The thermoregulatory mechanism of melatonin-induced hypothermia in chicken

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The thermoregulatory mechanism of melatonin-induced hypothermia in chicken. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R232–R236, 1998.—The involvement of melatonin (Mel) in body temperature (Tb) regulation was studied in White Leghorn layers. In experiment 1, 35 hens were injected intraperitoneally with seven doses of Mel (0, 5, 10, 20, 40, 80, or 160 mg Mel/kg body wt) dissolved in ethanol. Within 1 h, Mel had caused a dose-dependent reduction in Tb. To eliminate a possible vehicle effect, 0, 80, and 160 mg/kg body wt Mel dissolved in N-methyl-2-pyrrolidone (NMP) was injected. NMP had no effect on Tb with Mel again causing a dose-dependent hypothermia. In experiment 2 (n = 30), Mel injected before exposure of layers to heat reduced Tb and prevented heat-induced hyperthermia. Injection after heat stress had begun did not prevent hyperthermia. Under cold stress, Mel induced hypothermia, which was not observed in controls. In experiment 3 (n = 12), Mel injection reduced Tb and increased metatarsal and comb temperatures (but not feathered-skin temperature), respiratory rate, and evaporative water loss. Heart rate rose and then declined, and blood pressure increased 1 h after Mel injection. Heat production rose slightly during the first hour, then decreased in parallel to the Tb decline. We conclude that pharmacological doses of Mel induce hypothermia in hens by increasing nonevaporative skin heat losses and slightly increasing respiratory evaporation.

The pineal organ and its metabolites play an important role in circadian thermoregulatory adjustments of body temperature (Tb) in many species. Pinealectomy resulted in the loss of endogenous circadian rhythm of locomotor activity in the house sparrow and abolished Tb rhythm in sparrows kept in complete darkness (4, 12). Melatonin (Mel) administration lowers Tb by 2–3°C in mice (9). This latter observation correlates with the reduction in plasma L-thyroxine concentration observed in pinealectomized White Leghorn roosters, suggesting a possible link between Mel and regulation of metabolic rate (16). Pinealectomy or blinding did not abolish the circadian rhythm of locomotor activity in pigeons; however, when these treatments were combined, this rhythm disappeared under constant or dim light (10). In pigeons, Mel is synthesized not only by the pineal gland but also in the retina (21, 27). Accordingly, daily administration of Mel to pinealectomized and blinded pigeons restored circadian rhythms of locomotor activity, and Tb was correlated to Mel injections (20).

Materials and Methods

Experimental Procedure

White Leghorn (Loman) laying hens, kindly provided by Kibbutz Haetz Hayim in Israel, were housed in individual cages in an open-house pen, with artificial light supplement giving the birds a total of 16 h of light/day. Commercial feed and water were provided ad libitum. In the three experiments detailed further on, Tb was measured; the rest of the studied variables were measured in experiment 3 as detailed in previous studies (19, 29). Briefly, comb, metatarsal, and trunk (feathered skin) temperatures, as well as Tb were recorded by telothermometer (Yellow Springs Instruments, Yellow Springs, OH). Blood pressure and heart rate were continuously monitored with a pressure transducer (Statham, Hato Rey, Puerto Rico), connected to a polygraph (Gilion, Middletown, WI) and to a vinyl catheter inserted in the sciatic artery under local anesthesia (lidocaine, 2%). Oxygen consumption was measured by placing the hen's head in a head mask through which 4 l/min was circulated, and 150 ml air/min aliquots were bypassed through an oxygen analyzer (Servomex 1100, East Sussex, UK). Respiratory rate was recorded continuously by connecting the head mask to a pressure transducer (Mercury, Glasgow, Scotland) connected to the polygraph. Evaporative water loss was determined by measuring air-flow humidity with a hygrometer (Novasina, Pfaffikon, Switzerland) connected to the head mask. Evaporative water loss and metabolic heat production were calculated and expressed in W/kg body wt using routine procedures.

Experimental Protocols

Experiment 1. Hens (n = 35) were divided into seven treatment groups (n = 5), held under thermoneutral conditions (air temperature 25°C, relative humidity 50%). Each was injected intraperitoneally with either 0 (control), 5, 10, 20, 40, 80, or 160 mg Mel/kg body wt (Janssen Chimica, Geel, Belgium) dissolved in 0.5 ml ethanol. Tb was recorded immediately before injection, every 20 min during the first 170 min of the experiment, and every 60 min thereafter to 290 min, when the experiment was terminated. Because ethanol slightly (NS) reduced Tb, another vehicle, N-methyl-2-pyrrolidone (NMP) was tested. Twenty-four layers were divided into four groups (n = 6): untreated controls, NMP...
vehicle (0.6 ml) controls, and 80 or 160 mg Mel/kg body wt dissolved in 0.6 ml NMP.

Experiment 2. Hens (n = 36) were exposed to either acute heat (37°C) or cold (10°C) stress for 5 h. In each thermal treatment, NMP-vehicle controls were compared with hens injected with 160 mg Mel/kg body wt; injection was performed either immediately before or 30 min after the start of heat or cold exposure.

Experiment 3. Twelve hens divided equally into two groups, NMP controls and hens injected with 160 mg Mel/kg body wt, were held under thermoneutral conditions. Body, metatarsal, comb, and feathered trunk-skin temperatures; respiratory rate; oxygen consumption; evaporative water loss; heart rate; and blood pressure were recorded 30 min before injection and every 10 min after injection until termination of the experiment.

Data Analysis

All data were analyzed by a repeated-measurements procedure using the SAS General Linear Model Procedure (SAS Users Guide).

RESULTS

Experiment 1: Thermoneutral State

Ethanol-dissolved Mel administered to normothermic laying hens reduced (P < 0.05) T<sub>b</sub> in a dose-dependent manner (Fig. 1A). The lowest T<sub>b</sub> (39.2 ± 0.2°C) was recorded 60 min after injection of 160 mg Mel/kg body wt, followed by 80 mg Mel/kg body wt (39.8 ± 0.5°C) and 40 mg Mel/kg body wt (40.0 ± 0.2°C). The lower doses (5–20 mg Mel/kg body wt) also lowered T<sub>b</sub> after 60 min by 0.5°C (P, 0.05), but these subsequently rose to control values (NS). The correlation coefficient between Mel dose and lowest T<sub>b</sub> recorded after injection was 0.96 (P, 0.05). Administration of NMP vehicle did not change T<sub>b</sub> (Fig. 1B). Similar to Mel dissolved in ethanol, administration of NMP-dissolved Mel resulted in a significant hypothermic response (Fig. 1B), reaching a nadir 60 min after injection (39.9 ± 0.3 and 39.6 ± 0.1°C for 80 and 160 mg Mel/kg body wt, respectively, P, 0.05).

Experiment 2: Cold and Heat Stresses

Mel injection either before or 30 min after the start of cold exposure resulted in a hypothermic response (P < 0.05; Fig. 2A). Although pattern of the two curves did not differ from one another, the minimum T<sub>b</sub> value (37.5°C, 60 min) in the subgroup injected with Mel after the start of cold stress was lower than the corresponding value in the other subgroup (P < 0.05). After 5 h of cold exposure, T<sub>b</sub> in both Mel-injected hens was lower (P < 0.05) than that in controls. It is worth noting that,
under cold conditions, panting was not visible, as it was under thermoneutral or hot conditions. In contrast to cold stress, during heat exposure $T_b$ curves differed significantly ($P < 0.05$) between hens injected with Mel before or 30 min after the start of heat exposure (Fig. 2B, $P < 0.05$). Mel injected before the start of heat stress induced a typical hypothermic response with minimal values (40.7°C) higher than those recorded in thermoneutral or cold states (Figs. 1 and 2A). Mel eliminated the hyperthermic response recorded in control hens. At 150 min after injection, $T_b$ of Mel-pretreated hens returned to normothermic values (41.6°C). Injection of Mel 30 min after initiation of heat exposure did not prevent hyperthermia, but shortened its duration (Fig. 2B, $P < 0.05$).

Experiment 3: Thermal and Cardiorespiratory Responses

Figure 3, A-D, presents the changes in body core and peripheral temperatures following Mel injection under thermoneutral conditions. The typical hypothermic response to Mel was recorded; $T_b$ dropped by 2°C 60 min and more after injection (Fig. 3A). Although Mel did not affect feathered-skin temperature (Fig. 3B), it caused a significant biphasic response in the unfeathered skin: comb and metatarsal temperatures (Fig. 3, C and D, respectively) peaked within 20–30 min ($P < 0.05$), stayed high until 60 min after Mel injection, and decreased below and then stabilized at initial values.

Mel induced an immediate rise in respiratory rate (Fig. 4A, $P < 0.05$), which peaked 30 min after injection, then dropped, reaching a basal level at 100 min, and stabilized. The pattern of evaporative heat loss was similar to that of respiratory rate. It rose ($P < 0.05$) immediately after Mel injection, decreased, then climbed back up to initial values 60 min later (Fig. 4B). During the first 60 min, metabolic heat production in Mel-treated hens increased by ~7% above the control level, but this rise was not statistically significant. Heat production then decreased and stabilized toward the end of the experiment (Fig. 4C). In control hens, heat production was stable during the first 60 min, then rose slightly. The different heat production patterns in control vs. Mel hens (ascending and descending patterns, respectively) was reflected in a treatment-by-time interaction ($P < 0.05$). Mel induced a transient rise in heart rate immediately after injection, which was followed by a decrease ($P < 0.05$) of ~10% thereafter (Fig. 5A). Mean blood pressure (Fig. 5B) was not altered during the first 60 min; it then rose ($P < 0.05$) in Mel-treated birds by ~10% above control values.

**DISCUSSION**

A single injection of Mel caused a significant dose-dependent hypothermic response in laying hens. Such a response to Mel has been found previously in sparrows (5, 12), chicks (2), and several mammalian species (15, 22), including humans (6, 7, 8, 25). The present study revealed two phases following Mel injection: the first lasted 1 h, during which the maximal hypothermic response occurred associated with enhancement of heat-loss mechanisms; the second phase lasted at least 3 h, during which the birds were hypothermic but heat-loss mechanisms were no longer active. The hypothermic response was not related to the type of Mel-dissolving vehicle, because neither vehicle induced hypothermia on its own. The use of NMP as a solvent in vivo has been previously reported (3).
This study showed that heat-loss mechanisms are responsible for Mel-induced hypothermia. Peripheral vasodilatation (indicated by the 3–5°C rise in skin temperature and recorded only in the unfeathered-skin extremities) caused a marked rise in nonevaporative heat loss. In contrast, under thermoneutral conditions, Mel did not increase feathered-skin temperatures of the body trunk above initial values (38°C), the latter being higher than those in the comb and metatarsus. In fact, convective and conductive heat loss from the skin seemed to be the major heat-loss pathway inducing hypothermia. Although respiration rate rose to 140 breaths/min, evaporative water loss rose in Mel hens by only 13% above control values during the first hour after Mel injection; this value was much lower than expected for panting White Leghorn hens (1, 28). Moreover, the extra heat loss by evaporation during the first hour after Mel injection (2.9 W/kg body wt) was similar to the slight rise (3.0 W/kg body wt; NS) in heat production recorded during the same time period; the latter was due to the increased respiratory-muscle activity during panting. It is not clear, however, why enhanced respiratory evaporation induced by Mel is ineffective in terms of heat loss.

In agreement with the finding that nonevaporative heat loss is the main heat-loss pathway causing hypothermia in Mel birds, we found that under cold conditions (Fig. 2A) $T_b$ reached a nadir of $\approx 1°C$ lower than under thermoneutral conditions (Fig. 1, A and B). This can only be attributed to an increase in sensible heat loss from the skin under cold conditions; as indicated, panting was not observed in cold-stressed Mel hens. In Japanese quails, exposure to cold significantly lowered plasma, pineal, and retinal Mel levels and depressed the usual Mel rise in the dark (17). The latter parameters had been suggested to be a manifestation of a natural mechanism that protects the animal from severe hypothermia during cold nights.

Of particular interest are the responses to Mel under heat stress. Mel administered 30 min after the start of heat exposure did not cause the typical immediate hypothermia (Fig. 2B). This is probably because heat exposure induced skin vasodilatation and panting that Mel injected 30 min later could not enhance further. In contrast, in birds injected with Mel just before the start of heat exposure, the immediate skin vasodilatation and rise in respiratory rate induced hypothermia even though hens were exposed to heat. It is worth noting that under heat stress, Mel lowered $T_b$ below control values not only during the first hour after Mel injection but also during the last 2 h of the experiment. This finding could be developed into a tool to prevent or reduce the heavy losses of domestic fowl during hot conditions.

![Fig. 4](image-url) Effect of Mel administration (160 mg/kg body wt) on respiratory rate (A), evaporative heat loss (B), and heat production (C). Points represent means ± SE. Bw, body wt.

![Fig. 5](image-url) Effect of Mel administration (160 mg/kg body wt) on heart rate (A) and mean blood pressure (BP; B). Points represent means ± SE.
spells in hot countries, provided that Mel is administered before air temperatures rise.

The pattern of heat production in response to Mel followed that of T_b, except during the first hour after Mel injection. During that time heat production did not fall with T_b because of panting activity. However, later, when panting ceased, the drop in heat production followed the reduction in T_b (Q_{10} effect). The rise in heat production after 140 min followed a similar rise in T_b. An almost similar pattern was noted for heart rate as well (Fig. 5A). The similar responses were expected and it is well established that a rise in metabolic rate is associated with a rise in heart rate, representing a rise in cardiac output. The 15% drop in heart rate occurred associated with a rise in heart rate, representing a rise in metabolic rate when metabolic rate was at a minimum, and later in cardiac output. The 15% drop in heart rate occurred when metabolic rate was at a minimum, and later (after 140 min), the rise in heart rate corresponded to the increase in both T_b and heat production. The slight decline in Tb (Q_{10} effect). The rise in heat production during the morning and early afternoon hours, when endogenous Mel secretion is low; responses may change if Mel is administered at night hours when Mel secretion is high.

In relation to a “set-point” model, Mel may act by lowering the central set-point temperature, thereby causing immediate stimulation of heat-loss mechanisms. These immediate changes, which last about an hour, are long enough to induce deep hypothermia, which then lasts for several additional hours.

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REFERENCES


