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Melatonin effects on megakaryocyte membrane patch-clamp outward K^+ current

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: This study was carried out to evaluate the influence of melatonin concentration on rat bone marrow megakaryocyte outward K^+ current and its implications with regard to platelet production. It is the Authors' view that the greatly extended development of megakaryocyte membrane, together with its ion channels, makes the choice of this topic particularly pertinent.

Material/Methods: Megakaryocytes from fresh Wistar rat bone marrow were clampaged with patch–clamp technique and the examination of membrane outward current was performed when melatonin dissolved in external or internal standard solution was used.

Results: On the basis of this study, melatonin does reduce outward K^+ current intensity, the more the higher the melatonin concentration. In quantitative terms, whereas no change is noticed when dissolved melatonin concentration in external standard solution is smaller than 25 μM, at 50 μM only a delayed outward current decrease appears. The effect on the outward current intensity is reversible at least until melatonin concentration reaches 500 μM; when melatonin concentration is higher than 1000 μM the effect is demonstrably irreversible. The presence on the megakaryocyte membrane of internal standard melatonin solution does reduce the outward current even more sharply.

Conclusion: There seems to be a positive correlation between cationic pump and megakaryocyte intimate processes of platelet production.

key words: **melatonin • megakaryocyte • patch–clamp • outward K^+ current • thrombocytogenesis**

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BACKGROUND

Histochemical platelet (PLT) origin is confirmed not only by histomorphological criteria but also by electrophysiological outward current means [1]. We have here analyzed the influence of melatonin (MLT) on the outward K^+ current of megakaryocyte (MEG) membrane.

MLT is synthesized in pineal gland from L-tryptophan, that is hydroxylated and decarboxylated to serotonin (5-HT). N-acetyltransferase (NAT) converts 5-HT to N-acetylserotonin, by transferring acetyl group from acetyl CoA to serotonin-NH2; lastly N-acetylserotonin is methylated to MLT by hydroxyindole-O-methyltransferase (HIOMT) [2].

MLT is implicated in the MEG membrane evolution as well as in the demarcation membrane system (DMS) [3]; PLT formation is indeed influenced by microtubular systems, which are activated by 5-HT [4]. In several human haemathopaties an abnormal PLT microtubular system has been observed [5] as well as voltage-gate K^+ currents changes in the course of human MEG development [6].

It could then be concluded that MLT is closely linked to MEG outward current, thus promoting PLT production.

MATERIAL AND METHODS

Cell preparation

Fresh Wistar rat bone marrow was suspended in a Ca^{2+}-free solution (NaCl 135 mM; KCl 5 mM; $MgCl_2$ 1 mM; D-Glucose 10 mM; Hepes 10 mM; pH 7.3) [7] and stored at 4°C for at least 4 hours before the experiment. A fragment was then removed and dismembered into the external standard solution (NaCl 133 mM; KCl 5 mM; $CaCl_2$ 2 mM; $MgCl_2$ 1 mM; D-Glucose 10 mM; Hepes 10 mM; pH 7.3) on the inside space of the recording chamber where, in advance, a cell-tak® adhesive polypeptide (Bedford, MA) was coated. The length of time between this operation and the acquisition of whole-cell membrane current is no more than five hours.

Patch-clamp procedure

The clampage was performed on whole-cell at –60 mV and voltage protocols (the membrane was depolarized in 10 mV steps from –60 to +50 mV; 180 ms pulse duration) were monitored by an oscilloscope HM 204-2 (Germany), recorded with a patch-clamp amplifier (Axopatch model 1D, Axon Instrument, U.S.A.) and analyzed by a Pentium computer equipped with a digital Digidata 1200 acquisition system and pClamp software (Axon Instrument, U.S.A.). Signal filtering and digitization were adjusted according to the specific features of the membrane currents. Patch pipettes were obtained by pulling 1.5 mm capillary glass (Baxter, U.S.A.) in a two-stage vertical puller (Narishige, Japan). The pipette resistance, when filled with the internal standard solution (KCl 150 mM; $MgCl_2$ 1 mM; Hepes 10 mM; pH 7.2), was usually 2÷6 MΩ. The polar silver electrode (–50÷+50mV), put into a patch-pipette filled with internal standard solution, was then applied on MEG membrane through the movable stage of an inverted microscope (Leitz DM IL, Leica Microscope, Wetzlar-Germany). External and internal standard solutions were titrated with NaOH and KOH 1M respectively. The osmolalities of the external and internal salines were measured by an automatic micro-osmometer using freezing-point depression (Roebling, Mestechnik, Berlin, Germany) and their values were 282±3.1 (mean±S.D.; n=21) and 286±4.2 (n=11) mOsm/Kg H_2O respectively. The experiments were performed at room temperature.

Solutions and reagents

MLT (Sigma-Aldrich, Milan, Italy) was dissolved in the external or internal standard solution just before use. Various concentrations (5-10-25-50-100-500-1000 and 2500 μM) of MLT in the external and (5-25-100–500 μM) in the internal standard solution were used. The gravity-fed volume of external standard solution was controlled by a six-valve multisolenoid (General Valve Corp, Fairfild, NJ) and introduced through a common inlet into the recording chamber. With this technique, the solution surrounding a MEG could be normally exchanged within 20 sec. The external standard melatonin solution was subjected to perfusion for a time between 30 and 300 seconds and then washed out with the same solution. The MLT dissolved in the internal standard solution was placed into the patch-pipette by means of a microsyringe.

RESULTS

Megakaryocytes and relative membrane properties

The mean diameter of our MEG was 40±9 m (n=50). After membrane breakage, the mean resting potential (V_0) ranged from –17 to –65 mV (–35±15; n=50) and

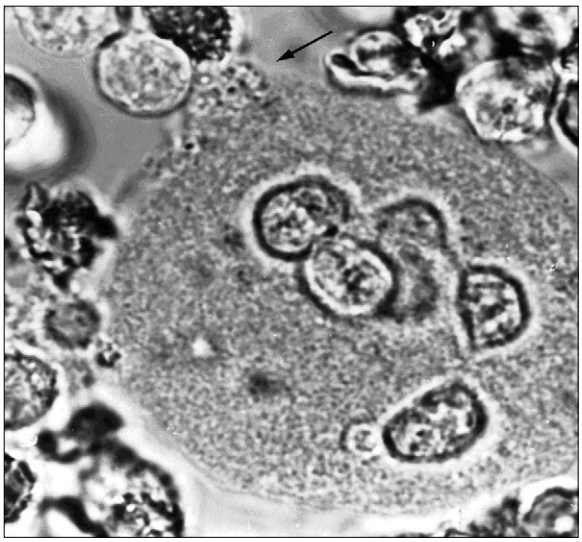

Figure 1. Megakaryocyte in melatonin solution (1200x). A remarkable granulated cytoplasmic lump is evident; melatonin causes a progressive increase of cytoplasm granulations and the megakaryocyte membrane becomes more and more thin.

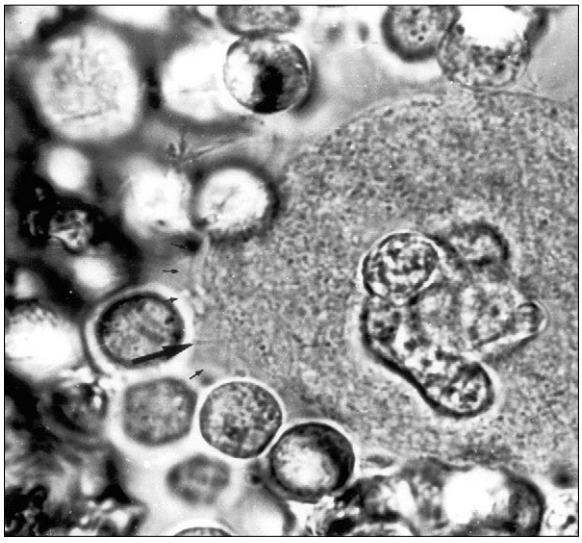

Figure 2. Megakaryocyte in melatonin solution (1200x). Afterwards, any trace of membrane seems to disappear to be replaced by platelets; the arrows point at such phenomenon.

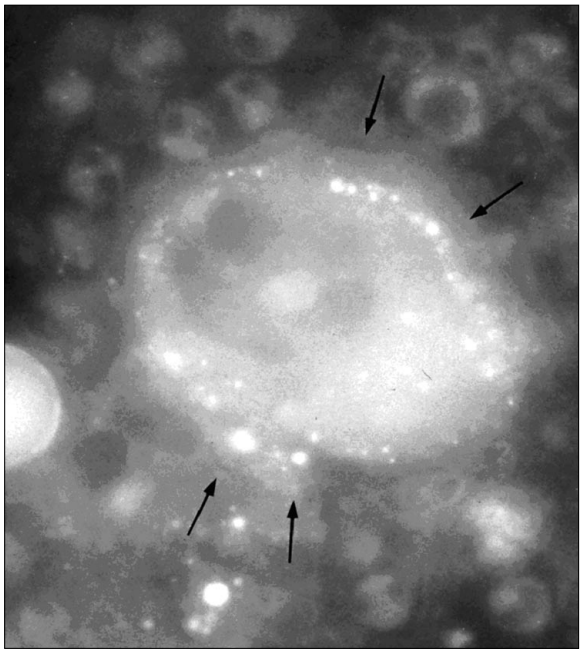

Figure 3. Megakaryocyte after perfusion with melatonin and NAT-inhibitor solution (1200x). Many fluorescent (510 nm) platelets appear on the surface of megakaryocyte membrane; the HIOMT-inhibitor seems to be less active on melatonin-induced thrombocytogenesis than NAT-inhibitor; a less intense fluorescence involves the probable nucleus area.

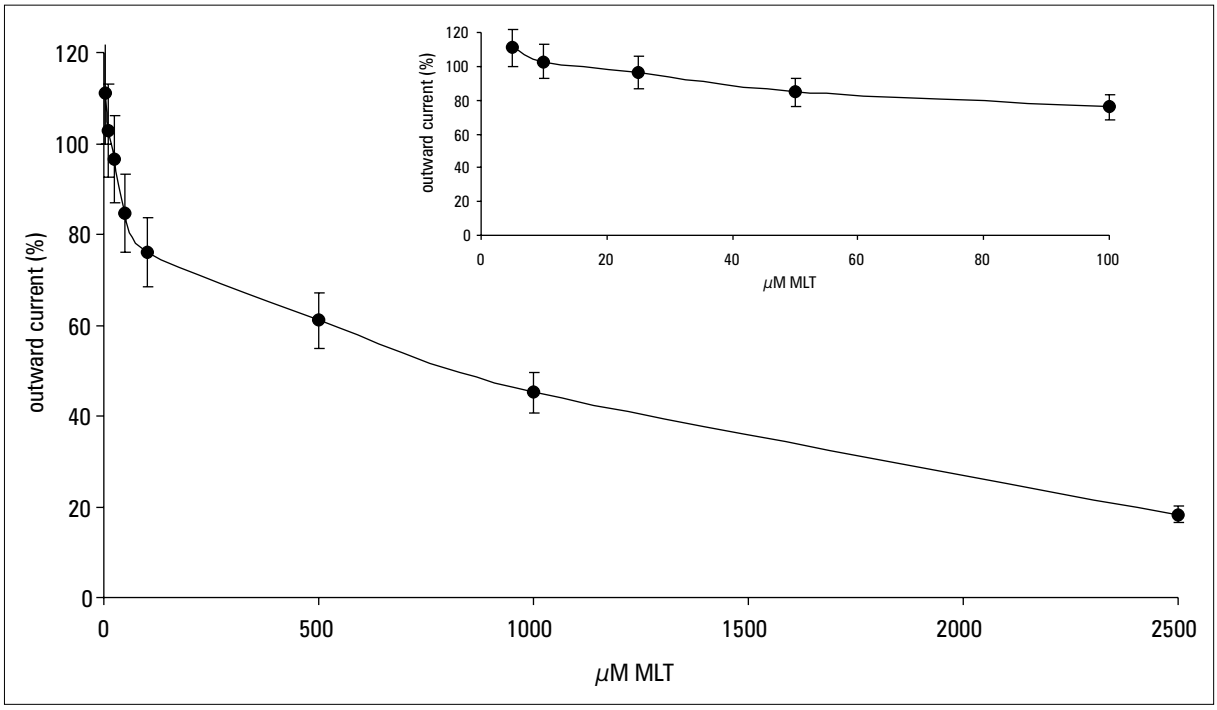

Figure 4. Experimental data report the outward current behaviour, after 2 minutes perfusion, when different external standard melatonin concentrations (5-10-25-50-100-500-1000-2500 μM) are used. Top right – magnified detail of figure 4 (mean±s.d.; n=5). The outward current does not change at melatonin concentration lower than 25 μM, afterwards the outward current decreases so more so more concentrated is melatonin solution.

the input resistance measured 0.6 to 2.6 GΩ (1.6±1.2; n=50).

Voltage-gate K^+ current

Outward K^+ current, as noticed in preliminary observations, was negatively affected by externally applied 6 mM TBA (tetrabutylammonium chloride hydrate) (Sigma-Aldrich, Milan, Italy) or only partially by 50 mM TEA (tetraethylammonium chloride) (Sigma-Aldrich) solution. The outward K^+ current could be also moderately reduced by external application of 10 mM $CoCl_2$ even if no change in K^+ current occurred. Yet no influence seems to exist relative to Ca^{2+}-mediate K^+ conductance [8].

Melatonin influence

MLT induced, in a fresh bone marrow suspension, granulation appearance over MEG membrane, followed by a final PLT production (Fig. 1,2) [9]. Same images are observed at scanning electron microscope. PLT fluorescence may be induced to become more intense by MLT together with NAT-inhibitor (4-1naphtylvinil-pyridine) (Fig. 3) rather than by MLT plus HIOMT-inhibitor (S-adenosyl-L-cysteine) [10,11].

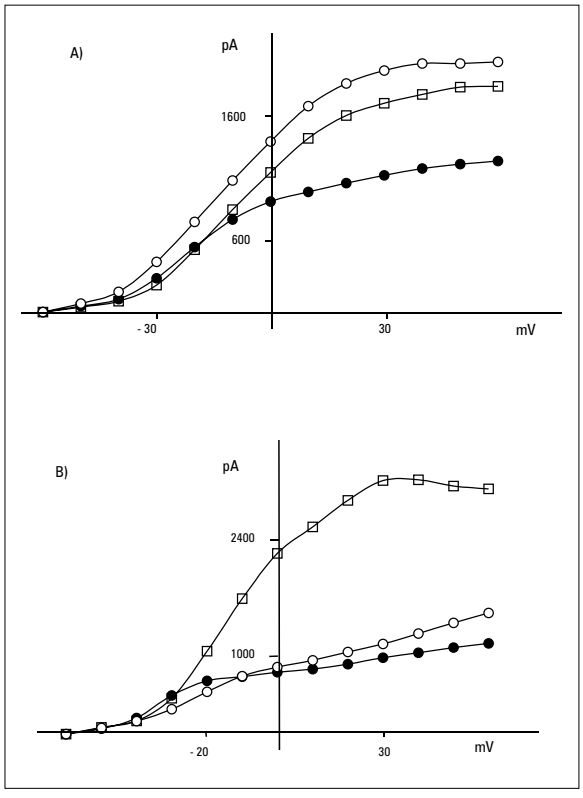

Figure 5. Current-voltage (I/V) relationships before (□), during 120' application of melatonin dissolved in external standard solution (●) and after washout (○). The current-voltage reversibly declines at 500 μM (A) or lower melatonin concentration, at 1000 μM (B) or higher the current-voltage declines irreversibly.

In this study MLT dissolved in external or internal standard solutions reduced MEG membrane outward K^+ current. MEG membrane outward current did not change when MLT concentration was reduced to less than 25 μM; on the contrary, the higher the MLT concentration the greater the outward current intensity reduction (Fig. 4).

At 50 μM MLT only a delayed outward current decrease appeared; at 100 μM MLT the outward current declined more precociously and more slowly; at 500 μM the outward current reduction was more intense and reversible; at 1000 μM MLT the outward current intensity reduction could no longer be reversed (Fig. 5).

The influence of MLT concentration on the outward current intensity appeared earlier and was more intense when MLT was dissolved in patch-pipette internal standard solution at the growing concentrations of 5-25-100-500 μM.

Discussion

MEGs produce PLTs with a poorly understood process. The relative constancy of blood PLT number is the result of equivalent processes of production and destruction. PLT production develops on the MEG DMS, with different modalities at the periphery and at the perinuclear area [3,12,13].

MLT induces MEG membrane to grow thinner and thinner and PLT membrane to appear. MLT and NAT- or HIOMT-inhibitors raise the fluorescence of PLTs, that appears as leaning on MEG membrane [10,11,14,15]. The less intense fluorescence over the relative nucleus area is a probable equivalent of cytoplasm MLT concentration. Such a view would suggest MLT demethylation and following desacetylation on MEG contractile membrane system. These complex biochemical and physical processes lead to PLT appearance on the MEG DMS.

MLT does also inhibit PLT aggregation by ADP [10,16,17], probably through its binding with PLT tubulin [18,19].

The left-over MLT can diffuse over the nucleus area and carry out a β-cytochalasin-like effect of endoreduplication inhibition process [11].

Filament could then influence MEG membrane outward current through MLT. An interesting result concerns the correlation between cationic membrane charge and PLT production.

Conclusions

The conclusions confirm the extreme complexity in PLT production by MEG membrane system and the relevant MLT trophic role on the membrane itself.

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REFERENCES:

1. Hamill OP, Marty A, Neher E et al: Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch, 1981; 391: 85-100
2. Reiter RJ: Neuroendocrinology of melatonin. In: Melatonin - Clinical Perspectives, edited by Miles et al: Oxford University Press, 1988; 1-42
3. Behnke O: An electron microscope study of the rat megacaryocyte. II. Some aspects of platelet release and microtubules. J Ultrastruct Res, 1969; 26: 111-29
4. Martha E, Fedorko ME: The functional capacity of guinea pig megakaryocytes. Laboratory Investigation, 1977; 36: 310-20
5. Stenberg PE, McDonald TP, Jackson CW: Disruption of microtubules in vivo by vincristine induces large membrane complexes and other cytoplasmatic abnormalities in megakaryocytes and platelets of normal rats those in human and Wistar Furth rat hereditary macrothrombocytopenias. J Cell Physiol, 1995; 162: 86-102
6. Kapural L, Fein A: Changes in the expression of voltage-gated K+ currents during development of human megakaryocytic cells. Biochim Biophys Acta, 1997; 1326: 319-28
7. Kawa K: Voltage-gated calcium and potassium currents in megakaryocytes dissociated from guinea-pig bone marrow. J Physiol, 1990; 431: 187-206
8. Romero R, Sullivan R: Complexity of the outward K+ current of the rat megakaryocyte. Am J of Physiol, 1997; 272: C1525-31
9. Di Bella L, Gualano L, Rossi MT, Scalera G: Azione della Melatonina (MLT) sulla piastrinogenesi in vitro. Boll Soc it Biol Sper, 1979; 55: 323-6
10. Rossi MT, Di Bella L, Gualano L, Scalera G: Il sistema megacariociti-piastrine, organo bersaglio della Melatonina (MLT). Boll Soc it Biol Sper, 1981; 57: 25-6
11. Rossi MT, Di Bella L: Melatonin in thrombocytogenesis. In: The pineal gland and cancer, edited by Gupta et al: Brain Research Promotion, 1988; 183-94
12. Topp KS, Tablin F, Levin J: Culture of isolate bovine megakaryocytes on reconstituted basement membrane matrix leads to proplatelet process formation. Blood, 1990; 76: 912-24
13. Cramer EM, Norol F, Guichard J et al: Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand. Blood, 1997; 89: 2336-46
14. Lemaitre BJ, Leunay JM, Dreux C et al: Melatonin content, uptake and synthesis by blood platelets. EPSG 2nd Colloq Giessen, 1981; 42
15. Tan D, Manchester LC, Reiter RJ et al: Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. Biochimica et Biophysica Acta, 1999; 1472: 206-14
16. Di Bella L, Scalera G, Rossi MT, Gualano L: L'aggregazione piastrinica in presenza di Melatonina (MLT). S I F Florence, 1979; 25-26
17. Gimeno MF, Ritta MN, Bonacossa A et al: Inhibition by melatonin of prostaglandin synthesis in hypothalamus, uterus and platelets. In: Melatonin: Current Status and Perspective, edited by Birau et al: Pergamon Press, 1980; 147-50
18. Cardinali DP: Molecular Biology of Melatonin: Assessment of the 'Microtubule Hypothesis of Melatonin Action'. In: Melatonin: Current Status and Perspective, edited by. Birau et al: Pergamon Press, 1980; 247-56
19. Benitez-King G, Anton-Tay F: Calmodulin mediates melatonin cytoskeletal effects. Experientia, 1993; 49: 635-41

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