PLASMA MELATONIN LEVELS IN THE SCINCID LIZARD
TRACHYDOSAURUS RUGOSUS

THE EFFECTS OF PARIETAL EYE AND LATERAL EYE
IMPAIRMENT

BY B. T. FIRTH* AND D. J. KENNAWAY

Department of Anatomy and Histology and Department of Obstetrics and Gynaecology,
University of Adelaide, Adelaide, South Australia, 5001, Australia

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SUMMARY

1. At a constant temperature of 24 °C there was a diel fluctuation in plasma melatonin concentration; highest levels occurring in the scotophase of a reversed daily light-cycle. Parietalectomy did not appear to affect melatonin titres under these conditions.
2. When lizards were subjected to a photoperiod together with a thermo-period (31 °C in the photophase, 24 °C in the scotophase), nocturnal plasma melatonin levels were almost twice as high as those in animals subjected to a photoperiod at constant temperature.
3. Capping the lateral eyes of T. rugosus under these conditions did not alter the phase or amplitude of the rhythm in plasma melatonin content. However, removal of the parietal eye abolished the rhythm, owing mainly to reduced levels during the mid-scotophase and elevated levels during the mid-photophase.
4. It is concluded that plasma melatonin levels are regulated extraretinally, and that the parietal eye may help to mediate environmental input to centres secreting melatonin. It is suggested that the parietal eye may mediate thermal as well as photic information.

INTRODUCTION

The pineal organ forms part of the diencephalic brain roof of most vertebrates. It may be directly photosensory, as in fishes, amphibians, and some reptiles, or it may receive photic information indirectly via sympathetic neural pathways, as in birds, mammals and certain reptiles (Collin, 1971; Kappers, 1971). Some frogs and lizards may, in addition to the pineal, possess an extracranial parapineal organ (frontal organ and parietal eye respectively), the photoreceptive nature of which has been determined from ultrastructural (Eakin, 1973) and neurophysiological (Dodt, 1973) studies.

* Present Address. Department of Zoology, University of New England, Armidale, N.S.W. 2351, Australia.
The function of the lizard parietal eye remains uncertain, but it has been suggested that it may be a radiation dosimeter (Stebbins & Wilhoft, 1966) or, more appropriately, an illuminometer (Packard & Packard, 1972). In support of this hypothesis, field and laboratory studies of lizards have shown that parietal eye impairment results in more frequent exposure to bright light, increased basking time and increased activity (Glaser, 1958; Stebbins, 1963, 1970; Palenschat, 1964; Packard & Packard, 1972; Stebbins & Cohen, 1973). The ‘eye’ therefore might help to regulate seasonal physiological cycles, such as reproduction and metabolic activity, which depend to some degree upon environmental light cues for their synchronization (Stebbins & Wilhoft, 1966). Indeed, there is evidence that reproductive cycles (Stebbins, 1970; Stebbins & Cohen, 1973) and seasonal thyroid activity cycles (Stebbins & Eakin, 1958; Stebbins & Wilhoft, 1966; Stebbins & Cohen, 1973) are accelerated by parietal eye impairment.

Recent studies suggest a thermoregulatory function for the parietal eye. Parietectomy or parietal nerve disruption causes lizards to behaviourally select higher temperatures in thermal or photothermal laboratory gradients (Hutchison & Kosh, 1974; Engbretson & Hutchison, 1976; Roth & Ralph, 1976, 1977). Parietectomy also depresses thermal tolerance, as measured by the critical thermal maximum (Kosh & Hutchison, 1972) and the threshold for thermal panting (Firth & Heatwole, 1976).

The vertebrate pineal organ appears to be the principal, though not the sole source of secretion of the indoleamine N-acetyl-5-methoxytryptamine, or melatonin (Ozaki & Lynch, 1976; Kennaway et al. 1977; Gern & Ralph, 1979; Gern, Owens & Ralph, 1978a). The levels of this substance vary rhythmically within the blood of fish (Gern, Owens & Ralph, 1978b), amphibians (Gern & Norris, 1979), lizards (Kennaway et al. 1977; Firth, Kennaway & Rozenbilda, 1979), turtles (Owens, Gern & Ralph, 1978), birds and mammals (Ralph, 1976), with maximal levels occurring during the dark phase (scotophase) of a daily light cycle. Melatonin is believed to be a chemical transducer of the photic environment, and has been implicated in a number of functions, including reproduction (Reiter, 1978), circadian activity rhythms (Turek, McMillan & Menaker, 1976) and thermoregulation (Ralph et al. 1979).

It has been suggested that there is a functional interaction between the parietal eye and pineal organ of lizards. Neuropharmacological studies show that in Crotaphytus collaris the electrical activity of the parietal eye is modified by efferent feedback from the pineal organ (Engbretson & Lent, 1976). Also, in Sceloporus occidentalis, the activity of pineal hydroxyindole-O-methyltransferase (HIOMT), the final enzyme in the melatonin biosynthetic pathway, is altered by parietectomy (Quay et al. 1971; Bethea & Walker, 1978).

The present study examines the role of the parietal eye and the lateral eyes in mediating the photothermal regulation of diel rhythms in plasma melatonin levels in the scincid lizard, Trachydosaurus rugosus.

MATERIALS AND METHODS

General

Lizards (Trachydosaurus rugosus Gray) were collected in the vicinity of Adelaide, South Australia, in the spring of 1976 and were housed in enclosures, partly exposed to the natural environment. Animals were marked by toe-clipping, and the sex, snout-vent length (S-V) and weight recorded.
Prior to each experiment, lizards were acclimated in an environmentally controlled, light-proof box. The photothermal regimen for each experiment is specified below. Blood sampling and anaesthetic procedures have been described previously (Firth et al. 1979).

Lizards were parietalectomized under tribromoethanol anaesthesia, according to the following procedure. The interparietal scale was removed, and the bony border surrounding the exposed parietal eye was pared away with a dental drill. The 'eye' was removed with watchmaker forceps, care being taken not to puncture the underlying meninges. Sham parietalectomy consisted of drilling a small wound 1 mm lateral to the interparietal scale.

Photic input to the lateral eyes was eliminated by capping the eyes with surgical tape overlaid with aluminium foil, and sealed at the edges with contact cement. The control animals for this experiment had contact cement placed above the eyes.

Melatonin levels were determined by radioimmunoassay. This involved incubation with an antibody raised against N-acetyl serotonin-BSA, following extraction in borate buffer and chloroform and in columns of Lipidex 5000 (see Kennaway et al. 1977, and Firth et al. 1979 for details).

**Experiment 1**

Twenty lizards were parietalectomized and sham-parietalectomized (five males and five females in each treatment group) in November. They were acclimated for 12 days at a constant 24 °C, with a photoperiod of 13 h light, 11 h dark (13L:11D; photophase, 05.30-18.30 h, 240 lx incandescent). On the 12th night of acclimation, blood samples were taken around midnight from half of the lizards in each treatment group. The remaining half were sampled on the 13th night of acclimation. Precautions were taken to prevent the second group of lizards from being exposed to light while sampling the first group.

**Experiment 2**

Twenty-eight lizards were parietalectomized and sham-parietalectomized in April (the Austral Autumn). They were acclimated to a constant 24 °C and a reversed photoperiod of 13L:11D (photophase, 17.30-06.30; 240 lx incandescent) for 12 days. In early May one-half of each treatment group was sampled for blood around midnight (24.00 h) and the remaining half around mid-scotophase (12.00 h).

The lizards were then returned to their enclosures for 2 weeks, following which they were reacclimated to the above conditions for 12 days. The animals were subjected to a similar blood sampling procedure as before, except that those previously sampled at 12.00 h were sampled at 24.00 h and vice versa.

**Experiment 3**

Male and female *T. rugosus* were divided into four treatment groups: parietalectomized (P), lateral eyes shielded (E), parietalectomized and lateral eyes shielded (PE) and a control group (C) which was subjected to both sham-parietalectomy and sham eye-shielding.

Beginning in November, the lizards were acclimated for 12 days to a 13L:11D photoperiod delayed by 6 h (photophase, 11.30-00.30 h.) The intensity of the in-
candescent light source was increased to 830 lx, and the temperature in the photophase elevated to 31 °C. The scotophase temperature remained at 24 °C.

Blood samples were collected over a period of 1 h bracketing each of the following times: 06.00 h, 12.00 h, 18.00 h and 24.00 h. Because of the large number of animals sampled, the experiment was staggered over a period of 4 weeks (6 Nov. to 6 Dec.).

Statistical procedures

Experiment 1 was analysed by a factorial two-way analysis of variance with sex and experimental treatment as the factors.

In experiments 2 and 3, a three-way factorial analysis of variance was performed on log10 transformed data. Time, experimental treatment and sex were the factors. Where appropriate, a Tukey's multiple comparison test was applied (Sokal & Rohlf, 1969).

An analysis of covariance was administered on all data to test for the effect of size on plasma melatonin levels.

RESULTS

In no experiment was a significant sexual difference ($P > 0.05$) in plasma melatonin concentrations evident. Similarly, the covariate of size (snout-vent length) did not significantly affect melatonin levels ($P > 0.05$).

(A) Effect of parietalectomy on plasma melatonin levels at constant temperature

Fig. 1A shows plasma melatonin levels measured at midnight (mid-scotophase) in sham-parietalectomized and parietalectomized T. rugosus subjected to normal 13L:11D photoperiod. Parietalectomy did not significantly alter melatonin levels ($P > 0.05$).

Reversing the photoperiod resulted in a concomitant shift in plasma melatonin concentration, mid-scotophase (midday) levels being higher than those at mid-photophase (midnight) ($P < 0.001$). Fig. 1B represents the data for the sampling periods of May and June combined, since analysis of variance with repeated measures indicated no significant difference ($P > 0.05$) between the two sets of data.

There was no significant difference ($P > 0.05$) in plasma melatonin concentration between parietalectomized and sham-parietalectomized lizards either at mid-scotophase or at mid-photophase.

(B) Effect of parietalectomy and lateral eye-shielding on plasma melatonin levels in a fluctuating photothermal environment.

Fig. 2 illustrates the plasma melatonin concentrations of T. rugosus subjected to various experimental treatments and exposed to a delayed 13L:11D photoperiod accompanied by a thermoperiod of 31 °C (in the photophase) and 24 °C (scotophase). Analysis of variance on control and eye-shielded (C and E) lizards indicated that there was a diel fluctuation in plasma melatonin levels ($P < 0.001$), the peak of which coincided with the middle of the scotophase and the trough of the thermoperiod. Further analysis with a $Q$ test (Sokal & Rohlf, 1969) revealed that the melatonin levels at 06:00 h were greater than at all other times ($P < 0.05$) and that the levels at
Figure 1. Mean plasma melatonin levels of sham-parietalectomized and parietalectomized *T. rugosus* in 13L:11D, in (A) normal photoperiod and (B) reversed photoperiod. The vertical lines represent ± S.E.M. and the numbers above them the sample size, *N*. Shaded areas at the top of the figure represent the dark period of the lighting cycle.

24.00 h were higher than those at 12.00 h (*P* < 0.05). A similar analysis of the parietalectomized and parietalectomized-eye shielded (P, PE) groups, however, did not indicate any diel fluctuation in melatonin concentrations (*P* > 0.1). The analysis also demonstrated that experimental treatment did not significantly affect melatonin levels when separately considering the C and E groups (*P* > 0.7) and the P and PE groups (*P* > 0.3). However, a grouping of the data based on the presence or absence of the parietal eye (i.e. C and E animals combined and P and PE animals combined) showed that parietalectomy significantly lowered (*P* < 0.01) mid-scotophase levels of plasma melatonin and elevated them (although not significantly so) at 18.00 h (Fig. 3).
Fig. 2. Mean plasma melatonin levels of control, eye-capped, parietectomized and eye capped-parietectomized *T. rugosus* subjected to 13L:11D photoperiod delayed 6 h, and a thermoperiod of 31 and 24 °C. The sample at 24.00 h indicated by an asterisk represents a control group of lizards sampled immediately following 'lights-off'. C, Control; E, eyes capped; P, parietectomized; PE, eyes capped and parietectomized. Other conventions as in Fig. 1.

Fig. 3. Mean plasma melatonin levels of *T. rugosus*. Aggregated data based on Fig. 2, grouped according to the presence or absence of the parietal eye. Conventions as Figs. 1 and 2.
DISCUSSION

The present study confirms two previous observations in connexion with plasma melatonin levels in *T. rugosus* (Kennaway *et al.* 1977; Firth *et al.* 1979). At constant temperature there was (i) a light-dependent diel rhythm, with scotophase levels exceeding those in the photophase and (ii) an apparent seasonal fluctuation, with scotophase levels in spring being lower than those at other seasons. Nocturnal melatonin levels described here for winter (May; around 100 pg/ml) are slightly higher than spring levels (November; 60–80 pg/ml) but not as high as those reported by Kennaway *et al.* (1977) for mid-summer (January; 240 pg/ml). A similar seasonal rhythm in melatonin content (with peak levels in mid-summer and lower levels in spring) has been found in the pineal gland of the tortoise, *Testudo hermanni* (Vivien-Roels, Arendt & Bradtke, 1979).

It is well documented that, in birds and mammals, melatonin secretion is closely coupled to the environmental lighting cycle (Ralph, 1976). The present demonstration of a photoperiod-dependent shift in plasma melatonin levels in *T. rugosus* confirms that this is true for lizards. Under a reversed photoperiod, there is a complete phase reversal of melatonin titres within 12 days. A similar light-dependent rhythm in pineal HIOMT activity in the lizard *Lampropholis guichenoti* has been shown to become entrained to an altered light-cycle within 5 days (Joss, 1978) and in rats a 12 h shift in environmental lighting has been shown to re-entrain the melatonin excretion rhythm within 5–7 days (Adler, Lynch & Wurtman, 1979).

The low nocturnal levels of plasma melatonin in *T. rugosus* in November (Fig. 1 A) were comparable to those previously recorded for that month (Firth *et al.* 1979). However, the nocturnal melatonin titres of control and eye-shielded lizards exposed simultaneously to a photoperiod and thermoperiod in November (Figs. 2, 3) were almost double those subjected to a photoperiod at constant temperature during the same month. Such elevated nocturnal melatonin concentrations may have been due to one or both of two factors: (i) the higher intensity of illumination during the photophase (830 lx v. 240 lx) or (ii) the higher photophase temperature (31 °C versus 24 °C). As to the first possibility, constant light intensity has been shown to be inversely related to pineal HIOMT activity (Bethea & Walker, 1978), but no studies have been conducted to test whether photophase light intensity may influence scotophasic pineal or plasma melatonin content. However, there is some precedent for suggesting that ambient temperature may influence either the secretion, release or degradation of melatonin. For example, the activity of pineal HIOMT in field populations of lizards (*Sceloporus occidentalis*) has been observed to be lower on cold days than on warm days (Quay *et al.* 1971). Similarly, Eichler & Moore (1975) showed that diencephalic HIOMT activity in the frog *Rana pipiens* was temperature-sensitive. Under a photoperiod and a thermoperiod (12 h light, 27 °C:12 h dark, 22 °C), a diel rhythm in HIOMT activity was present, but this rhythm was abolished if the same lighting conditions were imposed at a constant 22 °C. In rats, the activities of two of the enzymes involved in pineal melatonin synthesis, HIOMT and N-acetyltransferase (NAT) are depressed by a high temperature of 33 °C (Nir, Hirschmann & Sulman, 1975; Nir & Hirschmann, 1978). Furthermore, in 12-day-old suckling rats, the activity of pineal NAT is more sensitive to temperature changes than to light
fluctuations, although pineal HIOMT activity is unresponsive to both environmental variables (Ulrich et al. 1973). Such thermal sensitivity may be related to the poikilothermic nature of suckling rats, since artificial cycles in environmental temperature do not affect the daily rhythm of melatonin excretion in adult animals (Adler et al. 1979).

The observation that an alteration of photophase temperature affects melatonin levels in the scotophase (Figs. 2, 3) suggests the possibility of a photoperiod–temperature interaction in the regulation of rhythms in plasma melatonin concentration in *T. rugosus*. Recent studies on plasma melatonin levels of salamanders subjected to a variety of photothermal regimens suggest that such a phenomenon is true for ectothermic vertebrates in general. W. A. Gern & D. O. Norris (personal communication) have found in *Ambystoma tigrinum* that the rhythm in serum melatonin concentration was most prominent when the temperature was highest (20 °C) during the photophase. As in *T. rugosus*, the diel rhythm in melatonin levels was present in the absence of a temperature cycle. However, this was only obvious at low, constant temperature (10 °C).

Shielding the lateral eyes of *T. rugosus* did not significantly alter the rhythm in plasma melatonin content from that of controls. It is unlikely that such lateral eye impairment simulated the effect of constant darkness, since the latter appears to inhibit the rhythm of plasma melatonin concentration in this species (Firth et al. 1979). The most probable explanation is that, in *T. rugosus*, melatonin synthesis and/or release is extraretinally mediated. Studies in optic tract-sectioned trout have similarly shown persistent plasma melatonin rhythms, indicating a direct photosensory input to the melatonin-secreting source(s) (Gern et al. 1978a). These observations are consistent with the view that, in many ectothermic vertebrates, the pineal organ, which is presumed to be the major source of melatonin secretion, perceives light directly (Collin, 1971; Kappers, 1971) rather than via accessory optic tracts as in mammals (Moore, 1978).

Our study indicates that, in *T. rugosus*, at least one extraretinal photoreceptive structure, the parietal eye, may help to mediate environmental information for the regulation of rhythms in plasma melatonin content. The exact nature of the environmental information mediated by the parietal eye for this purpose requires further investigation. However, the observation that parietalectomy altered plasma melatonin titres only under fluctuating ambient temperatures suggests that the parietal eye may mediate changes in plasma melatonin levels that are induced photothermally or thermally. At least two studies indirectly support the notion that the parietal eye might mediate photothermal factors influencing melatonin production. Parietalectomy in *S. occidentalis*, subjected to constant light, has been shown to elevate pineal HIOMT activity, especially at high light intensities (Bethea & Walker, 1978). In a natural population of the same species, Quay et al. (1971) showed that pineal HIOMT activity was lowered by parietalectomy on cold days, but not on warm days. However, since pineal melatonin levels may not identically parallel pineal HIOMT activity (Klein & Weller, 1970; Binkley, 1976), no firm conclusions can be drawn from these studies with respect to plasma melatonin. It would also need to be presumed that the parietal eye exerts its main influence over the pineal organ rather than other possible
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melatonin sources, although pharmacological studies indicate a parietal–pineal interaction (Engbretson & Lent, 1976). The possibility also remains that the parietal eye itself produces melatonin, since high HIOMT activity has been demonstrated in the parietal eye of *Iguana iguana* (Quay, 1965).

The above data point to the possibility that the parietal eye acts as a relay for the processing of photothermal environmental information, and that melatonin may be a chemical transducer of such information. The ultrastructural appearance of the ‘eye’ (Eakin, 1973) and certain behavioural studies (Stebbins, 1970) have led to the conclusion that this structure is purely photoreceptive. On the other hand, studies on thermoregulatory behaviour (Hutchison & Kosh, 1974; Engbretson & Hutchison, 1976; Roth & Ralph, 1976, 1977) and thermal tolerance (Kosh & Hutchison, 1972; Firth & Heatwole, 1976) indicate that the parietal eye may be sensitive to thermal as well as photic information.

Licht (1972) has stressed the importance of temperature, particularly in the spring, in regulating reptilian seasonal physiological cycles. Indeed, in some species there is a complex interaction between temperature and photoperiod in determining gonadal cycles (Licht, 1967a, b, 1969). Consequently, factors such as the apparent seasonal sensitivity of the parietal eye (Firth & Heatwole, 1976) and its possible mediation of thermal as well as photic input should be considered in the design of future experiments concerning the seasonal aspects of this organ’s function.

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