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Diurnal Fluctuations in Acetylserotonin Methyltransferase (ASMT) Activity in the Pineal Gland of the Steelhead Trout (*Salmo gairdneri*) (38359)

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In 1958 Lerner isolated a "blanching principle" from bovine pineal extracts (1), which proved to be 5-methoxy-*N*-acetyltryptamine (melatonin). Subsequent investigations into the properties of melatonin showed effects upon the plasma levels of prolactin and thyroid stimulating hormone (2,3). The importance of these two hormones in mammals is well appreciated, and in teleosts they are strongly implicated in the physiological predisposition for migration (4, 5). Another important element in migratory behavior is the influence of photoperiod. Since the pineal gland of teleosts is capable of photoreception (6), and is a prime source of melatonin, the pineal may act as a photochemical transducer, instrumental in the proper timing of essential endocrine adjustments prior to migration. Photoperiod dependence of the activity of pineal acetylserotonin methyltransferase (ASMT), the enzyme catalyzing the final step in melatonin synthesis, has already been shown to exist in rats (7).

It was the purpose of this study to determine whether a diurnal fluctuation in ASMT activity exists in the pineal of the migratory species of trout, *Salmo gairdneri* (steelhead trout), when exposed to natural photoperiod.

Methods and Materials. Juvenile steelhead trout averaging 20 cm in length were maintained in an outdoor tank at the Alsea Trout Hatchery near Alsea, Oregon.

Sampling was done by weighing and sacrificing the fish, and then excising the top of the skull. Two such skull fragments were placed in a single vial that was kept on dry ice. The two skull fragments later yielded two pineals that were pooled and assayed as a single sample. Pineal removal usually began at noon and was repeated every 4 hours over a 24 hr period. At each 4 hour sampling time ten fish were processed as described above. This procedure was repeated on five separate days over a 1 month period. In order to minimize the obvious problem of changing photoperiod over a 4 week period, all sampling was done approximately two weeks before and two weeks after the summer solstice.

The procedure employed for the assay of ASMT activity was a modification of a procedure previously described by Hafeez and Quay (8). It relies upon the formation of ^{14}C-melatonin when a pineal homogenate is incubated with *N*-acetylserotonin and ^{14}C-*S*-adenosylmethionine. The pineals employed in this study were homogenized in 0.35 ml of a sodium phosphate buffer, pH 7.9. Samples were kept at 4° until incubation. A ground-glass, hand-operated tissue grinder was employed for all homogenizations. Aliquots (0.1 ml) of homogenate were transferred to two 15 ml glass-stoppered centrifuge tubes, and 0.05 ml of a 1 μg/ml solution of *N*-acetylserotonin (Regis Chemical Co., Chicago, IL.) was added to one of the tubes, while the control tube received an equal volume of doubly-distilled water. Both tubes then received 50 mμCi of ^{14}C-*S*-adenosylmethionine (48 mCi/mmole, Amersham/Searle, Arlington Heights) and were transferred to a Dubnoff shaking incubator. After incubation for 15 min at 25°, 0.35 ml of a 0.2 *M* borate buffer, pH 10, and 2.5 ml of an 80–20% toluene–isoamyl alcohol solution were added to the centrifuge tubes. The samples were hand-shaken for 2 min and then the particulate matter separated by centrifugation. Two milliliters of the organic phase was transferred from each tube to separate scintillation vials and one milliliter of 95% ethanol and 10 ml of a liquid scintillation fluid (339, 6 g naphthalene, 24.75 g 2,5-diphenyloxazole, 0.3081 g dimethyl POPOP, in three liters of 1,4-dioxane) was

added to each vial. All counting was done on a Packard Tri-Carb liquid scintillation counter (Model 3380). A third 0.1 ml aliquot from the same homogenate was used to determine total sample protein as described by Lowry *et al.* (9). Identification of labeled melatonin was accomplished by thin layer chromatography as described by Klein and Notides (10). The Student's *t*-test was employed for all statistical analysis.

Results. A diurnal fluctuation in pineal ASMT activity was detected in juvenile steelhead trout. This rhythm is evident when ASMT activity is expressed as a function of total sample protein. The maximum activity occurred at 4:00 AM and the minimum activity was observed at 8:00 PM (Table I). These points were significantly different ($P < 0.05$). No relationship between ASMT activity and mean body weight was evident (Table I).

Discussion. At the time of year this study was conducted the peak ASMT activity occurred about six or seven hours after onset of darkness, and the low activity occurred about one hour before the onset of darkness. The timing in steelhead was similar to that observed by Axelrod in rats (7), which strongly suggests an influence of photoperiod upon steelhead pineal ASMT activity.

The magnitude of the diurnal fluctuation reported by Axelrod was from 1.6- to threefold (7), whereas the change reported here is twofold. The greater fluctuations seen in rats might be due to an accumulative effect of the artificial lighting regimen or, perhaps, larger changes in steelhead ASMT might have been masked by the variability encountered. Lighting undoubtly played a role since all fish were held outdoors under natural lighting conditions, which meant

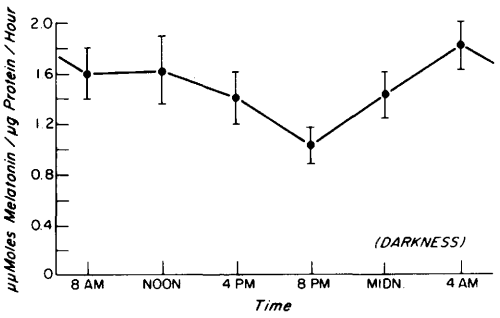

FIG. 1. Melatonin production in homogenates of pineal glands collected at six different sampling times over a 24 hr period. Points represent mean values ± SE. The approximate period of darkness is indicated at the bottom.

the photoperiod was changing from day to day. Another source of variation may also have been sex differences, since the sex of the fish was not determined.

The inconsistency encountered when the pineal ASMT activity was evaluated as a function of mean body weight could be attributable to (a) incomplete pineal removal, (b) the size variations in the fish used, (c) the absence of any strict correlation between pineal size and (or) activity and body weight.

The presence of a diurnal fluctuation in pineal ASMT activity in juvenile steelhead trout, which may be photoperiod oriented can lead to some interesting speculations. Evidence from mammalian studies linking melatonin with the pituitary hormones TSH and prolactin (2,3), coupled with strong evidence that these hormones are responsible for such things as salinity preference (11) and osmoregulation (12) in anadromous fish, suggests a photoperiod-pineal-endocrine relationship in the migratory behavior of teleosts. Hafeez and Quay were unable to find a light

TABLE I. Melatonin Production over a 24 hr Time Period Expressed as a Function of Total Pineal Protein and Mean Body Weight.*

Time	Number of samples	Moles ($\times 10^{-12}$) melatonin hr/μg pineal protein	Number of samples	Moles ($\times 10^{-12}$) melatonin hr/mg mean body wt.
NOON	16	1.49 ± 0.54	20	0.51 ± 0.19
4 PM	12	1.34 ± 0.31	21	0.80 ± 0.39
8 PM	15	0.98 ± 0.11**	23	0.52 ± 0.17
MIDN.	22	1.45 ± 0.32	23	0.50 ± 0.14
4 AM	20	1.91 ± 0.46**	22	0.90 ± 0.28
8 AM	21	1.59 ± 0.61	23	0.97 ± 0.44

*Values given are means ± standard error.

**The two values with this superscript were found to be significantly different using the Student *t* test ($P < 0.05$).

response in pineal ASMT activity in rainbow trout, a non-migratory form of *Salmo gairdneri*. Perhaps the steelhead pineal is more responsive to photoperiod, which could contribute to its migratory behavior.

In conclusion, it should be noted that all assays included in this study were conducted under similar conditions and within a short period of time, indicating that the changes observed might be a result of the amount of enzyme present, a conclusion made by Axelrod (7) on the basis of experiments in which puromycin blocked the dark-induced rise in ASMT activity in rats.

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