition of L-type Ca^{2+} current in sinoatrial node cells of rabbits. Am J Physiol 1995; 268:1053-60.
12. Aiello EA, Cingolani HE. Angiotensin II stimulates cardiac L-type Ca^{2+} current by a Ca^{2+}- and protein kinase C-dependent mechanism. Am J Physiol Heart Circ Physiol 2001; 280:1528-36.
13. Ichiyanagi O, Ishii K, Endoh M. Angiotensin II increases L-type Ca^{2+} current in gramicidin D-perforated adult rabbit ventricular myocytes: comparison with conventional patch-clamp method. Pflugers Arch 2002; 444:107-16.
14. Conforti L, Sumii K, Sperelakis N. Dioctanoyl-glycerol inhibits L-type calcium current in embryonic chick cardiomyocytes independent of protein kinase C activation. J Mol Cell Cardiol 1995; 27:1219-24.
15. Weiss S, Doan T, Bernstein KE, Dascal N. Modulation of cardiac Ca^{2+} channel by G_q-activating neurotransmitters reconstituted in Xenopus oocytes. J Biol Chem 2004; 279:12503-10.
16. Suh BC, Leal K, Hille B. Modulation of high-voltage activated Ca^{2+} channels by membrane phosphatidylinositol-4,5-bisphosphate. Neuron 2010; 67:224-38.
17. Liu L, Heneghan JF, Michael GJ, Stanish LF, Egertova M, Rittenhouse AR. L- and N-current but not M-current inhibition by M1 muscarinic receptors requires DAG lipase activity. J Cell Physiol 2008; 216:91-100.
18. Davies JN, Zamponi GW. Old proteins, developing roles: The regulation of calcium channels by synaptic proteins. Channels (Austin) 2008; 2:130-8.
19. Minor DL Jr, Findeisen F. Progress in the structural understanding of voltage-gated calcium channel (Ca_V) function and modulation. Channels (Austin) 2010; 4:459-74.
20. Yasuda T, Chen L, Barr W, McRory JE, Lewis RJ, Adams DJ, Zamponi GW. Auxiliary subunit regulation of high-voltage activated calcium channels expressed in mammalian cells. Eur J Neurosci 2004; 20:1-13.
21. Buraei Z, Yang J. The betas subunit of voltage-gated Ca^{2+} channels. Physiol Rev 2010; 90:1461-506.
22. Colecraft HM, Alseikhan B, Takahashi SX, Chaudhuri D, Mittman S, Yegnasubramanian V, et al. Novel functional properties of Ca^{2+} channel beta subunits revealed by their expression in adult rat heart cells. J Physiol 2002; 541:435-52.
23. Gomez JP, Potreau D, Branka JE, Raymond G. Developmental changes in Ca^{2+} currents from newborn rat cardiomyocytes in primary culture. Pflugers Arch 1994; 428:241-9.
24. Chien AJ, Carr KM, Shirokov RE, Rios E, Hosey MM. Identification of palmitoylation sites within the L-type calcium channel beta2a subunit and effects on channel function. J Biol Chem 1996; 271:26465-8.
25. Feng ZP, Arnot MI, Doering CJ, Zamponi GW. Calcium channel beta subunits differentially regulate the inhibition of N-type channels by individual Gbeta isoforms. J Biol Chem 2001; 276:45051-8.
26. Nabauer M, Callewaert G, Cleemann L, Morad M. Regulation of calcium release is gated by calcium current, not gating charge, in cardiac myocytes. Science 1989; 244:800-3.
27. Dolphin AC. Beta subunits of voltage-gated calcium channels. J Bioenerg Biomembr 2003; 35:599-620.
28. Miriyala J, Nguyen T, Yue DT, Colecraft HM. Role of Ca_Vbeta subunits and lack of functional reserve, in protein kinase A modulation of cardiac $Ca_V1.2$ channels. Circ Res 2008; 102:54-64.
29. Heneghan JF, Mitra-Ganguli T, Stanish LF, Liu L, Zhao R, Rittenhouse AR. The Ca^{2+} channel beta subunit determines whether stimulation of G_q-coupled receptors enhances or inhibits N current. J Gen Physiol 2009; 134:369-84.
30. Roberts-Crowley ML, Rittenhouse AR. Arachidonic acid inhibition of L-type calcium ($Ca_V1.3b$) channels varies with accessory Ca_Vbeta subunits. J Gen Physiol 2009; 133:387-403.
31. Link S, Meissner M, Held B, Beck A, Weissgerber P, Freichel M, Flockerzi V. Diversity and developmental expression of L-type calcium channel beta2 proteins and their influence on calcium current in murine heart. J Biol Chem 2009; 284:30129-37.
32. Chu PJ, Larsen JK, Chen CC, Best PM. Distribution and relative expression levels of calcium channel beta subunits within the chambers of the rat heart. J Mol Cell Cardiol 2004; 36:423-34.
33. Chen X, Nakayama H, Zhang X, Ai X, Harris DM, Tang M, et al. Calcium influx through $Ca_V1.2$ is a proximal signal for pathological cardiomyocyte hypertrophy. J Mol Cell Cardiol 2011; 50:460-70.
34. Schaub MC, Hefti MA, Harder BA, Eppenberger HM. Various hypertrophic stimuli induce distinct phenotypes in cardiomyocytes. J Mol Med 1997; 75:901-20.
35. Marrus SB, Nerbonne JM. Mechanisms linking short- and long-term electrical remodeling in the heart… is it a stretch? Channels (Austin) 2008; 2:117-24.
36. Nakayama H, Chen X, Baines CP, Klevitsky R, Zhang X, Zhang H, et al. Ca^{2+}- and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure. J Clin Invest 2007; 117:2431-44.
37. Harary I, Farley B. In vitro studies of single isolated beating heart cells. Science 1960; 131:1674-5.
38. Heron SE, Khosravani H, Varela D, Bladen C, Williams TC, Newman MR, et al. Extended spectrum of idiopathic generalized epilepsies associated with CACNA1H functional variants. Ann Neurol 2007; 62:560-8.
39. Horn R, Marty A. Muscarinic activation of ionic currents measured by a new whole-cell recording method. J Gen Physiol 1988; 92:145-59.
40. Barry PH. JPCalc, a software package for calculating liquid junction potential corrections in patch-clamp, intracellular, epithelial and bilayer measurements and for correcting junction potential measurements. J Neurosci Methods 1994; 51:107-16.
41. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985; 260:3440-50.