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Short communication

Modulation of the voltage-gated potassium channel Kv2.1 by the anti-tumor alkylphospholipid perifosine

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ABSTRACT

Background: The aim of the present study was to assess the effects of perifosine—a third generation alkylphospholipid analog with anti-tumor properties—on the activity of Kv2.1 channels.
Methods: The whole-cell patch clamp technique was applied to follow the modulatory effect of perifosine on Kv2.1 channels expressed in HEK293 cells.
Results: Obtained data provide evidence that perifosine application decreases the whole cell Kv2.1 currents in a concentration-independent manner. Perifosine induces a hyperpolarizing shift in the voltage dependence of Kv2.1 channels inactivation without altering the voltage dependence of channels activation. The kinetics of Kv2.1 closed-state inactivation was accelerated by perifosine, with no significant effects on the recovery rate from inactivation.
Conclusions: Taken together, these results show that perifosine modified the Kv2.1 inactivation gating resulting in a decrease of the current amplitude. These data will help to elucidate the mechanism of action of this promising anti-cancer drug on ion channels and their possible implications.

Introduction

Perifosine (octadecyl-(1,1-dimethylpiperidinio-4-yl)-phosphate) is a synthetic anti-tumor alkylphospholipid analog with promising results against a variety of cancers [1]. Perifosine is structurally related to membrane lipids and therefore target cellular membranes [2], where it accumulates into lipid rafts [3]. Lipid rafts are membrane microdomains enriched in cholesterol and sphingolipids and are the platform of several signaling pathways, including the Akt pathway [4], one of the most frequently hyperactivated signaling pathways in human cancers and an important target for the prevention of this disease. Interestingly, it was demonstrated that perifosine induces a reorganization of lipid rafts and as a consequence inhibits the Akt signaling pathway [4]. The inhibition of the Akt signaling by disrupting lipid rafts is the principal mechanism of the anti-tumor activity of perifosine.

Increasing evidence point to the important role of ion channels in cell proliferation, migration, apoptosis and differentiation and is increasingly being suggested that they contribute to cancer progression [5].

Voltage-gated potassium (Kv) channels are potassium selective membrane proteins formed by the assembly of four homologous subunits [6]. In response to a membrane depolarization, Kv channels open, allowing potassium ions to permeate. Multiple studies have reported dysregulated expression of several Kv channels in human cancer [5]. Kv2.1 is a member of this large family that has been detected in cancer cells, including uterine cancer cells [7], gastric cancer cells [8] and medulloblastoma [9]; and it has been reported that Kv2.1 channels inhibition decreases proliferation of various uterine cancer cells [7].

Previous studies have reported the preferential localization of Kv2.1 in lipids rafts [10], and disruption of these microdomains has an influence on the function of this channel [10]. Hence, we wished to investigate if perifosine, a drug targeting lipid rafts, has an influence in the functioning of Kv2.1 channels. In this study, we report that perifosine modulates the Kv2.1 inactivation gating resulting in a decrease of the current amplitude.

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Material and methods

Drugs

Perifosine was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in water to get a 20 mM stock perifosine solution. The stock solution was diluted to the final concentrations in bath solution for the patch clamp recordings.

Cell culture and transfection

Human embryonic kidney (HEK) 293 cells were grown in 60-mm tissue culture dishes (Corning, Corning, NY, USA) in DMEM with 10% fetal bovine serum and 1% antibiotic anti-mycotic solution (Sigma-Aldrich, St. Louis, MO, USA) in a humidifier incubator at 37 °C (5% CO_2). Kv2.1 in the pXoom vector was gratefully received from Dr. D. Logothetis (Virginia Commonwealth University, Virginia, VA, USA). Kv2.1 channels were transfected into HEK 293 cells with the use of Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Electrophysiological recordings

Current recordings in HEK 293 cells were performed at room temperature (22–24 °C) by using the whole-cell configuration of the patch clamp technique with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Data acquisition and generation of voltage-clamp pulse protocols were carried out with a Digidata 1440A interface (Molecular Devices) controlled by the pCLAMP 10 software (Molecular Devices). Micropipettes were pulled from borosilicate glass capillary tubes (World Precision Instruments, Sarasota, FL, USA) on a programmable puller (Sutter Instruments, Novato, CA, USA). When micropipettes were filled with the pipette solution, tip resistance ranged from 1.5 to 2.5 mΩ. The external solution contained (in mM): 130 NaCl, 4 KCl, 1 $MgCl_2$, 10 HEPES, 1.8 $CaCl_2$, and 10 glucose (pH was adjusted to 7.35 with NaOH). The pipette solution contained (in mM): 5 K_4BAPTA, 100 KCl, 5 $MgCl_2$, and 5 K_2ATP (pH adjusted to 7.2 with KOH).

Data analysis

Patch clamp data were processed using Clampfit 10 (Molecular Devices), and analyzed in Origin 8.6 (OriginLab Corp., Northampton, MA, USA).

Conductance–voltage (G–V) relationships were determined based on the equation:

$$G = \frac{I_p}{V - V_{rev}},$$

where I_p is the peak current amplitude at the test potential V and V_{rev} is the potassium reversal potential. The voltage dependence of Kv2.1 channel activation was determined from the G–V relationships fitted to a Boltzmann equation:

$$y = \frac{1}{1 + \exp[-(V - V_{1/2})/K]},$$

where V represents the test potential, and $V_{1/2}$ and K are the potential at which the conductance was half-activated and the slope, respectively. The voltage dependence of Kv2.1 channel steady-state inactivation was determined using a three step protocol applied every 30 s [11]. From a holding potential of −100 mV, a depolarizing 100 ms step to +80 mV was applied (P_1), after a brief repolarization to the holding potential, a 6 s conditioning pulse to potentials between −100 and +80 mV were applied (P_2) followed by a final pulse to +80 mV (P_3). The normalized current was calculated dividing the current in P_3 by the current in P_1 and plotted *vs.* the conditioning potential (P_2). The resulting steady-state inactivation data were fitted with the Boltzmann equation:

$$y = \frac{1}{1 + \exp(V - V_{1/2})/K},$$

where V is the conditional potential, and $V_{1/2}$ and K are the potential at which the conductance was half-inactivated and the slope, respectively.

Data are presented as mean ± standard error of the mean (SEM) (n = number of cells). Statistical significance was evaluated by Student's t-test. A value of $p < 0.05$ was considered statistically significant.

Results

Perifosine inhibits the Kv2.1 channels in a concentration-independent manner

Whole-cell voltage clamp experiments were performed to investigate the effects of perifosine on Kv2.1 channels heterologously expressed in HEK293 cells. Representative current traces recorded at +60 mV under control conditions and after application of 0.3 μM (A) and 3 μM (B) of perifosine are shown in Fig. 1. Perifosine decreased the peak and end-pulse current amplitude by 10.74 ± 1.3% and 23.46 ± 1.2% (n = 10), and 10.49 ± 3.5% and 21.36 ± 1.13% (n = 6) in the presence of 0.3 and 3 μM of perifosine, respectively (Fig. 1C and D). There were no significant differences among the concentrations of perifosine tested (Fig. 1C and D). Hereinafter, we use the 0.3 μM concentration for subsequent experiments.

Effect of perifosine on steady-state activation and inactivation of Kv2.1 channels

Fig. 2A shows the steady-state activation curves of Kv2.1 currents under control conditions and the presence of perifosine. The potential of the $V_{1/2}$ and K of the steady-state activation curves were 11.01 ± 1.01 mV and 15.87 ± 0.57 for the control, and 10.6 ± 0.95 mV and 19.2 ± 0.7 for perifosine (0.3 μM) (n = 8), respectively. The steady-state inactivation curve for Kv2.1 under control conditions was −24.47 ± 0.99 mV and a K of 6.19 ± 1.33. Perifosine significantly shifted the inactivation curve to hyperpolarized potentials ($V_{1/2}$ = −34.14 ± 0.89 mV) with no significant change in K (6.34 ± 0.17) (Fig. 2B) (n = 9, $p < 0.05$).

Recovery kinetics from inactivation of Kv2.1 channels

Recovery from inactivation was measured by using the three-pulse protocol shown in the top of Fig. 3. Fig. 3 shows families of current traces in absence (A) and presence of perifosine (B). The recovery kinetics of Kv2.1 current was best fit by a double exponential function that was not altered by perifosine (Fig. 3C). The recovery time constants were 0.39 ± 0.07 and 2.27 ± 0.41 s for control, and 0.27 ± 0.03 and 2.65 ± 0.54 s in the presence of perifosine (n = 7).

Effect of perifosine on closed-state inactivation of Kv2.1 channels

Previous studies have shown that Kv2.1 channels inactivate from the closed state without opening in the sub-threshold voltage range [11]. We tested the effect of perifosine (0.3 μM) on the closed-state inactivation. The kinetics of closed-state inactivation