VERSITA

CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 12 (2007) pp 220 - 230
http://www.cmbl.org.pl

DOI: 10.2478/s11658-006-0067-6

Received: 27 July 2006
Revised form accepted: 12 October 2006
Published online: 12 December 2006

THE INFLUENCE OF PROTONS AND ZINC IONS ON THE STEADY-STATE INACTIVATION OF Kv1.3 POTASSIUM CHANNELS

ANDRZEJ TEISSEYRE* and JERZY W. MOZRZYMAS
Department of Biophysics, Wrocław Medical University,
ul. Chałubińskiego 10, 50-368 Wrocław, Poland

Abstract: Using the whole-cell patch-clamp technique, we investigated the influence of extracellular pH and zinc ions (Zn^{2+}) on the steady-state inactivation of Kv1.3 channels expressed in human lymphocytes. The obtained data showed that lowering the extracellular pH from 7.35 to 6.8 shifted the inactivation midpoint (V_i) by 17.4 ± 1.12 mV (n = 6) towards positive membrane potentials. This shift was statistically significant ($p < 0.05$). Applying 100 μM Zn^{2+} at pH 6.8 further shifted the V_i value by 16.55 ± 1.80 mV (n = 6) towards positive membrane potentials. This shift was also statistically significant ($p < 0.05$). The total shift of the V_i by protons and Zn^{2+} was 33.95 ± 1.90 mV (n = 6), which was significantly higher ($p < 0.05$) than the shift caused by Zn^{2+} alone. The Zn^{2+}-induced shift of the V_i at pH 6.8 was almost identical to the shift at pH = 7.35. Thus, the proton- and Zn^{2+}-induced shifts of the V_i value were additive. The steady-state inactivation curves as a function of membrane voltage were compared with the functions of the steady-state activation. The total shift of the steady-state inactivation was almost identical to the total shift of the steady-state activation (32.01 ± 2.10 mV, n = 10). As a result, the "windows" of membrane potentials in which the channels can be active under physiological conditions were also markedly shifted towards positive membrane potentials. The values of membrane voltage and the normalised chord conductance corresponding to the points of intersection of the curves of steady-state activation and inactivation were also calculated. The possible physiological significance of the observed modulatory effects is discussed herein.

---

* Author for correspondence: ateiss@biofiz.am.wroc.pl

Abbreviations used: gK_{norm} – normalised relative chord conductance; gK_{SSnorm} – steady-state normalised relative chord conductance; k_i – steepness of the voltage dependence (inactivation); k_n – steepness of the voltage dependence (activation); pH_o – extracellular pH; TL – human T lymphocytes; V_i – inactivation midpoint; V_n – activation midpoint; Zn^{2+} – zinc ions

Key words: Zinc, Lymphocyte, Potassium channels, Patch-clamp, pH, Neuronal excitability

INTRODUCTION

Zinc ions (Zn^{2+}) are important endogenous regulators of the functions of many proteins, including ion channels. It is known that Zn^{2+} is present in the central nervous system at concentrations of up to 100-300 μM [1, 2]. The results of electrophysiological studies provide evidence that Zn^{2+} modulates the activity of many different types of ion channels [2]. Among them are $GABA_A$ and NMDA receptors, voltage-gated sodium and calcium channels, voltage-gated and ATP-dependent potassium channels, and voltage- and ligand-gated chloride channels [2]. Importantly, it was shown that the modulatory effect of Zn^{2+} on ion channels was due to specific interactions with binding sites on the channels, and not due to a compensation of negative surface charges [2].

Voltage-gated potassium channels Kv1.3 also belong to the group of ion channels modulated by Zn^{2+}. These channels are expressed abundantly in human T lymphocytes (TL), where they play an important role in setting the resting membrane potential, cell mitogenesis, apoptosis and volume regulation [3-6]. Kv1.3 channels are also present in the rat central nervous system, especially in olfactory bulb neurons, where they play a modulatory role in action potential generation [7-9]. The channels are also expressed in human alveolar macrophages [10], rat choroid plexus epithelial cells [11], rabbit kidney and colon epithelial cells [12], human gliomas [13], and rat prostate cancer cell lines [14].

The modulatory effect of Zn^{2+} on Kv1.3 channels was studied, and the results were published [15]. The obtained data provided evidence that applying 10-100 μM Zn^{2+} caused a shift in the voltage dependence of both steady-state activation and inactivation towards positive membrane potentials. There was also a significant slowing of the current activation rate. Raising the Zn^{2+} concentration from 100 μM to 2.6 mM caused a concentration-dependent decrease in the current amplitude to about 20% of the control value without any further changes in the voltage-dependence of the steady-state activation and inactivation or in the activation kinetics. The modulatory effect of Zn^{2+} was not due to the compensation of negative surface charges by Zn [15].

The results of our recent experiments showed that the magnitude of the shift of the steady-state activation and the degree of current inhibition by Zn^{2+} were independent of extracellular pH (pH_o) in the range from 6.4 to 8.4 [16]. The modulatory effects of Zn^{2+} on Kv1.3 channels also did not depend on changes in the intracellular pH in this range [Teisseyre – unpublished observations]. Nevertheless, it was shown that lowering the pH_o from 7.35 to 6.4 significantly slowed the current activation rate, shifted the activation midpoint by about 16 mV towards positive membrane potentials and reduced the current amplitude to about 0.55 of the control value. By contrast, raising the pH_o from 7.35 to 8.4 did not significantly affect the activation midpoint and current amplitude [16]. The

modulatory effects of protons at pH_o 6.4 and Zn^{2+} were additive [16]. The additive modulatory effects of protons and Zn^{2+} on the steady-state activation and activation kinetics of Kv1.3 channels were also observed at pH_o 6.8 and at a Zn^{2+} concentration of 100 μM, as both values lie within a physiologically relevant range [17].

Nevertheless, some aspects of the modulatory effects of protons and Zn on Kv1.3 channels are still unknown. In particular, it remains unknown whether lowering the pH_o affects the steady-state inactivation of Kv1.3 channels and whether the modulatory effects of protons and Zn^{2+} on the steady-state inactivation are additive. The available literature does not provide any information on this issue [16-18]. Steady-state inactivation is an important parameter of the activity of Kv1.3 channels [3-6]. It is known that the channels are capable of activity under physiological conditions only within a "window" of membrane potentials where they can open and are not inactivated [3]. Since it is known that the steady-state inactivation of Kv1.3 channels was markedly shifted towards positive membrane potentials upon Zn^{2+} application [15] and because the modulatory effect of protons on the channels resembled the effect exerted by Zn^{2+} [16], it is possible that the steady-state inactivation was also shifted by protons towards positive membrane potentials. Such a hypothesis was presented in our recently published article [16]. However, because no experiments were performed, this hypothesis remained speculation.

In this study, the influence of protons and Zn^{2+} on the steady-state inactivation of Kv1.3 channels was examined. Since Kv1.3 channels are expressed abundantly and predominantly in human TL [3-6], these cells were used in our experiments as a model system.

The obtained results demonstrate that lowering the pH_o from 7.35 to 6.8 shifted the steady-state inactivation curve of the channels towards positive membrane potentials. The shifts in the steady-state inactivation caused by protons and Zn^{2+} were additive, such as in the case of the steady-state activation. Because of the shifts in the steady-state activation and inactivation towards positive membrane potentials, the "window" of the membrane potential in which the channels can be active under physiological conditions was also shifted towards positive potentials.

MATERIALS AND METHODS

Cell separation, solutions and pipettes

Human TL were separated from peripheral blood samples from 10 healthy donors using a standard method described elsewhere [19]. For the experiment, the cells were placed in an external solution containing (in mM): 150 NaCl, 4.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 Glucose, pH = 7.35, adjusted with NaOH, 300 mOsm. The pipette solution contained (in mM): 150 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 EGTA; pH = 7.2, adjusted with KOH, 300 mOsm. The concentration of free calcium in the internal solution was below 100 nM, assuming a dissociation constant for EGTA of 10^{-7} M at pH = 7.2 [20]. This low

calcium concentration was used in order to prevent the activation of calcium-activated IKCa1 channels [20]. The reagents were provided by the Polish Chemical Company (POCH, Gliwice, Poland), except for HEPES, EGTA and $ZnCl_2$, which were purchased from SIGMA. Dishes with cells were placed under an inverted Olympus IMT-2 microscope. External solutions containing Zn were applied using the RSC 200 fast perfusion system (Bio-Logic, Grenoble, France). The pipettes were pulled from a borosilicate glass (Hilgenberg, Germany) and fire-polished before the experiment. The pipette resistance was in the range of 3-5 MΩ.

Electrophysiological recordings

Whole-cell potassium currents in TL were recorded applying the patch-clamp technique [21]. The currents were recorded using an EPC-7 Amplifier (List Electronics, Darmstadt, Germany), low-pass filtered at 3 kHz, digitised using the CED Micro 1401 (Cambridge, UK) analogue-to-digital converter with a sampling rate of 10 kHz. The linear (ohmic) component of the current was subtracted off-line from the final record. The data was analysed using the WCP J. Dempster Program.

The results of our earlier studies demonstrate that the currents recorded in TL in the whole-cell configuration are predominantly due to the activation of Kv1.3 channels [15]. The steady-state inactivation of the currents was investigated applying the following experimental protocol: the examined cells were patch-clamped in the whole-cell configuration at various holding potentials from -80 mV to 0 mV (at 10 mV increments) for 40 sec. Then, Kv1.3 currents were evoked by applying 50-ms voltage pulses to +60 mV. Leak currents were subtracted from the records during the off-line analysis. Since the magntitude of the currents varied significantly from cell to cell, the normalised relative peak currents were used for calculations of the steady state-inactivation. This current was calculated by dividing the peak current recorded from a given holding potential by the peak current recorded in the same cell from the holding potential of -80 mV. Since all the currents were recorded at the potential of +60 mV, the calculated relative peak current was equal to the normalised relative chord conductance (gK_{norm} – see below). The gK_{norm} was defined by the equation: $gK_{norm} = gK/gK_{-80}$, where gK is the chord conductance of the current recorded from a given holding potential, and gK_{-80} is the chord conductance of the current recorded from the holding potential of -80 mV. The chord conductance was calculated according to: $gK = I_p/(V-V_{rev})$, where I_p is the amplitude of the current, V is the membrane potential at which the current was recorded (here +60 mV for all the currents), and V_{rev} is the reversal potential of the current, which is -75 mV. Thus, $gK = I_p/135$ mV for all the currents, and therefore $gK/gK_{-80} = I_p/I_{p(-80)}$. The voltage dependence of the steady-state inactivation was fitted by a Boltzmann function given by the equation: $gK_{norm}(V) = 1/[1+\exp-(V-V_i)/k_i]$, where V_i is the inactivation midpoint, and k_i is the steepness of the voltage dependence. To obtain the "windows" of the membrane potential in which the Kv1.3 currents can be activated under physiological conditions, the voltage

dependence of steady-state activation was also fitted to the data obtained in our previous experiments. This was done by applying a similar Boltzmann function: $gK_{norm}(V) = 1/[1+\exp-(V-V_n)/k_n]$, where V_n is the activation midpoint, and k_n is the steepness of the voltage dependence. In this case, the gK_{norm} was defined by the equation: $gK_{norm} = gK/gK_{60}$, where gK is the chord conductance of the current recorded at a given membrane potential, and gK_{60} is the chord conductance of the current recorded at the potential of +60 mV. The values of the membrane voltage that correspond to the points of intersection of the curves were calculated assuming that at the point of intersection $(V-V_i)/k_i = (V-V_n)/k_n$. As the values of V_i, k_i, V_n and k_n are known, V could be calculated by rearranging this equation to: $V = (V_ik_n - V_nk_i)/(k_n - k_i)$.
The data is given as the mean ± standard error. All the experiments were carried out at room temperature (22-24ºC).

RESULTS AND DISCUSSION

Fig. 1 depicts the steady-state inactivation of Kv1.3 currents as a function of the holding membrane potential. Under control conditions (pH_o 7.35, no Zn^{2+}) the value of V_i was -56.66 ± 0.61 mV (n = 10). When pH_o was lowered to 6.8 in the absence of Zn^{2+}, the steady-state inactivation curve was shifted markedly towards positive membrane potentials. The value of V_i at pH_o 6.8 was -39.2 ± 0.51 mV (n = 6), which was significantly ($p < 0.05$, Student's t-test) more positive than

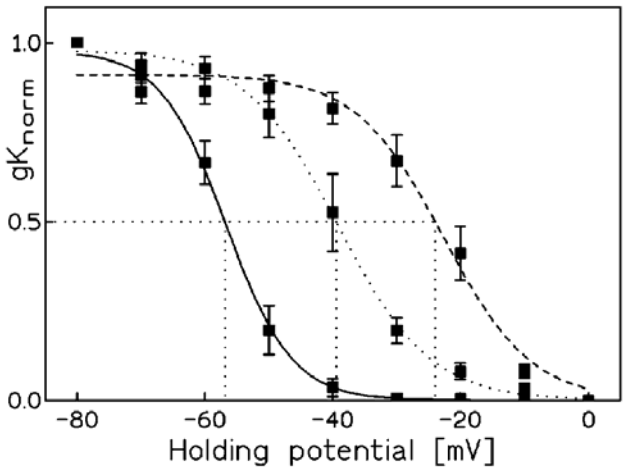

Fig. 1. Steady-state inactivation of Kv1.3 currents in terms of the normalised relative chord conductance (gK_{norm}) as a function of the holding potential: filled squares, solid line – control conditions (pH_o 7.35, no Zn^{2+}, n=10); filled squares, dotted line – pH_o 6.8, no Zn^{2+}, (n=6); filled squares, dashed line – pH_o 6.8, 100 μM Zn^{2+}, (n=6). The values of the holding potential corresponding to V_i are drawn as vertical dotted lines.

under the control conditions. The application of 100 μM Zn^{2+} at pH_o 6.8 caused a further shift of the steady-state inactivation of the currents towards positive membrane potentials. The value of V_i upon Zn^{2+} application at pH_o 6.8 was -22.65 ± 1.29 mV (n = 6), which was significantly ($p < 0.05$, Student's t-test) more positive than in the absence of Zn at pH_o 6.8. The values of the steepness parameter (k_i) were -5.14 ± 0.49 mV (n = 10), -7.23 ± 0.43 mV (n = 6) and -6.86 ± 1.29 mV (n = 6), respectively under control conditions, at pH_o 6.8 and upon the application of 100 μM Zn^{2+} at pH_o 6.8 (not shown). These values were not statistically different from each other ($p > 0.05$, one-way ANOVA).

Fig. 2 shows the value of the inactivation midpoint (V_i) shift upon the lowering of pH_o to 6.8 and upon the application of 100 μM Zn^{2+} at pH_o 6.8, and the total shift caused by the two factors. The average value of the shift upon the lowering of pH_o was 17.4 ± 1.12 mV (n = 6), and the application of Zn^{2+} at low pH_o shifted the inactivation midpoint by a further 16.55 ± 1.80 mV (n = 6) towards positive membrane potentials. The total value of the shift was 33.95 ± 1.90 mV (n = 6), which was significantly ($p < 0.05$, Student's t-test) higher than the shift caused by Zn^{2+} application.

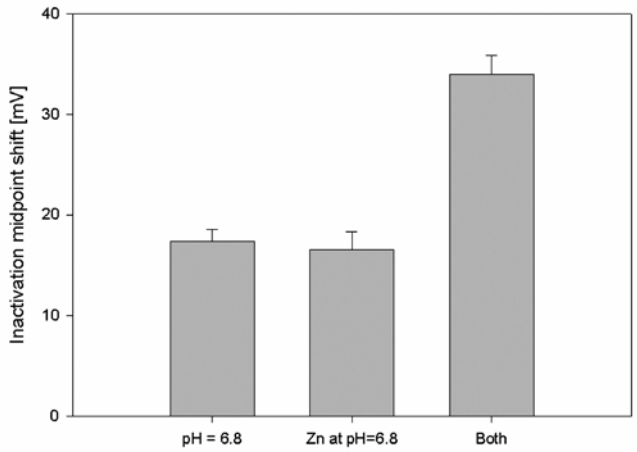

Fig. 2. The average inactivation midpoint shift caused by protons at pH_o 6.8, with 100 μM Zn^{2+} applied at pH_o 6.8, and the total shift caused by both ions.

Fig. 3 compares the average values of the inactivation midpoint shifts upon application of 100 μM Zn^{2+} at pH_o 6.8 and 7.35. The value obtained for pH_o 7.35 was calculated on the basis of our previously published data [15]. Apparently, the shifts induced by Zn^{2+} at pH_o 6.8 (16.55 ± 1.80 mV; n = 6) and pH_o 7.35 (17.01 ± 0.92 mV; n = 10) were not significantly different from each other ($p > 0.05$, Student's t-test). Thus, the obtained results demonstrate that the Zn^{2+}-induced shift of the steady-state inactivation of Kv1.3 currents was not affected by the lowering of the pH_o from 7.35 to 6.8.

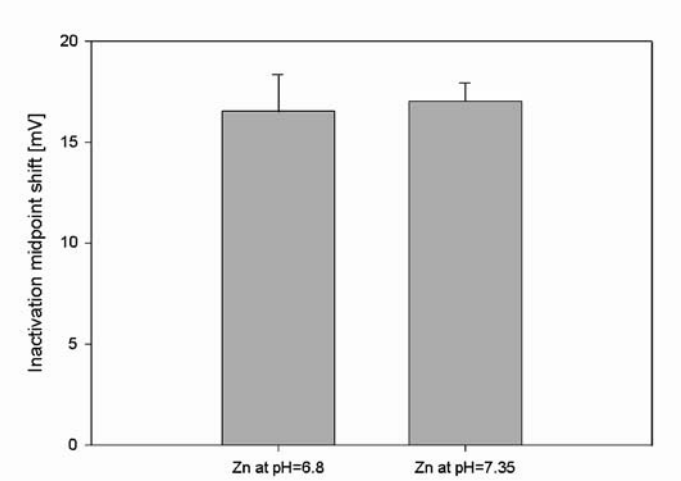

Fig. 3. Average inactivation midpoint shift caused by 100 μM Zn^{2+} applied at pH_o 6.8 and 7.35.

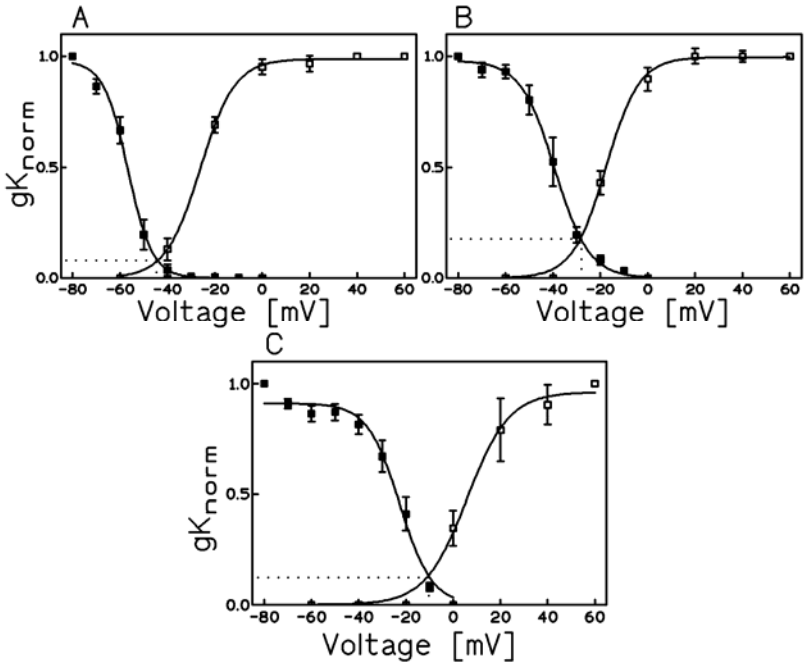

Fig. 4. Curves of steady-state inactivation (filled squares) and steady-state activation (open squares) as a function of voltage. A – control conditions (pH_o 7.35, no Zn^{2+}, n=10); B – pH_o 6.8, no Zn^{2+} (n=6); C – pH_o 6.8, 100 μM Zn^{2+} (n=6). The values of voltage and gK_{norm} corresponding to the points of intersection of the curves are indicated by vertical and horizontal lines, respectively.

As mentioned above, Kv1.3 channels can be activated under physiological conditions only within a "window" of membrane potentials where they are open and not inactivated. Such a "window" can be estimated by putting together the curves decribing the voltage dependence of the steady-state activation and inactivation. The channels can be activated under physiological conditions only when the curves of steady-state activation and inactivation intersect with each other: the point of intersection is the point where the probability for the channels to be open and not inactivated is the highest. The "frames" of the "window" are defined by those parts of these curves which lie below the point of intersection. We estimated the "windows" of the membrane potentials for Kv1.3 channels under control conditions, at pH_o 6.8 and upon the application of 100 μM Zn^{2+} at pH_o 6.8 by putting the curves of steady-state inactivation presented in Fig. 1 together with the curves of steady-state activation obtained by fitting our previously recorded data with the Boltzmann function defined in the Materials and Methods section. The results are presented in Fig. 4.

Apparently, the steady-state inactivation and activation curves were both shifted towards positive membrane potentials when the pH_o was lowered from 7.35 to 6.8 (Fig. 4B) and when 100 μM Zn^{2+} was applied at pH_o 6.8 (Fig. 4C). Therefore, the "windows" were also shifted towards positive membrane potentials. The values of the membrane voltage which correspond to the points of intersection of the curves were calculated. The values were -44.12 mV for the control conditions (pH_o 7.35, no Zn^{2+}), -28.3 mV at pH_o 6.8 with no Zn^{2+} and -10.23 mV when 100 μM Zn^{2+} was applied at pH_o 6.8. The value of the gK_{norm}, which corresponds to the voltage at the point of the curve intersection, defines the steady-state normalised chord conductance gK_{SSnorm}. The calculated values of gK_{SSnorm} were 0.08 for control conditions (pH_o 7.35, no Zn^{2+}), 0.18 at pH_o 6.8 with no Zn^{2+}, and 0.14 when 100 μM Zn^{2+} was applied at pH_o 6.8.

Interestingly, the value of gK_{SSnorm} was much higher at pH_o 6.8 than under the control conditions. This was because lowering the pH_o shifted the steady-state inactivation curve towards positive membrane potentials more significantly than the steady-state activation curve (Fig. 4B). In fact, the calculations showed that at pH_o 6.8, the inactivation midpoint (V_i) was shifted by 17.4 ± 1.12 mV (n = 6) towards positive membrane potentials, whereas the activation midpoint (V_n) was shifted only by 8.5 ± 1.28 mV (n = 10). The difference in the values of the shifts was statistically significant ($p < 0.05$, Student's t-test). By contrast, application of 100 μM Zn^{2+} at pH_o 6.8 shifted the V_i value by 16.55 ± 1.80 mV (n = 6) towards positive membrane potentials, whereas the V_n value was shifted by 23.56 ± 2.40 mV (n = 10) towards positive membrane potentials. Thus, applying Zn^{2+} shifted the value of V_n significantly more ($p < 0.05$, Student's t-test) than the value of V_i. Therefore, the value of gK_{SSnorm} was lower in the presence of 100 μM Zn^{2+} at pH_o 6.8 than in the absence of Zn^{2+} at this value of pH_o. The average total shift in the V_i value caused by protons at pH_o 6.8 and 100 μM Zn^{2+} at pH_o 6.8 was 33.95 ± 1.90 mV (n = 6), and it was almost identical ($p > 0.05$, Student's t-test) to the total shift in the V_n value (32.01 ± 2.10 mV, n = 10).

The results of this study show that the Zn^{2+}-induced shift in the steady-state inactivation of Kv1.3 channels remained unchanged when the pH_o was lowered from 7.35 to 6.8. Lowering the pH_o without Zn^{2+} application caused an additional significant shift in the steady-state inactivation towards positive membrane potentials. The magnitude of the total shift in the steady-state inactivation was significantly higher than the shift caused by Zn^{2+} application.

The obtained results demonstrate that the modulatory effects of Zn^{2+} and protons on the steady-state inactivation of Kv1.3 channels were additive. It should be pointed out that both a pH_o of 6.8 and a Zn^{2+} concentration of 100 μM are values within a physiologically relevant range. Thus, additive modulatory effects of protons and Zn^{2+} on Kv1.3 channel steady-state inactivation are likely to occur under physiolgical conditions. Similar results were obtained recently in the case of the steady-state activation [17].

The results demonstrated that the modulatory effects of protons and Zn^{2+} on the steady-state activation and inactivation were different. In the case of protons, the steady-state inactivation was shifted towards positive membrane potentials much more than the steady-state activation. When Zn^{2+} was applied, the situation was reversed: the steady-state activation was shifted much more than the steady-state inactivation. These results might support the hypothesis presented in our previous article [16] that the mechanisms of the modulatory effects of protons and Zn^{2+} on Kv1.3 channels were distinct. The fact that the proton-induced shift in the steady-state inactivation was much more pronounced than in the case of the steady-state activation might be surprising. This is because it is thought that the modulatory effect of protons on Kv1.3 channels was primarily due to the compensation of negative surface charges by protons [18]. In such a case, the steady-state activation and inactivation should be equally shifted towards positive membrane potentials. Nevertheless, it was shown that the effect of protons on the inactivation kinetics of Kv1.3 channels was more complex and included some specific interactions of protons with the histidine residue His399 [22]. It is possible that such interactions might also increase the shift of the steady-state inactivation towards positive membrane potentials.

The results of this study might be of physiological significance. Since it is known that the resting membrane potential in TL is set primarily by the activity of Kv1.3 channels, and that it is close to the "window" of membrane potentials [3], it could be suggested that the proton- and Zn^{2+}-induced shift of the "window" towards positive membrane potentials caused the membrane depolarisation. It is known that application of Zn^{2+} *in vitro* at concentrations up to 200 μM stimulated the proliferation of TL [23]. This stimulation could be related to the Zn^{2+}-induced depolarisation of TL, as suggested in our previously published papers [15, 16]. More studies are necessary to investigate the influence of protons and Zn^{2+} on the resting membrane potential in TL and to correlate possible changes in the resting potential with TL mitogenesis.

It is also known that Kv1.3 channels are present in the central nervous system, especially in olfactory bulb neurons [7-9], where they stabilise tonic firing of

action potentials [8]. Kv1.3 channels can stabilise the action potential generation in the "window" of membrane potentials, where they can be active. Because this "window" is shifted towards positive membrane potentials due to the modulatory effects of protons and Zn, the range of membrane potential where Kv1.3 channels can stabilise the action potential generation would also be shifted in the positive direction. How such a shift influences the Kv1.3-induced stabilisation of the action potential generation remains to be elucidated.

Acknowledgements. We would like to express our sincere gratitude to our colleague from the Biophysics Department, Dr. Andrzej Poła for his kind help in providing blood samples for lymphocyte isolation. This study was supported by the Polish State Committee for Scientific Research (KBN), Grant No. 2 PO5A 010 27. The experimental procedure is in accordance with the Good Medical Practice guidelines and was approved by the Committee of Bioethics at Wrocław Medical University.

REFERENCES

1. Frederickson, C., Klitenick, M., Menton, W. and Kirpatrick, J. Cytoarchitectonic distribution of zinc in the hippocampus of man and the rat. **Brain Res.** 273 (1983) 335-339.
2. Harrison, N. and Gibbons, S. Zinc: an endogenous modulator of ligand and voltage- gated ion channels. **Neuropharmacol.** 33 (1994) 935-952.
3. Lewis, R. and Cahalan, M. Potassium and calcium channels in lymphocytes. **Annu. Rev. Immunol.** 13 (1995) 623-653.
4. Teisseyre, A. Voltage-gated potassium channels in T lymphocytes – physiological role and changes in channel properties in diseases. **Cell. Mol. Biol. Lett.** 1 (1996) 337-351.
5. Shieh, Ch., Coghlan, M., Sullivan, J. and Gopalakrishan, M. Potassium channels: molecular defects, diseases and therapeutic opportunities. **Pharmacol. Rev.** 52 (2000) 557-593.
6. Cahalan, M., Wulff, H. and Chandy, K. Molecular properties and physiological roles of ion channels in the immune system. **J. Clin. Immunol.** 21 (2001) 235-252.
7. Veh, R., Lichtinghagen, R., Sewing, S., Wunder, F., Grumbach, I. and Pongs, O. Immunohistochemical localization of five members of the Kv1 channel subunits: contrasting subcellular locations and neuron-specific co-localizations in rat brain. **Eur. J. Neurosci.** 7 (1995) 2189-2205.
8. Kupper, J., Prinz, A. and Fromherz, P. Recombinant Kv1.3 potassium channels stabilize tonic firing of cultured rat hippocampal neurons. **Pflügers Arch.** 443 (2002) 541-547.
9. Colley, B., Tucker, K. and Fadool, D. Comparison of modulation of Kv1.3 channel by two receptor tyrosine kinases in olfactory bulb neurons of rodents. **Rec. Channels** 10 (2004) 25-36.

10. Mackenzie, A., Chirakkal, H. and North, A. Kv1.3 potassium channels in human alveolar macrophages. **Am. J. Physiol. Lung Cell. Mol. Physiol.** 285 (2003) L862-L868.
11. Speake, T., Kibble, J. and Brown, P. Kv1.1 and Kv1.3 channels contribute to the delayed-rectifying conductance in rat choroid plexus epithelial cells. **Am. J. Physiol. Cell Physiol.** 286 (2004) C611-C620.
12. Grunnet, M., Rasmussen, H., Hay-Schmidt, A. and Klaerke, D. The voltage-gated potassium channel subunit, Kv1.3, is expressed in epithelia. **Biochim. Biophys. Acta** 1616 (2003) 85-94.
13. Preußat, K., Beetz, Ch., Schrey, M., Kraft, R., Wölfl, S., Kalff, R. and Patt, S. Expression of voltage-gated potassium channels Kv1.3 and Kv1.5 in human gliomas. **Neurosci. Lett.** 346 (2003) 33-36.
14. Fraser, S., Grimes, J., Diss, J., Stewart, D., Dolly, J. and Djamgoz, M. Predominant expression of Kv1.3 voltage-gated K+ channel subunit in rat prostate cancer cell lines: electrophysiological, pharmacological and molecular characterisation. **Pflügers Arch**. 446 (2003) 559-571.
15. Teisseyre, A. and Mozrzymas, J.W. Inhibition of the activity of T lymphocyte Kv1.3 channels by extracellular zinc. Biochem. Pharmacol. 64 (2002) 595-607.
16. Teisseyre, A. and Mozrzymas, J.W. Influence of extracellular pH on the modulatory effect of zinc ions on Kv1.3 potassium channels. J. Physiol. Pharmacol. 57 (2006) 131-147.
17. Teisseyre, A. and Mozrzymas, J.W. [Influence of pH on the modulatory effect of zinc ions on the activity of Kv1.3 potassium channels] **Acta Universitatis Lodziensis, Folia Biologica et Oecologica** [Proc. V-Polish wide Conference: "Electrophysiological techniques in investigations of bioelectrical phenomena: from ion channels to neuronal networks"], Łódź, Poland, 2006, 31-40.
18. Deutsch, C. and Lee, S. Modulation of K+ currents in human lymphocytes by pH. J. Physiol. 413 (1989) 399-413.
19. Hirano, T., Kuritani, T., Kishimoto, Y. and Yamamura, Y. T cell dependency of PWM-induced Ig production by B cells. **J. Immunol.** 119 (1977) 1235-1242.
20. Grissmer, S., Nguyen, A. and Cahalan, M. Calcium-activated potassium channels in resting and activated human T lymphocytes. **J. Gen. Physiol.** 102 (1993) 601-630.
21. Hamill, O., Marty, A., Neher, E., Sakmann, B. and Sigworth, F. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. **Pflügers Arch.** 391 (1981) 85-100.
22. Somodi, S., Varga, Z., Hajdu, P., Starkus, J., Levy, D., Gaspar, R. and Panyi, G. PH-dependent modulation of Kv1.3 inactivation: role of His 399. **Am. J. Physiol. Cell Physiol.** 287 (2004) C1067-C1076.
23. Reardon, C. and Lucas, D. Heavy-metal mitogenesis: Zn and Hg induce cellular cytotoxicity and interferon production in murine T lymphocytes. **Immunobiology** 175 (1987) 455-469.