PHARMACOLOGY AND TOXICOLOGY

Melatonin Modifies the Rhythm of Protein Synthesis

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Melatonin (5 nM) added to medium with primary hepatocyte cultures shifted the phase of circahoralian rhythm of protein synthesis and hence, can be a factor synchronizing fluctuations in protein synthesis and rhythm organizer in the hepatocyte population. Blockade of melatonin receptors with luzindole (20 nM) arrested rhythm organization of protein synthesis by melatonin. Prospects of studying biochemical mechanisms of protein synthesis rhythm organization with other drugs (calcium agonists, similarly to melatonin) are discussed.

Key Words: *cell-cell interactions; circahoralian biorhythms; protein synthesis; melatonin*

One of the recent cytological findings is synchronization of organ functions by direct cell-cell interactions; impairment of relationships between the cells triggers the apoptosis mechanism [6]. One of the indicators of cell-cell interactions and synchronization of the cell population is circahoralian rhythm of protein synthesis in cell cultures. One of the studied signal molecules and rhythm synchronizers is melatonin, effectively synchronizing the protein synthesis rhythm being used in nanomolar doses [1,2]. Melatonin is used in cardiology and gastroenterology, as an antistress factor, and as a means for normalization of sleep duration and quality [4]. The chain of processes leading to synchronization of protein synthesis by melatonin includes its modification of the cytoplasmic calcium [2], stage 1 of the signal factor work.

The aim of our study is to clear out whether melatonin is involved in the realization of the final stage of synchronization, modification of fluctuations of protein synthesis rate.

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MATERIALS AND METHODS

The study was carried out on primary cultures of rat hepatocytes [1,2,7,8]. Solid cultures with cells lying close to each other were used. These cultures autosynchronize 10-15 min after replacement of the medium. Cultures without melatonin (control) were compared to cultures treated with melatonin alone or melatonin against the background of luzindole (melatonin receptor blocker).

Isolated rat hepatocytes were cultured on collagen-coated slides in medium 199 with 0.2 mg/ml albumin and 0.5 μg/ml insulin with the gaseous phase consisting of 95% air and 5% CO_2 [7,8]. Solid cultures with densely lying hepatocytes were obtained by inoculating about 10^6 cell/ml into Petri dishes. After 2 h, the slides with adhesive cells were washed and left in a thermostat for 24 h longer, then washed again and the study was started. Specimens of 3 cultures were collected every 10 min. Incorporation of ^{3}H-leucine and the pool of free leucine were measured in the protein and acid-soluble fraction, respectively, of each culture. Leucine incorporation I_{corr}, characterizing the intensity of protein synthesis was calculated by the formula:

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$I_{corr}=I_i \times P_v/P_i$ (cpm), where I_l is leucine incorporation in the proteins for 10 min, P_i is common radioactivity of a certain culture; $P_i=I_i+p_i$, where p_i is radioactivity of the pool in the same culture; P_v is the mean radioactivity of cultures of a certain experiment (36 cultures for 2 h). The I_{corr} value represents variations in the pool and cell counts in different cultures.

RESULTS

Melatonin synchronizes the fluctuations of protein synthesis rhythm by modulating the rhythm phase. Solid cultures of young rat hepatocytes were incubated in a serum-free medium, common for our studies (Fig. 1). Similar cultures were incubated in two dishes. After 60 min, 5 nM melatonin was added into one dish and left there until the end of the experiment (240 min). The protein synthesis kinetics in the two cultures (in two dishes) was virtually the same during the first 60 min: 20 min minimum was followed by 50 min maximum. We previously showed that 5-min treatment with melatonin is sufficient for synchronization of protein synthesis rhythm; permanent presence of melatonin in the medium is inessential for hepatocyte status [1]. The rhythm pattern in the cultures changed immediately after melatonin addition. High synthesis rate persisted during minutes 70-80 in cultures to which melatonin was added in comparison with the minimum control rhythm. This was followed by the minimum in experimental cultures, which occurred during the maximum rhythm in the control. Then the control curve minimum (minutes 110-120) coincided with the maximum rhythm of melatonin treated cultures. The peaks of control curves at minute 130 and minute 170 coincided with the minimum values for curves reflecting the time course of protein synthesis in cultures treated with melatonin. The same correlations were observed later until minute 240 of observation.

The next experiment showed that the shift of protein synthesis rhythm phase was really caused by melatonin. Melatonin treatment was preceded by addition

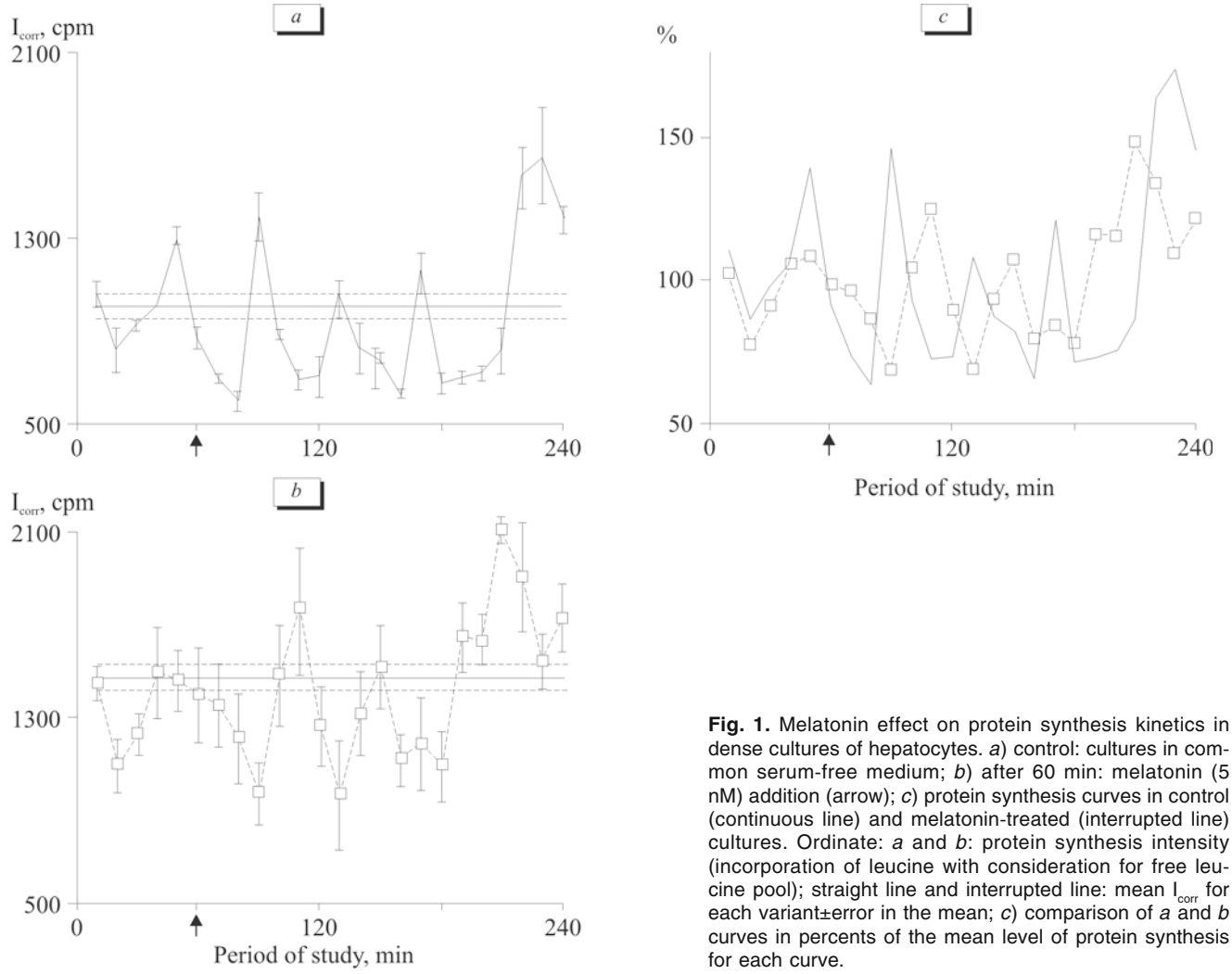

Fig. 1. Melatonin effect on protein synthesis kinetics in dense cultures of hepatocytes. *a*) control: cultures in common serum-free medium; *b*) after 60 min: melatonin (5 nM) addition (arrow); *c*) protein synthesis curves in control (continuous line) and melatonin-treated (interrupted line) cultures. Ordinate: *a* and *b*: protein synthesis intensity (incorporation of leucine with consideration for free leucine pool); straight line and interrupted line: mean I_{corr} for each variant±error in the mean; *c*) comparison of *a* and *b* curves in percents of the mean level of protein synthesis for each curve.

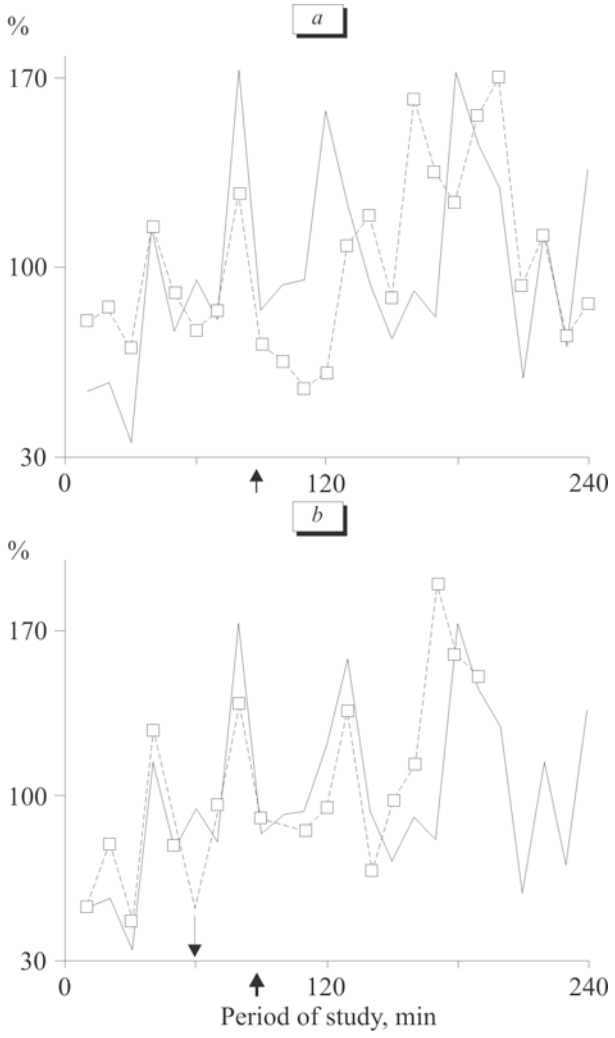

Fig. 2. Effects of melatonin and melatonin+luzindole, added into media with solid cultures of hepatocytes, on the kinetics of protein synthesis. Similar hepatocyte cultures were incubated in three dishes. *a*) comparison of kinetics of protein synthesis in control cultures (continuous line) and in cultures with melatonin (interrupted line); *b*) comparison of protein synthesis kinetics in control cultures (continuous line) and in cultures with melatonin added after luzindole (interrupted line). Ordinate: protein synthesis kinetics in percent of the mean level of synthesis for each curve. ↓ addition of 20 nM luzindole after 60 min, ↑ 5 nM melatonin after 90 min.

of luzindole (specific blocker of melatonin receptors [12]). Similar dense cultures of young rat hepatocytes were incubated in three dishes. In dish 1 (control), the cultures were incubated in common serum-free medium without any additives. In dish 2, melatonin (5 nM) was added after 90 min and left in the culture. In dish 3, luzindole (20 nM) was added after 60 min and left their until the end of observation and after the next 30 min melatonin (5 nM) was added. The three curves virtually coincided by their peaks and minimum values of protein synthesis rhythm before minute 90 of culturing. In cultures incubated in dish 2, the rhythm phase shifted directly after addition of melatonin into the medium (similarly as in experiment 1; Fig. 1). Melatonin did not modify the kinetics of protein synthesis after luzindole blockade of its receptors: the protein synthesis curve in the dish with luzindole and melatonin did not differ from that of the control dish by maximum and minimum values (Fig. 2).

Hence, melatonin, modulating the rhythm phase, can really initiate synchronization of fluctuations of protein synthesis rate. Previously we have demonstrated a shift of protein synthesis phase in hepatocyte cultures treated with phorbol ether (protein kinase stimulant) [8]. Shift of circahoralian rhythm phases for activities of some enzymes were observed in experiments on transformed cells after addition of insulin (stimulating protein phosphorylation) [10] or retinoic acid [9] to the medium. The yeast respiration circahoralian rhythm phase is shifted under the effect of acetaldehyde [11]. Signal factors initiate the chain of processes, detected in our experiments as exemplified by protein synthesis rhythm organization in hepatocyte culture [3,5,8].

Our study introduces melatonin in the family of synchronizing signals. Like the signal factors we studied previously, it is a calcium agonist [12]. Blockade of changes in cytoplasmic calcium with BAPTA-AM [2] leads to liquidation of protein synthesis rhythm, initiated by melatonin in the control. Other drugs, modulating the concentration of calcium ions in cells and stimulating protein kinase activities, can be potential signal factors involved in protein synthesis rhythm organization. In our studies [3,7,8] gangliosides, norepinephrine, and serotonin proved to be the natural signal factors; of the drugs these properties were exhibited by melatonin (natural hormone) and phenylephrine (synthetic norepinephrine).

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