Long lasting facilitation of the rabbit cardiac Ca^{2+} channel: correlation with the coupling efficiency between charge movement and pore opening

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Abstract Facilitation of Ca^{2+} entry into cells enhances Ca^{2+}-activated events such as transmitter release and stimulation of second messenger systems. We have found a long lasting prepulse facilitation (up to 3-fold) of the cardiac Ca^{2+} channel $\alpha_{1C}\beta_{1b}$ that lasts for tens of seconds without altering current kinetics. The voltage- and time-dependence of the installation of facilitation was characterized as well as the time- and use-dependence of the decay of facilitation. The degree of facilitation was correlated with the coupling efficiency between the charge movement and pore opening channels that were poorly coupled prior to facilitation exhibited the largest facilitation.

Key words: L-type Ca^{2+} channel; β subunit; Facilitation; Charge movement

1. Introduction

High voltage-activated (HVA) Ca^{2+} channels consist of a pore forming α_1 subunit and accessory β, α_2-δ, and γ subunits. There are at least six different classes of mammalian HVA Ca^{2+} channel α_1 subunits, and at least four different classes of β subunits, the classifications are based on the gene that encodes the subunit [1]. The α_{1C} channel is found in the heart, brain, aorta, lung and in hormone secreting cells including adrenal chromaffin cells. The α_{1C} subunit can function alone as a Ca^{2+} channel, although the coexpression of accessory subunits greatly affects the magnitude and kinetics of expressed currents [2,3]. The β subunit has been shown to confer specific changes to α_1 channel activation [4] and inactivation depending on the identity of the β subunit that is coexpressed [5].

The facilitation of HVA Ca^{2+} currents by a depolarizing prepulse has been reported in numerous preparations including adrenal chromaffin cells [6], sympathetic neurons [7], cerebral cortical neurons [8], dorsal root ganglion neurons [9], hippocampal neurons [10], and in cardiac and skeletal muscle [11,12] (for a recent review see [13]). Cloned cardiac and smooth muscle α_{1C} channels exhibit facilitation up to 3.5-fold [14,15]. Facilitation of α_{1C} cardiac channels is phosphorylation dependent and short lived with exponential time constants of decay of 12 and 100 milliseconds. Facilitation of neuronal and cardiac $\alpha_{1C}+\beta_{1b}$ channels has been reported to be phosphorylation dependent [16], and dependent on the identity of the β subunit; coexpression of the β_1, β_3 or β_4 subunits cause facilitation while the β_2 subunit does not [17]. We expand on these findings and report a very long lasting facilitation of the $\alpha_{1C}+\beta_{1b}$ channel with exponential time constants of decay of 3 and 20 s. The facilitation varies in magnitude up to a 3-fold increase in currents, and is correlated with the coupling efficiency measured as the ratio between the limiting charge movement and the magnitude of the ionic current, poorly coupled channels exhibit the greatest facilitation.

2. Materials and methods

2.1. Molecular biology and oocyte preparation

The cRNAs were prepared from two plasmids bearing the *a*-splice variant of the rabbit cardiac type C or α_{1C} (α_{1C-a}) and the rat brain type 1b β subunit here referred to simply as α_{1C} and β_{1b}. α_{1C} cDNA was digested with *Hin*dIII as described [18] and β cDNA with *Not*I [19]. cRNAs of β_{1b} subunits and of cardiac α_{1C} subunits were synthesized in vitro by T7 RNA polymerase using mMessage mMachine kit (Ambion) at 37°C for 1–2 h. After two LiCl precipitations, the cRNAs were suspended at a concentration of 0.5 mg/ml and then 50–75 nl were injected into *Xenopus* oocytes 5–12 days before electrophysiological recordings.

Xenopus frogs were anesthetized by immersion in water containing 0.15% tricane methanesulphonate (Sigma) for about 20 min until the animal was fully immobile. The ovaries were surgically removed under sterile conditions by abdominal incision and the animal killed by decapitation. Animal protocols were performed with the approval of the Institutional Animal Care Committee of the University of California, Los Angeles. Oocytes were maintained at 19.5°C in a modified Barth's solution containing (in mM) 90 NaCl, 2 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 50 μg/ml gentamicin, titrated to pH 7.2 with NaOH. Twenty four hours prior to injection, oocytes were defolliculated by collagenase treatment (type I, 2 mg/ml, Sigma) for 40 min at room temperature.

2.2. Electrophysiology

The cut open oocyte voltage clamp technique was used to measure currents as described previously [20]. To minimize the endogenous Ca^{2+}-activated Cl^- currents, oocytes were injected with 35–70 μl of 50 mM BAPTA $(Na)_4$[1,2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetate] (RBI) before recording. The bath solution contained (in mM) 10 Ba^{2+}, 96 Na^+, 0.1 ouabain, and 10 HEPES titrated to pH 7.0 with methanesulfonic acid (CH_3SO_3H) (Aldrich). The oocyte interior solution was 110 mM potassium glutamate and 10 mM

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Abbreviations: HVA, high voltage-activated; BAPTA, $(Na)_4$[1,2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetate]

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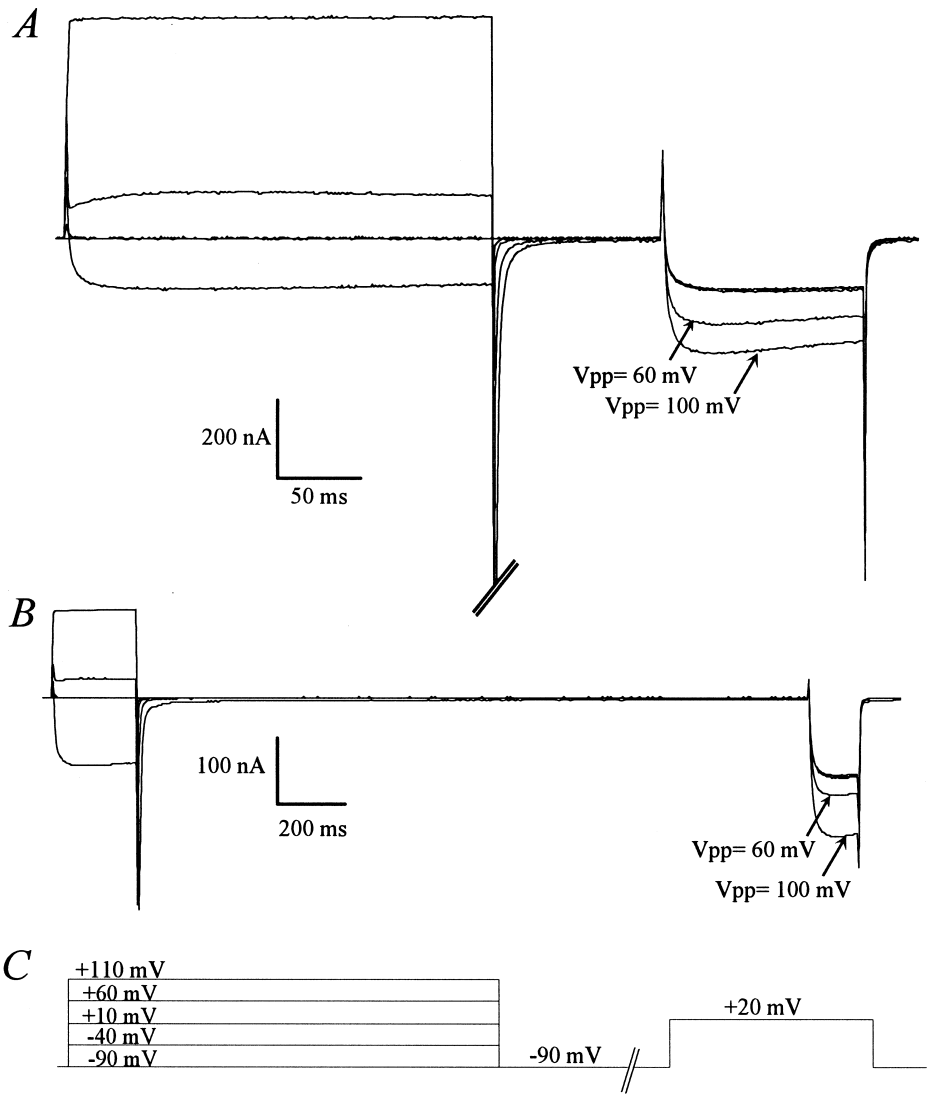

Fig. 1. Voltage-dependent facilitation of the $\alpha_{1C}+\beta_{1b}$ channel. A: The inter-pulse interval (i.p.i.) is 100 ms. B: The i.p.i. is 2 s. C: The voltage pulse protocol used to measure facilitation in A, the holding potential is −90 mV, the prepulse voltages (V_{pp}) are shown. The i.p.i. is lengthened to obtain the traces in B.

HEPES titrated to pH 7.0 with KOH. The voltage recording electrode was filled with 3 M NaCl and had a resistance of 0.1–0.5 mΩ. pClamp software (Axon Instruments) was used for collection and analysis of current recordings.

2.3. Determination of coupling efficiency and fractional facilitation

Coupling efficiency between the charge movement and the ionic current was determined by measuring the integral of the gating current at the reversal potential for Ba^{2+} and dividing the value obtained by the magnitude of the ionic current measured at 30 mV, 40 ms after pulse onset. The value of the charge movement at the Ba^{2+} reversal potential, measured between 40 mV and 60 mV, was close to its limiting value as seen in the charge-voltage curve [21]. Fractional facilitation (Fig. 2C) was calculated by dividing the amount of facilitation measured at each prepulse duration by the maximal facilitation obtained at longer prepulse durations.

3. Results

3.1. $\alpha_{1C}+\beta_{1b}$ channels exhibited a large magnitude time- and voltage-dependent facilitation

Fig. 1A and B show facilitation of $\alpha_{1C}+\beta_{1b}$ channels expressed in *Xenopus* oocytes using 10 mM Ba^{2+} as the charge carrier. The inter-pulse interval was 100 ms in Fig. 1A and 2 s in Fig. 1B, demonstrating the long lasting nature of the facilitation. Currents were facilitated by 2.6- and 1.8-fold in Fig. 1A and B, respectively.

The time- and voltage-dependence of the installation of facilitation was studied and the results are shown in Fig. 2. The time-dependence was determined by delivering a prepulse to 110 mV, increasing the duration with subsequent pairs of pulses, while keeping the inter-pulse interval fixed at one second (Fig. 2A–C). Prepulse induced currents are shown in Fig. 2A; the facilitation of the inward tail currents occurred with increasing prepulse duration. Corresponding test-pulse currents are in Fig. 2B; the first test-pulse current has no prepulse, subsequent currents have prepulses with incrementing duration. Due to the long lasting nature of the facilitation we delivered each pair of pulses once per minute. The time-dependence of the installation of facilitation is plotted in Fig. 2C; data are from three different oocytes. Increasing the duration of the prepulse caused increased facilitation, prepulse

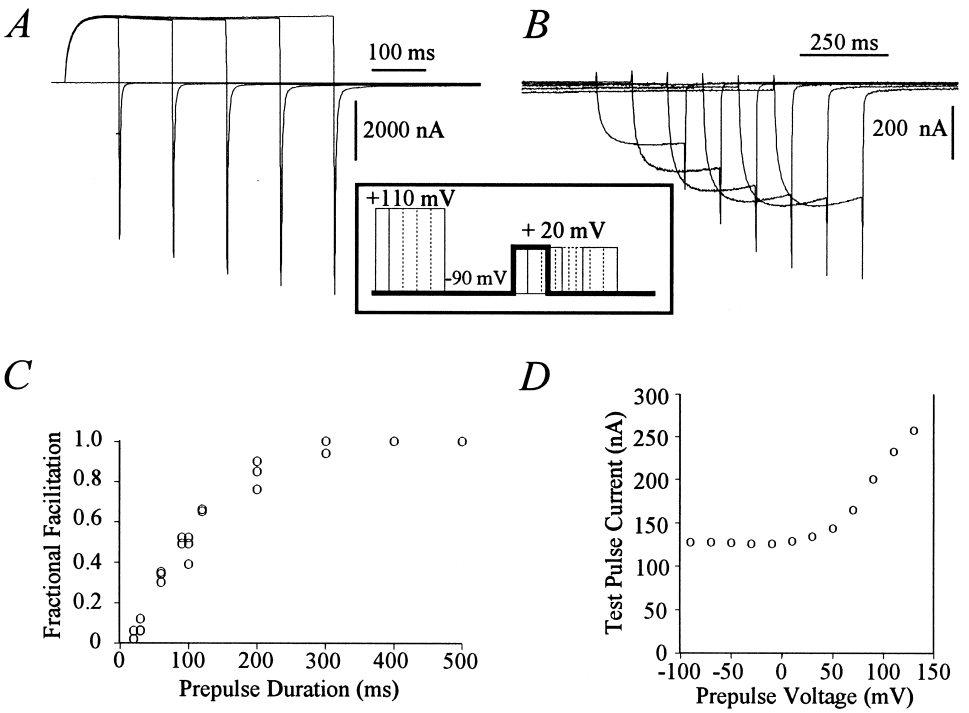

Fig. 2. Time- and voltage-dependence of facilitation. A: Prepulse currents to 110 mV with increasing durations. B: Test-pulse currents to 20 mV elicited by the voltage step protocol shown in the inset. The i.p.i. was maintained constant at 1 s. C: Plot of fractional facilitation versus prepulse duration. D: The voltage-dependence of facilitation.

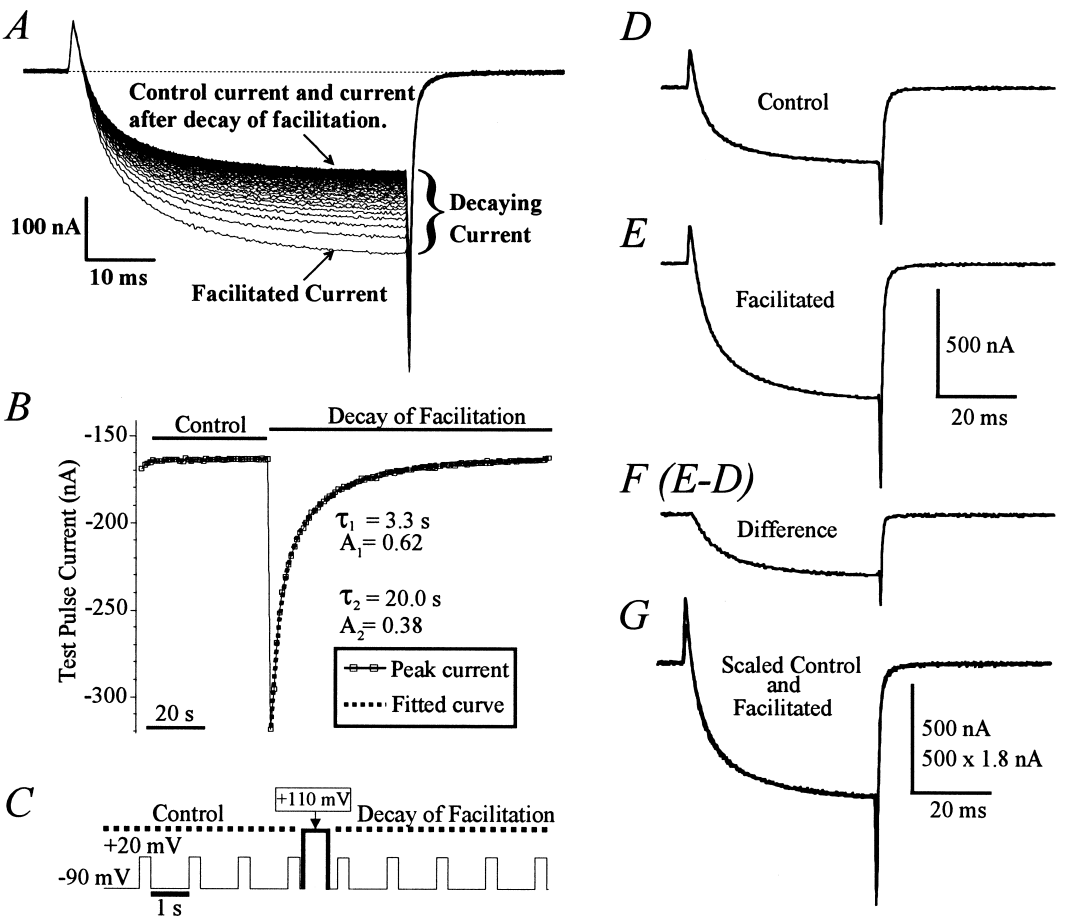

Fig. 3. Time course of the decay of facilitation. A: Control, facilitated and current during the decay of facilitation. B: Plot of peak currents versus time. C: Voltage pulse protocol, a single facilitating pulse is delivered during a train of 1 Hz test-pulses. D: Current prior to facilitation. E: Facilitated current. F: The difference current obtained by subtracting D from E. G: The current kinetics are unchanged by facilitation.

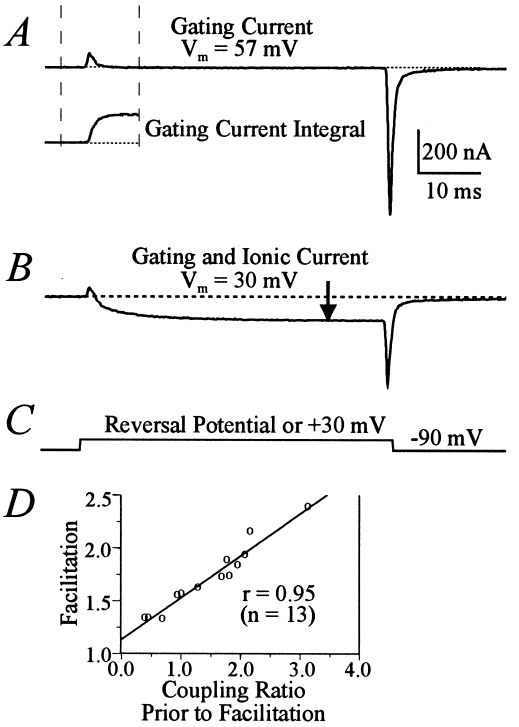

Fig. 4. The magnitude of the facilitation depends on the coupling efficiency between the charge movement and ionic current. A: The gating current measured at the ionic reversal potential, the measurement of the gating current integral (inset). B: The gating and ionic currents measured at 30 mV, the arrow indicates 40 ms after pulse onset. C: Voltage pulse protocol. D: Plot of facilitation versus the coupling ratio (efficiency). The correlation coefficient is 0.95.

durations longer than 300 ms did not further increase facilitation. The voltage-dependence of facilitation was studied by delivering a 250 ms prepulse to different voltages, a 100 ms inter-pulse interval to −90 mV, followed by a 100 ms test-pulse (to 20 mV). Fig. 2D plots the results from a typical oocyte, facilitation begins to occur with test-pulses above 10 mV and increases through 130 mV, we were unable to obtain values at prepulse voltages higher than this due to the appearance of persistent tail currents.

3.2. The $\alpha_{1C}+\beta_{1b}$ facilitation is extremely long lasting and does not alter the kinetics of the currents

The long lasting nature of the facilitation was investigated by delivering a train of test-pulses to 20 mV at a frequency of 1 Hz (Fig. 3A–C). The test-pulse train in itself did not change the magnitude or kinetics of the current. Test-pulse currents were then facilitated by delivering a single voltage pulse to 110 mV during the train of test-pulses (Fig. 3C). The long lasting nature of the facilitation was quantified by measuring the decay of the peak currents after the facilitating pulse. A typical experiment is shown here, current traces are displayed in Fig. 3A and a plot of the peak currents measured during a stable control period and during the peak, and decay, of facilitation (Fig. 3B). The line fitted to the data points is the sum of two exponential curves with time constants of 3.3 and 20.0 s. The decay of facilitation was not use dependent, if a single test-pulse was delivered 1 min after the facilitating pulse was delivered, there was no facilitation.

The kinetics of the ionic current during the test-pulse to 20 mV were not altered by the facilitation; control and facilitated currents from the same experiment as in Fig. 3A are plotted in Fig. 3D and E, respectively. Subtraction of the control current from the facilitated current resulted in the difference current (Fig. 3F), this is the current that was added to the test-pulse current by the 110 mV prepulse. Fig. 3G replots the traces from Fig. 3A and B with the control current scaled to match the facilitated current, the two traces are indistinguishable in terms of the kinetics of the ionic current during the first 50 ms of activation.

3.3. The magnitude of the facilitation is dependent on the initial coupling efficiency of the voltage sensor to the ionic channel

During the course of performing these experiments the degree of facilitation varied from a 1.2- to a 3.0-fold increase in the magnitude of ionic currents. The amount of facilitation that occurred, appeared to be dependent on the relative sizes of the ionic current compared to the gating current. The $\alpha_{1C}+\beta_{1b}$ currents that had small ionic currents relative to the gating current facilitated to a larger degree. This was studied in more detail by quantifying the size of the gating current relative to the ionic current (i.e. the coupling efficiency) for each oocyte at the beginning of each experiment. In $\alpha_{1C}+\beta_{1b}$ injected oocytes ($n=13$) from three different *Xenopus* frogs, we measured the size of the ionic current at 30 mV as well as the gating current at the reversal potential for the ionic current. The integral of the gating current was measured and divided by the magnitude of the ionic current at 30 mV to obtain the ratio (coupling efficiency) prior to facilitation. Fig. 4A and B are an example of the analysis. Facilitation was measured using a 250 ms prepulse to 110 mV, a 200 ms inter-pulse potential, and a test-pulse to 20 mV. The results are plotted in Fig. 4D ($n=13$), the size of the coupling ratio prior to facilitation was correlated to the amount of facilitation that was obtained. Thus, the $\alpha_{1C}+\beta_{1b}$ currents that were poorly coupled, with relatively small ionic currents, had a larger degree of facilitation.

4. Discussion

Ca^{2+} entry into cells following membrane depolarization triggers many important cytosolic events, including activation of second messengers and vesicle secretion. The transduction of the electrical signal, into the chemical signal of a rise in intracellular Ca^{2+}, is largely achieved by HVA Ca^{2+} channels. Facilitation of these channels occurs after a strong depolarization, more Ca^{2+} is admitted for each subsequent depolarization until the facilitation decays. Thus, facilitation is a temporal summation of strong depolarizing events. The α_{1C} subunit expressed alone has been reported to exhibit a short lived facilitation that decays exponentially with time constants of 12 and 100 ms [14]. The long lasting facilitation we report here decays exponentially with time constants of 3 and 20 s, rendering the $\alpha_{1C}+\beta_{1b}$ channel capable of temporally summating strong depolarizing events over a 200-fold longer period of time.

HVA Ca^{2+} channels have been shown to be modulated by several different means including phosphorylation and dephosphorylation, G-proteins and voltage. These systems do not necessarily work independently, there may be some degree of cross-talk and redundancy in the modulation of Ca^{2+} channels. A fine tuning of the amount of Ca^{2+} that is admitted

into a cell could be achieved by cross-talk of second messenger systems in the cytoplasm or membrane, or at the level of the ion channel protein itself. We believe that the correlation between the facilitation and the coupling efficiency may be an indication that the α_{1C}+β_{1b} channel has a self regulating mechanism that senses the modulatory state of the cell via the channel itself. The coupling efficiency is a measure of the regulatory state of the channel before facilitation. Although we do not know the mechanism that is responsible for altering the levels of the coupling efficiency, we were, nevertheless, able to estimate the degree of facilitation to expect in each oocyte prior to facilitation. We believe that the channel protein can structurally sense the modulatory state of the channel, and attenuate the facilitation if the channel is already upregulated. The mechanism of the long lasting facilitation is unknown at this time, the relief of G-protein inhibition is typically accompanied by a change in current kinetics, which does not occur in this case. A phosphorylation site may be exposed during the prepulse, or the proximity of a protein kinase to the channel may be involved as suggested by the work of Johnson et al. [22,12] where an anchored kinase is involved.

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References

[1] Birnbaumer, L., Campbell, K.P., Catterall, W.A., Harpold, M.M., Hofmann, F., Horne, W.A., Mori, Y., Schwartz, A., Snutch, T.P., Tanabe, T. and Tsien, R.W. (1994) Neuron 13, 505–506.
[2] Lacerda, A.E., Kim, H.S., Ruth, P., Perez, R.E., Flockerzi, V., Hofmann, F., Birnbaumer, L. and Brown, A.M. (1991) Nature 352, 527–530.
[3] Singer, D., Biel, M., Lotan, I., Flockerzi, V., Hofmann, F. and Dascal, N. (1991) Science 253, 1553–1557.
[4] Neely, A., Wei, X., Olcese, R., Birnbaumer, L. and Stefani, E. (1993) Science 262, 575–578.
[5] Olcese, R., Qin, N., Schneider, T., Neely, A., Wei, X., Stefani, E. and Birnbaumer, L. (1994) Neuron 13, 1433–1438.
[6] Artalejo, C.R., Mogul, D.J., Perlman, R.L. and Fox, A.P. (1991) J. Physiol. (Lond.) 444, 213–240.
[7] Ikeda, S.R. (1991) J. Physiol. (Lond.) 439, 181–214.
[8] Swartz, K.J. (1993) Neuron 11, 305–320.
[9] Womack, M.D. and McCleskey, E.W. (1995) J. Neurophysiol. 73, 1793–1798.
[10] Kavalali, E.T. and Plummer, M.R. (1996) J. Neurosci. 16, 1072–1082.
[11] Zygmunt, A.C. and Maylie, J. (1990) J. Physiol. 428, 653–671.
[12] Johnson, B.D., Scheuer, T. and Catterall, W.A. (1994) Proc. Natl. Acad. Sci. USA 91, 11492–11496.
[13] Dolphin, A.C. (1996) Trends Neurosci. 19, 35–43.
[14] Sculptoreanu, A., Rotman, E., Takahashi, M., Scheuer, T. and Catterall, W.A. (1993) Proc. Natl. Acad. Sci. USA 90, 10135–10139.
[15] Kleppisch, T., Pedersen, K., Strubing, C., Bosse-Doenecke, E., Flockerzi, V., Hofmann, F. and Hescheler, J. (1994) EMBO J. 13, 2502–2507.
[16] Bourinet, E., Charnet, P., Tomlinson, W.J., Stea, A., Snutch, T.P. and Nargeot, J. (1994) EMBO J. 13, 5032–5039.
[17] Cens, T., Mangoni, M.E., Richard, S., Nargeot, J. and Charnet, P. (1996) Pflugers Arch. Eur. J. Physiol. 431, 771–774.
[18] Wei, X.Y., Perez-Reyes, E., Lacerda, A.E., Schuster, G., Brown, A.M. and Birnbaumer, L. (1991) J. Biol. Chem. 266, 21943–21947.
[19] Perez-Reyes, E., Castellano, A., Kim, H.S., Bertrand, P., Baggstrom, E., Lacerda, A.E., Wei, X.Y. and Birnbaumer, L. (1992) J. Biol. Chem. 267, 1792–1797.
[20] Taglialatela, M., Toro, L. and Stefani, E. (1992) Biophys. J. 61, 78–82.
[21] Olcese, R., Neely, A., Qin, N., Wei, X., Birnbaumer, L. and Stefani, E. (1996) J. Physiol. 497, 675–686.
[22] Johnson, B.D., Brousal, J.P., Peterson, B.Z., Gallombardo, P.A., Hockerman, G.H., Lai, Y., Scheuer, T. and Catterall, W.A. (1997) J. Neurosci. 17, 1243–1255.