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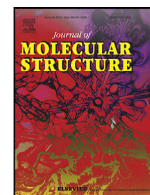

Statins as inhibitors of voltage-gated potassium channels Kv1.3 in cancer cells

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ABSTRACT

Voltage-gated potassium channels are integral membrane proteins selectively permeable for potassium ions and activated upon change of membrane potential. Voltage-gated potassium channels of the Kv1.3 type were discovered both in plasma membrane and in inner mitochondrial membrane (mito Kv1.3 channels). For some time Kv1.3 channels located both in plasma membrane and in mitochondria are considered as a potentially new molecular target in several pathologies including some cancer disorders. Lipophilic nontoxic organic inhibitors of Kv1.3 channels may potentially find a clinical application to support therapy of some cancer diseases such as breast, pancreas and lung cancer, melanoma or chronic lymphocytic leukaemia (B-CLL). Inhibition of T lymphocyte Kv1.3 channels may be also important in treatment of chronic and acute respiratory diseases including severe pulmonary complication in corona virus disease Covid 19, however further studies are needed to confirm this supposition.

Statins are small-molecule organic compounds, which are lipophilic and are widely used in treatment of hypercholesterolemia and atherosclerosis. Electrophysiological studies performed in our laboratory showed that statins: pravastatin, mevastatin and simvastatin are effective inhibitors of Kv1.3 channels in cancer cells of human T cell line Jurkat. We showed that application of the statins in the concentration range from 1.5 μM to 50 μM inhibited the channels in a concentration-dependent manner. The inhibitory effect was the most potent in case of simvastatin and the least potent in case of pravastatin. The inhibition was partially irreversible in case of simvastatin and fully reversible in case of pravastatin and mevastatin. It was accompanied by a significant acceleration of the current inactivation rate without any significant change of the activation rate. Mechanism of the inhibition is probably complex, including a direct interaction with the channel protein and perturbation of lipid bilayer structure, leading to stabilization of the inactivated state of the channels.

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1. Introduction

Voltage-gated potassium channels of the Kv1.3 type are widely expressed in many types of cells, both normal and cancer [1,2]. Activity of Kv1.3 channels plays important role, for example in setting resting membrane potential, cell proliferation, apoptosis and volume regulation [3,4]. Kv1.3 channels are expressed both in the plasma membrane and in the inner mitochondrial membrane (mito Kv1.3 channels) [4,5]. Specific blockers of Kv1.3 channels in human T lymphocytes potentially may be applied in selective immunosuppression [3,5] and in treatment of chronic respiratory diseases [6]. Recently formulated hypothesis claims that inhibition of T lymphocyte Kv1.3 channel might suppress the “cytokine storm” in severe cases of COVID-19 disease and this could be a novel therapeutic strategy to combat the disease [7]. The hypothesis was supported by the fact that chloroquine, which reduces both the viral replication and production of cytokines by leukocytes, also inhibits Kv1.3 channels in T lymphocytes [8] and this inhibition may exert a “cytokine storm” -suppressing immunosup pressive effect [7].

Several studies demonstrated an altered expression of Kv1.3 channels in some cancer disorders such as breast, colon, pancreas, smooth muscle, skeletal muscle, lung, kidney and prostate cancer [5,9-11]. Inhibitors of Kv1.3 channels may potentially find a clinical application in therapy of some cancer disorders characterized by an over-expression of Kv1.3 channels, such as for example: breast and lung cancer, melanoma, pancreatic ductal adenocarcinoma or

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chronic lymphocytic leukemia [10-12]. Among many inhibitors of Kv1.3 channels the most promising candidates for potential clinical application in cancer therapy could be lipophilic small-molecule organic compounds being able to simultaneously inhibit cancer cell proliferation (by inhibition of plasma membrane Kv1.3 channels) and to induce selective cancer cell death (by inhibition of mito Kv1.3 channels) [11].

Studies performed during in our laboratory showed that to this group of compounds may also belong some plant-derived flavonoids and substituted stilbenes, which exert anti-proliferative and pro-apoptotic effects on Kv1.3 channel-expressing cancer cells and combine high efficiency and specificity in cancer cell elimination with good bioavailability and low cytotoxicity [10,11]. We showed that genistein, a plant-derived is flavone known as a potent protein tyrosine kinase (PTK) inhibitor, and a substituted stilbene - resveratrol, a natural anti-cancer agent present at highest concentrations in red grapes and wine, both are inhibitors of Kv1.3 channels in human T lymphocytes [11]. Moreover, two synthetic methoxy-derivatives of flavonoid naringenin (4′, 7-dimethylether and 7-methylether) and one synthetic tetramethoxy-derivative of piceatannol also inhibit Kv1.3 channels in normal human T lymphocytes [11]. Another two flavonoids that inhibit Kv1.3 channels expressed both in normal human T lymphocytes and in Jurkat T cells are acacetin and chrysin [11].

Studies performed recently in our laboratory provide evidence that a natural derivative of a flavonoid naringenin isolated from common hops (*Humulus lupulus*) - 8-prenylnaringenin (8-PN), a potent phytoestrogen present in beer, inhibits Kv1.3 channels both in normal human T lymphocytes and in human Jurkat T cells when applied at low micromolar concentrations [11]. Ability to inhibit Kv1.3 channels in cancer cells is shared by other hops-derived prenylated compounds such as: xanthohumol, a prenylated chalcone and two prenylflavanones - isoxanthohumol and 6–prenylnaringenin (6-PN) [11]. All these compounds inhibited Kv1.3 channels much more strongly than all non-prenylated plant-derived compounds tested in our laboratory [11]. These results may confirm the hypothesis that the presence of prenyl group in a molecule is a factor that facilitates the inhibition of Kv1.3 channels by compounds from the groups of flavonoids and chalcones [11].

Other small-molecule organic compounds applied in medical therapy are statins [13]. These compounds are known as inhibitors of 3–hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. This enzyme catalyzes reduction of HMG-CoA to L-mevalonate, a key intermediate in biosynthesis of cholesterol and many isoprenoid metabolites. Thus, inhibition of this reductase strongly inhibits biosynthesis of cholesterol and isoprenoid metabolites. Therefore, statins are widely applied in treatment of hypercholesterolemia and atherosclerosis [13]. Recently formulated hypothesis claims that statins might also be key therapeutic agents in therapy of severe COVID-19 cases [14]. This is due to the HMG-CoA reductase inhibition, which leads to depletion of cellular and plasma membrane cholesterol. It was shown that this reduction of cholesterol content in cell membranes prevents SARS-CoV-2 virus entry into the host cell even if the viral spike protein is bound to the lipid raft ACE2 receptor [14]. Moreover, it was shown that statins – mevastatin and simvastatin exert antiproliferative, pro-apoptotic and reversing drug resistance effect in Kv1.3 channel-expressing human colon adenocarcinoma cell line LoVo and its doxorubicin-resistant subline LoVo/Dx [15]. Besides LoVo cells simvastatin also inhibits proliferation and induces apoptosis of other Kv1.3 channel-expressing cancer cells such as breast adenocarcinoma (MCF-7 and MDA-MB-231), leukaemia (Jurkat T and CEM) and promyelocytic leukaemia (HL60) [11,13] cells. Similarly, to simvastatin also pravastatin inhibits proliferation and induces apoptosis of Kv1.3 channel-expressing leukemic Jurkat T and CEM cells [13]. It is therefore possible that antiproliferative and proapoptotic effects of simvastatin, mevastatin and pravastatin on cancer cells may be, at least partially, due to inhibition of Kv1.3 channels.

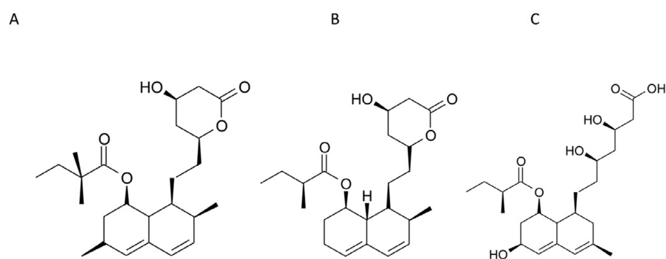

Fig. 1. Chemical structures of: simvastatin (A), mevastatin (B) and pravastatin (C).

However, a little is known about influence of statins on activity of Kv1.3 channels in cancer cells. Preliminary electrophysiological study applying the “patch-clamp” technique showed that pravastatin, lovastatin and simvastatin are inhibitors of Kv1.3 channels in non-tumor murine thymocytes [16]. A more detailed study performed with lovastatin showed that this compound inhibits Kv1.3 channels expressed both in normal human T lymphocytes and in cancer cells of human Jurkat T cell line [17]. Studies performed recently by Wang and co-workers have shown that simvastatin inhibits Kv1.3 channels in human monocytic leukaemia THP-1 cells in a concentration-dependent manner [18]. However, these studies were performed applying a voltage step protocol, in which the membrane voltage is changed in a stepwise manner [18]. This is in contrast to a gradual change of the voltage that occurs in case of the voltage ramp sequence applied in our studies (see below). Application of different experimental protocols leads to different values of recorded currents and different value of half-blocking concentration of the drug (see below). The influence of other statins on Kv1.3 channels in cancer cells was not studied yet.

The aim of our study was to investigate an influence of selected statins: simvastatin, mevastatin and pravastatin on activity of Kv1.3 channels in cancer cells. Structural formulas of selected compounds are shown on Fig. 1.

Since Kv1.3 channel is widely expressed in human leukemic T-cell line Jurkat [19,20] these cells were used in our study as a model system of cancer cells. Obtained results provide evidence that all selected statins effectively inhibit Kv1.3 channel in Jurkat T cells. Simvastatin was the most potent channel inhibitor, whereas pravastatin was the weakest one.

2. Materials and methods

2.1. Cell culture and solutions

The human leukemic T cell line, Jurkat (clone E6–1), was purchased from American Type Culture Collection (Manassas, VA). The Jurkat cells were grown in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) containing 10% heat-inactivated FBS, 10 mM HEPES and 2 mM glutamate. Cells were grown on culture plates at 37 °C in a 5% CO_2-humidified incubator. During the experiments cells were placed in the external solution containing in mM: 150 NaCl, 4.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES: pH of solution =7.35. The pipette solution contained in mM: 150 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 10 EGTA, pH of solution =7.2. The concentration of free calcium ions in the internal solution was below 100 nM, assuming the dissociation constant for EGTA at pH=7.2 of 10^{-7} M [21]. Such a low calcium concentration was applied to prevent the activation of calcium-activated K^+ channels $K_{Ca}2.2$ abundantly expressed in Jurkat T cells [22]. The chemicals were purchased from the Polish Chemical Company (POCH, Gliwice, Poland), except of HEPES and

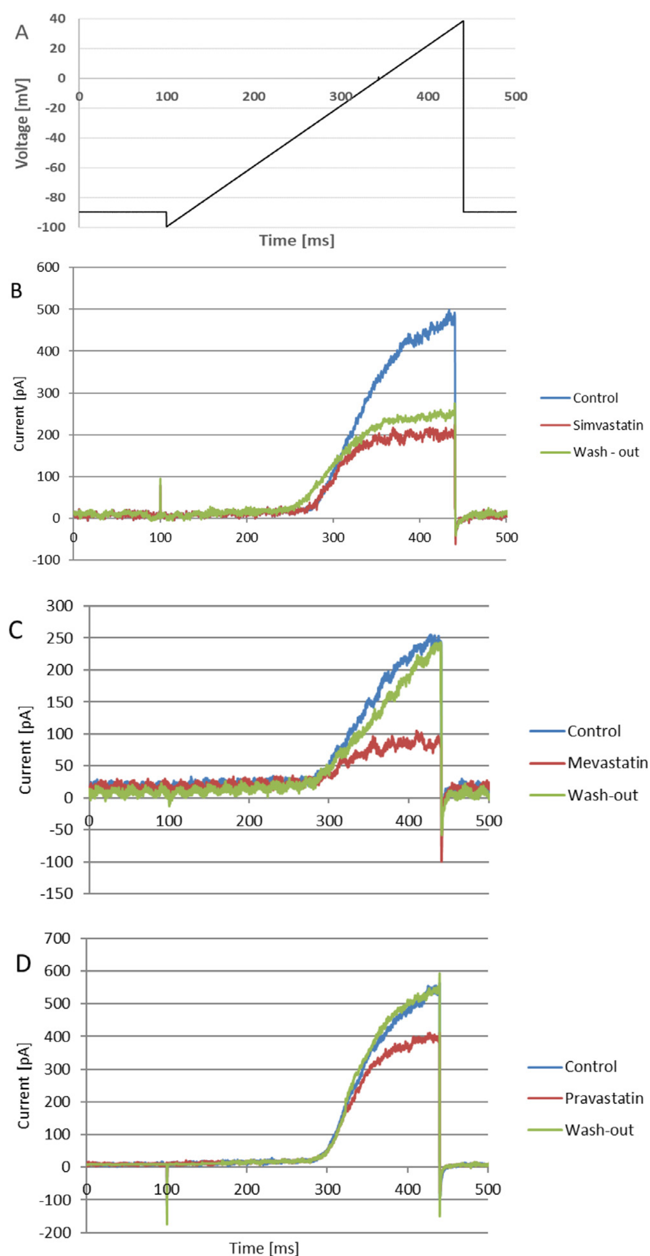

Fig. 2. The statins: simvastatin (B), mevastatin (C) and pravastatin (D) reduce the intensity of the whole-cell potassium currents recorded in Jurkat T cells. Currents were recorded applying the voltage ramp protocol (A), under control conditions, after 1 min of application of the compounds at concentration of 30 μM (simvastatin and mevastatin) or 50 μM (pravastatin), and after wash out of the statins.

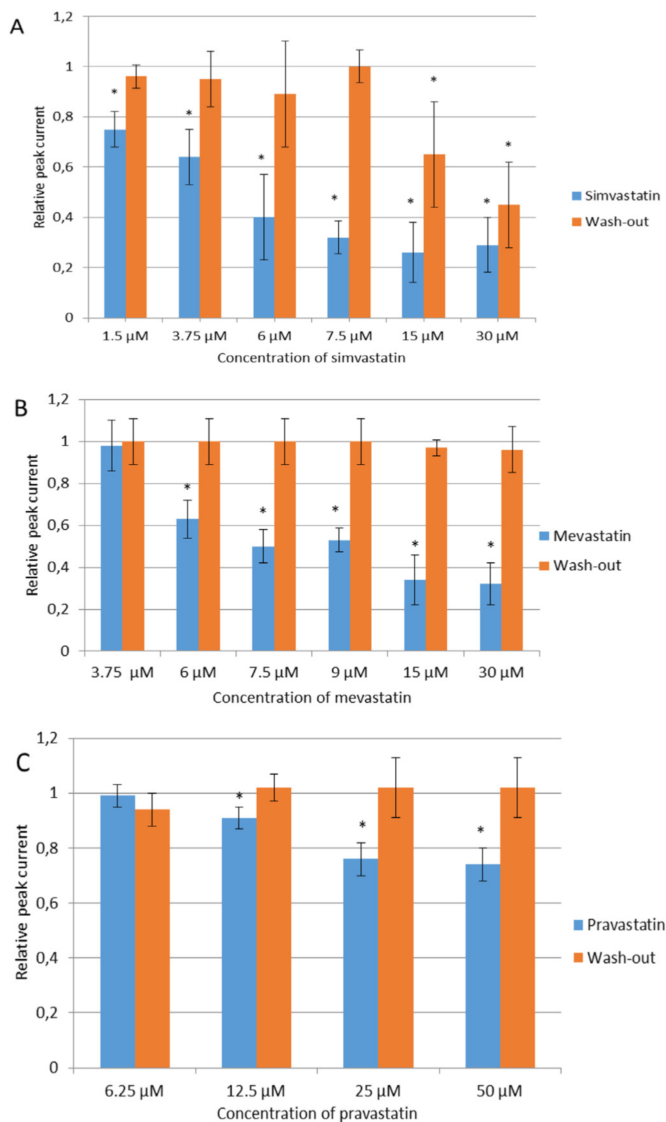

Fig. 3. The relative peak currents upon application of simvastatin (A), mevastatin (B) and pravastatin (C) in Jurkat T cells at the concentrations denoted below and after wash out of the statins. (*) – marks statistical significance.

EGTA that were purchased from SIGMA. The examined statins were purchased from Alexis Biochemicals (Lausen, Switzerland).

2.2. Patch-clamp recordings

Dishes with cells were placed under an inverted Olympus IMT-2 microscope. Solutions containing tested compounds were applied using eight-channel gravitation perfusion system (ALA Scientific Instruments, Farmingdale , NY, USA). 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.

Whole-cell potassium currents in TL were recorded applying the patch-clamp technique [23]. The currents were recorded using an EPC-7 Amplifier (HEKA, Germany), low-pass filtered at 3 kHz, digitised using a CED Micro 1401 analogue-to-digital converter (Cambridge, UK) with a sampling rate of 10 kHz. The influence of selected compounds on the activity of the channels was studied by applying the voltage ramp protocol. Voltage ramps gradually depolarising cell membranes from −100 mV up to +40 mV were applied every 30 s; the ramp duration was 340 ms and holding potential −90 mV (see below). Upon application of the voltage ramp protocol, potassium currents in Jurkat T cells could be stably recorded for at least 20 min after "break-in" to the whole-cell configuration. During the off-line analysis the value of Kv1.3 current at the end of a voltage ramp (+40 mV) was calculated. For this purpose, the leak current estimated at +40 mV was subtracted from the total ramp current recorded at this voltage.

In order to study the influence of selected compounds on the channel activation and inactivation kinetics in more detail another protocol of depolarising voltage stimuli was applied. This protocol contained a sequence of depolarising voltage steps from the holding potential of – 90 mV to +60 mV (500 ms step duration) applied every 30 s (see below).

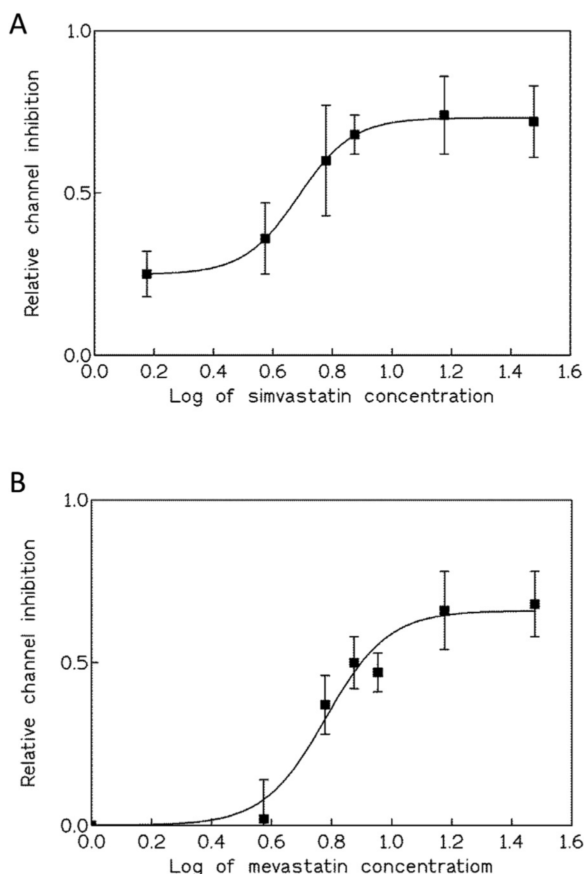

Fig. 4. The magnitude of the inhibition of Kv1.3 channels by simvastatin (A) and mevastatin (B) plotted as a function of a logarithm of the concentrations of the compounds (in μM). Data points were fitted by the Hill's equation (see Materials and Methods).

All experiments were carried out at room temperature (22–24 °C).

Unless otherwise stated the data are presented as mean ± standard deviation.

2.3. Data analysis

The inhibition of the channel is presented in terms of a relative current recorded upon application of the studied compounds, defined as I/I_{contr}; where: I – Kv1.3 current upon an application of an examined compound at + 40 mV, I_{contr} – Kv1.3 current recorded on the same cell at +40 mV under control conditions. For calculating the value of a half-blocking concentration (EC_{50}) and the Hill's coefficient the relative inhibition defined as 1- (I/I_{contr}) was taken in consideration. Both parameters were calculated applying the Hill's equation in a form: $y = a+(b-a)/(1+(x/c)^d)$, where: y – magnitude of the channels' inhibition, a – minimal value of the inhibitory effect, b – maximal value of the inhibitory effect, x – logarithm of the compound's concentration, c – logarithm of the EC_{50} value, d – Hill's coefficient. Inactivation kinetics were fitted by the single exponential function and described by the value of inactivation time constant. Activation kinetics were described by the time-to-peak parameter, which is determined as the time needed to reach the peak value of Kv1.3 current upon the channel activation during the application of a depolarising voltage step. Statistical analysis was performed applying the Student's unpaired t-test. The results were considered statistically significant when $p<0.05$.

3. Results

Examples of the whole-cell ramp currents recorded in Jurkat T cells applying voltage ramps (Fig. 2A) under control conditions, after1 min of application of the statins at the concentration of 30 μM (simvastatin and mevastatin) and 50 μM (pravastatin), and after wash-out of the compounds are presented in Fig. 2B-D.

This figure depicts the raw currents (without leak subtraction) that were recorded applying the voltage ramp protocol. The evoked currents contained two components: small linear and much bigger non-linear. The linear current was the unspecific leak current (reversal potential equal to 0 mV), whereas the non-linear component was due to activation of Kv1.3 channels [22,24].

Apparently, application of the statins significantly diminished the amplitude of Kv1.3 current. The reduction of the amplitude was more potent in case of simvastatin and mevastatin than in case of pravastatin, although the later compound was applied at higher concentration (Fig. 2B-D). Interestingly, the current did not recover completely after wash-out of simvastatin (Fig. 2B). This indicates that the inhibitory effect of simvastatin was partially irreversible. On the other hand, the currents recovered completely after wash-out of mevastatin and pravastatin (Fig. 2C and 2D). This indicates that the inhibitory effect of the compounds was reversible.

Fig. 3 depicts relative peak currents (defined in Materials and Methods) upon application of the statins at different concentrations and after wash-out of the compounds. An application of simvastatin at concentrations of 1.5 μM, 3.75 μM, 6 μM, 7.5 μM, 15 μM and 30 μM reduced relative peak currents to 0.75±0.07 (n = 22), 0.64±0.11 (n = 9), 0.40±0.17 (n = 38), 0.32±0.064 (n = 4), 0.26±0.12 (n = 9) and 0.28±0.11 (n = 9) of the control values, respectively (Fig. 3A). The reduction of the currents was statistically significant ($p < 0.05$, Student's t-test) for all the concentrations applied. The currents in the presence of simvastatin applied at concentrations of 6 μM, 7.5 μM, 15 μM and 30 μM did not significantly differ one from another ($p > 0.05$, one-way ANOVA). Thus, the magnitude of inhibition did not depend on the concentration of simvastatin in the range from 6 to 30 μM.

In accordance with the results shown in the Fig. 2, the currents did not fully recover after wash-out of simvastatin used at concentration of 30 μM. The relative peak current after wash-out was equal only to 0.45±0.17 (n = 4) of the control value and this reduction was statistically significant ($p < 0.05$, Student's t-test). Lowering of simvastatin concentration to 15 μM increased the relative current recovery upon wash-out to 0.65±0.21 (n = 4) of the control value, and this reduction was also statistically significant ($p<0.05$, Student's t-test). Only at concentrations of 7.5 μM or below the inhibitory effect of simvastatin was fully reversible (Fig. 3A).

Application of mevastatin at concentrations of 3.75 μM, 6 μM, 7.5 μM, 9 μM, 15 μM and 30 μM reduced relative peak currents to 0.98±0.12 (n = 25), 0.63±0.09 (n = 18), 0.50±0.08 (n = 8), 0.53±0.057 (n = 11), 0.34±0.12 (n = 8) and 0.32±0.10 (n = 12) of the control values, respectively (Fig. 3B). Reduction of the currents was statistically significant ($p < 0.05$, Student's t-test) for all concentrations applied, except for the lowest one. The currents in the presence of mevastatin applied at concentrations of 15 μM and 30 μM did not significantly differ one from another ($p > 0.05$, one-way ANOVA).

Thus, the magnitude of inhibition was not dependent on the concentration of mevastatin in the range 15 - 30 μM. The inhibitory effect exerted by mevastatin applied at concentrations of 15 and 30 μM was comparable to the effect of simvastatin. However, in contrast to what was observed in case of simvastatin, the inhibitory effect of mevastatin was fully reversible at these concentrations (Fig. 3B).

Application of pravastatin at concentrations of 6.25 μM, 12.5 μM, 25 μM and 50 μM reduced relative peak currents to 0.99±0.086 (n = 6), 0.91±0.04 (n = 4), 0.76±0.06 (n = 3) and 0.74±0.06 (n = 8) of the control values, respectively (Fig. 3C). The reduction of the currents was statistically significant ($p<0.05$, Student's t-test) for all the concentrations, except for the lowest one. The inhibitory effect of pravastatin was concentration-dependent. It was fully reversible at all applied concentrations.

In order to characterize the inhibitory effects of the statins on Kv1.3 currents in more detail, the magnitude of the inhibitory effect of simvastatin and mevastatin was plotted as a function of a logarithm of concentration (in μM) (Fig. 4A and 4B, respectively). The data was fitted applying the Hill's equation (see Materials and Methods). The values of a half-blocking concentration (EC_{50}) were equal to 4.85±0.011 μM for simvastatin and 6.04±0.4 μM (mean ± standard error) for mevastatin, whereas the Hill's coefficients were equal to 4.75±0.39 and 4.19±1.25 (mean ± standard error), for simvastatin and mevastatin, respectively. In case of pravastatin, the inhibitory effect on Kv1.3 channels was too small for application of the Hill's equation to calculate the value of EC_{50} and the Hill's coefficient. Therefore, the inhibitory effect of pravastatin was not characterised in detail, such as it was done for simvastatin and mevastatin.

In order to study the influence of selected compounds on the channel activation and inactivation kinetics in more detail a protocol of depolarising voltage steps was applied (Fig. 5A). Fig. 5B-D depicts normalised whole-cell potassium currents (defined in Materials and Methods) under control conditions, after1 min of application of the statins at the concentration of 30 μM (simvastatin and mevastatin) and 50μM (pravastatin) and after wash-out of the compounds.

Apparently, the inactivation was much more rapid in the presence of the statins than under control conditions. Interestingly, inactivation was still markedly accelerated after wash-out of simvastatin (Fig. 5B). This indicates that acceleration of inactivation by simvastatin was partially irreversible. On the other hand, inactivation acceleration was completely removed after wash-out of mevastatin and pravastatin (Fig. 5C and 5D). This indicates that acceleration of inactivation upon application of these compounds was reversible.

Fig. 6 depicts inactivation time constant (defined in Materials and Methods) under control conditions and upon application of the statins at different concentrations. Since inactivation was still markedly accelerated after wash-out of simvastatin, the values after wash-out of this statin were also depicted (Fig. 6A). Inactivation time constant values upon application of simvastatin at concentrations of 1.5 μM, 3.75 μM, 6 μM, 7.5 μM, 15 μM and 30 μM were equal to 59.35±26.05 ms (n = 22), 18.03±7.12 ms (n = 9), 16.95±4.2 ms (n = 31), 10.53±2.35 ms (n = 4), 9.19±1.60 ms (n = 8) and 12.81±4.80 ms (n = 8), respectively. The values calculated in the presence of simvastatin applied at concentrations of 7.5 μM, 15 μM and 30 μM did not significantly differ one from another ($p > 0.05$, one-way ANOVA) On the other hand, inactivation time constant under control conditions was equal to 241±54.42 ms (n = 15). The reduction of inactivation time constant upon application of simvastatin was statistically significant ($p<0.05$, Student's t-test) for all the concentrations applied. Inactivation time constant values after wash-out of simvastatin applied at concentrations of 1.5 μM, 3.75 μM, 6 μM, 7.5 μM, 15 μM and 30 μM were equal to 79.21±29.97 ms (n = 7), 67.60±24.90 ms (n = 6), 64.94±16.03 ms (n = 14), 118.27±51.24 ms (n = 3), 106.90±54.22 ms (n = 4) and 101.43±50.42 ms (n = 3), respectively.

All these values were significantly ($p<0.05$, Student's t-test) smaller than the inactivation time constant calculated for control conditions (Fig. 6A).

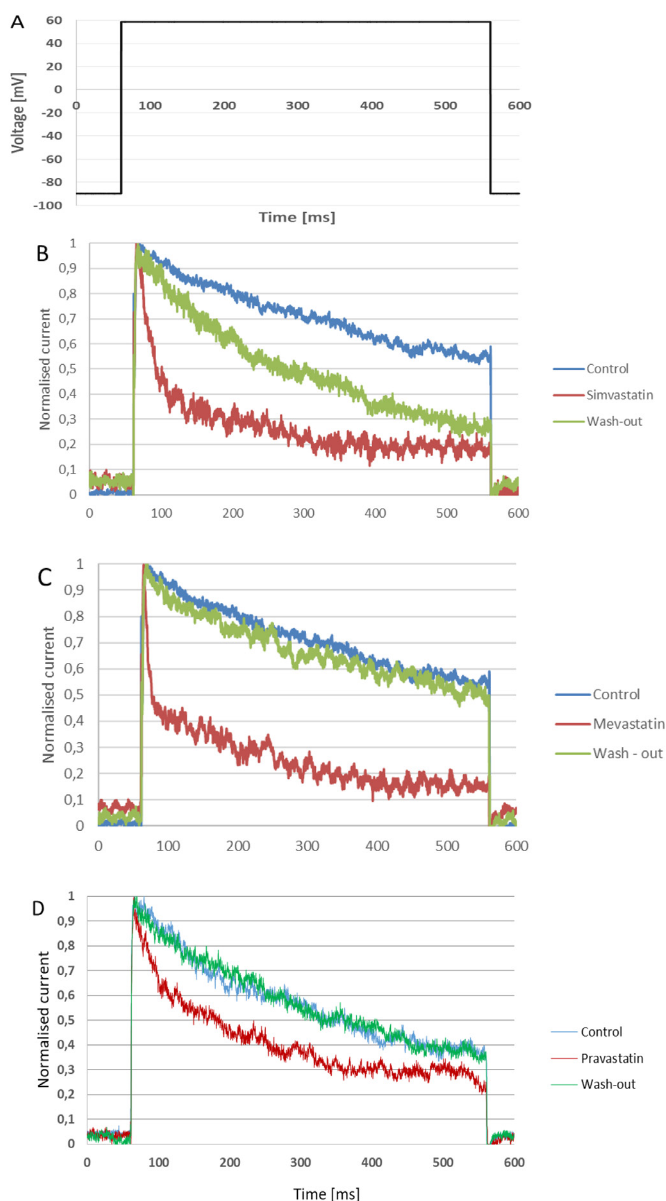

Fig. 5. Normalized whole-cell currents recorded in Jurkat T cells upon application simvastatin (B), mevastatin (C) and pravastatin (D). Currents were recorded applying the voltage pulse protocol (A), under control conditions, after1 min of application of the compounds at the concentration of 30 μM (simvastatin and mevastatin) or 50 μM (pravastatin), and after wash out of the statins.

Inactivation time constant values upon application of mevastatin at concentrations of 3.75 μM, 6 μM, 7.5 μM, 9 μM, 15 μM and 30 μM were equal to 137.83±42.73 ms (n = 9), 73.12±12.31 ms (n = 5), 19.83±5.63 ms (n = 4), 21.38±3.75 ms (n = 6), 11.08±3.79 ms (n = 4) and 11.96±4.62 ms (n = 9), respectively. The values calculated in the presence of mevastatin applied at concentrations of 15 μM and 30 μM did not significantly differ one from another ($p > 0.05$, one-way ANOVA). The reduction of inactivation time constant in relation to control conditions was statistically significant ($p < 0.05$, Student's t-test) for all the concentrations applied (Fig. 6B). Inactivation time constant values after wash-out of mevastatin applied at all concentrations were statistically not significantly ($p \geq 0.05$, Student'st-test) different from the control value (data not shown).

Inactivation time constant values upon application of pravastatin at concentrations of 6.25 μM, 12.5 μM, 25 μM and 50 μM

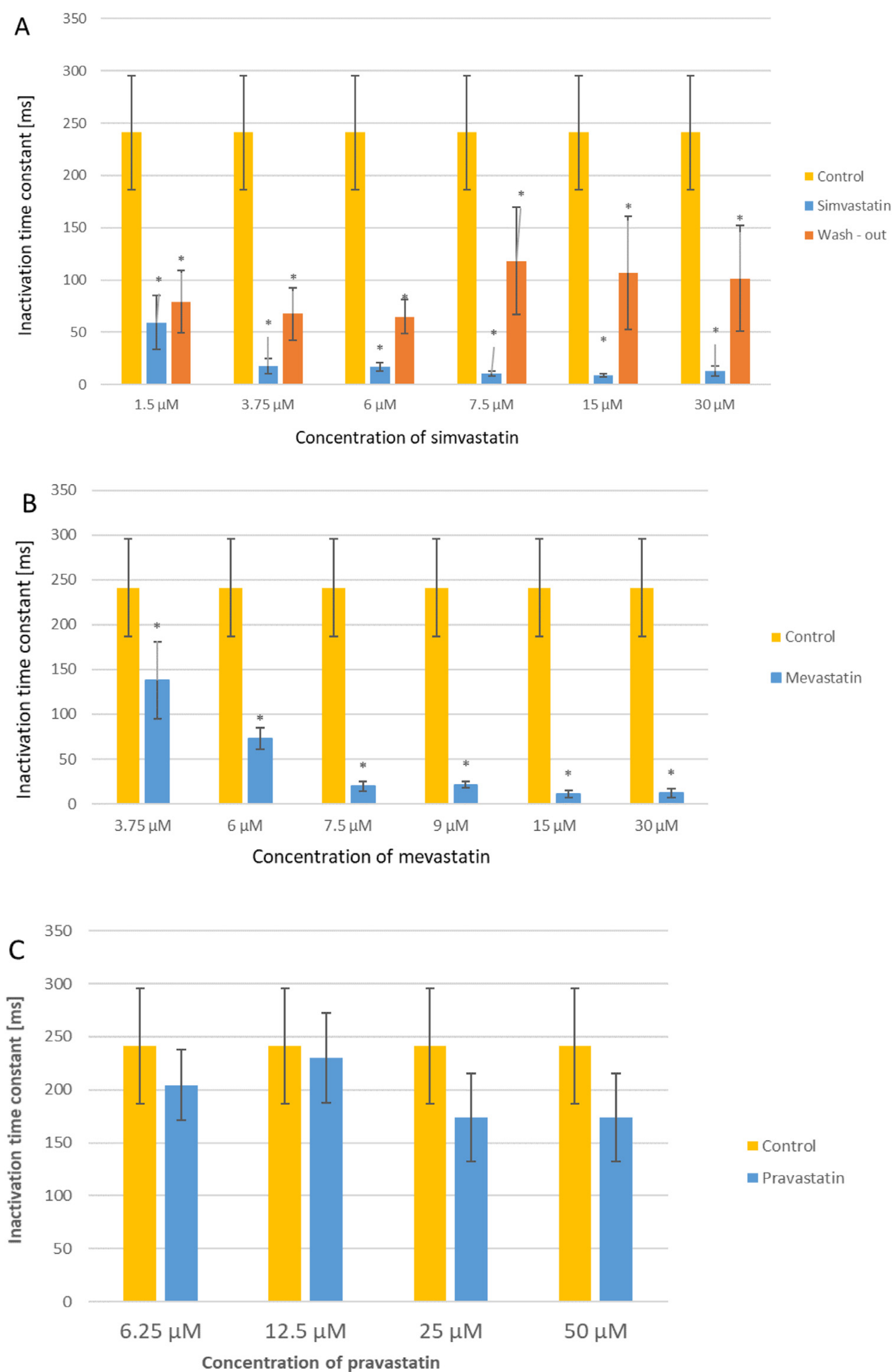

Fig. 6. The inactivation time constant upon application of simvastatin (A), mevastatin (B) and pravastatin (C) in Jurkat T cells at concentrations denoted below. For clarity, control values and values after wash out of simvastatin (A) were added. (*) – marks statistical significance.

were equal to 204.50±33.17 ms (n = 6), 230.37±42.43 ms (n = 2), 174.20±41.47 ms (n = 5) and 174.20±41.47 ms (n = 3), respectively. The reduction of inactivation time constant in relation to control conditions was statistically not significant ($p > 0.05$, Student's t-test) for all the concentrations applied (Fig. 6C).

Since the inactivation rate of the Kv1.3 currents was significantly higher after exposure to the statins, it was of interest to study the influence of the compound also on the channel activation kinetics. Fig. 7 depicts time-to-peak values (defined in Materials and Methods) under control conditions and upon application of the statins at the concentration of 30 μM (simvastatin and mevastatin) and 50 μM (pravastatin).

The value of time-to-peak parameter estimated for the currents recorded under control conditions was 5.17±1.04 ms (n = 23) and was not changed significantly upon application of the statins at the concentration of 30 μM (simvastatin and mevastatin) or 50 μM (pravastatin) (Fig. 7). No significant changes were also observed at lower concentrations of the statins (data not shown). Thus, in con-

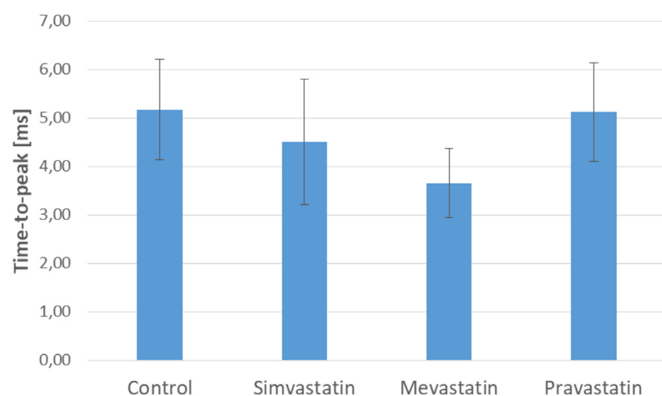

Fig. 7. The time-to-peak value calculated for the current recorded under control conditions and upon application of simvastatin (30 μM), mevastatin (30 μM) and pravastatin (50 μM) in Jurkat T cells.

trast to the influence on the inactivation kinetics, exposure to the statins did not change the activation kinetics significantly.

4. Discussion

It was shown that three examined statins: simvastatin, mevastatin and pravastatin are all inhibitors of Kv1.3 channels in human Jurkat T cells. The channel inhibition is accompanied by a significant acceleration of inactivation rate – by means of a significant decrease of the inactivation time constant. On the other hand, no significant change of the activation rate was observed (time-to-peak values did not change significantly upon application of the statins). The inhibitory effect of simvastatin is most potent, concentration-dependent and partially irreversible at concentrations of 15 and 30 μM. The inhibitory effect of mevastatin is less potent, concentration-dependent and reversible at all concentrations studied. The inhibitory effect of pravastatin is the weakest one, concentration-dependent and reversible at all concentrations.

The inhibitory effect of the two statins (simvastatin and mevastatin) on Kv1.3 channels was stronger than inhibition caused by the most of the non-prenylated flavonoids and stilbenes that were studied earlier in our laboratory [11]. We compared these two groups of compounds because both statins and flavonoids influence cholesterol synthesis pathway. Flavonoids inhibit biosynthesis of cholesterol by reducing activity of reductase HMG-CoA.

The estimated EC_{50} values in case of simvastatin (4.85 μM) and mevastatin (6.04 μM) are lower than those reported earlier for genistein (10–40 μM) and stilbene derivative - resveratrol (40 μM). The magnitude of inhibitory effect exerted by the statins applied at concentration of 30 μM is higher than in case of genistein, resveratrol, acacetin and chrysin applied at the same concentration [11].

The inhibitory effect of simvastatin and mevastatin was similar to the effect observed in case of application of lovastatin in Jurkat T cells [17]. It was shown that lovastatin inhibited Kv1.3 channels with the half-blocking concentration values equal to 39.81±5.11 μM and 6.92±0.95 μM, for the peak and end-of-pulse current, respectively [17]. Zhao and co-workers showed that lovastatin shared the binding site with other inhibitors of Kv1.3 channels, such as verapamil and internally applied tetraethyl-ammonium (TEA) [17]. Both compounds inhibit Kv1.3 channels by direct interaction with the channel protein via the "open channel block" mechanism [25]. The „open-channel block" mechanism was originally proposed by Armstrong (1966) for blocking of voltage gated potassium channels by internally applied quaternary ammonium ions (QA) [26]. According to the „open channel block" mechanism, the molecule of inhibitor blocks the channel from the inner side of membrane by interaction with binding site in the central channel cavity while the channel is open [26]. The "open channel block" mechanism is revealed by an observation of significant acceleration of current inactivation without a change in the activation rate [26]. It is known that extracellularly applied small-molecule lipophilic compounds, such as verapamil, can diffuse through the cell membrane and inhibit Kv1.3 channel from the inner side [25,27]. To the group of small-molecule lipophilic compounds belong also simvastatin and lovastatin [28]. Mevastatin is structurally related to simvastatin (see above). According to the "open channel block" mechanism, the inhibitory effect should be reversible. The inhibitory effect is reversible in case of mevastatin; however, in case of simvastatin the inhibitory effect is partially irreversible at concentrations of 15 μM and 30 μM. This is in accordance to what was observed by Kazama and co-workers, who reported irreversible inhibition of Kv1.3 channels in murine thymocytes by simvastatin applied at 10 μM concentration [16]. Such partial irreversibility could not be explained by the "open channel block" mechanism. This may be due to irreversible perturbations in structure of membrane lipid bilayer. Kazama and co-workers showed that application of simvastatin at 10 μM concentration caused a significant and irreversible decrease of the membrane capacitance [16]. This was probably due to irreversible increase of membrane thickness, which was a consequence of accumulation of the drug in the plasma membrane [16]. Accumulated drug molecules may directly or indirectly interact with channel protein irreversibly reducing whole-cell peak current and accelerating channel inactivation [16]. This may indicate that the inhibitory effect of simvastatin on Kv1.3 channels probably occurs via a complex mechanism including both direct interaction with the channel protein via the "open channel block" mechanism and interactions of simvastatin with cell membrane leading to perturbations of lipid bilayer structure due to accumulation of simvastatin molecules in the plasma membrane.

Studies performed recently by Wang and co-workers have shown that simvastatin inhibits Kv1.3 channels in human monocytic leukemia THP-1 cells in a concentration-dependent manner [18]. The estimated EC_{50} value was 8.75±1.25 μM [18], which was higher than that reported in our study (4.36±0.3 μM). This is not surprising, because such a difference may be due to application of different experimental protocol - voltage steps [18] different from the voltage ramp sequence applied in our studies. However, in a marked contrast to our results, no significant acceleration of the Kv1.3-current inactivation upon application of simvastatin was observed [18]. The reason for such a discrepancy is not clear.

In contrast to simvastatin and mevastatin, the inhibitory effect of pravastatin on Kv1.3 channels is relatively small. This was in accordance to what was reported by Kazama and co-workers [16]. Moreover, in contrast to what was observed in case of simvastatin and mevastatin, an application of pravastatin at concentrations up to 50 μM did not significantly accelerate inactivation of the currents (Fig. 6). Such a small effect may be explained by relatively small lipophilicity of pravastatin. It was shown that the octanol-water partition coefficient ($P_{o/w}$) value at physiological pH (7.4) is equal to 0.21±0.01 and 65±5 for pravastatin and simvastatin, respectively [28]. It means that pravastatin is 310 times less lipophilic than simvastatin. The mechanism of inhibitory effect of pravastatin on Kv1.3 channels remains to be elucidated. It is probably different than in case of simvastatin and mevastatin. It may involve interactions of drug molecule with external vestibule of the channel leading to inhibition of the current without a significant change of its inactivation rate.

The inhibition of Kv1.3 channels by statins may be related to anti-proliferative and pro-apoptotic influence of these compounds on cancer cells. It was shown that both simvastatin and mevastatin inhibited proliferation of colon cancer cell line LoVo and its doxorubicin-resistant sub-line LoVo/Dx [15]. It is known that LoVo

cells express Kv1.3 channels [11]. On the other hand, inhibition of proliferation of LoVo cells by simvastatin and mevastatin was observed at the concentration of 100 μM, the concentration high enough to inhibit most of Kv1.3 channels. The inhibitory effects of proliferation of LoVo cells exerted by simvastatin and mevastatin were comparable [15]. The same study showed that application of simvastatin and mevastatin at the same concentration (100 μM) induced apoptosis of LoVo and LoVo/Dx cells [15]. Mechanism of a possible contribution of inhibition of Kv1.3 channels to anti-proliferative and pro-apoptotic activity of the statins on Kv1.3 channel-expressing cancer cells remains to be elucidated.

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Declaration of Competing Interest

The authors: Andrzej Teisseyre, Anna Uryga and Krystyna Michalak, declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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