Inhibition of the Magnesium-Sensitive TRPM7-like Channel in Cardiac Myocytes by Nonhydrolysable GTP Analogs: Involvement of Phosphoinositide Metabolism

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Key Words

Cation channel • TRP • Guanine nucleotides • Phosphoinositides • Run-down

Conclusion: Guanine nucleotides can modulate cardiac TRPM7-like channels via a mechanism linked to G proteins and to PIP₂ metabolism.

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Abstract

Background/Aims: A magnesium-inhibited, transient receptor potential melastatin 7 (TRPM7)-like channel is expressed in cardiac cell membranes. The role and regulation of this channel by intracellular nucleotides and membrane components remain unclear. **Methods:** We used the whole-cell voltage-clamp technique in pig isolated ventricular myocytes to investigate the effect of non-hydrolysable guanine nucleotides. **Results:** The TRPM7-like current, induced by intracellular dialysis with low [Mg²⁺], remained stable when the intracellular solution contained GTP. Substituting GTP by GTP- γ -S or Gp-pNp, but not GDP- β -S, induced a run-down of the current. Under dialysis with GTP- γ -S, inhibiting phospholipase C by edelfosine or intracellularly adding exogenous phosphatidylinositol-4,5-bisphosphate (PIP₂) decreased run-down, whereas extracellularly applying carbachol and phenylephrine accelerated it. Pretreatment of cells with pertussis toxin did not prevent the run-down induced by GTP- γ -S.

Introduction

TRPM7 is a member of the melastatin subfamily of transient receptor protein (TRP) cation channels. TRPs are expressed in various tissues, where they mediate responses to various physiological stimuli [1], and where their abnormal behavior may contribute to pathological processes [2]. At present, the function and regulation of many members of this family of channels remain poorly understood. TRPM7 is ubiquitously expressed and appears to be critical for cell survival [3, 4]. It is especially highly expressed in renal and intestinal epithelia as well as in cardiac tissues. TRPM7 channels conduct inward fluxes of Mg²⁺, Ca²⁺ and other divalent cations [5], and may play a critical role in Mg²⁺ homeostasis [6].

We have previously shown that a cation current inhibited by intracellular Mg²⁺ (Mg²⁺_i) is present in cardiac myocytes, and that its electrophysiological and pharmacological properties resemble those of the current through TRPM7 channels [7, 8]. We have also shown

that the current decreases with time, i.e. it undergoes run-down, if ATP is absent from the intracellular medium. This run-down was attributed to a loss of phosphorylation and a depletion of membrane phospholipids, since either inhibition of lipid phosphatases or intracellular dialysis with exogenous phosphatidylinositol-4,5-bisphosphate (PIP₂) could prevent the run-down in ATP-depleted cells, whereas inhibition of PIP₂ synthesis by the phosphatidylinositol-4-kinase inhibitor wortmannin could induce run-down in ATP-containing cells. Beside the regulation by Mg²⁺_i and ATP, sensitivity of heterologously expressed TRPM7 channels to intracellular guanine nucleotides has also been demonstrated. This latter regulation involves an enhancement of the inhibitory effect of Mg²⁺_i by the nucleotides [9]. Here we report a different regulation: the loss of the TRPM7-like current during cell dialysis with nonhydrolysable GTP analogs, via a mechanism that is independent of intracellular ATP depletion and of the presence of free Mg²⁺_i but that may involve G protein-mediated PIP₂ depletion.

Materials and Methods

The study was approved by the Ethics Commission on Animal Experiments of the University of Leuven and carried out in accordance with the institutional guidelines.

Cell preparation

We used pig isolated cardiac ventricular myocytes. The methods for cell dissociation and measurement of whole-cell currents were similar to those described before [7, 8, 10]. Cells were kept in 0.18-mM Ca²⁺ Tyrode solution and were used on the same day. Most of them were used for the measurement of the TRPM7-like current. A few cells were used to measure the L-type Ca²⁺ current (I_{cal}) and its regulation by β -adrenergic and muscarinic agonists in order to assess the function of G proteins and the inactivation of G_i/G_o by pertussis toxin (PTX). In the latter case, cells were incubated with PTX (3.5 μ g/ml) for 1 hour at 37°C followed by at least 3 more hours at room temperature.

Electrophysiological measurements

During measurements, the myocytes were superfused at room temperature, with a solution containing (in mM): 135 NaCl, 5.4 CsCl, 0.9 MgCl₂, 1.8 CaCl₂, 0.33 NaH₂PO₄, 10 Hepes and 10 glucose (pH 7.4). Nifedipine (100 μ M) was included in the extracellular solutions to block L-type Ca²⁺ channels when measuring the TRPM7-like current. The standard pipette solution contained (in mM): 130 Cs-glutamate, 25 CsCl, 1 MgCl₂, 5 Na₂ATP, 1 EGTA, 0.1 Na₂GTP, 5 Hepes (pH 7.25). The pipette solution could be modified by omitting Mg²⁺, by substituting GTP with its non-hydrolysable analogs (guanosine-5'-O-3-thiotriphosphate, GTP- γ -S; guanylyl-5'- β , γ -imido-triphosphate, Gp-pNp; or guanosine 5-thiodiphosphate,

GDP- β -S), by substituting EGTA with 10 mM EDTA or BAPTA, or by adding specific drugs.

The holding potential was -80 mV. The voltage clamp protocols for measuring the TRPM7-like current consisted of ascending and descending ramps between -120 mV and +80 mV, and currents were measured during the descending limb. For measuring I_{cal}, a 400-ms depolarization to 0 mV was given after a 400-ms pre-step at -40 mV to inactivate the voltage-dependent Na⁺ current.

Materials

Drugs and chemicals were from Sigma-Aldrich (Bornem, Belgium) or Merck (Darmstadt, Germany), except for D-myo-phosphatidylinositol 4,5-bisphosphate (PIP₂) which was from Echelon Biosciences Inc (Salt Lake City, UT, USA). Edelfosine was prepared in dimethyl sulfoxide (which up to 1% has no effect on the TRPM7-like current [7]) and diluted to the desired final concentrations.

Statistics

Data are given as mean $\pm$ standard error of the mean (S.E.M), with *n* indicating the number of cells. Means were compared using the two-tailed Student's *t* test or ANOVA followed by post-hoc Bonferroni's test. *P* = 0.05 was taken as threshold for statistical significance.

Results

Figure 1A shows the time diary of currents measured at +80 mV and -80 mV during voltage ramps in a cell dialyzed with standard internal solution containing 100 μ M GTP, while the inset shows typical current-voltage relationships recorded in the same cell immediately after patch rupture and afterwards at 20 min and 60 min. Currents increased with time after patch rupture, due to the low free [Mg²⁺] (20-30 μ M) in the pipette solution, which removes the inhibition of TRPM7 by Mg²⁺_i [7, 11, 12]. The trace obtained at 20 min of dialysis displayed marked outward-going rectification, due to large outward monovalent cation fluxes in the presence of only small inward divalent cation fluxes [5, 7], typical of TRPM7 currents. During prolonged dialysis with GTP, the magnitude of the TRPM7-like current remained stable, and was the same after $\geq$ 60 min as at 20 min. Fig 1B (see also the inset) shows that, in contrast, in a cell where GTP was replaced by its non-hydrolysable analog GTP- γ -S (100 μ M), the TRPM7-like current subsided with prolonged dialysis after reaching its peak level. Similarly, substituting GTP by another nonhydrolysable GTP analog, Gp-pNp (300 μ M), also caused a loss of TRPM7-like current during prolonged cell dialysis (*n* = 3; see Fig. 1D, unfilled circles). Because guanine nucleotides can activate G proteins, the run-down of TRPM7-like

Fig. 1. TRPM7-like current in pig ventricular myocytes. Induction of run-down by GTP- γ -S or Gp-pNp but not GDP- β -S: A, B, C: Time diaries of whole-cell currents measured at +80 mV and -80 mV in cells dialyzed with either 100 μ M GTP (A), 100 μ M GTP- γ -S (B) or 300 μ M GDP- β -S (C). Insets: Current-voltage relationships in the same cells. Traces obtained using voltage ramps, at 1 min (○), 20 min (□) and 60 min (■) after patch rupture. D: Summary data of time diaries from cells dialyzed with GTP (filled squares; $n = 6$), GTP- γ -S (filled circles; $n = 14$), Gp-pNp (300 μ M; unfilled circles; $n = 3$) or GDP- β -S (filled triangles; $n = 7$). Notice stable TRPM7-like current under dialysis with GTP or GDP- β -S, but decreased current under dialysis with GTP- γ -S or Gp-pNp. In all panels, cells were superfused with standard solution containing 0.9 mM Mg^{2+} , except for a short exposure to the same solution but with 7.2 mM Mg^{2+} , indicated by a horizontal bar, in panel C.

current could be due to G protein-dependent processes. Consistent with this possibility, Fig. 1C (see also the inset) shows that the current remained stable in cells dialyzed with GDP- β -S (300 μ M), a nucleotide that stabilizes G proteins in their inactive form. Summary data obtained in cells dialyzed with GTP, GTP- γ -S, Gp-pNp or GDP- β -S are displayed in Fig. 1D. Relative to its maximum, the amplitude of the outward current at +80 mV after 60 min of dialysis was $95.5 \pm 1.8\%$ with GTP ($n = 6$), $14.2 \pm 4.5\%$ with GTP- γ -S ($n = 14$; $P < 0.001$ vs. GTP), $49.2 \pm 5.2\%$ with Gp-pNp ($n = 3$; $P < 0.001$ vs. GTP) and $69.3 \pm 10.3\%$ with GDP- β -S ($n = 7$; $P > 0.05$ vs. GTP). Thus, only the guanine nucleotide analogs capable of irreversibly activating G proteins were able to induce run-down. These data obtained on native cardiac channels are consistent with

previous, unexplained observations by others that GTP- γ -S suppresses heterologously expressed TRPM7 currents [13].

Inward currents in the presence of extracellular Ca^{2+} and Mg^{2+} displayed little or no run-down (see Fig 1B). However, large inward TRPM7 currents carried by monovalent cations in the absence of extracellular Ca^{2+} and Mg^{2+} [7, 14] displayed run-down with a time course similar to that of outward currents (Fig. 2). Since total currents were measured, the inward currents in the presence of extracellular Ca^{2+} and Mg^{2+} are likely due to non-TRPM7 currents contaminating very small TRPM7 currents. In support of this hypothesis, increasing extracellular Mg^{2+} largely suppressed outward currents, consistent with a pore block of TRPM7 by extracellular divalent cations [7, 14], while there was no or little

Fig. 2. GTP- γ -S-induced run-down of outward and inward monovalent cation TRPM7-like currents: A: Time diary of whole-cell currents measured at +80 mV and -120 mV in cells dialyzed with 100 μ M GTP- γ -S and extracellularly superfused with Ca^{2+} - and Mg^{2+} -free solution. Inset: Current-voltage relationships in the same cell. Traces obtained using voltage ramps, in the presence of extracellular Ca^{2+} and Mg^{2+} (control, $\bullet$), and at 7 min ($\circ$) and 55 min ($\square$) after removing the divalent cations. B: Summary data of time diaries from 5 cells under the same experimental conditions as in A.

Fig. 3. Preventing PIP_2 depletion delays/decreases run-down: A, B: Summary data of time diaries of currents measured at +80 mV and -80 mV during voltage ramps in cells dialyzed with 100 μ M GTP- γ -S plus either 10 μ M edelfosine (A; $n = 5$) or 20 μ M PIP_2 (B; $n = 7$). C: Relative amplitudes of currents measured at +80 mV at 60 min of dialysis as percent of the current at 20 min, in cells dialyzed with 100 μ M GTP- γ -S alone ($n = 14$) or with either 10 μ M edelfosine ($n = 5$) or 20 μ M PIP_2 ($n = 7$) added on top of GTP- γ -S. *: $P < 0.005$; #: $P < 0.001$ vs. GTP- γ -S alone.

effect on the inward current (see Fig. 1C).

Because the TRPM7-like current is inhibited by free Mg^{2+} , and because this inhibition can be enhanced by guanine nucleotides, we compared the GTP- γ -S-induced run-down in cells dialyzed with 20-30 μ M free $[\text{Mg}^{2+}]$

(our standard internal solution: 1 mM MgCl_2 + 1 mM EGTA + 5 mM Na_2ATP) to that of cells dialyzed with practically zero free $[\text{Mg}^{2+}]$ (0 mM MgCl_2 + 10 mM EDTA + 5 mM Na_2ATP). Run-down was similar in both groups of cells (after 60 min, decrease to 18 ± 3 % of

Fig. 4. Insensitivity of the run-down process to an inactivation of inhibitory G proteins: **A:** Time diary of the L-type Ca^{2+} current (I_{CaL}) measured during step depolarizations to 0 mV in a cell dialyzed with zero free Mg^{2+} (0 $MgCl_2$ + 10 mM EDTA + 5 mM ATP in the pipette solution). Isoproterenol (Iso, 10 nM) and carbachol (CCh, 10 μ M) were applied at the time indicated by horizontal bars. **B:** Summary data of time diaries of TRPM7-like currents measured at +80 mV during voltage ramps in 4 cells pretreated with pertussis toxin (PTX) and dialyzed with solution containing 100 μ M GTP- γ -S. Notice that PTX pretreatment does not prevent run-down. **C, D:** Time diaries (**C**) and summary data (**D**) of I_{CaL} measured as in **A**, in cells dialyzed with 0.51 mM free Mg^{2+} (5.5 mM $MgCl_2$ + 10 mM BAPTA). Upper panel of **C:** untreated cell. Lower panel of **C:** cell pretreated with PTX for 6.5 hours. Insets in **A** and **C:** I_{CaL} traces in control (in the absence of drugs), and in the presence of Iso or Iso + CCh. Voltage protocol: holding at -80 mV, conditioning at -40 mV, test at 0 mV.

maximum with zero free $[Mg^{2+}]$; $n = 5$, $P > 0.05$ vs. decay in standard pipette solution; see above). These data indicate that the GTP- γ -S-induced run-down was largely independent of the known inhibitory effect of free Mg^{2+} .

Since we have previously shown that maintaining the membrane PIP_2 pool is necessary for stable cardiac TRPM7-like channel activity [8], one possible mechanism underlying the GTP- γ -S- and Gp-pNp-induced run-down could be a G protein-dependent loss of this phosphoinositide. Therefore, we first tested the effect of inhibiting phospholipase C (PLC), the enzyme responsible for PIP_2 breakdown, in cells dialyzed with GTP- γ -S. The most widely used PLC inhibitor, U73122, was

unsuitable for this purpose because of its inhibitory effect on TRPM7, including in cardiac cells [8, 15, 16]. Instead we used edelfosine, an alkyl-lysophospholipid also shown to inhibit PLC [17]. Fig. 3A shows a time diary of currents in cells ($n = 5$) dialyzed with GTP- γ -S and edelfosine (10 μ M). Edelfosine did not prevent the development of the TRPM7-like current (even at a concentration of 40 μ M; not shown), suggesting that it does not inhibit the channel (see also [18]), in contrast to U73122. Interestingly, edelfosine suppressed or delayed run-down. No rundown was present in 2 cells up to more than 20 min after reaching peak value. In 5 other cells, run-down was present but slow, with a time of decay to half

Fig. 5. Agonist-induced acceleration of the rate of run-down of TRPM7-like current: A,B,C,D: Time diaries of currents measured at +80 mV during ramp depolarizations in cells dialyzed with solution containing 5 mM ATP + 100 μ M GTP + 0 GTP- γ -S (A and C), or 0 ATP + 100 μ M GTP + 0 GTP- γ -S (B), or 5 mM ATP + 0 GTP + 100 μ M GTP- γ -S (D). In C, wortmannin (10 μ M) was added to inhibit PIP₂ synthesis via phosphatidylinositol 4 kinase. In all panels, carbachol (CCh, 100 μ M) and phenylephrine (PE, 40 μ M) were added simultaneously in the extracellular solution. Insets in C: Estimated half-times of current run-down before (unfilled column) and during application of CCh + PE (filled column). Decay half-times ($t_{0.5}$) were estimated using initial amplitude and rate of decrease of the current in each condition, and assuming a first-order process ($t_{0.5} = 0.69 \times \text{amplitude}/\text{rate}$). In D: Measured $t_{0.5}$ of current run-down in cells dialyzed with 100 μ M GTP- γ -S without other treatment (unfilled column), or in cells further treated with CCh + PE (filled column). Notice run-down of the TRPM7-like current within 20 min after agonist application in D.

maximum current ($t_{0.5}$) of 28.9 ± 1.06 min ($n = 5$), which is significantly longer ($P = 0.0005$) than the corresponding time in untreated cells ($t_{0.5}$: 14.2 ± 1.97 min, $n = 14$; see inset of Fig. 5D). Relative to its maximum, the amplitude of the outward current at +80 mV after 60 min of dialysis with GTP- γ -S and 10 μ M edelfosine was $68.9 \pm 15.6\%$ ($P = 0.004$ vs. GTP- γ -S alone; $P = 0.04$ vs. GTP). This result suggests that the guanine nucleotide analogs cause run-down at least in part by activating PLC. Another way to maintain the membrane PIP₂ pool is to supply the phospholipid exogenously. Fig. 3B shows that the addition of 20 μ M PIP₂ to a GTP- γ -S-containing pipette solution

prevented run-down of the TRPM7-like current ($n = 7$). Relative to its maximum, the amplitude of the outward current at +80 mV after 60 min of dialysis with GTP- γ -S and 20 μ M PIP₂ was $88.4 \pm 26.9\%$ ($P < 0.001$ vs. GTP- γ -S alone; $P > 0.05$ vs. GTP) (see Fig. 3C). Altogether these results are consistent with the hypothesis that G-protein-mediated activation of PLC and a subsequent depletion of PIP₂ underlie the GTP- γ -S-induced run-down of TRPM7-like current.

Because normal G protein function requires intracellular Mg²⁺ whereas most of our experiments were carried out under conditions of low or zero free Mg²⁺,

it was necessary to establish that G proteins can be operative under these conditions. For this purpose, we used the regulation of I_{CaL} by β -adrenergic and muscarinic receptors as index of G protein function. Fig. 4A shows that the β -adrenergic agonist isoproterenol (Iso, 10 nM) could stimulate I_{CaL} , and that the muscarinic agonist carbachol (CCh, 10 μ M) could reverse this stimulation, as expected from their actions via G_s and G_i , respectively. We also tested for a possible involvement of PTX-sensitive G proteins in the GTP- γ -S-induced run-down of the TRPM7-like current. In 4 cells pre-treated with PTX for 6–8 hours, dialysis with GTP- γ -S could still induce run-down with a time course similar to that of untreated cells (Fig. 4B). Fig. 4C and Fig. 4D provide evidence that G_o/G_i was effectively inactivated in such cells, by showing that the CCh antagonism of the effect of Iso was abolished in PTX pre-treated cells. These results are not unexpected if run-down is caused by PIP_2 metabolism, which in cardiac cells is linked to PTX-insensitive G proteins [19].

As an additional test of our hypothesis of G-protein-mediated loss of PIP_2 as mechanism underlying the GTP- γ -S-induced run-down, we examined whether receptor activation in the presence of GTP- γ -S is associated with a faster current decrease. Because muscarinic agonists at high concentration and α -adrenergic agonists enhance PIP_2 metabolism, we applied extracellularly CCh at 100 μ M plus phenylephrine (PE, 40 μ M). Fig. 5A shows that these agonists do not cause measurable run-down in cells dialyzed with ATP in the absence of GTP- γ -S, as reported previously [8]. However, the run-down induced by PIP_2 depletion when dialyzing cells without ATP (Fig. 5B) or with wortmannin (10 μ M; Fig. 5C) [8] was accelerated by the agonists (see inset in Fig. 5C for estimated $t_{0.5}$ that decreased to 11.9 ± 4.62 min from 32.4 ± 6.30 min upon adding CCh + PE in cells dialyzed with wortmannin; $p = 0.018$, $n = 4$). In cells dialyzed with GTP- γ -S, the agonists were applied during the rising phase of the TRPM7-like current, before the start of GTP- γ -S-induced run-down. Fig. 5D shows that agonist application was immediately followed by a current decay that was complete within 20 min. To assess the effect of agonist application on the kinetics of run-down, half-times ($t_{0.5}$) of current decay in cells dialyzed with GTP- γ -S without further treatment ($n = 14$), or dialyzed with GTP- γ -S and in addition treated with CCh and PE ($n = 7$) are compared in the inset to Fig. 5D. Current decay was significantly accelerated in agonist-treated cells ($t_{0.5}$: 6.8 ± 0.88 min vs. 14.2 ± 1.97 min; $P < 0.05$), a result consistent with faster PIP_2 breakdown.

Discussion

In the present study we show that non-hydrolysable GTP analogs cause a run-down of the TRPM7-like current in cardiac cells and we have examined the underlying mechanisms. Run-down with non-hydrolysable GTP analogs could reflect a direct inhibitory action of these nucleotides on TRPM7 and similar channels [9]. Alternatively, since such nucleotides activate G proteins coupled to a variety of enzymes, including kinases, phosphatases or phospholipases, the run-down can also be a result either of changes in the phosphorylation of the channel or of an associated regulator, or the result of the catabolism of a channel-regulating lipid.

We have previously described a run-down of cardiac TRPM7-like current following depletion of intracellular ATP [8]. Changes in protein phosphorylation were not involved since activation or inhibition of protein kinases and protein phosphatases did not modify the time course of run-down. In contrast, lipid kinases and lipid phosphatases were shown to be implicated, and exogenous PIP_2 prevented the run-down. Thus run-down of the TRPM7-like current upon ATP depletion was attributed to a depletion of membrane PIP_2 [8]. The mechanisms studied in the present study are beyond the level of ATP supply since the nucleotide was always added to the intracellular solution. Because PIP_2 loss can be induced by activating PLC, the inhibitory effect of GTP- γ -S and Gp-pNp in the present study could be due to a G protein-mediated activation of this enzyme. A role of activated G proteins is suggested by the fact that GDP- β -S, in contrast to GTP- γ -S, could not induce run-down. A role of phospholipid depletion is suggested by the protective effects against run-down of inhibiting PLC with edelfosine or of supplying exogenous PIP_2 . The acceleration of run-down by a simultaneous muscarinic and α -adrenergic receptor activation also supports the involvement of G proteins and the phosphoinositide pathway. Thus we propose that G protein activation can affect cardiac TRPM7-like channels by modifying the PIP_2 availability. This mechanism is different from the reported accentuated TRPM7 inhibition by free Mg^{2+} in the presence of guanine nucleotides [9], and run-down could occur in the absence of Mg^{2+} in the pipette solution, with Mg^{2+} highly buffered by EDTA. In addition, an enhancement of Mg^{2+} inhibition of TRPM7 also occurs with guanine diphosphonucleotides [9]. If this mechanism were implicated, run-down should have been also obtained with GDP- β -S, in contrast to our findings.

Our results on cardiac TRPM7-like current are consistent with reports of TRPM7 regulation by PIP_2 [15, 20, 21]. In our experiments, we find only a slow (over several minutes) run-down using either intracellular ATP depletion [7, 8] or non-hydrolysable guanine nucleotides (this study). In addition, the acceleration of the GTP- γ -S-induced run-down by activating G_q -coupled receptors was modest: although faster compared to control conditions, run-down under agonist treatment still occurred relatively slowly compared to the known fast (within milliseconds) co-activation of G proteins and coupled receptors [22]. The slow run-down and the modest agonist effect may be due to the fact that G protein activation, directly with GTP- γ -S or indirectly via muscarinic and alpha-adrenergic receptors, mobilize only a fraction of the total membrane phospholipid pool in cardiac cells [23]. In addition, while the present data show an agonist-mediated accelerated run-down in the presence of intracellular GTP- γ -S (see Fig. 5D), or under conditions of intracellular ATP depletion (see Fig. 5B) or of treatment with wortmannin to inhibit PIP_2 resynthesis (see Fig. 5C), the same agonists did not cause run-down in cells dialyzed with ATP and GTP (see Fig. 5A and [8]). While enhanced PIP_2 breakdown should occur under the latter conditions too, it is likely that the PIP_2 pool is rapidly replenished by PIP_2 re-synthesis [24] or that the channels have such a high affinity for PIP_2 that the phospholipid needs to be decreased to extremely low levels in order to affect channel function [25]. Additional studies to monitor PIP_2 levels in the membrane are needed to clarify these issues. Finally, despite the protection against run-down for about 50 min in the presence of edelfosine, the TRPM7-like current decreased after prolonged time with the drug (see Fig 3A). The protection against PIP_2 depletion afforded by inhibiting PLC may be incomplete because PIP_2 degradation via the phosphatase pathway remains unaffected. More stable TRPM7-like current was obtained by supplying exogenous PIP_2 , probably due to faster replenishment of PIP_2 broken down via G_q and PLC.

Depletion of PIP_2 is associated with a loss of activity of many other channel types. For example, it has also been implicated in the desensitization of the muscarinic receptor-regulated K^+ current (I_{KACH}) during continued agonist application, as well as in the inhibition of I_{KACH} by various agonists coupled to G_q [26]. These effects are relatively rapid, occurring within seconds, in contrast to the run-down of TRPM7-like current described here, which took several minutes. However, beside the agonist-dependent rapid activation and desensitization, slow

activation followed by an even slower loss of I_{KACH} with time can be observed in atrial cells dialyzed with GTP- γ -S [27, 28]. The extent and kinetics of the secondary decrease are enhanced by agonists coupled to G_q [27], a result consistent with the view that it is due to PIP_2 depletion. Similar results have been obtained with ATP-sensitive K^+ channels [29]. Modulation of the PIP_2 pool therefore appears to be a widespread mechanism for controlling channels [30]. This widespread regulation of TRP and other channels by PIP_2 has been related to the presence of positively charged residues within or near conserved or common domains in the channels proteins, but there is as yet no consensus on the identity of the domains concerned (see [30]). In the TRPM subfamily, at least four members, namely TRPM4, -5, -7 and -8, are sensitive to membrane PIP_2 levels. For TRPM4 and -5, the lipid affects both the Ca^{2+}_i - and the voltage-dependence of the channel. For TRPM8, PIP_2 also affects the sensitivity to ligands such as menthol, and vice-versa, but the lipid is also able to gate the channel by itself, i.e. in the absence of other known activating ligand. For all these channels, rapid desensitization following activation has been linked to a depletion of membrane PIP_2 . For TRPM7, no clear gating mechanism has been identified. The apparent voltage-dependence, with outward rectification of the TRPM7 current in the presence of extracellular Ca^{2+} and Mg^{2+} , largely reflects permeation and/or block. The only known potential gating factor is intracellular Mg^{2+} , but the mechanisms by which it inhibits the activity of the TRPM7 channels are still unknown. The influence of PIP_2 on the Mg^{2+} sensitivity of TRPM7 remains unexplored, but the lipid has been reported to affect the Mg^{2+} affinity of TRPV5 [31], another Mg^{2+} -sensitive channel. Interaction between Mg^{2+}_i and PIP_2 on TRPM7 has been proposed to involve surface charges near the channels [21].

TRPM7 has been implicated in a variety of functions, including Mg^{2+} transport, stretch sensing, cell adhesion, cell proliferation or death, etc. (see [2]), but its role in cardiac cells remains unexplored. The experimental conditions used in our study are far from physiological. Particularly, intracellular $[\text{Mg}^{2+}]$ was kept at very low levels to allow measurements of the TRPM7-like current, or was set at practically zero level to study the regulation of the current independently of an effect mediated by this divalent cation. The current can modulate the action potential shape if Mg^{2+}_i is kept low (our unpublished results) but till now it remains unclear as to what extent the TRPM7-like current contributes to the total ion currents and to ion homeostasis under physiological

conditions in cardiac cells. While the present study provides additional evidence supporting the view that PIP_2 regulates the cardiac TRPM7-like channel, the question remains as to whether physiological or pathological conditions exist where the decreased channel activity following PIP_2 depletion can be of functional importance. Acute metabolic stress conditions such as ischemia and reperfusion may cause a change in phosphoinositide turnover (see [32]). In chronic diseases, reduced PIP_2 levels have been reported in hypertrophy, diabetic cardiomyopathy, and congestive heart failure [33]. Recent studies indicate that a loss of function of TRPM7 may underlie neurodegenerative diseases [13, 18], and in the case of familial Alzheimer's diseases,

the underlying mechanism seems to involve an abnormal turnover of PIP_2 with decreased membrane PIP_2 levels and decreased TRPM7 current [18]. Further studies are needed to determine whether similar mechanisms could be implicated in myocardial diseases.

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