

IN VITRO EFFECTS OF SOME BIO-INDOLES ON THE TRANSMEMBRANE POTENTIAL OF JURKAT E 6.1 LIMPHOBLASTS

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Abstract

In this paper we proposed to research the biochemical mechanisms of the bio-compounds with indolic structure, by investigating their biochemical effects on one very important physico-chemical parameter of the cell membrane, namely the (trans)membrane potential.

The aim of the study was the assessment of the membrane depolarization-repolarization processes induced by some bio-indoles, namely melatonin, serotonin, and tryptophan. To this effect we performed a spectrofluorimetric study using as experimental model the Jurkat E 6.1 lymphoblast cell line and the voltage-sensitive probe bis-(1,3 dibutylbarbituric acid) trimethine oxonol (DiBAC₄(3)). The obtained results showed that all the studied bio-indoles have a cell membrane hyperpolarization effect.

Rezumat

În această lucrare ne-am propus cercetarea mecanismelor biochimice de acțiune ale unor bio-compuși cu nucleu indolic, prin investigarea efectelor acestora asupra unui parametru fizico-chimic extrem de important al membranei celulare, și anume potențialul (trans)membranar.

Scopul acestui studiu a fost evaluarea fenomenelor de depolarizare-repolarizare a membranei celulare induse de unii bio-indoli, și anume: melatonina, serotonină și triptofanul. S-a realizat în acest scop un studiu spectrofluorimetric utilizând ca model experimental linia celulară limfoblastică Jurkat E 6.1 și sonda voltaj-sensibilă bis-(1,3 acid dibutilbarbituric) trimetin oxonol (DiBAC₄(3)). Rezultatele obținute au arătat că toți bio-indolii studiați conduc la hiperpolarizarea membranei celulare.

Keywords: bio-indoles, melatonin, Jurkat E 6.1, transmembrane potential, spectrofluorimetry, bis-oxonols

Introduction

The difference between ions distribution on both sides of cell membranes and organelles generates an electrical asymmetry that can be

outlined as an electric potential difference between the two sides of the cell membrane, called transmembrane potential (biophysical difference that is in the range of tens of millivolts) [5,12].

The transmembrane potential plays an important role in the transport processes between the extra and intracellular environment. The passive transport of ions through biological membranes is determined, on one hand, by simple diffusion phenomenon, and on the other hand, by the transmembrane potential difference, which imparts a drift movement among the ions.

What really constitutes a specific characteristic of living cells is the transport of substances through the membrane against ion concentration gradients, electric potential or osmotic pressure, the so-called *active transport*. The active transport is possible only by impulsing molecules transferred by cellular metabolism, by biochemical reactions resulting in the transfer of free energy[8,14,17].

Primary active ion transport can be achieved through specific mechanisms of the living cell, that received the generic name of *ionic pumps*. Ion pumps ensure that there is an active flow of ion, that counterbalance the effect of passive fluxes (due to concentration gradients and / or electric field).

Increases and decreases (in absolute value) of the transmembrane potential, designated as *hyperpolarization* and *depolarization*, respectively, play an important role in many physiological processes, including the nervous impulse propagation, muscle contraction, cell signaling and control of ionic channels [7,13,18]. In this respect, endogen compounds with indolic structure (mainly melatonin, serotonin and tryptophan) are factors that influence these processes. Recent studies in this field of research evaluated the influence of endogen bio-indoles on the electrostatic cell balance[14].

Therefore, this paper assessed the influence of melatonin, serotonin and tryptophan on cells membrane polarization. Thus, we used an *in vitro* experimental model and voltage-sensitive fluorescent probes in a steady-state fluorescence experiment[10].

Materials and Methods

The experimental research was carried out within the Biochemistry Department, of the Faculty of Pharmacy, Bucharest, Romania, using as experimental model the Jurkat E6.1 lymphoblastoma cell line, primary and secondary culture obtained at the “Victor Babeş” National Institute of Research and Development in the Field of Pathology and Biomedical

Sciences, Bucharest. The culture medium used for obtaining the cellular suspensions was RPMI-1640 supplemented with L-glutamine. There were obtained:

- a) the primary culture: $3-9 \times 10^5$ cells/mL in RPMI-1640 culture medium supplemented with fetal bovine serum (FBS) (10%), 2 mM L-glutamine; the culture was maintained for 3 days at 37°C, in controlled atmosphere (95% air, 5% CO₂);
- b) the secondary culture: was obtained by replacing the culture medium and the cells resuspension in RPMI- 1640, to a density of 10^5 viable cells/mL.

Reagents: RPMI 1640 medium was purchased from Promega, the fluorescent probe ((DiBAC₄(3))) was obtained from Molecular Probes; Melatonin, Serotonin, Tryptophan were purchased from Sigma-Aldrich.

Fluorescent probes: for evaluating the membrane potential changes it was used a slow-response potential-sensitive probe from the bis-oxonol class: bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC₄(3)), appropriate to be used with non-excitabile cells and for evaluating very small potential differences in the range of millivolts. We obtained a 2.5 mM stock solution in dimethylsulfoxide (DMSO), maintained between experiments at -20 °C.

Chemical stimuli: melatonin, serotonin and tryptophan solutions of the following concentrations: 1µM, 2,5µM, 5µM, 10µM, 20µM, 25µM, prepared in the culture medium RPMI-1640.

Equipment: LS50 B spectrofluorimeter Perkin Elmer, equipped with thermostated cell holder and magnetic stirring.

Transmembrane potential evaluation

1. Evaluation of basal membrane potential of Jurkat cell type

For assessing the potential changes under the influence of the chemical stimuli it was used a slow-response potential-sensitive probe from the bis-oxonol class: DiBAC₄(3).

In this respect, 2 µL of DiBAC₄(3) stock solution were added to 2 mL of the cell suspension and the cells were incubated with the probe at 37° C, under continuous magnetic stirring for 2 minutes, in order to allow the fluorescent probe to bind to the cell membrane. The fluorescence intensity of the cell suspension was recorded at 493 nm excitation wavelength, in the 500-600 nm range, excitation and emission slits of 5 nm, the spectrum scanning speed of 500 nm/min. Cell membrane depolarization has as a major effect the influx of the anionic probe, and consequently an increase of the fluorescent signal, while the hyperpolarization process determines a

decrease of the fluorescent signal, due to the probe efflux (out of the cell) through transmembrane ionic pumps.

2. *Cells stimulation with solutions of melatonin, serotonin, and tryptophan, with the concentration 1 μ M, 2.5 μ M, 5 μ M, 10 μ M, 20 μ M, 25 μ M*

In order to evaluate the effect of the bio-indoles, the cell suspension was incubated with 2 μ L of appropriate concentrations of indoles solutions to obtain the above mentioned stimuli concentration, for 20 minutes. After 20 minutes, the emission intensity of the cell suspension was registered again, under the same conditions, and changes in the membrane potential of Jurkat lymphoblastic cells were assessed.

Statistical analysis

Each sample was assessed 10 times, so the final value of each sample is the mean $\pm$ SD of 10 determinations. Statistical significance was determined using the SPSS software. Significance was set at $p \leq 0.05$.

Results and Discussion

Table I presents the experimental absolute fluorescence intensities, expressed as relative fluorescence units, analytical parameters that directly express the transmembrane potential dynamics.

Table I

The dynamics of the membrane potential under the effect of the studied bio-indoles

Indoles concentration	The transmembrane potential after melatonin stimulation	The transmembrane potential after serotonin stimulation	The transmembrane potential after tryptophan stimulation
0 μ M (without stimuli)	198.8 $\pm$ 7.41	196.7 $\pm$ 8.07	198.13 $\pm$ 8.26
1 μ M	152.5 $\pm$ 9.28	131.4 $\pm$ 7.96	180.13 $\pm$ 10.22
2.5 μ M	150.5 $\pm$ 9.11	132.05 $\pm$ 7.41	182.14 $\pm$ 10.58
5 μ M	152.3 $\pm$ 9.56	134.1 $\pm$ 7.39	181.6 $\pm$ 10.72
10 μ M	150.58 $\pm$ 8.48	131.6 $\pm$ 7.82	180.53 $\pm$ 10.95
20 μ M	155.65 $\pm$ 9.73	133.54 $\pm$ 7.17	182.08 $\pm$ 11.20
25 μ M	153.4 $\pm$ 9.04	132.6 $\pm$ 7.38	181.92 $\pm$ 10.73

The values presented in table I are plotted in figure 1.

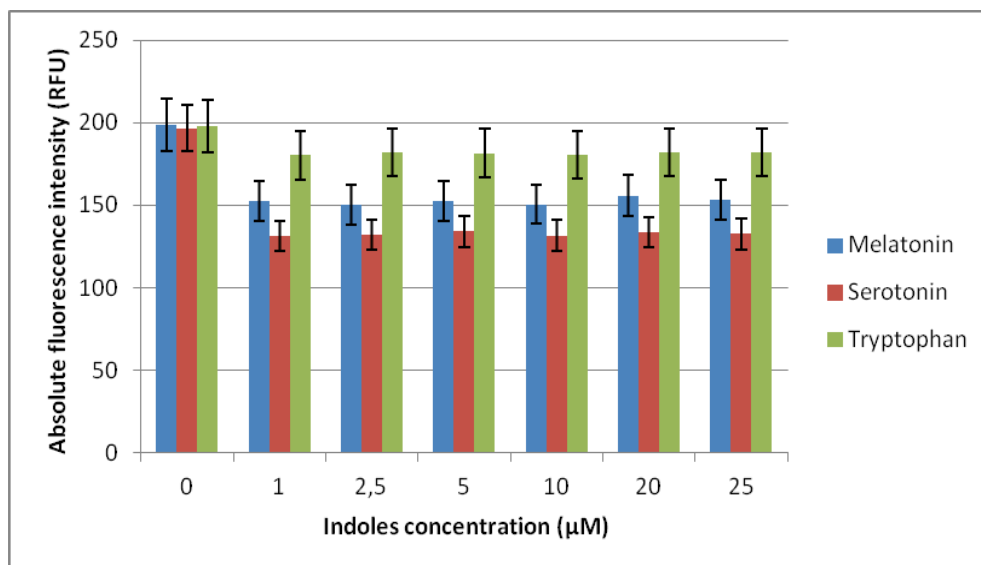


Figure 1

Comparative influence of three studied indolic biomolecules on the dynamics of the transmembrane potential

Analyzing the data obtained one can see that the three studied bio-indoles (melatonin, serotonin, tryptophan) induced a decrease in the fluorescence intensity regardless of the used dose (1 μM, 2.5 μM, 5 μM, 10 μM, 20 μM, 25 μM). The decrease in the fluorescence intensity of cell membranes labeled with the fluorescent probe DIBAC₄(3) represents a membrane hyperpolarization, as the higher the potential difference between the inner and outer cell membrane, the lesser fluorescent probe is bound to the membrane and, consequently, the smaller the fluorescent signal becomes [9]. The hyperpolarizing effect is not dose-dependent, but constant and stable for the three studied bio-indoles.

In addition, experimental results show an interesting dynamics of the fluorescence intensity, depending on the used indole (melatonin, serotonin, tryptophan).

Thus, the lowest values of fluorescence intensity (the strongest hyperpolarization) were obtained for serotonin and melatonin ($p < 0.001$ serotonin vs melatonin, and $p < 0.001$ melatonin vs tryptophan), for all studied doses (1 μM, 2.5 μM, 5 μM, 10 μM, 20 μM, 25 μM).

The lowest hyperpolarizant effect occurred under the action of tryptophan (the highest values of fluorescence intensity using the fluorescent probe DIBAC₄(3)) ($p < 0.001$ serotonin vs tryptophan).

In order to depict the hyperpolarization process, we presented in figure 2 some representative fluorescence spectra of Jurkat cells before and after incubation with melatonin solutions, in the presence of potential-sensitive probe DiBAC₄(3).

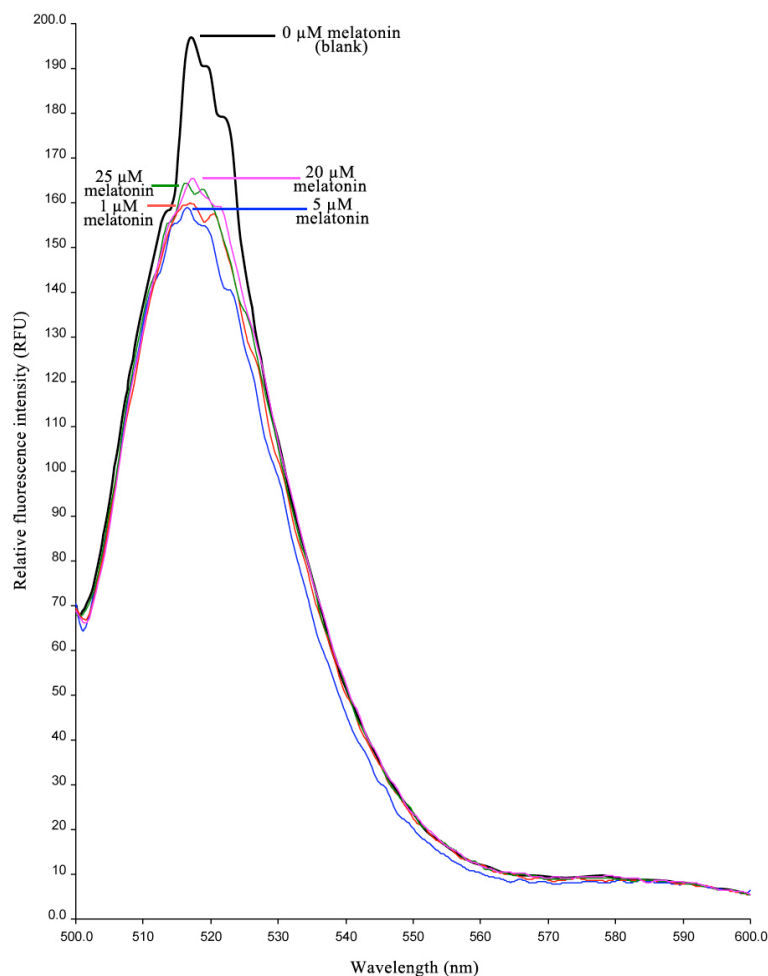


Figure 2

Fluorescence spectra of Jurkat cells before (cells incubated with DiBAC₄(3) only) and after incubation with melatonin solutions in the presence of the specific potential probe DiBAC₄(3).

A possible explanation for this hyperpolarization phenomenon can be found by correlating the experimental results obtained in the present study, with some observations in the scientific literature[1,2,3,4,11,16].

Mant and Elliot from Southampton University, England, published the first predictive results regarding the phosphorylation ("phosphorylation prediction") of the intracytoplasmatic domain of the K^+ channels [15]. They have demonstrated for the first time (in cell culture studies) that protein kinase A (PKA) phosphorylates the serine terminal residue of the polypeptidic intracytoplasmatic domain of the K^+ channels.

On the other hand, Drummond and Benson from Friedrich Miescher Institute, Basel, Switzerland, conducted *in vitro* studies on the mechanism of action of serotonin at the ganglia level [6]. This team found that serotonin and cAMP analogues produce a similar increase in K^+ channel conductance. The conclusion of their study was that there may be a biochemical link among serotonin - cAMP - K^+ channels.

Based on the obtained experimental results and from these literature data, a very interesting hypothesis might be issued regarding the mechanism of membrane hyperpolarization induced by serotonin and melatonin.

Thus, serotonin (through its receptors $5HT_1$, $5HT_4$, $5HT_5$, $5HT_6$, $5HT_7$) and melatonin (by its receptors MT_1 and MT_2) have, as effector system at the cellular level, the adenylate cyclase (AC) system. The activity of this enzyme determines the conversion of ATP to cAMP, which activates protein kinase A by fixing on its regulatory subunits (enzyme therefore dependent on the intracellular concentration of cAMP), and this one phosphorylates the serine terminal residue of the polypeptidic intracytoplasmatic domain of the K^+ channels.

Conclusions

The transmembrane potential is a biophysical parameter of great importance for cell physiology. Muscle contraction (including the heart muscle), nerve propagation, cell signaling, are just some examples of physiological processes dependent on the membrane potential.

From this point of view, cellular hyperpolarization, or a subsequent repolarization after depolarization, thus restoring transmembrane potential difference during the vital processes, are crucial for the proper functioning of the living cell. Also, any electrostatic imbalance of the cell can be the basis of very serious diseases (cardiovascular diseases).

The present experimental study demonstrated the positive (hyperpolarization) effect of melatonin, serotonin and tryptophan on the electric properties of the cell membranes. All studied bio-indoles induced the membrane hyperpolarization, with best results for serotonin and melatonin. A proposed mechanism for this effect suggests that melatonin

and serotonin, as effector systems at the cellular level, determine the conversion of ATP to cAMP, followed by the activation of protein kinase A, which phosphorylates the serine terminal residue of the polypeptidic intracytoplasmatic domain of K⁺ channels.

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