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INVOLVEMENT OF MELATONIN AND THYROID HORMONES IN THE CONTROL OF SLEEP, FOOD INTAKE AND ENERGY METABOLISM IN THE DOMESTIC FOWL

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SUMMARY

1. Growing male domestic fowl of an egg-laying strain were fed *ad libitum* and injected intraperitoneally with melatonin or intramuscularly with triiodothyronine (T3) to study the effects on sleep, food intake, blood glucose, e.e.g., oxygen consumption and carbon dioxide production.

2. Melatonin caused a dose-related depression of food intake with sleep and aphagia lasting for 2½ hr following 8 mg, drowsiness and greatly reduced intake following 4 and 2 mg and a slight reduction in food intake after 1 mg.

3. T3 injection was followed by increased feeding within the range 50–200 μg. The higher dose (200 μg) completely prevented the effects of 10 mg melatonin injected simultaneously.

4. Melatonin (10 mg) depressed plasma glucose levels whereas T3 (200 μg) elevated blood glucose.

5. Either darkness or melatonin (10 mg) caused an increase in amplitude and a decrease in frequency of the e.e.g.

6. Birds fasted for 3 hr before injection showed significantly lower oxygen consumption and carbon dioxide production when given melatonin (10 mg); T3 had no effect within the 4 hr period after injection and did not modify the effects of melatonin.

7. It is postulated that the rapid effects of melatonin and T3 which were observed result from direct effects of these hormones on the central nervous system.

INTRODUCTION

Many species of birds, including domestic fowl, do not normally eat during darkness, as long as this does not extend for more than about 12 hr (Savory, 1979). Nocturnal aphagia is unlikely to be due to an inability to find the food because fowl

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do eat during longer periods of darkness. There is more likely to be a dark-induced change in the bird's control systems which results in suppression of feeding. One such change is the great increase in the secretion of melatonin by the pineal gland during darkness (Pelham, 1975). In addition to the diurnal rhythms of feeding there is a cycle of metabolic rate in fowl, with oxygen consumption up to 1·7 times higher in the middle of the day compared to the middle of the night (Berman & Meltzer, 1978). Thyroid hormones play an important role in the control of metabolic rate (Singh, Reineke & Ringer, 1968) and the levels of thyroxine (T4) and triiodothyronine (T3) in blood show a consistent diurnal change, with high levels during the photoperiod (Sadovsky & Bensadoun, 1971; Klandorf, Sharp & Duncan, 1978).

Experiments have therefore been carried out on the interrelationships between melatonin, thyroid hormones, food intake and related phenomena. The results of experiments 1 and 2 have been briefly reported to a meeting of the Physiological Society (Forbes & Injidi, 1979).

METHODS

Male domestic fowl of an egg-laying strain (404, Mytholmroyd Hatcheries, Hebden Bridge, W. Yorks) were used in all of the experiments described. They were reared to 3 weeks of age in groups and then penned individually in wire-mesh cages, with poultry grower pellets (Linton Mill Ltd, Wintringham, Malton, Yorks) and drinking water offered *ad libitum*. Because natural light was not excluded from the room in which the birds were housed and as the environmental temperature was not closely controlled, the effects of these variables were minimized by using changeover experimental designs, and analysis of variance where appropriate. In order to minimize carry-over effects at least 48 hr elapsed between successive treatments in any one bird.

Melatonin (*N*-acetyl-5-methoxytryptamine, Sigma Chemical Company, St Louis, MO) was dissolved in 0·5 ethyl alcohol: 0·5 isotonic saline to give concentrations of 0–10 mg/ml. Triiodothyronine (3,3′,5-triiodo-L-thyronine, Sigma Chemical Company) was dissolved in sterile isotonic saline with a few drops of 0·1 N-sodium hydroxide to give concentrations of 0–200 μg/ml. Injections were made intraperitoneally for melatonin and subcutaneously for T3, following which the food containers were weighed to the nearest 0·5 g at 15 min intervals for 7 hr and after 24 hr.

Electroencephalography

Three birds aged three months were prepared for e.e.g. recording as follows: general anaesthesia was induced by intramuscular injection of Equithesin (Gandal, 1969) at 2·5 ml./kg. After 30 min the level of anaesthesia was checked and further 0·5 ml. doses of Equithesin administered as necessary to maintain moderately deep anaesthesia. The feathers on top of the head were plucked and the area was disinfected (Wardotrane, W. B. Veterinary Ltd., London). A mid-line incision was made extending approximately 15 mm backwards from the comb and the periosteum removed by scraping an area of 12 mm diameter. Two electrodes were screwed into the cranium. 3 mm to each side of the mid line with stainless-steel watchmakers' screws (1 mm diameter, 2 mm length); these electrodes were single-pole sockets (stock no. 444–062, Radiospares Ltd., London). The incision was sutured with silk thread and the birds allowed to recover for several days.

For recording, the bird was gently restrained in a box with its head protruding from a hole. Plugs were inserted into two sockets from which wires led to an amplifier and chart recorder (400 MD2C, George Washington Ltd., Sheerness, Kent). A silver disk smeared with conducting jelly and taped to the comb was used as the indifferent electrode. E.e.g. recordings were made before and after the injection of 10 mg melatonin/kg and in artificial light (200 lux) or in darkness.

Respiration calorimetry

Four birds were used, aged 12 weeks and with a mean weight of 1·5 kg. Each bird was transferred from its home cage into the respiration chamber for several hours on two or three occasions before the experiment.

During the course of the experiment each bird received the following treatments in Latin Square: 0 mg melatonin + 0 μg T3; 10 mg melatonin + 0 μg T3; 0 mg melatonin + 200 μg T3; 10 mg melatonin + 200 μg T3, all per kg of body weight.

On each experimental day the bird to be treated was placed in the respiration chamber without food at 10.00 hr and respiratory exchange was measured for 3 hr. The bird was then removed from the chamber, injected, immediately returned and the lid sealed in position to enable recording to continue. Records of oxygen consumption and carbon dioxide production were taken for a further 4 hr.

The respiration chamber was a box 45 × 60 × 65 cm high, made in transparent plastic sheet (Perspex, I.C.I. Ltd., Billingham). Air was drawn through the chamber at 10 l./min (pump, Lacy Hulbert RBI-5, Croyden, Surrey; flow meter, EALL-50K, Teledyne Hastings-Raydist, Hampton, VA, U.S.A.); and dried by passing through silica gel before analysis for oxygen (analyser type OA 184, Taylor Servomex Ltd., Crowborough, Sussex), and carbon dioxide (IRGA 126, G.P. Instrumentation, Newcastle-upon-Tyne). Continuous records of the differences in oxygen contents of air entering and leaving the chamber and of the carbon dioxide content of the air leaving the chamber were made on a chart recorder (Servoscribe 220, Smiths Industries, London). Mean oxygen consumption and carbon dioxide production were calculated for 1 hr before, and 4 successive hours after injection.

Statistical comparisons

Except where stated otherwise the results were subjected to analysis of variance from which the residual mean square was used to calculate the standard error appropriate to all treatment means in that analysis (Mather, 1965; pp. 78–79).

RESULTS

Effects of several doses of melatonin

In a preliminary experiment it was shown that 10 or 25 mg melatonin per kg body weight caused sleep and anorexia for approximately 3 hr.

Six birds aged three months and weighing 950–1050 g were therefore given the following doses of melatonin: 0, 0·5, 1·0, 2·0, 4·0 and 8·0 mg/kg body weight in an experiment of Latin Square design. Melatonin treatment at all doses except 0·5 mg significantly depressed food intake during the first hour after injection. Fig. 1 shows cumulative intakes during 7 hr after injection. The 8 mg treatment caused the birds to assume within 10 min a sleeping attitude from which they could be aroused by tapping on the cage: after about 2½ hr they awoke and started to feed. Following injection of 2 or 4 mg of melatonin the birds appeared drowsy but did not sleep, while there was no observeable change in behaviour following 0·5 or 1 mg.

Feeding following melatonin and triiodothyronine

The previous experiment showed a dose-related effect of melatonin on food intake, accompanied by drowsiness or sleep at higher levels. Because food intake is often related to energy requirements it is possible that melatonin depressed metabolic rate, which resulted in a lower intake of food. It was postulated that the active thyroid hormone, T3, a metabolic stimulant, might reverse the effects of melatonin.

Eight birds aged two months and weighing 725–805 g were used in a factorial experiment with two levels of melatonin (0 or 10 mg/kg) and four levels of T3 (0, 50, 100 or 200 μg/kg).

Melatonin again depressed food intake and induced sleep for at least 2 hr. This effect was reversed by T3 which gave a dose-related increase in food intake (Table 1) and

completely prevented sleep or drowsiness; there was no significant interaction between the effects of the two substances. The effect of melatonin became apparent within 10 min of injection and T3 blocked this early response; the speed of this effect suggests that T3 was acting via some other route than metabolic rate, which is affected more slowly by T3 (Singh, Reineke & Ringer, 1968).

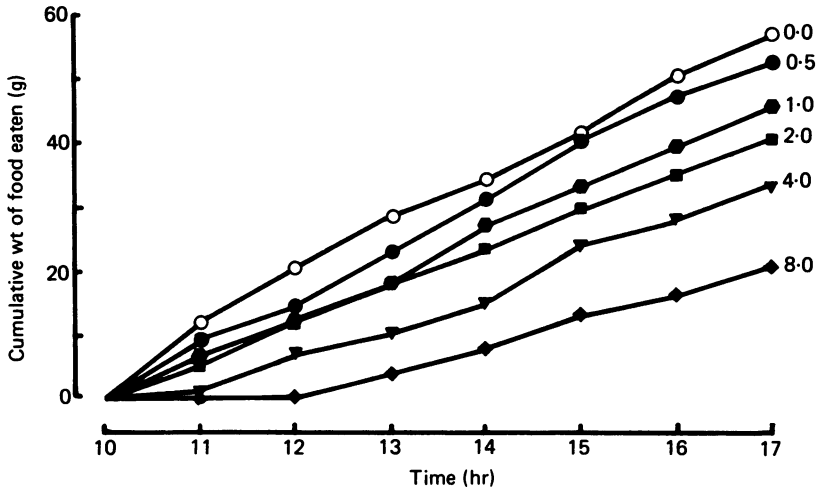

Fig. 1. Effect of melatonin, injected intraperitoneally, on the cumulative food intake of male fowl. Doses of 0, 0·5, 1, 2, 4 or 8 mg were given at 10.00 hr. Standard error of a treatment mean at 17.00 hr was ± 3·1.

TABLE 1. Weight of food eaten during 3 hr after injection of melatonin and T3 (g)

Dose of melatonin (mg/kg)	Dose of T3 (μg/kg)			
	0	50	100	200
0	$10{\cdot}4^{bc}$	$11{\cdot}1^{bc}$	$11{\cdot}9^{b}$	$16{\cdot}9^{a}$
10	$0{\cdot}6^{d}$	$1{\cdot}0^{d}$	$3{\cdot}2^{d}$	$8{\cdot}0^{c}$

Standard error of a treatment mean, ± 1·11. Means with different superscripts are significantly different ($P < 0{\cdot}05$).

Blood glucose following melatonin and triiodothyronine

Melatonin and T3 affect food intake in opposite directions. Because glucose metabolism has been postulated to play an important role in the control of food intake an experiment was carried out to examine the effects of melatonin and T3 on blood glucose levels.

Four birds aged 3 months, weighing 1500–1800 g were prepared under general anaesthesia (Equithesin; Gandal, 1969) with median wing vein catheters (polyvinyl NT2, Portex Ltd., Hythe, Kent) for the collection of blood samples. When they had recovered the pre-operative level of food intake they were subjected to three

treatments given according to a Latin Square design: 0 or 10 mg melatonin per kg body weight; 0 or 200 μg T3 per kg body weight. Blood samples (2 ml.) were taken at half-hourly intervals from 10.00 to 15.00 hr except at 11.00 hr when the injections were made. Samples were taken into heparinized tubes and immediately centrifuged. Plasma was stored at $-20°$ until analysed for glucose using the ferricyanide method on an autoanalyser (Technicon Instrument Corp., New York, Method N-26 1/11).

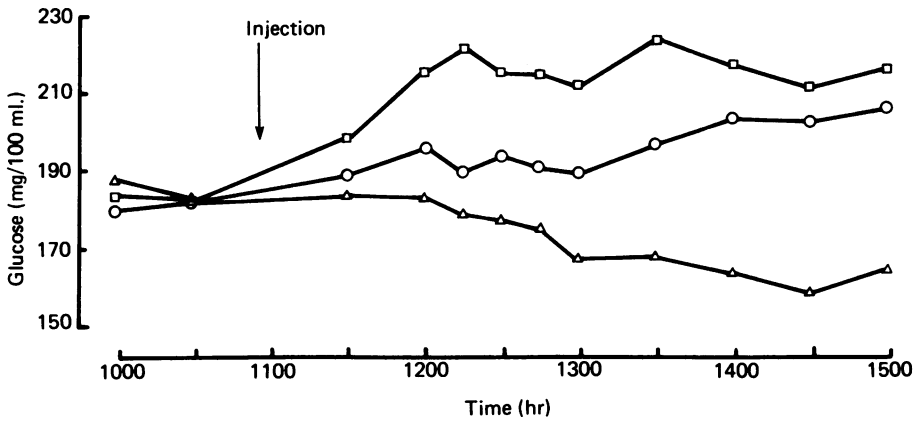

Fig. 2. Effect of melatonin or T3 on plasma glucose concentrations in male fowl. ○, control; □, 200 μg T3 injected intra-muscularly; △, 10 mg melatonin injected intraperitoneally, all at 11.00 hr.

Mean intakes during the 3 hr after injection were 14·6 g (controls), 3·7 g (melatonin) and 22·0 g (T3) with a standard error of $\pm 0{\cdot}86$; both treatments significantly affected food intake ($P < 0{\cdot}001$). Plasma glucose concentrations are shown in Fig. 2 from which it will be seen that within 1 hr of injection melatonin had significantly depressed, while T3 had significantly elevated the levels; these changes were parallel to the effects on food intake.

Electroencephalograph with darkness and melatonin

Recordings of the e.e.g. were made to compare the sleep induced by melatonin with the effects of darkness.

Fig. 3 shows extracts from the recordings made in one bird; results from the other two birds were very similar. In the light the bird was awake but resting; the e.e.g. had an amplitude of 4–40 μV and a frequency of 6–9 Hz. In darkness the amplitude increased to 50–230 μV but the frequency decreased to 4–6 Hz. These changes occurred within a few seconds of extinguishing the light and were reversed equally quickly after switching the light on.

Following melatonin injection there was a gradual change in e.e.g. but after 10 min it had settled down to a pattern which was very similar to that seen in darkness (amplitude, 50–250 μV; frequency, 3–6 Hz).

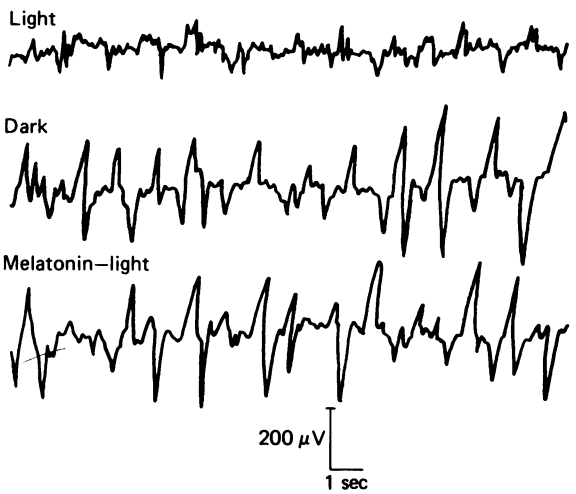

Fig. 3. Examples of the electroencephalogram of male fowl in daylight, 10 min after excluding light and 10 min after injecting 10 mg melatonin intraperitoneally.

TABLE 2. Oxygen consumption and carbon dioxide production of fowl injected at time 0 with melatonin (MT) or T3 or both. In any row means with different letters differ significantly ($P < 0.05$). S.E. standard error of a treatment mean derived from covariance analysis

Time relative to injection (min)	Treatment: 0 mg MT 0 μg T3	10 mg MT 0 μg T3	0 mg MT 200 μg T3	10 mg MT 200 μg T3	S.E.
		Oxygen consumption (l./hr)			
−60–0	1·70[a]	1·61[a]	1·83[a]	1·64[a]	±0·11
0–60	1·55[a]	1·29[b]	1·53[ab]	1·23[b]	±0·08
60–120	1·59[a]	1·31[b]	1·61[a]	1·29[b]	±0·06
120–180	1·60[a]	1·37[b]	1·74[a]	1·36[b]	±0·06
180–240	1·62[ac]	1·41[b]	1·71[a]	1·43[ab]	±0·07
		Carbon dioxide production (l./hr)			
−60–0	1·69[a]	1·70[a]	1·71[a]	1·73[a]	±0·05
0–60	1·43[a]	1·11[ab]	1·32[ab]	1·08[b]	±0·11
60–120	1·42[a]	1·05[b]	1·42[a]	1·07[b]	±0·09
120–180	1·40[a]	1·06[b]	1·49[a]	1·15[b]	±0·07
180–240	1·43[a]	1·13[b]	1·41[a]	1·24[ab]	±0·07

Oxygen consumption following melatonin and triiodothyronine

Measurements of oxygen consumption and carbon dioxide production were made in fasted birds after injection with melatonin, T3 or both substances to compare the effects on metabolic rate with those on food intake.

The results were subjected to covariance analysis with live weight as covariate, and are shown in Table 2 corrected to a body weight of 1·5 kg. Melatonin caused a significant depression in oxygen consumption and carbon dioxide production which

persisted for at least 4 hr after injection. When melatonin and T3 were given simultaneously the effect was similar to that of melatonin alone with inhibition of oxygen consumption and carbon dioxide production in the first 3 hr after injection with an increase towards control values in the fourth hour.

Respiratory quotients were not affected by treatment at any stage after injection but did tend to decline during the measurement period, presumably due to an increase in oxidation of lipids as the starvation progressed.

DISCUSSION

Melatonin induced a sleep-like state in growing fowl, as previously observed by Pang, Ralph & Petrozza (1976) and by Barchas, Da Costa & Spector (1967) in very young chicks. Sleep and feeding are mutually exclusive, so it is not surprising that we observed inhibition of feeding following the injection of melatonin. Hypophagia also occurred with doses of melatonin that were too low to cause obvious sleep but probably reduced the birds' reactions to stimuli. The rapid and severe effect of melatonin on behaviour strongly suggests that the site of action is in the central nervous system. This would account for the high dose (8 mg) required to induce sleep following intraperitoneal injection when only a very small proportion of the injected dose would reach the brain in contrast to the high proportion of melatonin secreted by the pineal gland which is likely to reach other parts of the brain directly via the cerebrospinal fluid.

The second experiment demonstrated that the rapid effects of exogenous melatonin on sleep and feeding were prevented by simultaneous intramuscular injection of T3. The response of metabolic rate to exogenous T3 is slow (at least 4 hr in the last experiment) so that it is possible that T3 was also acting directly on the C.N.S. to block the effects of melatonin. T3 alone caused increased food intake for 3 hr after injection and it has been shown that thyroxine and T3 levels in plasma are reduced by exogenous melatonin (M. H. Injidi, J. M. Forbes, H. Klandorf & P. J. Sharp, unpublished observations); it follows that the effects of melatonin might be mediated, in part at least, by a suppressing effect on thyroid secretion rate. Such a possibility would not however, account in full for the effects of melatonin injections, because thyroidectomy does not cause continuous sleep. T3 concentrations are depressed in fasted chickens (May, 1978) and it is possible that the decline in thyroxine and T3 levels following melatonin were due in part to lack of food.

Although the original glucostatic theory of the control of food intake in mammals (Mayer, 1955) has now developed into an energostatic theory (Booth, 1972) glucose is still an important component of the energy supply and high blood glucose levels might be expected to cause low food intake and vice versa. In birds, however, peripheral glucose infusions or insulin injections have not caused changes in food intake (Smith & Baranowski-Kish, 1979), although there is recent evidence that infusion of glucose into the hepatic portal vein of chickens depresses food intake (Shurlock & Forbes, 1981). The changes in blood glucose following injection of melatonin or T3 were in the same direction as the effects on food intake and were thus likely to be caused by these changes in food intake, rather than being responsible for them.

The effects of melatonin on e.e.g. agree unequivocally with previous observations (Ookawa, 1972; Pang *et al.* 1976), that environmental darkness rapidly causes an increased amplitude and decreased frequency (dusk is also quickly followed by an increased endogenous melatonin secretion). Melatonin injection caused similar changes in e.e.g. to those induced by darkness, but part of the response to melatonin is closure of the eyelids as part of the sleep response. Thus it is difficult to establish the causal relationship between darkness, melatonin and e.e.g. Some of the difficulties of interpretation of the results of experiments reported here can be alleviated by the use of pinealectomized birds, and such studies are reported elsewhere (Injidi, 1981).

The results of the final experiment demonstrated a depressing effect of melatonin on fasting metabolic rate which was not prevented by simultaneous injection of T3 at a dose which clearly did block the effects of melatonin on sleeping and feeding. This is further evidence that the effects of these two substances on the weight of food eaten during 3 hr after injection were not directly due to their effects on metabolic rate and, therefore, energy demand.

In conclusion, melatonin injection intraperitoneally at a dose of 10 mg per kg body weight into growing male domestic fowl causes sleep and aphagia, both of which are prevented by simultaneous intramuscular injection of T3 at 200 μg per kg; the rapidity of these effects suggests mediation via the central nervous system and this is supported by observations of changes in e.e.g. The effects of these treatments on blood glucose and oxygen consumption do not suggest that the short-term effects of melatonin and T3 on feeding are via peripheral mechanisms.

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