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Melatonin affects voltage-dependent calcium and potassium currents in MCF-7 cell line cultured either in growth or differentiation medium

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ABSTRACT

Big efforts have been dedicated up to now to identify novel targets for cancer treatment. The peculiar biophysical profile and the atypical ionic channels activity shown by diverse types of human cancers suggest that ion channels may be possible targets in cancer therapy. Earlier studies have shown that melatonin exerts an oncostatic action on different tumors. In particular, it was shown that melatonin was able to inhibit growth/viability and proliferation, to reduce the invasiveness and metastatic properties of human estrogen-sensitive breast adenocarcinoma MCF-7 cell line cultured in growth medium, with substantial impairments of epidermal growth factor (EGF) and Notch-1-mediated signaling. The purpose of this work was to evaluate on MCF-7 cells the possible effects of melatonin on the biophysical features known to have a role in proliferation and differentiation, by using the patch-clamp technique. Our results show that in cells cultured in growth as well as in differentiation medium melatonin caused a hyperpolarization of resting membrane potential paralleled by significant changes of the inward Ca^{2+} currents (T- and L-type), outward delayed rectifier K^+ currents and cell capacitance. All these effects are involved in MCF-7 growth and differentiation. These findings strongly suggest that melatonin, acting as a modulator of different voltage-dependent ion channels, might be considered a new promising tool for specifically disrupting cell viability and differentiation pathways in tumour cells with possible beneficial effects on cancer therapy.

1. Introduction

Conventional chemotherapy is not always successful and usually accompanied by systemic toxicity and detrimental side effects. Thus, growing attempts are made to identify novel targets for cancer therapy.

The diverse types of human cancers may show a peculiar biophysical profile with an atypical ion channels activity (Abdul et al., 2003; Arcangeli et al., 2009). In such perspective, it might be worth to better characterize this issue with the aim of designing efficient ionic channel inhibitors/modulators that may potentially affect cancer therapy. Functional relationship exists between resting membrane potential, K^+ channel type expression and cell functions such as proliferation and differentiation (Asher et al., 2010; Cos and Sánchez-Barceló, 2003; Enomoto et al., 1986; Wang, 2004). Resting membrane potential is typically more depolarized in undifferentiated respect to differentiated cells (O'Grady and Lee, 2005; Pardo, 2004) and in cancer cells respect to terminally differentiated cells (Kunzelmann, 2005). Of interest, resting membrane potential and the type and amount of K^+ and Ca^{2+} currents change during both cell cycle and differentiation (Blackiston et al., 2009; Sundelacruz et al., 2009) and are atypically modified in cancer cells (Becchetti, 2011; Bielanska et al., 2009; Haren et al., 2010; Lang et al., 2003; Ohkubo and Yamazaki, 2012; Ouadid-Ahidouch and Ahidouch, 2008).

By multidisciplinary approach, it was demonstrated on MCF-7 cell line (Margheri et al., 2012) that growth/viability and proliferation is significantly reduced by the treatment with melatonin, the hormone mostly produced by the pineal gland (Jablonska et al., 2013).

Moreover, preclinical in vitro and in vivo studies and clinical trials showed that melatonin was able to exert an oncostatic action on different tumors (Girgert et al., 2009; Hill et al., 2009, 2011a,b; Jung and Ahmad, 2006; Sánchez-Barceló et al., 2012; Srinivasan et al., 2008), to increase the efficacy of chemotherapy when used

Abbreviations: α-DTX, α-dendrotoxin; Chr, chromanol; HP, holding potential; IbTx, iberiotoxin; I_{BK}, voltage- and Ca^{2+}-dependent delayed-rectifier iberiotoxin-sensitive K^+ current; IKCa, intermediate-conductance Ca^{2+}-activated K^+ channels; $I_{Ca,L}$, L-type Ca^{2+} current; $I_{Ca,T}$, T-type Ca^{2+} current; I_{Ks}, slowly activating chromanol-sensitive K^+ current; $I_{K(V)}$, fast activating α-dendrotoxin-sensitive K^+ current; MLT, melatonin; MT1, melatonin receptor 1; MTS, 3-(4.5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium assay; RMP, resting membrane potential

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in adjuvant settings and to decrease the side effects (Vijayalaxmi et al., 2002). Since proliferation and differentiation are typical of any cell system and are carefully regulated in the course of physiological development and life cycle, tumor evolution must imply a deregulation of their morphofunctional features.

In the present study, we evaluated the electrophysiological properties and the effects of melatonin on MCF-7 cells cultured in either growth or differentiation medium focusing our attention on ion channels as possible novel targets involved in cancer pathophysiology. In particular, by electrophysiological technique, we evaluated the action of melatonin on resting membrane potential and on voltage-dependent ionic channels known to play a role in proliferation (Baglioni et al., 2012; Bertolesi et al., 2002; Currò, 2014; Enomoto et al., 1986; Gamper et al., 2002; Girgert et al., 2009; Grant et al., 2009; Gray et al., 2004; Haren et al., 2010; Kunzelmann, 2005).

The rationale for this research originates from the evidence that several ionic channels have a key role in proliferation and differentiation and can be therefore considered as new targets for cancer therapy (Arcangeli et al., 2009).

2. Materials and methods

2.1. Cell culture and treatments

Human hormone-sensitive breast adenocarcinoma cell line MCF-7 was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured as previously described (Margheri et al., 2012). Briefly, cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma, Milan, Italy) supplemented with 10% Fetal Bovine Serum (FBS, Sigma), 100 U/mL penicillin–streptomycin and 1% L-glutamine 200 mM (Sigma) in a humidified 5% CO_2 atmosphere at 37 °C. This medium was suitable to allow cells growth/proliferation. In all the experiments melatonin 100 μM (Sigma) was used. This dose was proved to be the most effective to reduce cell viability and proliferation in this experimental model (Margheri et al., 2012). MCF-7 cells were cultured in growth medium in the presence of 100 μM melatonin for 24, 48, 72 h. In parallel experiments, the cells were cultured for 72 h in growth medium in the presence of melatonin and/or the following channel blockers (Sigma): nifedipine (10 μM) and $NiCl_2$ (50 μM) for L- and T-type Ca^{2+} channels; α-dendrotoxin, α-DTX (10 nM) for $K_{(V)}$, iberiotoxin, IbTx (100 nM) for BK and chromanol, Chr (50 μM) for K_s channels. The treatments lasted up to 72 h and the media were daily replaced. In other experiments the cells were cultured for 72 h in differentiation medium (supplemented with 2% FBS) and treated with channel blockers as above described.

2.2. Cell growth/viability assay (MTS)

Cell growth/viability was evaluated by 3-(4.5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium (MTS) assay (Promega, Madison, WI, USA). MCF-7 cells were plated into 96-multiwell-plates and then treated with melatonin 100 μM and/or the above-mentioned channel blockers for 72 h. Then, the cells were shifted in 100 μl of fresh medium phenol red-free and 20 μl of MTS test solution was added to each well. After 4 h of incubation, the color reaction was measured using a multiwell scanning spectrophotometer (Enzyme-Linked ImmunoSorbent Assay-ELISA- reader) (Amersham, Pharmacia Biotech, Cambridge, UK) at a wavelength of 490 nm. The values were expressed as mean ± S.D. obtained from five independent experiments carried out in triplicates.

In some experiments MCF-7 were treated with melatonin for 72 h in growth medium and, in a parallel set of experiments, in differentiation medium and treated with different channel blockers.

2.3. Confocal immunofluorescence

To detect proliferation activity, untreated and treated MCF-7 cells grown on glass coverslips were fixed with 0.5% buffered paraformaldehyde for 10 min at room temperature. After permeabilization with cold acetone for 3 min, the fixed cells were blocked with 0.5% bovine serum albumin (BSA; Sigma) and 3% glycerol in PBS for 20 min and then immunostained with rabbit polyclonal anti-Ki-67 (1:100; Abcam, Cambridge, UK). The immunoreaction was revealed by incubation with specific anti-rabbit Alexa Fluor 488-conjugated IgG (1:200; Molecular Probes, Eugene, OR, USA), for 1 h at room temperature. After washing in PBS, the sections were mounted with an anti-fade mounting medium (Biomeda Gel mount, Electron Microscopy Sciences, Foster City, CA, USA). A negative control was performed by replacing the primary antibody with non-immune mouse serum. Sections were examined with a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystem, Mannheim, DE) equipped with a HeNe/Argon laser source for fluorescence measurements. Fluorescence was collected using a Leica PlanApo x63 oil-immersion objective. Optical sections (1024 × 1024 pixels) at intervals of 0.8 μm were obtained and superimposed to create a single composite image. When needed, a single optical fluorescent section and DIC images were merged to view the precise distribution of the immunostaining. Quantification of Ki-67 expression was performed on digitized images by counting the number of positive cells over the total cell number.

2.4. Confocal analysis of intracellular Ca^{2+} concentration

The analysis of $[Ca^{2+}]_i$ was performed as previously reported (Formigli et al., 2002). Briefly, to reveal the resting intracellular calcium concentration in MCF-7 cells in the absence or presence of melatonin (100 μM), ~2×10^4 cells were plated on glass coverslips and incubated at 37 °C for 10 min in DMEM with Fluo 3-acetoxymethyl ester (Molecular Probes) as fluorescent Ca^{2+} indicator at a final concentration of 10 μM and 0.1% anhydrous dimethyl sulfoxide and Pluronic F-127 (0.01% wt/vol) as dispersing agent (Molecular Probes). After being washed, the cells were placed in open slide flow-loading chambers mounted on the stage of the confocal laser scanning microscope. Optical sections (1024 × 1024 pixels) at intervals of 0.8 μm were obtained. A variable number of cells ranging from 15 to 30 were analysed for each cell preparation. Multiple regions of interest (ROIs) of 25 μm^2 were selected within the cells to monitor Ca^{2+} signals, and outside the cells as baseline. Fluorescence signals were expressed as fractional changes above the resting baseline, $\Delta F/F$, where F was the averaged baseline fluorescence and ΔF represented the fluorescence changes from the baseline.

2.5. Electrophysiological experiments

The electrophysiological characteristics of different voltage-gated ionic channels of cultured MCF-7 were investigated on glass coverslip-adherent single cells at room temperature (20–23 °C) by the whole-cell patch-clamp technique (Baglioni et al., 2009; Margheri et al., 2012); both voltage-clamp and current-clamp modes were used.

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