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Short report

Intake of melatonin increases tryptophan hydroxylase type 1 activity in aged rats: Preliminary study

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ABSTRACT

Pineal melatonin is important not only for synchronization of biological rhythms, but also in the ageing process as a potential drug to relieve oxidative damage. During ageing, the nocturnal melatonin production decreases resulting in an increased incidence of disorders. Present in vivo experiments were performed to study the effects of exogenous melatonin chronically administered to old rats on the pineal biosynthesis of melatonin and the precursor serotonin (5-HT) mediated by tryptophan hydroxylase type 1 (TPH-1). Accumulation of 5-hydroxytryptophan (5-HTP) after decarboxylase inhibition was used as a measure of the TPH-1 activity. 5-HT and its metabolite 5-HIAA were also quantified by HPLC-ED. As expected, ageing resulted in worsening of different neurochemical parameters. However, chronic intake of melatonin (1 mg/kg/day, diluted in drinking water, 4 weeks) increased TPH-1 activity and significantly improved the age-induced deficits in nocturnal melatonin content in the pineal gland. Results suggest that melatonin intake (or melatonin rich foods) may contribute to recover the pineal function preventing the nocturnal descent of 5-HT and melatonin biosynthesis that normally occur in pineal gland as a consequence of ageing.

1. Introduction

Melatonin is the major secretory product of the pineal gland and its production is highly controlled by circadian rhythms in response to neural inputs originated in the suprachiasmatic nuclei (Borjigin et al., 2012). Beneficial effects of melatonin on human health are well-known and are commonly associated with the attenuation of oxidative damage (Galano et al., 2011, 2013). However, a morphological and functional deterioration of the pineal gland occur during ageing, highlighting the gradual decline in nocturnal melatonin production (Reiter et al., 1980, 1981), that usually causes important consequences in the circadian pacemaker and chronobiological disorders (Poeggeler, 2005). Moreover, current evidence suggests that internal desynchronization is closely associated with an increased risk of developing or worsening certain diseases like premature ageing, cancer and cardiovascular diseases (Erren and Reiter, 2008). The synthesis of melatonin starts with the essential amino acid tryptophan. Tryptophan hydroxylase (TPH, EC 1.14.16.4) is the first enzyme of this pathway that catalyzes tryptophan conversion into 5-hydroxytryptophan, being the rate-limiting step in the serotonin (5-HT, 5-hydroxytryptamine) synthesis. Two tryptophan hydroxylase isoforms have been described to date: TPH-1 is mainly expressed in the pineal gland and in gut enterochromaffin cells, while TPH-2 is preferentially expressed in the brain (Walther et al., 2003). The TPH activity regulation is an important research area due to its central role in 5-HT formation and its importance in numerous pathophysiological processes. Recently, chronic treatment with melatonin has proven effective in increasing brain TPH-2 activity in old rats (Esteban et al., 2010). Therefore, and due to the role of 5-HT as a precursor in the synthesis of melatonin, this study examined changes in nocturnal melatonin and 5-HT synthesis in the pineal gland mediated by TPH-1 resulting from ageing and exogenous melatonin administration.

2. Methods and materials

2.1. Animals and treatments

Young (3 months, $n = 5$) and old (18 months, $n = 11$) male Sprague–Dawley rats were maintained under controlled environmental conditions (20 ± 2 °C, 70% humidity, 12-h light/dark cycle, lights on at 08:00 h) and free access to standard food and water. The dose of melatonin was chosen from previous studies (Esteban et al., 2010). A group of old rats received melatonin (1 mg/kg/day, $n = 6$) diluted in the drinking water for 4 weeks. To increase melatonin solubility in water, it was solubilized in ethanol to a final concentration of 1% alcohol in drinking water. Control young and old animals also received

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1% alcohol in drinking water. At the end of the treatments, animals received the aromatic l-amino acid decarboxylase inhibitor NSD 1015 (3-hydroxybenzylhydrazine, 100 mg/kg, i.p.) 2 h after lights off (22:00 h). Animals were sacrificed by decapitation 30 min after NSD 1015 administration to determine the TPH-1 activity by measuring the in vivo 5-HTP accumulation in the pineal gland (see below). Pineal glands were quickly removed, dissected on an ice-cold plate and stored at −80 °C until assays. Experimental procedures were conducted according to standard ethical guidelines (European Communities Council Directive 86/609/EEC; Bioethical Committee of University of Balearic Islands).

2.2. Tryptophan hydroxylase type 1 activity (synthesis of 5-HT in pineal gland)

Limiting step in 5-HT synthesis is the transformation of tryptophan into 5-HTP that requires the enzyme TPH-1 in the pineal gland. The accumulation of 5-HTP within 30 min after inhibition of the aromatic L-amino acid decarboxylase, by a maximally effective dose of NSD 1015 is the most commonly used assay system to monitor the in-vivo rate of tryptophan hydroxylation. Pineal glands were placed individually into cold tubes, homogenized by sonication 10 s in 120 μl mobile phase, and centrifuged at 10,000 rpm, 5 min at 4 °C. The resulting supernatant was filtered through 0.45 μm syringe filters (Spartan-3, Sigma–Aldrich). Levels of 5-HTP, 5-HT and its metabolite 5-HIAA were measured by HPLC. Aliquots of supernatants (15–20 μl) were subjected to HPLC on a reversed-phase column (Spherisorb S3 ODS1 C18; 3-μm particle size range; 4.6 mm × 10 cm). For representative chromatograms (see Garau et al., 2006). Mobile phase consisted of 0.1 M KH_2PO_4, 2.1 mM octane sulfonic acid, 0.1 mM K_2EDTA, 2 mM NaCl and 12% methanol (pH 2.7–2.8, adjusted with 85% H_3PO_4) at a flow rate of 0.8 ml/min with a Waters M-510 solvent delivery system (Waters, Barcelona, Spain). The compounds were detected electrochemically by means of a cell with a glassy working carbon electrode with an applied oxidation potential of +0.75 V against an in situ Ag/AgCl reference electrode (Waters Concorde Electrochemical Detector).

2.3. Determination of melatonin in pineal gland

To determine melatonin levels, the chromatographic system was operated with the following phase at 21 °C: 0.1 M sodium acetate, 0.1 M citric acid, 0.15 mM NaEDTA, 12% methanol, pH 3.7 at a constant flow rate of 1 ml/min. The electrochemical detector potential was adjusted to a steady value of +0.92 V. Compound concentrations were calculated using the software Brezze (Waters).

2.4. Statistics

Results are expressed as mean ± SEM. One-way ANOVA followed by Bonferroni's test was used for statistical evaluations. Level of significance was set at $p < 0.05$.

3. Results

Melatonin levels in rat pineal gland, 2.5 h after lights off were 65% lower in aged rats compared with young controls (Fig. 1). However, old rats chronically treated with melatonin (administered in drinking water) presented higher melatonin levels in pineal glands (93%) than control old rats. We also observed age-related changes when analysing the activity of the limiting enzyme TPH-1 in rat pineal gland. Thus, the accumulation of 5-HTP in old rats was 32% of that found in young animals (Fig. 2), but showed a significant increase in old animals treated with melatonin (74%) (Fig. 2). We could postulate that an increase in TPH-1 activity in old animals treated with melatonin, may be accompanied by increased levels of 5-HT. However, melatonin treatment did not induce changes in the levels of 5-HT or 5-HIAA in the pineal gland (Fig. 2). In this way, one might suggest that 5-HT synthesized during the first hours of the night (before NSD inhibition) was probably intended for the melatonin synthesis.

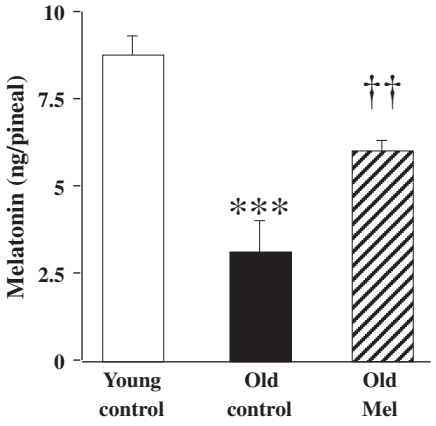

Fig. 1. Chronic effect of exogenous melatonin (Mel) on the nocturnal melatonin content in pineal gland. Columns are mean (ng/pineal) ± S.E.M. derived from pineal glands analysis from young controls ($n = 5$), old controls ($n = 5$) and a group of old rats receiving melatonin diluted in drinking water for 4 weeks (1 mg/kg/day, $n = 6$). One way ANOVA followed by Bonferroni's test was used for statistical evaluation: ***$P < 0.001$ when compared with the young control group; †† $P < 0.01$ compared to the old control group.

4. Discussion

Ageing causes deterioration in the pineal gland function and nocturnal melatonin production (Reiter et al., 1980, 1981), accompanied by an increased risk of certain disorders (Erren and Reiter, 2008). In this short report, we focus on the effects of exogenous melatonin (chronically administered in the drinking water) on the nocturnal melatonin content and the synthesis of the precursor 5-HT mediated by TPH-1 in the pineal gland of aged animals. The most conclusive result of this preliminary study was the improvement on TPH-1 activity after melatonin treatment in correlation with increased levels of melatonin in the pineal gland of aged rats.

There is a peak of the 5-HT output during the early night which is followed by a quick decline associated with melatonin production (Liu and Borjigin, 2006). This early increment of nocturnal synthesis of 5-HT is driven by the increase of the TPH-1 enzyme activity (Shibuya et al., 1977) which needs to be stabilized by phosphorylation of serine-58 in order to elevate 5-HT production at the beginning of the night (Huang et al., 2008). With this information we decided to perform the study 2.5 h after lights off. In this study, accumulation of 5-HTP (after inhibition of aromatic amino acid decarboxylase) determined TPH-1 activity (see materials and methods). Thus, 5-HTP levels decreased significantly in the pineal gland of aged rats, suggesting a decline in TPH-1 activity and impairment in 5-HT synthesis with age. However, we observed a significant increase in TPH-1 activity (74%) in old rats treated with melatonin compared to old controls. This result is in good agreement with previous results from our laboratory showing similar improvement in TPH-2 isoform when aged rats were repeatedly treated with melatonin or its precursor L-tryptophan (Esteban et al., 2010).

In the current work, melatonin levels quantified in the pineal of young animals were similar to that described by other authors in young rats with minimal differences that could be related to the timing of the photoperiod in which the animals were killed or to the strains of rats used (Liu and Borjigin, 2006). Moreover, we observed a decrease in nocturnal melatonin levels in the pineal gland of aged rats compared with young controls which is in agreement with previous reports (Reiter et al., 1980, 1981). Nevertheless, the chronic treatment with melatonin (administered in the drinking water) increased considerably