Acta Biologica Hungarica 52 (1), pp. 1–7 (2001)

A REPRODUCTIVE PHASE-DEPENDENT EFFECT OF DIETARY L-TRYPTOPHAN ON PINEAL GLAND AND GONAD OF A NOCTURNAL BIRD, INDIAN SPOTTED OWLET *ATHENE BRAMA*

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(Received: May 20, 1999; accepted: November 27, 1999)

Unlike other temperate owls, Indian spotted owlet *Athene brama* possesses a well-developed pineal gland that secrets moderate amount of hydroxy- (serotonin) and methoxy- (melatonin) indoles in circulation. However, in this study, we have reported the response of this endocrine gland to exogenous L-Tryptophan (precursor of the above indoles), and also its effect on gonads of this nocturnal bird. During breeding phase or pineal inactive phase (March), oral treatment of L-Trp (0.5 mg/100 g Bwt/day) significantly increased the pineal gland wt and plasma melatonin (MEL) level, while decreased the gonadal wt and plasma sex steroids levels (estradiol and progesterone in female and testosterone in male). Interestingly, during reproductively quiescent phase or pineal active phase (August), similar amount of L-Trp significantly decreased the plasma MEL level, while increased the above sex steroid levels in plasma. Finally, the results show a clear reproductive phase-dependent inverse effect of L-Trp on pineal gland and gonads for both sexes of the spotted owlets, and suggest that the therapeutic use of this amino acid would be a great advantage for controlling the reproduction of these economically important birds.

Keywords: L-Tryptophan – pineal – melatonin – gonad – sex steroid – spotted owlet-reproductive phase

INTRODUCTION

L-Tryptophan (L-Trp) is an essential amino acid for most of the organisms. It is obtained through food materials because it cannot be synthesized physiologically. Like the other amino acids, L-Trp synthesizes different structural and functional proteins and regulates different cellular activities of an organism. However, the properties like transamination, decarboxylation, hydroxylation and methoxylation of L-Trp into different indoleamines are the quite different phenomena, which have been reported extensively in pineal gland and other brain as well as peripheral tissues of the vertebrates. The pineal gland and other brain tissues take up L-Trp from circulation and synthesize serotonin (5-HT), which is converted into melatonin (MEL) following a complex process of N-acetylation and subsequent O-methylation [12]. Some *in vitro* and *in vivo* studies explained that exogenous L-Trp can regulate the

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biosynthesis of 5-HT [9, 13] and MEL [2, 7, 15, 17] in diurnal birds and mammals, nevertheless, it remains unclear in any nocturnal animal particularly in bird. Until now, particularly the L-Trp regulation of pineal gland function and in turns the pineal modulation of reproduction in any seasonally breeding bird is also unknown.

Therefore, in this present study we have emphasized on detailed investigation of the pineal gland function and pineal control of gonadal physiology of a nocturnal bird, Indian spotted owlet *A. brama* following L-Trp treatment. This study is also having a great interest, since such an attempt is first in any Strigiformes bird, in which earlier it was known that the birds do not have a functional pineal gland [16, 19]. However, with the help of morphological and endocrine observations, we for the first time suggested that the tropical Strigiformes, *A. brama* possesses a well-developed pineal gland [6, 8].

MATERIALS AND METHODS

Collection and maintenance of owlets

Adult owlets (average body weight 100 ± 11 g) were collected from the vicinity of Varanasi (Lat; 25°, 18' N; Long; 83°, 1'E) during the 1st week of March (NDL 11.30 h; 0630–1800 h) and mid-August (NDL 13.30 h; 0530–1900 h). The reproductive phases were studied earlier by our group [20]. In owlets, the breeding phase starts from Feb and retains unto mid-April, and then the gonads start to regress and enter into quiescent stage that persists unto Nov-end. We have noted earlier that during the beginning of March, the ovary shows general enlargement of stroma and follicles with little deposition of yolk, similarly the testes shows large glandular leydig cells with little intracellular cholesterol, and both the gonads start gamatogenesis during end of March, while during mid-August the above cells are existed in smallest size [4].

Both sexes were separated following laparotomy, since the birds lack sexual dimorphism. In the same time, depending on gonadal size, reproductively mature birds were selected for experiments during respective phases. Birds were acclimatized for a week in an outdoor aviary. Wooden hutment was provided for the purpose of hiding during the daytime. They were fed with fresh meat and water *ad libitum.*

Experimental design

After acclimatization during both the phases, the birds were divided into two groups i.e. (i) control and (ii) experimental, having two sub-groups of each sex (male and female). Experimental birds were administered with L-Trp (Sigma, USA) orally by dissolving first in few drops of ethanol and then in drinking water. Based on preliminary observation of the quantity of water intake by each bird, i.e. approximately 2.5 ml water/day/bird, 20 mg of L-Trp was dissolved in 400 µl of ethanol which were then mixed with 100 ml of drinking water to make it 0.5 mg/day/100 g B wt. The

control birds were received similar amount of drinking water mixed with 400 µl of ethanol. The mentioned does or volume was ingested via feeding syringe at 1200 h to each bird. After 20 days of continuous treatment the experiments were terminated.

Studied parameters, methodologies and analysis

After termination of experiments during both reproductive phases, only 7 birds from each sub-group were bled through pectoral vein at 0200 h (for MEL estimation, as the hormone has been noted in highest value during both the months at this particular hour [6]) and then sacrificed by decapitation at 1030–1100 h on the following day. The trunk blood was collected for sex steroids assays. The pineal gland, ovary, testes were dissected out carefully and weighed in a microelectronic balance (Sartorius). Collected plasma stored at was –20 °C until different hormonal assays were performed. The hormones, i.e. MEL, estradiol, progesterone of females, and MEL and testosterone of males were estimated following different quantitative assay methodologies [1, 3, 10], which are enumerated detailed in Table 1. Data were analysed with the help of Student '*t*' test.

RESULTS

A) Breeding Phase

Changes in pineal, ovary and testes weights (Fig. 1)

In comparison to controls, dietary L-Trp treatment significantly increased the pineal gland wt in females ($P < 0.05$) and in males ($P < 0.05$), on the other hand, it significantly decreased the ovary wt ($P < 0.05$) and testis wt ($P < 0.05$).

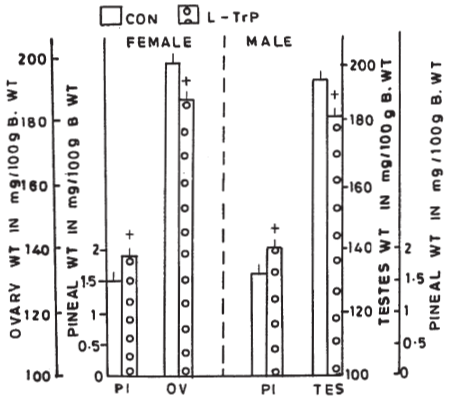

Fig. 1. Effect of dietary L-Tryptophan (L-Trp) on pineal gland, ovary and testes weight of spotted owlet during breeding phase. Vertical bars present standard error (M ± SE) +, $P < 0.05$ when compared with control

Hormonal profiles in plasma (Fig. 2)

When compared with controls, the MEL level of L-Trp treated birds was increased significantly ($P < 0.01$ for both sexes). On the other hand, L-Trp treatment significantly decreased the estradiol ($P < 0.05$) and progesterone ($P < 0.05$) levels in females, and testosterone level in males ($P < 0.05$) when compered with respective controls.

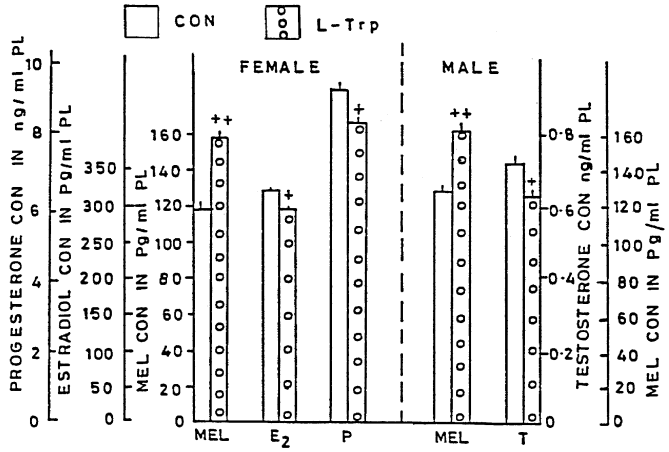

Fig. 2. Effect of dietary L-Trp on plasma melatonin (MEL), estradiol (E_2), progesterone (P) in female and melatonin, testosterone (T) level in male spotted owlets during breeding phase. +, $P<0.05$ and ++, $P < 0.01$ when compared with control

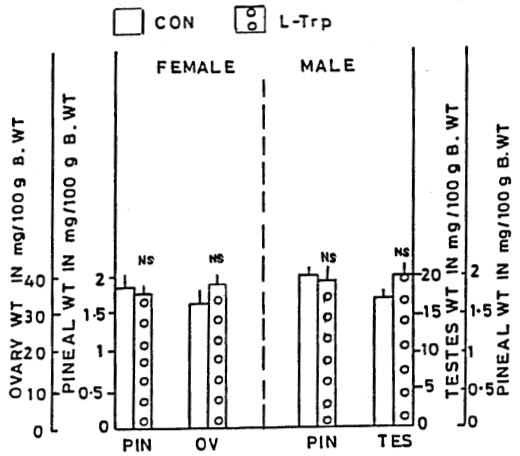

Fig. 3. Effect of dietary L-Trp on pineal gland, ovary and testes weight of spotted owlet during reproductively quiescent phase. NS = Non significant when compared with control

B) Quiescent phase

Changes in pineal, ovary and testes weights (Fig. 3)

In comparison to controls, no significant change was noted in pineal gland wt of L-Trp treated females (P = NS) and males (P = NS). On the other hand, the treatment showed an increasing trend in ovary and testes wt when compared with controls.

Hormonal profiles in plasma (Fig. 4)

When compared with controls, the L-Trp treatment significantly decreased the MEL level in females ($P < 0.05$) and in males ($P < 0.01$). On the other hand, when compared with control, L-Trp treatment significantly increased the estradiol ($P < 0.01$) and progesterone ($P < 0.05$) levels in females, and testosterone level in males ($P < 0.05$).

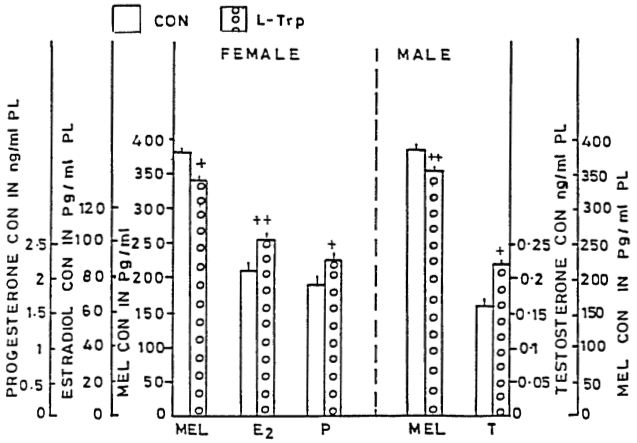

Fig. 4. Effect of dietary L-Trp on plasma melatonin, estradiol, progesterone in female and melatonin, testosterone in male spotted owlets during reproductively quiescent phase. +, $P < 0.05$ and ++, $P < 0.01$ when compared with control

DISCUSSION

Unlike the other temperate owls [19], Indian spotted owlet possesses a well-developed intermediate form of tubulofollicular and solid types pineal gland, which secrets moderate amount of 5-HT and MEL in plasma with a circannual cyclicity [6, 8]. In this study, we have noted the response of pineal gland to exogenous L-Trp (precursor of pineal indoles) stimuli during two crucial reproductive phases, breeding and quiescent, when the pineal gland was existing in completely inverse conditions with the gonads [4]. We also noted the changes in endocrine physiology of the gonads in response to the changes in pineal gland activities induced by L-Trp.

During breeding phase, a physiological dose of L-Trp (0.5 mg/day/100 g B wt) significantly increased the pineal gland wt and plasma MEL level in both sexes of the owlets (Figs 1, 2). During this reproductive phase, the pineal gland was existing in minimum state of its activity having less amount of secretory product like 5-HT and MEL [4, 8], which could be due to the less availability of L-Trp (precursor) into the cytosol of pinealocytes. Therefore, when L-Trp was administered during this phase, the physiologically inactive pineal gland could have taken up it from circulation for restoration of its intracellular activities, since it is well reported that this amino acid is essential for pineal gland activity and the gland takes up major percent of the circulating L-Trp than any other brain tissues [11]. Thus, the integration of L-Trp into cytosol of pinealocytes could have stimulated the intrapineal cell growth and MEL synthesis in treated owlets. In addition, the phenomena of integration of circulating L-Trp into extra pineal brain region particularly into hypothalamus and then its transformation into 5-HT, could be the another reason for pineal hyperactivity in treated owlets, since it has been reported earlier in rat that acting as a neurotransmitter hypothalamic 5-HT stimulates the pineal gland activity and increases MEL synthesis [13, 18].

When the experiment was repeated during reproductively quiescent phase, the same amount of exogenous L-Trp significantly decreased the plasma MEL level in both sexes, although no such significant effect was noted in pineal gland wt (Figs 3, 4). During this reproductive phase, the owlet's pineal gland was physiologically active along with maximum mass and secretory products [4, 8]. It could be that the pinealocytes were already having moderate amount of L-Trp, therefore, further ingestion of this amino acid caused the impairment of pineal gland function and affected MEL synthesis and secretion in these treated owlets. Our result in this nocturnal bird resembles the earlier reports [14, 15], in which comparatively less NAT (N-acetyltransferase) enzyme activity and less MEL concentration were noted into the pineal gland following L-Trp treatment.

On the other hand, during breeding phase the exogenous L-Trp significantly decreased the ovary wt and ovarian steroids (i.e. estradiol and progesterone) levels in plasma. Similarly, the testes wt and plasma testosterone concentration were also decreased in males following this treatment (Figs 1, 2). It could be due to the strong inhibitory influence of MEL on hypothalamo-hypophyseal-gonadal (HHG) axis of these birds [5] because the L-Trp treatment increased their pineal gland activity and MEL synthesis significantly.

While, during quiescent phase completely inverse phenomenon was noted in testicular as well as ovarian physiology following the L-Trp treatment. The gonadal wt and sex steroids (estradiol and progesterone in female and testosterone in male) were increased (Figs 3, 4). It could be due to the suppression of inhibitory influence of MEL on HHG axis, since the exogenous L-Trp inhibited the pineal MEL synthesis significantly during this phase (Fig. 4).

Finally, we find that independently of sex, exogenous L-Trp (at least 0.5 mg/day/100 g B wt) inhibits the gonadal function in sexually active owlets by stimulating the pineal MEL production, and terminates gonadal quiescency by suppressing the MEL. Hence, we suggest that the therapeutic use of L-Trp would be a great

advantage for controlling the reproduction of these economically important birds by manipulating their pineal gland activities.

ACKNOWLEDGEMENTS

Dr. B. Furr, ICI Ltd. Cheshire, UK, generously provided the antiserum for testosterone. The antiserum of estradiol was obtained from Dr. G. D. Niswender Colorado State University, Colorado.

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