The Effect of Melatonin Administration on Circulating Plasma Luteinizing Hormone Concentration in Castrated White Leghorn Roosters

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ABSTRACT Melatonin (MLT) has a significant role in mammalian reproduction, with little or no effect in birds. In the present study we studied the role of MLT in regulation of luteinizing hormone (LH) secretion in castrated White Leghorn (WL) roosters. In Experiment 1, castrated WL roosters (n = 30) were divided into three groups, and each group (n = 10) was subdivided into two subgroups (n = 5). Birds in one subgroup received an injection of MLT at 5 mg (MLT-5), 20 mg (MLT-20), or 80 mg (MLT-80)/kg BW. Birds in the second subgroup were vehicle-injected and served as controls. Each dose of MLT was administered on a separate day at 1100 h. Blood was sampled 30 min before and 10, 30, 60, and 120 min after MLT or vehicle administration. Ten minutes after MLT administration, a significant reduction in plasma LH was observed in the MLT-20 and -80 groups, i.e., 70.3 ± 8.3% and 62.2 ± 4.1% of control values, respectively. In the MLT-80 group, plasma LH further declined to 42.1 ± 9.7% of control values 60 min after injection. In Experiment 2, 18 castrated WL roosters were divided into three groups of six birds each. Two groups were injected with 80 mg MLT/kg BW at the beginning of the experiment; the second group received an additional dose of 80 mg MLT/kg BW 140 min after the first injection. The third group was injected twice (as in second group) with vehicle and served as control. Blood was sampled 30 min before and 30, 60, 120, 170, 200, and 240 min after injection. Repeated MLT injection maintained low levels of plasma LH level until the end of the experiment. In Experiment 3, 10 castrated WL roosters were divided into two groups (n = 5). The first group was injected daily, for 10 d, with 80 mg MLT/kg BW, the second group was vehicle-treated and served as a control. At Day 3, there was a significant reduction in plasma LH level in the MLT-treated group, which continued for 7 d. This study demonstrates that, in birds, MLT suppresses LH secretion in a dose- and a time-related manner.

(Key words: melatonin, luteinizing hormone, reproduction, pituitary)

INTRODUCTION

The pineal gland plays an important role in the reproductive activities of mammalian species. Subcutaneous melatonin (MLT) injection of male rats had no effect on natural luteinizing hormone (LH) secretion but inhibited (84%) LH release in response to naloxone (Shacoori et al., 1996). Melatonin was found to inhibit gonadotropin releasing-hormone-induced LH release in plasma of male rats (Vanecek and Klein, 1995). In addition, in Syrian hamsters, prostaglandin E2 was shown to play an essential role in the gonad-inhibiting effect of MLT by transforming the neuroendocrine signal to gonadal reactivity (Nir et al., 1994). Furthermore, in women the amplitude of LH pulses and mean LH levels were increased by MLT in the follicular, but not in the luteal, menstrual phase (Cagnacci et al., 1995).

Studies conducted in wild birds have shown a significant role for MLT in reproductive activity. In male Indian finches (Estrilda amandava), administration of MLT for 30 d induced complete inhibition of seasonal gonadal growth (Gupta et al., 1987). In blossom-headed parakeets (Psittacula cyanocephala) and Indian weaver birds (Ploceus philippinus), the pineal gland is inactive during the breeding season. Administration of MLT (250 µg/100 g BW) for 10 consecutive d resulted in a significant involution of testes during the breeding phase (Chakraborty, 1993). In jungle bush quail (Perdicula asiatica), an inverse relationship between pineal gland activity and ovarian activity was demonstrated when tested over a yearly reproductive cycle (Dubey and Haldar, 1997). In contrast, MLT administration to domesticated Japanese

Abbreviation Key: LH = luteinizing hormone; MLT = melatonin; NMP = N-methyl-2-pyrrolidone; WL = White Leghorn.
quail had no effect on photostimulatory response to increased light duration (Juss et al., 1993).

Whereas MLT has been found to affect thermoregulation in domestic fowl (Rozenboim et al., 1997), there are few reports as to its possible effects on reproductive activity in chickens. In the present study, the possible effect of MLT on LH secretion was studied in castrated White Leghorn (WL) roosters.

MATERIALS AND METHODS

Experiment 1

Surgically castrated WL roosters (n = 30), 6 mo of age, were used in this experiment. Castration of birds was conducted at 8 wk of age under pentobarbital anesthesia. The procedure was done to elevate plasma LH levels by eliminating negative feedback. Birds were reared under photostimulatory conditions (16L:8D), using warm white fluorescent lamps, at an intensity of 0.1 W/m² at bird head level. Birds were maintained in individual cages located in an open-sided house in each experiment. The birds were divided into three treatment groups (n = 10). Each treatment group was then divided into two subgroups (n = 5); the first subgroup was injected i.p. with 5 mg (MLT-5), 20 mg (MLT-20), or 80 mg (MLT-80) MLT/kg BW at 1100 h. The second subgroup in each group was used as control and injected with the same volume of vehicle [N-methyl-2-pyrrolidone (NMP) saline 1:10 vol/vol] as the matched experimental ones and at the same time. The experiment lasted 3 d, and on each day, one dose was tested. Heparinized blood samples (2 mL) were drawn from ulnar vein 30 min before and 10, 30, 60, and 120 min after the administration of MLT or the vehicle. Plasma samples were stored at −20 C pending assay.

Experiment 2

Castrated WL roosters (n = 18) at the age of 6 mo were used in this experiment. Castration of birds was conducted at 8 wk of age under pentobarbital anesthesia. Birds were maintained as described in Experiment 1 and were divided into three treatment groups (n = 6). The first and second groups were injected i.p. with 80 mg MLT/kg BW 1100 h; in addition, the second group was reinjected with 80 mg MLT/kg BW 140 min thereafter. The third group was injected with NMP at both times and served as controls. Heparinized blood samples were collected 30 min before the first injection and at 30, 60, 120, 170, 200, and 240 min thereafter. Plasma samples were stored at −20 C pending assay.

Experiment 3

Ten castrated WL roosters at the age of 6 mo were used in this experiment. Birds were castrated at 8 wk of age under pentobarbital anesthesia and were main-
FIGURE 2. Percentage change (mean ± SE) in plasma luteinizing hormone (LH) level of White Leghorn roosters injected i.p. with 0 (control) or 80 (1st MLT) mg melatonin/kg BW or reinjected after 140 min (2nd MLT) with an additional 80 mg melatonin/kg BW. a,bValues marked with different letters are significantly different ($P < 0.05$).

tained as described in Experiment 1. Birds were divided into two treatment groups (n = 5). For 10 d, the first group received i.p. injections of 80 mg MLT/kg BW per day, and the second group was NMP-treated and served as control. All injections were at 1100 h, and blood samples were taken 240 min later. Plasma samples were stored at −20 C pending assay.

**Plasma LH Determination.** Plasma LH levels were measured in two assays, one for the first experiment, and a second for Experiments 2 and 3, according to the method of Krishnan et al. (1994). The intra-assay coefficient of variation was 8%, and the interassay coefficient of variation was 10%. Reagents for the assays were kindly provided by J. A. Proudman (USDA/ARS, Beltsville, MD). Statistical analyses of the data were by analysis of variance, in the repeated measurement model of SAS software (SAS Institute, 1987).

**RESULTS**

**Experiment 1**

Administration of 5 mg of MLT/kg BW had no significant effect on plasma LH level; however, a significant reduction in plasma LH level was observed 10 min after administration of 20 or 80 mg/kg BW MLT (70.3 ± 8.3% and 62.2 ± 4.1%, respectively). Plasma LH declined further (42.1 ± 9.7%) in the MLT-80 group, 60 min postinjec-

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Time (min)</th>
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<tbody>
<tr>
<td></td>
<td>−30 0 10 30 60 120</td>
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<tr>
<td>Control</td>
<td>76.8 ± 4.5 $^{xx}$ 79.8 ± 5.4 $^{xx}$ 85.1 ± 8.5 $^{xx}$ 75.9 ± 10.2 $^{xx}$ 75.1 ± 10.4 $^{xx}$ 75.8 ± 10.2 $^{xx}$</td>
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<tr>
<td>MLT-5</td>
<td>70.6 ± 10.3 $^{xx}$ 72.8 ± 8.2 $^{xx}$ 56.2 ± 5.4 $^{by}$ 66.6 ± 4.1 $^{xx}$ 89.1 ± 12.3 $^{xx}$ 67.8 ± 8.2 $^{xx}$</td>
</tr>
<tr>
<td>MLT-20</td>
<td>77.6 ± 8.2 $^{xx}$ 72.5 ± 5.4 $^{xx}$ 54.2 ± 9.2 $^{by}$ 47.4 ± 5.6 $^{by}$ 47.4 ± 6.1 $^{by}$ 56.8 ± 7.0 $^{by}$</td>
</tr>
<tr>
<td>MLT-80</td>
<td>79.2 ± 10.2 $^{xx}$ 76.5 ± 6.6 $^{xx}$ 53.8 ± 8.3 $^{by}$ 40.3 ± 5.2 $^{by}$ 41.2 ± 5.1 $^{by}$ 53.8 ± 5.5 $^{by}$</td>
</tr>
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$^{xx}$Means within times with different superscripts are significantly different ($P < 0.05$).

$^{by}$Means over times with different superscripts are significantly different ($P < 0.05$).

$^{1}$Data are means ± SE.
FIGURE 3. Percentage change (mean ± SE) in plasma luteinizing hormone (LH) level of White Leghorn castrated roosters injected daily for 10 d i.p. with 0 (control) or 80 MLT mg melatonin/kg BW. \(^{ab}\) Values marked with different letters are significantly different (\(P < 0.05\)). Percentage change (mean ± SE) in plasma luteinizing hormone (LH) level of White Leghorn castrated roosters injected daily for 10 d i.p. with 0 (control) or 80 (MLT 80) mg melatonin/kg BW. \(^{ab}\)Values marked with different letters are significantly different (\(P < 0.05\)).

Experiment 2

A significant reduction in plasma LH was observed 30 min after administration of 80 mg/kg BW MLT (56.6 ± 7.5%) compared to the vehicle control group (Figure 2). The second injection of MLT reduced plasma LH further to 40.4 ± 4.7% of control values 110 min thereafter, whereas plasma LH levels of roosters that were injected only once returned to control values (Table 2).

Experiment 3

Daily administration of MLT at 80 mg MLT/kg BW resulted in a reduction in plasma LH that became significant by the third day of MLT treatment (42 ± 5.8% compared to 99.4 ± 28.9%, respectively; Figure 3). Except for Day 6, this suppression in plasma LH continued for the 10-d MLT treatment (Table 3).

DISCUSSION

The results of this study demonstrate for the first time that MLT has a suppressive effect on plasma LH in a

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<th>Treatment group</th>
<th>Time (min)</th>
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<th>Time (min)</th>
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<th>Time (min)</th>
<th>Time (min)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-30</td>
<td>0</td>
<td>30</td>
<td>60</td>
<td>120</td>
<td>170</td>
<td>200</td>
<td>240</td>
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<td></td>
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<tr>
<td>MLT-80</td>
<td>83.2 ± 5.1(^{as})</td>
<td>83.8 ± 8.2(^{as})</td>
<td>58.3 ± 5.4(^{by})</td>
<td>47.8 ± 4.1(^{by})</td>
<td>59.3 ± 12.3(^{by})</td>
<td>64.0 ± 13.6</td>
<td>78.0 ± 12.1(^{as})</td>
<td>76.3 ± 2.6(^{as})</td>
<td></td>
<td></td>
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<tr>
<td>MLT-80 × 2</td>
<td>79.3 ± 5.5(^{as})</td>
<td>84.5 ± 5.4(^{as})</td>
<td>56.3 ± 9.2(^{by})</td>
<td>48.8 ± 5.6(^{by})</td>
<td>61.4 ± 6.1(^{by})</td>
<td>41.6 ± 7.4(^{by})</td>
<td>37.8 ± 6.7(^{by})</td>
<td>33.4 ± 6.5(^{by})</td>
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\(^{as}\)Means with different superscripts within times are significantly different (\(P < 0.05\)).

\(^{by}\)Means with different superscripts over time are significantly different (\(P < 0.05\)) by repeated sample analysis.

\(^{1}\)Data are means ± SE.
dose- and time-related manner in domestic fowl. Repeated MLT administration resulted in further reductions in plasma LH and for a longer duration.

There are two possible mechanisms by which MLT affects the gonad. Some experimental results have suggested that in birds MLT inhibits gonadal activity by a direct action on gonads. Specific MLT binding sites have been identified in the testes and ovaries of chickens, ducks, and quail (Ayre and Pang, 1994). In addition, administration of MLT to female quail have been shown to inhibit oviduct and ovarian growth in a nondose-related manner (Homma et al., 1967).

The second pathway might be via the central nervous system, because pinealectomy resulted in rapid oviduct growth in young quail reared under photostimulatory conditions but not in quail maintained under nonphotostimulatory lighting (Homma et al., 1967). In addition, oral supplementation of MLT to European quail caused a reversible decline in testicular development associated with photostimulation. Furthermore MLT had no effect on premigratory fat development suggesting that MLT is a regulatory factor in reproductive cycle of this bird (Guyomarc’h et al., 2001). Further evidence identified specific MLT receptors located in the preoptic area of quail, which plays a key role in the activation of male quail mating behavior and hosts a large population of gonadotropin-releasing hormone-containing neurons (Aste et al., 2001). Melatonin administration of the Indian spotted owlet decreased ovary weight plasma estrogen and progesterone levels in sexually active and inactive birds but more potently in active than inactive birds, suggesting a central effect of MLT on ovarian function (Guchhait and Haldar, 2000).

Melatonin is one of the major stimulants for migration in birds on one hand, but on the other hand it has a crucial effect on synchronization of reproductive response to environmental conditions (Pang et al., 1998).

Circannual rhythms are responsible for initiation of migration of birds in spring and autumn. Investigations on migratory birds have shown that the amplitude of the 24-h plasma MLT rhythm is reduced during the migratory seasons compared with nonmigratory seasons (Gwinner et al., 1997). Migration is associated with depression or initiation effects on the reproductive system (depending on the migration season); thus, its correlation with MLT fluctuation is important.

A study on the effect of MLT on ovarian granulosa cells suggested a direct action of MLT on those cells in hens that lowered the responsiveness to LH for progesterone production (Murayama et al., 1997). Studies on MLT receptors in the reproductive system, i.e., in the testes, epididymis, vas deferens, prostate, ovaries, and mammary glands, indicate that environment affects MLT synthesis and release. The multiple levels of MLT action in the gonads and other reproductive tissues may guarantee the role that MLT plays in the reproductive system (Pang et al., 1998).

The present study shows that in the domestic fowl, MLT may reduce gonadal activity by inhibiting LH secretion, which suggests that MLT has a gonadostatic role at the keel of pituitary, hypothalamus, or both. In WL layers, MLT was found to induce hypothermia in a dose-related manner (Rozenboim et al., 1997), supporting the hypothesis that the hypothalamus serves as a target organ for MLT.

### TABLE 3. Plasma luteinizing hormone level (ng/mL) of castrated White Leghorn roosters daily injected for 10 d with 0 or 80 mg melatonin/kg BW (control and MLT-80, respectively)1

<table>
<thead>
<tr>
<th>Treatment day</th>
<th>Control</th>
<th>MLT-80</th>
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<tbody>
<tr>
<td>0</td>
<td>102.8 ± 13.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>114.5 ± 15.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>84.2 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.6 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>80.6 ± 11.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.6 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>81.8 ± 9.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.6 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>88.0 ± 14.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.2 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>72.8 ± 9.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.5 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>58.6 ± 5.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.4 ± 8.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>74.4 ± 11.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.2 ± 7.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>8</td>
<td>69.6 ± 7.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.0 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>9</td>
<td>75.7 ± 2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.0 ± 12.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>92.3 ± 11.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.8 ± 11.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup>Means within time with different superscripts are significantly different (P < 0.05).

<sup>b</sup>Means over time with different superscripts are significantly different (P < 0.05) by repeated sample analysis.

<sup>1</sup>Data are means ± SE.
tion on the sexual development in European quail (*Coturnix coturnix*). Behav. Proc. 53:121–130.


