Physiology & Behavior 107 (2012) 458–465

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Melatonin modulates apoptosis and TRPM2 channels in transfected cells activated by oxidative stress

Ömer Çelik [a], Mustafa Nazıroğlu [a,b,*]

[a] Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey
[b] Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey

HIGHLIGHTS

► TRPM2 currents, apoptosis and Ca^{2+} influx values in transfected cells were increased compared with controls.
► However, melatonin modulated the values.
► The current study is the first to compare treatment with melatonin to TRPM2 currents in the transfected cells.

ARTICLE INFO

Article history:
Received 13 April 2012
Received in revised form 5 August 2012
Accepted 26 September 2012

Keywords:
Melatonin
Oxidative stress
Ca^{2+} signaling
TRPM2 channel antagonist
Apoptosis

ABSTRACT

Transient receptor potential melastatin-like 2 (TRPM2) is a non-selective Ca^{2+} permeable cation channel and is known to be activated by H_2O_2, one of the most important indicators of intracellular oxidative stress. A neurohormone melatonin may have a modulator role on TRPM2 channels activated by oxidative stress because it is a strong antioxidant. In this study we investigated the effects of melatonin on apoptosis, whole cell currents and Ca^{2+} influx arising from TRPM2 channels activated by H_2O_2.
In whole-cell patch clamp experiments, TRPM2 channels in transfected Chinese hamster ovary (CHO) cells were activated by H_2O_2. However, the currents were inhibited either by intracellular or by extracellular melatonin. When intracellular melatonin was introduced by pipette, TRPM2 channel currents were not activated by H_2O_2 although H_2O_2-induced Ca^{2+} gating and release were not blocked 2-aminoethyldiphenyl borate (2-APB). Cytosolic Ca^{2+} release was measured by Fura-2 and was higher in H_2O_2 groups than in control. Melatonin also inhibited apoptosis in the transfected cells.
In conclusion, we observed modulator roles of intracellular and extracellular melatonin on Ca^{2+} influx and apoptosis through a TRPM2 channel in transfected CHO cells.
© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Calcium ion (Ca^{2+}) homeostasis is one of the most important factors of cellular physiological function. It is involved in such diverse functions as cellular proliferation, apoptosis, physiological signal transduction and production of oxidative stress [1]. The cytosolic free Ca^{2+} $[Ca^{2+}]_i$ concentration is controlled by a number of membrane-bound ion channels located both on the plasma and intracellular membranes [2,3]. In fact, Ca^{2+} is the most ubiquitous and studied of second messenger and has enormous physiological and pathological implications in cellular function. Ca^{2+} signaling needs a complex system of refinement since Ca^{2+} is a simple cation with two positive charges that is able to translate a variety of extracellular signals in many different cell types to produce often very different responses [1].

Transient receptor potential (TRP) channels are a group of non-selective cation channels that have important functions in cellular systems [4]. One subgroup of the TRP channels is denoted as TRP melastatin 2 (TRPM2). The TRPM2 channel protein has two distinct domains with one functioning as an ion channel and the other as an ADP-ribose (ADPR)-specific pyrophosphatase [5]. The TRPM2 channel is also a redox-sensitive Ca^{2+}-permeable cation channel, and Ca^{2+} influx through TRPM2 is induced by H_2O_2 [6]. When expressed in the cell lines such as transfected Chinese Hamster Ovary (CHO) cells [7–9], transfected human embryonic kidney293 (HEK) cell lines [6] and dorsal root ganglion (DRG) neurons [10–12], TRPM2 channels can also be gated by oxidative stress.

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced and released by the pineal gland in association with the suprachiasmatic

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; ADPR, ADP-ribose; CHO, Chinese Hamster Ovary; DMSO, dimethyl sulfoxide; DRG, dorsal root ganglion; GFP, green fluorescent protein; GSH, glutathione; GSH-Px, glutathione peroxidase; LP, lipid peroxidation; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; TRP, transient receptor potential; TRPM2, transient receptor potential melastatin-like 2.

* Corresponding author at: Department of Biophysic, Medical Faculty, Süleyman Demirel University, Dekanlık Binasi, TR-32260 Isparta, Turkey. Tel.: +90 2462113708; fax: +90 246 2371165.
E-mail addresses: omercelik.vm@gmail.com (Ö. Çelik), mnaziroglu@med.sdu.edu.tr (M. Nazıroğlu).

0031-9384/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.physbeh.2012.09.013

nucleus and peripheral tissues and is considered a potent antioxidant that detoxifies a variety of ROS in many pathophysiological states [13]. Chemically, melatonin and its metabolites can function as endogenous free radical scavengers and broad-spectrum antioxidants [14,15]. Oxidative stress-induced pathophysiologic conditions such as ischemia/reperfusion injury, neuronal excitotoxicity, and chronic inflammation can be a direct cause of cell death but melatonin has been shown to counteract such pathophysologic conditions [13,16,17]. Melatonin has been reported to modulate the L-type voltage gated channelopathies [18,19], as well as apoptosis [15,20]. However, the mechanisms by which melatonin are involved in Ca^{2+} influx is continuing to be explored. We hypothesized that, if melatonin is a potent scavenger, it might prevent or ameliorate the experimental oxidative stress-induced cellular oxidative injury through regulation of TRPM2 channels and Ca^{2+} influx, counteracting the impairment of endogenous antioxidant systems.

The molecular mechanisms by which antioxidants lead to inhibition of TRPM2 Ca^{2+} channels need to be elucidated in detail. Therefore, the present study was aimed at elucidating the role of melatonin in modulation of the effects of H_2O_2-induced oxidative stress on TRPM2 cation channels and to our knowledge, there has been no prior study on the interaction of melatonin and TRPM2 channels in cell lines.

2. Materials and methods

2.1. Cell culture and transfection

CHO cells K1 were obtained from the Cell Culture Bank of Şap Institute (Ankara, Turkey) and cultured in Ham's F12 medium (1×) (Biochrome, Berlin, Germany), supplemented with 10% (v/v) fetal calf serum (Biochrome), and 4 mM L-glutamine (Biochrom, Berlin, Germany). Penicillin (50 U/ml)-streptomycin (50 mg/ml) combination in whole-cell experiments was added to the medium. Cells were seeded on glass cover slips (TPP Cell culture and Labour Technique, Trasadingen, Switzerland) at a density of $<10^3$ cells/mm^2 and grown for 24 h. Subsequently, the pcDNA3-EGFP-TRPM2/TRPM2ΔC expression (5 μg) constructs were transiently transfected into the CHO cells, using the Trans-Fast transfection reagent (7.5 μl; Promega, Mannheim, Germany). As controls, cells were transfected with pcDNA3-enhanced green fluorescent protein (GFP) vector alone. Electrophysiological studies were carried out 24–48 h after transfection in cells visibly positive for enhanced GFP.

2.2. Cell viability (MTT) assay

Cell viability in transfected CHO cells was evaluated by MTT assay based on the ability of viable cells to convert a water-soluble, yellow tetrazolium salt into a water-insoluble, purple formazan product. The enzymatic reduction of the tetrazolium salt happens only in living, metabolically active cells but not in dead cells. Cells were seeded on plates at a density of 1×10^6 cells per well. They were incubated at 37 °C with different doses (0.001, 0.010, 0.10 and 1.0 mM) of H_2O_2 and harvested at different incubation times (30 min, 1 h, 2 h, 5 h, 10 h and 24 h). The medium was removed and MTT (15 μl) was added into each well and then incubated for 45 min at 37 °C in a shaking water bath. The supernatant was discarded and dimethyl sulfoxide (DMSO, 500 μl) was added to dissolve the formazan crystals. Treatments were carried out in duplicate. Absorbance values were measured in a spectrofluorometer cuvette at 490 nm and 650 nm, and expressed as a percentage of the control.

2.3. Electrophysiology

Patch clamp techniques have been described in detail elsewhere [15,16]. The cells were studied with the patch-clamp technique in the whole-cell mode, using an EPC 10 USB equipped with a personal computer with patch-master USB software (HEKA, Lamprecht, Germany). Pipettes were made of borosilicate glass (Sutter Instrument Borosilicate Glass with filament. O.D.: 1.5 mm, I.D. 0.86 mm and 10 cm in length, Novato, CA, USA). The standard extracellular bath solution contained (in mM): 145 NaCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 5 KCl, 10 HEPES, 10 glucose, with the pH adjusted with KOH to 7.4. For Na^+ free solutions, Na^+ was replaced by 150 mM NMDG (*N*-methyl-D-glucamine) and the pH was adjusted with HCl. The osmolarity of the solution was 300 mosmol/l. The pipette solution contained in mM: 145 L-glutamic acid, 8 NaCl, 8 EDTA, 1 $MgCl_2$ and 10 HEPES (pH 7.2) (adjusted with CsOH). The calcium concentration was adjusted to 1 μM.

Cells were held at a potential of −60 mV, and current–voltage (I–V) relations were obtained from voltage ramps from −90 to +60 mV applied over 400 ms. H_2O_2 and melatonin and all other chemicals were obtained from Sigma. Melatonin was dissolved in DMSO, further diluted with PBS (final DMSO concentration <0.2%) and added to the medium for Ca^{2+} signaling and apoptosis experiments or to extracellular or intracellular buffers for electrophysiological experiments at the concentration to be tested, as previously described [20]. After addition of melatonin to standard extracellular bath solution, the pH of the solution was adjusted with KOH to 7.4. The melatonin was added to the cell dishes extracellularly (in the bath). In previous studies, melatonin at concentrations of 0.3–3.0 mM induced protective effects against H_2O_2-induced oxidative stress in cell culture [21,22]. For the present study, cell cultures were incubated with two different doses (0.3 and 1.0 mM) of melatonin for 2 h before H_2O_2 addition because transfer of the antioxidants into the cell is slow [21,22]. In some experiments, melatonin (0.2 mM) was also included in the patch pipette.

2.4. 2-aminoethyldiphenyl borate (2-APB) treatments

TRP channels can be indirectly blocked by certain chemicals. However, for most TRP channels including TRPM2, the range of suitable pharmacological modulators is limited. Recently, 2-aminoethyl diphenylborinate (2-APB) was described as a TRPM8 channel blocker in addition to its actions as an inositol 1,4,5-triphosphatase ($InsP_3$) receptor antagonist [23]. Thus, oxidative stress-induced Ca^{2+} influx in the TRPM2-transfected CHO cells might also be modulated by 2-APB. To test this idea, some cells in the Ca^{2+} signaling experiments were pre-incubated with 2-APB (0.05 mM) for 1 min [11].

Stock 2-APB was dissolved in DMSO and stored at −33 °C. Before the patch-clamp and Ca^{2+} signaling experiments, 2-APB in extracellular bath solutions was diluted to reach the required final concentrations. All experiments were carried out at room temperature (approx 22 °C). After addition of 2-APB to standard extracellular bath solution, the pH was adjusted to 7.4 with KOH. The 2-APB was added to the patch-chamber (in the bath).

2.5. Measurement of intracellular free calcium concentration ($[Ca^{2+}]_i$)

The CHO cells at a density of 1×10^5 cells per ml were loaded with 4 μM fura-2/AM in loading buffer and incubated for 45 min at 37 °C in the dark, then washed twice with phosphate buffer and incubated for an additional 30 min at 37 °C to complete probe de-esterification. The cells were then re-suspended in loading buffer at a density of 1×10^5 cells per ml according to a procedure published elsewhere [24,25]. The six experimental groups were exposed to H_2O_2 for stimulation of Ca^{2+} release and entry. Fluorescence was recorded from 2 ml aliquots of magnetically stirred cellular suspension at 37 °C by using a spectrofluorometer (Carry Eclipsys, Varian Inc, Sydney, Australia) with excitation wavelengths of 340 and 380 nm and emission at 505 nm. Changes in $[Ca^{2+}]_i$ were monitored by using the fura-2 340/380 nm fluorescence ratio and were calibrated according to the method of Grynkiewicz et al. [25].

Ca^{2+} mobilization in the CHO cells was estimated using the integral of the rise in $[Ca^{2+}]_i$ for 150 s after addition of H_2O_2 [26].

Download English Version:

https://daneshyari.com/en/article/5925191

Download Persian Version:

https://daneshyari.com/article/5925191

Daneshyari.com