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Short communication

Cytoprotective action of the potassium channel opener NS1619 under conditions of disrupted calcium homeostasis

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Abstract:
Cytoprotective properties of potassium channel openers (KCOs) have been demonstrated in several models of cell injury, mainly in ischemia-reperfusion-induced damage of cardiac muscle. The mechanism responsible for the observed cytoprotection and the relative contribution of plasma membrane or inner mitochondrial membrane potassium channels regarding the beneficial effects exerted by KCOs remain unclear.
Our work demonstrates the cytoprotective properties of NS1619, an opener of large-conductance calcium-activated potassium channels (BK_{Ca} channels), using C2C12 myoblasts injured by calcium ionophore A23187 treatment. Application of two BK_{Ca} channel inhibitors, paxilline and iberiotoxin, abolished this cytoprotective effect. At concentrations of 10–100 μM, NS1619 increased the respiration rate and decreased mitochondrial membrane potential ($\Delta\psi$) in C2C12 cells in a dose-dependent manner. At a concentration of 0.2 μM, paxilline, which effectively abolished the protective effect of NS1619, failed to counteract the opener-induced mitochondrial depolarization and increase in cellular respiration. This result indicates that the NS1619-mediated increase in the survival rate of A23187-treated C2C12 cells occurs in a manner distinct from its effect on mitochondrial functioning and suggests that activation of BK_{Ca} channels in the plasma membrane is the mechanism responsible for cytoprotection by NS1619.

Key words: BK_{Ca} channel, mitochondria, cytoprotection, NS1619, calcium

Abbreviations: BK_{Ca} channel – large-conductance calcium-activated potassium channel, K_{ATP} channel – ATP-regulated potassium channel, KCO – potassium channel opener, LDH – lactate dehydrogenase, MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, OGD – oxygen-glucose deprivation

Introduction

It is well documented that the administration of certain potassium channel openers (KCOs) prior to ischemic insult results in a strong inhibition of ischemia and reperfusion-induced tissue damage [1]. This cytoprotective effect has been extensively studied in cardiac muscle, but it has been observed also in other tissues, including neuronal tissue [20]. Cytoprotective activity has been reported for numerous activators of ATP-regulated potassium channels (K_{ATP} channels), such as diazoxide, pinacidil and nicorandil [1]. More recently, it has been shown that activators of large-conductance calcium-activated potassium channels (BK_{Ca} channels) also demonstrate cytoprotective effects [22].

K_{ATP} channels and BK_{Ca} channels have been identified both in the plasma membrane and in the inner mitochondrial membrane in numerous cell types [20]. As both plasmalemmal and mitochondrial channels are targets of KCOs action, it is still somewhat controversial as to which of these channels is responsible for the beneficial effects of the openers [21]. Currently, the prevailing view is that KCO-mediated cytoprotection is related to the stimulation of potassium influx into the mitochondria. Potassium influx can lead to mild uncoupling of mitochondria, which results in changes in reactive oxygen species generation and limitation of calcium uptake into the matrix. This, in turn, helps to preserve mitochondrial function under stress conditions [13–15]. Conversely, activation of potassium channels located in the plasma membrane results in cell membrane hyperpolarization and reduction in the influx of Ca^{2+} into the cytosol, both of which can also protect against ischemic damage [21]. It is also possible that plasmalemmal and mitochondrial potassium channels coactively contribute to ischemic preconditioning [21].

NS1619 is a BK_{Ca} channel opener that has demonstrated cytoprotective activity in various models of cell injury, including cardiac tissue under conditions of ischemia and reperfusion [4, 19, 22], cardiomyocytes challenged with metabolic inhibition and anoxia [4], neuronal cell injury due to oxygen-glucose deprivation, glutamate exposure or hydrogen peroxide treatment [8, 9] and C2C12 myoblasts injured by H_2O_2 treatment [18]. In the present study, we demonstrate that NS1619 increases the survival of C2C12 cells under conditions of disrupted calcium homeostasis induced by the calcium ionophore A23187.

Materials and Methods

Reagents

NS1619, paxilline and A23187 were purchased from Sigma. Iberiotoxin was obtained from Bachem. Stock solutions of NS1619 (33 mM), paxilline (10 mM) and A23187 (10 mM) were prepared in DMSO, and iberiotoxin was dissolved in water. Immediately prior to carrying out our experiments, the appropriate chemical dilutions were prepared in Dulbecco's Modified Eagle's Medium (DMEM) and then used for cell treatment. The DMSO concentration in cell culture medium used for cell treatment did not exceed 0.3%. At such a low concentration, the solvent did not affect the viability of C2C12 cells.

Cell culture conditions

C2C12 murine myoblasts were obtained from European Collection of Animal Cell Cultures (ECACC). Cells were cultured at 37°C in a humidified atmosphere with 5% CO_2 and maintained in DMEM supplemented with 20% fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. For cell injury tests, cells between the 5^{th} and 15^{th} passage were seeded onto 96-well plates, and experiments were performed the following day. Chemical incubations were conducted in culture medium containing 0.1% FBS (culture medium was switched to the medium containing 0.1% FBS directly before the experiment).

Determination of cell injury levels

Cells grown in 96-well plates (about 6×10^3 cells per well) were treated for 6 h with the calcium ionophore A23187. NS1619 was added 15 min prior to ionophore administration, and iberiotoxin or paxilline was added 30 min prior to ionophore administration. Following treatment, cell survival was assessed using either the lactate dehydrogenase (LDH) release test or the MTT reduction assay.

LDH release was measured using the Cytotoxicity Detection Kit (Roche Molecular Biochemicals). After incubation with the tested chemicals, the incubation medium was collected, and cell debris was removed by centrifugation. In parallel, 0.1% Triton X-100 was added to each well to lyse the attached cells. LDH activity in the cell lysates and in the incubation medium was measured colorimetrically according to the instructions provided by the manufacturer. The amount of LDH released from the cells was then calculated using the formula: % of LDH release = (LDH_{medium}/LDH_{total}) × 100%, where 'LDH_{medium}' is the LDH activity detected in the incubation medium, and 'LDH_{total}' is the sum of the LDH activities detected in the incubation medium and in the cell lysates.

Cell survival was also assessed using the MTT reduction assay. Following chemical treatment, the cell culture medium was discarded, and 50 μl of DMEM containing 0.5 mg/ml of MTT [3-(4,5-dimethyl-