CHAPTER X

DOPAMINE ANTAGONIST SPEEDS UP TAIL REGENERATION IN LIZARDS EXPOSED TO CONTINUOUS DARKNESS : EVIDENCE FOR PROLACTIN INVOLVEMENT

Prolactin (PRL) has been reported to have numerous activities in vertebrates including effects on osmoregulation, growth and development, and reproduction. However, the predominant osmoregulatory role played by PRL in fishes has prompted Nicoll (1981) to suggest that this action may have been the original vertebrate PRL regulatory function. In addition, he has proposed that PRL may have comparable osmoregulatory importance during the ontogeny of higher vertebrate groups. 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). It has been demonstrated that the plasma concentration of PRL is highest during the light period of the circadian cycle (Mattheij and Swarts, 1978). Indeed, it has been shown that serum levels of PRL are affected by the length of light and photoperiodic cycle. Long light periods elevate serum PRL levels while shorter lengths produce lower levels. (Leining et al., 1979). Previous studies with the lizards, Anolis carolinensis (Turner and Tipton, 1972) and Hemidactylus flaviviridis (Ndukuba and Ramachandran 1989c, Chapter 1) demonstrated

that long length photoperiod speeds up the rate of tail regeneration while a short period slows down the rate. Similar results were obtained in the newt, Notophthalmus viridescens forelimb regeneration using either continuous light or total darkness (Maier and Singer, 1977).

Neuroendocrine studies using neuropharmacological agents have amply demonstrated that dopamine has an inhibitory role in the control of PRL release. Studies with catecholamine synthesis inhibitors have unequivocally demonstrated that a catecholamine is involved in the inhibitory control of PRL release. (cf. Clemens, 1976). It is well documented that dopamine is the main regulator of pituitary PRL secretion and that it exerts its effects directly at the level of the lactotroph (Fernandez-Ruiz et al., 1987). Dopamine is known to modulate the light-evoked responses of horizontal cells in fish (Teranishi et al., 1983), turtle (Gerschenfeld et al., 1982) and Xenopus (Witkovsky et al., 1988) retinas. Dopamine receptor stimulation inhibits inositol phosphate production (Pizzi et al., 1988) and its neurons inhibit gene transcription for neuropeptides in rat (Normand et al., 1988). Noradrenaline is also involved in the control of PRL secretion, acting at the level of the hypothalamus (Day et al., 1982). The presence of both dopamine and noradrenaline in an anterior pituitary gland transplanted under the Kidney capsule has been reported (Iturriza et al., 1983; Fernandez-Ruiz et al., 1985). Moreover, results obtained in grafted rats treated chronically with dopamine agonists or antago-

nists (Esquifino et al., 1984) suggested the existence of catecholaminergic regulatory mechanisms of PRL secretion from the ectopic pituitary gland.

The catecholamine antagonists block the action of the catecholamine on its receptor. The antipsychotic drugs were a good source of catecholamine receptor blockers, because among most antipsychotic drugs, a positive correlation between dopamine receptor blocking ability and antipsychotic potency exists (Clemens, 1976). Table 2 (Clemens, 1976) summarizes the studies performed with the receptor blocking drugs. Out of this group of drugs, only pimozide is known to be a specific blocker of dopamine receptors over a limited dose range (Clemens et al., 1977). While all of the drugs listed appear to implicate some monoamine as being involved in the inhibitory control of PRL secretion, only the studies with pimozide clearly focus on dopamine as a monoamine that is involved in inhibiting PRL rease (Clemens et al., 1977)

Judging from the numeroud studies cited above, it is evident that an increasing number of reports have appeared in literature in which PRL and photoperiod were implicated in various physiological and endocrine processes in vertebrates, and as growth promoters in regenerating systems. However, to date, no investigation has attempted the use of a pharmacological agent (s) to identify the mechanism of PRL release during

lacertilian tail regeneration. Previous experimental evidence suggests that the injection of drugs that increase brain serotonin (5-HT) stimulated the release of pituitary PRL in avian species (Fehrer et al., 1983; El Halawani et al ; 1984; Hall et al., 1986) or intracerebroventricular injection of the neurotransmitter (Hargis and Burke, 1984). Parachlorophenylalanine (p-CPA), as well as other 5-HT antagonists, have been shown to decrease basal PRL levels when administered systematically to male chickens (Rabii et al., 1981), while quipazine maleate produces the opposite effect (Rabii et al., 1981). Quipazine maleate is a known 5-HT agonist (Hargis and Burke, 1984), but unlike 5-HT, it can easily cross the blood brain barrier and is not metabolized by monoamine oxidase. p-CPA is reported to deplete the 5-HT stores in the brain, peripheral tissues and blood in rats and dogs (Sloviter et al., 1978) and the drug acts by reducing serotonergic stimulation of PRL release in teleosts (Olcese et al., 1981). Thus, indirect evidence, from studies with 5-HT agonists and antagonists, indicates that 5-HT has a stimulatory role in the regulation of PRL secretion in teleosts, birds and mammals. There are no comparable reports in reptilian species (Ramachandran and Ndukuba, 1989b, Chapter 4) and present efforts, in this and other similar studies, are directed towards the establishment of the gekkonid lizard, as a model for the study of the mechanism of PRL release in reptiles. Hence, the present report demonstrates the enhancement of tail regeneration in the lizard, Hemidactylus flaviviridis exposed to continuous darkness, with daily intraperitoneal injection of the

antipsychotic drug, pimozide, a potent dopamine receptor blocker, possibly occurring by increased PRL release via the dopaminergic mechanism.

MATERIALS AND METHODS

A total of 30 lizards was used for this investigation, and they were balanced for size and sex in order to eliminate any possible error in the final statistical analysis due to size and sex differences. The animals were divided into three groups and exposed to LD 0:24 photoregime.

Group 1. Pimozide treated (50 µg/kg body wt).

The first groups of 10 lizards received once daily intraperitoneal injection of 50 µg/kg pimozide, 5 days prior to tail autotomy and 50 days thereafter.

Group 2. Control lizards (0.6 % sterile saline).

The second group of 10 lizards, which served as the control, received once daily intraperitoneal injection of

0.6% sterile saline, 5 days prior to tail autotomy and 50 days post-caudal autotomy.

Group 3. Control lizards (without saline)

A third group of 10 animals, which served as the second control, proceeded as in group 2 but without daily saline injection.

Preparation of pimozide (50 µg/kg)

Pimozide (orap by Ethnor Ltd., Bombay, India) was prepared and stored in a refrigerator at 4°C for daily use. The drug was dissolved in few drops of ethanol and then made up to the required concentration with warmed (40°C) sterile saline (0.6%). All animals in this group received daily intraperitoneal injection of 0.1 ml of the prepared solution, giving an approximate daily dose of 0.5 µg/animal.

Preparation of saline (0.6%)

0.6 gm of reagent grade sodium chloride (NaCl) was dissolved in 100ml re-distilled water with few drops of ethanol and stored in a refrigerator for daily use.

Experimental set up.

Tail autotomy was performed by pinching off the tail at the third segment from the vent. The length of tail removed from the animals varied from 50mm to 60 mm depending

on the length of each tail from the third segment to the tip of the tail. The animals were exposed to LD 0:24 and administered once daily intraperitoneal injection of 50 µg/kg pimozide and 0.6% sterile saline, in the case of the control group, for a period of 50 days after tail autotomy. Food and water were provided ad libitum throughout the entire period of experimentation. Except for a period of about 3 minutes daily exposure to dim red light for taking measurements and giving injections, all the animals were completely deprived of light. The investigation was conducted during the months of September and October and the average daily temperature at the level of the animals was 25°C.

Statistical analysis.

The length of new growth (regenerate), was measured and recorded daily with a measuring rule graduated in mm and the recorded measurements at fixed time intervals of 10, 20, 30, 40 and 50 days post-caudal autotomy were later used for morphometric calculation. The data on the length of tail regenerated and total percentage replacement were subjected to Students 't' test for statistical signifiances between pimozide treated and non saline/saline-injected animals. Values which were different at the $P < 0.01$ level were considered statistically significant.

Results

The results are clearly shown in table 1 and figures 1-3.

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN PIMOZIDE TREATED AND CONTROL LIZARDS, H.FLAVIVIRIDIS EXPOSED TO CONTINUOUS DARKNESS DURING THE MONSOON MONTHS OF SEPTEMBER AND OCTOBER.

EXPERIMENTAL ANIMALS	WOUND HEALING	BLASTEMA	EARLY DIFFEREN-TIATION	MID DIFFEREN-TIATION	LATE DIFFEREN-TIATION	GROWTH
PIMOZIDE TREATED	5	8 - 10	12 - 14	20	30	40*
SALINE TREATED (CONTROL)	7	12 - 14	18 - 20	25	35	45

* DAYS POST - CAUDAL AUTOTOMY.

TABLE 2. EFFECT OF CATECHOLAMINE RECEPTOR BLOCKING DRUGS ON PROLACTIN SECRETION (Cf.11).

DRUG	RECEPTORS BLOCKED	EFFECTS ON PROLACTIN SECRETION	REFERENCE
Chlorpromazine	Dopamine Norepinephrine Histamine 1	Increase	Lu et al. (1970) Kleinberg et al. (1971)
Perphenazine	Dopamine Norepinephrine Histamine 1	Increase	Ben-David et al. (1970) MacLeod et al. (1970) Ben-David et al. (1971)
Haloperidol	Dopamine Norepinephrine	Increase	Dickerman et al. (1972)
Cyproheptadine	Dopamine Serotonin	Increase	Clemens, unpublished
Promethazine	Histamine 1 serotonin	Decrease	Clemens et al. (1974)
Pimozide*	Dopamine	Increase	Clemens et al. (1974)
Sulpiride	Dopamine	Increase	Clemens et al. (1974)

* Highly potent

TABLE 3. LENGTH OF TAIL REGENERATED AND TOTAL PERCENTAGE REPLACEMENT IN PIMOZIDE TREATED AND CONTROL H. FLAVIVIRIDIS EXPOSED TO CONTINUOUS DARKNESS DURING THE MONSOON SEASON.

EXPERIMENTAL LIZARDS	DAY 10	DAY 20	DAY 30	DAY 40	DAY 50	TOTAL % TAIL REPLACE-MENT
PIMOZIDE TREATED (10)	6.2 ± 1.07	14.9 ± 1.70	24.6 ± 2.00	32.7 ± 2.23	36.5 ± 2.20	68.9%
SALINE TREATED (CONTROL) (10)	-	5.1 ± 1.57	43.8 ± 1.93	21.8 ± 2.31	26.3 ± 2.00	49.6%
SALINE TREATED AND EXPOSED TO LL (L) (40)	4.0 ± 0.16	7.0 ± 0.24	17.5 ± 0.30	26.5 ± 0.35	33.3 ± 0.32	62.3%

LL (L) - CONTINUOUS LIGHT OF LOW INTENSITY (638 lux)
(N) - NUMBER OF EXPERIMENTAL ANIMALS
* - TOTAL LENGTH OF REGENERATE IN MM.

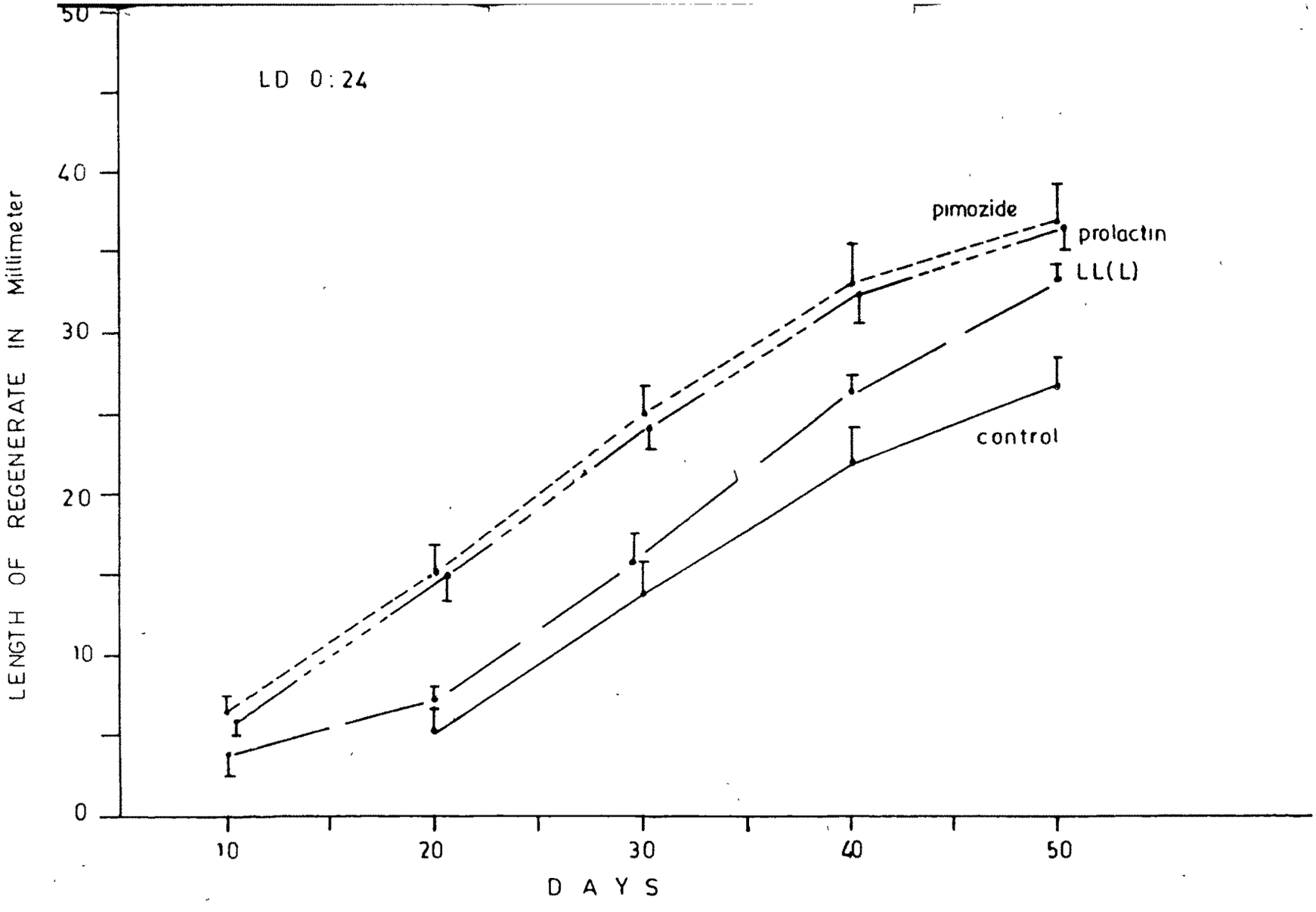

FIG.-1 LENGTH OF TAIL REGENERATED IN CONTROL AND PIMOZIDE TREATED H FLAVIVIRIDIS EXPOSED TO CONTINUOUS DARKNESS (LD 0:24), VERTICAL BARS REPRESENT (±) SD., PROLACTIN AND LL(L) CONTINUOUS LIGHT OF LOW INTENSITY (638 lux) DATA FROM NDUKUBA AND RAMACHANDRAN (1988 a, b).

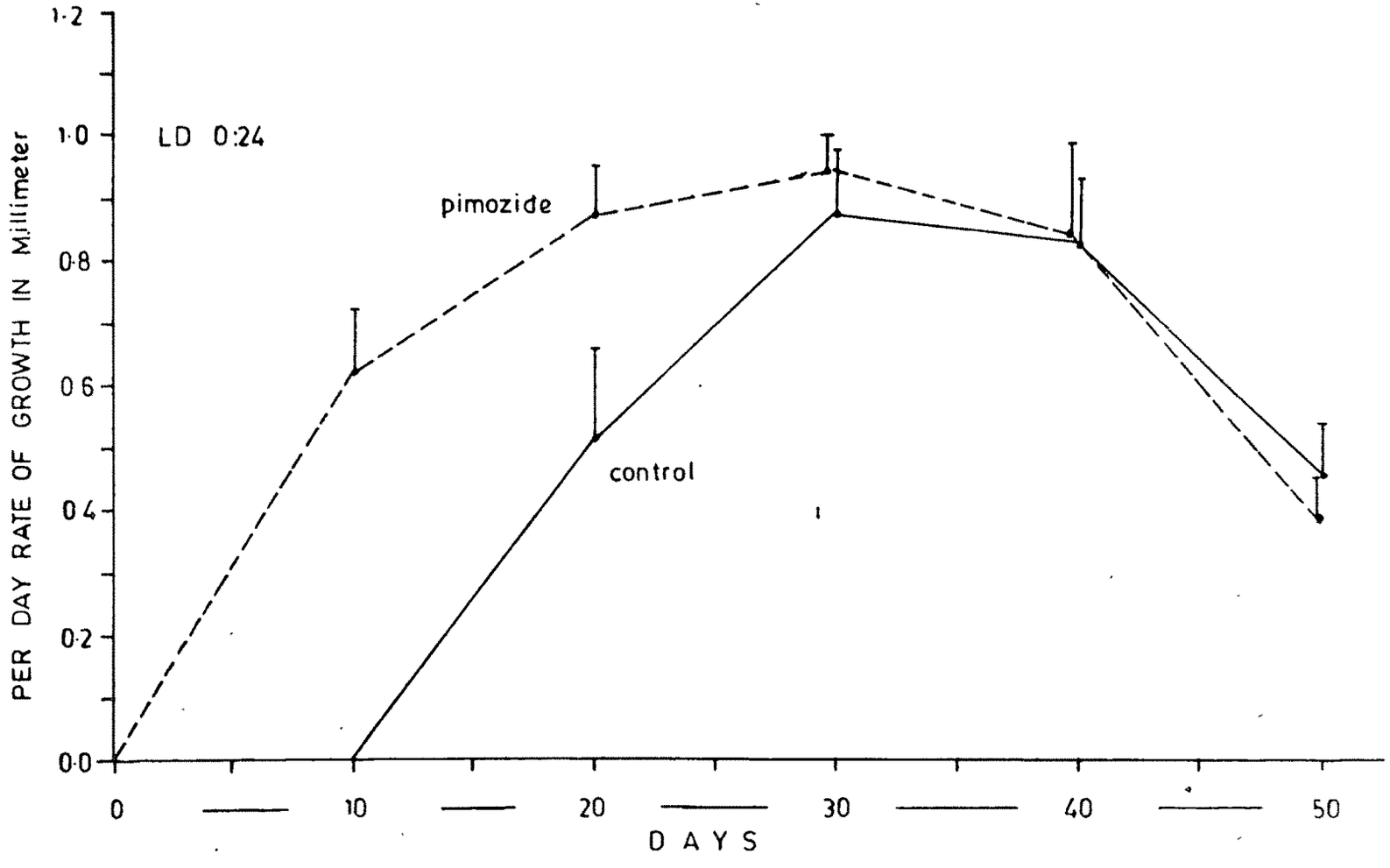

FIG. 2. PER DAY RATE OF GROWTH IN CONTROL AND PIMOZIDE TREATED H-FLAVIVIRIDIS EXPOSED TO CONTINUOUS DARKNESS (LD 0:24). VERTICAL BARS REPRESENT (±) S.D

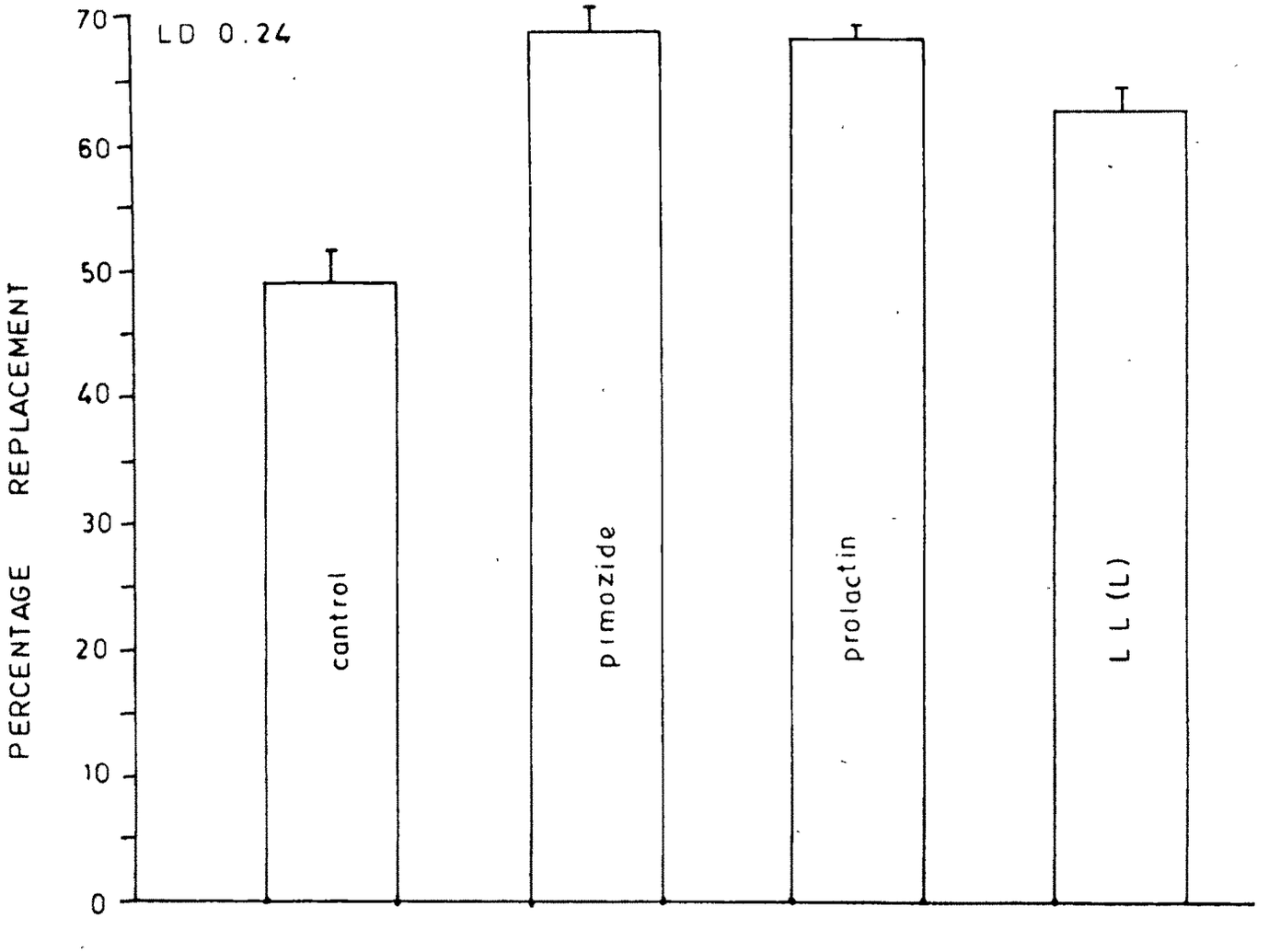

FIG-3 PERCENTAGE OF TAIL REPLACED IN CONTROL AND PIMOZIDE TREATED H FLAVIVIRIDIS EXPOSED TO CONTINUOUS DARKNESS (LD 0·24). VERTICAL BARS REPRESENT (±) SD PROTACTION AND LL(L) CONTINUOUS LIGHT OF LOW INTENSITY (638 lux) DATA FROM NDUKUBA AND RAMACHANDRAN (1988 a, b).

As there was no statistically significant difference between lizards with and without saline injection, it is thought pertinent to represent, for graphical purposes, only the saline-injected controls.

Growth rate, total length of tail regenerated and total percentage replacement. The regeneration blastema appeared in pimozide-treated lizards exposed to LD O:24 (Group 1) by day 8 to day 10 and in their counterparts injected with sterile saline (group 2) and those without saline (group 3) by day 12 to day 14 post-caudal autotomy. The total length of tail regenerated by the 50th day in intact animals injected with 50 µg/kg pimozide once daily was 36.9 mm, and those given once daily intraperitoneal injection of 0.6% sterile saline and those without saline was 26.3mm and 26.8mm which correspond to a replacement of 68.8%, 49.6% and 50.1% respectively (figs.1 and 3). The pattern of growth rate (fig.2) indicates a linear increase peaking at 30-40 days in pimozide- and saline-treated lizards. Comparisons between the three groups of animals (Student's 't' test) revealed statistical significance between pimozide-treated lizards on the one hand and saline/nonsaline treated animals on the other at the 1% level.

DISCUSSION

The results of this investigation demonstrate that the antipsychotic drug pimozide, a potent dopamine receptor antagonist, stimulated tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis maintained in LD O : 24

photoregime. The initiation of regeneration, the total length of new growth (regenerate) produced by day 50 and the total percentage replacement of the lost (autotomized) tails by the 50th day were all significantly enhanced in pimozide-treated animals when compared with those of their saline-injected counterparts, in fact, better than saline-treated lizards exposed to LD 24:0 of 638 lux-intensity (see figs. 1 and 3). The growth rate (fig.2) was enhanced during the early stages of tail regeneration (blastema and early differentiation) in pimozide-treated animals which accounted for the total better regenerative performance in this group of lizards.

PRL secretion is known to be under the tonic inhibitory control of the central nervous system. Suppression of this inhibitory influence by lesions in the median eminence of the hypothalamus (Welsch et al., 1971), transplantation of the pituitary gland under the kidney capsule (Everett, 1956) or transection of the pituitary stalk (Nikitorich-Winer, 1965) results in an increase of PRL secretion. Depletion of hypothalamic catecholamines by compounds which inhibit their synthesis resulted in a rise in serum PRL (Donoso et al., 1976). In contrast, pharmacological procedures which enhance the amine levels in brain either by the injection of monoamine oxidase inhibitors or L-DOPA, inhibit PRL release (Donoso et al., 1976; Lu and

Meites, 1971). It is generally accepted that inhibition of anterior pituitary PRL secretion is regulated mainly by dopamine (DA) released from median eminence terminals. (See Fernandez-Ruiz et al., 1987). The presence of DA in hypophysial protal blood (Plotsky et al., 1978) and the localization of DA receptor sites in the anterior pituitary gland (Caron et al., 1978) suggest that DA could act on membrane receptors in hypophysial anterior lobe. However, the presence of intracellular DA in the anterior pituitary gland (Rosenzweig and Kanwar; 1982) and the demonstration that its variations are inversely related to serum PRL (Chiocchio et al;, 1980) suggest a second level of DA action. The observation that an increase in circulating PRL is associated with a decrease in DA content in the pituitary gland and vice versa (Chiocchio et al., 1988) suggested that DA could be contained in the lactotroph cells. Other authors (Nansel et al., 1979), reported that DA and PRL were present in the same particle. The study by Gallardo et al.(1985) presents direct evidence of the accuracy of both contentions.

The present study is the first investigation which demonstrates the effect of the antipsychotic drug and a potent dopamine antagonist, on tail regeneration in a tropical saurian. In previous reports, it has been shown that continuous light stimulates tail regeneration while continuous darkness depresses the same in the lizard H. flaviviridis.

(Ndukuba and Ramachandran, 1989c, Chapter 1) and further, the lateral eyes, or retinae, do not participate in photoperiodically significant photoreception, since blinded lizards regenerate their lost (autotomized) tails like their sighted counterparts exposed to similar experimental photoperiodic schedules (Ndukuba and Ramachandran, 1988, Chapter 2). Moreoever, it has been demonstrated that the pineal organ is the principal site of extraretinal photoreception in Hemidactylus since both pinealectomy, as well as light deprivation to the pineal abolished the stimulatory influence of increasing lengths of light and significantly retarded the regeneration process (Ramachandran and Ndukuba, 1989a, Chapter 3) and exogenous PRL stimulated tail regeneration in intact but not pinealectomized lizards exposed to continuous darkness suggesting that the pineal is somehow linked with the favourable influence of light on tail regeneration in lacertilians (Ndukuba and Ramachandran, 1989a, Chapter 7). In our recent investigations, p-CPA, an inhibitor of tryptophan hydoxylase (Koe and Weisman, 1966; Walker, 1983) and an agent employed for chemicals pinealectomy, retarded tail regeneration in animals exposed to LD 24 : 0, indicating that lizards with physically intact pineals but deprived of their ability to synthesize 5-HT do not exhibit the favourable influence of light on tail regeneration in lizards (Ramachandran and Ndukuba, 1989b, Chapter 8). However, bromocriptine, a potent DA agonist, did not affect the regeneration process in lizards exposed to

either LD 24 : 0 or LD 0 : 24 photoregimes(Ramachandran and Ndukuba, 1989 Chapter-9).

Since bromocriptine failed to retard tail regeneration, it was thought pertinent to investigate with the potent DA receptor blocker, pimozide. Once daily intraperitoneal injection of pimozide (50 µg/kg) for 50 days post-caudal autotomy enhanced the regenerative performance of lizards maintained in LD 0:24 when compared with their saline injected counterparts. Interestingly, the regenerative performance of pimozide treated animals was similar to that of exogenous ovine PRL-treated lizards (Ndukuba and Ramachandran, 1989a Chapter 7). These results may suggest that in lizards, as in mammals, the anti-psychotic drug, pimozide, has the potency of blocking the inhibitory role of DA on PRL release. Several studies infer that, hypothalamic neurons which secrete prolactin inhibiting factor (PIF), are tonically stimulated by catecholaminergic fibres thus maintaining, in resting condition, a sustained release of PIF, and, as a consequence, PRL secretion is restrained (Caligaris et al., 1974). Suppression of the influence of catecholamines by pharmacological procedures results in the enhancement of PRL release (Caligaris et al., 1974). There is considerable evidence in favour of regulation of PRL cells by DA and 5-HT in mammals (Weiner and Ganong, 1978) and, to a lesser extent in other vertebrates (Ball, 1981). The drug

p-CPA, reduces 5-HT synthesis by inhibiting tryptophan hydroxylase and so blocking the conversion of tryptophan to 5-HT (Koe and Weisner 1966; Walker; 1983). That p-CPA does act by reducing serotonergic stimulation of PRL is indicated by the reported decline in brain 5-HT and pituitary cAMP levels in Salmo gairdneri following p-CPA treatment (Olcese et al., 1981). In addition, Olivereau (1978) has reported that p-CPA produced a decrease in PRL cell function in Anguilla anguilla, as indicated by reduced cell nuclear area and increased cytoplasmic granulation. In some teleosts, DA seems to be inhibitory (McKeown et al., 1980). The report of James and Wigham (1984) suggests that DA exerts an inhibitory influence on PRL cells in S. gairdneri. The ability of domperidone to stimulate PRL synthesis in S. gairdneri suggests that the DA receptors controlling PRL secretion are situated in the pituitary since this drug is unable to cross the blood-brain barrier. (Pourmand et al., 1980).

The earlier observation that bromocriptine had no effect on regenerating lizards exposed to either LD 24: 0 or 0:24 (Ramachandran and Ndukuba, 1989a, chapter 9), coupled with the present finding that pimozide stimulated tail regeneration in LD 0:24 exposed animals, may suggest that both serotonergic and dopaminergic systems of PRL release are operative on par at the intermediate photoperiodic regime of LD 12 : 12. With

increasing photoperiodism, there is a direct antagonism by 5-HT of the dopaminergic system that inhibits PRL release. One possible mechanism is that 5-HT may inhibit DA (or PIF) release from the median eminence of the hypothalamus. Caligaris and Teleisnik (1974) have suggested that such inhibition of DA neurons does occur and involves interneurons. In mammals, where the portal blood supply is the major functional link between the hypothalamus and the adenohypophysis (Holmes and Ball, 1974), there is good evidence that 5-HT acts at the hypothalamus, rather than directly on the pituitary (Weiner and Ganong, 1978). In birds, as in mammals, the secretion of anterior pitutitary hormone is under the influence of the hypothalamus (Daris and Follett, 1975) and the avian hypothalamus appears to exert predominantly stimulatory influence on the release of pituitary PRL (Harvey et al., 1979). Evidence exists that the activation of the turkey hypothalamus by electrical stimulation induces PRL release via serotonergic neurons within the ventromedial nucleus (El Halawani et al., 1988). It is, therefore, interesting to speculate on the possible similarity between mammals, birds and lizards in the hypothalamo-hypophysial circulatory system. Another possible mechanism involves the release by 5-HT of a PRL releasing factor (PRF) which then antagonizes the effect of DA (or PIF) at the pituitary level. The antagonistic effect of 5-HT (or PRF) on DA (or PIF) probably reaches the peak in the LD 24:0 condition with the serotonergic neurons fully

activated. With decreasing photoperiodism, the dopaminergic mechanism becomes activated and a direct antagonism by DA of the sertotonergic system that stimulates PRL release occurs. Alternatively, the release of DA or PIF antagonizes the effects of 5-HT or PRF at the level of the hypothalamus. This may attain its peak in the LD 0:24 photoperiodic schedule where the dopeminergic neurons are fully activated.

Viewed in this perspective, the observation that bromocriptine failed to influence tail regeneration in the LD 24:0 exposed animals indicates that the dopaminergic system does not function under this regimen since the antagonistic 5-HT/PRF mechanism of PRL release is functioning maximally. On the other hand, bromocriptine also did not affect the regeneration process in lizards maintained in the LD 0:24 schedule, probably because the DA/PIF mechanism is functioning maximally leading to the saturation of the dopaminergic receptors on lactotrophs and, consequently, the agonist has no available binding sites. However, pimozide, stimulated tail regeneration in the LD 0:24 exposed lizards (this report) confirming the proposition that the dopaminergic mechanism of PRL release may be operative under this conditions. And having previously shown that p-CPA, an ihibitor of 5-HT synthesis, retarded tail regeneration in the LD 24:0 condition (Ramachandran and Ndukuba, 1989b, Chapter 4), it was felt that it would be interesting to conclusively demonstrate the sertotonergic mechanism of PRL release during lacertilian

tail regeneration with the help of one of the well known 5-HT receptor antagonists, such as cyproheptadine, methysergide or SQ 10,631. This investigation is now in progress in our laboratory using methysergide, a potent 5-HT receptor antagonist. There is also an ongoing study in our laboratory in search of conclusive experimental evidence, to support the dopaminergic regulatory mechanism, with bromocriptine injection in lizards exposed to LD 12:12 where it has been suggested that both the sertonergic and dopaminergic mechanisms of PRL release may be functioning on par (this study).