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Diurnal impact of locomotory activity and melatonin and *N*-acetylserotonin treatment on blood metabolite levels in the rainbow trout

BY

T. M. JOHN, F. W. H. BEAMISH and J. C. GEORGE

(Department of Zoology, University of Guelph, Guelph, Ontario, Canada)

In rainbow trout forced to swim continuously at sustained speeds for six weeks, selected doses of melatonin or *N*-acetylserotonin (1.25 and 5.0 mg/kg body weight) injections caused no change in haematocrit. Melatonin did not produce any significant change in plasma glucose level either in the photophase or in the scotophase. However, diurnal variations were observed in the effect of melatonin on plasma free fatty acids (FFA). Melatonin was ineffective in causing any change in plasma FFA level during photophase but during scotophase, the higher dose (5.0 mg/kg) produced an increase in FFA while the lower dose (1.25 mg/kg) had no effect. *N*-acetylserotonin administration produced diurnal variation in its effect on both plasma glucose and FFA. The higher dose of *N*-acetylserotonin brought about a drop in plasma glucose level during photophase, but both doses were ineffective during scotophase. *N*-acetylserotonin produced no change in FFA during photophase, but during scotophase tended to lower FFA level. It is suggested that exercise shortens the time required to cause a hypoglycemic effect of *N*-acetylserotonin during photophase, blocks FFA release-inhibiting action of melatonin observed in photophase, and minimizes the time required for the FFA mobilizing action of melatonin in scotophase.

Introduction

The involvement of the pineal gland in the modulation of several neuroendocrine and behavioural mechanisms, has been well documented (see REITER, 1982). There is considerable experimental evidence to indicate that the pineal, through the mediation of its hormone, melatonin, is involved in physiological and behavioural thermoregulation in different Vertebrates (BINKLEY *et al.*, 1971; JOHN *et al.*, 1978; RALPH *et al.*, 1979; ERSKINE & HUTCHISON, 1981). In Fish, pinealectomy has been shown to disrupt diel rhythms of behavioural thermoregulation leading to an elevation of preferred environmental temperature (KAVALIERS & RALPH, 1980).

Of the several indole compounds present in the pineal gland, the most extensively studied are melatonin (5-methoxy-*N*-acetyltryptamine) and serotonin (5-hydroxytryptamine). Intraperitoneal injections of serotonin were found to cause an increase in locomotory activity in sockeye salmon, *Oncorhynchus nerka*, during scotophase whereas melatonin caused a decrease during photophase (BYRNE, 1970).

In order to see if the indoleamine-mediated changes in locomotory activity observed by BYRNE (1970), could be correlated with changes in the blood levels of glucose and free fatty acids (FFA), the main fuels used for muscular energy, we (1980) studied the effects of more or less identical dosage of the indoleamines as was used by BYRNE (1970), on these metabolites in the rainbow trout. It should be mentioned here that instead of serotonin (BYRNE, 1970) we preferred to use its immediate precursor, *N*-acetylserotonin (*N*-acetyl-5-hydroxytryptamine). In addition to the low dosage used by BYRNE (1970), we used a higher dosage (5.0 mg/kg body weight) which was very much lower than the dosage of melatonin (8.0 mg/kg body weight) used by ERSKINE & HUTCHISON (1981) for turtle.

In our previous study (1980) on non-exercised rainbow trout, we reported that melatonin did not alter plasma glucose level but lowered plasma FFA during photophase at 25 min post-injection. In scotophase, only the higher dose of melatonin (5.0 mg/kg body weight) was effective in causing an increase in plasma FFA at 90 min. The lower dose of *N*-acetylserotonin (1.25 mg/kg body weight) decreased plasma glucose level during photophase but the higher dose did so at 90 min post-injection in both photophase as well as scotophase. During scotophase, both doses of *N*-acetylserotonin caused an increase in plasma FFA at 90 min but had no effect at 25 min. Only with the higher dose was an increase in FFA obtained during photophase. These observations prompted us to suggest that *N*-acetylserotonin is more potent as a lipolytic agent in trout than melatonin.

In the present study, it was considered to be of interest to study the diurnal effect of the two indoleamines on the circulating levels of the two metabolites in continuously exercised rainbow trout under identical experimental conditions as in our (1980) previous investigation.

Materials and Methods

A total of 120 adult rainbow trout, each weighing between 230-250 g, were obtained from *Goosen's Trout Farm* Ltd., Otterville, Ontario. They were maintained indoors in an annular trough (45 cm wide × 20 cm deep × 1 000 cm circumference) supplied with non-chlorinated running water (10 °C). A paddle wheel installed in the channel and controlled by an infinitely variable hydraulic transmission was used to generate a water current of 25 cm/sec. The indoor lighting (supplied by incandescent light, 90 lux at the water surface) was set to provide a daily photoperiod of 16 h (08-24 h). Fish were fed to satiation each morning on a commercial trout food (*Martin Feed Mills* Ltd., Ontario). Oxygen concentration was at or near air saturation and ammonia below 0.1 mg/litre.

All fish were forced to swim continuously at the sustained speed of approximately one body length/sec (BEAMISH, 1978) in the channel for a period of 6 weeks, at the end of which they received intraperitoneal injections of the specific test substance. Two sets of experiments using 60 fish in each were performed to test for the metabolic effects of melatonin in one and for those of *N*-acetylserotonin in the other.

In the first set, three groups of 10 fish each were injected intraperitoneally with either 0 (control), 1.25 or 5.0 mg melatonin/kg body weight, in 0.3 ml of vehicle solution per fish. The injections in the above three groups were made during the latter half of the photophase (19-21 h). Following injection, each fish was kept separately in a 75 litre aquarium for 25 min following which it was removed and bled. This procedure was repeated using three other groups (10 each) of fish, with injections given during the latter half of the scotophase (05-07 h). An indirect dim light beam from an adjacent room was used to aid in capturing the fish from water during

the scotophase. During injections and bleeding in the scotophase, the fish were blind folded with the help of wet paper towels.

The experimental procedure for the second set of experiments was identical to the first except that melatonin was replaced by *N*-acetylserotonin.

In all the experiments bleeding was carried out by withdrawing blood from the anterior dorsal aorta by means of EDTA-coated syringes. A small amount of blood was used for haematocrit, while the rest was centrifuged and the plasma stored at −70 °C for the assay of glucose and FFA.

Plasma glucose was measured using the "enzymatic colorimetric" method of *Sigma* (Kit No. 510, *Sigma Chemical* Co., St. Louis, Missouri, USA) and FFA by employing the semi-automated colorimetric method of ANTONIS (1965) using a *Technicon* Auto Analyzer system.

Test Substances

Crystalline melatonin *(Sigma)* was dissolved in absolute ethyl alcohol and subsequently diluted with 0.6 % saline to a final alcohol concentration of 4.0 % (v/v). *N*-acetylserotonin *(Sigma)* was directly dissolved in 0.6 % saline. Both melatonin and *N*-acetylserotonin solutions were freshly prepared just prior to use. The control fish in each experiment received the corresponding vehicle solution.

Statistics

Analysis of variance (STEEL & TORRIE, 1960) was employed. $P < 0.05$ was considered acceptable as significant.

Results

Results obtained from injection of melatonin and that of *N*-acetylserotonin are presented in Tables I and II, respectively.

Neither melatonin nor *N*-acetylserotonin had any significant effect on haematocrit values either in the scotophase or photophase.

TABLE I. *Diurnal variation in the effect of melatonin injections on haematocrit, plasma glucose and free fatty-acid levels in exercised trout*, Salmo gairdneri. Blood samples were drawn at 25 min post-injection. Values represent mean ± SEM.

Parameter	Photophase			Scotophase		
	Control	Melatonin 1.25 mg/kg b.wt	Melatonin 5 mg/kg b.wt	Control	Melatonin 1.25 mg/kg b.wt	Melatonin 5 mg/kg b.wt
Haematocrit (%)	38.80 ±4.73	42.80 ±0.87	40.80 ±1.88	38.15 ±1.86	37.35 ±1.28	34.35 ±1.50
Plasma glucose (mg %) .	151.78 ±10.20	145.99 ±8.10	143.44 ±5.81	109.05 ±10.44	100.39 ±5.00	112.23 ±9.55
Plasma FFA (μEq/litre)..	209.34 ±7.45	171.22 ±37.94	215.03 ±10.48	121.81 ±13.60	132.24 ±13.74	157.52[a] ±9.82

[a] $P < 0.05$ when compared to the respective control.

TABLE II. *Diurnal variation in the effect of* N-*acetylserotonin on haematocrit, plasma glucose and free fatty-acid levels in exercised trout*, Salmo gairdneri. The blood was collected at 25 min post-injection. Values represent mean ± SEM.

Parameter	Photophase			Scotophase		
	Control	*N*-acetyl serotonin 1.25 mg/kg b.wt	*N*-acetyl serotonin 5 mg/kg b.wt	Control	*N*-acetyl serotonin 1.25 mg/kg b.wt	*N*-acetyl serotonin 5 mg/kg b.wt
Haematocrit (%)	39.50 ±0.78	38.88 ±1.23	43.80 ±1.94	38.70 ±2.21	38.05 ±2.36	39.00 ±1.72
Plasma glucose (mg %) .	104.80 ±5.80	97.28 ±4.58	83.80[a] ±4.65	85.00 ±5.97	84.59 ±4.95	84.37 ±5.37
Plasma FFA (μEq/litre)..	202.95 ±30.87	279.81 ±36.46	199.56 ±22.39	237.71 ±33.99	156.61[a] ±13.83	172.18 ±23.49

[a] $P < 0.05$ when compared to the control.

Melatonin did not produce any significant change in plasma glucose levels at either phase of the day-night cycle.

Neither dose of melatonin had any significant effect on FFA level during the photophase. During scotophase, however, the higher dose caused an increase in FFA level, while the lower dose produced no significant change.

The higher dose of *N*-acetylserotonin produced a significant drop in the plasma glucose level during the photophase, but both the doses were ineffective in producing any significant change during the scotophase.

Neither dose of *N*-acetylserotonin produced any significant alteration in the FFA level during the photophase, while a drop was obtained with the lower dose, during the scotophase. Although an apparent drop in FFA with the higher dose of *N*-acetylserotonin was observed during the scotophase, the difference was not statistically significant.

Discussion

In our previous investigation on rainbow trout (1980) we reported that neither melatonin nor *N*-acetylserotonin had any significant effect on haematocrit. In the present study too, using continuously swimming trout and with the same dosage of the test substances, we obtained no change in haematocrit during photophase or scotophase. This indicates that the indoleamines used did not cause any osmotic problem even in trout swimming at sustained speeds.

In non-exercised trout melatonin had no effect on plasma glucose levels either in the photophase or scotophase. The same was the case with exercised trout indicating that exercise had not influenced the effect of melatonin. In contrast, DELAHUNTY *et al.* (1978) reported hyperglycemia in non-exercised goldfish maintained on a 16-h daily photoperiod and given 16 daily injections of melatonin (250 μg/fish/day) at the middle of the photophase. Similarly, hyperglycemia was reported in the

pigeon in response to melatonin treatment (McKeown *et al.*, 1975). These differences in the effect of melatonin could be due to differences in the experimental conditions or may also be a case of species difference. It is possible that, since in long term continuous muscular activity in fish, lipid forms the main source of energy (see Bilinski, 1974; George & Stevens, 1978), carbohydrate is spared and consequently the hyperglycemic effect of melatonin also becomes less pronounced. However, with the higher dose of *N*-acetylserotonin given in the photophase at 25 min post-injection in exercised trout, we have obtained a significant drop in plasma glucose. Since such a hypoglycemic effect was observed only at 90 min post-injection in the non-exercised trout (John *et al.*, 1980), it may be inferred that perhaps exercise minimizes the time required by *N*-acetylserotonin to manifest the hypoglycemic effect. The hypoglycemic effect was obtained only during the photophase. A relatively high-potency for *N*-acetylserotonin action as a hypoglycemic agent during photophase was also observed in our previous study with non-exercised trout and we suggested that this difference was due to a possible diurnal variation in hydroxyindole-*O*-methyltransferase (HIOMT) activity (John *et al.*, 1980). This suggestion was based on the finding that pineal HIOMT activity (Smith & Weber, 1974 & 1976) and plasma melatonin concentration (Gern *et al.*, 1978) in trout are at their peak during scotophase and low during photophase. Since HIOMT is known to be the enzyme that catalyses the conversion of *N*-acetylserotonin to melatonin, the exogenous *N*-acetylserotonin given during scotophase when HIOMT was high, could rapidly get converted to melatonin before hypoglycemia sets in. On the other hand, *N*-acetylserotonin given during photophase when HIOMT was low could remain without conversion to melatonin for an extended period of time, thereby inducing hypoglycemia.

In our previous study (1980), with non-exercised trout, we reported a drop in plasma FFA level at 25 min post-injection with melatonin during photophase. In the present study with exercise, such a diminution in plasma FFA level was not obtained and the results were comparable to that obtained at 90 min post-injection during photophase in the non-exercised fish. This suggests that exercise either blocks the FFA release-inhibiting action of melatonin or brings about quick recovery from the initial inhibitory effect of melatonin on FFA release in photophase. At 25 min post-injection of both the high and low doses of melatonin during scotophase, there was no significant change in plasma FFA in non-exercised trout (John *et al.*, 1980) but in the exercised ones, there was a significant increase in plasma FFA following the administration of the higher dose. On the other hand, in the non-exercised fish, an increase in plasma FFA was observed at 90 min post-injection of the higher dose of melatonin (John *et al.*, 1980). Exercise, therefore, appears to have reduced the time required for producing the FFA mobilizing effect of melatonin. However, it is possible to state that melatonin, as was observed in the resting pigeon (John & George, 1976), causes FFA mobilization both in non-exercised as well as exercised trout only when administered in the scotophase.

As for the effect of *N*-acetylserotonin on plasma FFA, it was revealed that in exercised fish at 25 min post-injection during scotophase, FFA level actually dropped, whereas in the non-exercised fish (John *et al.*, 1980), there was no change, but with both the doses at 90 min post-injection in the scotophase, there was an increase in FFA (only the higher dose produced an increase in the photophase). The significance of the difference between the action of *N*-acetylserotonin in the exercised and non-exercised fish is not clear. It appears that the drop in plasma FFA at 25 min post-injection of *N*-acetylserotonin in exercised fish during scotophase was caused by

increased extraction of FFA by the muscles, especially the red muscle, perhaps prompted by increased fatty acid oxidation as well. It has been shown in the pigeon that after mid-scotophase, the plasma FFA decreases as the muscle FFA increases (JOHN & GEORGE, 1972). On the other hand, in our previous study (1980) with non-exercised trout, we observed that at 90 min post-injection with both doses, the effect of *N*-acetylserotonin showed up by registering an increase in plasma FFA during the scotophase. We had, therefore, suggested that *N*-acetylserotonin was more potent as a lipolytic agent than melatonin. In the light of these observations, it appears that, with a longer lapse of time such as 90 min post-injection, the lipolytic effect of *N*-acetylserotonin was manifested by the increase in plasma FFA in the non-exercised fish in which, unlike in the exercised fish, the muscle presumably was not actively extracting FFA.

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References

ANTONIS, A. (1965) *J. Lipid Res.* **6**, 307-312.
BEAMISH, F. W. H. (1978) in *Fish Physiology* (HOAR, W. S. & RANDALL, D. J., eds) vol. 7, pp. 101-137. Academic Press, New York.
BILINSKI, E. (1974) in *Biochemical and Biophysical Perspectives in Marine Biology* (MALINS, D. C. & SARGENT, J. R., eds) vol. 1, pp. 239-288. Academic Press, New York.
BINKLEY, S., KLUTH, E. & MENAKER, M. (1971) *Science (Washington)* **174**, 311-314.
BYRNE, J. E. (1970) *Can. J. Zool.* **48**, 1425-1427.
DELAHUNTY, G., BAUER, G., PRACK, M. & DE VLAMING, V. (1978) *Gen. Comp. Endocrinol.* **35**, 99-109.
ERSKINE, D. J. & HUTCHISON, V. H. (1981) *Physiol. Behav.* **26**, 991-994.
GEORGE, J. C. & STEVENS, E. D. (1978) *Env. Biol. Fish.* **3**, 185-191.
GERN, W. A., OWENS, D. W. & RALPH, C. L. (1978) *Gen. Comp. Endocrinol.* **34**, 453-458.
JOHN, T. M. & GEORGE, J. C. (1972) *J. Interdiscipl. Cycle Res.* **3**, 33-37.
JOHN, T. M. & GEORGE, J. C. (1976) *Endocr. Exper.* **10**, 131-137.
JOHN, T. M., ITOH, S. & GEORGE, J. C. (1978) *Horm. Res.* **9**, 41-56.
JOHN, T. M., BEAMISH, F. W. H. & GEORGE, J. C. (1980) *Comp. Biochem. Physiol.* **65***C*, 105-109.
KAVALIERS, M. & RALPH, C. L. (1980) *J. Exp. Zool.* **212**, 301-303.
MCKEOWN, B. A., JOHN, T. M. & GEORGE, J. C. (1975) *Endocr. Exp.* **9**, 263-268.
RALPH, C. L., FIRTH, B. T., GERN, W. A. & OWENS, D. W. (1979) *Biol. Rev.* **54**, 41-72.
REITER, R. J. (Ed.) (1982) *The Pineal and its Hormones.* Alan Liss Inc., New York.
SMITH, J. R. & WEBER, L. J. (1974) *Proc. Soc. Exp. Biol. Med.* **147**, 441-443.
SMITH, J. R. & WEBER, L. J. (1976) *Can. J. Zool.* **54**, 1530-1534.
STEEL, R. G. D. & TORRIE, J. H. (1960) *Principles and Procedures of Statistics.* McGraw-Hill, New York.

T. M. JOHN, F. W. H. BEAMISH & J. C. GEORGE
Department of Zoology, University of Guelph
Guelph, Ontario, Canada N1G 2W1.