REGULATION OF MELATONIN SYNTHESIS IN THE OVINE PINEAL GLAND

An in Vivo and in Vitro Study

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In all vertebrates, melatonin synthesis displays diurnal variations, being high at night (1). This nocturnal increase in melatonin production in the rat pineal gland is under the control of the circadian release of norepinephrine (NE) from sympathetic nerves terminating in the gland (2). Melatonin is produced from circulating tryptophan after a four-step enzymatic pathway involving successively the tryptophan hydroxylase (L-tryptophan tetrahydropteridin oxydoreductase, TPOH), the aromatic aminoacid decarboxylase, the serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase, NAT) and the hydroxyindole-O-methyltransferase (HIOMT).

Many studies on melatonin regulation have focused on the enzyme NAT which converts serotonin to N-acetylsertotonin in the penultimate step of the melatonin synthetic pathway (3,4). Little is known about the regulation of TPOH, the first enzyme in the melatonin synthesis and the rate limiting enzyme in serotonin synthesis. This enzyme is present at high levels in the pineal gland of several species and it has been suggested that NE may influence the expression of the TPOH gene in the gland. In the rat and chicken pineal gland, TPOH and NAT enzymatic activity and mRNA levels have been shown to follow a day/night rhythm (5–9).

In the present study, we have measured TPOH mRNA expression in the sheep pineal gland throughout the light/dark cycle by a semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis and compared it with the expression of NAT mRNAs. We showed a significant (p < 0.05, Mann-Whitney) nocturnal increase of 28.5 and 38% in TPOH and NAT mRNA expression, respectively (Figure...
1), which supports the existence of a transcriptional regulation of the melatonin synthesis in the ovine pineal gland.

Nevertheless, these transcriptional mechanisms can not entirely explain the 10-fold increase in plasma melatonin level measured during the dark period (data not shown).

In addition, we developed the culture of ovine pineal cells, in order to study the possible adrenergic modulation of melatonin synthesis and release. Incubation with isoproterenol, a β-adrenergic agonist, elevates to about 900% the production of melatonin (Table 1).

This stimulation is almost totally blocked by propranolol, a β-adrenergic antagonist, and p-chlorophenylalanine, which inhibits TPOH enzymatic activity. Thus, TPOH could be the rate-limiting enzyme in melatonin synthesis in the ovine pineal gland. The adrenergic stimulation of melatonin production is also partially inhibited by the presence of an inhibitor of transcription (actinomycin D) or translation (cycloheximide) in the culture medium. Transcriptional and post-transcriptional mechanisms seem to be implicated in the regulation of melatonin synthesis in the ovine pineal gland.

![Figure 1](image_url)

**Figure 1.** Day/night expression of TPOH and NAT mRNAs from ovine pineal gland. Quantitative RT-PCR of TPOH (day: n = 7; night: = 8) and NAT (day: n = 7; night: = 9) mRNAs from «day» and «night» ovine pineal glands. (*: p < 0.05)(bars: SEM). Male lambs (Ile de-France, 6-months old, from breeding of INRA (Nouzilly, France)), reared under defined lighting conditions (8L: 16D, lights on: 02:00 pm, off 10:00 pm), were sacrificed by decapitation during the light (10 animals at 05:30 pm) or dark (10 animals at 08:30 am) period. The RT-PCR reactions were performed with 0.1 µg of total RNA extracted from pineal gland using 4µl of RT products. After electrophoresis, DNAs were transferred and hybridized with appropriate internal 32P-labelled probes. The membrane sections were counted in a liquid scintillation counter. Experiments were repeated three times.