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Modulation of Ca^{2+}- and voltage-activated K^+ channels by internal Mg^{2+} in salivary acinar cells

Laura G. Squire and Ole H. Petersen

M.R.C. Secretory Control Research Group, Department of Physiology, University of Liverpool, Liverpool (U.K.)

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High-conductance K^+ channels are known to be activated by internal Ca^{2+} and membrane depolarization. The effects of changes in internal Mg^{2+} concentration have now been investigated in patch-clamp single-channel current experiments on excised membrane fragments from mouse parotid acinar cells. It is shown that Mg^{2+} in the concentration range 10^{-6}–10^{-3} M evokes a dose-dependent K^+ channel activation at a constant Ca^{2+} concentration of 10^{-8} M. The demonstration that changes in $[Mg^{2+}]_i$ between $2.5 \cdot 10^{-4}$ and $1.13 \cdot 10^{-3}$ M has effects on the channel open-state probability indicates that fluctuations in $[Mg^{2+}]_i$ in intact cells may influence the control of channel opening.

Introduction

Electrophysiologial events in the basolateral acinar membranes of mammalian salivary as well as other exocrine glands, have been reported to be dominated by currents through a high-conductance voltage- and Ca^{2+}-activated K^+ channel [1,2]. This channel has a conductance of around 250 pS when both sides of the membrane are bathed in high K^+ solutions (145 mM K^+). Channels of this type have been found in a variety of other tissues including endocrine cells [3–5] and muscle [6].

Single channel current events may be studied in two different ways. The patch-clamp method [7] allows ion channels to be observed while still part of the original membrane, either in intact cells or as small patches of membrane removed from the cell. The second method involves a reconstituted system in which ion channels are inserted into a model phospholipid bilayer [8]. The latter system has recently been used to study how Mg^{2+} modulates Ca^{2+} activation of the high-conductance K^+ channel found in rat skeletal muscle [9]. Mg^{2+} in the concentration range 1–10 mM was shown to activate the K^+ channels in the presence of Ca^{2+}, whereas this effect was not seen in its absence. Mg^{2+} was only effective when added to what corresponds to the cytoplasmic side of the channel. It was suggested that Mg^{2+} acts by increasing the affinity of the K^+ channel for Ca^{2+} [9].

The intracellular concentration of Mg^{2+} has been estimated to be between 0.4 and 3.0 mM using a variety of methods [10]. Solutions used to mimic the intracellular solution in cell-free patch-clamp experiments generally have a Mg^{2+} concentration in this range, 1.13 mM in our laboratory, added in the form of $MgCl_2$. In the following, we describe experiments in which the patch-clamp method [7] was used to study the effects of

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfphonic acid.

Correspondence: O.H. Petersen, M.R.C. Secretory Control Research Group, Department of Physiology, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, U.K.

changes in the bath fluid Mg^{2+} concentration (in the presence of a constant Ca^{2+} concentration) on the single-channel K^+ currents from excised inside-out membrane patches of basolateral acinar cell membranes. Changes in Mg^{2+} concentration within the range 1.13–10 mM had litte effect on the activity of the K^+ channels, whereas a concentration-dependent Mg^{2+} activation of channels was seen in the range 10^{-6}–10^{-3} M.

Materials and Methods

Adult male mice were killed by stunning and cervical dislocation. Parotid glands were removed and dissociated using a method similar to that described by Findlay [11] in which glands were serially treated with 3 mg/ml trypsin, 3 mg/ml trypsin inhibitor and 200 units/ml collagenase in a physiological saline solution. After digestion, the tissue was washed in physiological saline for at least 30 min before use. Aliquots of cells were placed in a 2 ml chamber on the stage of an Olympus CK inverted microscope and viewed at 400 × magnification. Single-channel currents from membrane patches were measured using an LM EPC 5 patch-clamp amplifier. The signal was displayed on a storage oscilloscope and simultaneously recorded on Ampex four track magnetic tape (Racal 4DS recorder).

The taped current records were analyzed using a laboratory microcomputer (BBC model B with 6502 second processor) connected to a Winchester Hard Disc System. The data was digitized (3 kHz), and an idealized current record obtained from computerized threshold analysis. The average open-state probability was determined from current records lasting 10–20 s.

Pipettes with resistances of 1 to 2 MΩ were used and filled, in all experiments, with a solution of the following composition: 140 mM KCl/1.13 mM $MgCl_2$/10 mM glucose/10 mM Hepes/1.2 mM $CaCl_2$ at pH 7.2. Gigaseals were obtained with cells in a standard extracellular solution containing 140 mM NaCl/4.8 mM KCl/1.13 mM $MgCl_2$/10 mM glucose/10 mM Hepes/1.2 mM $CaCl_2$ at pH 7.2. Patches of membrane were then excised by pulling the pipette away from the cell and passing it briefly through the air/bath solution interface. The pipette tip was then moved into a small adjoining chamber, allowing the solution in the main chamber to be changed without damage to the patch. The effects of changes in Mg^{2+} concentration were investigated by exposing the inside of the patch membrane to different high K^+ (140 mM) solutions, all containing 10^{-8} M Ca^{2+} and between 10^{-6} and 10^{-3} M Mg^{2+}.

The free concentrations of Ca^{2+} and Mg^{2+} in the bath solutions were determined by the addition of $CaCl_2$, EGTA and $MgCl_2$. The total concentrations of each, required to produce the desired free concentrations, were calculated using an iterative procedure on a BBC (model B) microcomputer using the stability constants for the reactions between Ca, Mg, H and EGTA [5,12] (Table I). In the case of reactions involving H^+ the constants were corrected so as to transform the

TABLE I

TOTAL CONCENTRATIONS OF CALCIUM, MAGNESIUM AND EGTA ADDED TO INTRACELLULAR SOLUTIONS TO PRODUCE THE REQUIRED FREE ION CONCENTRATIONS

Calculations are based on stability constants for the reactions between Ca, Mg, H and EGTA [12]. pH was kept constant at 7.2 (10 mM Hepes) in all solutions.

Total EGTA (M)	Total Ca^{2+} (M)	Total Mg^{2+} (M)	Calculated free Ca^{2+} (M)	Calculated free Mg^{2+} (M)
$2.2 \cdot 10^{-3}$	$1.34 \cdot 10^{-4}$	0 (estimated to be 10^{-6})	10^{-8}	$9.0 \cdot 10^{-7}$
$2.2 \cdot 10^{-3}$	$1.33 \cdot 10^{-4}$	$2.76 \cdot 10^{-4}$	10^{-8}	$2.5 \cdot 10^{-4}$
$2.2 \cdot 10^{-3}$	$1.29 \cdot 10^{-4}$	$8.25 \cdot 10^{-4}$	10^{-8}	$7.5 \cdot 10^{-4}$
$2.2 \cdot 10^{-3}$	$1.28 \cdot 10^{-4}$	$1.24 \cdot 10^{-3}$	10^{-8}	$1.13 \cdot 10^{-3}$
$2.2 \cdot 10^{-3}$	$9.11 \cdot 10^{-5}$	$1.07 \cdot 10^{-2}$	10^{-8}	10^{-2}

concentration constants into mixed constants including activity and concentration terms, to take account of the fact that pH measurement results in values for activity rather than concentration [13]. Ca-EGTA buffers were prepared using a titration method as described by Miller and Smith [14]. The content of $MgCl_2$ in hydrated $MgCl_2$ was estimated to be 90% on the basis of atomic absorption spectrometry.

Results

Giga seals were formed using pipettes with resistances in the region of 1–2 MΩ. Small clusters of 3 to 15 cells were used and it was assumed that the portions of membrane which were accessible in such a cluster were basolateral. After formation of the seal, with the pipette potential clamped at zero millivolts, inward single channel current pulses were observed which became more frequent and longer by the application of negative pipette potentials [1]. These inward currents reversed at a pipette potential of around −55 mV. After excision into the bath solution, which initially contained a high Na^+ solution, no reversal potential could be obtained and rectifying inward (K^+) currents were observed. After exchanging the bath solution for a high K^+ solution with 1.13 mM Mg^{2+} and 10^{-8} M Ca^{2+} (control), the activity of the channels was reduced and reversal of currents occurred at zero pipette potential.

Single-channel conductance and open-state probability were measured at different membrane potentials in excised patches. In an initial series the bath contained either control solution (1.13 mM Mg^{2+}) or a test solution with 10 mM Mg^{2+}. The mean unit conductances of the channels in the two different Mg^{2+} concentrations were approximately equal, 255 ± 46 (S.D.) pS in 10 mM Mg^{2+} and 243 ± 43 pS in 1.13 mM Mg^{2+} ($n = 12$). In 10 out of the 12 experiments there was little or no difference between the pattern of channel opening at the two different Mg^{2+} concentrations. In the two remaining experiments, the higher Mg^{2+} concentration blocked the channels. In all these, as well as the following experiments, the inside-out nature of the excised patch membrane was demonstrated by the activation of channels in response to application of negative pipette voltages.

In another series of experiments we investigated the effects of changes in Mg^{2+} concentration within the range 10^{-6}–$1.13 \cdot 10^{-3}$ M. The bath concentration of Ca^{2+} was maintained at 10^{-8} M in all cases. Fig. 1 shows the reversible activation of channels observed when the solution bathing the cytoplasmic side of the membrane was changed from one containing virtually no Mg^{2+} (approx. 10^{-6} M) to one containing 1.13 mM Mg^{2+}. The maximum number of channels to open simultaneously in this experiment in the virtual absence of Mg^{2+} was two, but in the presence of 1.13 mM Mg^{2+} up to four coincident channel openings were seen in the same patch. Fig. 2 quantifies the results of the three experiments that were sufficiently long to permit reversal of the Mg^{2+} effect to be observed. The mean conductances of the K^+ channels investigated in the two Mg^{2+} concentrations were similar, 261 ± 29 (S.D.)

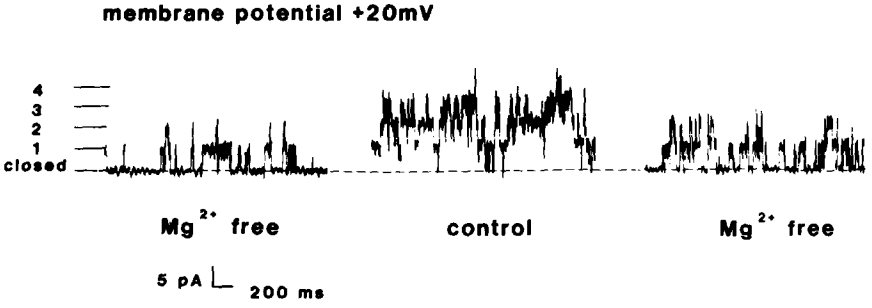

Fig. 1. Reversible activation of K^+ channels by 1.13 mM Mg^{2+}. The inside-out patch of basolateral membrane was in symmetrical high K^+ bath and pipette solutions. The concentration of Mg^{2+} in the bath was altered from about 10^{-6} M Mg^{2+} (Mg^{2+}-free) to 1.13 mM Mg^{2+} (control) and then returned to 'Mg^{2+}-free'. The Ca^{2+} concentration was maintained at 10^{-8} M with EGTA and pH at 7.2 with Hepes throughout the experiment.

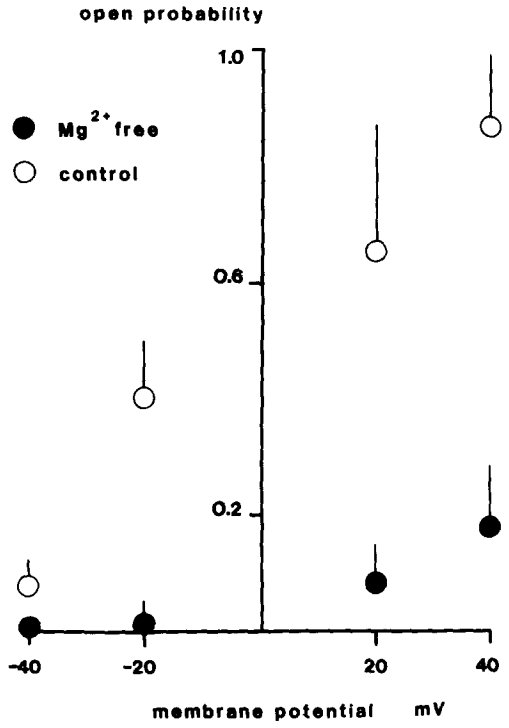

Fig. 2. Graphical representation of Mg^{2+}-activation of K^+ channels. Means and standard deviations of open probabilities in three experiments in which a reversible activation of K^+ channels in inside-out patches was achieved by changing the Mg^{2+}-free (approx. 10^{-6} M) bath solution to a solution containing 1.13 mM Mg^{2+}.

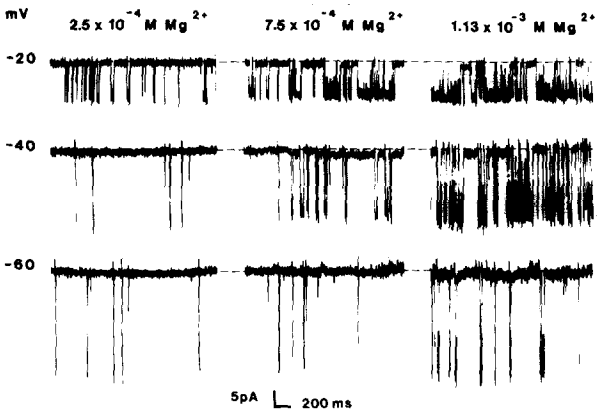

Fig. 3. Concentration dependence of Mg^{2+}-activation of K^+ channels. The inside-out patch of basolateral membrane was exposed to symmetrical 140 mM KCl solutions in the bath and pipette. The bath solution was changed from one containing $2.5 \cdot 10^{-4}$ M Mg^{2+} to $7.5 \cdot 10^{-4}$ M Mg^{2+} and then to one containing 1.13 mM Mg^{2+}. This series was reversed and repeated three times. All traces shown were obtained from the same membrane patch.

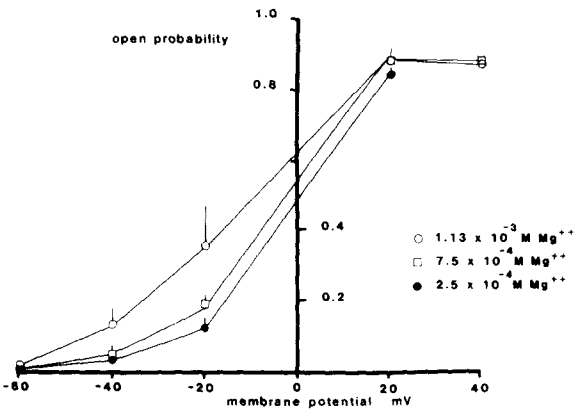

Fig. 4. Graphical representation of the concentration-dependence of Mg^{2+} activation of K^+ channels. Inside-out patches of membrane, in symmetrical 140 mM KCl bath and pipette solutions, were exposed to a series of bath solutions containing $2.5 \cdot 10^{-4}$ M, $7.5 \cdot 10^{-4}$ M and $1.13 \cdot 10^{-3}$ M Mg^{2+}. All solutions contained 10^{-8} M Ca^{2+}. The graph shows means and standard deviations from three repeats of this series on the same inside-out patch.

pS in 10^{-6} M Mg^{2+} and 260 ± 17 pS in 1.13 mM Mg^{2+}.

The dose dependence of the Mg^{2+}-activation was also investigated. Fig. 3 shows traces taken from an experiment in which the cytoplasmic side of the patch was exposed to high K^+ solutions containing 10^{-8} M Ca^{2+} and either $2.5 \cdot 10^{-4}$ M, $7.5 \cdot 10^{-4}$ M or $1.13 \cdot 10^{-3}$ M Mg^{2+}. Reversals of the effect of the two higher Mg^{2+} concentrations were observed after return to the lower Mg^{2+} concentration. Reversible channel activations by the two higher Mg^{2+} concentrations were repeated three times in this particular patch and the results are summarized in Fig. 4, showing that Mg^{2+} caused a dose-dependent increase in the open-state probability of the Ca^{2+}-activated K^+ channel.

Discussion

Our results demonstrate for the first time that the activity of the high-conductance voltage- and calcium-dependent K^+ channel in its normal membrane environment may be regulated by internal Mg^{2+}. The effect on the open-state probability at a constant internal Ca^{2+} concentration of 10^{-8} M is particularly pronounced in the concentration range 10^{-6} M–1.13 mM.

Mg^{2+}-activation of reconstituted K^+ channels from T-tubule membranes in rat skeletal muscle has recently been observed by Golowasch et al. [9] who reported that Mg^{2+} in the concentration range 1–10 mM increases the apparent affinity of the channel for Ca^{2+} and greatly enhances the sigmoidicity of the Ca^{2+}-activation curve studied [9] in the concentration range 10–100 μM. The high-conductance voltage- and calcium-activated K^+ channel has been characterized in native membranes from a variety of tissues, but the Ca^{2+}-activation curves differ markedly between tissue types [2]. In mammalian exocrine glands the maximum sensitivity to changes in internal Ca^{2+} concentration occurs between 10^{-8} M and 10^{-7} M [2,11,15,16], whereas in rat muscle cells the corresponding range is 10^{-6}–10^{-4} M [6]. The apparent insensitivity to Ca^{2+} of the channels in the reconstituted system of Golowasch et al. [9], is proposed by the authors to be due to the electroneutrality of the lipids used in their membranes as compared to the negative charges which would normally surround the calcium activation site of the channel in natural plasma membranes. Our finding that the range within which Mg^{2+} is effective in modulating channel activity is 10^{-6} M–1 mM, is consistent with the prediction of Golowasch et al. [9], that the Mg^{2+} effect in the reconstituted system might have been observed at lower concentrations by placing the channel in an environment of charged lipids.

The possibility that effects of changes in Mg^{2+} concentration on the outside of the plasma membrane might influence channel opening was not investigated in this study. Evidence from Rb^{86} efflux experiments [17] suggests, however, that changes in external Mg^{2+} concentration do not influence the activity of K^+ channels in the rat parotid gland.

The significance of our findings in relation to physiological regulation of cell activity is not clear, since it is unknown whether fluctuations in the intracellular salivary acinar Mg^{2+} concentration occur, but it has been suggested that Mg^{2+} may act as a second messenger and that its transport into some cells is hormonally regulated [18]. Our results are, however, at least important in the context of cell-free or excised patch-clamp single-channel current experiments in which parameters such as pH and free Ca^{2+} are varied and the activity of channels observed. In such experiments the results from observations on cell-free patches can be compared to those from cell-attached recordings in order to estimate, for example, the intracellular Ca^{2+} concentration [15]. Our findings show that the internal Mg^{2+} concentration is an additional factor to which careful attention should be paid in such experiments, and, when divalent cation chelators are used to buffer Ca^{2+}, their effect on Mg^{2+} should not be ignored.

Acknowledgements

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References

1 Maruyama, Y., Gallacher, D.V. and Petersen, O.H. (1983) Nature 302, 827–829
2 Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696
3 Marty, A. (1981) Nature (Lond.) 291, 497–500
4 Wong, S., Lecar, H. and Adler, M. (1982) Biophys. J. 39, 313–317
5 Findlay, I., Dunne, M.J. and Petersen, O.H. (1985) J. Membr. Biol. 83, 169–175
6 Barrett, J.N., Magleby, K.L. and Pallotta, B.S. (1982) J. Physiol. (Lond.) 331, 211–230
7 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth F.J. (1981) Pflügers Arch. 391, 85–100
8 Miller, C. (1986) Ion Channel Reconstitution, Plenum Press, New York
9 Golowasch, J., Kirkwood, A. and Miller C. (1986) J. Exp. Biol. 124, 5–13
10 Corkey, B.E., Duszynski, J., Rich, T.L., Matschinsky, B. and Williamson, J.R. (1986) J. Biol. Chem. 261, 2567–2574
11 Findlay, I. (1984) J. Physiol. 350, 179–195
12 Martell, A.E. and Smith, R.M. (1974) Critical Stability Constants, Vol. 1, Amino Acids, Plenum Press, New York
13 Fabiato, A. (1981) J. Gen. Physiol. 78, 457–497
14 Miller, D.J. and Smith, G.L. (1984) Am. J. Physiol. 246, C160–C166
15 Maruyama, Y., Petersen, O.H., Flanagan, P. and Pearson, G.T. (1983) Nature (Lond.) 305, 228–232
16 Gallacher, D.V. and Morris, A.P. (1986) J. Physiol. 373, 379–395
17 Putney, J.W., Jr. (1978) Am. J. Physiol. 235, C180–C187
18 Flatman, P.W. (1984) J. Membr. Biol. 80, 1–14