*J Endocrinol Reprod 14 (2010) 1 : 19 - 24* *Original Article*

# Melatonin and methoxytryptophol have temporal effect on tail elongation but not methoxytryptamine: Studies on tail regeneration in *Hemidactylus flaviviridis*

**Anju Kurup and A.V. Ramachandran**

Division of Developmental Biology, Department of Zoology, Faculty of Science, The M S University of Baroda, Vadodara – 390002, Gujarat, India.

## Summary

In order to evaluate the time-specific influence of pineal indoles on tail regeneration in lizards, melatonin (M), methoxytryptophol (ML) and methoxytryptamine (MT) were administered intraperitonealy to different groups of lizards at morning 7:00 hrs (m) or in the evening at 17:00 hrs (e). The Mm and MLe groups of lizards showed a delay in the attainment of various arbitrary stages of regeneration like wound healing, pre-blastema, blastema and initiation of growth while the Me and MLm groups of lizards showed hastened attainment. However, MTm and MTe treatments did not bring about any difference compared to the controls. The total length of tail regenerated and percentage replacement at the end of 30 days were significantly less in Mm and MLe groups of lizards. MTm and MTe treatments, however, did not affect the regeneration process. The results indicate that M and ML have opposite time-specific effects on regeneration, while MT has no effect. Apparently, M and ML are part of the photoperiodic and neuro-endocrine transduction mechanisms related to regenerative growth in lizards.

***Keywords :*** Lizard, melatonin, methoxytryptamine, methoxytryptophol, regeneration.

## Introduction

Previous study on tail regeneration in *Hemidactylus flaviviridis* had revealed an enhancing influence of longer photic schedules on overall growth (Ndukuba and Ramachandran, 1991a) with the favorable influence of light on regeneration not mediated *via* the eyes as blinded lizards regenerated their tail as well as the sighted controls (Ndukuba and Ramachandran, 1988). The alluded extra-retinal photoreceptors was identified as the pineal since both pinealectomized (PX) lizards and pineal-intact lizards exposed to continuous darkness (DD) depicted a 50% deficit in the total length of tail replaced (Ramachandran and Ndukuba, 1989; Ndukuba and Ramachandran, 1991). Of the many methoxyindoles produced by the pineal of vertebrates, the three most important ones that appear in circulation in the order of their importance are melatonin (M), methoxytryptophol (ML) and methoxytryptamine (MT).

Melatonin, by its rhythmic secretion, synchronizes seasonal and circadian activity in various vertebrates (Underwood, 1981, 1989; Bartness and Goldman, 1989). In addition, both ML and MT have influence on reproductive function by their circadian variations (Young and Silman, 1981; Pévet, 1983; Skene et al., 1986, 1987; Vivien-Roels et al., 1992). Light has been identified as an important stimulus that can affect the levels of pineal indoles (Skene et al., 1987), and experimental alterations in the duration of light have been shown to alter nocturnal melatonin levels in many non-mammalian vertebrates such as fishes (Duston and Bromage, 1987), reptiles (Firth et al., 1979; Menaker and Wisner, 1983, Vivien-Roels, 1985) and birds (Underwood and Siopes, 1985; Liou et al., 1987).

In view of the fact that both PX and experimental photic schedules affect tail regeneration in *Hemidactylus flaviviridis* and as pineal indoles serve as the essential photoneuroendocrine transducers, it was thought pertinent to test the influence of exogenous administration of pineal indoles on tail regeneration. Considering the fact that the effect of indoles administered exogenously on gonadal functions in both mammals and lizards is time-dependent (Reiter et al., 1976; Misra and Thapliyal, 1979; Reiter, 1980a, b), in the present study M, ML and MT were administered in the morning or evening to two separate groups of lizards to ascertain their time-dependent influence on tail regeneration.

## Materials and Methods

### Experimental animals

Adult lizards (*Hemidactylus flaviviridis*) of both the sexes, weighing 10±2 g and 70-80 mm snout - vent length were used for the experiments. The cages that housed the animals measured 18 cm X 15 cm X 10cm, with one side made of transparent glass and ventilated on three sides. Each cage housed six lizards balanced for size. The

**Correspondence to be addressed to** : Prof. A. V. Ramachandran, Ph.D.; Email: mailtoavrcn@yahoo.co.in

animals were maintained under a normal light-dark photoperiodic schedule in the monsoon months (22-26ºC) and were fed with nymphs of cockroach and water *ad libitum.* Autotomy was performed by pinching off the tail three segments from the vent.

### Preparation of methoxyindoles

The three methoxyindoles (Sigma Aldrich, Ltd, USA) were prepared fresh daily before injection. They were dissolved in a few drops of ethanol before being diluted with 0.6 % saline to the required concentration.

### Experimental schedule

Altogether eighty lizards, divided into 8 groups of 10 each, were subjected to different treatment schedules as follows:

#### *Groups I and II (M groups; Mm and Me)*

These two groups of lizards received daily intraperitoneal injections of 20µg M in 0.1 ml saline either at 07:00 hr or 17:00 hr, starting 5 days prior to tail autotomy and 30 days thereafter.

#### *Groups III and IV (ML groups; MLm and MLe)*

These two groups of lizards received daily intraperitoneal injections of 25µg ML in 0.1 ml saline at either 07:00 hr or 17:00 hr, starting 5 days prior to tail autotomy and 30 days thereafter.

#### *Groups V and VI (MT groups; MTm and MTe)*

These two groups of lizards received daily intraperitoneal injections of 20µg MT in 0.1 ml saline at either 07:00 hr or 17:00 hr starting 5 days prior to tail autotomy and 30 days thereafter.

#### *Groups VII and VIII (Controls)*

These two groups of lizards received daily intraperitoneal injections of 0.1 ml saline at either 07:00 hr or 17:00 hr starting 5 days prior to tail autotomy and 30 days thereafter.

### Parameters evaluated

The number of days taken to attain various arbitrary stages of regeneration such as wound healing (WH), pre-blastema (PB), blastema (B) and initiation of growth (IG), was recorded. The length of new growth (regenerate) was measured by using graduated scale. The measured length was recorded every alternate day and used for recording the tail length at fixed time intervals of 10, 15, 20, 25 and 30 days post-caudal autotomy. The per day growth rate and total percentage of tail replaced were calculated. The data were subjected to ANOVA and Duncan's multiple range test with an alpha level of both 0.05 and 0.01.

## Results

### Number of days taken to reach the various arbitrary stages of regeneration

All groups of lizards except Me and MLm completed wound healing by 6 days. This delay by a day was persistently shown by the other groups of lizard even while attaining the other stages like pre-blastema, blastema and initiation of growth (Table 1).

### Length of tail regenerated

The total length of tail regenerated at the end of 30 days was similar in control as well as MTm and MTe groups of lizards. In comparison, Mm and MLe groups of the lizards regenerated significantly lesser length of tail ($p<0.01$) and Me and MLm groups of lizard showed significantly greater length ($p<0.01$) (Fig. 1).

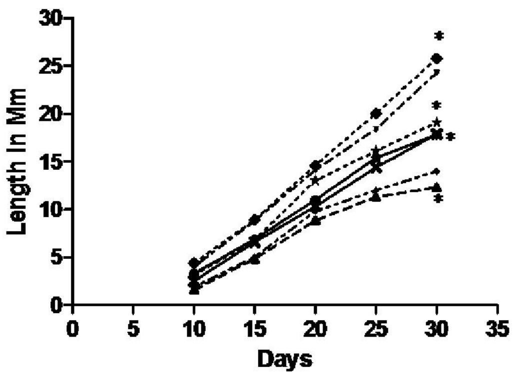


Fig.1: Length of tail regenerated in control and methoxyindoles treated lizards, *H. flaviviridis* exposed to L:D 12:12. Abbreviations, as in Table 1.

Data are expressed as Mean±SEM, where, $*p<0.05$, $**p<0.01$ and $***p<0.001$ compared to control

### Rate of growth and total percentage replacement

The rate of tail elongation calculated for blocks of 5 days showed a continuous increase in control, MTm and MTe groups of lizards, to reach a maximum of 0.88 mm between days 20-25 post- autotomy, where after the rate declined. The Mm and MLe groups of lizards however showed an initial slow growth rate between 5-10 days and reached maximum growth rates of 0.84 and 0.94 mm, respectively, between 15 and 20 days. Thereafter, both these groups of lizards showed significantly decreased growth rate. In contrast, Me and MLm, groups of lizards showed significantly greater initial growth rates of 0.88 and 0.76 mm respectively between 5-10 days. Thereafter, the growth rates continued to increase gradually to attain the maximum rates of 1.2 mm and 1.14 mm between 20-25 days, respectively. The percentage of tail replaced at the end of 30 days was significantly less in Mm and MLe groups of lizards when compared with control, MTm and MTe (25% v/s 35%) and significantly more in Me and MLm (50%) (Table 2; Fig. 2).

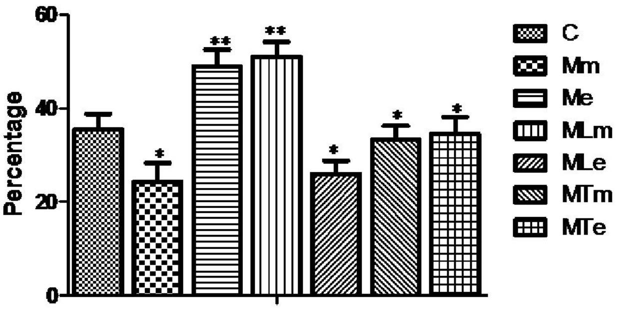


Fig.2: Percentage tail replacement in control and methoxyindoles treated lizards, *H. flaviviridis* exposed to L:D 12:12. Abbreviations, as in Table 1.

Data are expressed as Mean±SEM, where, *p<0.05, **p<0.01 and ***p<0.001 compared to control

## Discussion

The results show that pineal indoles have differential time-dependent influence on tail regeneration in *H. flaviviridis*. Of the three indoles tested, M and ML had opposite influence while MT had no effect on the process of tail regeneration. Whereas Mm depicted an anti-regenerative effect, Me produced a pro-regenerative effect. In contrast, MLm showed pro-regenerative effect while MLe had an anti-regenerative influence. Time-dependent influence of M on gonadal functions of mammals and lizards is well-documented (Underwood, 1981; Carter et al., 1982; Stetson et al., 1986). The anti-regenerative effect of Mm appears to be essentially due to the morning melatonin injection, causing the lizards to read it as an extended dark phase. This stands substantiated by the earlier observations of decreasing regenerative tail elongation with decreasing photic schedules (Ndukuba and Ramachandran, 1991).

The present observations on the effect of melatonin in lizards find support from the studies suggesting the critical importance of duration of melatonin for induction of photoperiodic effects in ewes (Wayne et al., 1988). Based on the contention that tail regeneration in Hemidactylus is stimulated by the growth-promoting influence of PRL and longer photic schedules lead to an increased PRL release and *vice versa* (Ramachandran and Ndukuba, 1989; Ndukuba and Ramachandran, 1991a), the anti-regenerative effect observed in Mm lizards could be related with reduced PRL release, a duration effect of melatonin. Further, the nullifying influence of PRL on the anti-regenerative effect of Mm has been demonstrated (Ndukuba and Ramachandran, 1991). Similar nullifying influence of PRL on melatonin effects have been reported

Table 1. Number of days taken to attain the different arbitrary stages of tail regeneration in control and methoxyindoles treated *H. flaviviridis* under LD 12:12

| Groups | Wound healing | Preblastema | Blastema | Initiation of growth |
|---|---|---|---|---|
| Control | 6.00±1.25 | 7.00±1.01 | 8.00±0.97 | 9.00±1.42 |
| Mm | 6.00±0.96 | 7.00±2.48 | 8.00±1.21 | 9.00±1.26 |
| Me | 5.00±0.92* | 6.00±1.35* | 7.00±1.52* | 8.00±1.09* |
| MLm | 5.00±0.92* | 6.00±1.68* | 7.00±1.028* | 8.00±1.09* |
| MLe | 6.00±1.04 | 7.00±0.98 | 8.00±1.07 | 9.00±1.12 |
| MTm | 6.00±1.08 | 7.00±0.68 | 8.00±1.11 | 9.00±0.98 |
| MTe | 6.00±0.86 | 7.00±0.68 | 8.00±1.42 | 9.00±1.06 |

Data are expressed as Mean±SEM, where, *p<0.05, **p<0.01 and ***p<0.001 compared to control
Mm- Morning melatonin, Me- Evening melatonin, MLm-morning methoxytryptophol, MLe-Evening methoxytryptophol, MTm-Morning methoxytryptamine, MTe- Evening methoxytryptamine. Values are mean±SD

Table 2. Per day growth rate calculated in blocks of 5 days of the 30 days post-caudal autotomy in control and in methoxyindoles injected *H. flaviviridis* under LD 12:12.

| Groups | BLOCKS OF DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 |
| Control | - | 0.70±0.02 | 0.60±0.08 | 0.80±0.06 | 0.88±0.11 | 0.68±0.10 |
| Mm | - | 0.32±0.08*** | 0.60±0.10 | 0.84±0.12 | 0.48±0.09** | 0.26±0.06*** |
| Me | - | 0.88±0.15* | 0.92±0.02** | 0.96±0.09* | 1.20±0.02*** | 1.12±0.06*** |
| MLm | - | 0.76±0.02 | 1.01±0.08*** | 1.02±0.06** | 0.92±0.11* | 1.14±0.10*** |
| MLe | - | 0.36±0.02*** | 0.64±0.06 | 0.94±0.06* | 0.28±0.11*** | 0.48±0.10** |
| MTm | - | 0.52±0.10** | 0.78±0.13* | 0.70±0.05 | 0.92±0.05* | 0.58±0.11** |
| MTe | - | 0.62±0.08* | 0.78±0.10* | 1.16±0.07** | 0.68±0.10* | 0.52±0.12** |

Data are expressed as Mean±SEM, where, *p<0.05, **p<0.01 and ***p<0.001 compared to control Abbreviations, as in Table 1.

in the mink, *Mustela vision* (Murphy et al., 1990). Enough evidence is available to link increasing photic schedule with PRL release (Matthiej and Swarts, 1978; Barrell and Lapwood, 1979; Brown and Forbes, 1980). There are also documented evidences to show that melatonin can interfere with PRL secretion (Kennaway et al., 1982; Symons et al., 1983; Murphy et al., 1990).

The pro-regenerative effect of Me appears to be mainly an amplitude effect of melatonin leading to greater PRL release in the early photo-phase. The high amplitude effect of melatonin can be related with a greater turnover of 5-HT in the hypothalamus, a potent secretogogue of PRL. The ability of 5-HT to induce PRL release in this context is very well documented (Lawson and Gala, 1976, 1978; Clemens et al., 1977; James and Wigham, 1984). Increased serotonergic activity, some 16-20 hrs after melatonin administration, has been reported in the gold fish (Olcese et al., 1981). Further evidence for the amplitude effect of melatonin is provided by our earlier observations of enhanced regenerative tail elongation in LD 12:12 during the summer months as compared to the monsoon and winter months (Ramachandran and Ndukuba, 1989; Ndukuba and Ramachandran, 1991). In this context, Vivien-Roels et al. (1992) have shown in the box turtle, *Terrapene carolina trianquis,* that while photoperiodism is responsible for duration of melatonin in the dark phase, the ambient temperature is responsible for greater amplitude of melatonin. Moreover, the contention that evening melatonin administration causes increased PRL release due to the greater amplitude of melatonin, is well supported by the reported elevation of PRL level subsequent to melatonin administration in the evening in rats (Vanage and Sheth, 1991). Additional evidence of 5-HT-induced PRL release in Me animals is provided by our findings that both para-chlorophenylalanine, a specific inhibitor of 5-HT synthesis and 5-HT antagonist, prevented the stimulatory influence of Me on tail regeneration (Ndukuba and Ramachandran, 1989; Ramachandran and Ndukuba, 1989). Another possible means by which Me might impact the course of regeneration could be by its *in loco* action of reducing inflammation and oxidative stress and help create an environment that contributes to regeneration promoting scarless wound healing (Wicker and Kamler, 2009). Further, favorable role of Me in this context could be by decreasing the expression of basic FGF and TGFB-1 post-caudal autotomy, which are known to contribute to regeneration inhibitory collagen formation, as has also been inferred by Turgut et al. (2006) in their studies on melatonin and sciatic nerve regeneration in rats.

The presently observed diametrically opposite influence of ML suggests two possibilities, (i) ML is an active pineal principle in lizards, and (ii) ML also has a time-dependent influence on PRL release. However, the diametrically opposite effect of ML as compared to M is understandable by the reported higher plasma levels of this indole during daytime (Vivian-Roels et al., 1999; Zavilska et al., 2000). Though the exact mechanisms are not clear, it is presumable that ML administered in the evening dampens PRL release while in the morning it potentiates PRL release. Some experiments in this context aiming at understanding the time-dependent influence on PRL release are necessary. Pertinently, Me, like M, has been shown to stimulate gene expression and, hence, may favor regeneration by bringing about the expression of regeneration conducive genes.

Though MT, like M, has found relevance as an active pineal indole with antigonadotrophic properties in mammals (Masson-Pévet et al., 1987; Saxena and Mehrotra, 1992), interestingly this indole was totally ineffective in influencing PRL release in lizards. Overall, the present study provides evidence for both M and ML as potent pineal agents capable of influencing PRL release in a time-dependent fashion and, thereby, influence the process of regenerative tail elongation. Further studies are needed to gauge the relative significance of the two indoles and their actions.

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