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Modulation of $Ca_v3.2$ T-type Ca^{2+} channels by protein kinase C

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Abstract **Although T-type Ca^{2+} channels have been implicated in numerous physiological functions, their regulations by protein kinases have been obscured by conflicting reports. We investigated the effects of protein kinase C (PKC) on $Ca_v3.2$ T-type channels reconstituted in Xenopus oocytes. Phorbol-12-myristate-13-acetate (PMA) strongly enhanced the amplitude of $Ca_v3.2$ channel currents (∼3-fold). The augmentation effects were not mimicked by 4α-PMA, an inactive stereoisomer of PMA, and abolished by preincubation with PKC inhibitors. Our findings suggest that PMA upregulates $Ca_v3.2$ channel activity via activation of oocyte PKC.**
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Key words: T-type calcium channel; α1H; Protein kinase C; Phorbol-12-myristate-13-acetate; Modulation

1. Introduction

T-type Ca^{2+} channels have been implicated in a wide range of physiological functions including neuronal firing, cardiac pacemaking, hormone secretion, muscle development and contraction, and fertilization (reviewed in [1]). Neuronal T-type channels are thought to contribute to many forms of neural excitability, such as low threshold spikes that trigger bursting firing of action potentials, rebound bursting following recovery from inactivation, and long-term potentiation [2,3]. Furthermore, abnormal overexpression of T-type channels has been implicated in animal models of epilepsy and cardiac hypertrophy [4–6].

Molecular cloning of three genes encoding T-type α1 subunits ($Ca_v3.1$, α1G; $Ca_v3.2$, α1H; $Ca_v3.3$, α1I) has provided the tools with which to study the distribution and regulation of T-type channels. The biophysical properties of expressed T-type channels were very similar to those of native T-type currents, suggesting the α1 subunit may function alone [1]. Among the three, the $Ca_v3.2$ showed nickel sensitive T-type currents with fast activating and inactivating kinetics [7]. Expression of $Ca_v3.2$ mRNA was broadly detected throughout the central nervous system, but also in peripheral organs including heart, kidney, liver, and adrenal gland [8,9]. Thus, $Ca_v3.2$ channels are thought to participate in neuronal excitability, myocardial pacemaking activity, smooth muscle contraction, hormone secretion, and muscle differentiation [1]. Regulation of T-type Ca^{2+} channels has been investigated to understand their relevance to those physiological functions. Although T-type channel activities are primarily regulated by dynamic changes in cell membrane potential, recombinant $Ca_v3.2$ channels have also been shown to be regulated by a number of diverse signaling molecules, such as G-proteins [10] and calmodulin-dependent protein kinase II [11].

The protein kinase C (PKC) family has been established to be a prominent modulator of ion channel activity. Binding of hormones or neurotransmitters to receptors can couple with activation of G_q-proteins. Activated G_q stimulates phospholipase C (PLC) which splits phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 3-phosphate (IP_3). The former activates PKC and the latter increases cytoplasmic calcium levels by calcium release from internal calcium stores [12]. Studies on PKC modulation of native T-type channels have produced conflicting results. Currents in rat neonatal ventricular myocytes were reported to be stimulated 30% by phorbol-12-myristate-13-acetate (PMA) or the related PKC stimulant, phorbol 12,13-dibutyrate (PdBu), while the inactive analog 4α-PdBu had no effect [13]. Studies on rat dorsal root ganglion (DRG) neurons found that PMA or 12-*O*-tetradecanoylphorbol-13-acetate (TPA), inhibited the T-type current by ∼30%, and again this effect was not observed with the inactive analog [14]. Inhibition of T-type currents by PKC stimulants has also been observed in NIH-3T3 fibroblasts [15]. Thus, we examined whether PKC can modulate human T-type $Ca_v3.2$ channels reconstituted in Xenopus oocytes. We report here that PMA, a strong stimulant of PKC, can strongly augment T-type $Ca_v3.2$ channel activities in Xenopus oocytes.

2. Materials and methods

2.1. Materials

4β-PMA, and calphostin C were obtained from Alexis (San Diego, CA, USA). Staurosporine, calphostin C, and chelerythrine were purchased from TOCRIS (Ballin, MO, USA). Most of the other chemicals used in this study were from Sigma (St. Louis, MO, USA).

2.2. Expression of the human $Ca_v3.2$ Ca^{2+} channel in Xenopus oocytes

The cloning of the human $Ca_v3.2$ cDNA was reported previously [8] and the $Ca_v3.2$ cDNA was subcloned into pGEM-HEA to improve its expression in Xenopus oocytes [7]. $Ca_v3.3$ cDNA was linearized at the 3′ end with *Afl*II. cRNA transcripts were synthesized in vitro using T7 RNA polymerase according to the supplier's protocol (Ambion, Austin, TX, USA).

Xenopus oocytes were prepared as described previously [16]. Briefly, ovary lobes were surgically removed from female *Xenopus*

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laevis (*Xenopus* One, Ann Arbor, MI, USA) and manually torn into small clusters of 5–10 oocytes in SOS solution (in mM: 100 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, 2.5 pyruvic acid, 50 μg/ml gentamicin, pH 7.6). The follicle membranes from oocytes were digested by agitating in Ca^{2+}-free OR2 solution (in mM: 82.5 NaCl, 2.5 KCl, 1 $MgCl_2$, 5 HEPES, pH 7.6) containing 2 mg/ml collagenase type 1A (Sigma, St. Louis, MO, USA). Each oocyte was injected with 1–5 ng of cRNA in a volume of 50 nl using a Drummond Nanoject pipette injector (Parkway, PA, USA) attached to a Narishige micromanipulator (Tokyo, Japan) under a dissecting microscope.

2.3. Electrophysiological recordings and data analysis

Barium currents (10 mM) were measured at room temperature 5–8 days after cRNA injection using a two-electrode voltage clamp amplifier (OC-725C, Warner Instruments, Hamden, CT, USA). Microelectrodes were pulled from borosilicate capillaries (Warner Instruments, Hamden, CT, USA) using a pipette puller. The electrode resistance was 0.2–1.0 MΩ after being filled with 3 M KCl. The Ba^{2+} bathing solution contained (in mM): 10 $Ba(OH)_2$, 90 NaOH, 1 KOH, 5 HEPES (pH 7.4 with methanesulfonic acid). The currents were normally sampled at 5 kHz and low pass filtered at 1 kHz using the pClamp system (Digidata 1320A and pClamp 8; Axon Instruments, Union City, CA, USA). Peak currents and exponential fits to currents were determined using Clampfit software (Axon instruments, Foster City, CA, USA) and graphing of the data was obtained using Prism software (GraphPad, San Diego, CA, USA). Statistical values are given as means ± S.E.M. Data were tested for significance using Student's paired or unpaired *t*-tests.

3. Results

We investigated PKC effects on human $Ca_v3.2$ T-type Ca^{2+} channels expressed in Xenopus oocytes. This expression system has been shown to be useful for studying the PKC modulation of recombinant Ca^{2+} channels [17]. Although oocytes have been reported to express endogenous high and low voltage-activated Ca^{2+} channels [18], we rarely detect any endogenous calcium channel activity in oocytes used for this study. In contrast, oocytes injected with $Ca_v3.2$ (α1H) cRNA led to the production of robust Ba^{2+} currents (> 500 nA) that could be measured using a two-electrode voltage clamp amplifier. Recorded currents displayed typical biophysical properties of T-type Ca^{2+} currents such as low voltage threshold for activation (about −60 mV), fast activation and inactivation current kinetics, and a criss-crossing pattern between current traces evoked by a voltage protocol. The current–voltage relationship also resembled that of native T-type channels, peaking at −20 mV during a voltage protocol, and displaying an apparent reversal potential around +40 mV.

To examine whether recombinant $Ca_v3.2$ T-type channels can be modulated by PKC, PMA (200 nM), a strong PKC stimulant, was applied to the chamber where an oocyte was located. Currents were evoked by a test potential to −30 mV from a holding potential of −90 mV every 15 s. Without any drug treatment, the current amplitude was stable with no significant run-up or run-down during the experimental period (20–30 min). Addition of 200 nM PMA led to an increase in current amplitude after a small lag (∼2 min; Fig. 1A). This lag is not due to solution exchange in the chamber, as control experiments with Ni^{2+} showed a lag of only 15 s [7]. PMA-induced stimulation of current amplitude continued for 20–30 min before reaching a maximum level, where they remained stable or sometimes ran down slowly (Figs. 1A and 3A). Representative traces before and 20 min after addition of 200 nM PMA effect were overlapped (Fig. 1B) for comparison, displaying that applied PMA strongly stimulated the amplitudes of T-type currents about 2.6-fold.

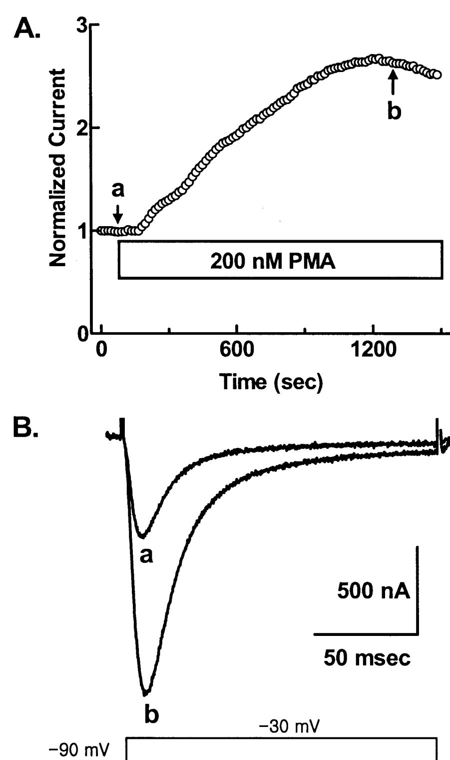

Fig. 1. Effects of PMA on human $Ca_v3.2$ channel currents. Recombinant $Ca_v3.2$ channels were expressed in Xenopus oocytes and their activity was measured using 10 mM Ba^{2+} as charge carrier. Test pulses were delivered every 15 s to a test potential of −30 mV from a holding potential of −90 mV. A: The time course of PMA's effect on peak current amplitude is plotted. B: Representative current traces taken before (a) and 20 min after (b) PMA treatment.

The effect of PMA on the $Ca_v3.2$ current–voltage (*I–V*) relationship was examined with depolarizing voltage steps in 10 mV increments from −70 to +40 mV from a holding potential of −90 mV. To control for variability in expression, the peak amplitudes measured during each voltage step were normalized to the maximum observed before addition of PMA (Fig. 2A). PMA stimulated current at all test potentials. To examine the effect of PMA on the *I–V* curve, the currents were also normalized to the maximum in each experimental condition (Fig. 2B). PMA appeared to induce a slight shift in the *I–V* towards more negative potentials. This effect can also be demonstrated by calculating the effect of PMA at each test potential (Fig. 2C). PMA stimulated currents recorded during test potentials to −60 mV and −50 mV by 5.3- and 3.7-fold, respectively (Fig. 2C), while currents recorded above −40 mV were stimulated significantly less (∼2.7-fold, $n = 10$, $P < 0.05$). Taken together, these data indicate that PMA produces a robust increase in $Ca_v3.2$ currents and induces a small negative shift in the current–voltage curve.

One of the questions raised from the above results is whether or not the enhancement effects of PMA were mediated by the activation of endogenous oocyte PKC. To address this question, we tested if 4α-PMA, the inactive stereoisomer of PMA, can mimic the stimulation effects of PMA on $Ca_v3.2$ currents. When 200 nM 4α-PMA was superfused onto oocytes expressing $Ca_v3.2$ channels, current amplitudes were not significantly altered (Fig. 3A).

Another way to test this hypothesis is to examine whether

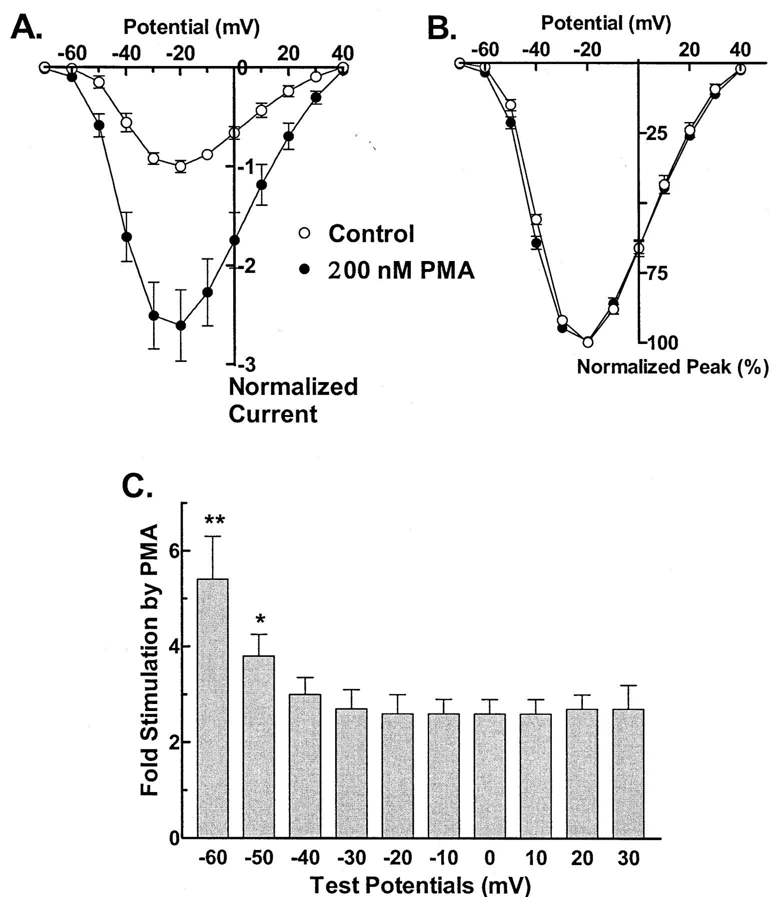

Fig. 2. Effect of PMA on the $Ca_v3.2$ current–voltage relationship. Peak currents elicited during test potentials to varying voltages were normalized to the maximum observed. A: Peak currents were normalized to the maximum observed before the application of PMA (control, open circles). Currents were also measured 20 min after exposure to 200 nM PMA, and normalized to the maximum observed under control conditions ($n = 10$). B: The data in A were normalized to the peak current observed in each experimental condition. C: From the same data we calculated the fold stimulation by PMA at each test potential. Significant differences where $P < 0.01$ are noted with **, while those at the $P < 0.05$ are noted with *.

PKC inhibitors, such as staurosporine, calphostin, or chelerythrine, are capable of abrogating the PMA response. To ensure complete inhibition of PKC, oocytes were preincubated for 5–10 h in the presence of one of the PKC inhibitors, 200 nM staurosporine, 200 nM calphostin, or 500 nM chelerythrine. Oocytes were then voltage clamped and the response to 200 nM PMA was measured. All three PKC inhibitors totally abolished the ability of PMA to stimulate current amplitudes (Fig. 3B,C, $n = 4$–5). These data provide strong support for the hypothesis that PMA stimulated recombinant T-type $Ca_v3.2$ currents via activation of endogenous oocyte PKC.

The effects of PMA on the biophysical properties of $Ca_v3.2$ channels were examined by comparing activation curves, steady-state inactivation curves, and activation and inactivation time constants. As detected in the I–V relationships, PMA shifted the activation curve toward the negative direction by 2.5 mV ($V_{50} = -36.61 \pm 0.78$ vs. -39.11 ± 0.72 V), which was statistically significant ($n = 10$, $P < 0.05$; Fig. 4A). Similarly, PMA also shifted the steady-state inactivation by -3 mV ($V_{50} = -61.1 \pm 0.5$ vs. -64.2 ± 0.49 V), which was statistically significant ($n = 10$, $P < 0.05$; Fig. 4B). Effects on current kinetics were evaluated by fitting the inward currents with two exponentials, where one corresponds to channel activation and the other inactivation. Despite differences in clamp speed, the values obtained from oocytes are very similar to those obtained during patch clamp recording of either native or recombinant T-type channels expressed in 293 cells (reviewed in [1]). In all cases, T-type channels activate and inactivate slowly near threshold (-50 mV), but both kinetics are faster at more depolarized potential, reaching apparent plateaus around 0 mV (Fig. 4C,D). PMA slowed activation kinetics at threshold voltages, with no effect on the voltage-independent value. In contrast, PMA-stimulated currents inactivated faster at all test potentials measured.

4. Discussion

Studies on PKC modulation of native T-type channels have reported conflicting results [13,19,20]. The recent cloning of T-type channels allowed us to re-examine this issue in the absence of background contaminating currents, and in a recording configuration that does not disturb the intracellular milieu. We report here that the $Ca_v3.2$ T-type calcium channels expressed in Xenopus oocytes can be potently modulated by the PKC stimulator PMA, as evidenced by: (i) robust increments of current amplitudes, (ii) lower threshold for $Ca_v3.2$ channel activation, (iii) modifications of activation

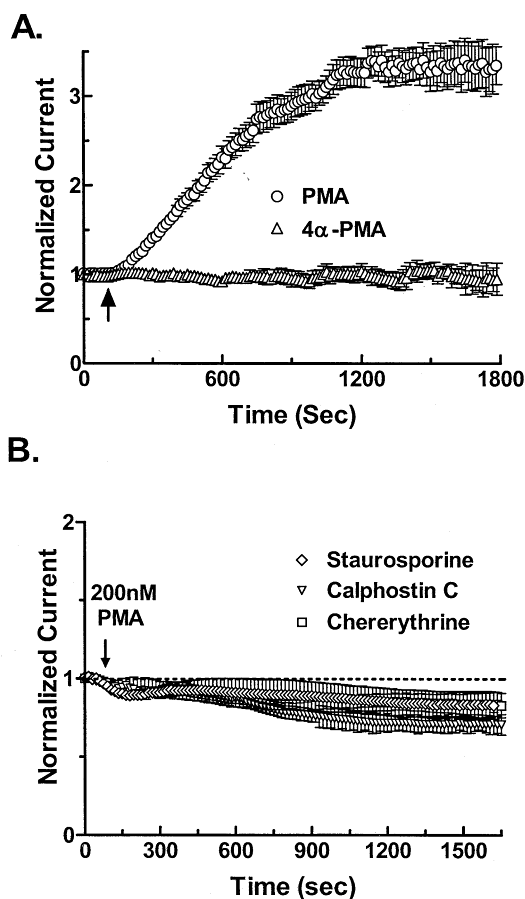

Fig. 3. Selectivity of the PMA response. A: Time course showing the responses to the stereoisomers of PMA. In contrast to PMA, which strongly augmented $Ca_v3.2$ currents, the inactive isomer of PMA, 4α-PMA, did not augment currents. B: Time course of PMA effects with oocytes preincubated with various PKC inhibitors. Oocytes expressing $Ca_v3.2$ channels were incubated for 5–10 h in SOS solution including either 200 nM staurosporine, 200 nM calphostin C, or 500 nM chelerythrine. The currents measured from these oocytes were normalized to the initial current amplitudes, and then averaged ($n = 4$–5).

and inactivation time constants, and (iv) a negative shift in the activation and steady-state inactivation curves. The stimulatory effects were not mimicked by application of 4α-PMA, an inactive isomer of PMA, and abolished after preincubation of PKC inhibitors. These results illustrate a new regulation pattern for T-type channels where PKC activation leads to potent upregulation of calcium entry through $Ca_v3.2$ channels. PKC has also been reported to upregulate recombinant high voltage-activated Ca^{2+} channels that contain either $Ca_v1.2$, $Ca_v1.3$, $Ca_v2.2$, or $Ca_v2.3$ α1 subunits [21–24].

The ability of PMA to shift the activation curve suggests that PKC-dependent regulation would be more effective at low potentials around resting membrane potentials. Similarly, calmodulin-dependent protein kinase II is also capable of stimulating recombinant $Ca_v3.2$ activity although to a lesser extent (30% at the peak of the *I–V*), and induces a shift in the *I–V* to more negative potentials [25]. Modulation of threshold currents may be of particular relevance to the ability of $Ca_v3.2$ channels to regulate basal calcium levels via window currents [26,27].

The whole cell current is a function of the number of channels in plasma membrane, their probability of opening, and their single channel conductance. PMA produced a profound effect on current amplitude, with only modest effects on kinetics, suggesting that the primary effect of PMA is to increase the apparent number of channels that open during test depolarizations. Two likely mechanisms for this effect are an increase in the number of channels in the plasma membrane, or an increase in their probability of opening. PKC activation has been reported to alter the structure of the plasma membrane of Xenopus oocytes, leading to a reduction in total cell capacitance [28] (but see [17]). This would lead to a decrease in T-type channel currents, rather than an increase. Studies on the calmodulin-dependent kinase II regulation of native $Ca_v3.2$ channels in bovine glomerulosa cells found that stimulation was caused by a decrease in the number of blank sweeps, with no change in single channel conductance, open time distributions, or burst lengths [29]. Therefore it is likely that PKC phosphorylation of $Ca_v3.2$ channels enhances their ability to respond to test depolarizations, although single channel studies are required to establish this mechanism.

One of the questions raised by our findings is whether the PKC-dependent augmentation was dependent on the expression system. To address this question, we tested whether PKC-dependent upregulation could be observed in mammalian cell lines (293 or COS cells) transfected with $Ca_v3.2$ cDNA. PMA application (200 nM) did not modulate $Ca_v3.2$ currents in these cells in the whole cell recording mode (results not shown). Therefore, as observed with native T-type channels, PKC regulation is dependent on the cell type used. Similarly, PKA-dependent modulation of $Ca_v1.2$ varies between cell types with no regulation in tracheal smooth muscle but robust regulation in cardiac muscle, and regulation has been difficult to recapitulate with recombinant channels (reviewed in [30]). Coexpression of an A-kinase anchor protein (AKAP) with recombinant L-type channels has been shown to restore PKA-dependent stimulation of $Ca_v1.1$ and $Ca_v1.2$ channels [31,32]. Similarly, PKC anchoring proteins called RACKs (receptors for activated C kinases) were also shown to localize PKC isoforms in the proximity to ion channels [32]. These precedent findings suggest the possibility that regulation of $Ca_v3.2$ channel activity by PKC might be dependent on the differential expression of RACKs between different cell types.

Alternatively divergent regulatory patterns of T-type Ca^{2+} channels might be due to one or more of the following factors: (i) presence or absence of specific PKC isoforms; (ii) presence of specific T-type channel isoforms; (iii) unidentified regulatory protein(s) such as RACKs; and (iv) differential expression of as yet to be identified auxiliary subunits of T-type channels. Among these possibilities, we would like to rule out the second one because both the rat $Ca_v3.1$ and $Ca_v3.3$ measured in Xenopus oocytes were also potently stimulated by PMA (Park and Lee, unpublished data). Taken together, this study provides strong evidence that T-type currents can be potently regulated by PKC in Xenopus oocytes. Future studies will examine the mechanisms for this regulation and why they differ between cell types, which may provide insights into the physiological role of T-type channels.

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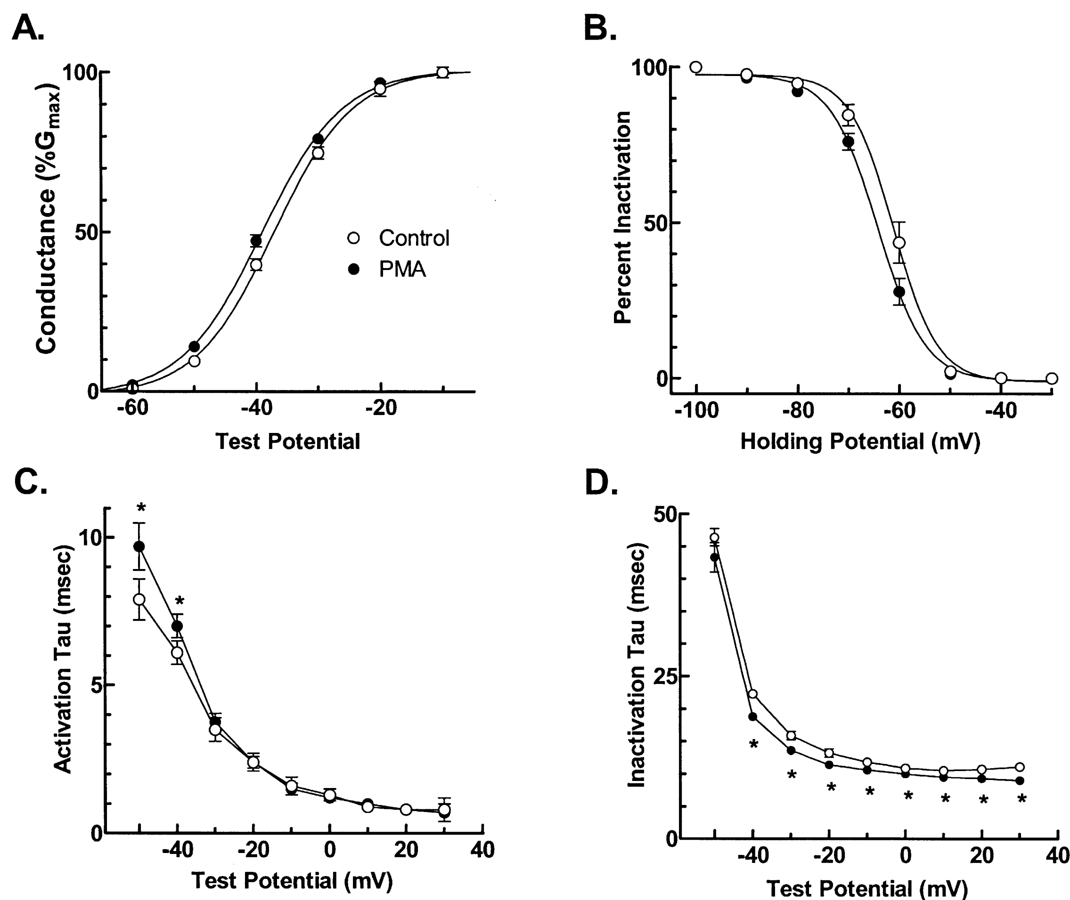

Fig. 4. Effect of PMA on the biophysical properties of $Ca_v3.2$ currents. Activation curves were obtained by the chord conductance method, where conductance (G) was calculated by dividing the current amplitude by the driving force (observed reversal potential minus the test potential). The data for each cell were normalized to the maximum observed in that cell. Smooth curves represent the fit to the data with the Boltzmann equation ($G = 1/[1+\exp(V_{50}-V)/k]$), where V_{50} is the half-activation voltage, and k is a slope factor. B: The effect of 200 nM PMA on steady-state inactivation curves. Channel availability was tested during voltage steps to −20 mV after 10 s prepulses to potentials varying between −100 mV and −20 mV in 10 mM increments. The data were normalized to the maximum current observed after prepulse to −100 mV, averaged, and fit with the same Boltzmann equation as in A. C and D: The activation and inactivation taus of the current traces were obtained by fitting the current traces with two exponentials simultaneously, one of which is for activation kinetics (C) and the other is for inactivation kinetics (D). Significant differences at the $P < 0.05$ are marked with *.

References

[1] Perez-Reyes, E. (2003) Physiol. Rev. 83, 117–161.
[2] Huguenard, J.R. (1996) Annu. Rev. Physiol. 58, 329–348.
[3] Song, D., Wang, Z. and Berger, T.W. (2002) Hippocampus 12, 689–697.
[4] Tsakiridou, E., Bertollini, L., de Curtis, M., Avanzini, G. and Pape, H.C. (1995) J. Neurosci. 15, 3110–3117.
[5] Su, H., Sochivko, D., Becker, A., Chen, J., Jiang, Y., Yaari, Y. and Beck, H. (2002) J. Neurosci. 22, 3645–3655.
[6] Martínez, M.L., Heredia, M.P. and Delgado, C. (1999) J. Mol. Cell. Cardiol. 31, 1617–1625.
[7] Lee, J.H., Gomora, J.C., Cribbs, L.L. and Perez-Reyes, E. (1999) Biophys. J. 77, 3034–3042.
[8] Cribbs, L.L., Lee, J.H., Yang, J., Satin, J., Zhang, Y., Daud, A., Barclay, J., Williamson, M.P., Fox, M., Rees, M. and Perez-Reyes, E. (1998) Circ. Res. 83, 103–109.
[9] Talley, E.M., Cribbs, L.L., Lee, J.H., Daud, A., Perez-Reyes, E. and Bayliss, D.A. (1999) J. Neurosci. 19, 1895–1911.
[10] Wolfe, J.T., Wang, H., Howard, J., Garrison, J.C. and Barrett, P.Q. (in press) Nature.
[11] Wolfe, J.T., Wang, H., Perez-Reyes, E. and Barrett, P.Q. (2002) J. Physiol. (Lond.) 538, 343–355.
[12] Newton, A.C. (2003) Biochem. J. 370, 361–372.
[13] Furukawa, T., Ito, H., Nitta, J., Tsujino, M., Adachi, S., Hiroe, M., Marumo, F., Sawanobori, T. and Hiraoka, M. (1992) Circ. Res. 71, 1242–1253.
[14] Schroeder, J.E., Fischbach, P.S. and McCleskey, E.W. (1990) J. Neurosci. 10, 947–951.
[15] Pemberton, K.E., Hill-Eubanks, L.J. and Jones, S.V. (2000) Pflügers Arch. 440, 452–461.
[16] Lee, J.H., Daud, A.N., Cribbs, L.L., Lacerda, A.E., Pereverzev, A., Klöckner, U., Schneider, T. and Perez-Reyes, E. (1999) J. Neurosci. 19, 1912–1921.
[17] Singer-Lahat, D., Gershon, E., Lotan, I., Hullin, R., Biel, M., Flockerzi, V., Hofmann, F. and Dascal, N. (1992) FEBS Lett. 306, 113–118.
[18] Lacerda, A.E., Perez-Reyes, E., Castellano, A. and Brown, A.M. (1994) Biophys. J. 66, 1833–1843.
[19] O'Dell, T.J. and Alger, B.E. (1991) J. Physiol. (Lond.) 436, 739–767.
[20] Tseng, G.N. and Boyden, P.A. (1991) Am. J. Physiol. Heart Circ. Physiol. 261, H364–H379.
[21] Blumenstein, Y., Kanevsky, N., Sahar, G., Barzilai, R., Ivanina, T. and Dascal, N. (2002) J. Biol. Chem. 277, 3419–3423.
[22] Scholze, A., Plant, T.D., Dolphin, A.C. and Nurnberg, B. (2001) Mol. Endocrinol. 15, 1211–1221.
[23] Cooper, C.B., Arnot, M.I., Feng, Z.P., Jarvis, S.E., Hamid, J. and Zamponi, G.W. (2000) J. Biol. Chem. 275, 40777–40781.

[24] Stea, A., Soong, T.W. and Snutch, T.P. (1995) Neuron 15, 929–940.
[25] Wolfe, J.T., Wang, H., Perez-Reyes, E. and Barrett, P.Q. (2002) J. Physiol. (Lond.) 538, 343–355.
[26] Chemin, J., Monteil, A., Briquaire, C., Richard, S., Perez-Reyes, E., Nargeot, J. and Lory, P. (2000) FEBS Lett. 478, 166–172.
[27] Bijlenga, P., Liu, J.H., Espinos, E., Haenggeli, C.A., Fischer-Lougheed, J., Bader, C.R. and Bernheim, L. (2000) Proc. Natl. Acad. Sci. USA 97, 7627–7632.
[28] Vasilets, L.A., Schmalzing, G., Madefessel, K., Haase, W. and Schwarz, W. (1990) J. Membr. Biol. 118, 131–142.
[29] Barrett, P.Q., Lu, H.K., Colbran, R., Czernik, A. and Pancrazio, J.J. (2000) Am. J. Physiol. Cell Physiol. 279, C1694–C1703.
[30] Hofmann, F., Biel, M. and Flockerzi, V. (1994) Annu. Rev. Neurosci. 17, 399–418.
[31] Kamp, T.J. and Hell, J.W. (2000) Circ. Res. 87, 1095–1102.
[32] Mochly-Rosen, D. and Gordon, A.S. (1998) FASEB J. 12, 35–42.