Pharmacology Biochemistry & Behavior, Vol. 12, pp. 843–845. Printed in the U.S.A.

Circadian Rhythm in the Effect of Theophylline on the Behavioral Thermoregulation of the White Sucker, *Catostomus commersoni*

MARTIN KAVALIERS

Department of Zoology and Entomology, Colorado State University, Fort Collins, CO 80523

Received 4 December 1979

KAVALIERS, M. *Circadian rhythm in the effect of theophylline on the behavioral thermoregulation of the white sucker,* Catostomus commersoni. PHARMAC. BIOCHEM. BEHAV. 12(6) 843–845, 1980.—The temperatures selected by white suckers placed in a thermal gradient were significantly altered by interperitoneal injections of theophylline (25 mg/kg). There was a circadian variation in the effect of theophylline on temperatures selected by individual fish held under constant illumination. Depending on injection time, there was either a significant increase or significant decrease in the temperatures selected. These results are considered in relation to the possible physiological and biochemical effects of theophylline on thermoregulation and circadian rhythms.

Circadian rhythm Theophylline Behavioral thermoregulation

ALTHOUGH there have been demonstrations of diel and circadian rhythms in the preferred temperatures selected by ectotherms [12, 17, 18, 19], relatively little is known about the physiological and biochemical controls of behavioral thermoregulation [4]. Similarities seen in responses to pyrogens [19,20], putative neurotransmitters [8, 9, 10] locations of thermally sensitive nuclei, and integration of thermal inputs [11,21] suggest that the control of behavioral thermoregulation in ectotherms may be similar to that of endotherms.

Theophylline, a methylxanthine which is known to increase cyclic adenosine 3′:5′-monophosphate (cAMP) and cyclic guanosine 3′:5′-monophosphate (cGMP) by inhibiting phosphodiesterases (PDE) [2,16], affects circadian rhythms in plants and animals [2, 6, 7, 13]. Theophylline alters the body temperature of mammals, there being a circadian rhythm in it's phase-shifting effects on the body temperature of rats [6]. However, the effects of theophylline on the behavioral thermoregulation of ectotherms are not known.

The present study was undertaken to determine whether: (1) theophylline affects the behavioral thermoregulation of white suckers, *Catostomus commersoni,* an ectotherm that displays a diel rhythm of temperature selection [12,17] and, (2) there are any circadian or chronotypic variations in the effects of theophylline on the preferred temperatures selected by white suckers held under constant illumination. This is part of an effort to ascertain the controls of, and relations between circadian rhythms and thermoregulation in ectotherms.

METHOD

White suckers (10–15 g; 10–20 cm total length) were captured during July-September 1979 from a small stream at Fort Collins CO (water temperature 18–23°C) and individually placed in thermal gradients under a 12 hour light:12 hour dark cycle (LD 12:12, L=600–1800 hr at an energy fluence rate of 420 μw cm^{-2} for 270–800 nm) for a week. They were fed at a random time of day along the entire length of the gradient a pelleted food prepared by Colorado Division of Wildlife (Brood Chow no. 4). The gradient chambers were isolated from external disturbances and consisted of a trough with Plexiglas sides and an aluminum base (120×15×15 cm) that was provided with a river gravel substrate. The gradient was produced by a constant external temperature bath (10°C) at one end and an immersion heater (30°C) isolated at the other end of the chamber. A constant flow of water (15 ml min^{-1}) prevented thermal stratification (13 cm water depth). Thermoregulatory behavior of individual fish was measured by continuously recording internal body temperature with a 34 ga copper-constantan thermocouple inserted approximately 2 cm into the cloaca of the animal, with the data presented on a strip-chart recorder. The thermocouple was anchored to the fish with a suture through the dorsal fin. Descriptions of the diel rhythm of temperature selection by individual fish under LD 12:12 are provided in Kavaliers and Ralph [12] with additional values in Reynolds and Casterlin [17]. Significantly higher ($p<0.01$, t-test) temperatures were selected during D than L, with the amplitude of the rhythm being approximately 2.0°C [12]. Visual observations of fish indicated that attachment of the thermocouple did not significantly affect their mobility in the chambers. In addition, with temperatures held constant fish did not show preferences for any particular locations in the chamber.

A dose-response curve for the effects of theophylline on

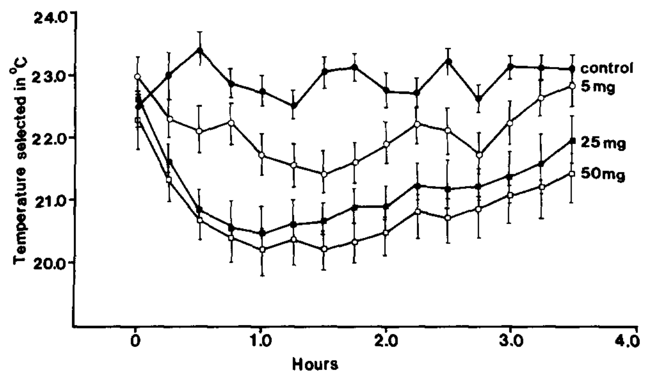

FIG. 1. Dose-response curves of the effects of theophylline on the temperatures selected by individual white suckers (n=10). Fish were maintained under LD 12:12 (L=600–1800 hr MST) and IP injected at 1300 hr. Temperature at Oh (1300 hr) was measured from fish before theophylline was administered. Vertical lines represent the standard errors of the temperatures selected.

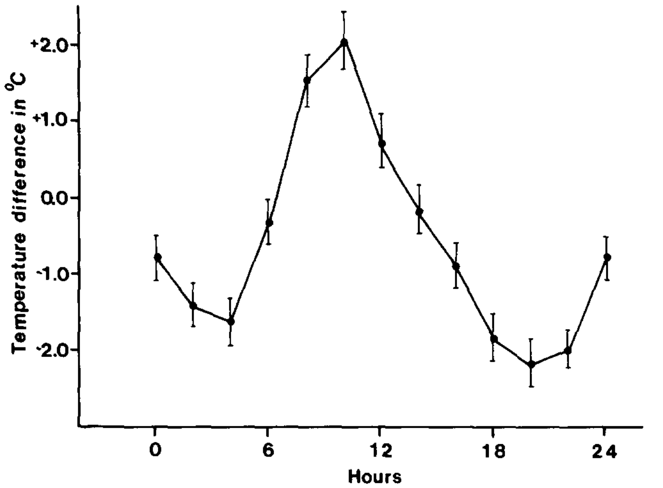

FIG. 2. Effects of theophylline (25 mg/kg^{-1}) on temperatures selected by white suckers under constant illumination over a 24 hr period. IP injections were given to different pairs of fish every 2 hr through a 24 hr period. Each point represents the mean difference in temperatures selected by five suckers 1 hr after receiving theophylline as compared to five fish receiving control (0.9% saline) injections (points are at actual injection times). Vertical lines represent the standard errors of the temperature differences.

the temperature selected by individual white suckers was established. On consecutive days ten individual fish were each interperitoneally (IP) injected at midphotophase (1300 hr M.S.T.) with 5, 25, 50, or 100 mg/kg^{-1} body weight theophylline in 250 μl saline and the temperatures the fish selected recorded for 12 hr. Saline (0.9% NaCl) injected fish were used as controls. Dose-response determinations were performed at midphotophase (L) because the smallest fluctuations in the temperatures selected occurred during this portion of the diel cycle [12]. Determinations were not carried out during the dark period because of the confounding effects that occur when fish experience brief light pulses.

After establishing an effective dose (25 mg/kg^{-1}) determinations were made of the responsiveness to theophylline of fish over a 24 hr period of constant illumination (LL). Individual fish were held under LD 12:12 for 7 days followed by constant illumination (LL 420 μw cm^{-2}) for 2 days. Fish were fed at a random time of day throughout. Under LL every 2 hr for a 24 hr time interval a different pair of fish, with each fish in it's own gradient, were given control and theophylline injections and the temperatures they selected were recorded for 2 hr. These procedures were repeated five times such that each of the 12 points that provided a measure of responsiveness for a 24 hr time period is based on 5 samples. Due to the phase-shifting effects of experimental manipulations on circadian rhythms the same pair of fish could not be given repetitive injections for a 24 hr period.

RESULTS

Dose-response curves of theophylline on the temperatures selected by individual white suckers are shown in Fig. 1. In the figure hour 0 is at midphotophase (1300 hr M.S.T.) and gives temperatures selected before injection. Theophylline caused a dose-dependent reduction in temperatures selected (Fig. 1). Lowest temperature selection occurred 1–2 hr after injection (ΔT -2.5°C for 25 and 50 mg/kg^{-1}), followed by a slow rise in temperatures selected. There were no significant differences in response between 25 and 50 mg/kg^{-1}. After 4–5 hr the normal diel pattern of temprature selection [12] was resumed. Control observations revealed no apparent long-term (12–24 hr) effects of theophylline on temperature selection.

Theophylline (25 mg/kg^{-1}) also affected the temperature selected by fish held under LL. An endogenous, apparently circadian rhythm, in the effects of theophylline on temperature selection is shown in the composite plot (Fig. 2) derived from all of the responses of the individual pairs of fish. The effects of theophylline on temperature selection, rather than the actual temperatures selected are shown. This eliminates any confounding effects arising from endogenous or random fluctuations in temperature selection. Data are presented as the absolute mean difference between temperatures selected by theophylline and control treated fish 1 hr after injection. For example, the response of the fish injected at hour 0 (MST) is seen as a decrease (ΔT -0.8°C) in temperatures selected 1 hr later. During a 24 hr period there was a rhythmic variation in the effects of theophylline, initially a depression of temperatures selected (ΔT -2.0 to -2.5°C) followed by a rise (ΔT $+2.0$ to $+2.5$°C) and then a decline.

DISCUSSION

The results show that theophylline affects the behavioral thermoregulation and preferred temperatures selected by white suckers in a circadian dependent fashion. In fish held under LL there was rhythmic variation in the effects of theophylline—either a significant increase or a significant decrease in the temperatures selected, depending on when theophylline was injected. The significant increases and decreases in temperatures selected under LL corresponded approximately to the light and dark portions, respectively, of the previous LD 12:12 cycle the fish were maintained under. Discrepancies in the relations arise from the use of a composite data plot as well as from differences in period length from LL and LD conditions. These observations, however, do suggest that there is an underlying circadian basis and, or mechanism in the preferred temperatures selected and behavioral thermoregulation of this teleost fish.

A number of other pharmacological agents, as well as pyretic and anti-pyretic substances, have been shown to affect the behavioral temperature selection of fishes [8, 9, 10, 15, 17, 18, 19, 20]. Pharmacological studies were primarily concerned with the manipulation and quantification of biogenic amine activity and did not examine the possibility of circadian variations in effects [8, 9, 10]. Theophylline has been shown to influence circadian rhythms of plants and animals. Pulses of theophylline shifted the phases of body temperature in rats [6] and leaf movement in plants [13,14]. These phase-shifting effects varied in a circadian fashion, there being either an advance or delay, depending on when theophylline was administered. These effects are similar to the circadian variations in the actions of theophylline on the temperature selection of white suckers. Pyrogens, which can induce behavioral fever in fishes [18, 19, 20], are also reported to show circadian variations in their ability to induce fever in rats [21]. This suggests the possibility of similar controls of diel and circadian variation in body temperatures of ectotherms and endotherms.

Theophylline is known to increase cAMP and cGMP by inhibiting PDE activity [2,16]. Injections of theophylline in goldfish, flounders, and trout significantly elevated their plasma levels [16]. Both cAMP and cGMP have been implicated in the control of body temperature region of mammals [4,11]. Cummings [5] has proposed a biochemical model of circadian rhythms in which cAMP, ATP, membrane bound adenyl cyclase (AC), PDE and AMP are major constituents. Limit-cycle oscillations are brought about by allosteric feedback actions of cAMP on AC and AMP. Perturbations of any of these components should result in phase-dependent shifts in circadian rhythmicity. However, there are also suggestions that the effects of theophylline are caused by alterations in cellular CA^{++} levels [2] or brain serotonin levels [1], both of which have been implicated in the control of thermoregulation [11]. cAMP, cGMP, $CA,^{++}$ and prostaglandins [11,19] have also been implicated in the actions of other thermoregulatory agents such as pyrogens [11], that have circadian variations in their effects in mammals [21]. Investigations with all of the aforementioned substances have to made before any definite conclusions can be drawn.

ACKNOWLEDGEMENTS

This work was supported by a NIH grant (NS 12257) to C. L. Ralph. I thank Drs. T. Gorell and C. L. Ralph for their reviews of the manuscript.

REFERENCES

1. Berkowitz, B. and J. Spector. The role of brain serotonin in the pharmacologic effects of methyl xanthines. In: *Serotonin and Behavior*, edited by J. Barchas and E. Usdin. New York: Academic Press, 1973, pp. 137–162.
2. Bolling, I., K. Mayer, W. Mayer and W. Engleman. Effects of cAMP, theophylline, imidazole, and 4-(3,4-Dimethoxybenzyl (-2-Imidasolidone)) on the leaf movement rhythm of *Trifolium repens*–a test of the c-AMP hypothesis of circadian rhythms. *Planta* **141:** 225–230, 1978.
3. Bünning, I. *The Physiological Clock*. New York: Springer-Verlag, 1977.
4. Crawshaw, L. I. Temperature regulation in vertebrates. *Ann. Rev. Physiol.* **42:** 473–491, 1980.
5. Cummings, F. W. A biochemical model for the circadian clock. *J. theor. Biol.* **55:** 455–470, 1975.
6. Ehert, C. F., V. R. Potter and K. W. Dobra. Chronotypic action of theophylline and of pentobarbitol as circadian zeitgebers in the rat. *Science* **188:** 1212–1215, 1975.
7. Feldman, J. F. Circadian periodicity in *Neurosporsa;* alterations by inhibitors of cyclic AMP phosphodiesterase. *Science* **190:** 789–790, 1975.
8. Fryer, J. N. and D. M. Ogilvie. Alterations of thermoregulatory behavior in fish by 5-hydroxytryptamine. *Pharmac. Biochem. Behav.* **8:** 129–132, 1978.
9. Fryer, J. N. and D. M. Ogilvie. Temperature selection responses of Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri*, after exposure to pentobarbitol. *Comp. gen. Pharmac.* **5:** 11–116, 1974.
10. Green, M. D. and P. Lomax. Behavioural thermoregulation and neuroamines in fish (*Chromus chromus*). *J. Thermal. Biol.* **1:** 237–240, 1977.
11. Hammel, H. T., L. I. Crawshaw and H. D. Cabanac. The activation of behavioral responses in the regulation of body temperature in vertebrates. In: *The Pharmacology of Thermoregulation*, edited by P. Lomax and E. Schonbaum. New York: Basel, pp. 33–45, 1973.
12. Kavaliers, M. and C. L. Ralph. Pineal involvement in the control of behavioral thermoregulation of the white sucker, *Catostomus commersoni*. *J. exp. Zool.*, in press.
13. Mayer, W., R. Gruner and H. Strubel. Periodenverlangerung und pasenverschiebungen der Circadianen Rhythmik van *Phaseolus coccineus* L. durch Theophyllin. *Planta* **125:** 141–148, 1975.
14. Mayer, W. and I. Scherer. Phase shifting effect of caffeine in the circadian rhythm of *Phaseolus coccineus* L. *Z. Naturforsch.* **30:** 855–856, 1975.
15. Ogilvie, D. M. and J. N. Fryer. Effect of sodium pentobarbitol on the temperature selection respones of guppies (*Poecilia reticulata*). *Can. J. Zool.* **49:** 949–951, 1971.
16. Porthe-Nibelle, J. and R. Lahlou. Cyclic AMP levels in plasma and in intestinal mucosa of fishes, with special reference to the effects of theophylline and catecholamines. *Gen. Comp. Endocr.* **36:** 609–617, 1978.
17. Reynolds, W. W. and M. E. Casterlin. Behavioral thermoregulation and diel activity in white sucker (*Catostomus commersoni*). *Comp. Biochem. Physiol.* **59:** 261–262, 1978.
18. Reynolds, W. W. and M. E. Casterlin. Behavioral thermoregulation and the 'final preferendum' paradigm. *Am. Zool.* **19:** 211–224, 1979.
19. Reynolds, W. W. and M. E. Casterlin. The pyrogenic responses of non-mammalian vertebrates. In: *Pyretics and Antipyretics. Handbook of Experimental Pharmacology*, edited by A. S. Milton. New York: Springer-Verlag, 1980.
20. Reynolds, W. W., M. E. Casterlin and J. B. Covert. Behavioral fever in teleost fishes. *Nature* **259:** 41–42, 1976.
21. Williams, B. A., R. L. George, P. E. Penn and M. J. Weber. Circadian effect of pyrogen induced fever in rats. *Fedn Proc.* **29:** 1077, 1980.