MELATONIN DOES NOT INFLUENCE THERMOREGULATORY BEHAVIOR IN *BUFO AMERICANUS* AND *BUFO MARINUS*.-

Behavioral thermoregulation is used by amphibians to exert some control over body temperature ($T_b$). Unlike reptiles that are often very precise thermoregulators, amphibians must balance the costs of dehydration incurred by high $T_b$s with benefits of elevating $T_b$. As a result, although many amphibians do thermoregulate, they do not do so to the extent observed in reptiles (Hutchison and Dupré, 1992; Tracy et al., 1993). Possibly because of this, we have less information on amphibian temperature preferences and control of thermoregulation than we have for reptiles.

Melatonin is produced by the pineal gland of vertebrates and affects thermoregulatory behavior in vertebrates such as turtles (Erskine
and Hutchison, 1981), lizards (Cothren and Hutchison, 1979), and salamanders (Hutchison, 1981). Its effects on amphibian thermoregulation have only been studied in the aquatic salamander Necturus maculosus where it causes a decrease in both preferred temperature (Hutchison, 1981) and thermal tolerance (Erskine and Hutchison, 1982). Chlorpromazine blocks the breakdown of melatonin (Hutchison and Dupré, 1992; Rawding and Hutchison, 1993), and therefore, it also lowers preferred temperature (Hutchison, 1981) and thermal tolerance (Erskine and Hutchison, 1982) of N. maculosus.

Although melatonin has a strong impact on behavioral thermoregulation in N. maculosus, it is unknown whether this effect is unique to N. maculosus or whether it is widespread in amphibians that behaviorally thermoregulate. Our purpose was to determine whether melatonin exerted control on thermoregulatory behavior in the toads Bufo americanus and Bufo marinus. We chose Bufo for studying the role of melatonin in controlling thermoregulation because many Bufo species behaviorally thermoregulate and more is known about thermoregulation in Bufo than in most other amphibian genera. Bufo readily bask under an incandescent light and use a thigmothermal source to elevate T_b in a laboratory setting. We hypothesized that melatonin would increase the selected T_b in Bufo.

Materials and methods.—Bufo americanus (87.5–151.8 g) were captured in Blount County, Tennessee, and maintained individually in the laboratory at Maryville College at room temperature (22.0 ± 3.0 C). Bufo marinus (112–300 g) were purchased from an animal supply company and maintained individually in the laboratory at the University of Oklahoma at 25 ± 1 C. An incandescent bulb above each end of each 33 x 22 x 22 cm plastic holding tank allowed the toads to elevate T_b during photophase. Bufo americanus were provided an LD 11:13 photoperiod with the photophase centered at 1230 h EST. Bufo marinus were maintained on an LD 12:12 photoperiod with the photophase centered at 1200 h CST. Water was provided ad lib, and crickets (Acheta) and/or mealworm larvae (Tenebrio) were provided three times per week to the B. americanus. Crickets, mealworm larvae, or neonatal mice (Mus) were fed to the B. marinus two times per week. Animals were cared for and handled in accordance with institutional guidelines.

Five days before measuring T_b, each toad was acclimated to room temperature and not allowed to bask. Each B. americanus was fasted for three days and each B. marinus for five days before and during the experimental period. Water was provided constantly throughout acclimation. The day before T_b was measured, each toad was placed into a linear thigmothermal gradient and allowed to habituate until the experiment began.

Each gradient used for the B. americanus measured 156 x 26 x 25 cm and for the B. marinus measured 210 x 18 x 18 cm. Each gradient had a floor of 0.3 cm thick aluminum and a top of clear acrylic. Water bowls were placed at either end of the gradient behind a styrofoam wall which prevented access to the water bowl. Strips of Handi-wipes (Softsoap Enterprises) sewn together covered the central two-thirds of the gradient floor. Each end of the Handi-wipes was placed in the water bowl. This provided a moist floor and prevented the toad from dehydrating. A subsurface heating pad maintained floor temperatures of 40 ± 1 C at one end of the gradient and room temperature in the environmental chamber kept the cold end at 15 ± 0.5 C for the B. americanus experiments and at 10 ± 0.5 C for the B. marinus experiments. The photoperiod during each experiment was the same as the acclimation photoperiod. During habituation and the experiment with B. marinus, light was provided by a point source of light directly above the gradient at the hot end. During habituation and experimentation with B. americanus, light was provided by fluorescent bulbs suspended over the gradients.

Before the experiment, the toad was removed from the gradient, induced to empty its urinary bladder, and weighed to the nearest g. At 1030 h EST, the B. americanus were injected intraperitoneally with one of the following: 0.4 mg melatonin/100 mg body mass, 2.5 mg chlorpromazine/100 mg body mass, or amphibian saline solution. The B. marinus were injected with either 0.4 mg melatonin/100 mg body mass or amphibian saline solution. In the fall, injections to B. marinus were made at 1730 h, and during the spring injections were made at 1130 h. The melatonin solution and the control saline solution for the melatonin treatment contained 1% ethanol. All solutions were made so that a volume of 1 ml was injected per 100 mg mass. These dosages of melatonin and chlorpromazine were chosen because they were the same as those used with Necturus maculosus (Hutchison, 1981). This allowed us to compare the responses of B. americanus and B. marinus with those of another amphibian.

A flexible Chromega-Alomega or copper-constantan thermocouple (Omega Engineering, Inc.) was inserted 1–2 cm into the toad's
Repeated measures ANOVA was used to determine whether \( T_b \) within a group varied over time. If it did, a Tukey test was performed to determine during which hours of the day \( T_b \) differed. Six \textit{B. americanus} or eight \textit{B. marinus} were used in each group. Values were considered significant at \( P \leq 0.05 \). Values are given as mean \( \pm \) SD.

**Results.**—Melatonin injections had no effect on mean \( T_b \) selected over the 24-h period in either \textit{B. americanus} or \textit{B. marinus}. There were no diel cycles of \( T_b \) in either the \textit{B. americanus} injected with melatonin or amphibian saline with ethanol (Fig. 1). In contrast, the \textit{B. marinus} injected with melatonin and the fall control group did select significantly different \( T_b \)s over time, but the control group during the spring did not (Fig. 2).

The \textit{B. americanus} injected with chlorpromazine selected slightly, but not significantly, higher (\( P = 0.058 \)) \( T_b \)s over the 24-h period than did the control without ethanol toads. Both groups selected \( T_b \)s that varied over time (\( P < 0.001 \)), and the pattern of temperature selection over time did not differ between the two groups (Fig. 1).

**Discussion.**—Melatonin and chlorpromazine did not decrease preferred temperature in \textit{Bufo}. This is in contrast to studies with \textit{N. maculatus} and \textit{Terrapene carolina} where the same dosages of melatonin and chlorpromazine caused a decrease in preferred temperature (Hutchison, 1981; Erskine and Hutchison, 1981). Since effects of melatonin and chlorpromazine on preferred temperature have only been published in one species of amphibian (Hutchison and Dupré, 1992), it is possible that only some species alter preferred temperature in response to chlorpromazine or exogenous melatonin. The season or time of injection of melatonin (1130 or 1730 h) had no effect on temperature selection. We used injection times at midphotophase and just prior to scotophase to test whether the time of injection of melatonin played a role in its effects on thermoregulation. This seemed likely since endogenous melatonin levels fluctuate over a 24-h period (Hutchison and Dupré, 1992). Both species of \textit{Bufo} tested to date showed no effect of melatonin on mean selected \( T_b \).

The diverse results observed in melatonin’s role on thermoregulation in \textit{Necturus} and \textit{Bufo} may be the result of phylogenetic differences. Another possibility is that \textit{Bufo}, like \textit{Necturus}, does use fluctuating endogenous melatonin levels as a thermoregulatory cue, but the pharmacological doses we used may have been ill-
appropriate to effect changes in thermoregulatory behavior. *Ambystoma tigrinum* on an LD 12:12 photoperiod at 10 C had plasma melatonin titres of approximately 140 pg/ml plasma (Gern et al., 1983). The pineal gland of *Anolis carolinensis* in scotophase had melatonin levels of several hundred pg/pineal (Underwood and Hyde, 1989). To our knowledge, plasma levels of melatonin have not been measured in *Bufo*.

Both the chlorpromazine and saline without ethanol control groups displayed differences in T_s over time. Although *B. americanus* is generally active at night (Johnson, 1987), it selected higher T_s during the last half of photophase than during late scotophase. A similar pattern was seen in the congeneric *B. cognatus* (Sievert, 1991). Adult *B. americanus* can be found in moist, warm retreats during the day, and possibly toads select for thermal as well as hydric aspects when selecting retreat sites during the day. Juvenile *B. americanus* are diurnal and thermoregulate when adequately hydrated (Tracy et al., 1993).

In contrast to other *Bufo* species, control without ethanol *B. americanus* selected cooler T_s than *B. boreas* (Lillywhite et al., 1973; Carey, 1978; Smits, 1984), *B. cognatus* (Sievert, 1991), *B. marinus* (Sievert, 1991; Mullens and Hutchison, 1992; present study), hydrated juvenile *B. americanus* (Tracy et al., 1993), and *B. valliceps* (Williams and Wygoda, 1993) in laboratory thermal gradients. Dehydration causes hypothermia in *B. valliceps* (Williams and Wygoda, 1993), but that was not the case in this study. Toads remained hydrated and frequently urinated during handling immediately after the experiment.

*Bufo americanus* did not show distinct diel cycles of temperature selection in either the melatonin or saline control with ethanol group, but *B. marinus* selected lower T_s during some portion of the afternoon than at night or early morning. Melatonin disrupts normal diel cycles of temperature selection in *N. maurus* (Hutchison, 1981) but not in the lizards *Crotaphythus collaris* (Cothran and Hutchison, 1979) or *Corvus vittifer* (Skinner, 1991). Since chlorpromazine injections of either 2 or 20 mg/Kg body mass elevated plasma melatonin levels in *N. maurus* (Rawling and Hutchison, 1993), it is interesting that diel cycles of T_s persisted in the chlorpromazine group but not in the melatonin group. In *N. maurus*, chlorpromazine de-
increased $T_o$ (Hutchison, 1981), but it did not have that effect in *B. americanus*. It was not until the second day of chlorpromazine injections that chlorpromazine obliterated the diel cycle in *N. maculosus* (Hutchison, 1981). Since we only recorded $T_o$ for 24 h after a single injection, we do not know whether multiple injections over time would have disrupted the diel cycles of temperature selection in *B. americanus*. Because of the paucity of data regarding the effect of melatonin on amphibian thermoregulatory behavior, more research is needed to elucidate the role of melatonin and the pineal gland on amphibian thermoregulation.

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**Literature Cited**


