Nocturnal plasma levels of melatonin in quails (*Coturnix japonica*) injected with l-5-hydroxy-tryptophan

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(With 1 figure)

**Abstract**

This study aimed to demonstrate the influence of the systemic administration of l-5-hydroxy-tryptophan (L-HTP) on the plasma levels of melatonin during the dark period in quails. Throughout daylight, the plasma levels of melatonin did not differ significantly, oscillating between 110.2 ± 15.8 pg.mL⁻¹ and 157.4 ± 34.8 pg.mL⁻¹, from 8 to 16 hours. L-HTP (25 mg.kg⁻¹, through the intracelomic route) administered at 18 hours lessened significantly the nocturnal increase of the plasma levels of melatonin (controls, 327.3 ± 20.1 and 315.8 ± 20.9 pg.mL⁻¹ vs. 242.1 ± 24.8 and 217.5 ± 21 pg.mL⁻¹, respectively, at 20 and 24 hours, P < 0.05). The results obtained showed that the administration of L-HTP reduced the nocturnal melatonin release, possibly by bringing about an increase in serotonin synthesis and synaptic release in the pineal. Therefore, the serotoninergic transmission from the raphe towards the pineal would constitute a mechanism of modulation of the synthesis and melatonin release in quails.

**Keywords:** melatonin, pineal, l-5-hydroxy-tryptophan, *Coturnix japonica*.

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**1. Introduction**

Melatonin is an indoleamine neurohormone synthesized by the pineal gland, located in the diencephalic roof (Hadley, 1996). Melatonin synthesis is dependent on the rhythm of norepinephrine release by postganglionic neurons projected from superior cervical ganglia. Noradrenergic stimulation of the pinealocytes is relayed from retinal excitation by absence of light. Therefore, the pineal gland is considered a photo-neuroendocrine transducer.

Melatonin synthesis is fulfilled from its precursor, L-tryptophan (L-TRP), after uptake by pinealocytes. LTRP is converted to l-5-hydroxy-tryptophan (L-HTP) by the enzyme l-5-hydroxytryptophan decarboxylase (L-HTPD) which acts on the L-HTP, producing 5-hydroxytryptamine (5-HT, serotonin) which is converted to N-acetyl-serotonin catalyzed by N-acetyltransferase (NAT) the activity of which is very much increased during the nocturnal cycle.
N-acetyl-serotonin is O-methylated by hydroxyindole-O-methyltransferase (HIOMT) to produce N-acetyl-5-methoxytryptamine (melatonin) (Hadley, 1996; Foulkes et al., 1997; Natesan et al., 2002).

We intend to investigate the influence of the systemic administration of L-HTP on the melatonin plasma levels during the nocturnal period. This experimental maneuver is widely used to evaluate the brain serotonin production and subsequent increase of the serotoninergic transmission (Fenstrom, 1983; Badauê-Passos et al., 2003; Reis et al., 2005). TPO is considered the rate-limiting enzyme of the serotonin synthesis in serotoninergic neurons (Tyce, 1990; Boadle-Biber, 1993). Administration of serotonin precursors (and thus leading to melatonin synthesis) has given rise to controversial results (Namboodiri et al., 1983; Sugden et al., 1985; Cavallo et al., 1987; Reiter et al., 1990; McIntyre and Oxenkrug, 1991; Nathan et al., 1998; Steardo et al., 2000; Huether et al., 1993). However, such a model has not been employed in birds to evaluate the effect of brain serotonin precursors on melatonin synthesis.

2. Material and Methods

2.1. Animals

Male quails weighing 145-175 g and raised under laboratorial conditions were used, under photoperiod control (lights on: 7 hours, lights off: 19 hours). Two experimental series of 5 groups of 8 birds each were constituted for determination of the plasma melatonin levels, at 8, 12, 16, 20 and 24 hours.

2.2. Experimental procedures

In a first series of 5 groups, 1-5-hydroxy-tryptophan (L-HTP, 25 mg.kg\(^{-1}\), by intracelomic route, ic) was administered at 18 hours. A second series of 5 groups was treated with isotonic saline (1 mL.kg\(^{-1}\), ic) in the same schedule. Samples of heparinized blood (2 mL) were collected from the cervical trunk, after decapitation of the birds and centrifuged to 2500 rpm for separation of the plasma. Collection of blood samples during the nocturnal period was made under red lighting.

2.3. Determination of the plasma levels of melatonin

Determination of the plasma levels of melatonin was made by direct radioimmunoassay (for more details, see Stokkan et al., 1991). Briefly, 250 µL of the sample or pattern were used (range from 2 pg.mL\(^{-1}\) to 1000 pg.mL\(^{-1}\) combined with 100 mL of antibody (initial dilution of 1:9000; Batch G/S/704-8483, Stockgrand Ltd. Guilford, UK) and 100 µL of \(^{[3]}\)H-melatonin (TRK 789, Amersham, approximately 2000 cpm/tube). Results were expressed in pg.mL\(^{-1}\).

2.4. Statistical analysis

Statistical analysis was made using ANOVA. For comparisons among the means obtained at 20 and 24 hours from the groups treated with saline vs. groups treated with L-HTP, the paired Student “t” test was used. Differences among the means were considered significant when P < 0.05.

3. Results and Discussion

Plasma levels of melatonin during the daylight (time pre-injection) did not differ between the two experimental series (controls, 110.2 ± 15.8; 110.5 ± 18.9 and 157.4 ± 34.8 pg.mL\(^{-1}\) vs. L-HTP, 119.5 ± 20.4; 132.1 ± 21.3 and 138.2 ± 23.1 pg.mL\(^{-1}\), at 8, 12 and 16 hours, respectively). On the contrary, after the treatment with L-HTP, a significant reduction was observed in the levels of nocturnal plasma melatonin (controls, 327.3 ± 20.1 and 315.6 ± 20.9 pg.mL\(^{-1}\), vs. L-HTP, 242.1 ± 24.8 and 217.5 ± 21 mg.mL\(^{-1}\), at 20 and 24 hours, respectively, P < 0.05) (Figure 1).

Results reached in our study revealed different data comparatively to those reported in the literature. Thus, systemic administration of L-TRP or serotonin releaser increased the plasma levels of melatonin during the 24 hours light and dark periods in rats (Huether et al., 1993). The authors imputed the increase of the melatonin synthesis to increase of the pool of free serotonin in pinealocyte cytoplasm. Besides, administration of L-HTP after continuous exposure to light by 72 hours increased the melatonin synthesis in young rats (McIntyre and Oxenkrug, 1991). In this sense, administration of L-HTP (20 or 200 mg.kg\(^{-1}\), ip) in ovine, during the light period, induced an increase of the glandular content and melatonin plasma levels (Namboodiri et al., 1983; Sugden et al., 1985). These results provided the suggestion of the employment of this model as the base of clinical tests to investigate the pineal function. However, attempts in this sense in human beings have not been successful (Cavallo et al., 1987). It should be emphasized that in this study the authors used the oral route and lower doses of L-HTP (5-12 mg.kg\(^{-1}\)).

On the other hand, Steardo et al. (2000) evidenced an increase in the NAT activity and of the glandular content...
of melatonin during the nocturnal period in rats treated with 5-HT2C agonists and thus it was suggested that these receptors are probably involved in the serotonergic modulation of the pineal activity. In our observations, the systemic administration of L-5HTP lessened significantly the melatonin plasma levels during the nocturnal period. L-5HTP is made available after the TPO stage and the increase in concentration of the product (serotonin) does not give rise to the negative feedback as effectively as that operated by the catecholamines on the tyrosine-hydroxylase (Tyce, 1990; Boadle-Biber, 1993).

Similar observations to ours were made by Reiter et al. (1990), who noted an increase in the glandular content of L-5HTP, 5-HT and acid 5-hydroxy-indol acetic (5-HIAA) after nocturnal overloads of L-TRP in rats. The authors attributed this observation to autocrine serotonin action (derived from an increase of the precursor availability) that would act in the pinealocytes, inhibiting melatonin synthesis. Alternatively, instead of autocrine action, the serotonin-originated mechanism could be worked from serotonergic neurons of the midbrain raphe (Matsuura and Sano, 1983; Leander et al., 1998). Therefore, serotonin synthesized from L-5HTP administration and released in synapses in the pineal could be the mediator of the responses described by Reiter et al. (1990). If this hypothesis is correct, the serotonergic output relayed from the midbrain raphe towards the pineal could constitute a nocturnal mechanism of modulation of NAT activity and therefore, of melatonin synthesis.

References


