CHAPTER IX

GENERAL DISCUSSION

It would appear from the present observations that there was no strict correlation between plasma LH levels and hypothalamic TH activity. Reduced LH levels were associated with increase in TH activity, while increase in both LH levels and TH activity was evident following intraventricular administration of acetylcholine and also after progesterone treatment.

Dopamine has been shown to have both stimulatory and inhibitory actions on LH release. In vitro studies demonstrated that DA stimulates LH RH release from hypothalamic fragments (Fotsztejn et al., 1976), hypothalamic synaptosomes (Bennet et al., 1975) and also from median eminence tissue (Negro-Vilar et al., 1978). Intraventricular injection of DA or its agonist apomorphine is
capable of releasing LH in OVX, steroid-primed rats (Vijayan and McCann, 1978) and also pulse iv injection of DA elevated LH in the steroid-primed and also in OVX animals (Vijayan and McCann, 1978). At the same time evidence for an inhibitory role of DA on LH release was put forward by Fuxe and Hokfelt (1969) who observed that decreased turnover of tuberoinfundibular dopaminergic neurons was associated with enhanced LH release and vice versa. TH activity in hypothalamus was significantly lowered at 1200h during proestrus and this decrease was followed by elevation of serum LH in the afternoon. Hypothalamic TH activity was significantly elevated following estrogen treatment to OVX rats, with concomitant decrease in plasma LH levels. Increased TH activity following acetylcholine administration is also observed in noradrenergic (NA) systems, suggesting the formation of noradrenaline (Lewander et al., 1975). Progesterone also enhances NA turnover (Simkins et al., 1978; Kaira et al., 1978). Noradrenaline, which strongly stimulates LH release is implicated in the physiological regulation of LH secretion (Sawyer, 1977; Martinovic and McCann, 1977; Vijayan and McCann, 1978). The increased luteinizing hormone release following acetylcholine and progesterone treatment may possibly be due to enhanced NA synthesis. NA synthesis actually enhances LH release since stimulation
of LH release by pre-optic stimulation can be blocked by inhibitors of noradrenaline synthesis (Kalra and McCann, 1973).

Paradoxically neurotransmitter/neuropeptide administration generally failed to show any effect on FSH release, while LH levels were modified by some. Inhibin is the only peptide, other than LHRH, that could alter the release of FSH. Intraventricular injection of ovine inhibin selectively suppressed plasma FSH levels without altering LH suggesting a hypothalamic site of action of inhibin. Inhibin preparations obtained from bovine spermatozoa (Lugaro et al., 1974), ram rete testis fluid (Lumpkin et al., 1981) also specifically inhibited FSH release, indicating that inhibin may selectively regulate FSH secretion possibly by controlling a follicle stimulating hormone releasing factor (FSHRF), different from LHRH. However, the FSH inhibin employed in the present study significantly suppressed both FSH and LH in immature rats. This preferential but non-specific effect has been observed previously in vivo and in vitro using preparations of inhibin extracted from bovine testis (Baker, et al., 1976), bull seminal plasma (Franchimont et al., 1975; 1977; Setchell et al., 1977) and ram rete testis fluid (Franchimont et al., 1977; Lee et al., 1977; Davies et al.
1978). It was suggested that these preparations are capable of reducing LH, usually in doses much higher than those which induce a significant decrease in FSH.

Though the existence of a separate FSHRF has been suggested by several studies involving purification of hypothalamic extracts (Bowers et al., 1973; Igarashi et al., 1974; McCann, et al., 1974), its chemical identity has not been conclusively demonstrated to date.

It may be seen that, in general, there was correlation between decreased PRL levels and increased hypothalamic TH activity. On the other hand, TH activity was unaffected in general, or elevated following steroid or reserpine treatment where PRL concentrations were also elevated.

The secretion of PRL by anterior pituitary is under the tonic inhibitory control of the hypothalamus. It is indicated that in mammals dopamine is the major PRL inhibitory hormone released from the terminals of tuberoinfundibular dopaminergic neurons in the median eminence, is transported via the hypothalamic-hypophyseal portal system to the anterior pituitary, where it directly inhibits PRL release from pituitary lactotrophes (Ojeda et al., 1974;
MacLeod and Lehmeyer, 1974; MacLeod, 1976). It is generally accepted that dopamine mediates the inhibition of PRL secretion by binding to specific pituitary receptors (Calabro and MacLeod, 1978; Caron et al., 1978; Cronin et al., 1973). Dopamine is also shown to be associated with PRL secretory granules (Nansel et al., 1979) and suggested to have an action in inhibiting PRL synthesis to some extent (MacLeod et al., 1980).

As dopamine has been now clearly demonstrated in portal vessels and that DA concentrations in hypophyseal portal plasma are sufficient to inhibit PRL release (Gibbs and Neill, 1978) has led some to propose that dopamine is the prolactin inhibiting factor (PIF). But complete deafferentation of medial basal hypothalamus is not followed by an elevation of PRL levels (Kruitich et al., 1975) and a strict inverse correlation between PRL levels and hypothalamic (including ME) DA levels is lacking. Though there was negative correlation between plasma PRL levels and hypothalamic TH activity during estrous cycle, estradiol stimulated PRL release and increased TH activity in OVX rats.

It is suggested that circulating PRL stimulates the activity of tubero-infundibular dopaminergic (TIDA) neurons.
This hormonal feedback effect is observed 12-24h following systemic or intracerebroventricular injection of PRL (Hökfelt and Fuxe, 1972; Gudelsky et al., 1976) or after endogenous PRL concentrations are increased by pharmacological or endocrinological manipulations (Moore and Wuerthele, 1979). Prolactin appears to activate the synthesis and release of dopamine in these neurons (Gudelsky and Porter, 1980 and Johnston et al., 1980). The increased hypothalamic TH activity following steroid or reserpine treatment may be attributed to the neuronal mediation of the prolactin autoregulatory feedback effects by TIDA system.

It appears from these observations that dopamine is probably the major prolactin inhibiting factor (PIF). However, since a precise inverse correlation between hypothalamic DA, as determined by TH activity, and circulating prolactin concentrations is lacking, some extra factors in addition to DA are suggested to modulate PRL secretion, including nondopaminergic PIF and prolactin releasing factor (PRF). Hypothalamic synaptosomes devoid of DA activity still showed a dose dependent inhibitory effect on PRL release (Enjalbert et al., 1977). A naturally occurring metabolite of TRH, histidyl-proline-diketopiperazine (DKP), is present in the median eminence and pituitary and shown to inhibit PRL release from normal pituitaries as well.
as cloned prolactin cells (Enjalbert et al., 1979; Bauer, 1978). It is possible that dopamine may stimulate the release of PIF, in addition to its direct action on pituitary (McCann et al., 1979).

In general the hypothalamic TH activity was not decreased with concomitant elevation of PRL levels (see Results). The activation of PRL secretion by stress or suckling is probably not via inhibition of dopaminergic system since both stimuli still activated PRL release after the inhibition of this system by pimozide or α-MPT (Marchlew ska-koj and Krulich, 1975). Other neurotransmitters like serotonin and histamine have PRL releasing activity (McCann et al., 1978). Hypothalamic extracts contain several components capable of stimulating PRL secretion (Boyd et al., 1976 and Szabo and Frohman, 1976). One of the components which is known to have a stimulatory effect on prolactin secretion is thyrotropin releasing hormone (TRH) (Tashjian, 1971). However, TRH is not likely to be the major or sole, physiological PRF (Shin, 1978). In addition to TRH, vasoactive intestinal peptide (Vijayan et al., 1979) and opioids, endorphins and enkephalins, are implicated as prolactin releasing factors. (Enjalbert et al., 1979). The effect of VIP on PRL release is still observed in the presence of dopamine antagonists (Enjalbert et al., 1980) and met-enkephalin
and β-endorphin are able to antagonize the inhibitory action of dopamine (Enjalbert et al., 1979). The physiological relevance of endogenous opiates in prolactin control is better established than that of the other prolactin releasing factors, since administration of opiate antagonists like naloxone or naltrexone suppresses prolactin release observed during stress or after suckling of lactating animals (Ferland et al., 1978; Grandison and Guidotti, 1977 and Van Vugt et al., 1978).

CONCLUSIONS:

It is evident from the present results that neurotransmitter GABA has a modulatory role in the control of prolactin release. However, its physiological significance in modifying LH release appears to be limited since only very high doses of intraventricular GABA resulted in stimulation of LH release. Acetylcholine appears to inhibit PRL release via stimulation of tuberoinfundibular dopaminergic neurons, which in turn release dopamine into hypophyseal portal system that can act directly on adenohypophysis. On the other hand, Ach stimulated LH release and this effect was inhibited by Ach receptor blocker, atropine. Acetylcholine may regulate circhoral LH release that occurs in the castrate.
Arginine vasotocin, a peptide which has been demonstrated recently by RIA, in mammalian pineal gland, strongly inhibited both LH and PRL following intraventricular injection in OVX, conscious rats and increased hypothalamic TH activity, suggesting that this pineal peptide has a role in the control of LH and PRL release. Another peptide bombesin, which was first isolated from anuran skin and recently demonstrated in mammalian CNS, produced a near total depletion of plasma PRL following its third ventricular administration without affecting gonadotropin release indicating that this peptide has a potent inhibitory action on PRL release in OVX, conscious rats. Hypothalamic dopaminergic mediation of this peptide is possible since TH activity was increased after its ivt injection. Third ventricular injection of gastrointestinal tract peptide, secretin, whose immunohistochemical demonstration indicated its presence in mammalian hypothalamus, pituitary gland, and other parts of the CNS, exhibited differential effects on PRL release. Secretin inhibited LH levels and increased hypothalamic TH activity at lower doses. Bombesin and secretin appear to act on both hypothalamus and pituitary showing opposite effects on PRL release i.e. an inhibitory effect by acting on a hypothalamic site, while they produced stimulatory effect
on pituitary lactotrophs in other studies (Westendorf and Schonbrunn, 1982; Enjalbert et al, 1980).

Luteinizing hormone releasing hormone showed inhibitory effects on pregnancy which appear to be exerted also at gonadal level. Inhibin, a non-steroidal gonadal factor, seems to be involved in the physiological regulation of FSH release by acting on hypothalamic as well as pituitary sites. Its site specificity in the selective regulation of FSH, however, requires further investigation.