EXPLANATION OF PHOTOGRAPH

DOSE - DEPENDENT EFFECT OF BROMOCRIPTINE ON TAIL REGENERATION IN LIZARDS EXPOSED TO 12L : 12D PHOTOREGIME.

A LOW DOSE (1 mg/Kg^{-1}) OF BROMOCRIPTINE HAD NO EFFECT ON LACERTILIAN TAIL REGENERATION WHILE A HIGH DOSE (2 mg/Kg^{-1}) OF THE DRUG SIGNIFICANTLY RETARDED TAIL REGENERATION IN LIZARDS EXPOSED TO 12L : 12D PHOTOREGIME.

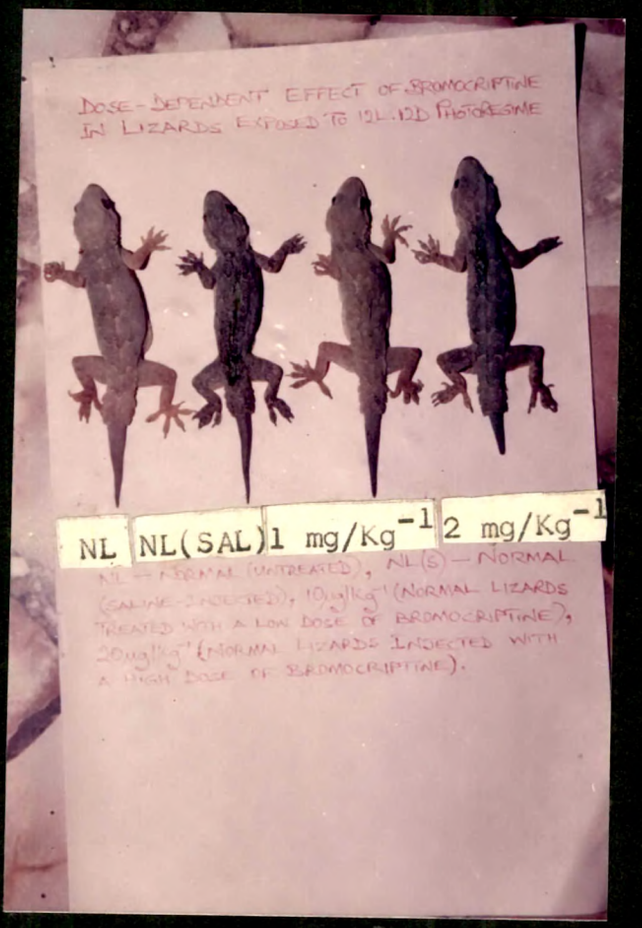
NL - NORMAL LIZARD WITHOUT INJECTION.

NL(SAL) - NORMAL LIZARD WITH DAILY SALINE INJECTION.

1 mg/Kg^{-1} - LIZARD INJECTED WITH 10 µg BROMOCRIPTINE/ DAY (LOW DOSE).

2mg/Kg^{-1} - LIZARD INJECTED WITH 20 µg BROMOCRIPTINE/ DAY (HIGH DOSE).

12L : 12D - 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS.


DOSE-DEPENDENT EFFECT OF BROMOCRIPTINE IN LIZARDS EXPOSED TO 12L:12D PHOTOPERIOD
NL
NL(SAL)
1 mg/Kg^{-1}
2 mg/Kg^{-1}
NL — NORMAL (UNTREATED), NL(S) — NORMAL (SALINE-INJECTED), 10 μg/Kg^{-1} (NORMAL LIZARDS TREATED WITH A LOW DOSE OF BROMOCRIPTINE), 20 μg/Kg^{-1} (NORMAL LIZARDS INJECTED WITH A HIGH DOSE OF BROMOCRIPTINE).

in control lizards exposed to 24L : OD and injected with 0.6% saline was 32.5mm, and their counterparts treated with 2mg. kg^{-1} bromocriptine, 32.2.mm, representing a replacement of 57.3% and 57.0% respectively. In animals exposed to 12L : 12D photoregime, the same was 25.1mm (in both 1mg. kg^{-1} bromocriptine and saline-injected control) and their counterparts treated with 2mg. kg^{-1} bromocriptine, 12.8mm which was a replacement of 48.4% and 24.6% respectively. The pattern of growth rate (Fig.2) indicates a biphasic growth rate curve in 24L : OD (one during the first 10 days and the second between 20-30 days) and a linear increase peaking on the 20th day in the 12L : 12D exposed animals. All possible comparisons between saline and 2mg. kg^{-1} bromocriptine-treated lizards in 24L : OD on one hand and between saline and 1mg. kg^{-1} bromocriptine - treated animals in 12L : 12D on the other (Student's *t* test) showed no statistically significant difference. However, all other comparisons other than these between saline and 2mg kg^{-1} bromocriptine-treated animals in 24L : OD and 12L: 12D, and between saline and 1mg. kg^{-1} bromocriptine-treated lizards in 12L:12D on one hand and 2mg. kg^{-1} bromocriptine-treated animals on the other (Duncan's multiple range test) were statistically significant at both 5% and 1% levels (Duncan, 1955) (Figs. 1 and 3).

DISCUSSION

The results of the present investigation demonstrate

that bromocriptine, a potent dopamine receptor agonist, retarded tail regeneration in the gekkonid lizard, Hemidactylus flaviviridis exposed to 12L : 12D photoperiodic condition. This study also shows that the retardation effect of bromocriptine is dose-dependent since a low dose of the drug(1mg kg^{-1}) did not affect the regeneration process while a high dose (2mg kg^{-1}) significantly retarded it. The onset of regeneration, the length of new growth (regenerate) produced by day 30, and the total percentage replacement of the lost (autotomized) tails at the end of 30 days were all significantly retarded in lizards treated daily with bromocriptine (20 µg/animal) when compared with the nonsaline/saline-injected controls as well as their counterparts treated daily with 10 µg/animal (Figs 1 and 3). The growth rate (Fig.2) showed a slower daily progression in lizards treated with the high dose of bromocriptine than the other three groups of animals.

PRL has been reported to have numerous activities in vertebrates including effects on osmoregulation, growth, and reproduction (Nicoll, 1981). PRL has been established as a growth promoter in developing organisms (Crim, 1975) and in regenerating systems (Maier and Singer, 1981; Ndukuba and Ramachandran, 1989a, Chapter 7) and has been shown to stimulate protein synthesis in developing tadpoles (Yamaguchi and Yasumasu, 1977).

In mammals, several lines of evidence suggest that inhibition of anterior pituitary PRL secretion is regulated mainly by DA released from median eminence terminals (for details and references see Fernandez-Ruis et al., 1987). PRL secretion in birds is believed to be controlled by a hypothalamic prolactin-releasing factor (PRF) (Kragt and Meites, 1965). Activation of the turkey hypothalamus by electrical stimulation induces PRL release (El Halawani et al., 1988). Several indications infer that control of PRL release in amphibians may involve both stimulatory and inhibitory hypothalamic influences (see Hall and Chadwick, 1979). Earlier, Kühn and Engelen (1976) have suggested that the amphibian hypothalamus may contain both PRF and prolactin-inhibiting factor (PIF). There are reports showing that teleostean pituitaries release large amounts of PRL in vivo indicating the involvement of hypothalamic PIF (Sage, 1966, 1968). Hall and Chadwick (1978) have reported that in the eel, Anguilla anguilla the hypothalamus contains PRL stimulating activity. In addition, James and Wigham (1984) have suggested that both an inhibitory dopaminergic and a stimulatory serotonergic system may be involved in PRL cell regulation in the trout, Salmo gairdneri. Little is known about the control of PRL secretion in reptiles. However, reptilian pituitaries have been found to release large amount of PRL in vitro, suggesting inhibitory control by the hypothalamus as in mammals

(Hall et al., 1978).

The basic aim of the present study was to ascertain whether both stimulatory and inhibitory mechanisms of PRL release operate in lizards. In attempting to solve this problem we are using an indirect method of treating the animals with pharmacological agents (drugs) that are well known agonists or antagonists of DA or serotonin (5-HT). Bromocriptine was injected in regenerating lacertilians exposed to 12L : 12D regime because recent studies did not produce any effect with the drug in lizards exposed to either 24L : OD or OL : 24D condition (Ramachandran and Ndukuba, 1989c, Chapter 9) nor in 24L : OD schedule presently. The failure of bromocriptine to affect tail regeneration in H. flaviviridis exposed to 24L : OD photoperiodic schedule, coupled with our earlier observation of a 50% retardation effect with p-CPA (Ramachandran and Ndukuba, 1989b, Chapter 8), suggest that a stimulatory serotonergic rather than an inhibitory dopaminergic system may be operative under this regime. Bromocriptine also did not affect the regeneration process in lizards kept in the OL : 24D condition (Ramachandran and Ndukuba, 1989c, Chapter 9). However, pimozide, a potent DA receptor antagonist, significantly enhanced tail regeneration in lizards maintained under OL : 24D (Ndukuba and Ramachandran, 1989b, Chapter 10), suggesting that the inhibitory dopaminergic rather than the stimulatory serotonergic system may be functioning in this regimen. We interpreted the failure of bromocriptine to affect tail regeneration in lizards, exposed to OL : 24D as indicative

of a state of full saturation of DA receptors on pituitary lactotrophs, thereby leaving no available sites for its agonist to bind. This interpretation is now positively supported by the evidence emanating from the present investigation which suggests the presence of the dopaminergic system in the 12L : 12D photoregime.

A concept on the regulatory mechanisms of PRL release in lacertilians can now be advanced. The concept is that both serotonergic and dopaminergic systems of PRL release are operative on par at the intermediate photoperiodic regimen of 12L : 12D. With increasing photoperiods, the stimulatory serotonergic system becomes more dominant. One possible mechanism is that 5-HT/serotonergic activity may inhibit DA or PIF release from the median eminence of the hypothalamus or inactivate the dopaminergic system. Caligaris and Taleisnik (1974) have suggested that such inhibition of DA neurons does occur and involves interneurons. Another possible mechanism involves the release by 5-HT of PRF which then antagonizes or suppresses the effect of DA (or PIF) at the pituitary level. The antagonistic effect of 5-HT (or PRF) on DA (or PIF) may reach the peak in the 24L : OD condition with the serotonergic neurons fully activated. With decreasing lengths of light, the dopaminergic mechanism becomes activated and a direct antagonism by DA of the serotonergic system that stimulates PRL release occurs. Alternatively, the release by DA of PIF antagonizes the effects of 5-HT or PRF at the level of the

hypothalamus. This may attain its peak in the OL : 24D photoregime where the dopaminergic neurons are fully activated.

In the light of the above concept, it is pertinent to point out that bromocriptine failed to influence tail regeneration in the 24L : OD exposed animals because the dopaminergic system does not function under this regime since the antagonistic 5-HT/PRF mechanism is functioning maximally. On the other hand, bromocriptine also did not affect the regeneration process in lizards maintained in the OL : 24D condition because the DA/PIF mechanism is functioning maximally, leading to the saturation of the DA receptors on lactotrophs and, consequently, the agonist has no available binding sites. Evidence now exists that bromocriptine retarded tail regeneration in lizards exposed to 12L : 12^{D} (this report,) providing neuropharmacological support for the existence of the dopaminergic system in this schedule. However, the fact that bromocriptine at the low dose of $1mg \cdot kg^{-1}$ did not affect the regeneration process, and also did not completely inhibit PRL release at the high dose of $2mg\ kg^{-1}$ (only 50% retardation effect was obtained), indicates that a second regulatory mechanism may be operating simultaneously in the 12L : 12D photoregime. It has earlier been suggested (Ndukuba and Ramachandran, 1989b, Chapter 10) that the second mechanism is serotonergic, probably functioning on par with the dopaminergic system under this condition. One possible experimental approach to conclusively demonstrate the existence of the stimulatory sertotonergic mechanism of PRL release in the 12L : 12D

exposed lizards is to test with a potent 5-HT receptor agonist. This proposal is suggested as the subject matter of an investigation in the near future, aimed at continuing the search for the total elucidation of the mechanisms of PRL release in reptiles.