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Large-conductance K^+ channel openers NS1619 and NS004 as inhibitors of mitochondrial function in glioma cells

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Abstract

Recently, it has been reported that large-conductance Ca^{2+}-activated potassium channels, also known as BK_{Ca}-type potassium channels, are present in the inner mitochondrial membrane of the human glioma LN229 cell line. Hence, in the present study, we have investigated whether BK_{Ca}-channel openers (BK_{Ca}COs), such as the benzimidazolone derivatives NS004 (5-trifluoromethyl-1-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-2*H*-benzimidazole-2-one) and NS1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2*H*-benzimidazol-2-one), affect the functioning of LN229 glioma cell mitochondria *in situ*. We examined the effect of BK_{Ca}COs on mitochondrial membrane potential, mitochondrial respiration and plasma membrane potassium current in human glioma cell line LN229. We found that BK_{Ca}COs decrease the mitochondrial membrane potential with an EC_{50} value of 3.6 ± 0.4 μM for NS1619 and 5.4 ± 0.8 μM for NS004. This mitochondrial depolarization was accompanied by an inhibition of the mitochondrial respiratory chain. Both BK_{Ca}COs induced whole-cell potassium current blocked by charybdotoxin, as measured by the patch-clamp technique. The BK_{Ca}COs had no effect on membrane bilayer conductance. Moreover, the inhibition of mitochondrial function by NS004 and NS1619 was without effect on cell survival, as measured by lactate dehydrogenase release from the cells.

Keywords: Glioma cells; Mitochondria; Respiratory chain; Ion channels; Potassium channel openers; Charybdotoxin

1. Introduction

The intracellular targets for KCOs have recently attracted attention mainly due to the interaction of the KCOs with mitochondria. Originally, KCOs were identified as openers of two classes of plasma membrane K^+ channels; K_{ATP} channels and large-conductance BK_{Ca} channels [1]. Some KCOs may directly interact with the potassium channels present in different intracellular membranes (for a review see [2]). In particular, mitochondrial ion channels seem to be important intracellular targets for KCOs. Mitochondrial ion channels play an important role in such cellular events as apoptosis [3], exocytosis [4] and synaptic transmission [5]. Different ion channels were identified in the inner mitochondrial membranes, including both potassium and chloride channels. Recently, it was found that KCOs acting on the mitoK_{ATP} channel may play an important role as inducers of ischemic preconditioning in the heart (for a review see [6]).

A BK_{Ca} channel was identified by patch-clamp techniques in the inner mitochondrial membrane (mitoBK_{Ca} channel) of the human glioma cell line LN229 [7]. This

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Abbreviations: mitoK_{ATP} channel, mitochondrial ATP-regulated potassium channel; K_{ATP} channel, plasma membrane ATP-regulated potassium channel; KCOs, potassium channel openers; BK_{Ca}COs, calcium-activated potassium channel openers; TMRE, tetramethylrhodamine ethyl ester; BK_{Ca} channel, calcium-activated potassium channel; mitoBK_{Ca}, mitochondrial calcium-activated potassium channel; NS004, 5-trifluoromethyl-1-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-2*H*-benzimidazole-2-one; NS1619, 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2*H*-benzimidazol-2-one; DNP, 2,4-dinitrophenol; mito-EYFP, mitochondrial enhanced yellow fluorescent protein; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; ChTx, charybdotoxin; DHS I, dehydrooyasaponin; PBS, phosphate buffered saline.

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channel has a conductance of 295 pS and is activated by Ca^{2+}. It is not known whether the Ca^{2+}-binding site is on the matrix or the cytosolic side of the mitochondrial membrane [7]. Like most BK-type channels, the mitochondrial channel is blocked by charybdotoxin in a voltage-dependent manner [7]. Recently, mitoBK_{Ca} channel was found in cardiac ventricular myocytes [8]. The function of the mitochondrial BK-type K_{Ca} channel is still unknown.

A variety of small synthetic molecules, for example, the benzimidazolones NS004 and NS1619 [1], and natural product-derived compounds, maxiKdiol and DHS I, have been identified as selective large-conductance BK_{Ca}COs [1]. The action of NS004 and NS1619 was observed in different cell types such as smooth muscle cells [9,10] and neural cells [11]. However, little is known about the intracellular site of interaction of these openers.

In this work, we report that the two BK_{Ca}COs, NS004 and NS1619 inhibit mitochondrial function in human glioma LN229 cells due to inhibition of the complex I of mitochondrial respiratory chain. We were not able to observe a direct activation of mitoBK_{Ca} channel by NS004 and NS1619. Moreover, we show that a BK_{Ca} channel activated by BK_{Ca}COs is present in the plasma membrane of the human glioma cell line LN229.

2. Materials and methods

2.1. Materials

All reagents for cell culture were purchased from Life Technologies, Inc. (GibcoBRL) and from Sigma–Aldrich Co Ltd. TMRE and 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazoylcarbocyanine iodide (JC-1) were from Molecular Probes. The potassium channel openers NS1619 and NS004 were provided by S.-P. Olesen (NeuroSearch). All other reagents were obtained from Sigma–Aldrich Co Ltd.

2.2. Cell culture

Glioma cell line LN229 was routinely cultured in DMEM supplemented with 4.5 g/L glucose and 5% heat-inactivated fetal calf serum, 4 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (standard conditions). The cells were incubated in an atmosphere containing 5% CO_2/95% air at 37°.

2.3. Transient transfection

One day before transfection, cells were seeded into culture dishes under standard conditions. Cells were grown on glass coverslips to 60–80% confluency before transfection. Transfection with the mito-EYFP construct was performed using LipofectAMINE Plus™ Reagent (Life Technologies, Inc.) according to the manufacturer's instructions. The yield of transfection was usually 40–70%.

2.4. Membrane potential measurements

The mitochondrial membrane potential ($\Delta\Psi_m$) in LN229 cells was measured using TMRE, a potential-sensitive dye, according to a previously described procedure [12]. Cultured cells were collected after treatment with 0.25% trypsin and suspended in medium (KR-Hepes) containing 121 mM NaCl, 25 mM HEPES, 5 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.0 mM $CaCl_2$, 10 mM glucose, pH 7.4. The measurements were made at room temperature in a 1 mL cuvette of a Shimadzu RF-5000 spectrofluorometer using 0.4 μM TMRE and 2×10^4 to 2.5×10^4 LN229 cells. The samples were excited at 548 nm and fluorescence was measured at 590 nm. The fluorescence values were normalized to the uncoupled fluorescence values using the following equation: $\Delta\text{TMRE} = (\log \text{Fl548} - \log \text{Fl unc548})/\log \text{Fl unc548}$ as previously described [12]. In this equation, ΔTMRE represents the change in TMRE fluorescence at 548 nm, associated with $\Delta\Psi_m$; log Fl548 represents the logarithm of the mean TMRE florescence and log Fl unc548 represents the logarithm of the mean TMRE fluorescence after uncoupling with CCCP. The log of the fluorescence was used, rather than the direct measure of fluorescence, since TMRE is accumulated according to the Nernst equation and, hence, the relationship between TMRE accumulation by mitochondria and $\Delta\Psi_m$ is logarithmic.

Additionally, changes in $\Delta\Psi_m$ were monitored by the uptake of the fluorescent dye JC-1. Cells were incubated with 0.5 μM JC-1 in DMEM medium for 15 min at 37°. After that cells were washed twice with PBS and resuspended in KR-Hepes buffer. Stained cells were immediately analyzed with a FACSCalibur instrument (Becton Dickinson) equipped with a 488 nm argon laser. The filter in front of the FL1 photomuliplier transmitted fluorescence at 530 nm, and the filter used in the FL2 channel transmitted fluorescence at 617 nm. The red fluorescence measured in the FL2 channel corresponded to a J-aggregates form of JC-1 present in the mitochondria with high membrane potential. Compensation FL1–FL2 was 3.7% and compensation for FL2–FL1 was 9.5%. A minimum of 10 000 cells per sample were acquired and analyzed with Cell Quest software package.

2.5. Confocal images

A Bio-Rad MRC1024 confocal system equipped with a 100 mW Ar and 15 mW Ar:Kr laser was used. For TMRE fluorescence, excitation was 568 nm, for EYFP 488 nm; the objective lens was 60× PlanApo NA 1.4. During imaging, cells were maintained in a microincubator (Life Sci. Res.) at 37°, in Krebs buffer. Image analysis was performed using Confocal Assistant (T. Brelje) software.

2.6. Cell respiration measurements

Cultured cells were collected after treatment with 0.5% trypsin and suspended in KR-Hepes medium. Cell respiration was measured at 30° with a Clark type oxygen electrode (Yellow Springs Instruments). Respiration of permeabilized LN229 cell was measured in the presence of 10 μg/mL digitonine.

2.7. Planar lipid bilayer technique

The planar lipid membrane was formed by spreading phospholipid dispersions (painted bilayer). Planar phospholipid bilayers were formed in a 250 μm diameter hole drilled in a Delrin partition separating two chambers (*cis* 3 mL and *trans* 4 mL internal volume). Both chambers contained 50 mM KCl, pH 7.4 (adjusted with Tris–Hepes). The outline of the aperture was coated with a lipid suspension and dried under N_2 prior to bilayer formation to improve membrane stability. Planar phospholipid bilayers were painted using a 1:1 mixed suspension of PS:PE in decane at a final concentration of 20 mg of lipid/mL. Formation and thinning of the bilayer was monitored by capacitance measurements. Final capacitance values ranged from 80 to 120 pF. Electrical connections were made by using Ag/AgCl electrodes and agar salt bridges (2 M KCl) to minimize liquid junction potentials. Solutions of NS1619 and NS004 in DMSO were added to the *trans* compartment connected to ground. All measurements were carried out at room temperature. The current was measured using a Bilayer Membrane Admittance Meter (model ID 562, IDB, Gwynadd). The signals were filtered at 0.2 kHz (Low Pass Bessel Filter 4 Pole, Warner Instrument Corp.), digitized (A/D converter 1401plus, Cambridge Electronic Design) and transferred to a PC for off-line analysis by Patch and Vclamp 6.09 software (Cambridge Electronic Design).

2.8. Electrophysiological measurements

Whole-cell currents were recorded using the patch-clamp technique. Cells were used 1–3 days after plating. Experiments were carried out at room temperature. A Nikon TE300 inverted microscope and Axopatch 200B amplifier were used. Experimental data were low-pass filtered at 1 kHz and compensated for whole-cell capacity transients. Series resistance was typically 5–10 MΩ. Data were transferred to a computer at a sampling frequency of 100 μs per point using Clampex 7 software and then analyzed with pClamp7 software (Axon Instruments). The extracellular solution (NES) consisted of: 140 mM NaCl, 2.8 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, pH 7.4. The pipette solution was: 144 mM KCl, 16 mM NaCl, 2 mM $MgCl_2$, 2 mM PIPES, 2 mM EGTA. Drugs were applied using gravitational flow through a perfusion pipette containing 20 μM NS1619 in NES or 20 μM NS1619 with 500 nM ChTx in NES.

2.9. Cell injury assay

Lactate dehydrogenase (LDH) activity was assayed using a commercial kit (Roche Molecular Biochemicals). In the first step, NAD^+ is reduced to $NADH/H^+$ by the LDH-catalyzed conversion of lactate to pyruvate. In the second step, a catalyst (diaphorase) transfers H^+/H from $NADH/H^+$ to a yellow tetrazolium salt that is reduced to a red formazan compound. The absorbance at 492 nm was measured with a spectrophotometer (UV-160A, Shimadzu). The reference wavelength was 690 nm. The release of LDH into the medium was assayed at the indicated times of incubation and expressed as an index of cell injury, defined as percentage of total releasable LDH, yielded by lysis of LN229 cells in 0.1% Triton X-100 solution.

Additionally, cell injury was measured using the Trypan blue exclusion assay. The cells were loaded with 2% Trypan blue at room temperature for 5 min. Viable cells exclude the dye and dead cells stain blue. Both live and dead cells were counted using light microscopy and the percentage of dead cells was calculated.

2.10. Statistical analysis

The unpaired Student's *t*-test was used to determine the statistical differences between various experimental and control groups. P values <0.05 were considered significant.

3. Results

Figure 1A shows the effect of NS1619 on mitochondrial membrane potential in LN229 glioma cells. To visualize changes of the mitochondrial potential after treatment with the BK-channel opener, cells were first loaded with the fluorescent dye TMRE and incubated in KR-Hepes medium. The addition of 31 μM NS1619 caused a nearly complete decrease in the membrane potential. As shown in Fig. 1B, the decrease of the mitochondrial membrane potential was concentration-dependent with an EC_{50} of 3.6 ± 0.4 μM ($N = 3$) for NS1619 and 5.4 ± 0.8 μM ($N = 3$) for NS004. Similar observations were made with the use of JC-1 fluorescent probe and flow cytometer measurements (see Section 2). Application of 30 μM NS1619 lowered amount of LN229 cells with high mitochondrial membrane potential from $56 \pm 14\%$ to $26 \pm 13\%$. The same was observed in the presence of 10 μM CCCP; $30 \pm 2\%$ of the cells had high mitochondrial potential. Additionally, 0.5 μM charybdotoxin, a BK_{Ca}-channel blocker, was without effect on depolarization induced by 30 μM NS1619; $28 \pm 5\%$ of cells had high mitochondrial potential. In order to confirm that TMRE fluorescence was localized in the glioma mitochondria, confocal images of LN229 cells were recorded. Figure 1C shows LN229 glioma cells transiently transfected with the

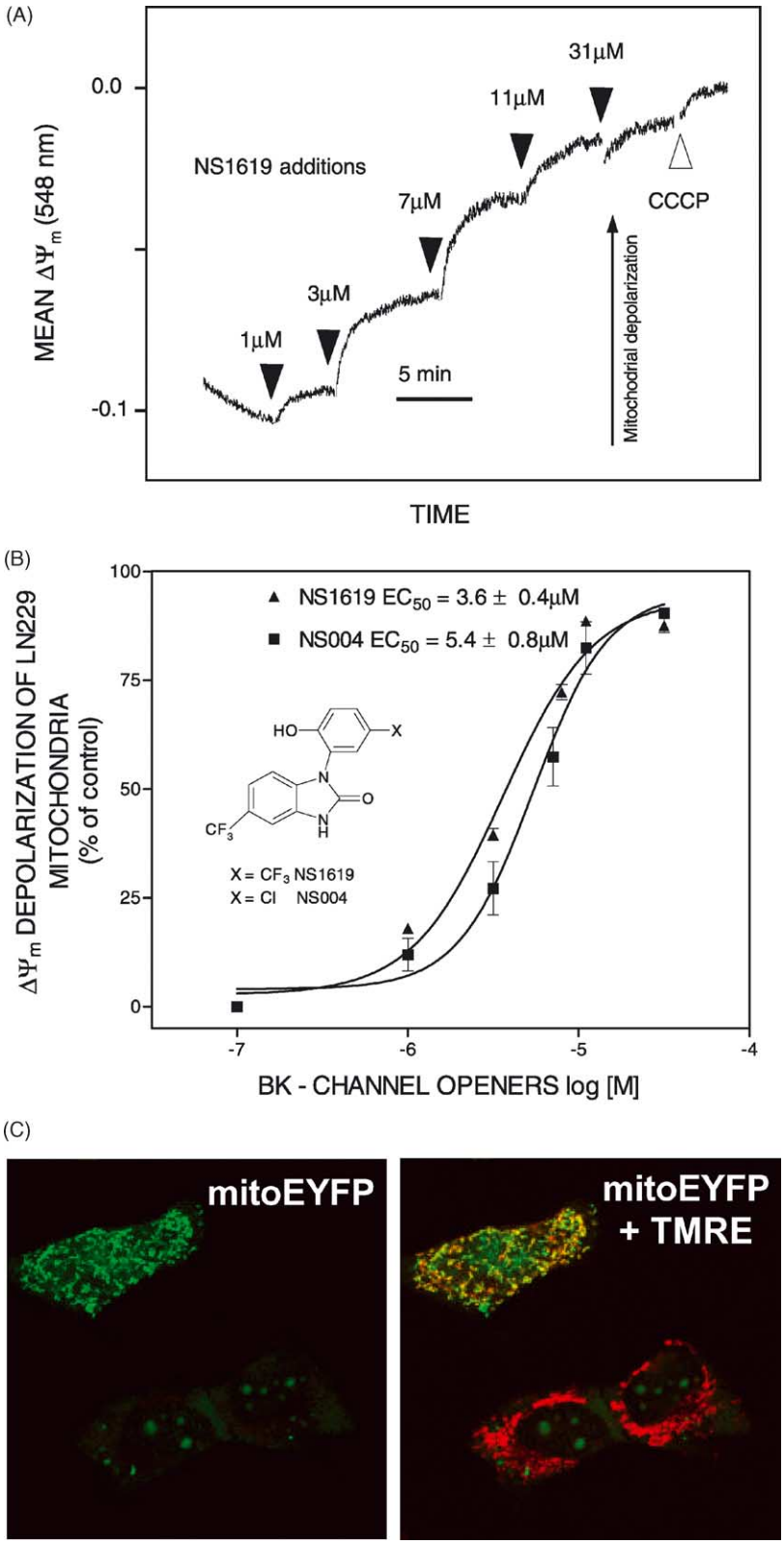

Fig. 1. Effect of BK channel openers NS1619 and NS004 on mitochondrial membrane potential in intact LN229 glioma cells. The membrane potential measurements in cell suspension (2.4×10^4 cells/mL) were performed using a fluorescent dye, TMRE. Assays were performed at 30° as described in Section 2. (A) Depolarization of mitochondrial membrane by the addition of NS1619 to a final concentration of 31 μM. Addition of 1.2 μM CCCP caused complete membrane depolarization. (B) Concentration dependence of NS1619- and NS004-induced mitochondrial membrane depolarization. The results are expressed as means ± SD (N = 3). (C) Confocal images of glioma cell line LN229 after transient transfection with a mito-EYFP construct (left panel) and colocalization of the green signal derived from mito-EYFP fluorescence and the red signal from TMRE fluorescence (right panel). TMRE was used at a final concentration of 200 nM. Assays were performed in KR-Hepes medium at room temperature.

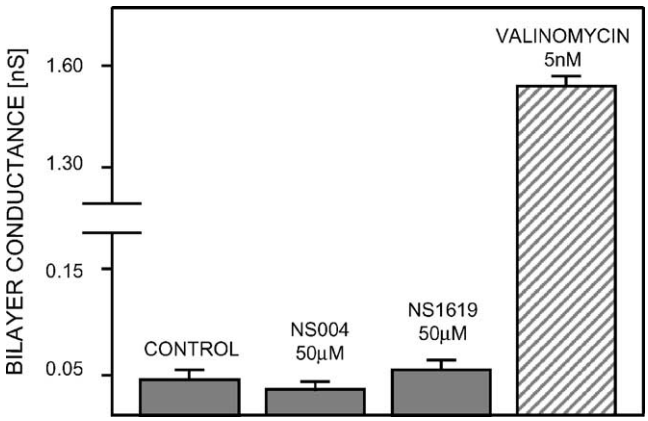

Fig. 2. Effect of NS1619 and NS004 on phospholipid bilayer conductance. Lipid bilayer conductance in the presence of 50 μM NS1619, 50 μM NS004 and 5 nM valinomycin. The assays were performed at room temperature as described in Section 2. Data are mean ± SD of three to four replicates.

mito-EYFP construct. As previously shown [13], mito-EYFP is expressed in mitochondria (Fig. 1C, left panel). A similar fluorescent pattern was observed in cells loaded with TMRE (a dye used to measure mitochondrial potential) (Fig. 1C, right panel). Moreover, the image in Fig. 1C (right panel) shows the co-localization of green and red signals derived from mito-EYFP fluorescence and TMRE fluorescence, respectively.

To exclude possible protonophoric or ionophoric properties of NS1619 and NS004, we investigated the effect of BK_{Ca}COs on membrane bilayer conductance. Figure 2 shows that both 50 μM NS1619 and 50 μM NS004 are unable to increase the membrane bilayer conductance (Fig. 2), as observed in the presence of 5 nM valinomycin which is known to increase membrane permeability.

Figure 3A shows that the addition of 100 μM DNP, an uncoupler of mitochondrial oxidative phosphorylation, accelerated mitochondrial respiration in glioma LN229. The addition of 10 μM NS1619, in the presence of 100 μM DNP, significantly decreases the respiration rate. As shown in Fig. 3B, the maximal uncoupled respiration after DNP treatment of LN229 cells decreased from 2.69 ± 0.70 ng O_2/min/10^6 cells to 0.89 ± 0.31 ng O_2/min/10^6 cells or 1.82 ± 0.32 ng O_2/min/10^6 cells after the addition of 10 μM NS1619 or 10 μM NS004, respectively. Similar measurements were performed with permeabilized LN229 cells. They were performed in the presence of 10 μg/mL digitonin and in the presence of various mitochondrial substrates; 10 mM glutamate and 5 mM malate or 5 mM succinate and 2 μM rotenone. A significant decrease in respiration rate, in the presence of 100 μM DNP, was observed after glioma cells were treated with 10 μM NS1619 or 20 μM NS1619, only in the medium supplemented with glutamate and malate (Fig. 3C).

In order to check whether the BK_{Ca}COs used in this study affect the plasma membrane potassium channels, we applied the patch-clamp technique in the whole-cell configuration. Glioma cells were voltage clamped at −60 mV and outward currents were elicited by 10 mV voltage steps from −60 to +40 mV, applied for 500 ms with 2 s intervals. Figure 4A shows a typical non-inactivating current evoked by depolarization to +40 mV. Administration of NS1619 to the bath increased the current by about 100% in 14 of 25 cells. It was accompanied by an increase of current noise (Fig. 4A, middle trace), indicating the presence of a high unitary conductance channel. The effect of NS1619 was blocked by simultaneous application of 500 nM ChTx ($N = 6$), a BK_{Ca}-channel blocker [8] (Fig. 4B).

Interestingly, incubation of LN229 glioma cells with 10 μM NS1619 or 10 μM NS004 for up to 24 hr was without effect on cell injury measured by LDH release from the cells (Fig. 5) or Trypan blue uptake (data not shown).

4. Discussion

Glioma cells express a variety of ion channels in the plasma membrane. These include voltage-gated K^+ [14] and Na^+ channels [15], Ca^{2+}-activated K^+ channels [15,16] and voltage-gated Cl^- channels [17]. BK_{Ca} channels were also described in human glioma cells [18]. Recently, the intracellular mitoBK_{Ca} channel has been shown to exist in human glioma LN229 cells [7].

It is believed that ion channels in glioma cells may contribute to invasive migration and proliferation, leading to the malignant behavior of these cells during brain tumor propagation [19,20]. Hence, the potassium channels expressed by glioma cells may represent novel therapeutic targets in the treatment of brain tumors. The mitoBK_{Ca} channel may be an important element of glioma cell ion homeostasis [21]. Changes of mitochondrial membrane conductance seem to be an early event in mitochondrial-derived apoptosis. The mitoBK_{Ca} channel activation could lead to mitochondrial depolarization, induction of permeability transition pore and further to apoptosis. Hence, it is important to clarify the mode of interaction of BK_{Ca}COs with glioma cell mitochondria.

The benzimidazolones NS004 and NS1619 were the first synthetic small molecules shown to activate BK_{Ca} channels [8]. The effect of these KCOs was potentiated by cytosolic Ca^{2+} but the gating was similar to that mediated by an increase of cytosolic Ca^{2+} concentration. Moreover, application of a BK_{Ca} inhibitor, such as charybdotoxin, attenuated the KCOs effects [8]. The lipophilicity of NS004 is sufficient to allow the molecule to cross the membrane but the intracellular action of NS004 is not well characterized [22]. Hence, we wanted to establish whether benzimidazolone BK_{Ca}COs affect mitochondrial function in the human glioma cell line LN229.

The depolarization of mitochondria observed in our studies upon application of the benzimidazolone BK_{Ca}COs could be explained by an influx of K^+ into the mitochondrial matrix, inhibition of the respiratory chain or by the uncoupling properties of the applied KCOs. This last possibility

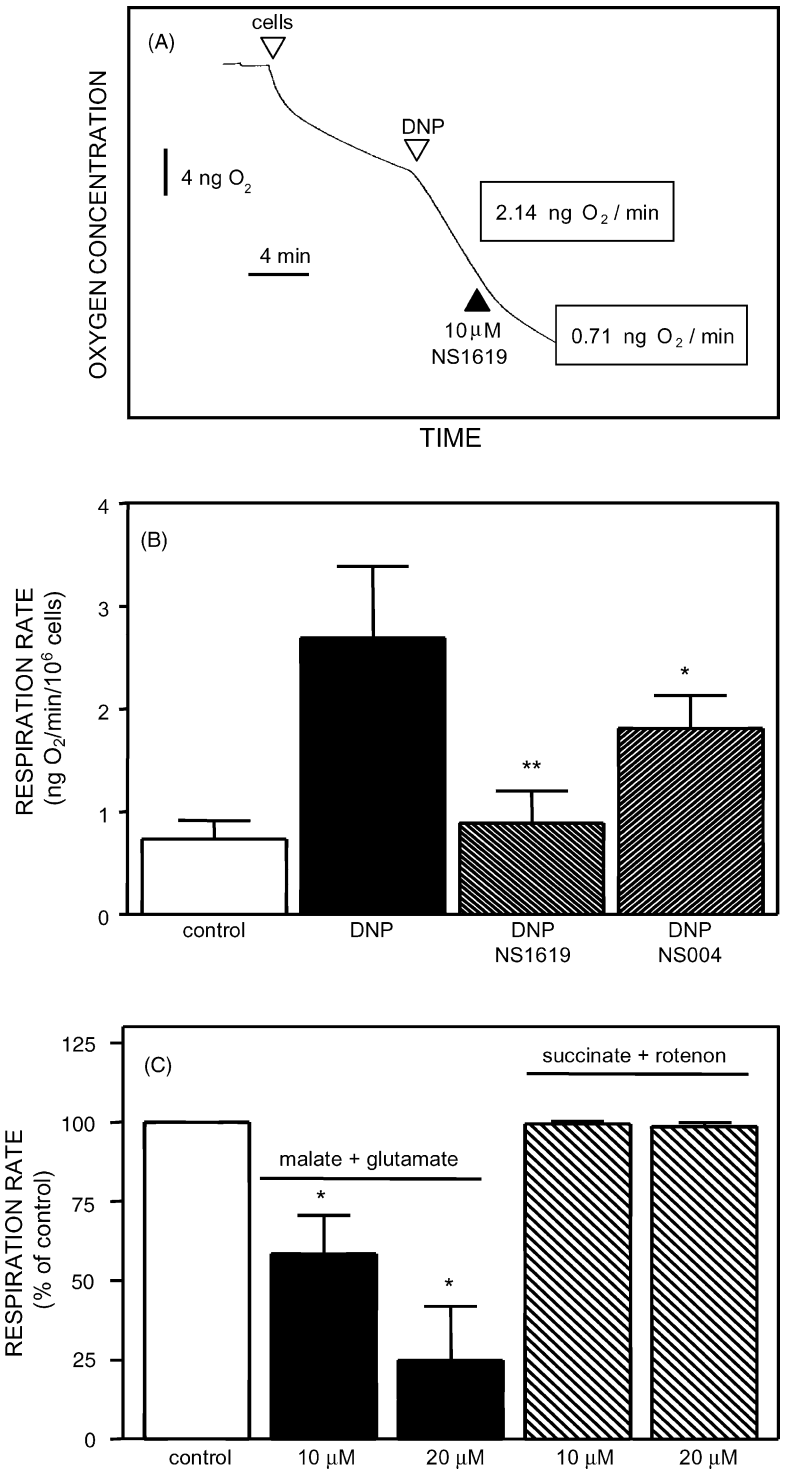

Fig. 3. The inhibitory effect of BK_{Ca}-channel openers on the respiration rate of LN229 cells. (A) Decrease of the maximal respiration rate, in the presence of 100 μM DNP, after the addition of NS1619, 10 μM final concentration. (B) Effect of 20 μM NS1619 and 20 μM NS004 on respiration in the presence of 100 μM DNP. As control, respiration in the absence of DNP was taken. Data represent the mean ± SD from three experiments. $^{*}P < 0.05$, $^{**}P < 0.005$ compared with maximal respiration rate after cell treatment with 100 μM DNP. (C) Effect of 10 and 20 μM NS1619 on respiration of permeabilized LN229 cells in the presence of 100 μM DNP and various mitochondrial substrates: 10 mM glutamate/5 mM malate or 5 mM succinate. As control, respiration in the presence of 100 μM DNP was taken. $^{*}P < 0.05$ compared with the respiration rate of control samples.

was excluded by the use of a planar lipid bilayer system. Despite the presence of an aromatic hydroxyl group in the benzimidazolone BK_{Ca}COs, no protonophoric or ionophoric properties of these compounds were detected.

Respiring mitochondria transfer H^+ from matrix to the cytosol, thus forming both transmembrane electric potential (negative inside) and a pH gradient (alkaline inside). Similar to the action of uncouplers such as CCCP, net K^+

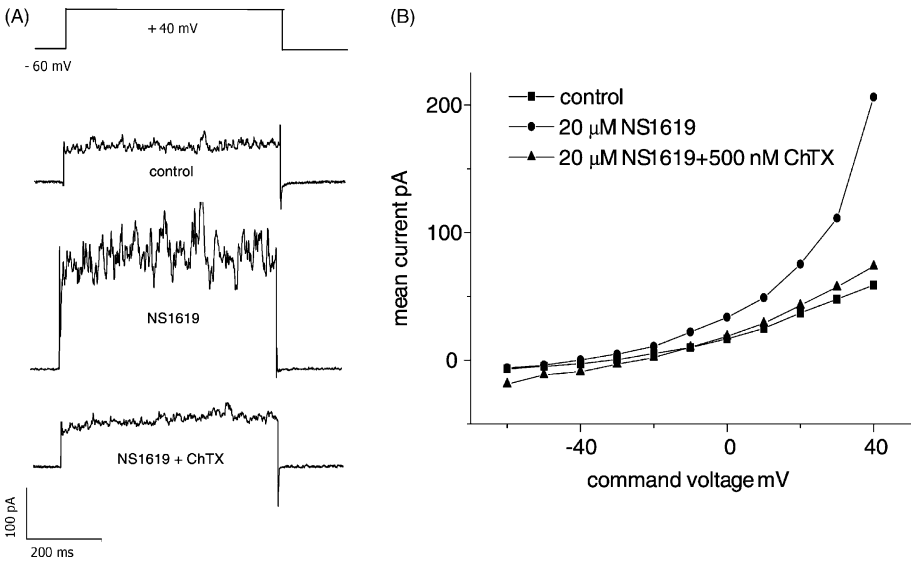

Fig. 4. The effect of BK channel activator NS1619 on cell current. (A) Selected current traces for the +40 mV step under control conditions, in the presence of 20 μM NS1619 and in the presence of 20 μM NS1619 supplemented with 500 nM ChTx in bath solution. The top trace represents the voltage clamp protocol applied to the cell. The presented traces were filtered with a 500 Hz Gaussian filter. (B) Mean current as a function of command voltage. All data were obtained from the same cell.

influx into mitochondria is accompanied by an increase of mitochondrial respiration. The transport of potassium ions into the mitochondrial matrix causes mitochondrial depolarization followed by increased electron flow through the respiratory chain. This is observed as an increase of mitochondrial respiration. In our studies, we observed that NS004 and NS1619, in a micromolar concentration range, caused an inhibition of mitochondrial respiration in glioma LN229 cells. Further studies with the application of various mitochondrial substrates to permeabilized LN229 cells revealed that, most probably, the benzimidazolone BK_{Ca}COs act by inhibiting the respiratory chain complex I. This was the most pronounced effect of these drugs that was detected in LN229 glioma cells. This interaction probably masked the stimulatory effect of the benzimidazolone channel openers (if any) on mitochondrial K^+ conductance due to the activation of the mitoBK_{Ca} channel.

Moreover, NS004 and NS1619 were able to activate the charybdotoxin sensitive potassium current in the plasma membrane of LN229 cells. This confirms the presence of

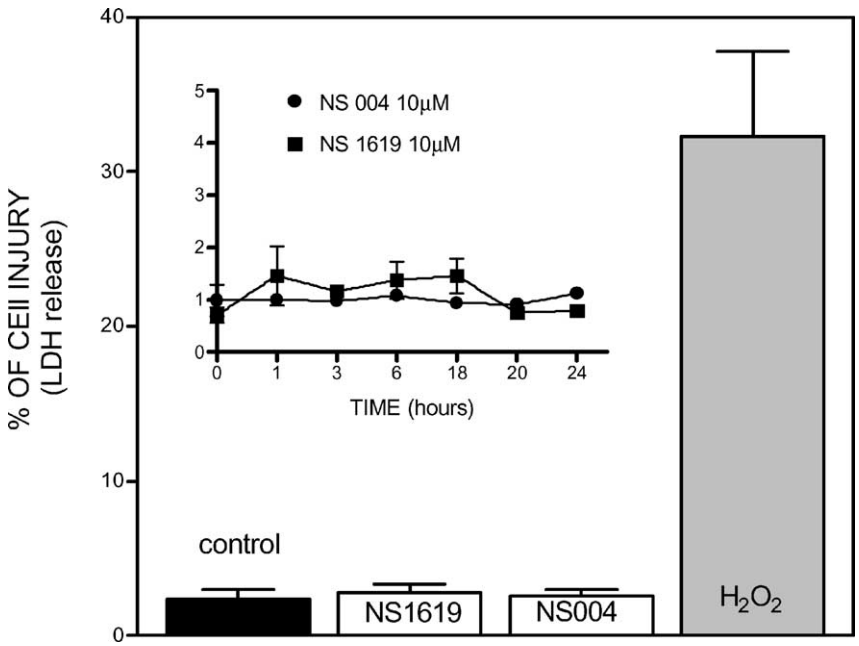

Fig. 5. Effects of BK channel openers on lactate dehydrogenase (LDH) release from the LN229 glioma cells. Cells were treated with 10 μM NS1619, 10 μM NS004 and 500 μM H_2O_2 for 18 hr. Data are mean ± SD of three replicates. Inset: Time course of LDH release from glioma cells caused of 10 μM NS1619 (squares) and 10 μM NS004 (circles). Data are mean ± SD of three replicates.

BK_{Ca} channels in LN229 glioma cells and supports the hypothesis that they are generally found in glioma cells [18]. The depolarization of mitochondria observed upon application of the benzimidazolone BK_{Ca}COs was rather not due to the activation of plasma membrane BK_{Ca} channels by NS004 and NS1619. First, the BK_{Ca}COs-induced depolarization was observed also in the presence of charybdotoxin, a BK_{Ca}-channel blocker. Second, depolarization of mitochondria was still observed after permeabilization of the plasma membrane by digitonin.

Potassium channels have been implicated in the growth and proliferation of several cancer cell lines. Cell death was observed both upon activation and inhibition of potassium channels. Apoptosis was observed due to the inhibition of outward rectifier K^+ channels in malignant astrocytoma cell lines [14] and due to the activation of the K^+ channel in leukemia ML-1 cells by UV [23]. The activity of voltage-gated K^+ channels has been demonstrated to regulate cell apoptosis and proliferation. Interestingly, both an increase of potassium current and depolarization of mitochondria were observed during apoptosis in pulmonary artery smooth muscle cells [24,25]. In our studies, the KCOs NS004 and NS1619 caused both an increase of potassium current and depolarization of mitochondria but without an effect on LN229 cells injury.

In summary, we have shown that the KCOs NS004 and NS1619, in addition to their known interactions with potassium and chloride channels [26], are able to inhibit mitochondrial function in the glioma cell line LN229. This effect was due to the interaction of BK_{Ca}COs with complex I of the mitochondrial respiratory chain. However, the mitochondrial depolarization, inhibition of mitochondrial respiration and activation of glioma plasma membrane BK_{Ca} channels were without effect on LN229 cell survival.

Acknowledgments

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