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Rapid report

A voltage-independent K^+ conductance activated by cell swelling in Ehrlich cells is modulated by a G-protein-mediated process

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Abstract

Cell swelling following hypoosmotic stress leads to the activation of volume-sensitive ion channels that allow a K^+ and Cl^- efflux accompanied by water loss. A Ca^{2+}-insensitive K^+ channel ($I_{K,vol}$) has been described in Ehrlich cells that can be activated by hypotonicity and leukotriene D_4 and is inhibited by clofilium. We have studied the activation and deactivation by osmotic stimuli of this channel. A G-protein appears to be involved in these processes since GTP-γ-S accelerates deactivation, while GDP-β-S blocks the channel in the open state, a result mimicked by pertussis toxin (PTX). In addition, PTX accelerates the onset of $I_{K,vol}$. We propose that $I_{K,vol}$ is tonically inhibited by a PTX-sensitive G-protein.

Keywords: G-protein; Cell swelling; Ehrlich cell

The early response of Ehrlich ascites tumour cells to a hypotonic stimulus is an efflux of K^+ and Cl^- ions accompanied by osmotically obliged water loss in a process known as regulatory volume decrease (RVD) [1]. Direct measurement of currents by the patch-clamp method in Ehrlich cells has revealed a K^+ current that becomes activated upon swelling. This volume-sensitive K^+ current ($I_{K,vol}$), is independent of both extra- and intracellular Ca^{2+} and is insensitive to many of the more common K^+-channel blockers (including apamin, charybdotoxin, tetraethylammonium and clotrimazole) but inhibited by clofilium. The current-voltage relation of $I_{K,vol}$ obeys the Goldman–Hodgkin–Katz formalism, suggesting that the channels involved lack intrinsic voltage-dependence, and are selective to K^+ and Rb^+, with $P_K > P_{Rb}$. In addition, this K^+ current is markedly dependent upon extracellular pH, being strongly inhibited at pH 6.4 and enhanced at pH 8.4, compared with the control at pH 7.4 [2–5]. $I_{K,vol}$ has been proposed to be mediated by TASK-2 (KCNK5) K^+ channels, which are present in the Ehrlich cells [6,7].

The considerable wealth of information about the biophysical characteristics of $I_{K,vol}$ is not matched by an understanding of how this current is regulated. It has been shown that leukotriene D_4 (LTD_4) is released from Ehrlich cells upon hypotonic stress [8], and that LTD_4 activates a K^+ conductance with similar properties to $I_{K,vol}$ [4]. These studies point to a significant role of LTD_4 in the activation of $I_{K,vol}$ and Hoffmann [1] proposes a model for intracellular signalling in the activation of $I_{K,vol}$ in Ehrlich cells. This model includes the translocation of cytosolic phospholipase A_2 to the nucleus, mobilisation of arachidonic acid and production of LTD_4 and its binding to a receptor which would activate $I_{K,vol}$.

This scheme, while it integrates the known facts about the channel activation into a coherent model, does not provide an explanation for its deactivation after returning to isotonicity. In this work, we present data which indicate the involvement of a pertussis toxin (PTX)-sensitive G-protein in this process.

GTP binding proteins have been proposed to play a role in the modulation of membrane permeability pathways mediating regulatory volume adjustments in several cell types. Most reports have focused on the role of G-proteins as mediators of the signalling cascade leading to the swelling-induced activation of anion channels [9–11] rather than on the volume-sensitive K^+ channels. Direct effects of G-proteins upon other ion channels are firmly established [12]. A well-researched case is that of the heart K^+ channel Kir, which is directly regulated by $G\alpha_i$ and $G\beta\gamma$ [13]. The voltage-dependent inhibition of type N Ca^{2+} channels is also

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thought to be mediated by the direct interaction of Gβγ with a channel subunit [14–17]. In addition, the discovery of a family of proteins termed regulators of G-protein signalling (RGS) that speed up the GTPase activity of $G\alpha_{i/o}$ and $G\alpha_q$-dependent pathways, has shed light on the activation and deactivation processes of GIRK channels [18]. Recently, an inhibitory effect of hormones and neurotransmitters on "background" K^+ currents, postulated to be carried by the 2P 4TM TASK-1 K^+ channel, has been described [19–21]. This inhibition, which enhances excitability, is assumed to be exerted through the action of a G-protein. A role for phospholipase C (PLC) in the signalling has been proposed for an angiotensin-receptor inhibition of the channels [22], but the downstream product(s) of G-protein activation are unknown.

In the present study, we investigate the involvement of G-proteins on activation and recovery of $I_{K,vol}$ and $I_{Cl,vol}$ after hypotonic swelling of Ehrlich cells. The currents

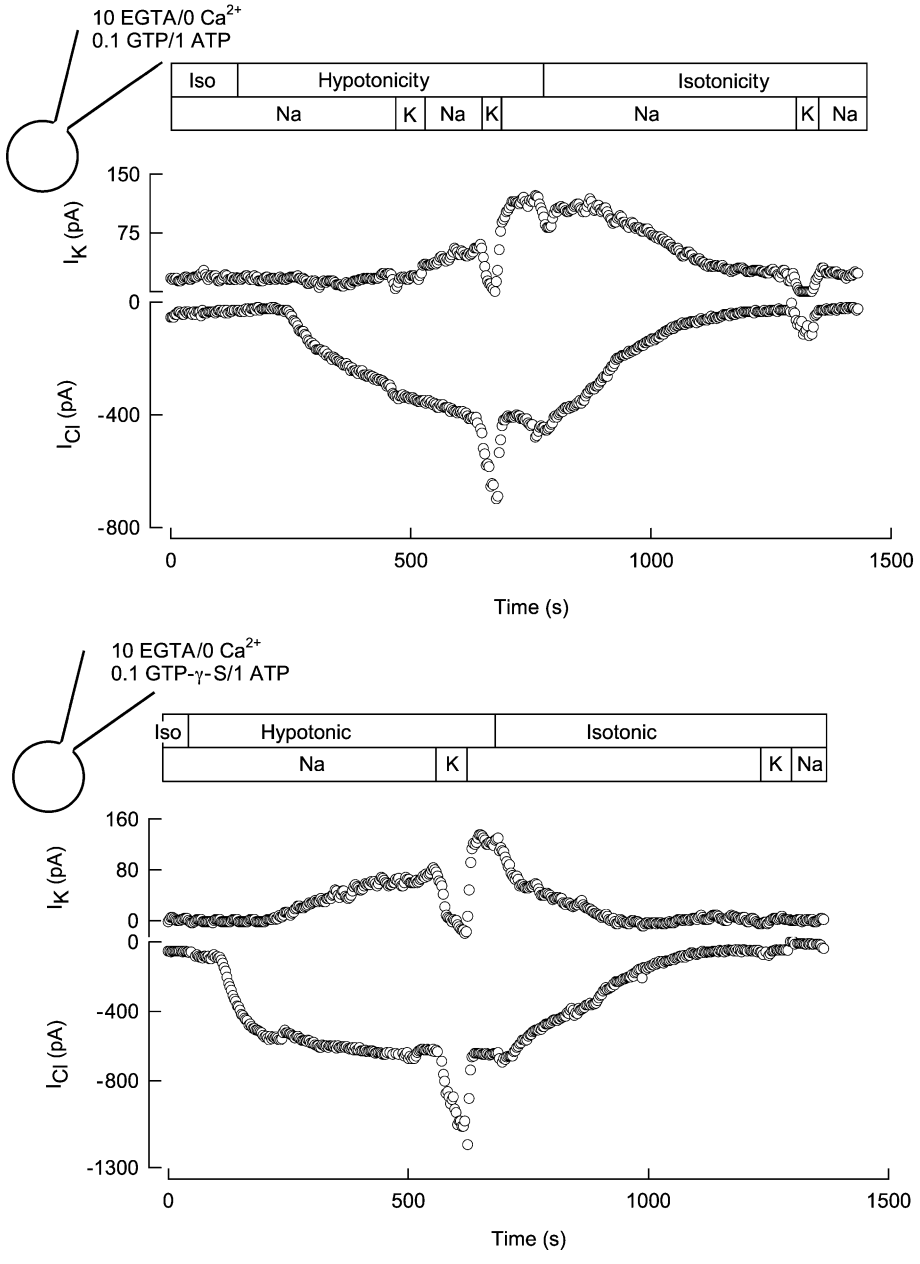

Fig. 1. Effect of GTP and GTP-γ-S, on swelling-activated K^+ current ($I_{K,vol}$) in Ehrlich cells. The composition of the pipette solution is (mM) NaCl 2, KCl 40, K gluconate 76, $MgCl_2$ 1.2, EGTA 10, Hepes 10, pH 7.4. Guanine nucleotides were added at 100 μM. Currents were measured in the whole-cell recording mode of the patch-clamp technique, using a holding potential of −30 mV and pulsing to −78 (E_K) or 0 mV (E_{Cl}) with square pulses of 500 ms duration with a 3 s period. Extracellular solution: NaCl 28, Na gluconate 62, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, D-mannitol 100, Hepes 10, pH 7.4. Tonicity was reduced by removal of D-mannitol to give a drop in osmolarity from 300 (isotonic) to ~200 mOsm/l (hypotonic). At the times indicated, all Na^+ in the solution was replaced by the K^+ salt. Note that the scales for the outward and inward currents are different.

arising from the exposure of cells to a hypotonic stimulus (total currents, in whole-cell voltage-clamp) were measured by dialysing the cell under study with GTP, GTP-γ-S, GDP-β-S or PTX in the pipette. Freshly collected cells from a 6-day tumour, were placed in a temperature-controlled micro-chamber and kept at 37 °C. Solution changes (composition in the legends to the figures) were effected by complete replacement of chamber volume. In order to determine K^+ and Cl^- currents developing during cell swelling, a two-pulse protocol was used, as explained in detail previously [2]. The voltage protocol was started 10–20 min after breaking into the cell, thus allowing adequate equilibration between the pipette containing the guanine nucleotides or PTX and the intracellular compartment. The membrane potential was clamped alternatively at E_K and E_{Cl} introducing a correction where exposure to hypotonicity leads to cell

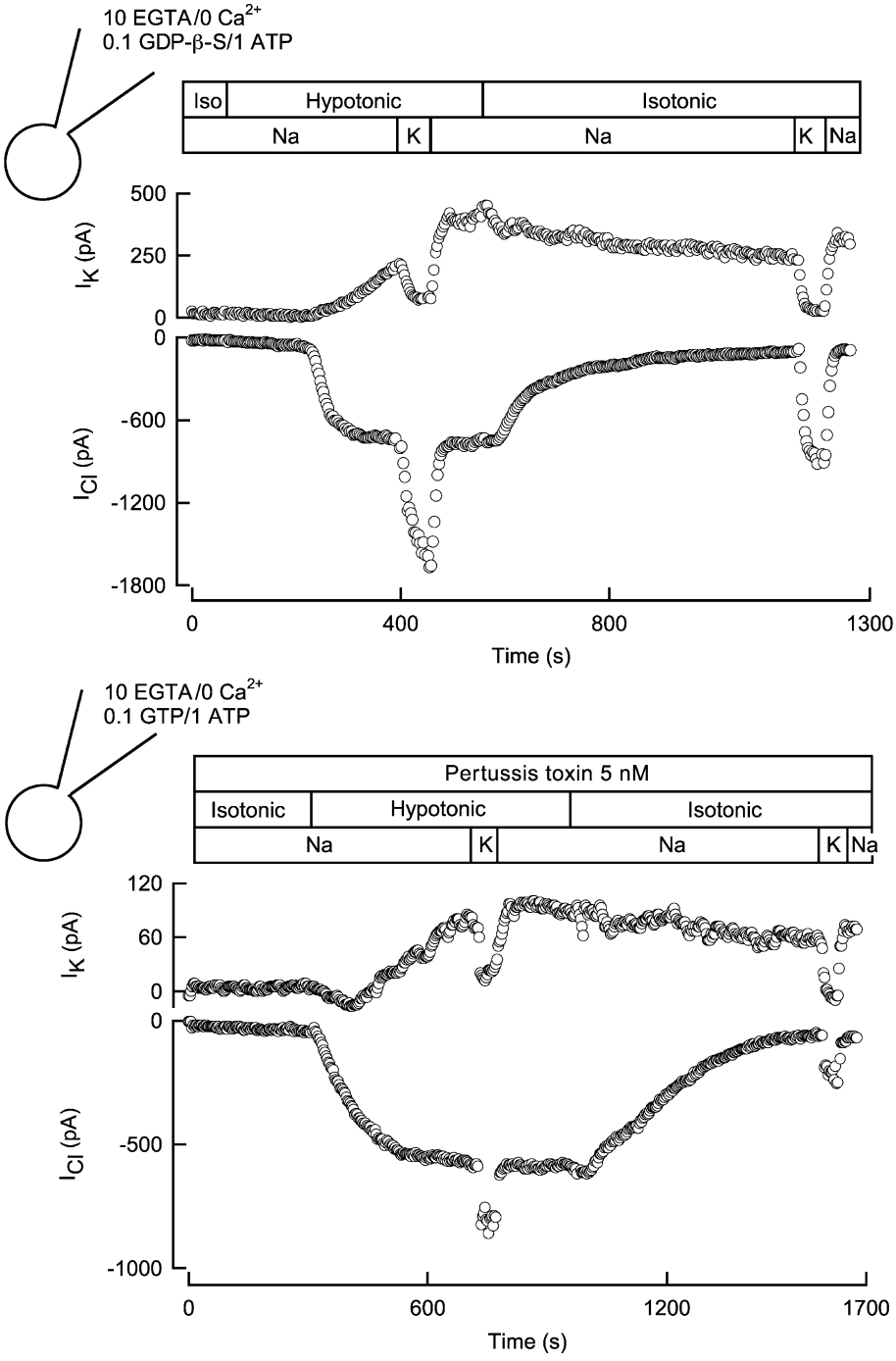

Fig. 2. Effect of GTP-β-S and PTX, on swelling-activated K^+ current ($I_{K,\ vol}$) in Ehrlich cells. Experimental protocol and solutions as in Fig. 1. Where indicated, 5 nM PTX was added to the pipette solution.

swelling and consequent dilution of intracellular ion concentrations [3].

Fig. 1 shows typical experiments designed to gauge the activation and deactivation of osmosensitive currents. In order to look for K^+ and Cl^- conductances activated by cell swelling, a 33% decrease in the tonicity of the extracellular medium was effected without altering the ionic composition (removal of mannitol). As seen in the figure, after a delay, Cl^- and K^+ currents developed when exposing the cells to the hypoosmotic solution. Subjecting the cells to a solution where the only monovalent cation is K^+, and at 0 mV (E_{Cl}), the driving force for a K^+-carried current is cancelled. Under these conditions, outward current is abolished and a large extra inward current appears at -78 mV (E_K). This is consistent with the outward current seen in external Na^+-rich solution being carried by K^+ and demonstrates that the conductance allows sizeable flow of ions in the inward direction. Once this K^+ current attains a stable value, the external solution is changed back to isoosmolarity. The upper panel of Fig. 1 shows an experiment where the pipette contained 100 μM GTP. Initially, there was no detectable K^+ or Cl^- current but both $I_{K,vol}$ and $I_{Cl,vol}$ arose after delays described in Table 1. Upon switching back to isotonicity, $I_{Cl,vol}$ vanished after about 300 s while substantial $I_{K,vol}$ still remained. A marked reduction in $I_{K,vol}$ had taken place 600 s after removal of the stimulus, as seen from the small effect of increasing extracellular K^+ on outward current. The lower panel of Fig. 1 shows a similar experiment but with GTP-γ-S in the pipette. Activation of $I_{K,vol}$ and $I_{Cl,vol}$ was indistinguishable from the experiment done with intracellular GTP. The same was true for the re-establishment of basal $I_{Cl,vol}$, but $I_{K,vol}$ decreased to pre-stimulus levels much faster (about 200 s) after switching back to isotonicity. This result could be interpreted to indicate that a G-protein is involved in deactivating K^+ channels activated by hypotonicity-induced increase in cell volume.

To test this further, experiments were carried out using GDP-β-S in the pipette solution which, were a G-protein involved, would be expected to produce an effect opposite to that of GTP-γ-S. Fig. 2, upper panel, shows a typical experiment where GDP-β-S (100 μM) is included in the pipette. This nucleotide did not alter the development of neither $I_{Cl,vol}$ nor $I_{K,vol}$ on exposure to hypotonicity. Upon returning to isotonicity $I_{Cl,vol}$ recovered back to pre-challenge levels within 200 s. A large $I_{K,vol}$ however, remained more than 600 s after restoration of medium tonicity, as shown by the sustained current in Na^+-rich medium and by the robust inward current elicited by replacement with a high K^+ solution.

A PTX-sensitive G-protein has been suggested to play a role in RVD in platelets [23]. A possible effect of PTX (5 nM) was therefore assayed on the activation and recovery of $I_{Cl,vol}$ and $I_{K,vol}$ in Ehrlich cells. The lower panel of Fig. 2 shows such an experiment. $I_{K,vol}$ but not $I_{Cl,vol}$ developed faster than what was seen when the cell was dialysed with GDP-β-S. And while $I_{Cl,vol}$ dissipated rapidly after return to isotonicity, $I_{K,vol}$ remained activated for longer than 600 s in isotonic medium.

Table 1 summarises the effects of intracellular guanine nucleotides and PTX upon the development of $I_{K,vol}$ and $I_{Cl,vol}$ following hypoosmotic cell swelling and subsequent recovery in isotonicity. The following parameters have been tabulated [4]: the time taken from decreasing the tonicity in the extracellular medium to the start of increase in currents (t_0); time taken, from t_0, to achieve half of the maximal current attained ($t_{1/2act}$) and the maximal current attained ($I_{K,max}$ or $I_{Cl,max}$). Under a column that has been called ''deactivation,'' the time from restoring the medium to isotonicity to attaining a decrease of 50% of $I_{K,max}$ or $I_{Cl,max}$ has been termed $t_{1/2deact}$.

Briefly, the use of different guanine nucleotide analogues or the inclusion of PTX in the pipette had no effect on the activation or deactivation parameters of the volume-sensitive Cl^- current in Ehrlich cells. In contrast, the half time for the deactivation of $I_{K,vol}$ is significantly reduced when GTP-γ-S is present in the pipette, while GDP-β-S or PTX efficiently blocked recovery. In addition, the presence of PTX in the pipette significantly reduced the onset (~ 4 times shorter) of $I_{K,vol}$.

The data presented here are best explained by speculating that the volume-sensitive K^+ channel is kept under tonic inhibition by an active PTX-sensitive Gα in isotonic conditions. Upon hypoosmotic challenge, a cascade of events would lead to an acceleration of the GTPase activity of Gα and thus deactivation of the G-protein with a concomitant release of the K^+ channel into the open state. PTX would

Table 1
Effect of intracellular guanine nucleotide analogues or PTX on $I_{K,vol}$ and $I_{Cl,\ vol}$ in Ehrlich cells

Activation and deactivation of $I_{K,\ vol}$ after hypotonic cell swelling				
Treatment (*n*)	Activation			Deactivation
	t_0 (s)	$t_{1/2act}$ (s)	I_K max (pA)	$t_{1/2deact}$ (s)
GTP (6)	118 ± 43	202 ± 25	264 ± 83	435 ± 84
GTP-γ-S (6)	232 ± 40	198 ± 30	157 ± 21	82 ± 31[a]
GDP-β-S (6)	164 ± 37	198 ± 14	269 ± 64	446, >600 (5)[b]
PTX (10)	31 ± 14[c]	138 ± 29	253 ± 38	383 (3), >600 (7)[d]

Activation and deactivation of $I_{Cl,\ vol}$ after hypotonic cell swelling				
Treatment (*n*)	Activation			Deactivation
	t_0 (s)	$t_{1/2act}$ (s)	I_{Cl} max (pA)	$t_{1/2deact}$ (s)
GTP (6)	73 ± 18	83 ± 28	− 592 ± 61	350 ± 75
GTP-γ-S (6)	90 ± 12	40 ± 7	− 545 ± 60	273 ± 44
GDP-β-S (6)	96 ± 21	73 ± 14	− 677 ± 57	233 ± 43
PTX (10)	75 ± 16	88 ± 21	− 532 ± 57	316 ± 65

[a] $P = 0.01$ for comparison to GTP.
[b] In five out of six experiments, the current had not recovered by 50% after 600 s in isotonicity.
[c] $P = 0.03$ for comparison with GTP.
[d] In 7 out of 10 experiments, the current had not recovered by 50% after 600 s in isotonicity.

block the G-protein into the deactivated form, thus keeping the K^+ channel open, and a similar effect would be exerted by GDP-β-S. These agents would therefore impede the reactivation of the G-protein, and the channel closure, after the disappearance of the hypoosmotic stimulus. GTP-γ-S, on the contrary, would accelerate the return of the channels to the closed state by favouring the activated state of the G-protein.

It is known from previous work on swelling activated K^+ channels in Ehrlich cells that a Ca^{2+}-independent action of LTD_4 can mimic the cell swelling effect [4,24]. LTD_4 effect on Ehrlich cells could be mediated through a G-protein GTPase-activating protein (GAP), which act allosterically on Gα subunits. Although GAPs do not contribute directly to the chemistry of GTP hydrolysis, they can accelerate hydrolysis >2000-fold [25]. The allosteric action of GAPs on Gα also would explain the reduction in $I_{K,vol}$ t_0 by PTX and not by GDP-β-S. It is interesting that all the $G\alpha_i$ (PTX-sensitive) subfamily of Gα, but not $G\alpha_s$, are substrate for two GAPs found in rat brain [26].

Neither the kinetics of activation or deactivation of $I_{Cl,vol}$ or its magnitude at steady state were affected by the nucleotide analogue treatments used here. This is surprising in view of the previously reported effects on cell swelling-induced anion currents reported before [11,27–29]. GTP-γ-S failed to activate $I_{Cl,swell}$ of Ehrlich cells under similar conditions as those used in previous investigations on neuroblastoma, endothelial and chromaffin cells. Cell-specific factors, including perhaps cell heterogeneity in the type of channels expressed, might explain the discrepancy.

More work needs to be done to understand fully the triggering cascades operating in RVD. The identification of $I_{K,vol}$ with TASK-2 and narrowing the search to the PTX-sensitive G-proteins will help in screening the known G-protein subunits to reveal the puzzle.

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References

[1] E.K. Hoffmann, Cell. Physiol. Biochem. 10 (2000) 273–288.
[2] G. Riquelme, F.V. Sepúlveda, F. Jørgensen, S. Pedersen, E.K. Hoffmann, Biochim. Biophys. Acta 1371 (1998) 101–106.
[3] M.I. Niemeyer, C. Hougaard, E.K. Hoffmann, F. Jørgensen, A. Stutzin, F.V. Sepúlveda, J. Physiol. (London) 524 (2000) 757–767.
[4] C. Hougaard, M.I. Niemeyer, E.K. Hoffmann, F.V. Sepúlveda, Pfluegers Arch. 440 (2000) 283–294.
[5] C. Hougaard, F. Jørgensen, E.K. Hoffmann, Pfluegers Arch. 442 (2001) 622–633.
[6] M.I. Niemeyer, L.P. Cid, F.V. Sepúlveda, Comp. Biochem. Physiol., Part A 130 (2001) 565–575.
[7] M.I. Niemeyer, L.P. Cid, L.F. Barros, F.V. Sepúlveda, J. Biol. Chem. 276 (2001) 43166–43174.
[8] I.H. Lambert, J. Membr. Biol. 98 (1987) 207–221.
[9] B.C. Tilly, M. Kansen, P.G. van Gageldonk, N. van den Berghe, H. Galjaard, J. Bijman, H.R. de Jonge, J. Biol. Chem. 266 (1991) 2036–2040.
[10] B. Nilius, T. Voets, J. Prenen, H. Barth, K. Aktories, K. Kaibuchi, G. Droogmans, J. Eggermont, J. Physiol. (London) 516 (1999) 67–74.
[11] A.Y. Estévez, T. Bond, K. Strange, Am. J. Physiol. 281 (2001) C89–C98.
[12] N. Dascal, Trends Endocrinol. Metab. 12 (2001) 391–398.
[13] D.E. Clapham, Annu. Rev. Neurosci. 17 (1994) 441–464.
[14] A.C. Dolphin, J. Physiol. (London) 506 (1998) 3–11.
[15] S. Herlitze, D.E. Garcia, K. Mackie, B. Hille, T. Scheuer, W.A. Catterall, Nature 380 (1996) 258–262.
[16] S.R. Ikeda, Nature 380 (1996) 255–258.
[17] S.R. Ikeda, K. Dunlap, Adv. Second Messenger Phosphoprotein Res. 33 (1999) 131–151.
[18] S. Fujita, A. Inanobe, M. Chachin, Y. Aizawa, Y. Kurachi, J. Physiol. (London) 526 (2000) 341–347.
[19] E.M. Talley, Q. Lei, J.E. Sirois, D.A. Bayliss, Neuron 25 (2000) 399–410.
[20] J.A. Millar, L. Barratt, A.P. Southan, K.M. Page, R.E. Fyffe, B. Robertson, A. Mathie, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 3614–3618.
[21] G. Czirják, T. Fischer, A. Spät, F. Lesage, P. Enyedi, Mol. Endocrinol. 14 (2000) 863–874.
[22] G. Czirják, G.L. Petheõ, A. Spät, P. Enyedi, Am. J. Physiol.: Cell Physiol. 281 (2001) C700–C708.
[23] A. Margalit, A.A. Livne, J. Funder, Y. Granot, J. Membr. Biol. 136 (1993) 303–311.
[24] E.K. Hoffmann, Pfluegers Arch. 438 (1999) 263–268.
[25] E.M. Ross, T.M. Wilkie, Annu. Rev. Biochem. 69 (2000) 795–827.
[26] D.M. Berman, T.M. Wilkie, A.G. Gilman, Cell 86 (1996) 445–452.
[27] T. van der Wijk, S.F. Tomassen, H.R. de Jonge, B.C. Tilly, Cell. Physiol. Biochem. 10 (2000) 289–296.
[28] T. Voets, V. Manolopoulos, J. Eggermont, C. Ellory, G. Droogmans, B. Nilius, J. Physiol. (London) 506 (1998) 341–352.
[29] P. Doroshenko, R. Penner, E. Neher, J. Physiol. (London) 436 (1991) 711–724.