THE ROLE OF EXCITATORY AMINO ACID NEUROTRANSMITTERS IN THE CENTRAL REGULATION OF PROLACTIN SECRETION IN NONHUMAN PRIMATES

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CERTIFICATE

This thesis by Sumera Sajjad is accepted in its present form by the Department of Biological Sciences as satisfying the thesis requirement for the degree of Doctor of Philosophy in Biology (Reproductive Physiology/Neuroendocrinology).

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Dated: 19.4.2005

DEDICATED TO
THE LOVE
AND
SACRIFICE
OF
MY PARENTS

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STUDY 3 *INTERACTION OF EXCITATORY AMINO ACID NEUROTRANSMITTERS WITH ADRENERGIC PATHWAY FOR THE REGULATION OF PROLACTIN*

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SUMERA SAJJAD

LIST OF ABBREVIATIONS

ARC	Arcuate Nucleus
ANOVA	Analysis of variance
CNQX	Cyano-3, 3-dihydro-7-nitroquinoxaline
CNS	Central Nervous System
DA	Dopamine
DNQX	6,7-Dinitroquinoxline2,3-dion
EAA	Excitatory Amino Acid
EOP	Endogenous Opioid Peptide
FSH	Follicle Stimulating Hormone.
GABA	Gamma Amino Butyric Acid
HA	Histamine
hGH	Human Growth Hormone
hPRL	Human Prolactin
LH	Lutenizing Hormone
MK-801	5-methyl-10, 11-dihydro-5H-dibenzo-cyclohepten-5,10-imine meleate
NaCl	Sodium Chloride
NAL	Naloxone
NE	Norepinephrine
NMA	N-methyl-D-Aspartic Acid
NMDA	N-methyl-D-aspartate
Ph.a.	Phentolamine
PIF	Prolactin Inhibiting Factors
PMSG	Pregnant Mare Serum Gonadotropin
PRF	Prolactin Releasing Hormone
PVN	Paraventricula Nucleus
SCN	Suprachiasmatic Nucleus
TH	Tyrosine Hydroxylase
THDA	Tuberhypophyseal Dopaminergic Neurons

TIDA	Tuberoinfundibular Dopaminergic Neurons
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone
VIP	Vasoactive Intestinal Peptide

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

Prolactin (PRL) is secreted by the anterior pituitary gland from cells called mammotrophs or lactotropes. It is the most versatile and diverse of all the pituitary hormones in its physiological actions (Nicoll, 1974; De Velming, 1979; Leong *et al.*, 1983). It serves several functions including osmoregulation, growth, development and reproduction (Nicoll, 1974; Clark and Bern, 1980; Nicoll *et al.*, 1986). The PRL molecule is a single polypeptide containing 198 amino acid residues with a molecular weight (MW) of 22,000 (Shome and Parlow, 1977). The structure is folded to form a globular shape, and three disulphide bonds connect the folds. The hPRL gene was cloned in 1981 (Cooke *et al.*, 1981).

The lactotrope of the adenohypophysis is the cell that synthesizes and secretes PRL. However, immunohistochemical studies indicate that some pituitary cells contain human growth hormone (hGH) as well as human prolactin (hPRL), suggesting that both hormones may be produced and secreted by a single cell (Zimmerman *et al.*, 1974). In normal pituitaries, lactotropes constitute at least 20 % of the pituitary cell population and aggregated mainly in the posterior lateral wing of the adenohypophysis (Zimmerman *et al.*, 1974).

Of all pituitary hormones, PRL has the most diverse actions. According to Nicoll and Bern (1971) there are six distinct functional categories including control of water and electrolyte balance, regulation of growth and development, metabolic effects, control of reproductive functions, effects on integument and ectodermal structures and synergism with steroids.

Nicoll in 1980 reported that within the above six categories PRL may have at least 227 different effects. For example death by inhibition of sodium loss through the gills in hypophysectomized killi fish (*Fundulus heterclitus*) is prevented by PRL (Pickford et al, 1970). PRL stimulates growth of the tail and tail fin in tadpole of frog and its treatment results in a doubling of body weight and a five-fold increase in the length of larval *Rana pipiens* (Dent, 1975).

The concept that PRL is a metabolic hormone was advanced by Riddle in 1963. PRL has some of the effects attributed to growth hormone (GH). PRL promotes the growth of the visceral organs of birds. Production of crop milk and stimulation of

brooding behavior are examples of the ability of PRL to control reproductive functions in birds (Hodson, 1982).

In 1980 Nicoll reported that there were 67 actions of PRL on the integument (Nicoll, 1980). Some examples are hair growth, sebaceous gland activity and mammary gland alterations in mammals, pigmentation in amphibians, cornifications of the reptilian skin and secretion of mucus by fish skin glands (Dent, 1975).

PRL has been known as a luteotropic hormone especially in rodents. It is involved in initiating luteinization of granulosa cells, in maintaining their levels of progesterone synthesis in luteal cells and inhibiting the activity of progesterone categorizing enzyme particularly in rodents (Rothchild, 1981). PRL has been demonstrated to enhance progesterone production in cultured granulosa cells of rats (Crisp, 1977) and porcine (Veldhius and Hammond, 1980) pre-ovulatory follicles. The appearance of specific receptors in granulosa cells, late follicular development and their induction by follicle stimulating hormone (FSH) in culture indicates the likelihood that PRL may exert a physiological action on granulosa cells at the stage of terminal differentiation when they are transformed into luteal cells. PRL injections (Advis *et al.*, 1981) or hyperprolactinemia induced by *in vivo* administration of dopaminergic receptors blocker (Siegal *et al.*, 1976; Gay *et al.*, 1970) have been found to induce precocious puberty, as well as to increase ovarian responsiveness to LH in immature rats. In contrast to the stimulatory action of PRL on progesterone secretion, progesterone production by granulosa cells from small immature porcine follicle was markedly inhibited by physiological concentration of PRL (Bex and Goodman, 1975) and can be reversed by estradiol exposure (De Paolo *et al.*, 1979).

Another inhibitory effect of PRL on estradiol secretion was reported for cultured rat granulosa cells obtained from follicles at both pre-antral and pre-ovulatory stages (Fujii *et al.*, 1983; Sauder *et al.*, 1984). Decreased estradiol secretion *in vitro* appears to be due, at least in part, to an inhibiting action of PRL on FSH induction of aromatase activity (Welschen *et al.*, 1980; Chappel and Selker, 1979). PRL has been reported to suppress basal and gonadotropin-stimulated estradiol secretion by human ovaries perfuse *in vitro* (Lee, 1983).

The ability of PRL to affect the spermatogenesis and growth of male accessory reproductive glands (Bartke, 1976) was described long before it was possible to quantitate peripheral levels of PRL in the male or demonstrate the presence of PRL receptors in tissues thought to respond directly to the action of this hormone. The

early suggestions that PRL can act directly on the male reproductive system received strong support from the demonstration that specific PRL receptors are present in the interstitial compartment of the testis (Aragona *et al.*, 1977; Charreau *et al.*, 1977) and in the male accessory reproductive glands (Aragona *et al.*, 1977; Charreau *et al.*, 1977; Kledzik *et al.*, 1976).

The ability of PRL to influence testicular function can most readily be demonstrated in PRL-deficient animals. In the golden hamster exposure to a short photoperiod or complete darkness causes a drastic reduction in PRL levels in the pituitary and in peripheral plasma and a more modest reduction in leutinizing hormone (LH) and FSH levels (Berndtson and Desjardins, 1974; Reiter and Johnson, 1974). This is accompanied by testicular atrophy, loss of libido and infertility. Administration of PRL to dwarf mice and to hamsters with photoperiod-induced testicular atrophy stimulates growth of testes and accessory reproductive glands, increases testicular testosterone production and spermatogenesis and induces fertility (Bartke *et al.*, 1977; Bex *et al.*, 1978)

The mechanism responsible for the stimulation of testicular function by PRL was suggested by the results obtained in hypophysectomized animals. In hypophysectomized rats and mice, PRL significantly augmented the effects of exogenous LH on biosynthesis of testosterone and spermatogenesis (Bartke, 1971; Hafiez *et al.*, 1972). In contrast, PRL did not potentiate the action of exogenous testosterone on spermatogenesis and had little, if any effect when administered alone (Bartke, 1971; Hafiez *et al.*, 1972). It was also demonstrated that treatment of hypophysectomized rat with PRL increases their ability to produce testosterone in response to acute LH stimulation (Bartke *et al.*, 1978). These results suggest that PRL can act on the Leydig cells to increase their responsiveness to LH stimulation. This action of PRL appears to be particularly important during the seasonal changes in gonadal function in the golden hamster. In this species, PRL can both prevent and reverse testicular atrophy induced by binding or by exposure to a short photoperiod (Bex *et al.*, 1978; Matthews *et al.*, 1978).

PRL increases the sensitivity of the testes to LH stimulation by increasing the ability of the leydig cells to bind LH. PRL deficiency in hereditary dwarf mice, in hamsters exposed to short photoperiod and in rats treated with an inhibitor of PRL release is associated with loss of testicular LH receptors (Aragona *et al.*, 1977; Bex and Bartke, 1977; Bohnet and Friesen, 1976). Treatment with PRL increases

concentration of LH receptors in the testes of dwarf mice (Aragona *et al.*, 1977; Golder *et al.*, 1972), hamster (Bex and Bartke, 1977) and hypophysectomized rats (Zipf *et al.*, 1978). In addition to its effects on testicular LH receptors, PRL can stimulate accumulation of esterified cholesterol and the activities of 3β- and 17β-hydroxysteroid dehydrogenases in the testes (Bartke, 1976).

It has been documented that PRL can potentiate the effects of exogenous androgens on the growth of male accessory reproductive glands in castrated animals (Thomas and Keenan, 1976). Administration of PRL alone to castrated males causes a small but detectable increase in the weight of accessory reproductive glands and it has been shown that this effect of PRL is not mediated through the pituitary or the adrenal gland (Bartke and Lloyed, 1970; Negro-Vilar *et al.*, 1977). The fact that PRL binding to prostatic membranes and cytosol is androgen-dependent (Charreau *et al.*, 1977; Kledzik *et al.*, 1976) provides an explanation for the greatly reduced responsiveness of accessory reproductive glands to PRL in the absence of endogenous or exogenous testosterone. Evidence also suggests that PRL may affect the number of LH receptors in the ovary and thus modulate steroidogenesis in the follicular cells (Zipf *et al.*, 1978).

It appears that the ability of PRL to stimulate growth of accessory reproductive glands in castrated males may be related to physiological action of PRL in intact males. Suppression of endogenous PRL levels by active immunization with heterologous PRL or by treatment with anti-PRL serum can decrease weight and secretory activity of accessory reproductive glands in rabbit (Asano *et al.*, 1971), mouse (Bartke 1974), rat (Hostetter *et al.*, 1977) and ram (Ravault *et al.*, 1977).

Several lines of evidence suggest that PRL can also affect the function of the male reproductive system indirectly, by altering the release of pituitary gonadotropins. In two types of genetically dwarfed mice, treatment with ovine PRL or with PRL producing ectopic pituitary homografts caused a significant increase in peripheral FSH levels (Bartke *et al.*, 1977). The ability of PRL to stimulate FSH release may account for some of its effects on the testis because FSH can increase testicular LH binding and potentiate the effects of LH on testosterone production (Bartke *et al.*, 1978). The PRL-induced FSH release could also explain why effects of PRL on the testes of hypophysectomized animals are less striking than those observed in intact males with PRL deficiency.

Excessive PRL release in men with anterior pituitary tumors is typically associated with a drastic decline in libido and potency and can be accompanied also by various degrees of hypogonadism (Thorner and Besser, 1978). Hyperprolactinemia appears to be responsible for the decline in sexual and reproductive functions, because suppression of PRL release usually results in rapid improvement of libido and potency and often appears to stimulate testicular function (Thorner and Besser, 1978). The results indicate that sustained elevation of plasma PRL in adult male rats does not affect plasma testosterone levels, testicular weight or fertility, that it significantly reduces LH release in response to LH releasing hormone (LHRH) administration or gonadectomy. If donors of pituitary grafts are of same inbred strain as the recipient, basal levels of LH and FSH in the plasma are also significantly suppressed (Bartke *et al.*, 1977; McNeilly *et al.*, 1978).

PRL appears to be normally involved in the pituitary control of male reproductive functions in a variety of mammalian species. In addition to influencing pituitary gonadotropin release and growth of male accessory reproductive glands, PRL acts directly on the leydig cells and increase their ability to bind and respond to LH by increased testosterone production (Bartke, 1980).

Pathologic elevation of PRL release can suppress male reproductive behavior and testicular function by inducing as yet unidentified changes in the function of central nervous system. Hyperprolactinemia in the human is associated with impaired fertility and is accompanied by decreased circulating levels of testosterone and libido in men and by amenorrhea in women.

PRL like all anterior pituitary hormones is secreted episodically, with a distinctive 24-hour pattern. There are about 14 pulses of PRL secretion in 24 hours in normal human approximately one each 95 minutes (Van Cauter *et al.*, 1981). Super-imposed upon this pattern is bimodal 24-hour pattern of secretion, with a major nocturnal peak beginning after sleep onset and peaking in mid sleep. Minimal levels were observed during noon and maximum in the evening (Sassin *et al.*, 1973; Van Cauter *et al.*, 1981). The high levels during night are due to increase in the amplitude of each pulse, unaccompanied by an increase in pulse frequency (Valdhuis and Johnson, 1988). PRL secretion remains pulsatile in patients with prolactinomas, whereas circadian variation is abolished. PRL is released in pulses of 8 –10 minutes intervals in rats anterior pituitaries transplanted under the pituitary capsule of hypophysectomized rats. Because hypothalamic connections have been severed, this

short periodicity appears to be intrinsic to the lactotroph (Shin and Reifel, 1981). These short pulses are not controlled by the hypothalamus in human and rat studies but within gland (Samuels *et al.*, 1991).

Among the pituitary hormones, PRL shares with GH the distinction of operating without direct feedback control by signals from peripheral target tissues, and both PRL and GH are under direct hypothalamic control. Although the role of hypothalamic inhibiting factors in the control of PRL release is now well-established but substantial evidence suggests that stimulating factors also play an important role. Thus PRL, like GH, is under a complex dual regulatory system that involves both inhibitory and stimulatory control by the hypothalamic-pituitary system via neuroendocrine, autocrine and paracrine mechanisms.

Regulation Of Prolactin

There are number of physiological factors which regulate the PRL secretion

Dopamine

PRL secretion is tonically inhibited by the hypothalamus and its secretion is increased when the pituitary is transplanted or when the median eminence of the hypothalamus is destroyed (Everett, 1954; McCann and Friedman, 1960). When hypothalamic extracts or pieces were incubated with pituitary gland *in vitro*, they reduce the release of PRL (Pasteels, 1961,1963). The confirmation of the existence of PRL inhibiting factor (PIF) came from the study of Meites and his colleagues in 1963. It was observed by Fuxe that Dopamine (DA) was present in high concentrations in the median eminence (Fuxe, 1965) and than Macleod provided much experimental evidence to support that DA is PIF (Macleod, 1976). DA has been detected and its concentration has been measured in hypophysial portal blood (Ben-Jonathan *et al.*, 1977) and when the same concentration of DA was infused, PRL secretion is inhibited (Gibbs and Neill, 1978). The DA in the hypophysial portal vessels is released from tuberoinfudibular dopaminergic neuron system (TIDA) of hypothalamus.

Based on the observations that drugs affecting catecholamine metabolism also alter PRL secretion (Barraclough and Sawyer, 1959; Kanematsu *et al.*, 1963; Coppola, 1986) and that DA is present in high concentration in both the median eminance (Fuxe, 1965) and the hypophysial stalk plasma (Ben-Jonathan *et al.*, 1977; Gibbs and Neill, 1978; Plotsky *et al.*, 1978), several investigators concluded that DA

is the hypothalamic PIF. Subsequently, receptors for DA have been detected on pituitary membrane (Brown *et al.*, 1976; Creese *et al.*, 1977; Goldsmith *et al.*, 1979; Caron *et al.*, 1986) and more recently we have learned the structure of these receptors (Bunzow *et al.*, 1988). Thus sufficient evidence is available to support the strong conclusion that DA is the major physiological hypothalamic PIF.

Despite the seeming sufficiency of hypothalamic DA to fully inhibit PRL release, other PIFs have also been reported to exist.

Gamma amino butyric acid (GABA)

Gamma amino butyric acid (GABA) directly inhibits the release of PRL (Schally *et al.*, 1977; Enjalbert *et al.*, 1979; Racagni *et al.*, 1979). Its receptors are present on adenohypophysial cells (Grandison and Guidotti, 1979; Grandison *et al.*, 1982) and GABA neurons have been visualized in the median eminance by immunohistochemistry using an antibody against glutamate decarboxylase, an enzyme involved in GABA synthesis (Tappaz *et al.*, 1977; Vincent *et al.*, 1982). Enhancement of endogenous GABAergic tone induced by sodium volproate (an inhibitor of GABA-transaminase that degrades GABA at central and peripheral sites) reduces basal and breast-stimulated PRL release in women (Melis *et al.*, 1982; Melis *et al.*, 1984).

The inhibitory activity of DA is far greater than for GABA (Matsushita *et al.*, 1983). It has been proposed that unlike DA, GABA as a PIF may function episodically in response to certain stimuli rather than being constantly secreted into portal blood (Leong *et al.*, 1983).

Endotheline-1 and 3

Endotheline-1 and endotheline-3 also have been reported to inhibit PRL release *in vitro* in a dose-dependent manner and to be unaffected by D_2 DA receptor agonists (Samson and Skala, 1992; Domae *et al.*, 1992). Both peptides are present in all three lobes of the pituitary gland and their concentrations are sufficiently high compared with other regions of brain, to postulate autocrine or paracrine inhibitory roles for these peptides in the control of PRL secretion.

Prolactin (Auto regulation)

PRL acts on the hypothalamus to inhibit its own secretion. Prolonged hyperprolactinemia resulting from transplantable PRL secreting tumors results in reduced *in situ* pituitary PRL content. The reduction can be reversed by blocking the hypothalamic catecholamine synthesis (Furth and Clifton, 1966; Chen and Meites, 1970; Tashjian et.al., 1971 and Macleod, 1976;). The intracerebroventricular injection of PRL results in an increase in both DA turnovers in the median eminance and in the DA concentration in the portal blood (Gudelsky and Porter, 1980). The high turnover rate of DA in the median eminance, found during lactation and pregnancy, is reduced by hypophysectomy or by reducing PRL secretion through a direct pituitary inhibition with bromocryptine administration (Hokfelt and Fuxe, 1972; Bybee *et al.*, 1983).

Suckling

The suckling stimulus from the young is the most powerful physiological signal to increase PRL secretion in mammalian species. The control of PRL secretion during lactation involves increased input from PRL releasing factor(s) (Samson *et al.*, 1986; Murai and Ben-Jonathan, 1987; Samson *et al.*, 1989) and decreased TIDA neuronal activity (Ben-Jonathan *et al.*, 1980; Selmanoff and Wise, 1981; Plotsky and Neill, 1982; Demarest *et al.*, 1983; Wang *et al.*, 1993; Arbogast and Voogt, 1996). The perikarya of the TIDA neurons are located in the dorsomedial arcuate nucleus and the adjacent periventricular nucleus and axonal projections to the external layer of the median eminance (Moore and Lookingland, 1995). It is believed that the suckling-induced PRL increase occurs due to the rapid, transient decrease in TIDA neuronal activity, which sensitizes the lactotrophs to releasing factors (Plotsky and Neill, 1982; Rondeel *et al.*, 1988; Grosvenor *et al.*, 1980; Grosonver and Mena, 1980; Neill and Nagy, 1994). While the TIDA system is considered to be a major regulator of PRL secretion, the tuberohypophyseal dopaminergic (THDA) system, also plays an important role in the regulation of PRL release during lactation (Peters *et al.*, 1981; Murai and Ben-Jonathan, 1986; Murai *et al.*, 1989; Nagy *et al.*, 1992; Vecsernyes *et al.*, 1997; Nagy *et al.*, 1998). These neurons arise from the rostral portion of the arcuate nucleus and terminate in the intermediate and neural lobes of the pituitary gland (Moore and Lookingland, 1995). The periventricular-hypophyseal dopaminergic neurons, which arise in the periventricular nucleus and terminate in the

intermediate lobe, only, do not appear to be involved in PRL regulation during lactation (Nagy *et al.*, 1998; Nagy *et al.*, 1992; Peters *et al.*, 1981; Vecsernyes *et al.*, 1997; Goudreau *et al.*, 1992). The effect of suckling on dopaminergic activity is illustrated most dramatically by the large increase in neuronal activity seen after separation from the pups. The changes in neuronal activity after pup removal are manifested by increased dopamine secretion in hypophysial portal blood (Ben-Jonathan *et al.*, 1980), increased dihydroxyphenylalanin (DOPA) accumulation in the median eminance (Demarest *et al.*, 1983; Arbogast and Voogt, 1996), increased tyrosine hydroxylase (TH) messenger RNA (mRNA) levels in the arcuate nucleus (Wang *et al.*, 1993; Arbogast and Voogt, 1996).

Thyrotropin releasing hormone (TRH)

The thyrotropin releasing hormone (TRH) was originally isolated as a hypophysiotropic factor that stimulates thyroid stimulating hormone (TSH) secreted from pituitary cells (Schally et.al., 1966). Subsequently TRH was shown to stimulate PRL release from lactotropes and its effect was dose related both in *vitro* and *in vivo* (Tashjian et. al., 1971; Bowers et. al., 1971; Blake, 1974; Kato et. al., 1985). TRH is secreted into hypophysial stalk blood (Eskay et. al., 1975; Fink et.al.,1982) and its receptors are present on pituitary cells (Martin and Tashjian, 1977) evidently on lactotropes (Hinkel and Tashjian, 1975). TRH stimulates PRL mRNA sequences and the release of PRL. Ca^{2+} is the intracellular messenger for TRH-mediated PRL release (Gershengorn, 1982). The action of TRH on mammotropes is altered by estrogen and DA-TRH action is facilitated by estrogen and inhibited by DA or its agonists (McGuire and Lisk, 1971; Labrie *et al.*, 1980).

Acetylcholine

Acetylcholine injection into the ventricles of the brain or systemic injection of acetylcholine agonists reduces PRL secretion. The inhibitory effects of acetylcholine on PRL secretion are apparently mediated by catecholamines because acetylcholine cannot prevent PRL release when hypothalamic catecholamine activity is inhibited. A role of acetylcholine in the control of PRL secretion is suggested because the acetylcholine agonist Pilocarpine prevents stress and suckling induced PRL release (Grandison and Meites, 1976; Meites, 1977; Enroth *et al.*, 1977).

Serotonin

Serotonin, its precursors 5-hydroxytryptophan and tryptophan, or its metabolite melanin stimulate PRL release (Kamberi et al., 1971; Kordon et al., 1974; Muller et al., 1976). Blockade of serotonin receptors or synthesis prevents the release of PRL in response to the stimuli of suckling or estrogen injections (Kordon et al., 1974; Gallo et al., 1975). Acute PRL release occurs in men after infusion or ingestion o 5-10 mg of L-tryptophan, the substrate for biosynthesis of serotonin. The effects of serotonin are independent of DA (Clemens et al., 1978). It is suggested that serotonergic neurons release a PRF because serotonin agonists release PRL more rapidly than does blockade of the brain catecholaminergic system (Clemens et al., 1978; Clemens and Shaar, 1980).

Vasoactive intestinal peptide (VIP)

Vasoactive intestinal peptide (VIP) was isolated originally from porcine small intestine (Said and Mutt, 1970) and demonstrated to occur in the hypothalamic paraventricular nuclei and median eminance (Larson *et al.*, 1976; Besson et. al., 1979; Pelletier et. al., 1981). VIP stimulates PRL release both in vivo and in vitro (Kato *et al.*, 1978; Reburg *et al.*, 1978; Vijayan *et al.*, 1979; Shaar *et al.*, 1979) through a direct action on VIP receptors in anterior pituitary cells (Bataille *et al.*, 1979). VIP stimulates PRL release in vitro in a dose-related manner. The peptide exists in the portal blood (Said and Porter, 1979; Shimatsu et. al., 1981; Shimatsu *et al.*, 1982; Shimatsu *et al.*, 1983;) in concentration about 10 times higher than that found in the general circulation. The concentrations in the portal blood are sufficiently high to stimulate PRL release from pituitary cells. The PRL-releasing action of VIP appears to be mediated through its antagonistic effect on the inhibitory action of DA and GABA on adenylate cyclase (Matsushita *et al.*, 1983). The involvement of the adenylate cyclase in the intracellular mechanisms by which DA and VIP regulate PRL secrtion supports VIP as a PRF, but the physiological role of VIP remains to be determined.

Histamine

Histamine is a putative hypothalamic transmitter found in highest concentrations in the median eminance (Brownstein *et al.*, 1979). There is evidence

that histamine binds to two types of receptors, H_1 and H_2; binding to the former stimulates PRL release, whereas binding to latter inhibits it (Knigge *et al.*, 1982). In the rat, intracerebroventricular administration of histamine induces a prompt PRL release whereas diphenhydramine, an H_1-recptor blocker abolishes stress-induced PRL release (Libertun and McCann, 1976). Cimetidine, an H_2-receptor antagonist, has been shown to induce PRL release and galactorrhea in humans (Carlson and Ippoliti, 1977). The effect of HA on PRL secretion has been suggested to be mediated via an inhibition of tuberoinfundibular dopaminergic neurons, since HA decreased the concentration of dopamine in blood obtained from the long pituitary portal vessel in female and male rats (Gibbs *et al.*, 1979; Knigge *et al.*, 1988). However, in other studies, HA did not affect the turnover of dopamine in the median eminanace (Seltzer and Donoso, 1986; Fleckenstein *et al.*, 1992). Other studies have indicated that the effect of HA is mediated via interaction with vasopressinergic as well as serotonergic neurons (Jorgensen *et al.*, 1996; Kjaer *et al.*, 1991; Kjaer *et al.*, 1993; Knigge *et al.*, 1988). Although histamin is known to diminish the release of hypothalamic DA, this effect is insufficient to account for the cimetidine-induced PRL elevation. Furthermore, cimetidine has been shown to inhibit the central nervous system H_2-receptor pathway, which is independent of the dopaminergic system (Gonzalez *et al.*, 1980). This suggests that histamine may be involved in certain PRF activity.

Estrogens

The testis produces estradiol, and high concentrations of specific receptors for this steroid are present in the Leydig cells (Mulder *et al.*, 1976). Estrogens stimulate the synthesis and the release of PRL in rats and other species by acting at both adenohypophysis and hypothalamus (Chen and Meites, 1970). This effect appears to be dose and duration-dependent. Administration of pharmacologic doses of estrogen induces within 2 days, a rapid and profound rise in PRL release in women (Yen *et al.*, 1974) and in men (Frantz *et al.*, 1972) with a corresponding suppression of serum LH and FSH levels. The increasing level of PRL during estrogen treatment appears to be maintained by an increase in the magnitude of episodic PRL release throughout the 24-hour period (Velekemans and Robyn, 1975). This positive influence of estrogen on PRL dynamics is due to a direct stimulatory action on the lactotrope; it induces DNA synthesis of mRNA leading to enhance synthesis of PRL secretion (Maurer, 1982).

Goodman (1988) demonstrated that estrogen increases the number of lactotropes and their PRL content. Estrogen also has an antidopaminergic effect and markedly decreases the ability of DA to inhibit PRL secretion (Raymond *et al.*, 1978). This antidopaminergic activity is also seen at hypothalamic level (Cramer *et al.*, 1979; Gottschall *et al.*, 1986). Murai and Jonathan (1990) have demonstrated the presence of PRF in the posterior pituitary of female rates, is the primary site that mediates the acute effects of estradiol on PRL release. An increase in the size and number of PRL cells or lactotropes has been documented in rats (Pasteels, 1963) and man (Pasteels *et al.*, 1972) following administration of estrogen, progesterone (Pasteels, 1963).

Norepinephrine

One neurotransmitter that may modulate the cellular activity of putative PRFs within the paraventricular nucleus of hypothalamus (PVN) is norepinephrine (NE) as both magnocellular and parvocellular divisions of the PVN receive dense afferent projections from noradrenergic cells (A1 and A2) located in the ventrolateral medulla and nucleus of the solitary tract (Dotti and Teleisnik, 1982; Swanson and Morgenson, 1981; Swanson *et al.*, 1986). Variations in noradrenergic activity with in the PVN have been shown to occur in concert with fluctuations in circulating levels of PRL. For example in the Siberian hamster, photoperiodic-driven differences in PRL may be due to seasonal fluctuations in noradrenergic activity within the PVN, as hamsters exposed to a short-day photoperiod demonstrated significantly higher levels of noradrenergic activity within the PVN, and lower basal levels of PRL, when compared to their long-day counterparts (Dodge and Badura, 2001).

NE simulation of PRL release is different from its inhibitory effects at the pituitary gland level. In the pituitary, NE binds to dopamine receptors on the mammtrophs and blocks PRL release. In contrast, *in vivo* administration of L-dopa, which increases brain NE content, results in increased PRL secretion. (Donoso *et al.*, 1971). Administration of a α_2-adrenergic agonist clonidine at high doses results in an increased PRL secretion (Lawson and Gala, 1975) as do iv injections of NE (Vijayan and McCann, 1978). Administration of disulfran (Donoso *et al.*, 1973) (an inhibitor of norepinephrine synthesis and 6-hydroxydopamine) (Fenske and Wuttke, 1976) causes selective destruction of noradrenergic neurons and results in reduced PRL secretion. These results suggest that noradrenergic neurons stimulate PRL release, although the

role of these neurons is not resolved. The demonstration of α-1 and α-2 receptors in the brain makes interpretation of the drug studies and the role of noradrenergic neurons in the control of PRL secretion difficult to resolve (Clemens and Shaar, 1980). Together these studies suggest that the α_2-receptors may have a role in modulating dopamine activity within the arcuate, and subsequently, circulating levels of PRL.

Opioids

The endogenous opiates (enkaphaline and endorphins) and morphine cause a rapid increase in PRL secretion when given by systemic or intraventricular injection (Van Vugt and Meites, 1980). Studies with morphine and methadone in man (Tolis *et al.*, 1975; Kleber and Gold, 1978) and endogenous opioid peptides (EOP) in rodents (Lien *et al.*, 1976; Rivier *et al.*, 1978; Cusan *et al.*, 1977; Ferland *et al.*, 1977; Cocchi *et al.*, 1977) have shown that stimulation of opiate receptor sites causes an increase in serum PRL. Pretreatment with the opiate antagonist naloxone, blocks the increase in serum PRL (Tolis *et al.*, 1975; Kleber and Gold, 1978) normally seen after opiate administration. Pure opiate antagonists, like Naloxone (NAL), block and reverse the effects of opiates and displace the endorphins at the opiate receptor sites in the brain. The reversal or attenuation of behavioral or neurochemical effects by NAL would then be taken as neuropharmacological evidence that the effects were mediated by opiate receptors and endorphins. In lower mammals, NAL has generally been found to have no effects of its own other than to block or reverse the effects of opiate agonists. However, NAL has been reported to decrease basal serum PRL in rodents (Bruni *et al.*, 1977; Shaar *et al.*, 1977) and nonhuman primates (Gold *et al.*, 1978). In pigs EOP can increase secretion of PRL (Barb *et al.*, 1991).

Administration of NAL or naltraxone, prevent PRL release in response to stress or suckling and reduce basal PRL secretion (Bruni *et al.*, 1977; Van Vugt *et al.*, 1978). The acute suckling-induced PRL rise is blocked by NAL (Selmanoff and Gregerson, 1986; Baumann and Rabii, 1991), as well as specific μ and κ opioid receptor antagonists (Baumann and Rabii, 1991).

The EOP do not act directly on the pituitary gland. They may inhibit the activity of the TIDA system (Van Vugt *et al.*, 1978) perhaps through cholinergic neurons (Shaar and Clemens, 1980). A number of independent lines of scientific

investigations support an opiate or endorphin modulation of DA activity similar to DA receptor-blocking antipsychotic medications (Lal, 1975; Edelberg, 1976; Gold *et al.*, 1977; Kleber and Gold, 1978). Potent antipsychotic medications block DA receptors in the brain (Synder *et al.*, 1974) and stimulate PRL secretion (Clemens *et al.*, 1974; Meltzer *et al.*, 1977). The arcuate nucleus is a major source of both β-endorphin (Mezey *et al.*, 1985) and TIDA neurons (Moore and Lookingland, 1995). Contacts between β-endorphin axon terminals and TIDA neurons in the arcuate nucleus have been described (Horvath *et al.*, 1992; Morel and Pelletier, 1986). Opioid μ, δ and κ receptors and /or their mRNA are abundantly distributed in the hypothalamus (Mansour *et al.*, 1995). EOPs may exert their stimulatory action on PRL secretion by inhibiting TIDA neuronal activity. Existing data support a role for EOPs in influencing hypothalamic DA neuronal activity and DA synthesis, release and turnover (Van Loon *et al.*, 1980; Gudelsky and Porter, 1979; Arita and Kimura, 1988).

Excitatory Amino Acids

The role and function of excitatory amino acids (EAAs) in the CNS have been an area of intense research over the past years. It is now generally accepted that EAA receptors are the main transmitter receptors mediating synaptic excitation in the CNS (Brann and Mahesh, 1993; Brann and Mahesh, 1993; Cotman *et al.*, 1989; Cotman and Iverson, 1987; Fonnum, 1984). EAAs are involved in many physiological phenomenons ranging from the processing of sensory information to the regulation of neuronal survival, synaptogenesis and synaptic plasticity. As such EAAs have been suggested to play an important role in shaping neuronal circuitry, in mediating synaptic excitation, and in the processes of learning and memory. EAA involvement in dysfunctional neurodegenerative disorders has been implicated in a variety of pathological situations such as Huntington's disease, Parkinson's disease and Alzheimer's disease (Cotman *et al.*, 1989; Chapman, 1992; Greenamyre *et al.*, 1992). Since EAAs, such as L-glutamate and L-aspartate, appear to be the major excitatory neurotransmitters in CNS, synaptic excitation through EAA neurotransmission may also underlie many of the normal physiological processes that occur in the brain.

The mediation of EAA neurotransmission in the CNS is achieved primarily by the acidic amino acids glutamate and aspartate (Cotman and Iverson, 1987; Hanson

and Krogsgaard-Larsen, 1990; Monaghan *et al.*, 1989). Glutamate is the most abundant amino acid in the brain, and in addition to its transmitter role, glutamate functions in intermediary metabolism in neuron (Fonnum, 1984). As a transmitter stored in synaptic vesicles, glutamate is known to be release from presynaptic terminals by depolarization in a Ca^{2+} dependent manner. Concentration of glutamate in the synaptic cleft reportedly can reach millimolar levels (Erecinska and Silver, 1990; Fonnum, 1984).

Once released into the synaptic cleft, EAAs binds to specific postsynaptic neuronal receptors and induce excitation of the postsynaptic neurons. Glutamate receptors can be categorized into two principal groups: 1) Ionotropic and 2) Metabotropic. Ionotropic receptors contain integral, cation-specific ion channels, whereas metabotropic receptors are coupled to G-proteins and modulate the production of second messenger. N-methyl-D-aspartate (NMDA) receptors are included in ionotropic category and activation of these ionotrpic receptors leads directly to the opening of a group of ion channels that are typified by different permeabilities to Na^+, K^+, and Ca^{2+} ions. Stimulation of these "ionotropic" receptors underlies rapid glutamate mediated excitatory synaptic transmission in the CNS. NMDA receptors also exhibit the unique feature of being regulated by Mg^{2+} and glycine (Barnes and Henley, 1992; Nowak *et al.*, 1984; Hanson and Krogsgaard-Larsen, 1990; Reynolds *et al.*, 1987).

Involvement of EAAs to stimulate PRL secretion has been demonstrated by NMDA administration in primates (Wilson and Knobil, 1983; Gay and Plant, 1987), intact and castrated male rats (Arslan *et al.*, 1991; Strobl *et al.*, 1993) as well as cycling female rats (Pohl *et al.*, 1989; Abbud and Smith, 1991; Luderer *et al.*, 1993). Kainate administration via the third ventricle, but not iv, was also found to stimulate PRL release in the cycling female rat (Abbud and Smith, 1991). Regulation of PRL secretion by both NMDA and non-NMDA receptors is evidenced from a number of studies utilizing specific antagonist. For instance, Brann and Mahesh (Brann and Mahesh, 1991) have shown that administration of the NMDA antagonist MK-801 blocks the proestrous PRL surge in the female rat. Likewise, Brann *et al.* (Brann *et al.*, 1993) have shown that treatment with the non-NMDA antagonist DNQX significantly attenuates the preovulatory PRL surge in the pregnant mare serum gonadotropin (PMSG)-primed immature rat. Suckling-induced PRL release in the lactating rat has been blocked by the administration of CNQX, a non-NMDA

antagonist, but not by administration of NMDA antagonists (Parker and Crowley, 1993).

NMDA induce c-Fos immunoreactivity in two hypothalamic regions known to regulate PRL secretion: the paraventricular nuclei (PVN) which is the site of TRH cell bodies and the ARC which is the site of dopamine cell bodies (Abbud and Smith, 1991; Lee *et al.*, 1993). Hence NMDA could act to regulate PRL via regulation of these PRL releasing / or inhibiting factors, such as VIP and oxytocin (from the SCN and ARC respectively) may also be regulated by EAAs. Wilson and Knobil (1983) have reported that TRH serum levels are unaffected by weather TRH is involved in NMDA's effect on PRL. Dopamine neurons in the ARC may be more likely site of EAA regulation in the control of PRL release. In support of this possibility, NMDA receptors have been reported to regulate dopamine release in the hypothalamus (Wagner *et al.*, 1993).

In view of the above discussion present work is primarily designed to investigate the role of EAAs in the regulation of PRL secretion in non-human primate and what are their mechanism of action. The present work has some specific objectives.

The general OBJECTIVE of the study was to investigate the role of excitatory amino acids in the regulation of PRL in non-human primates and what is the mechanism of action of EAAs?

The study has TWO specific Objectives:

1. To investigate the physiological involvement of endogenous EAA neurotransmitters in the control of PRL secretion under physiologically stimulated conditions.
2. To investigate the interaction of EAA with various neurotransmitters and peptides that affect PRL secretion. For this purpose EAA-dependent PRL response under adrenergic & opioidergic receptor blockade conditions was studied.

ABSTRACT

The contribution of endogenous excitatory amino acid neurotransmitters was determined during insulin-induced hypoglycemia for the regulation of Prolactin (PRL) in non-human primates Rhesus monkeys (*macaca mulatta*). Four adult male monkeys were used for this purpose, which were provided with standard colony conditions and were acclimatized to chair restrain for a period of four weeks prior to the experiment. Animals were anaesthetized with the ketamine hydrochloride (5mg/Kg) and two teflon canulae were inserted in the sephanous veins for blood sampling and drug administration. Blood samples were collected with an interval of 15 minutes for a period of 3 hrs with heparinized syringes and than immediately centrifuged. Plasma thus separated stored at –15°C for assay with specific assay system.

Four sets of experiments were performed. In the first sets of experiment, which was the control experiment, saline (5ml) was injected to all the animals. The saline administration caused significant ($p<0.01$) reduction in the plasma PRL level. In the second sets of experiment, MK-801 (0.1mg/Kg), an NMDA receptor antagonist was administered to four adult male monkeys, which caused a highly significant ($p<0.001$) reduction in plasma PRL concentration. Regression analysis of variance showed a highly significant decrease in plasma PRL level. In the third sets of experiments all the four animals received insulin injection (1.0 unit/Kg = 25μl/Kg). This hypoglycemic stress caused a significant ($p<0.05$) increase in plasma PRL concentration for a period of 45 minutes and then PRL level declined. Regression analysis of variance showed a highly significant decrease in plasma PRL level. In the final sets of experiments all the four animals were challenged with insulin and MK-801 simultaneously. It was observed that this combined treatment caused a

highly significant ($p<0.001$) increase in plasma PRL concentration and according to regression analysis of variance there was a significant negative trend in the mean plasma PRL levels.

These results indicate that insulin-induced hypoglycemia caused a significant release of plasma PRL from lactotropes but endogenous excitatory amino acids do not involve in the release of PRL during insulin-induced hypoglycemia. During physiologically stimulated conditions (hypoglycemia) the release of PRL may be through the inhibition of dopamine release, which causes a significant rise in PRL level.

STUDY 1

INVOLVEMENT OF ENDOGENOUS EXCITATORY AMINO ACID NEUROTRANSMITTERS IN THE REGULATION OF BASAL / STIMULATED PROLACTIN SECRETION

INTRODUCTION

INTRODUCTION

Of all pituitary hormones, PRL has the most diverse actions. According to Nicoll and Bern (1971) there are six distinct functional categories including control of water and electrolyte balance, regulation of growth and development, metabolic effects, control of reproductive functions, effects on integument and ectodermal structures and synergism with steroids.

The ability of PRL to affect the spermatogenesis and growth of male accessory reproductive glands (Bartke, 1976) was described long before it was possible to quantitate peripheral levels of PRL in the male or demonstrate the presence of PRL receptors in tissues thought to respond directly to the action of this hormone. The early suggestions that PRL can act directly on the male reproductive system received strong support from the demonstration that specific PRL receptors are present in the interstitial compartment of the testis (Aragona *et al.*, 1977; Charreau *et al.*, 1977) and in the male accessary reproductive glands (Aragona *et al.*, 1977; Charreau *et al.*, 1977; Kledzik et al., 1976).

The mechanism responsible for the stimulation of testicular function by PRL was suggested by the results obtained in hypophysectomized animals. In hypophysectomized rats and mice, PRL significantly augmented the effects of exogenous LH on biosynthesis of testosterone and spermatogenesis (Bartke 1971; Hafiez *et al.*, 1972). In contrast, PRL did not potentiate the action of exogenous testosterone on spermatogenesis and had little, if any effect when administered alone (Bartke 1971; Hafiez *et al.*, 1972). It was also demonstrated that treatment of hypophysectomized rat with PRL increases their ability to produce testosterone in response to acute LH stimulation (Bartke *et al.*, 1978). These results suggest that PRL can act on the Leydig cells to increase their responsiveness to LH stimulation. This action of PRL appears to be particularly important during the seasonal changes in gonadal function in the golden hamster. In this species, PRL can both prevent and reverse testicular atrophy induced by binding or by exposure to a short photoperiod (Bex *et al.*, 1978; Matthews *et al.*, 1978).

It has been documented that PRL can potentiate the effects of exogenous androgens on the growth of male accessory reproductive glands in castrated animals (Thomas and Keenan, 1976). Administration of PRL alone to castrated males causes a

small but detectable increase in the weight of accessory reproductive glands and it has been shown that this effect of PRL is not mediated through the pituitary or the adrenal (Bartke and Lloyed, 1970; Negro-Vilar et al., 1977). The fact that PRL binding to prostatic membranes and cytosol is androgen-dependent (Charreau *et al.* 1977; Kledzik *et al.* 1976), provides an explanation for the greatly reduced responsiveness of accessory reproductive glands to PRL in the absence of endogenous or exogenous testosterone. Evidence also suggests that PRL may affect the number of LH receptors in the ovary and thus modulate steroidogenesis in the follicular cells (Zipf *et al.*, 1978)

The dicarboxylic amino acids aspartate and glutamate, often referred to as neuroexcitatory amino acids, act as neurotransmitters in the central nervous system (Mayer and Westbrook, 1987). Their stimulatory effects are exerted through a variety of receptor subtypes classified according to their responsiveness to specific agonist. One such subtype is the N-methyl – D – aspartic acid (NMDA) receptor, so named because NMDA is a potent agonist for this receptor subtype. There is increasing evidence that receptors for neuroexcitatory amino acids of the NMDA subtype are an important component of the LH surge induced by ovarian hormones (Carbone *et al.*, 1992). The release of LH is stimulated by N-methyl – D – aspartic acid (NMDA) in rodents, primates and sheep apparently via increased release of gonadotropin-releasing (GnRH) from the hypothalamus (Gay and Plant, 1987, Bourgiugnon *et al.*, 1989). In addition to LH secretion, FSH secretion is also stimulated by NMA administered to young rats (Carbone *et al.*, 1992). There are also reports that NMA can elevate plasma concentrations of PRL (Gay and Plant, 1987; Pohl *et al.*, 1989; Barb *et al.*, 1992) and GH (Gay and Plant 1987; Estienne *et al.*, 1989; 1993; Barb *et al.*, 1992). NMA can act via the NMDA receptor because antagonists for this receptor will partially block responses initiated by aspartic acid, NMDA or NMA (Watkins and Evans, 1981).

Involvement of EAAs to stimulate PRL secretion has been demonstrated by NMDA administration in rodents and primates (Olney and Price, 1980; Wilson and Knobil, 1982; Wilson and Knobil, 1983; Gay and Plant, 1987), intact and castrated male rats (Arslan *et al.*, 1992; Strobl *et al.*, 1993) as well as cycling female rats (Abbud and Smith, 1991; Luderer *et al.*, 1993; Pohl *et al.*, 1989). Kainate administration via the third ventricle, but not iv, was also found to stimulate PRL release in the cycling female rat (Abbud and Smith, 1991). Regulation of PRL

secretion by both NMDA and non-NMDA receptors is evidenced from a number of studies utilizing specific antagonist (Brann and Mahesh, 1991; Brann *et al.*, 1993; Park and Crowley, 1993, Wagner *et al.*, 1993). For instance, Brann and Mahesh (Brann and Mahesh, 1991) have shown that administration of the NMDA antagonist MK-801 blocks the proestrous PRL surge in the female rat. Likewise, Brann *et al.* (Brann *et al.*, 1993) have shown that treatment with the non-NMDA antagonist DNQX significantly attenuates the preovulatory PRL surge in the PMSG-primed immature rat. Suckling-induced PRL release in the lactating rat has been blocked by the administration of CNQX, a non-NMDA antagonist, but not by administration of NMDA antagonists (Parker and Crowley, 1993).

Insulin-induced hypoglycemia (Fish *et al.*, 1986; Garber *et al.*, 1976) and the resulting neuroglucopenia (Hourani *et al.*, 1992) result in significant activation of various neuroendocrine pathways involved in producing peripheral hormonal and metabolic responses aimed at restoring euglycemia. The hypothalamic-pituitary-adrenal axis is known to be among the initial and predominant systems involved in substrate mobilization, enhanced hepatic glycogenolysis and gluconeogenesis which are essential components of the counter-regulatory response to an acute decrease in blood glucose. However, many additional, redundant systems are involved in glucose homeostasis, including direct neural stimulation (Havel *et al.*, 1996; Paramore *et al.*, 1999; Hevener *et al.*, 2000), histaminergic (Molina *et al.*, 1997) and endorphinergic systems (Radosevich *et al.*, 1988; Paramore *et al.*, 1999)

In addition to the classical hormones and neurotransmitters involved in glucoregulation, studies have demonstrated that in the adult brain, extracellular fluid concentrations of excitatory amino acids (EAA; glutamate and aspartate) raise 4-10 fold in response to hypoglycemia. This excessive efflux of EAA has been suggested to contribute to the pathogenesis of hypoglycemia-induced neuronal necrosis (Weiloch, 1985). EAA receptors are the main transmitter receptors mediating synaptic excitation in the CNS (Watkins and Evans, 1984). Two broad groups of EAA receptors have been recognized, namely ionotropic and metabotropic receptors (Brann and Mahesh, 1994) and these have been localized in a variety of areas of the brain including hypothalamus. Stimulation of the ionotropic receptors underlies rapid glutamate-mediated synaptic transmission in the CNS, while activation of metabotropic receptors is characterized by prolonged synaptic modulation (through second messenger system). Recent data strongly suggest a key role for glutamate in

modulating the descending autonomic pathways (Daftary *et al.*, 1998) that result in excitation of noradrenergic fibers (Yousef *et al.*, 1994).

The involvement of various EAA in the CNS control of peripheral carbohydrate metabolism has been proposed and supported by number of studies (Molina *et al.*, 1994; Yousef *et al.*, 1994; Lang and Ajmal, 1995). Stimulation of both ionotropic (with either NMDA or Kainate) or metabotropic glutamate receptors resulted in marked hyperglycemia. The increased glucose concentrations produced by central intracerebroventricular (i.c.v.) injection of NMDA kainate was associated with decreased circulating insulin levels, and with elevated concentrations of corticosterone, glucagon and catecholamines (Yousef *et al.*, 1994). These findings suggested that ionotropic glutamate receptor agonist modulate secretion of pancreatic hormones. However, the role of EAA in the modulation of the autonomic efferent pathways that are activated during hypoglycemia is less clear.

Based on the above findings, we hypothesized that endogenous EAA involve in modulating peripheral hormones in response to hypoglycemia. In this study we examined the effect of hypoglycemic condition on the release of endogenous EAA and their effect on pituitary gland to release and regulate PRL. Rhesus monkey (*macaca mulatta*) is a very good model to study such type of hypothalamic and pituitary interactions.

MATERIALS AND METHODS

MATERIALS AND METHODS

ANIMALS

The animals used in the study were adult male rhesus monkeys (*Macaca mulatta*). All of them were of the same age (3+ years). They were housed in individual cages and maintained under standard colony conditions at the Primate Facility of the Quaid-I-Azam University, Islamabad. They were provided with standard monkey food supplemented with fresh fruits and vegetables. Water was available *ad-libitum.*

PHARAMACOLOGIC AGENTS

The following drugs were used in the present study:

1.	**Ketamine hydrochloride**	(ketavat; park Davis, Berlin, FRG).
2.	**Mk-801**:	Sigma Chemical Co. (St. Louis, MO, USA).
3.	**Insulin**:	Humulin (Eli Lilly, Lilly France S.A., F-67640 Fegersheim, France)
4.	**Normal Saline (0.9% NaCl)**	Plasaline, Otsuka Pakistan Ltd., F/4-9, H.I.T.E., Hub, Balochistan, Pakistan.
5	**Dextrose 10%**	Paksol, M.S. Enterprises Ltd., 3.5 km Raiwind, Kot Radha Kishan Road, Distt. Kasur, Pakistan.

CHAIR RESTRAINING

All the animals were chair restrained daily for about four hours for a period of twenty days prior to initiation of experiment.

CATHETERIZATION

Before handling, the animals were anaesthtised with ketamine hydrochloride (5mg/kg; ketavet, Parke-Davis, Freiburg, FRG) and while under ketamine anaesthesia, two teflon cannulae (Vasocan Brannule 0.8 mm/22 G, O.D., b, Braun Melsangen AG, Belgium) were inserted in the sephanous veins for blood sampling and drug or neuropeptide infusion. The dose of ketamine used was not enough to induce narcosis but was sufficient to immobilize the animals.

BLEEDINGS

Sequential blood samples (2.0 ml) were obtained at 15-min interval in heparinized syringes. Following withdrawal of each sample, an equal volume of heparinized (5 IU/ml) saline was injected into the tubing. All bleedings were carried out between 900-1600 h to minimize diurnal variations. Blood samples were immediately centrifuged at 3000 rmp for 10 min. Plasma was separated and stored at –15 °C until analyzed.

EXPERIMENTAL PROTOCOL

A single group of adult male rhesus monkeys accustomed to chair-restrain was subjected to the following treatments with an interval of 1 to 2 weeks.

a) VEHICLE ADMINISTRATION:

The animals were bled at 15 minutes interval for a period of 6 hours through an indwelling 22-guage Teflon cannula implanted in the sephanous vein. The animals were given 5 ml of vehicle (0.9% NaCl) at 1hr of the blood sampling.

b) MK-801 ADMINISTRATION:

The animals were bled as above. The animals received intravenous bolus injection of MK-801 (0.1mg/kg BW), a specific NMDA receptor antagonist at 1hr of the sampling. MK-801 was dissolved in 5 ml of normal saline immediately before use.

c) INSULIN ADMINISTRATION:

The animals were bled for a period of 4 hr at 15-min intervals. At 1 hr, the animals were challenged with a single bolus injection of insulin (1.0 unit/kg BW = 25 μl/kg BW).

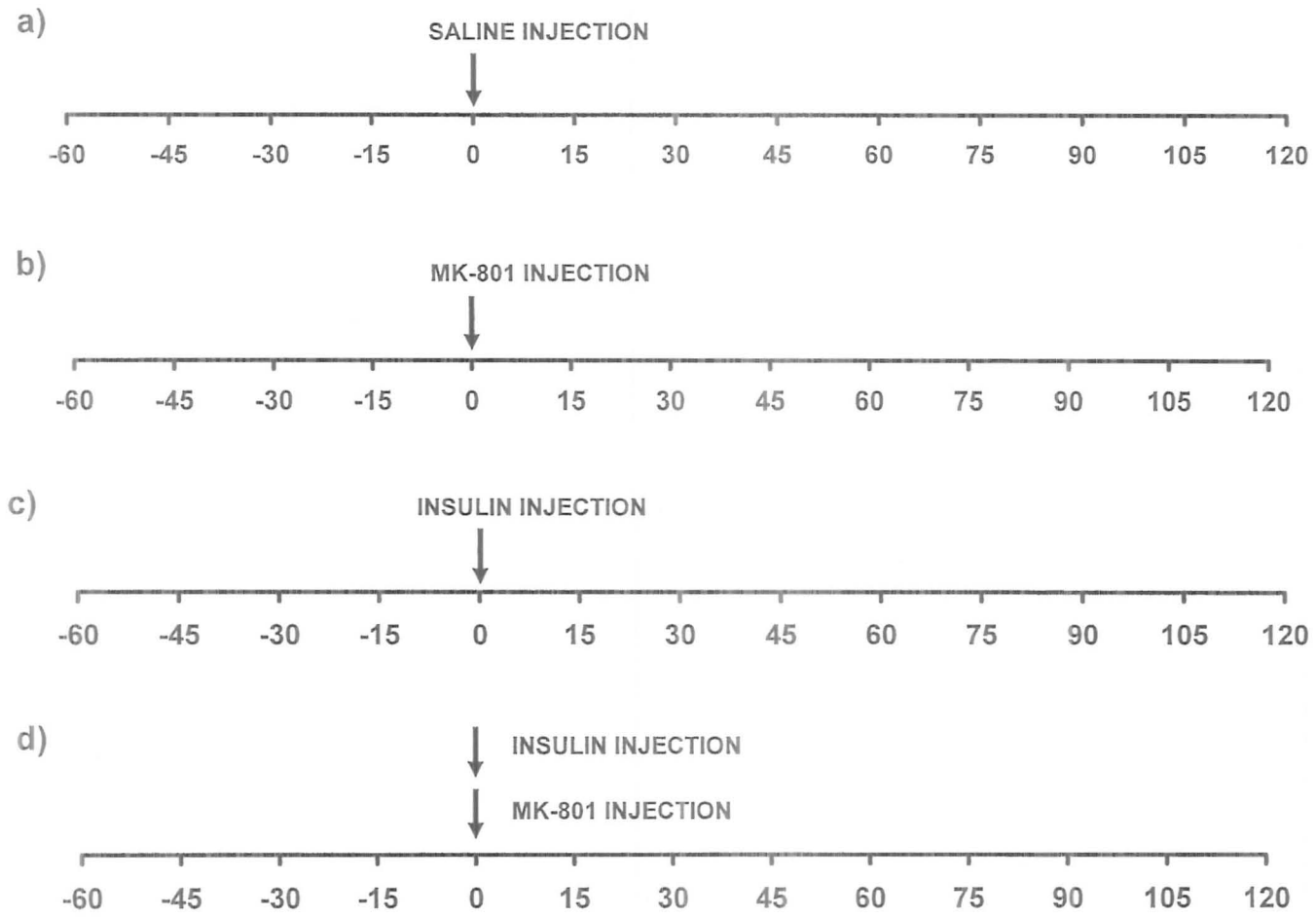

Fig. 1. Experimental Protocol showing the administration of a) Saline b) MK-801 c) Insulin d) MK-801 + Insulin to adult male monkeys (n =4).

d) INSULIN + MK-801 ADMINISTRATION:

The animals bled at 15-min interval for a period of 4 hr. At 1 hr, animals received iv bolus of insulin (1.0U/Kg BW). Immediately following insulin, bolus iv injection of MK-801 (0.01mg /Kg BW) at 1hr of the sampling administered.

HORMONE DETERMINATION

Plasma PRL was determined by using enzymimmunoassay (EIA) system.

PROLACTIN ENZYME IMMUNOASSAY

The enzymeimmunoassy (EIA) system, presently employed, was developed for the Special Programme of Research in Human Reproduction of the World Health Organization and is intended for the measurement of PRL in plasma. The time required to complete an assay is approximately 5 hrs. The concentration range covered by standards was 0-2500 mIU/L (WHO IPR 84/500). The sensitivity of the assay was 20 mIU/L.

The assay is of immunometric ("sandwich") design, utilizing two anti Prolactin antibodies. The first is a polyclonal antibody and is attached to a magnetic particle. The second is a monoclonal antibody and is labeled with alkaline phosphate particle. The assay has three main stages.

IMMUNOEXTRACTION

Sample is incubated with magnetic anti-Prolactin antibody for 30 minutes at 37°C. Prolactin in the sample binds to the magnetic particles. Other serum components are removed by decantation following a magnetic separation that includes one wash step.

LABELED ANTIBODY REACTION.

The solid phase is incubated with alkaline phosphatase labeled anti-Prolactin for 2 hrs at 37°C. The labeled antibody reacts with any PRL bound to the magnetic particles after immunoextraction. Excess-labeled antibody is removed by decantation following a magnetic separation that includes two wash steps.

COLOR DEVELOPMENT.

The solid phase is incubated with a colored enzyme substrate for 1 hr at 37°C. The presence of alkaline phosphatase causes a color change from yellow to pink. The intensity of the pink color produced is a measure of the amount of alkaline phosphatase labeled antibody and hence PRL bound to the magnetic particles. The reaction is terminated by addition of Stop Buffer and the optical density of all tubes is measured. The intensity of the color formed by enzyme reaction is directly proportional, within the working range of the assay, to the concentration of PRL in the sample. The concentration of PRL in a sample or control can be determined directly by interpolation from the standard curve. Results were calculated according to the WHO Immunoassay Processing Programme. The intra-and inter assay coefficients of variation were 7% and 11%.

STATISTICAL ANALYSIS

For comparison of baseline PRL secretion before treatment, hormone levels were calculated by averaging all the concentrations before treatment. On the other hand PRL responsiveness to the drugs induced was determined by comparing basal levels of the hormone calculated by averaging the concentrations immediately before the injection at 0 min and the levels worked out by averaging the concentration of hormone 15 min after inducing the drug. Student's t-test was used to determine differences between the means of basal and stimulated levels. The data were also subjected to regression analysis of variance. P values are mentioned for t-test applied. Where analysis of variance is carried out both values for F and P are given.

RESULTS

RESULTS

Body Weight:

Mean body weight of all the four adult male rhesus monkeys (*Macaca mulatta*) included in the experiment are given in Table 1.

Behavioral Reactions

All the four adult male monkeys showed similar type of behavioral reaction after the administration of the drugs. Administration of MK-801 caused sedation in all the animals for a period of 1-2 hours with shallow respiration and slow reflexes. Almost all the animals started salivation after the administration of MK-801. Administration of Insulin caused restlessness in all the animals. Animals were thirsty throughout the bleeding after the injection of Insulin. Administration of Insulin also caused slow reflexes. Some animals got fits in the form of jerk during the bleeding hours after insulin administration. All the animals received 50 ml of Dextrose (10%) injection at the end of experiment.

Effect of Vehicle (Saline) Injection on Plasma PRL

In the control part of the experiment four adult male monkeys received 5 ml of saline (0.9% NaCl) injection. Individual and mean plasma PRL concentration (mIU/L) after saline injection is shown in Table 2 and Fig 2. Saline was injected at 0 minutes to each of four animals. Blood samples were collected one hour before and 2 hours after saline injection with an interval of 15 minutes. Mean plasma PRL concentration was 292.45 ± 52.40 mIU/L when collection of blood samples was started at –60 minutes and after one hour the mean plasma PRL levels were 254.10 ± 94.96 mIU/L. Regression analysis of variance showed a non-significant negative trend in mean plasma PRL levels ($b = -10.735 \pm 3.92$, $F_{(1,3)} = 7.48$, $P = 0.07$, Table 2.1, Fig 2.1).

At 0 minute time, 5 ml saline (0.9% NaCl) was injected and blood samples were collected after 15 minutes interval. Mean plasma PRL concentration was 261.50 ± 83.79 mIU/L after 15 minutes time. After an hour of saline injection the mean plasma PRL levels were 197.35 ± 48.48 mIU/L and after 2 hours (at 120 minutes) the levels were

TABLE 1

Body Weight (kg) of Rhesus monkeys treated with Insulin, Mk-801 and Insuline + Mk-801

Animal nos.	Saline	Mk-801	Insulin	Mk801+Insulin
9601	4.4	4.4	4.5	4.6
9602	4.4	4.5	-----	-----
9609	3.2	3.2	3.2	3.7
9611	3.7	3.7	3.7	2.6
9613	-----	-----	2.7	3.2
Mean ± S.E.M.	3.93 ± 0.29	3.95 ±0.31	3.53 ± 0.33	3.53 ± 0.42

TABLE 2

Effect of iv injection of Saline (V) on plasma PRL Concentration (mIU/L) in adult male rhesus monkeys

Time (min)	Animal nos. 9601	9602	9609	9611	Mean		S.E.M.
-60	288.8	246.0	198.0	437.4	292.55	±	52.56
-45	253.2	276.0	192.1	469.4	297.67	±	76.43
-30	220.5	253.2	115.2	442.3	257.80	±	78.45
-15	199.9	263.2	166.0	442.9	267.98	±	85.93
0	178.2	276.2	115.2	446.8	254.10	±	94.96
15	193.4	249.2	174.3	430.9	261.93	±	83.95
30	163.8	196.7	184.1	462.3	251.72	±	105.55
45	187.2	166.2	168.7	433.8	238.95	±	87.19
60	171.9	139.0	169.8	308.7	197.35	±	48.34
75	175.2	109.3	118.7	351.3	188.61	±	62.26
90	168.5	114.7	182.3	307.5	193.24	±	49.15
105	135.3	137.2	197.0	292.5	190.50	±	55.55
120	133.1	166.6	164.6	267.4	182.91	±	47.46

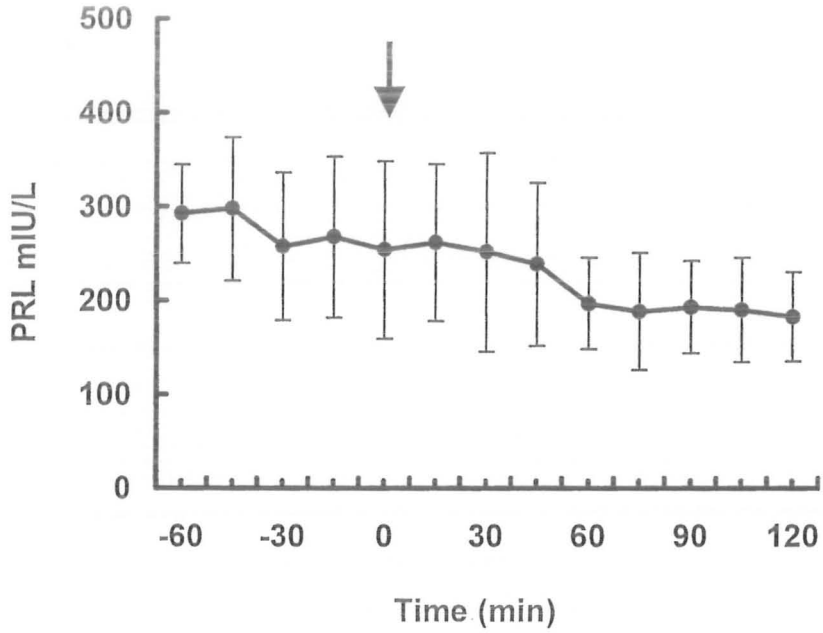

Fig. 2.

Effect of iv injection of Saline (↓) at 0 min on plasma PRL concentration (mIU/L) in male adult rhesus monkeys

TABLE 2.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before Saline injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	1152.6	1152.56	7.4817	0.0716
Residual	3	462.15	154.051		
Total	4	1614.7			
B	-10.74	± 3.924			

TABLE 2.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after Saline injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	6021.9	6021.87	30.133	0.0015
Residual	6	1199.1	199.843		
Total	7	7220.9			
B	-11.97	± 2.181			

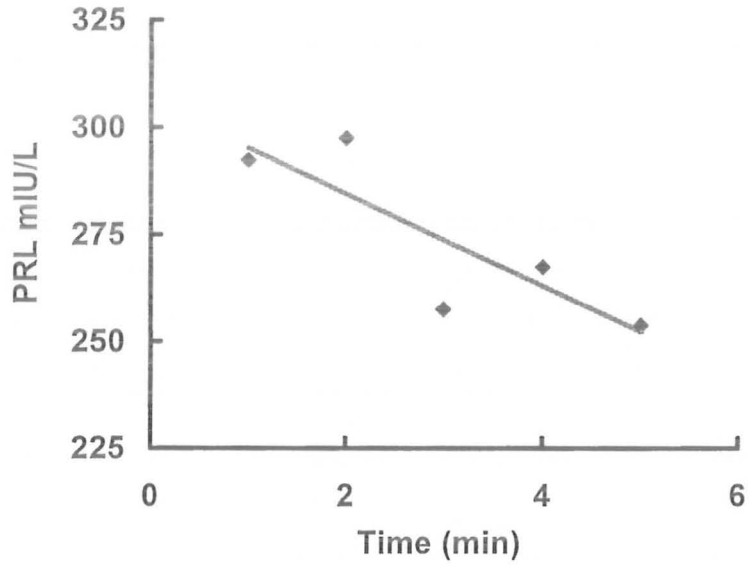

Fig. 2.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before Saline injection

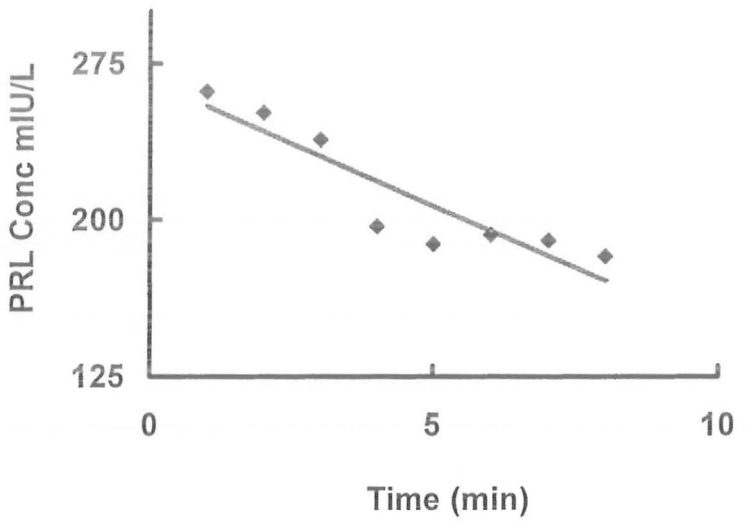

Fig. 2.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after saline injection

182.91 ± 47.46 mIU/L. Regression analysis of variance showed that mean plasma PRL levels decreased significantly (b = -11.97, $F_{(1,6)} = 30.13$, P = 0.001 Table 2.2 Fig 2.2). Plasma PRL levels reduced significantly ($p<0.01$) after the saline injection (Table 5.3 Fig 5.3).

Effect of MK-801 Injection on Plasma PRL

In order to block the endogenous EAA, an injection of MK-801 was administered to each of the four adult male rhesus monkeys. Mean plasma PRL levels (mIU/L) were recorded one hour before and two hours after the injection of MK-801 with an interval of 15 minutes (Table 3 and Fig 3). Mean plasma PRL level was 147.25 ± 14.09 mIU/L at –60 minutes time and within an hour the level increased to 190.25 ± 7.78 mIU/L. Regression analysis of variance showed that mean plasma PRL level increased very highly significantly during pretreatment hour (b = 10.700, $F_{(1,3)} =$ 523.66, P = 0.0001 Table 3.1 Fig 3.1).

NMDA receptor antagonist, MK-801 (dose = 0.1mg/kg BW dissolved in 5 ml saline) was injected at 0 minutes and blood samples were collected after an interval of 15 minutes for a period of 2 hours. After 15 minutes of the injection of MK-801, mean plasma PRL concentration was 122.75 ± 3.54 mIU/L which then decreased highly significantly ($p<0.001$) with the passage of time (Table 5.3 Fig 5.3). After one hour of the administration of drug the mean plasma PRL concentration was 99.50 ± 2.83 mIU/L and after another one hour time (120 minutes) the level reduced to 91.00 ± 4.95 mIU/L. Regression analysis of variance showed highly significant decrease in the mean plasma PRL level (b = -4.208, $F_{(1,6)} = 32.09$, P = 0.001 Table 3.2 Fig 3.2). Analysis of variance (ANOVA) also showed a highly significant ($p<0.05$) reduction in plasma PRL level after the administration of MK-801 in all the animals (Table 5.4).

Effect of Insulin on plasma PRL

In order to create a physiological stress in the form of hypoglycemia, insulin was injected to four adult male rhesus monkeys. Table 4 and Fig 4 show the observations made one hour before and two hours after the injection of insulin with an interval of 15 minutes. Initially when collection of blood samples was started (-60 minutes) mean plasma PRL concentration was 194.27 ± 70.29 mIU/L and after one hour it decreased to 175.45 ± 2.84 mIU/L.

TABLE 3

Effect of iv injection of Mk-801 on plasma PRL concentration (mIU/L) in four adult male adult rhesus monkeys

Time (min)	Animal nos. 9601	9602	9609	9611	Mean		S.E.M.
-60	122.8	166.7	136.9	162.6	147.25	±	14.09
-45	153.0	163.5	147.4	160.3	156.04	±	2.58
-30	169.3	177.2	142.0	180.9	167.35	±	4.13
-15	172.8	182.4	171.5	182.3	177.23	±	3.34
0	177.0	196.2	189.5	199.5	190.55	±	7.95
15	119.9	123.7	120.5	129.2	123.30	±	3.30
30	109.1	110.8	101.4	111.7	108.22	±	0.90
45	106.4	102.0	99.5	110.5	104.58	±	1.45
60	108.7	98.7	92.7	100.5	100.16	±	2.92
75	101.3	97.5	90.0	91.8	95.13	±	3.35
90	99.4	87.7	88.2	88.2	90.87	±	3.97
105	98.2	88.4	94.9	83.8	91.31	±	5.09
120	98.5	91.5	91.8	84.8	91.66	±	4.85

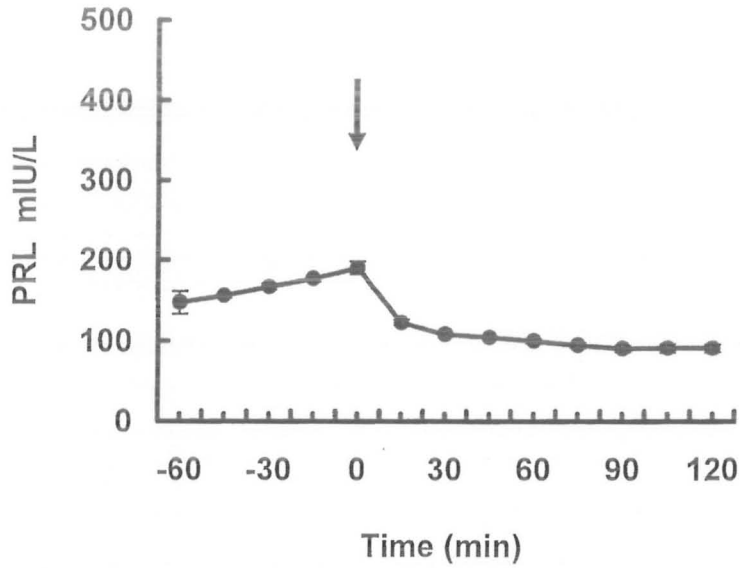

Fig. 3.

Effect of iv injection of Mk 801 (↓) on plasma PRL concentration (mIU/L) in male adult rhesus monkeys.

TABLE 3.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before Mk-801 injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	1145	1145.01	523.67	0.0002
Residual	3	6.5595	2.1865		
Total	4	1151.6			
B	10.701	± 0.467			

TABLE 3.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after Mk-801 injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	743.823	743.8229	32.0954	0.0013
Residual	6	139.052	23.1753		
Total	7	882.875			
B	-4.208	± 0.742			

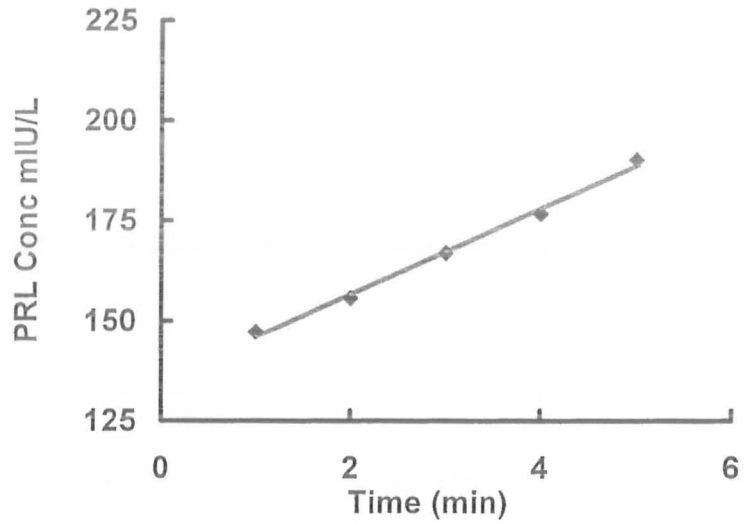

Fig. 3.1

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before Mk-801 injection.

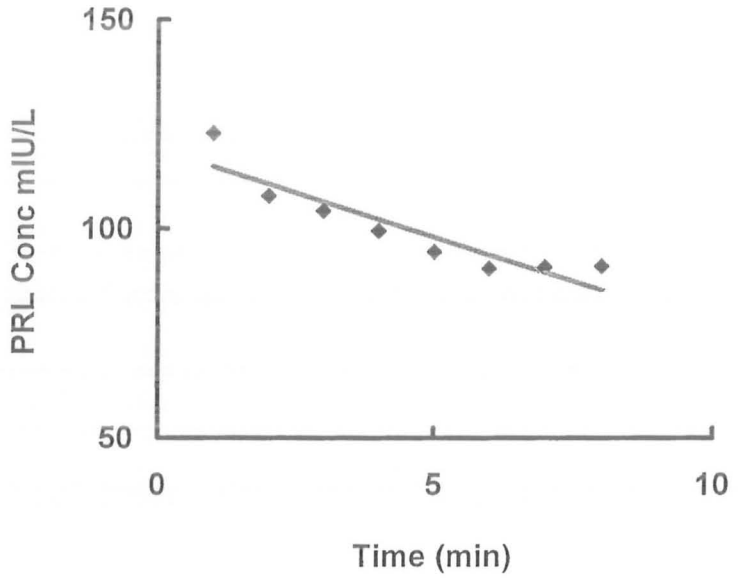

Fig. 3.2

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after Mk-801 injection

Regression analysis of variance showed that there was a non-significant negative trend in the mean pre-treatment plasma PRL levels (b = -3.55 ± 1.376, $F_{(1,3)} = 6.672$, P = 0.08 Table 4.1 Fig 4.1)

Insulin (dose = 1 unit / kg BW) was administered at 0 minutes and blood samples were collected after 15 minutes interval for a period of two hours. Mean plasma PRL level after 15 minutes of the insulin injection was 326.78 ± 17.14 mIU/L and after one hour (60 minutes time) the level declined to 200.02 ± 15.25 mIU/L. Hypoglycemia produced by the administration of insulin caused a significant ($p<0.05$) increase in plasma PRL level for 45 minutes. After another one hour time the mean plasma PRL level reduced to 143.31 ± 7.22 mIU/L. Regression analysis of variance showed a very highly significant decrease in mean plasma PRL levels (b = -26.79 ± 3.637, $F_{(1,6)} = 54.25$, P = 0.0003 Table 4.2 Fig 4.2). ANOVA showed a very highly significant ($p<0.0004$) increase in all the animals after insulin injection (Table 5.4).

Effect of MK-801 and Insulin on Plasma PRL

Four adult male rhesus monkeys were injected insulin and MK-801 simultaneously to study the involvement of EAA for the regulation of PRL during stimulated conditions. Table 5 and Fig 5 show the individual and mean plasma PRL concentration before and after the administration of insulin and MK-801. Blood samples were collected one hour before and two hours after the administration of the drugs with an interval of 15 minutes. Mean plasma PRL level mIU/L one hour before the administration of any drug was 126.36 ± 41.28 mIU/L and after one hour the level reached 166.66 ± 20.84 mIU/L. Regression analysis of variance showed a non-significant increase in mean plasma PRL level (b = 7.381 ± 5.031, $F_{(1,3)} = 2.152$, P = 0.23, Table 5.1 Fig 5.1).

NMDA receptor antagonist MK-801 (dose = 0.1mg/kg BW dissolved in 5 ml saline) and insulin (dose = 1unit/kg BW) were injected simultaneously at 0 minutes. Blood samples were collected with an interval of 15 minutes for two hours after the

TABLE 4

Effect of iv Insulin injection on plasma PRL Concentration (mIU/L) in male adult rhesus monkeys

Time (min)	Animal nos. 9601	9602	9609	9611	Mean		S.E.M.
-60	152.6	106.0	222.4	286.3	191.83	±	47.27
-45	159.8	156.0	265.0	117.2	174.50	±	15.06
-30	143.2	149.0	269.0	149.4	177.65	±	2.21
-15	136.6	115.0	256.0	153.7	165.32	±	6.05
0	153.1	126.0	214.0	162.1	163.82	±	3.18
15	279.3	217.0	356.0	225.1	269.36	±	19.16
30	275.7	208.0	341.0	210.2	258.73	±	23.15
45	145.4	156.0	238.7	198.0	184.51	±	18.59
60	99.8	141.0	276.7	148.0	166.38	±	17.05
75	108.4	164.0	250.9	133.6	164.20	±	8.92
90	159.8	108.0	256.3	157.9	170.51	±	0.67
105	163.2	114.0	223.8	121.7	155.66	±	14.65
120	114.8	117.0	225.1	92.0	137.23	±	8.08

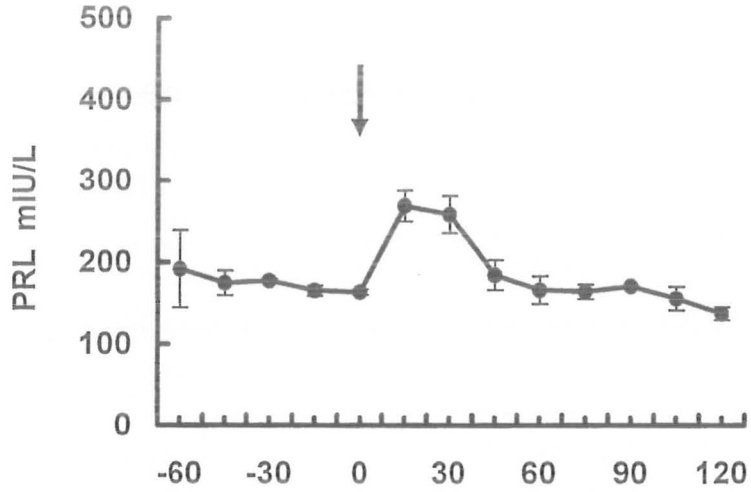

Fig. 4.

Effect of iv Insulin injection (↓) on plasma PRL concentration (mIU/L) in male adult rhesus monkeys.

TABLE 4.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before Insulin injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	6021.9	6021.87	30.133	0.0015
Residual	6	1199.1	199.843		
Total	7	7220.9			
b	-11.97	± 2.181			

TABLE 4.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after Insulin injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	13116.7	13116.706	22.6112	0.003
Residual	6	3480.59	580.10		
Total	7	16597.3			
b	-17.67	± 3.716			

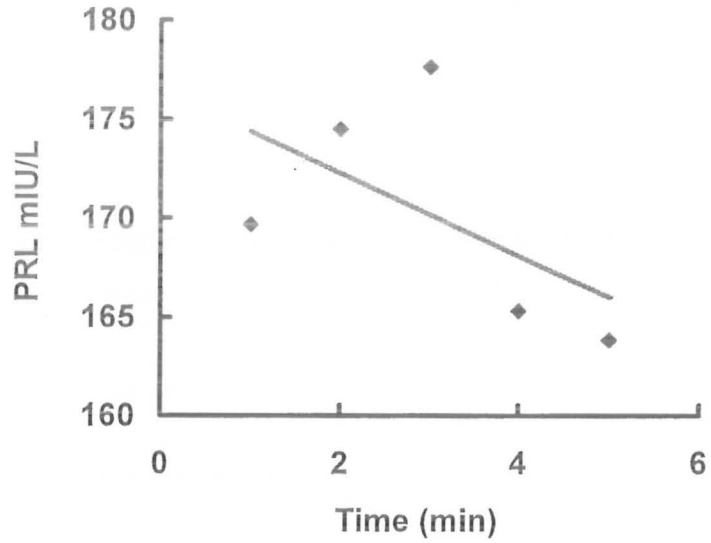

Fig. 4.1,

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before Insulin injection

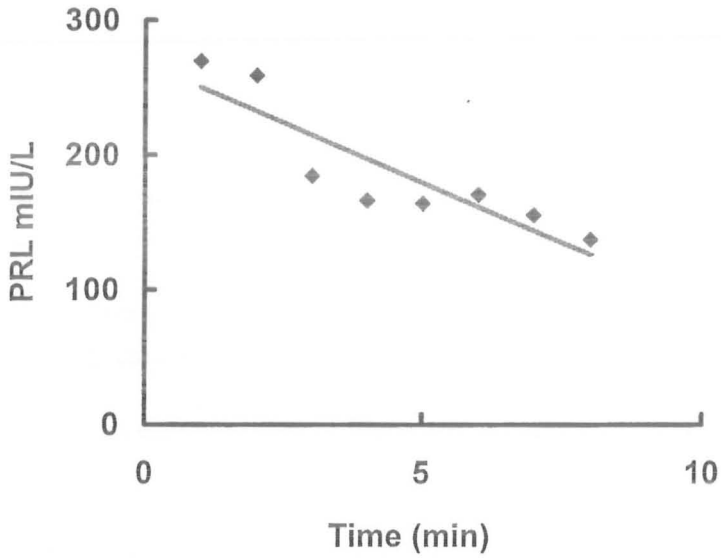

Fig. 4.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after Insulin injection.

TABLE 5

Effect of Insulin + MK-801 on plasma PRL Concentration (mIU/L) in adult male rhesus monkeys

Time (min)	Animal nos. 9601	9602	9609	9611	Mean		S.E.M.
-60	180.8	193.0	67.7	64.0	126.36	±	41.28
-45	253.8	175.0	181.7	64.0	168.64	±	67.11
-30	217.4	223.4	155.4	75.9	168.04	±	50.02
-15	210.4	195.5	125.7	115.9	161.85	±	33.43
0	207.4	179.8	131.0	148.5	166.66	±	20.84
15	421.9	251.0	571.0	291.0	383.72	±	46.28
30	382.6	112.3	488.0	160.0	285.73	±	78.71
45	388.2	116.1	420.8	158.0	270.76	±	81.39
60	237.5	103.8	391.5	96.7	207.37	±	49.80
75	229.5	77.2	572.9	86.7	241.57	±	50.46
90	232.5	86.7	561.7	64.0	236.23	±	59.56
105	170.0	81.0	521.4	64.0	209.11	±	37.48
120	144.8	74.4	712.7	64.0	248.72	±	28.57

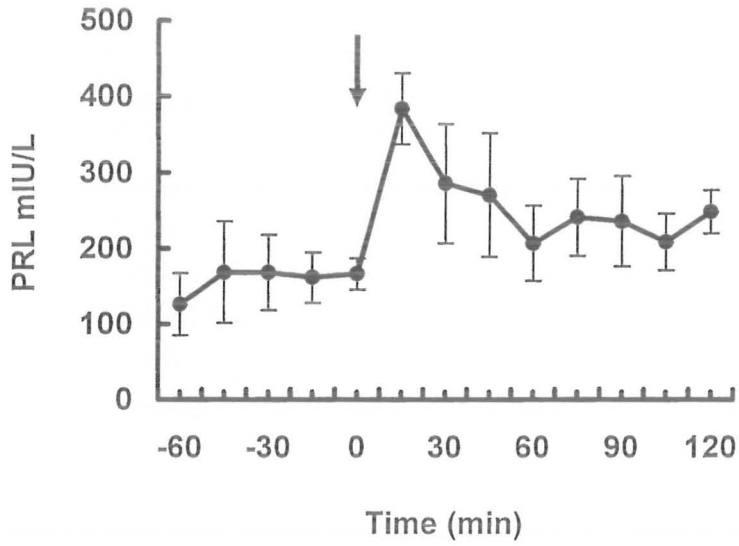

Fig. 5.

Effect of Insulin + MK-801 (↓) on plasma PRL concentration (mIU/L) in male adult rhesus monkeys.

TABLE 5.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before Mk-801 and Insulin administration with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	544.87	544.87	2.15	0.24
Residual	3	759.44	253.15		
Total	4	1304.31			
b	7.381 ± 5.031				

TABLE 5.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after Mk-801 and Insulin administration with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	11625.5	11625.54	6.429	0.044
Residual	6	10850.3	1808.38		
Total	7	22475.8			
b	-16.637 ± 6.561				

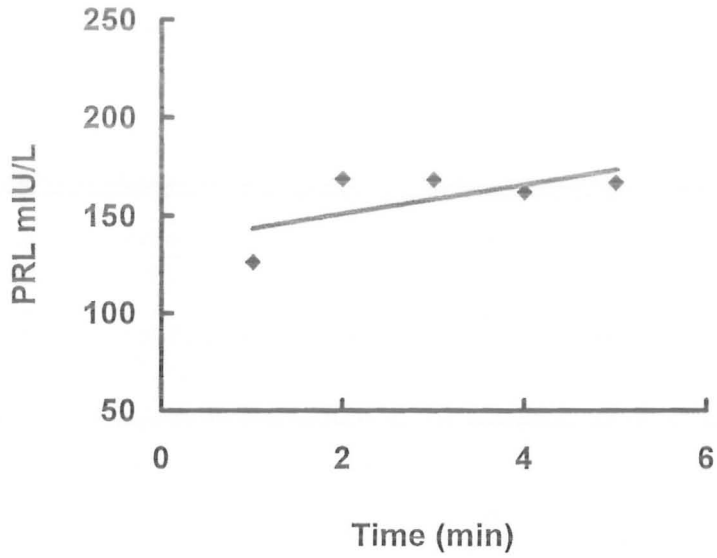

Fig. 5.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before the administration of Mk-801 and Insulin

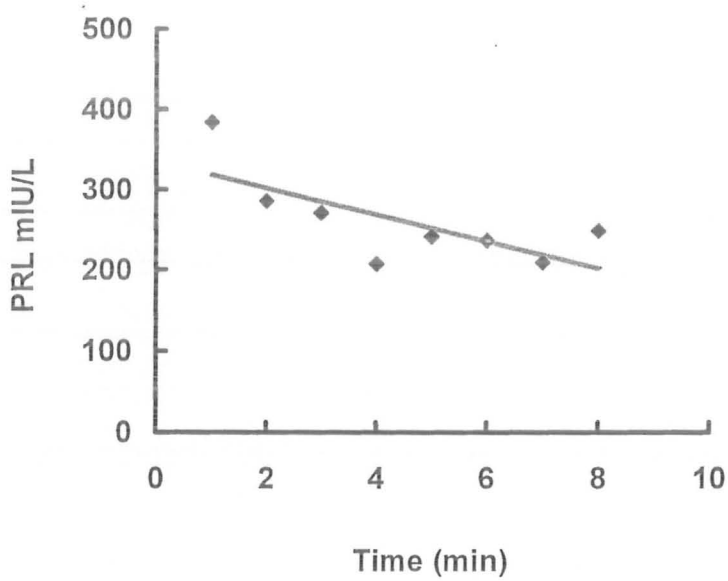

Fig. 5.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after the administration of Mk-801 and Insulin.

TABLE 5.3

Mean plasma PRL concentration (mIU/L) before and after different treatments

Treatments	*Before Treatment*		*After Treatment*	
	Mean	*S.E.M*	*Mean*	*S.E.M*
Saline	273.78 ± 8.99		*212.71 ± 11.36	
MK-801	167.40 ± 7.59		**100.125 ± 3.97	
Insulin	170.19 ± 2.64		188.32 ± 17.22	
Insulin + MK-801	158.31 ± 8.08		**260.39 ± 20.03	

p<0.01*

p<0.001**

TABLE 5.4

Analysis of variance showing the effect of different treatments on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

Treatments	*F- value*	*P-value*
MK-801 Treatment	69.24	2.57E-16
Insulin Treatment	5.12	0.0004
MK-801 + Insulin	2.36	0.041
Mk-801 Vs Mk-801 + Insulin	4.40	0.001
Insulin Vs MK-801 + Insulin	3.02	0.013

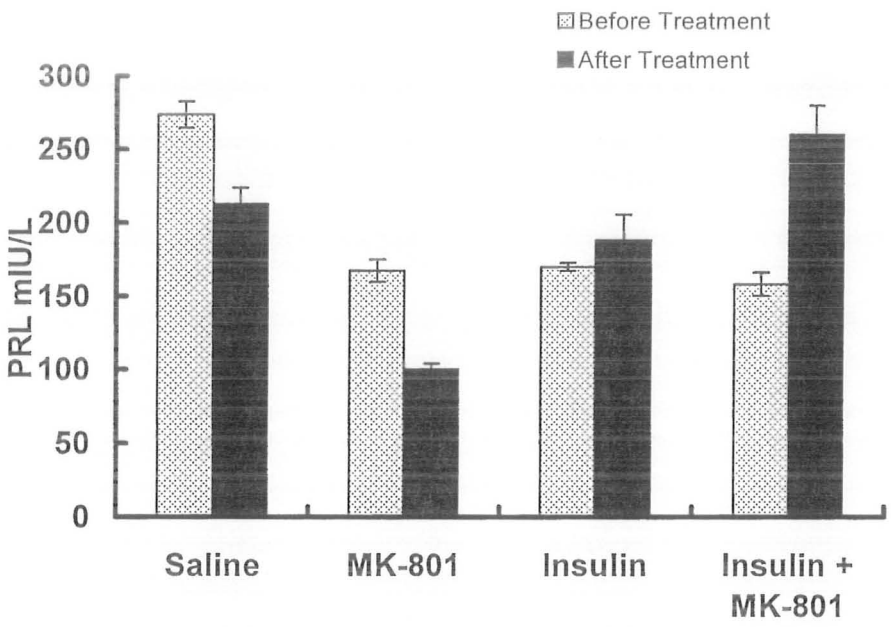

Fig. 5.3.

Plasma PRL concentration mIU/L before and after different treatments in four adult male monkeys.

administration of both the drugs. Mean plasma PRL concentrations 15 minutes after the administration of MK-801 and insulin were 383.72 ± 46.28 mIU/L and after one hour (at 60 minutes) the level reduced to 207.37 ± 49.80 mIU/L. After this the mean level of plasma PRL was fluctuating as the time proceeded and reached 248.72 ± 28.57 mIU/L at 120 minutes time. Regression analysis of variance showed that there was a significant negative trend in the mean plasma PRL levels ($b = -16.63 \pm 6.561$, $F_{(1,6)} = 6.42$, $P = 0.04$, Table 5.2 Fig 5.2).

Combined treatment of MK-801 and insulin caused a highly significant ($p<0.001$) increase in the basal plasma PRL level. Comparison showed that combined treatment of both the drugs caused highly significant ($p<0.001$) increase when compared to MK-801 (Table 3 Fig 3) as well as to the insulin ($p<0.05$) alone (Table 4 Fig 4). Concentration of plasma PRL after MK-801 (Table 3) and after combined treatment of MK-801 and Insulin (Table 5) was compared with two-way analysis of variance (ANOVA). The result showed a very highly significant ($p<0.001$) difference in the plasma PRL concentration. Similarly when plasma PRL levels after Insulin treatment (Table 4) was compared with plasma PRL after combined treatment of MK-801 and insulin (Table 5), a very highly significant ($p<0.01$) difference was observed.

DISCUSSION

The present work was designed to study the role and involvement of endogenous EAA in the regulation of PRL secretion during stimulated conditions. For this purpose four adult male Rhesus monkeys (*Macaca mulatta*) were used. In order to investigate the effect of endogenous EAA on PRL secretion, the endogenous EAA are blocked by an injection of NMDA receptor antagonist MK-801. Administration of the drug caused a very highly significant ($p<0.001$) reduction in the basal circulating plasma PRL levels immediately after its administration. Regression analysis of variance was applied which showed a highly significant negative trend in the plasma PRL levels. Excitatory amino acid neurotransmitters appear to be potent modulator of PRL secretion in rodents and primates (Wilson and Knobil, 1983; Gay and Plant, 1987; Olney and Price, 1980; Phol *et al.*, 1989). Administration of NMDA antagonist, MK-801 significantly attenuates the pro-estrous gonadotropin and PRL surge in immature and adult cycling female rats. Our results are consistent with the previous reports demonstrating that MK-801 markedly decreased basal PRL secretion in both female and male rats (Edward *et al.*, 1993). NMDA and non-NMDA receptor-induced PRL secretion is evidenced from a number of studies utilizing specific antagonist (Brann and Mahesh, 1991; Brann *et al.*, 1993; Parker and Crowley, 1993; Wagner *et al.*, 1993). Brann and Mahesh (1991) demonstrated that in female rat administration of NMDA receptor antagonist MK-801 blocks the proestrous PRL surge while that of non-NMDA receptor antagonist DNQX significantly attenuates the preovulatory PRL surge in the PMSG-primed immature rat (Brann *et al.*, 1993). It was also observed that suckling-induced PRL release in the lactating rat has been blocked by the administration of a non-NMDA antagonist (CNQX), but not by administration of NMDA antagonists (Parker and Crowley, 1993). MK-801 has also been reported to reduce the rate of PRL release from primary cultures of rat anterior pituitary cells (Login, 1990) thus suggesting a direct inhibitory effect of MK-801 at the level of lactotroph. It was also observed that direct administration of kainate to the third ventricle was also found to stimulate PRL release in the cycling female rat (Abbud and Smith, 1991). NMDA induce c-Fos immunoreactivity in two hypothalamic regions known to regulate PRL secretion: the paraventricular nuclei (PVN) which is the site of TRH cell bodies and the arcuate nuclei (ARC) which is the site of dopamine cell bodies (Abbud and Smith, 1991; Lee *et al.*, 1993). Hence NMDA could

act to regulate PRL via regulation of these PRL releasing/or-inhibiting factors, such as VIP and oxytocin (from the SCN and ARC respectively). EAAs are more likely to control PRL release by regulating dopamine neurons in the ARC. Wagner *et al.*, (1993) demonstrated that NMDA receptors are involved in the regulation of dopamine release from the hypothalamus and that DA released from TIDA nerve terminals in the median eminence travels through the hypophyseal long portal vessels to the anterior pituitary where activation of D_2 receptors on lactotrophs cause inhibition of PRL secretion from the anterior pituitary gland (Freeman *et al.*, 2000). It was previously observed by Toney *et al.* (1992) that removal of tonic stimulatory effects of endogenous PRL in female rats decreases TIDA neuronal activity and that MK-801 was also able to decrease TIDA neuronal activity in the absence of the tonic stimulatory effect of PRL following immunoneutralization of endogenous PRL (Edward *et al.*, 1993), thus suggesting that the inhibitory effect of MK-801 on TIDA neurons occurs independently of its inhibitory effect on PRL secretion. In contrast to MK-801, the competitive NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid has been shown to increase rather than decrease PRL secretion in male rats (Arslan *et al.*, 1991). This disparity in the effect of NMDA receptor antagonists on PRL secretion could be attributed to the ability of MK-801 to block voltage-gated ion channels (Wamil and McLean, 1992) thereby disrupting stimulus-secretion coupling in the lactotroph. A comparable decrease in the activity of TIDA neurons is seen.

In the present study a physiological stress in the form of hypoglycemia was induced by the administration of a single injection of insulin in four adult male monkeys. It was observed that administration of insulin caused a significant ($p<0.05$) increase in plasma PRL levels immediately after its administration for less then one hour, after which the levels were reduced to pre-treatment levels. These results are consistent with the previous studies that acute stress increased PRL secretion by mechanisms involving either increased secretion of PRFs or inhibition of dopamine release (Johnston and Negro-Vilar, 1986), which suggested that hypoglycemia is a well-defined stress stimulus, that generates a signal in glucosensitive cells of the central nervous system that activates neuroendocrine counterregulation in the hypothalamus. Glucosensitive cell neurons (glucostat) for individual counter regulatory functions are not localized in the same brain area and that glucoreceptors which generate impulses for PRL release during hypoglycemia are localized in a structure that is not protected by blood-brain barrier (Vigas *et al.*, 1990). Various

neuroendocrine pathways involved in producing peripheral hormonal and metabolic responses for restoring euglycemia as a result of insulin-induced hypoglycemia (Fish *et al.*, 1986; Garber *et al.*, 1976) and the resulting neuroglucopenia (Hourani *et al.*, 1992). The hypothalamic-pituitary-adrenal axis is known to be among the initial and predominant systems involved in substrate mobilization enhanced hepatic glycogenolysis and gluconeogenesis which are essential components of the counter-regulatory response to an acute decrease in blood glucose. However, many additional, redundant systems are involved in glucose homeostasis, including direct neural stimulation (Havel *et al.*, 1996; Paramore *et al.*, 1999; Hevener *et al.*, 2000), histaminergic (Molina *et al.*, 1997) and endorphinergic systems (Radosevich *et al.*, 1988; Paramore *et al.*, 1999). In addition to the classical hormones and neurotransmitters involved in glucoregulation, studies have demonstrated that in the adult brain, extracellular fluid concentrations of EAA (glutamate and aspartate) raise 4-10 fold in response to hypoglycemia. This excessive efflux of EAA has been suggested to contribute to the pathogenesis of hypoglycemia–induced neuronal necrosis (Wieloch, 1985). EAA receptors are the main transmitter receptors mediating synaptic excitation in the CNS (Watkins and Evans, 1984).

In the present study the involvement of endogenous EAAs during physiologically stimulated condition (hypoglycemia) was studied by simultaneous administration of the MK-801 and insulin in four adult male monkeys. Combined treatment of MK-801 plus insulin surprisingly caused a highly significant ($p<0.05$) rise in the basal plasma PRL levels and the levels remained high. Previous reports demonstrated that MK-801 markedly decreased basal PRL secretion in both female and male rats (Edward *et al.*, 1993). MK-801 has also been reported to reduce the rate of PRL release from primary cultures of rat anterior pituitary cells (Login, 1990) thus suggesting a direct inhibitory effect of MK-801 at the level of lactotroph. EAAs are more likely to control PRL release by regulating dopamine neurons in the ARC. Wagner *et al.* (1993) demonstrated that NMDA receptors are involved in the regulation of dopamine release from the hypothalamus and that DA released from TIDA nerve terminals in the median eminence travels through the hypophyseal long portal vessels to the anterior pituitary where activation of D_2 receptors on lactotrophs cause inhibition of PRL secretion from the anterior pituitary gland (Freeman *et al.*, 2000). It was also previously observed that acute stress increased PRL secretion by mechanisms involving either increased secretion of PRFs or inhibition of dopamine

release (Johnston and Negro-Vilar, 1986), which suggested that hypoglycemia is a well-defined stress stimulus, that generates a signal in glucosensitive cells of the central nervous system that activates neuroendocrine counterregulation in the hypothalamus. Studies have also demonstrated that in the adult brain, extracellular fluid concentrations of EAA (glutamate and aspartate) raise 4-10 fold in response to hypoglycemia. But the administration of MK-801 may certainly blocked the EAA pathway and the increase in PRL even after the administration of MK-801 is because acute stress increased PRL secretion by mechanisms involving either increased secretion of PRFs or inhibition of dopamine release (Johnston and Negro-Vilar, 1986), which suggested that hypoglycemia is a well-defined stress stimulus, that generates a signal in glucosensitive cells of the central nervous system that activates neuroendocrine counterregulation in the hypothalamus.

These results indicate that insulin cause an increase in plasma PRL level through a pathway, which might be through the involvement of endogenous excitatory amino acids in non-human primates. During physiologically stimulated conditions (hypoglycemia) the release of PRL may be through inhibition of dopamine release, which causes a significant rise in PRL level.

STUDY 2

INTERACTION OF EXCITATORY AMINO ACID NEUROTRANSMITTERS WITH ENDOGENOUS OPIOID PEPTIDES FOR THE REGULATION OF PROLACTIN

ABSTRACT

The present study was designed to investigate the interaction of N-methyl-D-Aspartic Acid (NMA) with opioids in the regulation of PRL release. Five adult male monkeys (*macaca mulatta*) were used for this purpose, which were maintained under the standard colony conditions. Experiments were carried out after acclimatizing the animals for chair restraining for a period of four weeks. Two teflon cannulae were inserted to the sephanous veins under the ketamine hydrochloride (5mg/kg) anaesthesia. Blood samples were collected for a period of four hrs with an interval of 15 minutes and plasma was separated after centrifugation and stored at -15°C until assayed through a special assay system.

Four sets of experiments were performed. In the control experiment all the animals were treated with an infusion of saline (3 ml/hr) for a period of 3 hrs. This saline infusion caused no significant change in plasma PRL levels and regression analysis of variance showed a non-significant negative trend in the plasma PRL levels. In the second sets of experiments two NMA injections were administered with an interval of one hr. Both the injection caused a highly significant ($p<0.05$) increase in plasma PRL levels. Regression analysis of variance showed that plasma PRL levels declined highly significantly ($p<0.0005$) as the time advanced. In the third sets of experiment all the five animals were given infusion along with a bolus injection of NAL (an opioid antagonist) for 3 hrs. This infusion caused a highly significant ($p<0.001$) decrease in plasma PRL levels. Regression analysis of variance showed a very highly significant ($p<0.0001$) negative trend in the plasma PRL levels. In the last set of experiments two NMA injections were administered during the bolus and infusion of NAL. Both NMA injections were failed to produce any significant increase in plasma PRL concentrations. Regression analysis of

variance showed a non-significant negative trend in plasma PRL level and NAL suppressed the plasma PRL response to NMA and attenuation of NMA induced PRL secretion during NAL infusion was greater after second NMA injection.

The results showed an involvement of opioid peptides in the central regulation of PRL in male monkeys and that endogenous excitatory amino acids act through endogenous opioids for the regulation of PRL from lactotropes of pituitary.

INTRODUCTION

The endogenous opiates (enkaphaline and endorphins) and morphine cause a rapid increase in PRL secretion when given by systemic or intraventricular injection (Van Vugt and Meites, 1980). Studies with morphine and methadone in man (Tolis *et al.*, 1975; Kleber and Gold, 1978) and endogenous opioid peptides (EOP) in rodents (Lien *et al.*, 1976; Cusan *et al.*, 1977; Ferland *et al.*, 1977; Cocchi *et al.*, 1977; Rivier *et al.*, 1978) have shown that stimulation of opiate receptor sites causes an increase in serum PRL. Pretreatment with the opiate antagonist Naloxone (NAL), blocks the increase in serum PRL (Tolis *et al.*, 1975; Kleber and Gold, 1978), normally seen after opiate administration. Pure opiate antagonists, like NAL, block and reverse the effects of opiates and displace the endorphins at the opiate receptor sites in the brain. The reversal or attenuation of behavioral or neurochemical effects by NAL would then be taken as neuropharmacological evidence that the effects were mediated by opiate receptors and endorphins. In lower mammals, NAL has generally been found to have no effects of its own other than to block or reverse the effects of opiate agonists. However, NAL has been reported to decrease basal serum PRL in rodents (Bruni *et al.*, 1977; Shaar *et al.*, 1977) and nonhuman primates (Gold *et al.*, 1978).

Administration of NAL or naltraxone also prevent PRL release in response to stress or suckling and reduce basal PRL secretion (Bruni *et al.*, 1977; Van Vugt *et al.*, 1978). The acute suckling-induced PRL rise is blocked by NAL (Selmanoff and Gregerson, 1986; Baumann and Rabii, 1991), as well as specific μ and κ opioid receptor antagonists (Baumann and Rabii, 1991). Although it is not known which of the EOP contribute to the suckling-induced PRL release. β-Endorphin (Selmanoff and Gregerson, 1986; Kehoe *et al.*, 1993), as well as specific μ-selective opioid peptides (Baumann and Rabii, 1990), can acutely increase PRL release in postpartum rats. NAL as well as μ, κ and δ receptor antagonists can block β-endorphin-induced PRL release on postpartum rats.

The EOP do not act directly on the pituitary gland. They may inhibit the activity of the TIDA system (Van Vugt et. al., 1978) perhaps through cholinergic neurons (Shaar and Clemens, 1980). A number of independent lines of scientific investigations support an opiate or endorphin modulation of DA activity similar to DA receptor-blocking antipsychotic medications block DA receptors in the brain

(Snyder *et al.*, 1974; Eidelberg, 1976; Gold *et al.*, 1977; Kleber and Gold, 1978) and stimulate PRL secretion (Clemens *et al.*, 1974; Meltzer *et al.*, 1977). The arcuate nucleus is a major source of both β-endorphin (Mezey *et al.*, 1985) and TIDA neurons (Moore and Lookingland, 1995). Contacts between β-endorphin axon terminals and TIDA neurons in the arcuate nucleus have been described by a number of investigators (Horvath *et al.*, 1992; Morel and Pelletier, 1986). Opioid μ, δ and κ receptors and /or their mRNA are abundantly distributed in the hypothalamus (Mansour *et al.*, 1995). EOPs may exert their stimulatory action on PRL secretion by inhibiting TIDA neuronal activity. Existing data support a role for EOPs in influencing hypothalamic DA neuronal activity and DA synthesis, release and turnover (Van Loon *et al.*, 1980; Gudelsky and Porter, 1979; Arita and Kimura, 1988).

β-Endorphin stimulated PRL secretion in postpartum and virgin female rats to levels that mimicked the suckling-induced PRL increase. This response was abolished by antagonizing the μ_1 (Janik *et al.*, 1992), μ, δ or κ sites (Kehoe *et al.*, 1993) indicating that β-endorphin activates a pathway involving multiple receptor subtypes. In lactating female rats, antagonism of either the μ or κ sites receptor site inhibited PRL release during suckling (Baumann and Rabii, 1990), but only the μ site seemed to mediate inhibition of hypothalamic dopaminergic neural activity (Callahan *et al.*, 1996). Arbogast and Voogt (1998) recently reported that opioidergic input was essential for normal lactation due to the effects on the TIDA neurons.

Numerous studies have revealed a high concentration of opioid peptides and receptors throughout the hypothalamus. Autoradiographic studies have shown that μ receptors are densely localized throughout the limbic system (Goodman *et al.*, 1988). In addition, Unterwald and coworkers (1991) identified moderate concentrations of κ_1 and κ_2 receptors subtypes in rat hypothalamus. More recently, in situ hybridization studies revealed opiate receptor mRNA for all three receptor subtypes in hypothalamus (Mansour *et al.*, 1995), but the hypothalamus distributions were different and distinct in hypothalamic nuclei (George *et al.*, 1994). There was no mRNA for either the μ, δ or κ receptor subtype in the anterior, intermediate or neural lobe of the pituitary (Mansour *et al.*, 1995). However, autoradiograghy studies revealed some κ_1 receptor binding in the neural lobe, possibly due to receptor transport (Mansour *et al.*, 1995). Loose *et al.* (1991) demonstrated that μ-specific

agonists inhibited spontaneous firing from arcuate nucleus neurons and that β-endorphin was immunocytochemically localized in this hypothalamic region. Horvath *et al.* (1992) detected β-endorphin-immunoreactive cells throughout the medial basal hypothalamus. Light and electron microscopy revealed that these β-endorphin-immunoreactive cells projected to tyrosine hydroxylase (TH)-positive cells, which are presumably dopaminergic neurons. A major portion of the β-endorphin-targeted TH cells were in the periventricular anterior hypothalamic regions, however, previous results indicate that β-endorphin did not inhibit TIDA neurons during suckling (Jaworski-Parman *et al.*, 1997). Enkephalin-containing neurons have been identified throughout the hypothalamus, including the arcuate nucleus and periventricular area (Khachaturian *et al.*, 1983; Zamir *et al.*, 1985).

Clearly, opiate receptor and peptide localization studies, as well as, physiological studies, indicate that EOP play an important and complex role in the regulation of anterior pituitary hormone secretion.

EAAs, such as L-glutamate and L-aspartate, appear to be the major excitatory neurotransmitters in CNS, synaptic excitation through EAA neurotransmission may also underlie many of the normal physiological processes that occur in the brain.

The mediation of EAA neurotransmission in the CNS is achieved primarily by the acidic amino acids glutamate and aspartate (Cotman and Iverson, 1987; Hanson and Krogsgaard-Larsen, 1990; Monaghan et. al., 1989). Their stimulatory effects are exerted through a variety of receptor subtypes classified according to their responsiveness to specific agonist. One such subtype is the N-methyl – D – aspartic acid (NMDA) receptor, so named because NMDA is a potent agonist for this receptor subtype. There is increasing evidence that receptors for neuroexcitatory amino acids of the NMDA subtype are an important component of the LH surge induced by ovarian hormones (Carbone *et al.*, 1992).

Involvement of EAAs to stimulate PRL secretion has been demonstrated by NMDA administration in rodents and primates (Wilson and Knobil, 1982; Gay and Plant, 1987; Olney and Price, 1980), intact and castrated male rats (Arslan *et al.*, 1992; Strobl *et al.*, 1993) as well as cycling female rats (Abbud and Smith, 1991; Luderer *et al.*, 1993; Pohl *et al.*, 1989).

NMDA induce c-Fos immunoreactivity in two hypothalamic regions known to regulate PRL secretion: the paraventricular nuclei (PVN) which is the site of TRH cell

bodies and the arcuate nucleus (ARC) which is the site of dopamine cell bodies (Abbud and Smith, 1991; Lee *et al.*, 1993). Hence NMDA could act to regulate PRL via regulation of these PRL releasing / or inhibiting factors, such as VIP and oxytocin (from the SCN and ARC respectively) may also be regulated by EAAs. Wilson and Knobil have reported that TRH serum levels are unaffected by whether TRH is involved in NMDA's effect on PRL. Dopamine neurons in the ARC may be more likely site of EAA regulation in the control of PRL release. In support of this possibility, NMDA receptors have been reported to regulate dopamine release in the hypothalamus (Wagner *et al.*, 1993).

In view of these facts the present study is designed to investigate the interaction of N-methyl-D-Aspartic acid (NMA) with opioids in the regulation of PRL release. Adult male rhesus monkeys are used for this specific objective.

MATERIALS AND METHODS

MATERIALS AND METHODS

ANIMALS

Same as in study 1.

PHARAMACOLOGIC AGENTS

The following drugs were used in the present study:

1. **Ketamine hydrochloride** (ketavat; park Davis, Berlin, FRG).
2. **N-methyl-D, L-aspartic acid**: (NMA Sigma Chemical Co. (St. Louis, Mo, 63178, USA).
3. **Naloxone:** Sigma Chemical Co. (St. Louis, Mo, 63178, USA).
4. **Normal Saline (0.9 % NaCl)**: Plasaline, Otsuka Pakistan Ltd. F/4-9. H.I.T.E., Hub, Balochistan, Pakistan.

CHAIR RESTRAINING

Same as in study 1.

CATHETERIZATION

Same as in study 1.

BLEEDINGS

Same as in study 1.

EXPERIMENTAL PROTOCOL

A treatment with opioidergic antagonist was carried out after an interval of 1-2 weeks:

a) Vehicle administration:

The animals were bled for a period of 4 hours at an interval of 15 minutes. All the animals were infused 6 ml of vehicle (0.9% NaCl, 3 ml/hr) at one hour of the blood sampling for 2 hours.

b) NMA

The animals were bled as above and two injections of NMA (15 mg/kg BW) were given at 1 and 2 hr of blood sampling. NMA was dissolved in normal saline immediately before use.

c) Naloxone:

The animals were bled as above and after 1 hr the animals received a bolus iv injection of naloxone (5 mg/3ml), an opioid receptor antagonist. Immediately following bolus naloxone, the animals were administered an infusion of naloxone (5 mg/3ml/hr) for a period of 2 hrs.

d) NMA + Naloxon

The animals were bled and administered naloxone treatment as in above experiment (c). Additionally, animals were challenged with two NMA injections (15 mg/kg BW, iv) 30 min after start of nalaxone infusion and 30 min before termination of infusion.

HORMONE DETERMINATION

As in study1.

STATISTICAL ANALYSIS

For comparison of baseline PRL secretion before treatment, hormone levels were calculated by averaging all the concentrations before treatment. On the other hand PRL responsiveness to the drugs induced was determined by comparing basal levels of the hormone calculated by averaging the concentrations immediately before the injection at 0 min and the levels worked out by averaging the concentration of hormone 15 min after inducing the drug. Student's t-test was used to determine differences between the means

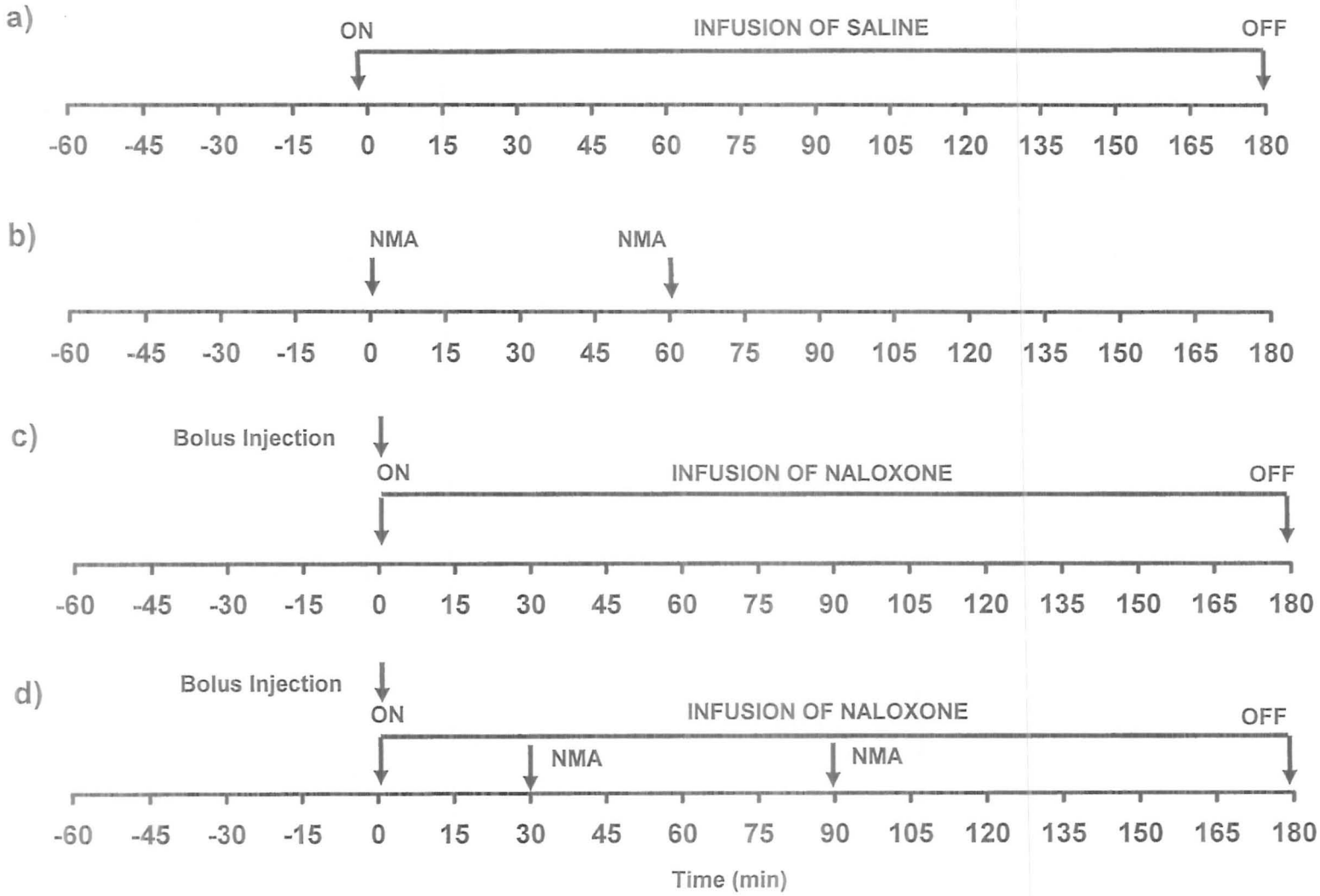

Fig. 6. Experimental Protocol showing the administration of a) Saline b) NMA c) Naloxone d) NMA + Naloxone to adult male monkeys (n = 5)

of basal and stimulated levels. The data were also subjected to two-way analysis of variance (ANOVA) and linear regression. P values are mentioned for t-test applied. Where analysis of variance is carried out both values for F and P are given.

RESULTS

RESULTS

Body Weight

Mean body weight of five adult male rhesus monkeys (*Macaca mulatta*) used in the study is given in the Table 6.

Behavioral Reaction

The treated animals remained calm after the administration of Nalaxon and saline (vehicle). Although the administration of first NMA injection produced no significant change in the behavior but all animals vomited after the administration of second NMA injection.

Effect of Vehicle (Saline) Infusion in adult male monkeys

In the control experiment the effect of saline infusion on mean plasma PRL concentration (mIU/L) was studied in five adult male monkeys and the observation are shown in the Table 7 and Fig 7. Pre-treatment levels of mean plasma PRL concentration, one hour before infusion was also recorded. At the start of the blood sampling the mean plasma PRL level was 192.6 ± 8.9 mIU/L and after an hour before the start of infusion the levels reached 219.1 ± 8.9 mIU/L. It shows an increase in mean plasma PRL concentration but this increase was not significant (b = 7.39 ± 3.500, $F_{(1,3)} = 4.45$, P = 0.12, Table 7.1 and Fig 7.1).

Infusion of saline was started at 0 minutes and samples were collected after 15 minutes time. Initial levels of mean plasma PRL concentrations after 15 minutes were 201.5 ± 27.4 mIU/L. Infusion was stopped at 180 minutes (after 2 hours) and the levels of plasma PRL concentration was 204.5 ± 16.9 mIU/L. There was a non-significant change in plasma PRL level was observed after the infusion of saline. Regression analysis of variance showed that negative trend in PRL concentration was not significant (b = -0.540 ± 0.82, $F_{(1,10)} = 0.433$, P = 0.52, Table 7.2, Fig 7.2).

TABLE 6

Body Weight (kg) of Rhesus monkeys treated with Saline, NMA, Naloxone and Naloxone + NMA

Animal nos.	Saline	NMA	NAL	NAL + NMA
9305	11.1	11.1	11.1	11.1
9311	9.12	9.12	9.2	9.2
9318	10.4	10.4	10.4	10.6
9319	7.6	7.6	7.7	7.7
9321	10.4	10.4	10.4	10.4
Mean ± S.E.M.	9.72 ± 0.62	9.76 ±0.60	9.80 ± 0.61	9.80 ± 0.61

TABLE 7

Effect of iv infusion of Saline (V) on plasma PRL concentration (mIU/L) in adult male rhesus monkeys

Time (min)	Animal nos. 9318	9319	9305	9311	9321	Mean		S.E.M.
-60	225.0	226.0	190.0	183.2	199.0	204.63	±	8.88
-45	219.0	182.0	176.4	167.6	187.0	186.39	±	8.77
-30	235.0	205.0	194.1	177.0	179.0	198.02	±	10.57
-15	204.0	300.7	158.7	147.1	196.0	201.29	±	27.09
0	189.0	358.1	163.6	199.0	186.0	219.13	±	35.22
15	169.0	296.0	221.4	152.2	154.0	198.51	±	27.42
30	187.0	244.2	219.0	161.0	188.2	199.87	±	14.39
45	172.9	239.0	253.7	181.9	200.0	209.49	±	15.84
60	192.0	258.0	290.0	151.0	184.5	215.10	±	25.52
75	160.0	121.0	339.5	120.0	167.6	181.62	±	40.65
90	168.0	161.0	341.2	149.6	200.0	203.95	±	35.31
105	168.0	125.1	281.4	105.7	205.4	177.12	±	31.28
120	150.0	159.0	268.4	113.1	199.2	177.94	±	26.43
135	125.0	191.0	289.0	125.6	155.7	177.26	±	30.45
150	150.0	192.0	279.0	122.0	144.0	177.40	±	27.81
165	184.0	183.0	251.0	124.3	128.9	174.24	±	23.04
180	144.7	216.1	198.0	158.6	125.1	168.48	±	16.86

TABLE 7.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before Saline infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	546.12	546.12	4.46	0.13
Residual	3	367.55	122.52		
Total	4	913.67			
b	7.389	± 3.500			

TABLE 7.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after Saline infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	41.83	41.83	0.433	0.525
Residual	10	965.42	96.54		
Total	11	1007.25			
b	-0.541	± 0.821			

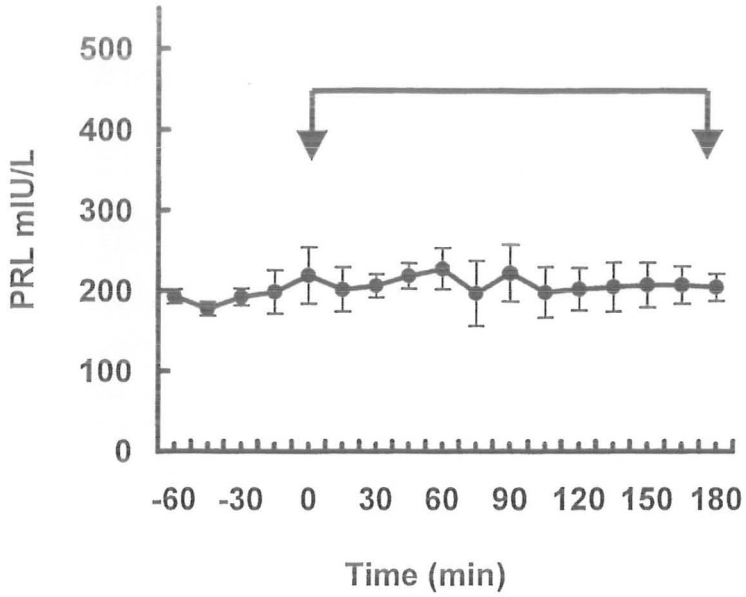

Fig. 7.

Effect of iv infusion of Saline (↓——↓) on plasma PRL concentration (mIU/L) in male adult rhesus monkeys.

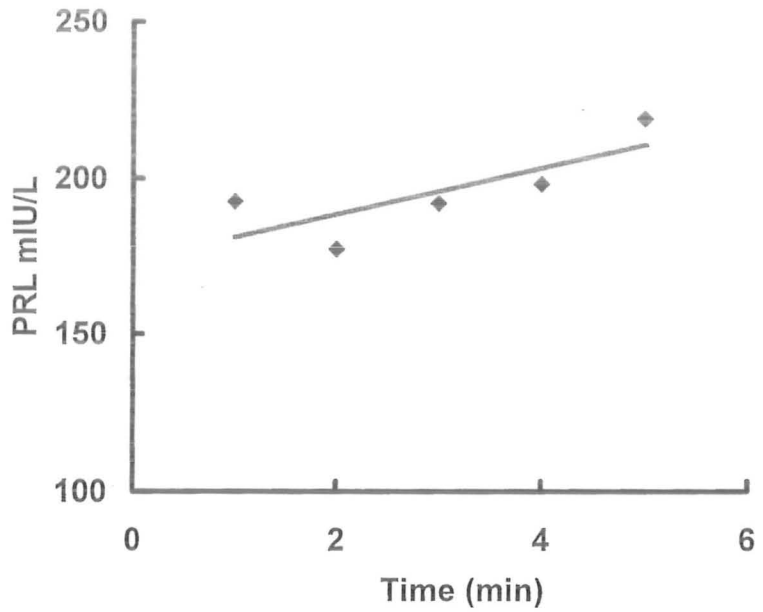

Fig. 7.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before Saline infusion.

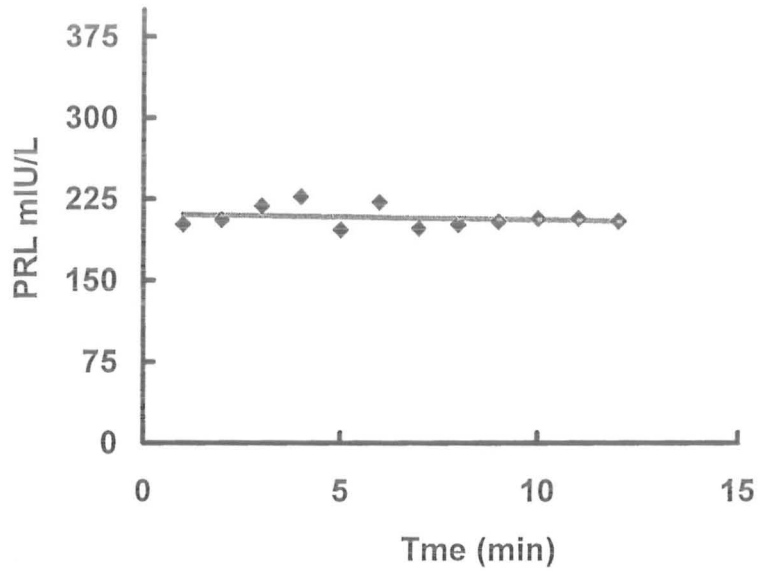

Fig. 7.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after Saline infusion.

Effect of Two NMA Injections on Plasma PRL Level

Two NMA injections were given at 0 minutes and 60 minutes stage. The effect of NMA injections on mean plasma PRL concentration in each animal was recoded (Table 8 and Fig 8). Mean plasma PRL level (mIU/L) recorded one hour before NMA injection (pretreatment levels) decreased with time. Initially mean plasma PRL level was 229.41 ± 47.35 mIU/L (-60 minutes) and after an hour (0 minutes) the level reached 206.65 ± 55.54 mIU/L. Regression analysis of variance showed that there was a highly significant negative trend in these levels (b = -5.654 ± 1.215, $F_{(1,3)}$ = 21.6, P = 0.01 Table 8.1, Fig 8.1).

First NMA injection (15 mg/kg BW) was administered at 0 minutes and immediately after 15 minutes the administration of NMA injection, a high mean plasma PRL level (424.20 ± 84.74 mIU/L) was observed which started decreasing as the time proceeded and reached 266.10 ± 54.39 mIU/L after 60 minutes of the injection. Regression analysis of variance showed a significant negative trend in plasma PRL level (b = -59.00 ± 13.434, $F_{(1,2)}$ = 19.29, P = 0.04 Table 8.2, Fig 8.2). NMA caused a significant ($p<0.05$) elevation in plasma PRL level as compared to pre-treatment level (Table 10.5 Fig 10.5).

Second NMA injection was given at 60 minutes when the plasma PRL level was 266.10 ± 54.39 mIU/L. Again there was an abrupt rise in plasma PRL levels after 15 minutes of the administration of NMA injection (388.95 ± 69.89 mIU/L) and after an hour (at 120 minutes) the levels reached 262.55 ± 36.78 mIU/L and to 213.91 ± 26.89 mIU/L after two hours (at 180 minutes) time. Administration of second NMA injection also caused a significant ($p<0.05$) increase in the plasma PRL level (Table 10.5 Fig 10.5).

Regression analysis of variance carried out for second NMA injection (75-180 minutes) also showed that plasma PRL concentrations decreased very highly significantly as the time advanced (b = -26.393 ± 4.266, $F_{(1,6)}$ = 38.2, P = 0.0008 Table 8.3, Fig 8.3). Regression analysis of variance showed that there is highly significant reduction in mean plasma PRL concentration (15-180 minutes) after the administration of two NMA injections (b = -17.460 ± 3.916, $F_{(1,10)}$ = 19.89, P = 0.001, Table 8.4, Fig 8.4). There was a significant ($p<0.0002$) difference observed between the circulating PRL levels after the administration of two NMA injections.

TABLE 8

Effect of two NMA injections on plasma PRL concentration (mIU/L) in adult male rhesus monkeys

Time (min)	Animal nos. 9318	9319	9305	9311	9321	Mean		S.E.M.
-60	423.0	187.0	194.1	159.8	243.1	241.41	±	47.35
-45	440.0	206.1	181.0	162.4	205.4	238.97	±	50.91
-30	454.4	196.0	186.0	156.0	153.3	229.14	±	56.92
-15	413.0	219.4	179.7	142.0	155.7	221.95	±	49.54
0	427.0	164.0	166.8	126.9	148.6	206.65	±	55.54
15	728.0	297.0	484.0	297.0	300.0	421.20	±	84.74
30	798.0	233.0	478.6	231.0	263.0	400.72	±	109.50
45	471.0	163.0	383.6	173.0	219.0	281.93	±	61.67
60	406.0	169.0	366.5	156.0	173.0	254.10	±	54.39
75	629.0	257.0	414.8	259.0	310.0	373.95	±	69.89
90	610.0	276.0	446.4	268.0	210.0	362.08	±	73.47
105	356.0	215.0	368.2	205.0	173.0	263.45	±	40.92
120	310.0	233.0	332.8	169.0	148.0	238.55	±	36.78
135	301.0	199.0	316.1	159.8	140.0	223.18	±	36.20
150	287.0	191.0	248.9	176.0	125.0	205.57	±	28.36
165	233.6	164.0	213.4	166.3	125.4	180.54	±	19.25
180	278.0	167.0	155.5	172.7	116.3	177.91	±	26.89

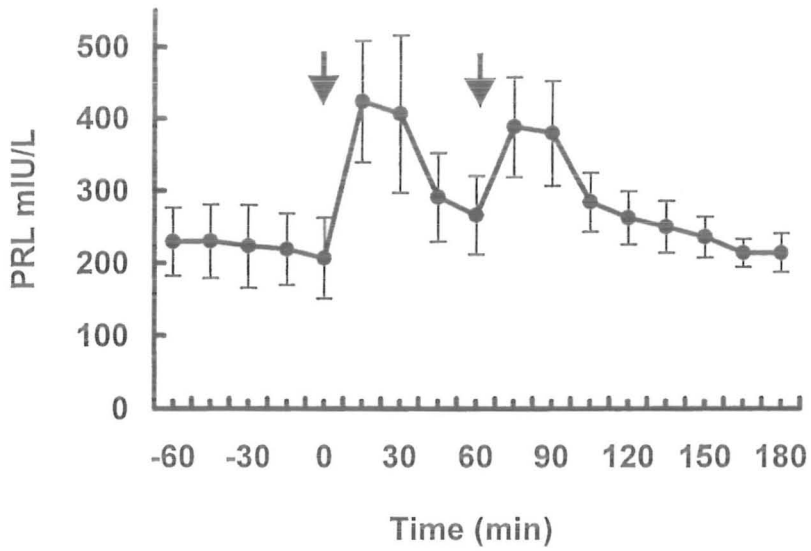

Fig. 8.

Effect of two NMA injections (↓) at 0 and 60 min on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

TABLE 8.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before two NMA injections with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	319.70	319.70	21.639	0.02
Residual	3	44.32	14.77		
Total	4	364.02			
b	-5.65	± 1.215			

TABLE 8.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after first NMA Injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	17411	17411	19.293	0.048
Residual	2	1804.8	902.42		
Total	3	19215			
b	-59.01	± 13.43			

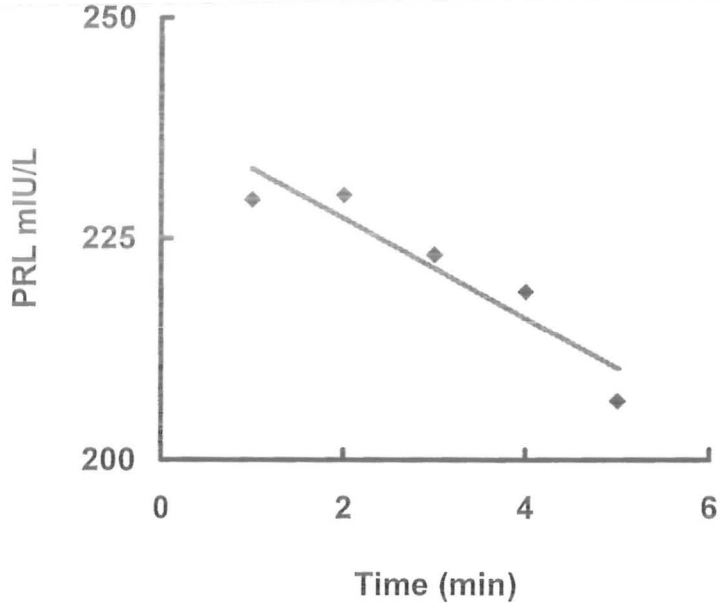

Fig. 8.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before two NMA injections.

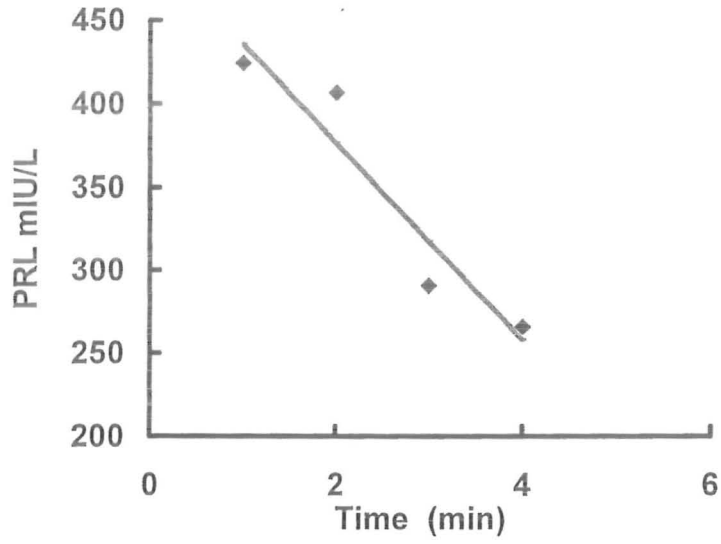

Fig. 8.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after first NMA injection.

TABLE 8.3

Regression analysis of variance of plasma PRL concentration (mIU/L) after second NMA Injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	29258	29258	38.263	0.0008
Residual	6	4587.9	764.65		
Total	7	33846			
b	-26.39	± 4.266			

TABLE 8.4

Regression analysis of variance of plasma PRL concentration (mIU/L) after two NMA injections with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	43598	43598	19.89	0.001
Residual	10	21915	2191.5		
Total	11	65513			
b	-17.46	± 3.914			

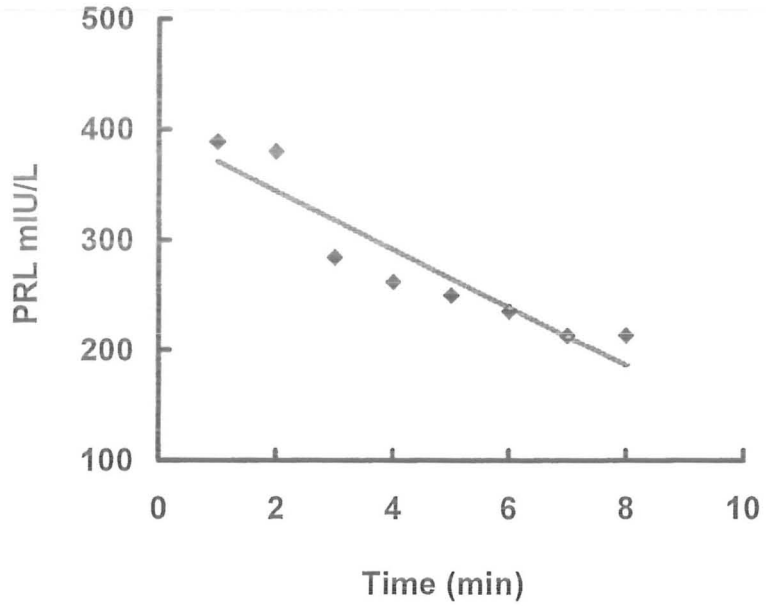

Fig. 8.3.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after second NMA injection.

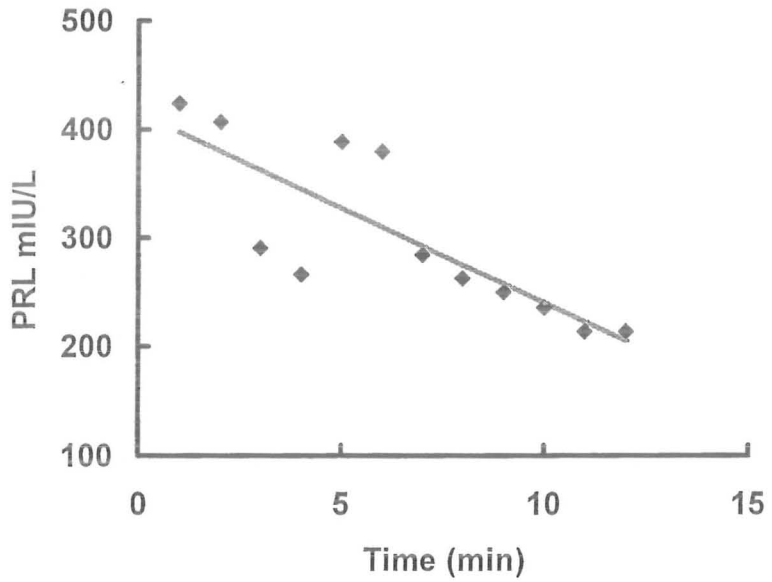

Fig. 8.4.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after two NMA injections.

Effect Of Naloxone Bolus and Infusion on Plasma PRL Level

Naloxone (NAL), an opiate antagonist, was given to five adult male monkeys. Table 9 and Fig 9 show the effect of NAL administration on mean plasma PRL concentration (mIU/L). Samples were collected one hour before the administration of NAL with an interval of 15 minutes. Pre-treatment values showed that mean plasma PRL concentration decreased with time from 181.15 ± 28.56 mIU/L to 147.34 ± 26.66 mIU/L, but this decrease in levels was non-significant although have a negative trend ($b = -7.428 \pm 1.99$, $F_{(1,3)} = 13.90$, $P = 0.03$, Table 9.1, Fig 9.1).

NAL bolus (5mg/3ml) and infusion (dose: 10 mg/6ml for all body weight, rate = 3 ml/hr) was started simultaneously at 0 minutes and blood samples were collected after an interval of 15 minutes. After 15 minutes of bolus and infusion plasma PRL concentration started decreasing (116.41 ± 56.34 mIU/L) and at 180 minutes when infusion was stopped the levels reduced to 74.86 ± 17.17 mIU/L. NAL caused very highly significant reduction in plasma PRL level (Table 10.6). Regression analysis showed a highly significant decrease in plasma PRL concentration as time advanced under the influence of opiate antagonist ($b = -3.815 \pm 0.338$, $F_{(1,10)} = 127.3$, $P = <0.0001$, Table 9.2, Fig 9.2). Bolus and infusion of NAL caused a highly significant ($p<0.001$) decrease in basal plasma PRL level (Table 10.5, Fig 10.5).

Effect of Two NMA Injections Under the Shadow of Naloxone Bolus and Infusion

Two injections of NMA were given during the administration of bolus and infusion of NAL to five adult male rhesus monkeys. Table 10 and Fig 10 show the effect of this combined treatment on individual and mean plasma PRL concentration (mIU/L). Blood samples were collected one hour before the NAL and NMA administration with an interval of 15 minutes each. The mean plasma PRL concentration before NAL and NMA administration was 338.68 ± 119.22 mIU/L and after 60 minutes the level was 316.24 ± 122.06 mIU/L. Regression analysis of variance showed non-significant negative trend in mean plasma PRL concentration during the pretreatment hour ($b = 0.491 \pm 2.643$, $F_{(1,3)} = 0.034$, $P = 0.8$ Table 10.1 and Fig 10.1).

TABLE 9

Effect of iv bolus and infusion of Naloxone on plasma PRL concentration (mIU/L) in adult male rhesus monkeys

Time (min)	Animal nos. 9318	9319	9305	9311	9321	Mean		S.E.M.
-60	280.2	194.0	129.0	106.7	189.9	179.95	±	28.56
-45	277.8	174.7	117.0	112.0	173.4	170.99	±	33.01
-30	274.0	185.9	125.0	105.0	200.0	177.97	±	23.40
-15	266.1	182.0	119.0	116.0	202.5	177.12	±	20.12
0	272.0	197.0	114.0	107.0	189.0	175.80	±	26.25
15	192.0	96.2	99.0	64.0	64.0	103.03	±	40.48
30	176.0	73.6	91.0	64.0	64.0	93.73	±	35.42
45	164.0	80.0	83.0	64.0	64.0	91.00	±	31.62
60	145.0	64.0	86.0	64.0	64.0	84.60	±	25.61
75	143.0	64.0	74.0	64.0	64.0	81.80	±	24.98
90	137.0	64.0	70.0	64.0	64.0	79.80	±	23.08
105	132.0	64.0	64.0	64.0	64.0	77.60	±	21.50
120	126.0	64.0	64.0	64.0	64.0	76.40	±	19.61
135	123.0	64.0	64.0	64.0	64.0	75.80	±	18.66
150	127.0	64.0	64.0	64.0	64.0	76.60	±	19.92
165	121.2	86.2	64.0	69.1	64.0	80.90	±	18.09
180	118.3	64.0	64.0	64.0	64.0	74.86	±	17.17

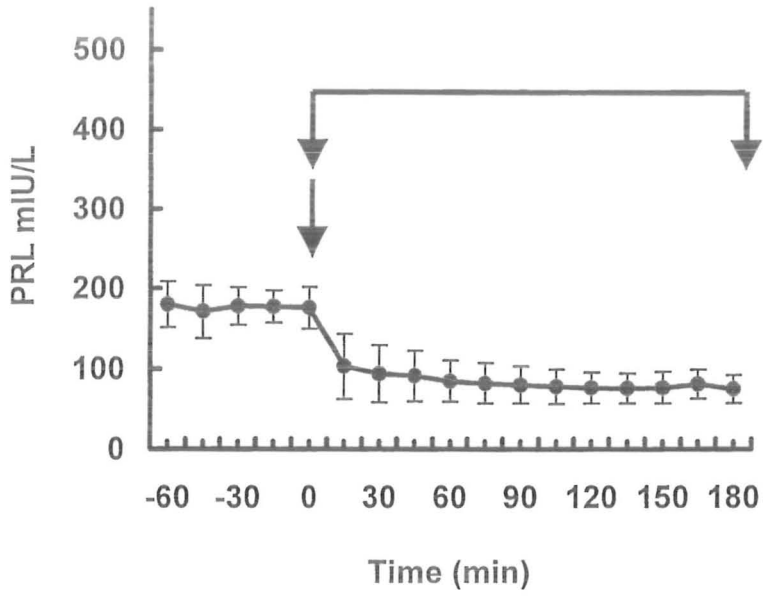

Fig. 9.

Effect of iv bolus (↓) and infusion (↓——↓) of Naloxone on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

TABLE 9.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before NAL bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	0.4666	0.4666	0.0313	0.871
Residual	3	44.766	14.922		
Total	4	45.232			
b	-0.216	± 1.221			

TABLE 9.2

Regression analysis of variance of plasma PRL concentration (mIU/L) during NAL bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	605.54	605.54	26.878	0.00041
Residual	10	225.29	22.529		
Total	11	830.83			
b	-2.058	± 0.396			

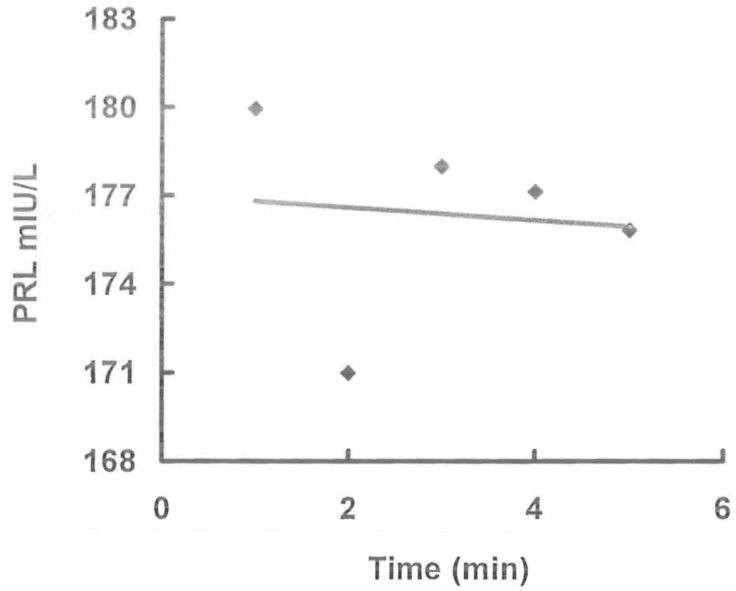

Fig. 9.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before Naloxone infusion.

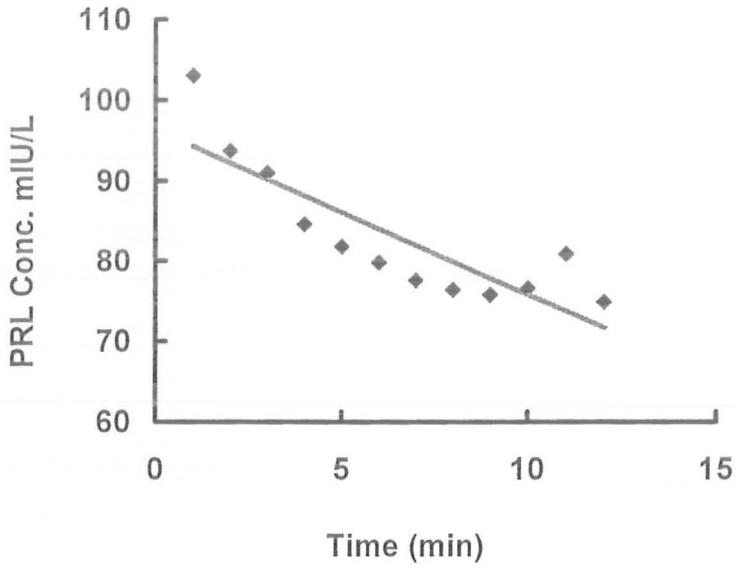

Fig. 9.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after Naloxone infusion.

TABLE 10

Effect of two NMA injections at 30 and 90 min during Naloxone bolus and infusion on plasma PRL concentration mIU/L in adult male rhesus monkeys.

Time (min)	Animal nos. 9318	9319	9305	9311	9321	Mean		S.E.M.
-60	637.0	251.4	375.0	170.0	260.0	338.68	±	119.22
-45	669.2	235.0	351.0	175.0	255.0	337.03	±	130.99
-30	646.6	220.0	419.1	191.0	242.0	343.73	±	127.93
-15	694.8	224.6	410.3	195.0	204.0	345.75	±	155.22
0	583.0	205.0	381.2	215.0	197.0	316.24	±	122.06
15	546.8	224.6	401.5	217.0	186.7	315.29	±	113.89
30	523.0	298.7	343.8	224.5	200.0	318.00	±	102.14
45	548.1	281.7	351.0	210.3	179.0	314.00	±	116.71
60	319.6	221.5	223.0	163.9	105.4	206.70	±	67.73
75	385.1	113.3	157.3	143.0	163.1	192.35	±	70.20
90	480.8	122.7	169.0	105.2	171.0	209.74	±	97.97
105	501.7	181.8	150.0	207.1	174.0	242.93	±	103.64
120	450.4	181.8	146.0	213.4	180.1	234.34	±	85.48
135	309.5	170.0	131.2	144.5	145.0	180.02	±	52.00
150	363.0	175.7	120.0	154.9	132.0	189.13	±	73.05
165	485.7	111.2	117.0	156.4	127.0	199.46	±	113.43
180	400.0	123.7	131.2	162.4	115.0	186.47	±	90.12

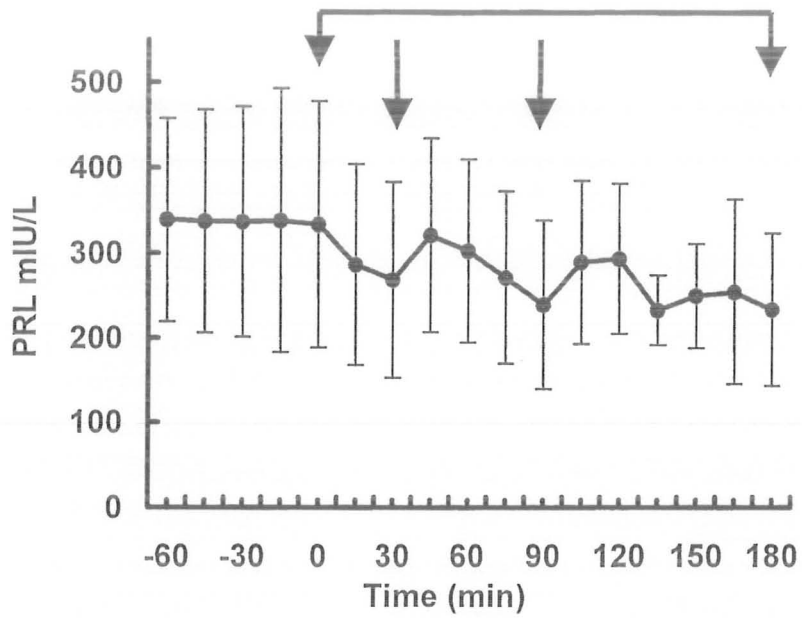

Fig. 10.

Effect of two NMA injections (↓) at 30 and 90 min during Naloxone bolus (↓) and infusion (↓——↓) on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

TABLE 10.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before two NMA injections during NAL bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	9.274	9.274	4.092	0.136
Residual	3	6.799	2.266		
Total	4	16.073			
b	-0.963	± 0.476			

TABLE 10.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after first NMA injection during NAL bolus and infusion with an interval of 15 minutes.

	df	SS	MS	F	Significance F
Regression	1	0.4666	0.4666	0.0313	0.871
Residual	3	44.766	14.922		
Total	4	45.232			
b	-0.216	± 1.221			

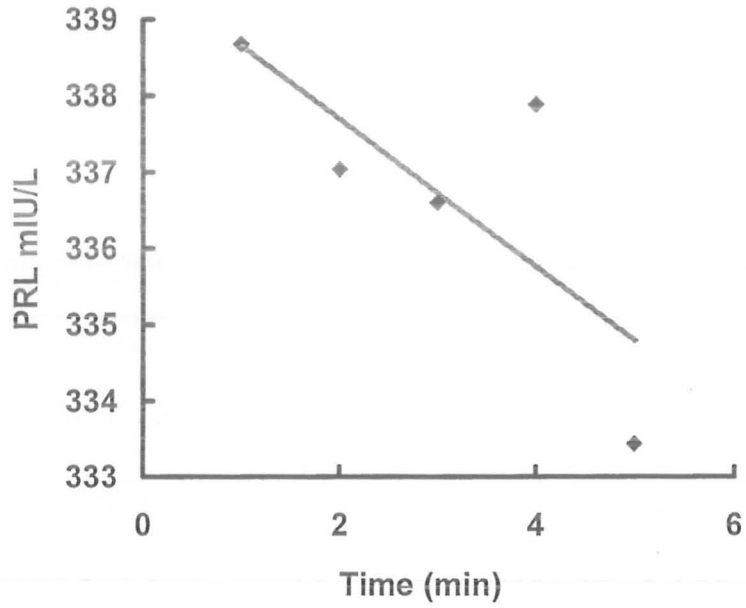

Fig. 10.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before two NMA injections during bolus and infusion of Naloxone.

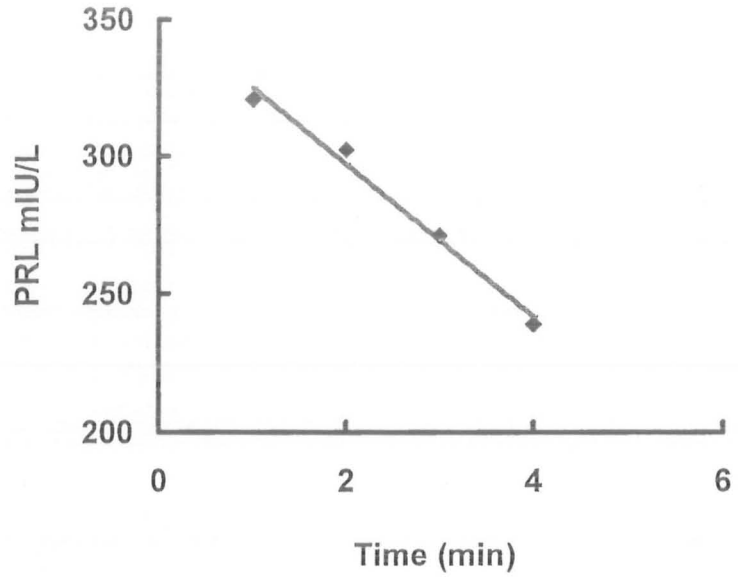

Fig. 10.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after first NMA injection during bolus and infusion of Naloxone.

TABLE 10.3

Regression analysis of variance of plasma PRL concentration (mIU/L) after second NMA injection during NAL bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	605.54	605.54	26.878	0.00041
Residual	10	225.29	22.529		
Total	11	830.83			
b	-2.058	± 0.396			

TABLE 10.4

Regression analysis of variance of plasma PRL concentration (mIU/L) after two NMA injections during NAL bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	3836.39	3836.39	141.29	0.007
Residual	2	54.30	27.15		
Total	3	3890.70			
b	-27.700	± 2.330			

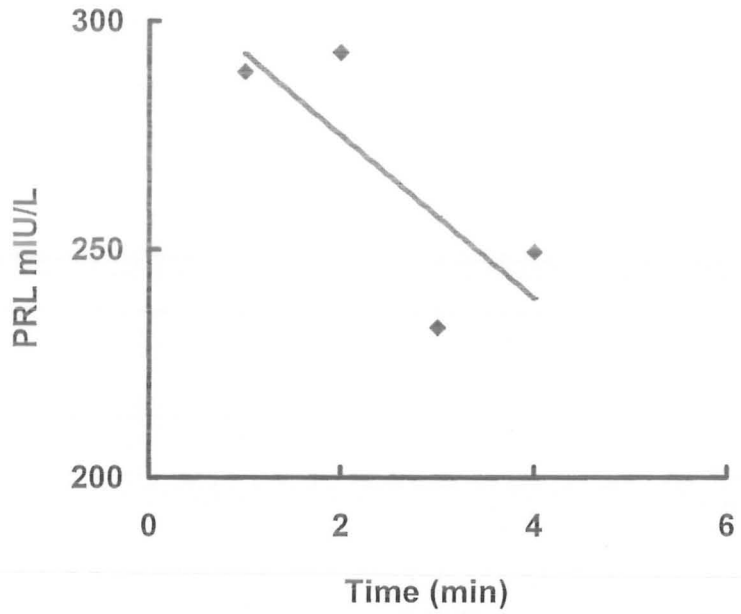

Fig. 10.3.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after second NMA injection during bolus and infusion of Naloxone.

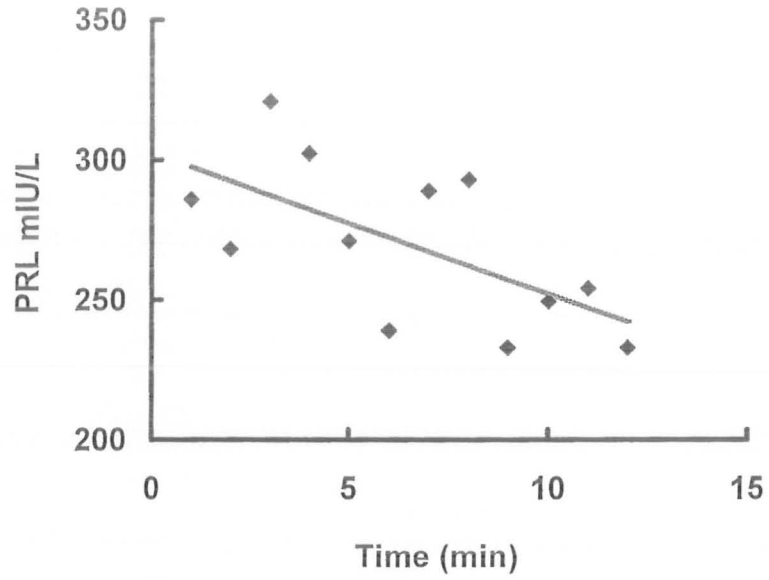

Fig. 10.4.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after two NMA injections during bolus and infusion of Naloxone.

A bolus injection of NAL (5mg/3ml) and infusion (dose: 10 mg/6ml for all body weight, rate = 3 ml/hr) was simultaneously given at 0 minutes and samples were collected after every 15 minutes interval. There was no appreciable change in plasma PRL concentration 30 minutes after this treatment. At 30 minutes stage an injection of NMA (15 mg/kg BW) was given and with 15 minutes interval mean plasma PRL concentration was recorded. From the time of injection (30 minutes stage) to one hour after injection (90 minutes stage) rise and fall in plasma PRL concentration was observed but overall there was a significant ($p<0.05$) decrease in plasma PRL level (Table 10.5, Fig 10.5). Regression analysis of variance showed a non-significant negative trend in mean plasma PRL concentration after the NMA injection ($b = -32.710$, $F_{(1,2)} = 2.626$, $P = 0.246$, Table 10.2, Fig 10.2).

Another injection of NMA (15 mg/kg BW) was given at 90 minutes stage. Again, plasma PRL concentration was recorded after every 15 minutes. An increased concentration of plasma PRL was noted at 105 minutes stages (242.93 ± 103.64 mIU/L). After this a highly significant ($p<0.001$) decrease in plasma PRL concentration was observed until infusion was switched off at 180 minutes time (186.47 ± 90.12 mIU/L). Regression analysis of variance showed a non-significant negative trend in mean plasma PRL levels ($b = -21.673$, $F_{(1,2)} = 6.904$, $P = 0.1$, Table 10.3 Fig 10.3).

The two NMA injections elevated plasma PRL level significantly ($p<0.05$) when compared with the pre-treatment level. However, NAL suppressed the PRL response to NMA. Furthermore, attenuation of NMA-induced PRL secretion during NAL infusion was greater after second NMA injection ($p<0.001$) (Table 10.5 Fig 10.5). Concentration of plasma PRL after both injections of NMA (Table 8) and plasma PRL concentration with two NMA injections during NAL infusion (Table 10) were compared applying two-way analysis of variance. The results showed that there was highly significant decrease in plasma PRL level when NMA was given during the NAL infusion (Table 10.6). When two-way analysis of variance was applied to analyze the difference in plasma PRL concentration after first NMA injection (Table 8) and first NMA injection during NAL infusion, results showed that there was a significant ($p<0.000006$) decrease in the plasma PRL level after NMA injection during NAL infusion (Table 10.6). When second NMA injection (Table8) was compared with the second NMA injection during NAL infusion (Table 10) with two-

TABLE 10.5

Mean plasma PRL concentration (mIU/L) before and after different treatments

Treatments	*Before Treatment*		*After Treatment*	
	Mean	*S.E.M*	*Mean*	*S.E.M*
Saline	195.89 ± 6.75		207.91 ± 2.76	
1st NMA Injection	221.62 ± 4.26		*346.98 ± 40.01	
2nd NMA Injection	221.62 ± 4.26		*329.01± 32.40	
Nalaxone	176.36 ± 1.50		**83.01 ± 2.50	
1st NMA Injection + NAL	336.28 ± 5.25		*259.34 ± 25.34	
2nd NMA Injection + NAL	336.28 ± 5.25		**205.39 ± 10.87	

*p<0.05
**p<0.001

TABLE 10.6

Analysis of variance showing the effect of different treatments on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

Treatments	*F- value*	*P-value*
Pre and Post First NMA	7.416	0.00001
Pre and Post Second NMA	6.023	0.00009
1st & 2nd NMA injections	6.040	0.0002
Pre and Post Nalaxone	18.214	6.586E-11
1st NMA Vs 1st NMA + NAL	9.351	6.114E-06
2nd NMA Vs 2nd NMA + NAL	6.220	0.0001
1st NMA + NAL Vs 2nd NMA + NAL	3.540	0.007

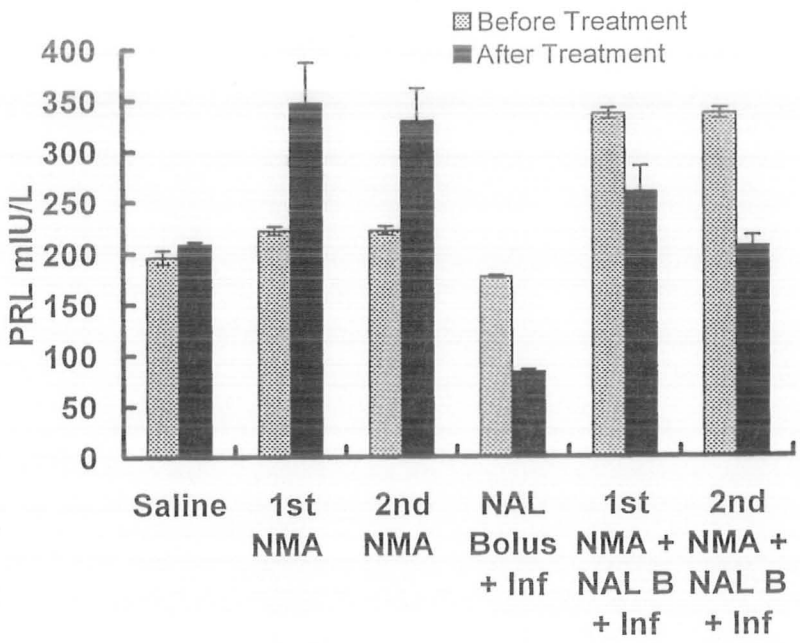

Fig. 10.5.

Mean plasma PRL concentration (mIU/L) before and after different treatments.

way analysis of variance results showed that there is a highly significant decrease ($p<0.0001$) in plasma PRL concentration because of NAL infusion (Table 10.6). When the two NMA injections during NAL infusion were compared there was a significant ($p<0.007$) difference observed between the effects of the two injections.

DISCUSSION

DISCUSSION

The present study was designed to investigate the interaction of excitatory amino acids neurotransmitters with the opioids for the regulation of PRL secretion in non-human primates. Adult male Rhesus monkeys (*Maccaca mulatta*) were used for this purpose. Two NMA injections were administered with an interval of one hour (at 0 and 60 min) in all the animals. First NMA injection administered at 0 minute time caused a significant ($p<0.05$) increase in the basal plasma PRL levels after 15 minutes of its administration. The plasma PRL level remained high till 30 minutes after the injection after which the levels started decreasing. Regression analysis of variance showed that there was a significant negative trend showing that the levels decreased significantly after one hour but remained significantly ($p<0.01$) higher than the pre-treatment level. In order to check the releasable pool of the pituitary lactotrops, the second injection of NMA was given after one hour of the first NMA injection to check the releasable pool of PRL from pituitary lactotropes. The second NMA injection also caused significant ($p<0.05$) rise in circulating plasma PRL level 15 min of its administration and remained high till 30 minutes, then started decreasing gradually. After one hour of the NMA injection the plasma PRL level was comparable to pre-treatment level, as there was a non-significant difference observed in both the levels. Regression analysis of variance applied here showed a significant negative trend with significant reduction in the levels after one hour of the second NMA injection. Evidence that both NMDA and non-NMDA receptors play a physiologically important role in the regulation of PRL secretion (Brann and Mahesh, 1991; Brann *et al.*, 1993; Parker and Crowley, 1993; Wagner *et al.*, 1993). Brann and Mahesh (1991) observed that administration of the NMDA antagonist MK-801 blocks the proestrous PRL surge in the female rat and that treatment with the non-NMDA antagonist DNQX significantly attenuates the preovulatory PRL surge in the pregnant mare serum gonadotropin (PMSG)-primed immature rat (Brann *et al.*, 1993). Suckling- induced PRL release in the lactating rat has been reported to be blocked by the administration of CNQX, a non-NMDA antagonist, but not by administration of NMDA antagonists (Parker and Crowley, 1993).

To elucidate the role of endogenous opiates (EOP) in the regulation of PRL secretion in male monkeys in the present study, an opiate antagonist Naloxone (NAL)

was infused which highly significantly ($p<0.001$) suppressed the basal plasma PRL levels. These observations are in accordance with the previous investigations that the pre-treatment of opiate antagonist NAL, blocks the increase in serum PRL (Tolis *et al.*, 1975; Kleber and Gold, 1978) which normally seen soon after its administration. Administration of opiate antagonists such as naloxone or naltraxone, prevent PRL release in response to stress or suckling and reduce basal PRL secretion (Bruni *et al.*, 1977; Van Vugt *et al.*, 1978). Systemic or intraventricular injection of opioid peptides like enkaphaline, endorphins, and morphine cause a rapid increase in PRL secretion (Van Vugt and Meites, 1980). Morphine and methadone treatment in man (Tolis *et al.*, 1975; Kleber and Gold, 1978) as well as the stimulation of endogenous opiate receptor sites in rodents (Lien *et al.*, 1976; Cusan *et al.*, 1977; Ferland *et al.*, 1977; Cocchi *et al.*, 1977; Rivier *et al.*, 1978) cause rapid increase in serum PRL level. Barb *et al.*, 1991; 1992) also demonstrated that the involvement of EOP in PRL release in pigs.

The endogenous opioid peptides (EOP) do not act directly on the pituitary gland. They may inhibit the activity of the TIDA system (Van Vugt et. al., 1978). A number of independent lines of scientific investigations support an opiate or endorphin modulation of DA activity similar to DA receptor-blocking antipsychotic medications that block DA receptors in the brain (Synder *et al.*, 1974) and stimulate PRL secretion (Clemens *et al.*, 1974; Meltzer *et al.*, 1977). Methadone and other opiate agonists which stimulate PRL secretion (Tolis *et al.*, 1975; Kleber and Gold, 1978) are potent inhibitors of DA-sensitive adenylate cyclase, produce a dose related increase in central DA metabolites and produce catalepsy (DiChiara *et al.*, 1972) which is reversed by low doses of the central DA receptor-stimulating agent apomorphine (Gessa and Taliamonte, 1975; Minneman and Iversen, 1977).

These neurochemical data support the interpretation that the opiate agonists may interfere with the functional action of DA systems to increase serum PRL. NAL would augment DA activity in the hypothalamus by blocking endorphin-mediated inhibition of DA activity. Ferland *et al.*, (1977) demonstrated that the increase in serum PRL induced by the endorphin enkephalin was accompanied by a decrease in DA release or turnover in the median eminance incontarary to this Lien *et al.* (1976) demonstrating a direct pituitary effect Behavioral (Lal, 1975) and neurochemical data support the similar net effect of opiates and DA-blocking drugs on serum PRL and DA turnover (Sasame *et al.*, 1972; Gessa and Taliamonte, 1975; Minneman and

Iversen, 1977; Ageri *et al.*, 1977; Kleber and Gold, 1978). In vitro studies indicate (Rivier *et al.*, 1977; Shaar *et al.*, 1977) that endorphin-mediated increase in serum PRL is not due to its direct effect on anterior pituitary cells.

The present study was designed to investigate the interaction of EOP system with EAA in the regulation of PRL secretion in non-human male primates. For this purpose two NMA injections were administered during NAL bolus and infusion. First NMA injection was administered 30 minutes after the start of NAL bolus and infusion. Pretreatment of NAL suppressed NMA induced plasma PRL secretions in all the five the monkeys and a non-significant increase was observed after the first NMA injection. While the Second NMA injection was administered at 90 minutes after the start of infusion (one hour later the first NMA injection), which also failed to produce any change in circulating PRL level in all the animals. NAL infusion suppressed the PRL response to NMA. It is possible that NMA may stimulate PRL secretion via EOP inhibition of dopaminergic neuronal activity. A similar role for EOP in modulating PRL secretion has been previously reported for the lactating sow (Barb *et al.*, 1991) and gilts (Chang *et al.*, 1993).

Systemic or intraventricular injection of opioid peptides like enkaphaline, endorphins, and morphine cause a rapid increase in PRL secretion (Van Vugt and Meites, 1980). Administration of opiate antagonists such as naloxone or naltraxone, prevent PRL release in response to stress or suckling and reduce basal PRL secretion (Bruni *et al.*, 1977; Van Vugt *et al.*, 1978). It was also reported that EOP inhibit the activity of the TIDA system (Van Vugt et. al., 1978) but do not act directly on the pituitary gland. Similarly neurochemical data support the interpretation that the opiate agonists may interfere with the functional action of DA systems to increase serum PRL. NAL would augment DA activity in the hypothalamus by blocking endorphin-mediated inhibition of DA activity. Ferland *et al.*, (1977) demonstrated that the increase in serum PRL induced by the endorphin enkephalin was accompanied by a decrease in DA release or turnover in the median eminance incontarary to this Lien *et al.* (1976) demonstrating a direct pituitary effect Behavioral (Lal, H., 1975) and neurochemical data support similar net effect of opiates and DA-blocking drugs on serum PRL and DA turnover (Sasame *et al.*, 1972; Gessa and Taliamonte, 1975; Minneman and Iversen, 1977; Ageri *et al.*, 1977; Kleber and Gold, 1978). In vitro studies reported (Rivier *et al.*, 1977; Shaar *et al.*, 1977) that endorphin-mediated increase in serum PRL is not due to its direct effect on anterior pituitary cells.

Arbogast and Voogt (1998) have reported that an endogenous opioid peptide decreases TIDA neuronal activity during lactation and thus contributes to the elevated PRL levels essential for normal lactation. Their data indicate that many aspects of the TIDA neurons are attenuated by the opioidergic inputs including tyrosine hydroxylase (TH) gene expression at the molecular level. Infusion of NAL caused a marked increase in TH activity in the stalk median eminence (SME) and TH mRNA in the arcuate nucleus. This augmented TIDA neuronal activity was associated with suppression of both the high PRL levels associated with a constant suckling stimulus and the acute suckling-induced PRL rise after pup separation. The NAL-induced suppression of PRL secretion had physiological consequences, in terms of reduced pup weight gain during suckling (Arbogast and Voogt, 1998). Horvath *et al.* (1992) have described the contacts between beta-endorphin axon terminals and TIDA neurons in the arcuate nucleus although they make up only a small proportion of opioid synapses on TIDA neurons (Fitzsimmons *et al.*, 1992). More recently Andrews and Grattan (2002) have reported that continuous infusion of the NAL during the night preceding parturition completely abolished the *antepartum* PRL surge and significantly increased TIDA neuronal activity, indicating the role of EOP in facilitating PRL secretion at the end of pregnancy by suppressing TIDA neuronal activity. These results are in agreement with the previous observations that NMA elicits PRL secretion in adult rats, (Olney and Price, 1980; Arslan *et al.*, 1988) monkeys, (Wilson and Knobil, 1982; Arslan et al, 1991) and pigs (Barb *et al.*, 1992). NMA has also been demonstrated to stimulate PRL secretion in rodents, primates (Olney and Price, 1980; Wilson and Knobil, 1982; Wilson and Knobil, 1983; Gay and Plant, 1987), intact and castrated male rats (Arslan *et al.*, 1992, Strobl *et al.*, 1993) as well as cycling female rats (Pohl *et al.*, 1989; Abbud and Smith, 1991; Luderer *et al.*, 1993). NMDA induce c-Fos immunoreactivity in two hypothalamic regions known to regulate PRL secretion: the paraventricular nuclei (PVN) which is the site of TRH cell bodies and the arcuate nuclei (ARC) which is the site of dopamine cell bodies (Abbud and Smith, 1991; Lee *et al.*, 1993). Hence NMDA could act to regulate PRL via regulation of these PRL releasing/or-inhibiting factors, such as VIP and oxytocin (from the SCN and ARC respectively). EAAs are more likely to control PRL release by regulating dopamine neurons in the ARC. Wagner *et al.* (1993) demonstrated that NMDA receptors are involved in the regulation of dopamine release from the hypothalamus and that DA released from TIDA nerve terminals in the median

eminence travels through the hypophyseal long portal vessels to the anterior pituitary where activation of D_2 receptors on lactotrophs cause inhibition of PRL secretion from the anterior pituitary gland (Freeman *et al.*, 2000).

It is possible that NMA may stimulate PRL secretion via EOP inhibition of dopaminergic neuronal activity. Results of the present study support this hypothesis since in the present study pretreatment of NAL suppressed plasma PRL response to NMA and a very slight increase (non-significant) is observed after both the NMA injections. Similar results were found by Chang *et al.* (1993), who observed that pretreatment of NAL blunted PRL response to NMA in gilts. A similar role for EOP in modulating PRL secretion has also been reported for the lactating sow (Barb *et al.*, 1991).

Taken together as a whole the present data indicate that there is an interaction between excitatory amino acids and EOP in modulating PRL secretion from pituitary lactotropes and this interaction could be through dopaminergic neurons in non-human male primates.

STUDY 3

INTERACTION OF EXCITATORY AMINO ACID NEUROTRANSMITTERS WITH ADRENERGIC PATHWAY FOR THE REGULATION OF PROLACTIN

ABSTRACT

The present study was designed to investigate the interaction of N-methyl-D-Aspartic acid (NMA) with adrenergic pathway for the regulation of PRL in non-human primates. Four adult male rhesus monkeys (macaca mulatta) were used for this purpose, which were maintained under the standard colony conditions. Experiments were performed after acclimatizing the animals for chair restraining for a period of 4 weeks. Two teflon cannulae were inserted to the sephanous veins under the ketamine hydrochloride (5mg/kg) anaesthsia. Blood samples were collected for a period of 4 hrs with an interval of 15 minutes and plasma was separated after centrifugation and stored at –15°C until assayed through a special assay system.

Four sets of experiments were performed. In the control experiment all the animals were treated with an infusion of saline (5ml/Kg) for a period of 3 hrs. Infusion of saline caused no significant change in the plasma PRL level. In the second set of experiment two NMA injections were administered with an interval of 1 hr. Both the injection caused a highly significant ($p<0.01$, $p<0.05$ respectively) increase in plasma PRL concentration. Regression analysis of variance showed a highly significant ($p<0.001$) decline in plasma PRL level. In the third set of experiment all the four animals were given infusion along with bolus of phentolamine (an α_2-adrenergic receptor blocker) for a period of 3 hrs. Bolus injection caused a highly significant ($p<0.001$) increase after 15 min of its administration and infusion has maintained this rise in circulating PRL level for 75 min. Then the levels started decreasing showing a non-significant negative trend. In the last set of experiment two NMA injections were administered with an interval of 1 hr during the bolus and infusion of Ph.a. The bolus injection of Ph.a caused a significant ($p<0.001$) increase in plasma PRL levels. First NMA injection significantly ($p<0.05$) elevated plasma PRL

level while the second NMA injection was failed to produce any increase in the circulating PRL level during the infusion of adrenergic receptor blocker. The PRL levels reduced significantly ($p<0.01$) until the end of the infusion at 180 minutes.

These results showed that adrenergic receptors play an important role in excitatory amino acid mediated PRL regulation in non-human primates.

INTRODUCTION

INTRODUCTION

Prolactin secretion is tonically inhibited by the hypothalamus and its secretion is increased when the pituitary is transplanted or when the median eminence of the hypothalamus is destroyed (Everett, 1954; McCann and Friedman, 1960). Attenuation of basal PRL occurs primarily through the inhibitory actions of the TIDA and THDA neurons whose cell bodies lie within the periventicular and arcuate nuclei of the hypothalamus (Moore and Demarest, 1982; Ben-Jonathan *et al.*, 1989). Because variations in DA activity cannot fully account for surges in circulating levels of PRL, such as those produced by estrogen, stress, or lactation, it has been hypothesized that a prolactin–releasing factor (PRF) or factors may also be contributing to the regulation of circulating levels of PRL (Boyd *et al.*, 1976; Shin, 1979; Shin, 1980). Research supports the existence of multiple PRFs, each of which may become active during different physiological states.

One neurotransmitter that may modulate the cellular activity of putative PRFs within the paraventricular nucleus of hypothalamus (PVN) is norepinephrine (NE) as both magnocellular and parvocellular divisions of the PVN receive dense afferent projections from noradrenergic cells (A1 and A2) located in the ventrolateral medulla and nucleus of the solitary tract (Swanson and Morgenson, 1981; Dotti and Teleisnik, 1982; Swanson *et al.*, 1986). Variations in noradrenergic activity with in the PVN have been shown to occur in concert with fluctuations in circulating levels of PRL. For example in the Siberian hamster, photoperiodic-driven differences in PRL may be due to seasonal fluctuations in noradrenergic activity within the PVN, as hamsters exposed to a short-day photoperiod demonstrated significantly higher levels of noradrenergic activity within the PVN, and lower basal levels of PRL, when compared to their long-day counterparts (Dodge and Badura, 2001). According to Dodge and Badura (2002), it could be the α_2-receptor within PVN, which mediates NE's influence on PRF cellular activity.

The α_2-receptor has been traditionally labeled as the adrenergic autoreceptor. However it has also been shown to regulate the activity of noradrenergic cells (Raiteri *et al.*, 1983). Activation of the α_2-receptor subtype presumably inhibits the cellular activity of its target cells by impairing adenyl cyclase activity (Lopez-Sanudo and Arilla, 1994; Kurose and Lefkowitz, 1994; Aantaa *et al.*, 1995). Radioligand binding

studies completed in rats have demonstrated α_2-receptor expression within the PVN (Leibowitz *et al.*, 1982) and intraventricular administration of α_2-adrenergic drugs have been shown to induce significant changes in circulating levels of PRL (Lawson and Gala, 1975; Subramanian and Gala, 1976; Gold *et al.*, 1979; Meltzer *et al.*, 1982; Lein *et al.*, 1986).

Despite a vast amount of research that supports a role for the α_2-receptor in modulating circulating levels of PRL, its relative role remains unclear. Paradoxically, α_2-antagonists have been shown to both augment and diminish circulating levels of PRL. The effects of these α_2-antagonists are apparently dependent upon the physiological condition present at the time of elevated PRL levels during basal conditions (Subramanian and Gala, 1976; Lawson and Gala, 1975), but attenuate them during surge conditions (Gold *et al.*, 1979; Meltzer *et al.*, 1982; Lein *et al.*, 1986). The mechanisms responsible for the divergent influence of α_2-antagonists on circulating levels of PRL are unknown. It is possible that the effects of α_2-antagonists on circulating levels of PRL are dependent upon their central site of action (i.e., if they are acting on stimulatory or inhibitory component of the PRL regulatory system).

Antagonism of α_2-receptor-mediated inhibition of PRF cellular activity (i.e., promotion of PRF activity) would theoretically induce an elevation in circulating levels of PRL, whereas antagonism of α_2-receptor-mediated inhibition of dopamine cellular activity (promotion of dopamine activity) would initiate a decrease in circulating levels of PRL. Alternatively, the α_2-receptor may be only influencing one component of the PRL regulatory system, but the relative role of the α_2-receptor may fluctuate from one physiological condition to the next. The former hypothesis is supported by radioligand binding experiments completed in the rat that have demonstrated α_2-receptor expression within central components of the PRL regulatory system outside the PVN (e.g., the periventricular nucleus, arcuate nucleus, median eminence and anterior pituitary) (Leibowitz *et al.*, 1982). In addition deafferentation of noradrenergic input to the medial basal hypothalamus (arcuate) may impair α_2-receptor-mediated inhibition of dopamine cellular activity (i.e., promote dopamine activity) to consequently, attenuate circulating levels of PRL (Blake *et al.*, 1972; Weiner *et al.*, 1972). Variations in noradrenergic activity within the arcuate have been correlated with elevations in circulating levels of PRL. For example, whole tissue content studies completed in rats and guinea pigs have demonstrated that NE turnover

in the arcuate significantly increases on the afternoon of proestrus (Honma and Wuttke, 1980; Wise *et al.*, 1981). In addition, microinjection of NE into the medial basal hypothalamus of male baboons has been shown to initiate a significant elevation in serum levels of PRL (Steiner *et al.*, 1978).

NE simulation of PRL release is different from the inhibitory effects of norepinephrine at the pituitary gland level. In the pituitary, norepinephrine binds to dopamine receptors on the mammotrophs and blocks PRL release. In contrast, *in vivo* administration of L-dopa, which increases brain norepinephrine content, results in increased PRL secretion. (Donoso *et al.*, 1971). Administration of a α_2-adrenergic agonist clonidine at high doses results in an increased PRL secretion (Lawson and Gala, 1975) as do iv injections of norepinephrine (Vijayan and McCann, 1978). Administration of disulfran (an inhibitor of norepinephrine synthesis and 6-hydroxydopamine) causes selective destruction of noradrenergic neurons and results in reduced PRL secretion (Donoso *et al.*, 1973; Fenske and Wuttke, 1976).

These results suggest that noradrenergic neurons be also involved in the PRL regulation, although the role of these neurons is not resolved. The demonstration of α-1 and α-2 receptors in the brain makes interpretation of the drug studies and the role of noradrenergic neurons in the control of PRL secretion difficult to resolve (Clemens and Shaar, 1980). Together these studies suggest that the α_2-receptors may have a role in modulating dopamine activity within the arcuate, and subsequently, circulating levels of PRL.

The role and function of excitatory amino acids (EAAs) in the CNS have been an area of intense research over the past years. It is now generally accepted that EAA receptors are the main transmitter receptors mediating synaptic excitation in the CNS (Brann and Mahesh, 1993; Brann and Mahesh, 1993; Cotman et. al., 1989; Cotman and Iverson, 1987; Fonnum, 1984). Regulation of PRL secretion by both NMDA and non-NMDA receptors is evidenced from a number of studies utilizing specific antagonist (Brann and Mahesh, 1991; Brann *et al.*, 1993; Parker and Crowley, 1993, Wagner *et al.*, 1993). For instance, Brann and Mahesh (1991) have shown that administration of the NMDA antagonist MK-801 blocks the proestrous PRL surge in the female rat. Likewise, Brann and colleagues (Brann *et al.*, 1993) have shown that treatment with the non-NMDA antagonist DNQX significantly attenuates the preovulatory PRL surge in the PMSG-primed immature rat.

The purpose of the present study was to elucidate the involvement of excitatory amino acids for the regulation of PRL through adrenergic pathway. The adult male monkeys were used in this study. Limited data are available regarding the role of EAA for the regulation of PRL through adrenergic pathways. Therefore the present investigation was undertaken to study some aspects of prolactin regulation under different physiological states.

MATERIALS AND METHODS

MATERIALS AND METHODS

ANIMALS

Same as in study 1.

PHARAMACOLOGIC AGENTS

The following drugs were used in the present study:

1. **Ketamine hydrochloride** (ketavat; park Davis, Berlin, FRG).
2. **N-methyl-D,L-aspartic acid**: (NMA Sigma Chemical Co. (St. Louis, Mo, 63178, USA).
3. **Phentolamine**: Sigma Chemical Co. (St. Louis, Mo, 63178, USA
4. **Normal Saline (0.9 % NaCl):** Plasaline, Otsuka Pakistan Ltd. F/4-9. H.I.T.E., Hub, Balochistan, Pakistan.

CHAIR RESTRAINING

Same as in study 1.

CATHETERIZATION

Same as in study 1.

BLEEDINGS

Same as in study 1.

EXPERIMENTAL PROTOCOL

A treatment with α-adrenergic antagonist was carried out after an interval of 1-2 weeks:

a) Vehicle administration:

The animals were bled for a period of 4hours at an interval of 15 minutes. All the animals were injected 5 ml of vehicle (0.9% NaCl) at one hour of the blood sampling.

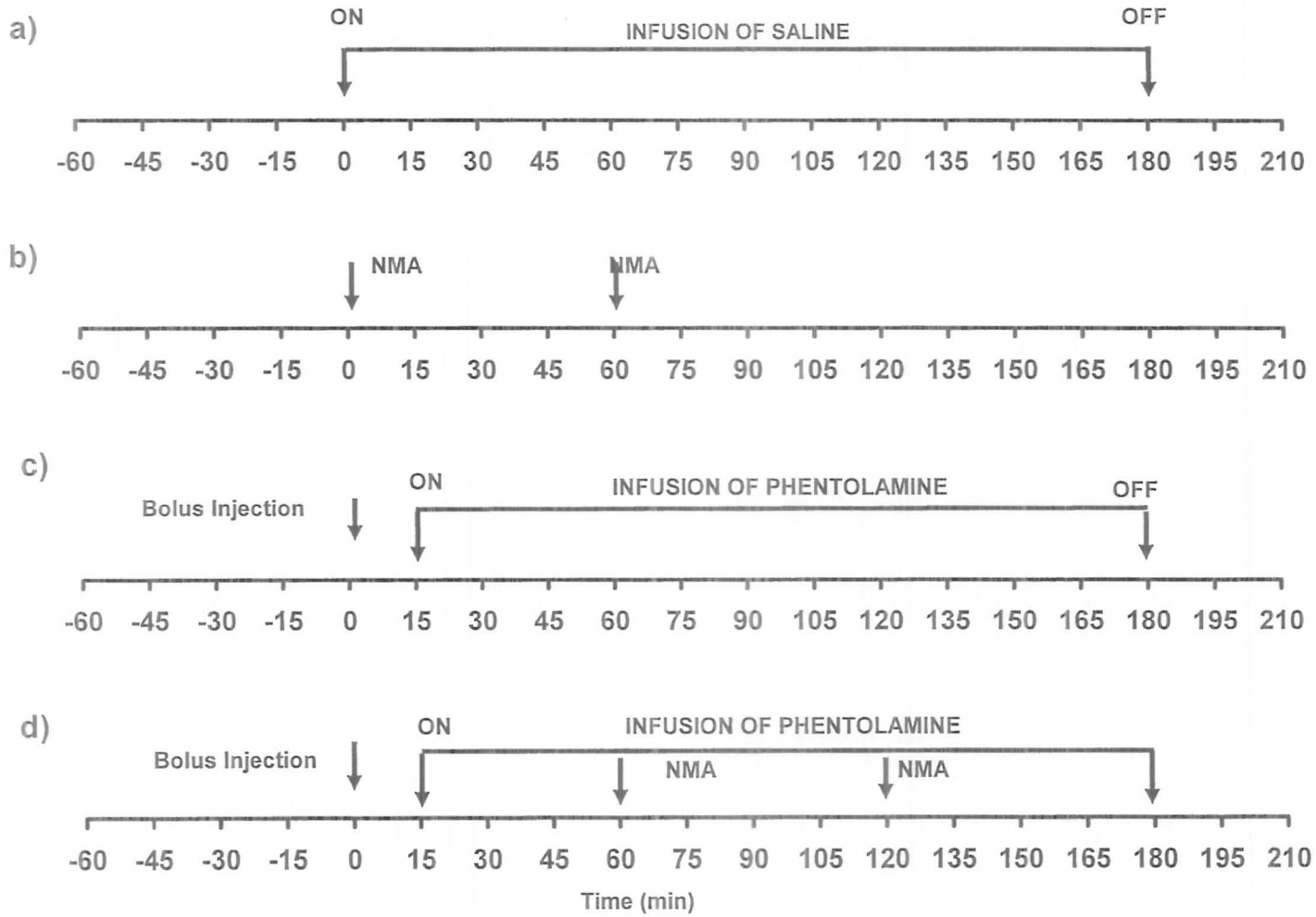

Fig. 11 Experimental Protocol showing the administration of a) Saline b) NMA c) Phentolamine d) NMA + Phentolamine to adult male monkeys.

b) NMA

The animals were bled as above and two injections of NMA (15mg/kg BW) were given at 1 and 2 hr of sampling. NMA was dissolved in normal saline immediately before use.

c) Phentolamine

The animals were bled as above and were administered phentolamine (5mg/5ml bolus injection and 1mg/kg/hr infusion), an alpha-adrenergic antagonist at 1 hr of sampling. Infusion (rate: 4ml/hr) stared along with or immediately after the bolus injection.

d) Phentolamine + NMA

Animals were bled as above, however, in addition to the bolus and infusion of phentolamine (5mg/5ml bolus injection and 1mg/kg/hr infusion), two injections of NMA (15 mg/kg BW) at 60 and 120 min of bleeding were also given. Infusion was terminated at 180min.

HORMONE DETERMINATION

As in study 1.

STATISTICAL ANALYSIS

For comparison of baseline PRL secretion before treatment, hormone levels were calculated by averaging all the concentrations before treatment. On the other hand PRL responsiveness to the drugs induced was determined by comparing basal levels of the hormone calculated by averaging the concentrations immediately before the injection at 0 min and the levels worked out by averaging the concentration of hormone 15 min after inducing the drug. Student's t-test was used to determine differences between the means of basal and stimulated levels. The data were also subjected to regression analysis of variance. P values are mentioned for t-test applied. Where analysis of variance is carried out both values for F and P are given.

RESULTS

RESULTS

Body Weight:

Mean body weight of all the four adult male rhesus monkeys (*Macaca mulatta*) are given in the Table no. 11.

Behavioral Reactions

All the animals showed a specific type of behavior after the administration of the drug in the form of bolus and infusion. Almost all the animals showed shallow respiration after the bolus administration. After the start of infusion animals became drowsier and remained in this condition throughout the infusion. Respiration became slow. Animals vomited sometimes after first NMA injection and sometimes after second NMA injection.

Effect of Vehicle (Saline) Infusion on plasma PRL in adult male monkeys

The effect of iv infusion of normal saline on mean plasma PRL concentrations (mIU/L) in four adult male monkeys for a period of three hours is shown in Table 12 and Fig 12. Pre-treatment levels of mean plasma PRL concentration one hour before the infusion was also recorded. Mean plasma PRL concentrations at the start of the sampling (-60 minutes) was 156.00 ± 8.17 (mIU/L). After an hour, with an interval of 15 minutes, before the initiation of infusion the level of plasma PRL reached 179.33 ± 40.25 mIU/L. Mean pre-treatment plasma PRL concentration showed a non-significant increase ($b = 7.166 \pm 2.719$, $F_{(1,3)} = 6.94$, $P = 0.07$, Table 12.1 and Fig 12.1). At the start of infusion (0 minutes) mean plasma PRL concentration was 179.33 ± 40.25 mIU/L. Mean plasma PRL concentration was recorded with an interval of 15 minutes in each monkey and the first record after 15 minutes of infusion showed decrease in mean plasma PRL concentration (171.08 ± 28.69 mIU/L). After the infusion the mean plasma PRL concentration was recorded up to 180 minutes and at this stage the infusion was stopped. Mean plasma PRL concentration was fluctuating with a significant ($p<0.01$) increase (Table 15.5, Fig 15.5). However, regression analysis of variance showed that there was a non-significant change

TABLE 11

Body Weight (kg) of Rhesus monkeys treated with Saline, NMA, Phentolamine and NMA + Phentolamine

Animal nos.	Saline	NMA	Ph.a.	Ph.a. + NMA
9305	11.10	11.10	11.30	11.30
9318	10.40	10.40	11.30	11.30
9319	7.60	7.70	7.40	7.00
9321	9.10	10.40	10.60	10.60
Mean ± S.E.M.	9.55 ± 0.77	9.90 ±0.75	10.15 ± 0.93	10.05 ± 1.03

TABLE 12

Effect of iv infusion of Saline on plasma PRL concentration (mIU/L) in adult male rhesus monkeys

Time (min)	Animal nos. 9318	9319	9305	9321	Mean		S.E.M.
-60	225.0	226.0	190.0	199.0	210.00	±	9.14
-45	219.0	182.0	176.4	187.0	191.10	±	9.55
-30	235.0	205.0	194.1	179.0	203.27	±	11.84
-15	204.0	300.7	158.7	196.0	214.86	±	30.27
0	189.0	358.1	163.6	186.0	224.16	±	45.00
15	169.0	296.0	221.4	154.0	210.10	±	32.08
30	187.0	244.2	219.0	188.2	209.59	±	13.71
45	172.9	239.0	253.7	200.0	216.40	±	18.41
60	192.0	258.0	290.0	184.5	231.13	±	25.65
75	160.0	121.0	339.5	167.6	197.02	±	48.57
90	168.0	161.0	341.2	200.0	217.53	±	42.07
105	168.0	125.1	281.4	205.4	194.98	±	33.15
120	150.0	159.0	268.4	199.2	194.14	±	26.95
135	125.0	191.0	289.0	155.7	190.17	±	35.60
150	150.0	192.0	279.0	144.0	191.25	±	31.14
165	184.0	183.0	251.0	128.9	186.72	±	25.00
180	144.7	216.1	198.0	125.1	170.97	±	21.53

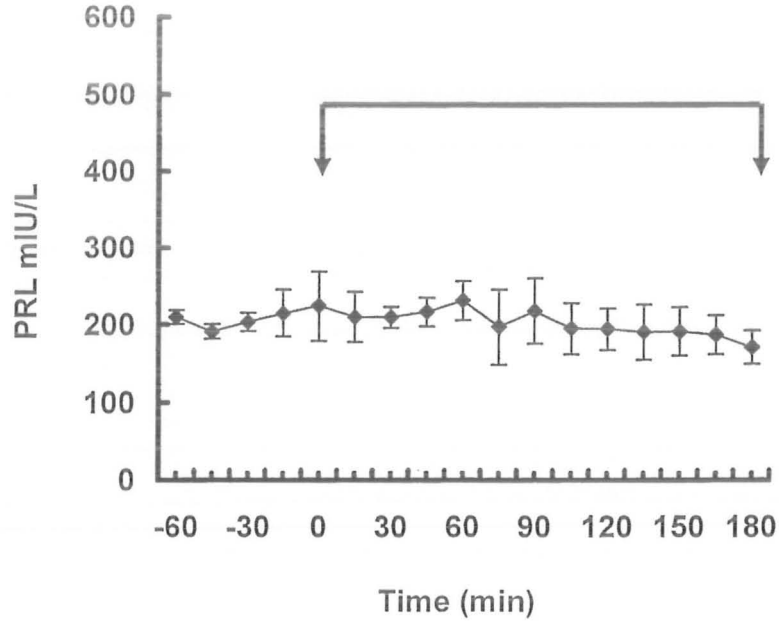

Fig. 12.

Effect of iv infusion (⌐¬)of Saline on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

TABLE 12.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before Saline infusion with an interval of 15 minutes

	Df	SS	MS	F	Significance F
Regression	1	513.544	513.544	6.943	0.0780
Residual	3	221.894	73.965		
Total	4	735.438			
b	7.166	± 2.719			

TABLE 12.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after Saline infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	0.653	0.653	0.010	0.924
Residual	10	685.129	68.513		
Total	11	685.783			
b	0.068	± 0.692			

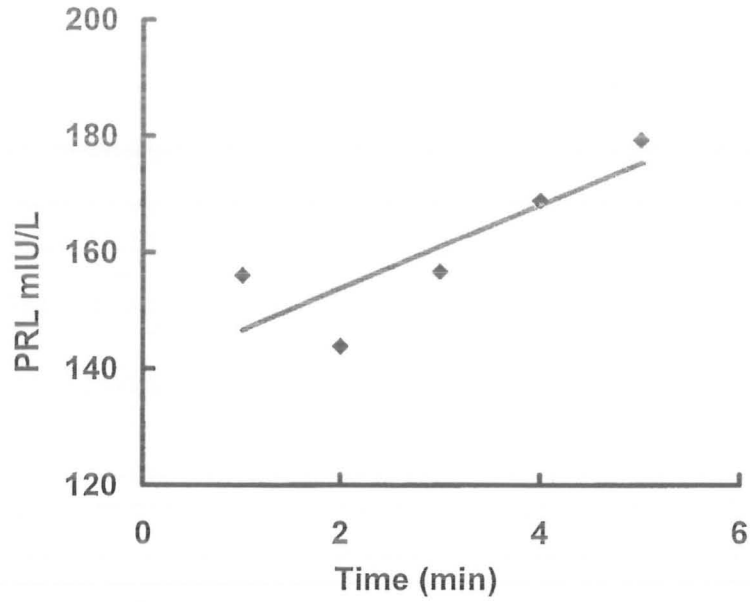

Fig. 12.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before Saline Infusion.

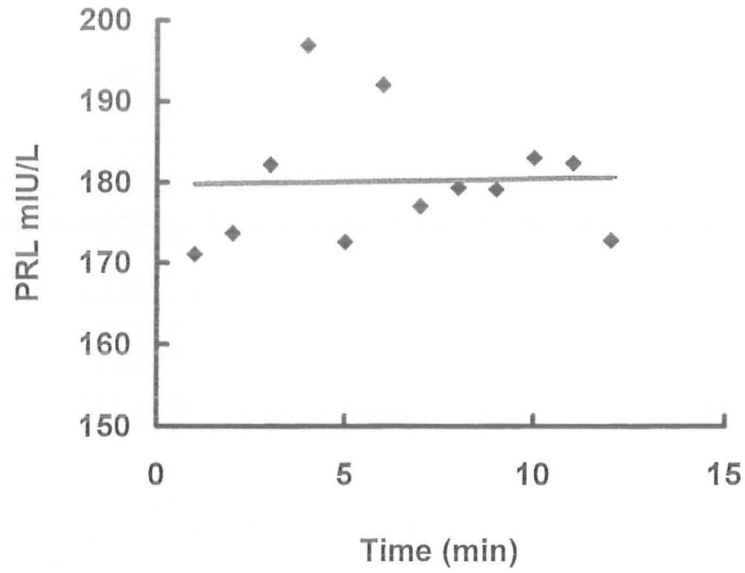

Fig. 12.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after Saline Infusion.

in these levels (b = 0.067 ± 0.692, $F_{(1,10)}$ = 0.0095, P = 0.9, Table 12.2, Fig 12.2) with the advance in time after infusion.

Effect of two NMA injections on PRL:

The effect of two NMA injections on individual and mean plasma PRL levels (mIU/L) administered at 0 and 60 min respectively in four adult male monkeys is shown Table 13 and Fig 13. Initially, when collection of blood samples was started the levels were 197.44 ± 49.34 mIU/L and after an hour the levels of mean plasma PRL concentration were 181.28 ± 59.86 mIU/L (at 0 minutes). Regression analysis of variance showed non-significant negative trend in pre-treatment plasma PRL level (b = -5.215 ± 1.505, $F_{(1,3)}$ = 12.001, P = 0.04 Table 13.1, Fig 13.1). After 15 minutes of administration of NMA (15 mg/kg BW) injection at 0 min a high mean concentration of plasma PRL (364.80 ± 91.04 mIU/L) was observed. NMA caused a significant ($p<0.01$) increase in all the monkeys (Table 15.5, Fig 15.5). Mean plasma PRL concentrations started decreasing and it was 234.91 ± 56.05 mIU/L after 60 minutes of this injection. ANOVA showed a highly significant ($p<0.0002$) increase in plasma PRL level (Table 15.6).

To further confirm the effect of EAA on pituitary lactotropes to release PRL at 60 minutes stage another NMA injection was given. At this stage mean plasma PRL concentration was 234.91 ± 56.05 mIU/L. This second NMA injection also produced an abrupt increase of plasma PRL and after 15 minutes (at 75 minutes of first injection) the levels were 337.2 ± 73.6 mIU/L. Administration of the second NMA injection also caused a significant ($p<0.05$) increase in plasma PRL concentration (Table 15.5, Fig 15.5). After this, levels started decreasing as the time proceeded and at 180 minutes the mean plasma concentration of PRL was 179.36 ± 31.01 mIU/L.

Regression analysis of variance was carried out at different time intervals. Mean plasma PRL concentration regresses non-significantly with time showing a negative trend after first NMA injection at 0 minute time (b = -49.39 ± 13.20, $F_{(1,2)}$ = 14.00, P = 0.0646 Table 13.2, Fig 13.2). Regression analysis was also carried out for 75-180 minutes interval segment showing that mean plasma PRL concentration decreases highly

TABLE 13

Effect of two NMA injections at 0 and 60 min on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

Time (min)	Animal nos. 9318	9319	9305	9321	Mean		S.E.M.
-60	423.0	187.0	194.1	243.1	261.80	±	55.16
-45	440.0	206.1	181.0	205.4	258.12	±	60.90
-30	454.4	196.0	186.0	153.3	247.43	±	69.59
-15	413.0	155.0	179.7	155.7	225.84	±	62.65
0	427.0	164.0	166.8	148.6	226.60	±	66.92
15	728.0	297.0	484.0	300.0	452.26	±	101.79
30	798.0	233.0	478.6	263.0	443.15	±	130.32
45	471.0	163.0	383.6	219.0	309.16	±	71.43
60	406.0	169.0	366.5	173.0	278.63	±	62.67
75	629.0	257.0	414.8	310.0	402.69	±	82.25
90	610.0	276.0	446.4	210.0	385.61	±	89.86
105	356.0	215.0	368.2	173.0	278.06	±	49.35
120	310.0	233.0	332.8	148.0	255.94	±	41.84
135	301.0	199.0	316.1	140.0	239.02	±	42.02
150	287.0	191.0	248.9	125.0	212.97	±	35.34
165	233.6	164.0	213.4	125.4	184.11	±	24.42
180	278.0	167.0	155.5	116.3	179.20	±	34.67

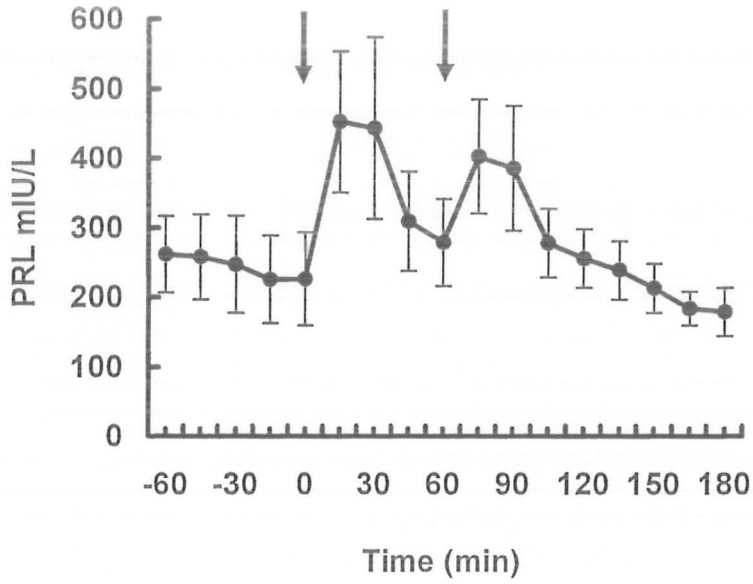

Fig. 13.

Effect of two NMA injections (↓)at 0 and 60 min on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

TABLE 13.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before two NMA injections with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	271.962	271.962	12.0013	0.0405
Residual	3	67.983	22.661		
Total	4	339.945			
b	-5.215	± 1.505			

TABLE 13.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after first NMA injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	12196.27	12196.27	14.0019	0.0646
Residual	2	1742.08	871.041		
Total	3	13938.4			
b	-49.389	± 13.198			

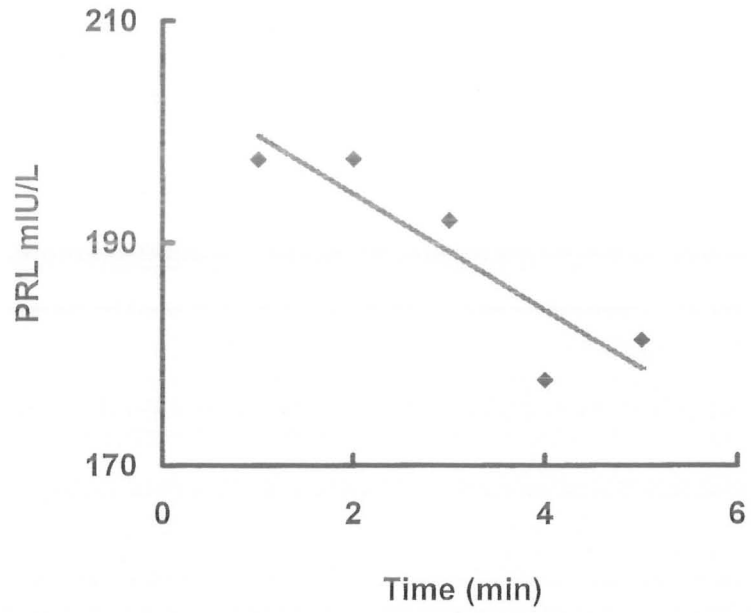

Fig. 13.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before two NMA Injections.

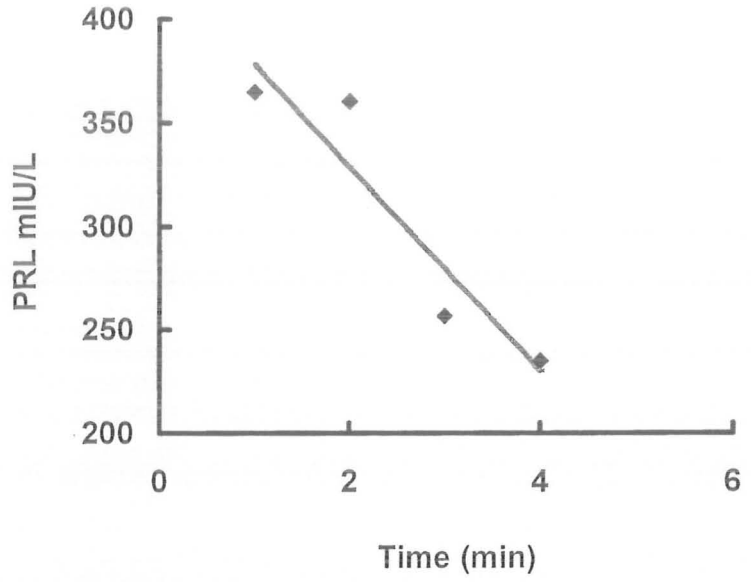

Fig. 13.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after first NMA Injection.

TABLE 13.3

Regression analysis of variance of plasma PRL concentration (mIU/L) after second NMA injection with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	23225.39	23225.39	44.738	0.00054
Residual	6	3114.857	519.143		
Total	7	26340.25			
b	-23.516	± 3.515			

TABLE 13.4

Regression analysis of variance of plasma PRL concentration (mIU/L) after two NMA injections with an interval of 15 minutes.

	df	SS	MS	F	Significance F
Regression	1	271.962	271.962	12.0013	0.0405
Residual	3	67.983	22.661		
Total	4	339.945			
b	-5.215	± 1.505			

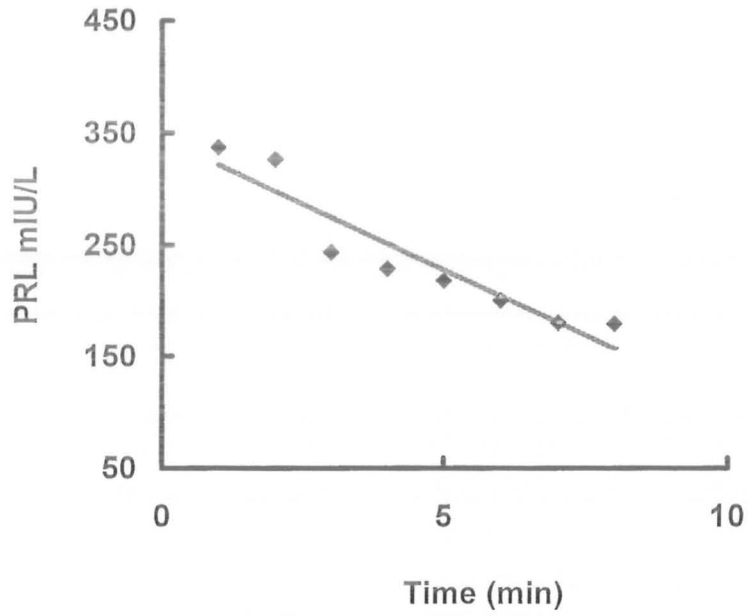

Fig. 13.3.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after second NMA Injection.

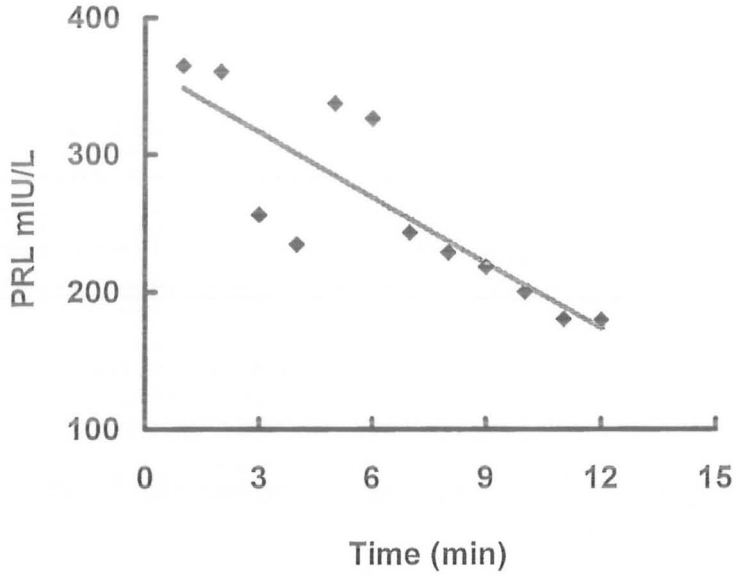

Fig. 13.4.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after two NMA Injections.

significantly when the experiment was allowed to proceed for a longer period of time ($b = -23.51 \pm 3.519$, $F_{(1,6)} = 44.66$, $P = 0.0005$ Table 13.3, Fig 13.3).

Effect of Phentolamine bolus and infusion on PRL:

To study the role of adrenergic pathway for the regulation of PRL, α-adrenergic receptor blocker phentolamine was administered to four adult male monkeys. The effect of iv bolus injection and infusion of Phentolamine on mean plasma PRL concentration (mIU/L) is shown in Table 14 and Fig 14. One hour before treatment concentration of mean plasma PRL was recorded with an interval of 15 minutes. Pre-treatment records showed that mean plasma PRL concentration increased with time. The mean plasma PRL concentration at –60 minutes was 241.15 ± 10.40 mIU/L and after one hour (at 0 minutes) before treatment the levels were 258.88 ± 18.00 mIU/L. This showed an increase in concentration of mean plasma PRL but this increase was not significant ($b = 3.959 \pm 2.124$, $F_{(1,3)} = 3.475$, $P = 0.159$, Table 14.1 and Fig 14.1).

Phentolamine bolus (5 mg/5ml) was administered at 0 minutes and infusion (Dose = 1 mg/kg BW, Rate = 4 ml/hr) was started after 15 minutes of the bolus injection. Phentolamine produced a significant ($p<0.001$) increase in circulating plasma PRL concentration (382.05 ± 9.14 mIU/L) and the levels remained high throughout the infusion. Infusion was stopped at 180 minutes time but the blood samples were collected 45 minutes after switching off the infusion. Plasma PRL levels decreased after switching off the infusion and were comparable to pre-treatment levels. A negative trend in mean plasma PRL concentration was observed until the end of the infusion. Record of mean plasma PRL concentration was made after every 15 minutes of interval. Regression analysis of variance showed a non-significant negative trend in mean plasma PRL concentration as the time advanced ($b = -7.1503 \pm 0.691$; $F_{(1,13)} = 107.05$; $P = 1.209$; Table 14.2 and Fig 14.2).

TABLE 14

Effect of iv bolus and infusion of Phentolamine on plasma PRL concentration (mIU/L) in adult male rhesus monkeys

Time (min)	Animal nos. 9318	9319	9305	9321	Mean		S.E.M.
-60	506.3	156.0	133.3	168.9	241.15	±	10.40
-45	538.0	149.0	166.9	189.0	260.74	±	11.57
-30	559.0	146.0	144.3	185.0	258.57	±	13.29
-15	542.0	135.0	161.4	197.1	258.88	±	18.00
0	535.0	124.0	201.0	187.6	261.88	±	23.74
15	622.3	291.7	320.2	294.0	382.05	±	9.14
30	639.5	213.7	320.8	296.9	367.71	±	32.47
45	615.7	233.0	313.2	310.4	368.09	±	26.29
60	627.3	218.0	293.5	316.9	363.93	±	29.85
75	642.6	223.3	287.1	345.8	374.69	±	35.36
90	639.3	219.5	283.9	335.6	369.57	±	33.58
105	648.8	201.7	288.9	339.2	369.66	±	40.16
120	600.6	192.9	275.6	348.3	354.35	±	44.91
135	585.3	196.6	268.9	326.1	344.23	±	37.48
150	540.3	184.2	263.4	321.0	327.23	±	39.66
165	492.3	186.7	255.6	317.6	313.02	±	37.81
180	485.3	182.8	259.8	311.5	309.85	±	37.37
195	474.5	180.7	247.3	301.9	301.11	±	35.04
210	461.5	174.3	242.5	290.1	292.09	±	33.62
225	426.4	164.5	251.0	285.2	281.76	±	35.91

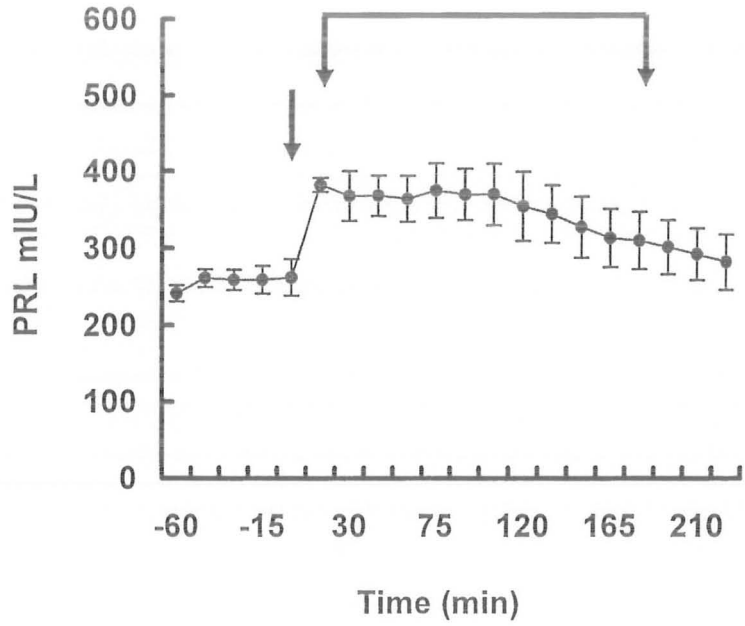

Fig.14.

Effect of iv bolus (↓) and infusion (↓——↓) of Phentolamine on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

TABLE 14.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before Ph.a. bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	156.72	156.72	3.4746	0.15919
Residual	3	135.31	45.103		
Total	4	292.03			
b	3.959	± 2.123			

TABLE 14.2

Regression analysis of variance of plasma PRL concentration (mIU/L) during Ph.a. bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	14316	14316	107.05	0.00000001
Residual	13	1738.4	133.72		
Total	14	16054			
b	-7.15	± 0.691			

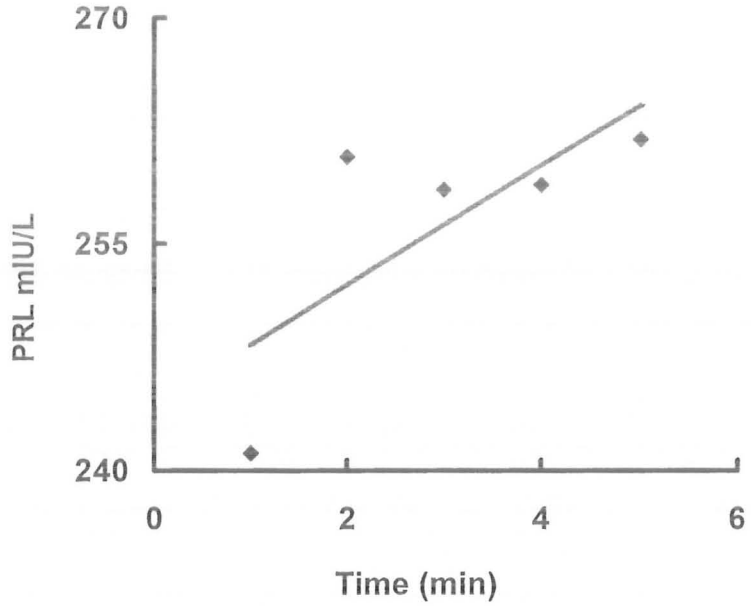

Fig. 14.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before Phentolamine infusion.

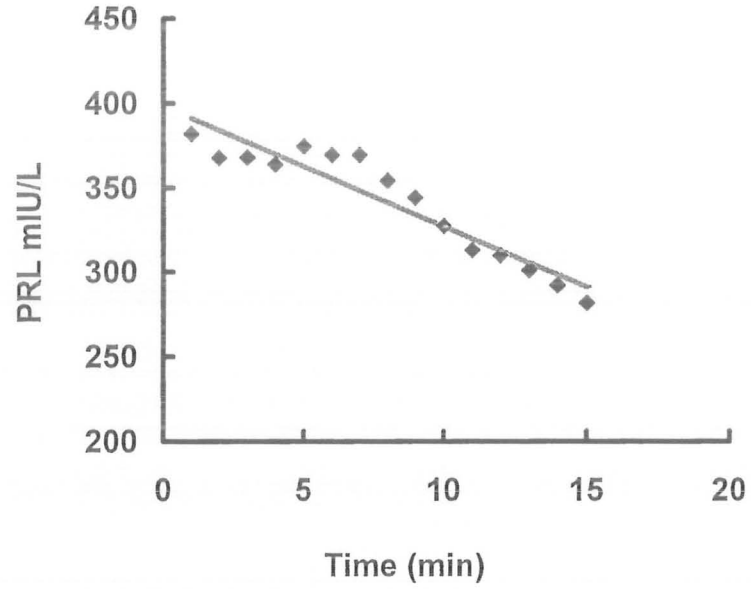

Fig. 14.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after Phentolamine infusion.

Effect of two NMA injections under the shadow of Phentolamine infusion

To study the interaction of EAA with adrenergic pathway for the regulation of PRL secretion, NMA was administered during the infusion of phentolamine. Mean plasma PRL concentration as a result of two NMA injections during the bolus and infusion of phentolamine in four adult male monkeys is given in Table 15 and Fig 15. Mean plasma PRL concentration (mIU/L) at the start of the sampling was 315.47 ± 74.94 mIU/L. Samples were collected after every 15 minutes and observations showed that the pre-treatment level reached 305.53 ± 73.89 mIU/L after one hour. But this decrease in mean plasma PRL concentration was not significant although has a negative trend (b = -3.186 ± 1.299, $F_{(1,3)} = 6.020$. P = 0.09 Table 15.1 Fig 15.1). At 0 minutes bolus injection of phentolamine (5 mg/5ml) was given to all the four monkeys and the level of mean plasma PRL concentration decreased from initial value of 368.27 ± 52.76 mIU/L to 304.35 ± 84.07 mIU/L after 15 minutes. The infusion of Phentolamine was started at the rate of 4 ml/hr after 15 minutes of the bolus injection.

First NMA injection (15 mg/kg BW) was given at 60 minutes (after one hour of bolus and 45 minutes of Phentolamine infusion). When NMA was given the level of mean plasma PRL concentration was 346.40 ± 67.53 mIU/L. NMA caused a significant ($p<0.05$) increase in mean plasma PRL level reaching 487.59 ± 75.75 mIU/L after 15 minutes of the NMA administration. After 60 minutes of NMA injection (at 120 minutes stage) the levels reduced to 340.03 ± 63.67 mIU/L. Regression analysis of variance showed a significant decrease in circulating plasma PRL level (b = -51.87 ± 5.87, $F_{(1,2)} = 77.97$, P = 0.01; Table 15.2 Fig 15.2) since the administration of NMA injection.

Another injection of NMA was given at 120 minutes (after an hour of the first injection). This second NMA injection was failed to release PRL and the level of plasma PRL significantly ($p<0.01$) decreased, (Table 15.5, Fig 15.5). The mean plasma PRL concentration was 291.03 ± 79.58 mIU/L after 15 minutes of NMA administration. After an hour of second NMA injection the mean plasma PRL level declined to 286.61 ± 77.00 mIU/L showing that this NMA injection was not able to release PRL during phentolamine infusion. Regression analysis of variance was carried out on mean plasma

TABLE 15

Effect of two injections of NMA at 60 and 120 min during bolus and infusion of Phentolamine on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

Time (min)	Animal nos. 9318	9319	9305	9321	Mean		S.E.M.
-60	529.2	185.8	299.6	247.3	315.47	±	74.94
-45	534.0	171.3	281.5	275.7	315.64	±	77.07
-30	530.3	164.8	274.0	246.6	303.93	±	78.94
-15	526.0	164.8	285.0	238.8	303.66	±	78.14
0	508.4	153.7	279.3	280.7	305.53	±	73.89
15	594.1	277.9	327.4	443.0	410.60	±	70.27
30	585.9	255.7	346.5	398.0	396.52	±	69.65
45	553.6	228.3	336.3	320.4	359.63	±	68.90
60	528.4	229.7	367.8	259.7	346.40	±	67.53
75	599.9	326.0	632.4	392.0	487.59	±	75.75
90	492.9	276.0	634.0	387.0	447.48	±	76.33
105	495.3	243.0	507.4	239.8	371.38	±	75.08
120	457.2	234.0	443.1	225.8	340.03	±	63.67
135	445.4	215.4	398.9	104.5	291.03	±	79.58
150	422.0	182.0	375.9	147.7	281.92	±	68.59
165	403.5	164.0	357.3	183.5	277.07	±	60.52
180	419.7	142.0	419.8	165.0	286.61	±	77.00
195	438.4	142.0	433.8	118.3	283.12	±	88.45
210	353.2	94.2	396.6	118.3	240.57	±	78.20
225	299.0	98.1	410.5	107.0	228.64	±	76.29

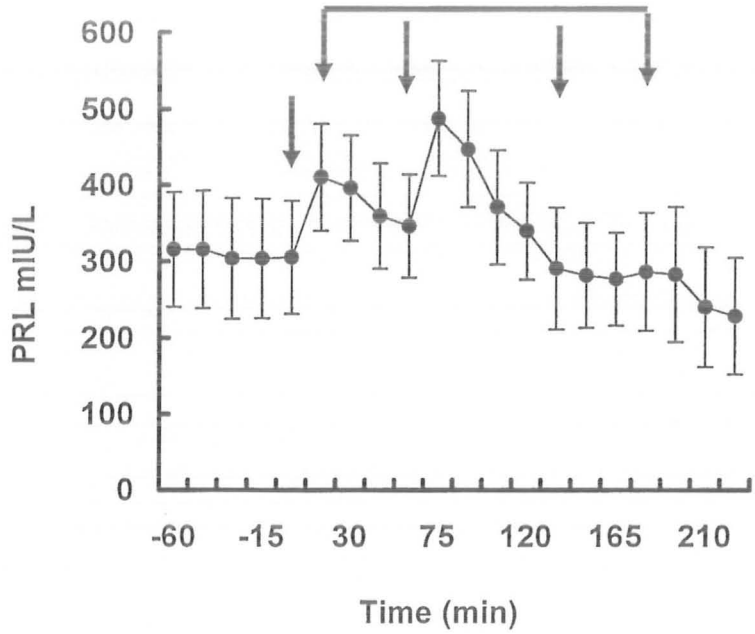

Fig. 15.

Effect of two injections (↓)of NMA at 60 and 120 min during bolus (↓) and infusion (↓——↓) of Phentolamine on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

TABLE 15.1

Regression analysis of variance of plasma PRL concentration (mIU/L) before two NMA injections during Ph.a. bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	101.506	101.506	6.020	0.091
Residual	3	50.588	16.863		
Total	4	152.094			
b	-3.186	± 1.298			

TABLE 15.2

Regression analysis of variance of plasma PRL concentration (mIU/L) after first NMA Injection during Ph.a. bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	13457.024	13457.024	77.979	0.013
Residual	2	345.144	172.572		
Total	3	13802.168			
b	-51.879	± 5.874			

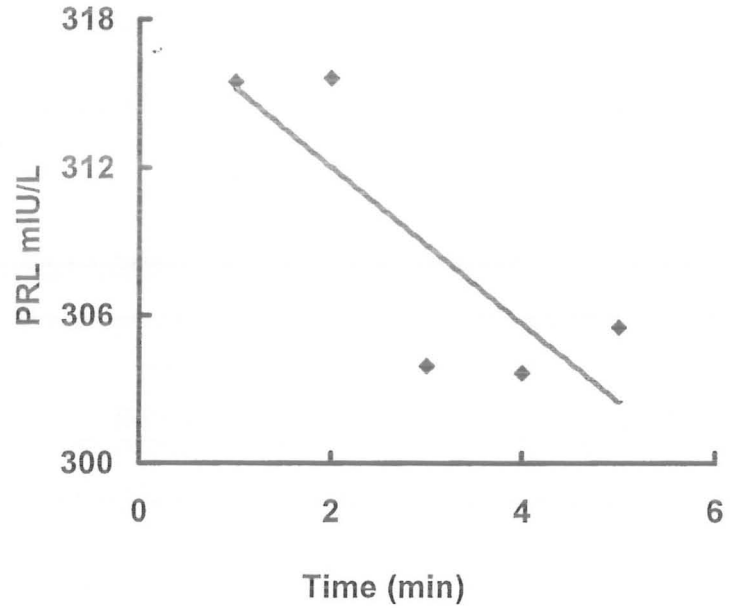

Fig. 15.1.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time before two NMA injections during phentolamine infusion.

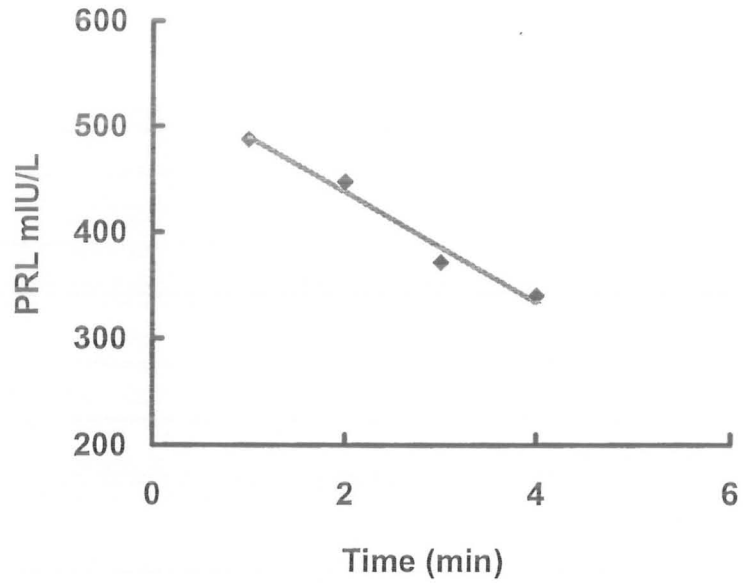

Fig. 15.2.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after first NMA injection during phentolamine infusion.

PRL levels from the start of second NMA injection and up to levels recorded after an hour of administration of NMA injection (135 minutes to 180 minutes). The analysis showed significant decrease in mean plasma PRL concentration ($b = -1.80 \pm 3.03$, $F_{(1,2)} = 0.35$, $P = 0.61$; Table 15.3 Fig 15.3).

Phentolamine infusion was discontinued at 180 minutes and the levels decreased further and reached 228.64 ± 76.29 mIU/L at 225 minutes stage. Regression analysis of variance applied after two NMA injections during phentolamine bolus plus infusion showed a very highly significant ($p<0.0002$) reduction after switching off the infusion in circulating plasma PRL level (Table 15.4, Fig 15.4). There was a very highly significant ($p<0.0000002$) difference observed between the effects of two NMA injections (Table 15.6).

TABLE 15.3

Regression analysis of variance of plasma PRL concentration (mIU/L) after second NMA injection during Ph.a. bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	16.367	16.367	0.355	0.612
Residual	2	92.154	46.077		
Total	3	108.521			
b	-1.809	± 3.035			

TABLE 15.4

Regression analysis of variance of plasma PRL concentration (mIU/L) after two NMA Injections during Ph.a. bolus and infusion with an interval of 15 minutes

	df	SS	MS	F	Significance F
Regression	1	53603.15	53603.15	25.5729	0.0002
Residual	13	27249.15	2096.089		
Total	14	80852.31			
b	-13.836	± 2.736			

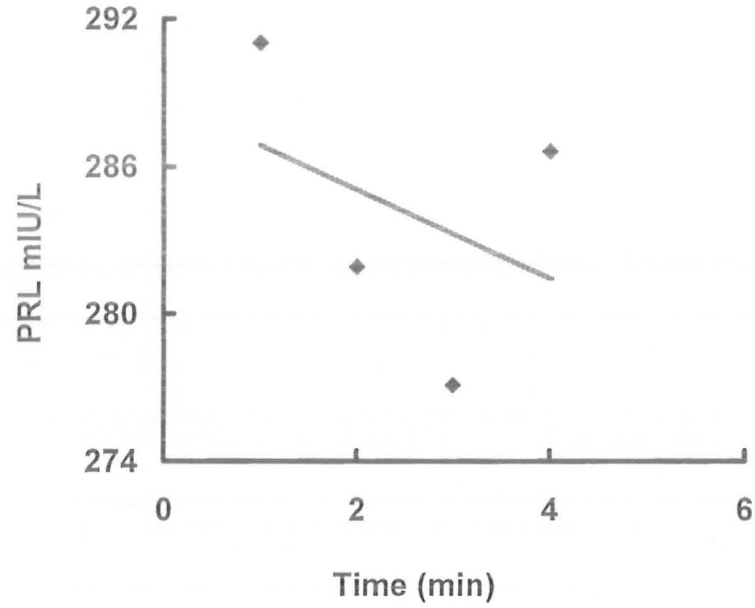

Fig. 15.3.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after second NMA injection during phentolamine infusion.

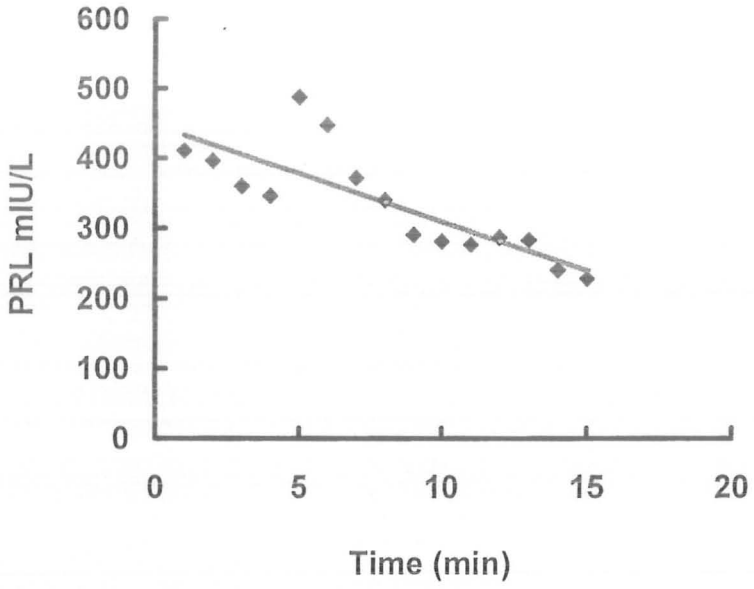

Fig. 15.4.

Calculated regression line indicating plasma PRL concentration (mIU/L) against time after two NMA injections during phentolamine infusion.

TABLE 15.5

Mean plasma PRL concentration (mIU/L) before and after different treatments

Treatments	*Before Treatment*		*After Treatment*	
	Mean	*S.E.M*	*Mean*	*S.E.M*
Saline	160.94	± 6.06	**180.16	± 2.27
1st NMA Injection	189.16	± 4.12	**304.13	± 34.08
2nd NMA Injection	189.16	± 4.12	*239.25	± 21.68
Phentolamine	256.24	± 3.82	***341.28	± 8.74
1st NMA Injection + Ph.a.	308.84	± 2.75	***394.95	± 18.30
2nd NMA Injection + Ph.a.	308.84	± 2.75	**269.85	± 9.33

*p<0.05
**p<0.01
***p<0.001

TABLE 15.6

Analysis of variance showing the effect of different treatments on plasma PRL concentration (mIU/L) in adult male rhesus monkeys.

Treatments	*F- value*	*P-value*
Pre and Post 1st NMA	2.355	0.0002
Pre and Post 2nd NMA	2.355	0.002
Pre and Post Ph.a.	2.250	4.641E-11
1st & 2nd NMA injections	2.487	0.002
1st NMA Vs 1st NMA + Ph.a.	2.487	0.003
2nd NMA Vs 2nd NMA + Ph.a.	2.487	0.003
1st NMA + Ph.a. Vs 2nd NMA + Ph.a.	2.487	2.310E-07

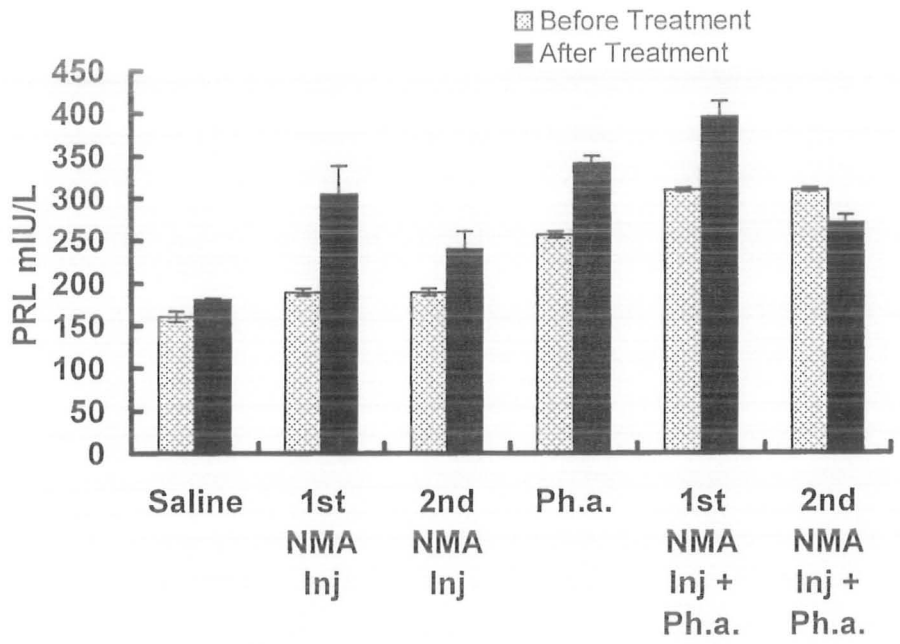

Fig. 15.5.

Mean plasma PRL concentration (mIU/L) before and after different treatments.

DISCUSSION

DISCUSSION

In the present work the result of the interaction of EAA with adrenergic pathway for the regulation of PRL was studied. Four adult male Rhesus monkeys (*Macaca mulatta*) were used for this purpose. Two NMA injections with one-hour interval were administered to all the four monkeys. Both the injections caused a significant ($p<0.01$and $p<0.05$ respectively) increase in the circulating plasma PRL level after 15 minutes of its administration. PRL levels remained high for 30 minutes and then started decreasing gradually showing negative trend in its decrease but were still higher after one hour then the pre-treatment level. These findings are consistent with the previous observations showing the existence for a stimulatory role of excitatory amino acids in the secretion of PRL both in vitro (Login, 1990) and in vivo (Pohl *et al.*, 1989; Arslan *et al.*, 1991). EAA have been implicated in the preovulatory surge of PRL in the female rat (Brann and Mahesh, 1991), as well as in the suckling-induced surge of PRL in the lactating female rat (Pohl *et al.*, 1989). NMA has proved to be a potent secretagogue of PRL in female rhesus monkeys (Wilson and Knobil, 1982; Gay and Plant, 1987), rodents (D'Aniello *et al.*, 2000), pigs (Chang *et al.*, 1993). Brann *et al.* (1993) have shown that treatment with the non-NMDA antagonist DNQX significantly attenuates the preovulatory PRL surge in the PMSG-primed immature rat. Suckling-induced PRL release in the lactating rat has been blocked by the administration of CNQX, a non-NMDA antagonist, but not by administration of NMDA antagonists (Parker and Crowley, 1993).

The involvement of NMA in the PRL release is further supported by the results of other authors who have demonstrated by immunohistochemical studies that receptors for NMDA have been localized in anterior pituitary hormone cell types, including PRL (Bhat *et al.*, 1995) as well as in the hypothalamus (Petralia *et al.*, 1994), that are associated with GnRH neurons ((Bhat *et al.*, 1995). However, it is also reported that in some particular physiological conditions, NMDA can induce an inhibitory effect on PRL release and secretion *i.e.* in female rats during lactation (Abbud and Smith, 1993), in prepubertal female rats (Pinilla *et al.*, 1996,) in hypoprolactinaemic female rats (Pinilla *et al.*, 1998), and in oestrogenized male rats (Pinilla *et al.*, 1995).

NMDA induce c-Fos immunoreactivity in two hypothalamic regions known to regulate PRL secretion: the paraventricular nuclei (PVN) which is the site of TRH cell

bodies and the arcuate nuclei (ARC) which is the site of dopamine cell bodies (Abbud and Smith, 1991; Lee *et al.*, 1993). Hence NMDA could act to regulate PRL via regulation of these PRL releasing/or-inhibiting factors, such as VIP and oxytocin (from the SCN and ARC respectively). EAAs are more likely to control PRL release by regulating dopamine neurons in the ARC. Wagner *et al.* (1993) demonstrated that NMDA receptors are involved in the regulation of dopamine release from the hypothalamus and that DA released from TIDA nerve terminals in the median eminence travels through the hypophyseal long portal vessels to the anterior pituitary where activation of D_2 receptors on lactotrophs cause inhibition of PRL secretion from the anterior pituitary gland (Freeman *et al.*, 2000).

In the present investigation α-adrenergic receptor blocker phentolamine was administered in the form of bolus and infusion for a period of 3 hours to block the adrenergic receptors. It was observed that the PRL secretion was significantly ($p<0.001$) increased 15 min after the administration of phentolamine bolus and remained high throughout the infusion. Infusion was stopped after three hours and the levels were observed 45 minutes afterwards. Regression analysis of variance also showed a non-significant negative trend in the circulating PRL level. These observations are in consistent with the previous results, which showed that the α-adrenergic antagonists are involved in both augmenting and diminishing circulating levels of PRL. The effects of the α_2-antagonists are apparently dependent upon the physiological condition present at the time of elevated PRL levels their administration. α_2-Antagonists have been shown to elevate PRL levels during basal conditions (Lawson and Gala, 1975; Subramanian and Gala, 1976), but attenuate them during surge conditions (Gold *et al.*, 1979; Meltzer *et al.*, 1982; Lein *et al.*, 1986). The mechanisms responsible for the divergent influence of α_2-antagonists on circulating levels of PRL are unknown. It is possible that the effects of α_2-antagonists on circulating levels of PRL are dependent upon their central site of action (i.e., if they are acting on stimulatory or inhibitory component of the PRL regulatory system).

Antagonism of α_2-receptor-mediated inhibition of PRF cellular activity (i.e., promotion of PRF activity) would theoretically induce an elevation in circulating levels of PRL, whereas antagonism of α_2-receptor-mediated inhibition of dopamine cellular activity (promotion of dopamine activity) would initiate a decrease in circulating levels of PRL. Alternatively, the α_2-receptor may be only influencing one

component of the PRL regulatory system, but the relative role of the α_2-receptor may fluctuate from one physiological condition to the next. The former hypothesis is supported by radioligand binding experiments completed in the rat that have demonstrated α_2-receptor expression within central components of the PRL regulatory system outside the PVN (e.g., the periventricular nucleus, arcuate nucleus, median eminence and anterior pituitary) (Leibowitz *et al.*, 1982). In addition deafferentation of noradrenergic input to the medial basal hypothalamus (arcuate) may impair α_2-receptor-mediated inhibition of dopamine cellular activity (i.e., promote dopamine activity) to consequently, attenuate circulating levels of PRL (Blake *et al.*, 1972; Weiner *et al.*, 1972). Variations in noradrenergic activity within the arcuate have been correlated with elevations in circulating levels of PRL. For example whole tissue content studies completed in both rats and guinea pigs have demonstrated that NE turnover in the arcuate significantly increases on the afternoon of proestrous (Weiner *et al.*, 1972; Honma and Wuttke, 1980)

Neurochemical regulation of PRL occurs primarily through the inhibitory actions of the tuberoinfundibular dopaminergic (TIDA) neurons whose cell bodies lie within the arcuate nucleus. These neurons release DA into portal vasculature via their terminals in the median eminence (Ben-Jonathan *et al.*, 1989). However, under certain physiological conditions, these TIDA neurons have been demonstrated to work in concert with hypothalamic factors that stimulate PRL release (Shin *et al.*, 1987). The PVN of the hypothalamus has been suggested to be the site of origin for the synthesis of several of these putative PRL-releasing factors. It has been demonstrated that knife cuts, which disrupt the afferent connections to the PVN, attenuate both lactation and stress-induced release of PRL (Watts *et al.*, 1989). The perikarya of TIDA neurons are also located in the arcuate nucleus of mediobasal hypothalamus. Their axons terminate in the external layer of the median eminence (Bjorklund and Nobin, 1973). DA released from these neurons is transported via the hypophysial portal vasculature to the anterior pituitary, where it activates D_2 receptors located on lactotropes and thereby inhibits the secretion of PRL (Ben-Jonathan, 1985).

In the present investigation involvement of adrenergic pathway for the regulation of EAA-induced PRL was also studied. For this purpose NMA was administered during the infusion of α-adrenergic receptor blocker. It was observed that PRL response to first NMA injection was significant ($p<0.05$) in all the monkeys.

The second NMA injection was administered after an interval of one hour during the infusion of phentolamine. This second NMA injection was failed to elevate circulating level of PRL. Instead of increased level of PRL there was a non-significant decrease in the plasma PRL level – an effect that was opposite to what it did when given alone without phentolamine. Regression analysis was also applied here to observe the trend of plasma PRL that was also not significant. First NMA injection may cause an increase in plasma PRL level through already stimulated adrenergic pathway. α_2-Antagonists have been shown to elevate PRL levels during basal conditions (Lawson and Gala, 1975; Subramanian and Gala, 1976). In the present study antagonism of adrenergic receptors with phentolamine bolus elevates the basal PRL level. Second NMA injection failed to produce any increase in the plasma PRL level and the level remained suppress during the infusion of phentolamine showing the involvement of adrenergic pathway for NMA induced regulation of PRL. Our results confirm the previous observations that antagonism of α_2-antagonists attenuate the PRL response during surge conditions (Gold *et al.*, 1979; Meltzer *et al.*, 1982; Lein *et al.*, 1986). These observations showed that NMA may cause an elevation in circulating PRL level through adrenergic pathway and blocking of this pathway through infusion attenuates the PRL response to NMA. Antagonism of α_2-receptor-mediated inhibition of PRF cellular activity (i.e., promotion of PRF activity) would theoretically induce an elevation in circulating levels of PRL, whereas antagonism of α_2-receptor-mediated inhibition of dopamine cellular activity (promotion of dopamine activity) would initiate a decrease in circulating levels of PRL. Alternatively, the α_2-receptor may be only influencing one component of the PRL regulatory system, but the relative role of the α_2-receptor may fluctuate from one physiological condition to the next. Dodge and Badura (2002) showed that antagonism of the α_2-receptor within a stimulatory component (i.e., PVN) of the PRL regulatory system initiates a significant elevation in circulating levels of PRL, whereas antagonism of the α_2-receptor within inhibitory component (i.e., arcuate) induce a significant decline in basal levels of PRL in male Siberian hamster.

These observations showed an involvement of adrenergic pathway for the regulation of NMA induced PRL from pituitary lactotropes. Dopamine neurons in the ARC may be more likely site of EAA regulation in the control of PRL release. In support of this possibility, NMDA receptors have been reported to regulate dopamine

release in the hypothalamus (Wagner *et al.*, 1993). Furthermore, NMDA induce c-Fos immunoreactivity in two hypothalamic regions known to regulate PRL secretion: the paraventricular nuclei (PVN) which is the site of TRH cell bodies and the arcuate nuclei (ARC) which is the site of dopamine cell bodies (Abbud and Smith, 1991; Saitoh *et al.*, 1991; Lee *et al.*, 1993). Hence NMDA could act to regulate PRL via regulation of PRL releasing/or-inhibiting factors from these regions. Dodge and Badura (2002) showed that the α_2-receptor might also have a role at the level of the arcuate in modulating circulating PRL in the Siberian hamster. They showed that antagonism of α_2-receptor-mediated inhibition of TIDA and THDA cellular activity (i.e., promotion of dopamine activity) within the arcuate may account for the circulating levels of PRL. Yavich et al (1997) have reported that α_2-receptor agonist has been shown to decrease dopamine outflow in the mouse striatum in a dose-dependent fashion and this effect can be prevented by co-administration with α_2-receptor antagonist. In addition, α_2-receptor antagonists have been shown to increase dopamine activity in the nucleus accumbens (De Villiers *et al.*, 1995).

In conclusion, present findings are noteworthy in demonstrating the involvement of adrenergic pathways in NMA-induced PRL regulation may be through TIDA neurons in non-human primates.

CONCLUSION

CONCLUSION

Based on the previous knowledge regarding the involvement of EAA in the regulation of pituitary hormones, we hypothesized that endogenous EAAs are involved in modulating peripheral hormones in response to hypoglycemia. In this study we examined the effect of hypoglycemic condition on the release of endogenous EAA and their effect on pituitary gland to release and regulate PRL. Our results indicate that insulin causes an increase in plasma PRL level through a pathway, which might be through the involvement of endogenous excitatory amino acids in non-human primates. During physiologically stimulated conditions (hypoglycemia) the release of PRL may be through inhibition of dopamine release, which causes a significant rise in PRL level or it might be through the involvement of different pathways like adrenergic and opioidergic pathways (Fig. 16.1).

Involvement of EAAs to stimulate PRL secretion has been demonstrated by NMDA administration in rodents, primates, intact and castrated male rats, as well as cycling female rats. The endogenous opiates (enkaphaline and endorphins) and morphine cause a rapid increase in PRL secretion when given by systemic or intraventricular injection. In view of these facts this study was designed to investigate the interaction of N-methyl-D-Aspartic acid (NMA) with opioids in the regulation of PRL release. The results of our study point towards the interaction between excitatory amino acids and EOP in modulating PRL secretion from pituitary lactotropes and this interaction could be through dopaminergic neurons in non-human male primates (Fig. 16.2).

Another neurotransmitter that may modulate the cellular activity of putative PRFs within the hypothalamus is norepinephrine (NE). Variations in noradrenergic activity with in the PVN have been shown to occur in concert with fluctuations in circulating levels of PRL. The purpose of our third study was to elucidate the involvement of excitatory amino acids for the regulation of PRL through adrenergic pathway. The observations showed that NMA may cause an elevation in circulating PRL level through adrenergic pathway and blocking of this pathway through infusion attenuates the PRL response to NMA. These observations showed an involvement of adrenergic pathway for the regulation of NMA induced PRL from pituitary lactotropes (Fig. 16.2).

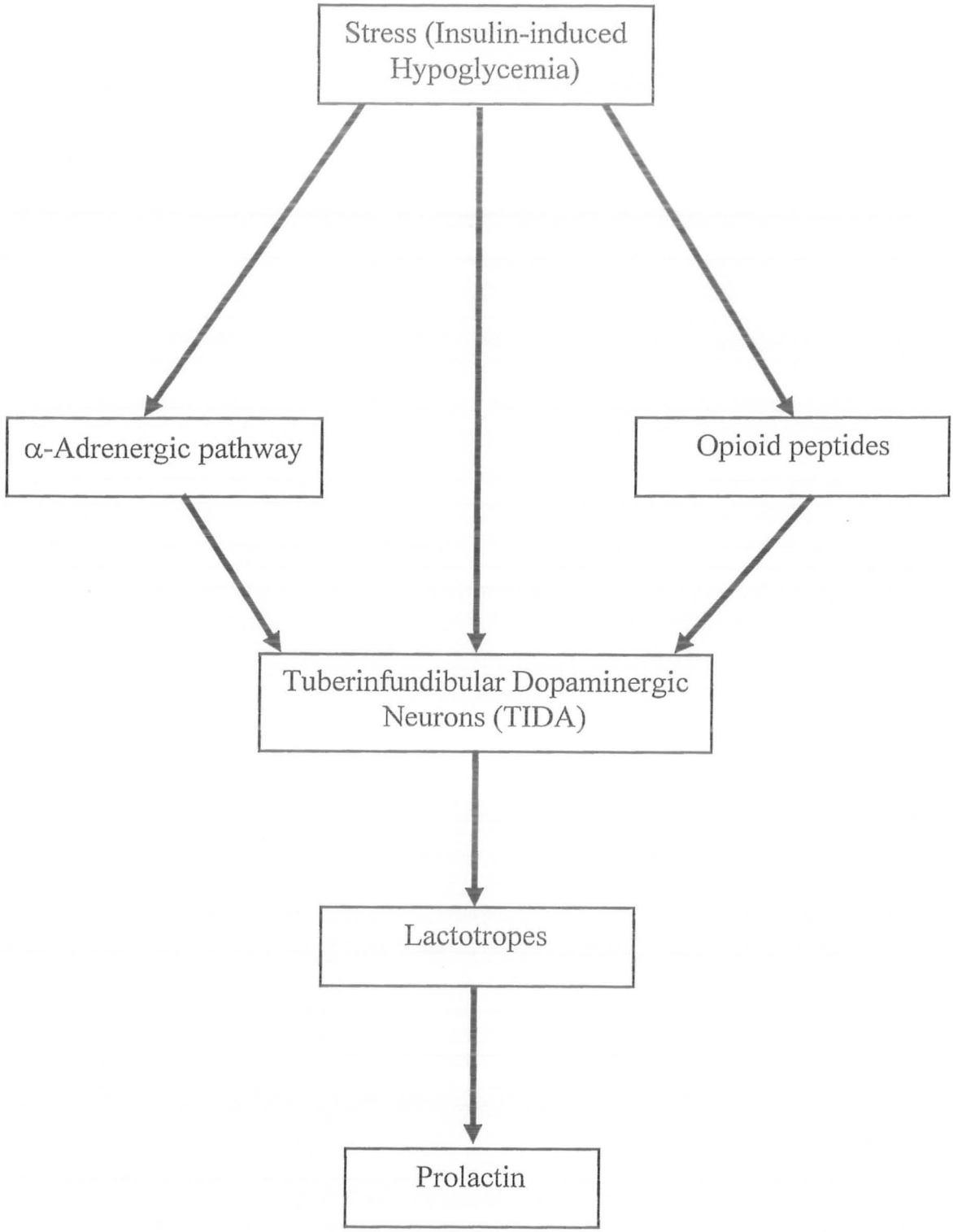

Fig. 16.1 Schematic diagram representing possible regulation of PRL through different pathways during stimulated conditions induced by physiological hypoglycemia (Insulin).

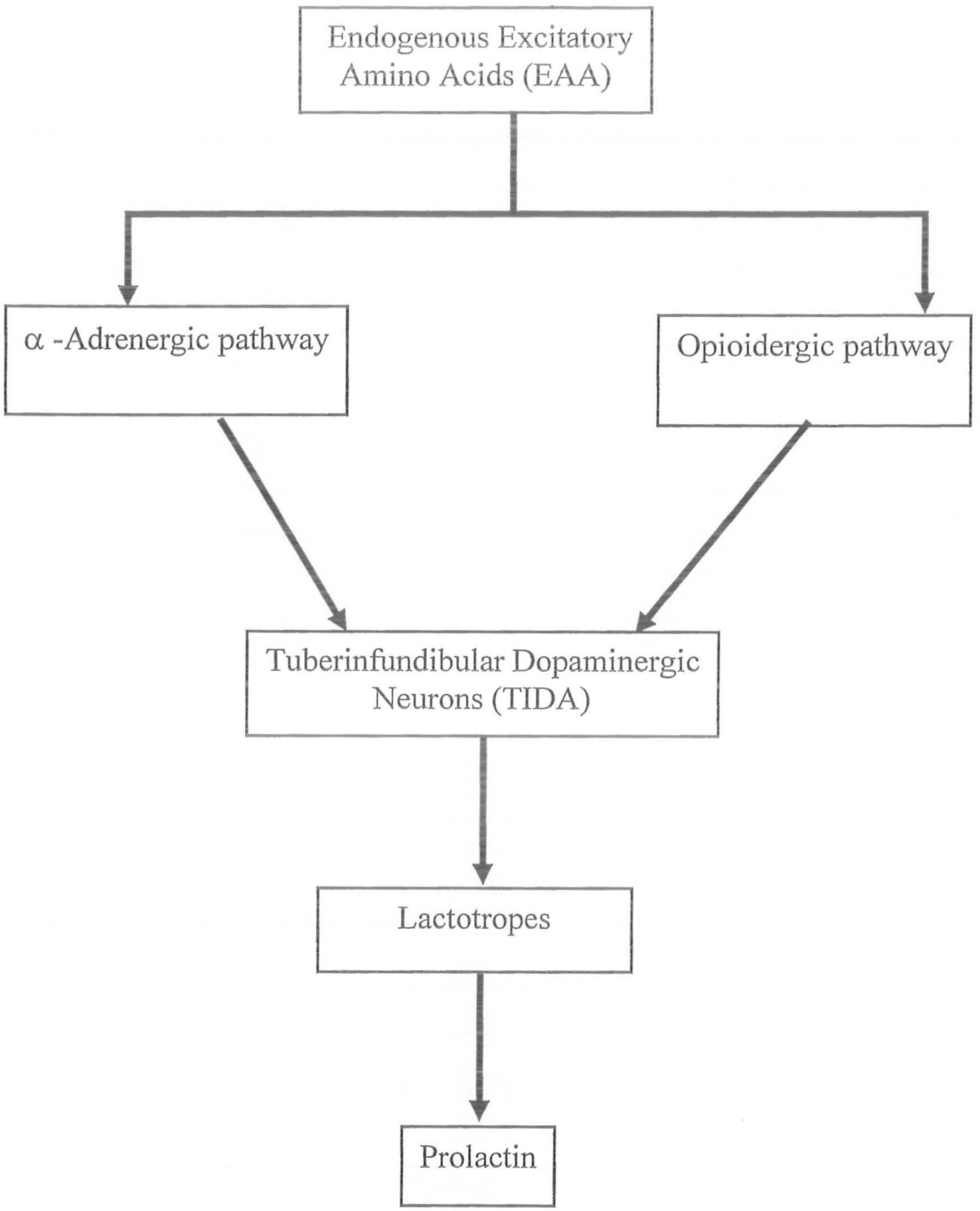

Fig. 16.2. Schematic diagram indicating the involvement of excitatory amino acid (EAA) in the regulation of PRL through adrenergic and opioidergic pathways.

REFERENCES

REFERENCES

Aantaa, R., Marjamaki, A. and Scheinin, M. 1995. Molecular pharmacology of α_2-adrenoceptors subtypes. Ann. Med. 27: 439-449.

Abbud, R. and Smith, M.S. 1991. Difference in the luteinizing hormone and prolactin responses to multiple injections of kainite as compared to N-methyl-D,L aspartate, in cycling rats. Endocrinology. 129: 3254-3258.

Abbud, R. and Smith, M.S. 1993. Altered luteinizing hormone and prolactin response to excitatory amino acids during lactation. Neuroendocrinology. 58: 454-464.

Advis, J. P., Hall, T. R., Hodson, C. A., Muller, G. P. and Meites, J. 1977. Temporal relationship and role of dopamine in short loop feedback of prolactin. Proc. Soc. Exp. Biol. Med. 155: 567.

Advis, J.P., Richard, J.S. and Ojeda, S.R. 1981. Hyperprolactinemia-induced precocious puberty: studies on the mechanism(s) by which prolactin enhances ovarian progesterone responsiveness to gonadotropins in prepubertal rats. Endocrinology. 198: 1333-1342.

Algeri, S., Calderini, G., Consolazione, A. and Garattini, S. 1977. The effect of methionin-enkephalin and d-alanin methionine-enkephalinamide on the concentration of dopamine metabolites in rat striatum. Eur. J. Pharmacol. 45: 207-209.

Andrews, Z.B. and Grattan, D.R. 2002. Opioid control of prolactin secretion in late pregnant rats is mediated by tuberoinfundibular dopamine neurons. Neurosci. Letter. 328: 60-64.

Aragona, C., Bohnet, H.G. and Friesen, H.G. 1977. Localization of prolactin binding in prostrate and testis. The role of serum prolactin concentration on the testicular LH receptors. Acta Endocrinol. 84: 402-409

Arbogast, L.A. and Voogt, J.L. 1998. Endogenous opioid peptide contribute to suckling-induced prolactin release by suppressing tyrosine hydroxylase activity and messenger ribonucleic acid levels in tuberoinfundibular dopaminergic neurons. Endocrinology. 139 (6): 2857-2862.

Arita, J. and Kimura, F. 1988. Enkephalin inhibits dopamine synthesis in vitro in the median eminence portion of rat hypothalamic slices. Endocrinology. 123: 694-699.

Arslan, M., Pohl, C. Smith, M.S. and Plant, T.M. 1991. Studies of the N-methyl-D-aspartate receptor in the hypothalamic control of prolactin secretion. Life Sci. 50: 295-300.

Asano, M., Kanzaki, S., Sekiguchi, E. and Tasaka, T. 1971. Inhibition of prostatic growth in rabbits with antiovine prolactin serum. J. Urol. 106: 248-252.

Barb, C.R., Derocher, G.M., Johnson, B., Utley, R.V., Chang, W.J., Rampacek, G.B. and Krealing, R.R. 1992. N-methyl-D, L-Aspartate stimulates growth hormone and prolactin but inhibits luteinizing hormone secretion in the pig. Dom. Anim. Endocrinol. 9(3): 225-232.

Barnes, J. and Hanley, J.M. 1992. Molecular characteristics of excitatory amino acid receptors. Prog. Neurobiol. 39: 113-133.

Barraclough, C.A. and Sawyer, C.H. 1959. Induction of pseudopregnancy in the rat by reserpine and chlorpromazine. Endocrinolgy. 65: 563-571.

Bartke, A. 1971. Effects of prolactin on spermatogenesis in hypophysectomized mice. J. Endocrinol. 49: 311-316.

Bartke, A. 1974. Effects of inhibitors of pituitary prolactin release on testicular cholesterol stores, seminal vesicles weight, fertility and lactation in mice. Biol. Reprod. 11: 319-325.

Bartke, A. 1976. Pituitary-testis relationships: Role of prolactin in the regulation of testicular function. Prog. Reprod. Biol. 1: 136-150.

Bartke, A. 1980. Role of prolactin in reproduction in male mammals. Federation Proc. 39: 2577-2581.

Bartke, A. and Lloyd, C.W. 1970. The influence of pituitary homografts on the weight of sex accessories in castrated male mice and rats and on mating behavior in male mice. J. Endocrinol. 46: 313-320.

Bartke, A., Goldman, B.D., Bex, F. and Dalterio, S. 1977. Effects of prolactin (PRL) on pituitary and testicular function in mice with hereditary PRL deficiency. Endocrinology. 101: 1760-1766.

Bartke, A., Hafiz, A.A., Bex, F.J. and Delterio, S. 1978. Hormonal interactions in regulation of androgen secretion. Biol. Reprod. 18: 44-45.

Bartke, A., Smith, M.S., Michael, S.D., Peron, F.G. and Delterio, S. 1977. Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology. 100: 182-186.

Bataille, D., Peillon, F., Besson, J. and Rosselin, G., 1979. Vasoactive intestinal peptide (VIP): recepteurs specifiques et activation de l'adenylate cyclase dans une tumeur hypophysaire humaine a prolactine. C.R. Acad. Sci. 228: 1315-1317.

Baumann, M.H. and Rabii, J. 1990. μ-Selective opioid peptides stimulate PRL release in lactating rats. J. Neuroendocrinol. 2: 271-276.

Baumann, M.H. and Rabii, J. 1991. Inhibition of suckling-induced prolactin release by mu-and kappa-opioid antagonists. Brain Res. 567: 224-230.

Ben- Jonathan, N., Oliver, C., Weiner, H.J., Mical, R.S. and Porter, J.C. 1977. Dopamine in the hypophysial portal plasma of the rat during the estrous cycle and during pregnancy. Endocrinology. 100: 452-458.

Ben-Jonathan, N. 1985. Dopamine: a prolactin-inhibiting hormone. Endocr. Rev. 6: 564-589.

Ben-Jonathan, N., Arbogast, L.A. and Hyde, J.F. 1989. Neuroendocrine regulation of prolactin release. Prog. Neurobiol. 33: 399-447.

Berndtson, W.E. and Desjardins, C. 1974. Circulating LH and FSH levels and testicular function in hamsters during light deprivation and subsequent photoperiodic stimulation. 95: 195-205.

Besson, J., Rotsztejn, W., Laburthe, M., Epelbaum, J., Beaudet, A., Kordon, C. and Rosselin, G. 1979. Vasoactive intestinal peptide (VIP): brain distribution, subcellular localization and effect of deafferentation of the hypothalamus in male rats. Brain Res. 165: 79-85.

Bex, F., Bartke, A., Goldman, B.D. and Dalterio, S.1978. Prolactin, growth hormone, luteinizing hormone receptors and seasonal changes in testicular activity in the golden hamster. Endocrinology. 103: 2069-2080.

Bex, F.J. and Bartke, A. 1977. Testicular LH binding in the hamster: modulation by photoperiod and prolactin. Endocrinology. 100: 1223-1226.

Bex, F.J. and Goodman, B.D. 1975. Serum gonadotropins and follicular development in the Syrian Hamster. Endocrinology. 96: 928-933.

Bhat, G.H., Mahesh, V.B., Chu, Z.W., Chorich, L.P., Zamorano, P.L. and Brann, D.W. 1995. Localization of the N-methyl-D-aspartate R1 receptor subunit in specific anterior pituitary hormone cell types of the female rat. Neuroendocrinology. 62: 178-186.

Bhat, G.K., Mahesh, V.B., Lamar, C.A., Ping, L., Aguan, K. and Brann, D.W. 1995. Histochemical localization of nitric oxide neurons in the hypothalamus: association with gonadotropin-releasing hormone neurons and co-localization with N-methyl-D-aspartate receptors. Neuroendocrinology. 62: 187-197.

Bjorkland, A. and Nobin, A. 1973. Fluorescence histochemical and microspectrofluorometric mapping of dopamine and noradrenaline cell groups in the rat diencephalon. Brain Res. 51: 171-191.

Blake, C. A. 1974. Stimulation of pituitary prolactin and TSH release in lactating and proestrous rats. Endocrinology. 94: 503-508.

Blake, C.A., Weiner, R.I. and Sawyer, C.H. 1972. Pituitary prolactin secretion in female rats made persistently estrous or diestrouds by hypothalamic deafferentation. Endocrinology. 90: 862-866.

Bohnet, H.G. and Friesen, H.G. 1976. Effect of prolactin and growth hormone on prolactin and LH receptors in the dwarf mouse. J. Reprod. Fertil. 48: 307-311.

Bourguignon, J., Gerard, A. and Franchimont, P. 1989. Direct activation of gonadotropin-hormone releasing hormone secretion through different receptors to neuroexcitatory amino acids. Neuroendocrinology. 49: 402-408.

Bowers, C. Y., Friesen, H.G., Hwang, P., Guyda, H.J. and Folkers, K.1971. Prolactin and thyrotropin release in man by synthetic pyroglutamyl-histidyl-prolinamide. Biochem. Biophys. Res. Commun. 45: 1033-1041.

Boyd, A.E. III, Spencer, E., Jackson, I.M.D. and Reichlin, S. 1976. Prolactin-releasing factor in porcine hypothalamus extract distinct from TRH. Endocrinology. 99: 861-871.

Brann, D.W. and Mahesh, V.B. 1991. Endogenous excitatory amino acid involvement in the preovulatory and steroid-induced surge of gonadotropins in the female rat. Endocrinology. 128: 1541-1547.

Brann, D.W. and Mahesh, V.B. 1992. Excitatory amino acid neurotransmission: Evidence for a role in neuroendocrine regulation. Trends Endocrinol. Metab. 3: 120-124.

Brann, D.W. and Mahesh, V.B. 1993. Neuroendocrine regulation of gonadotropin secretion: Role of excitatory amino acids. Assisted Reprod. Technol. Androl. 5: 201-212.

Brann, D.W. and Mahesh, V.B. 1994. Excitatory amino acids: function significance in reproduction and neuroendocrine regulation. Front. Neuroendocrinol. 15: 3-49.

Brann, D.W., Putnam, C.D. and Mahesh, V.B. 1993. Role of non-NMDA receptor neurotransmission in steroid and preovulatory gonadotropin surge expression in the female rat. Mol. Ce.. Neurosci. 4: 292-297.

Brown, G.M., Seeman, P. and Lee, T. 1976. Dopamine/ neuroleptic receptors in basal hypothalamus and pituitary. Endocrinology. 99: 1407-1416.

Brownstein, M.J., Saavedra, J.M., Palkovits, M. and Axelrod, J. 1974. Histamine content of hypothalamic nuclei of rat. Br. Res. 77(1): 151-156.

Bruni, J., Van Vugt, D.A., Marshall, S. and Meites, J. 1977. Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone. Life Sci. 21: 461-468.

Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A. and Civelli, O. 1988. Cloning and expression of a rat D2 dopamine receptor cDNA. Nature. 336: 783-787.

Bybee, D.E., Nakawatase, C., Szabo, M. and Frohman, L.A. 1983. Inhibitory feedback effects of prolactin on its secretion involve central nervous system dopaminergic mediation. Neuroendocrinology. 36: 27-32.

Callahan, P., Baumann, M.H. and Rabii, J. 1996. Inhibition of tuberoinfundibular dopaminergic neural activity during suckling: Involvement of μ and κ opiate receptor subtypes. J. Neuroendocrinol. 8: 771-776.

Carbone, S., Szwarcfarb, B., Losada, M. and Moguilevsky, J. 1992. Effects of ovarian steroids on the gonadotropin response to N-methyl-D-aspartate and on hypothalamic excitatory amino acid levels during dexual maturation in in female rats. Endocrinology. 130: 1365-1370.

Carbone, S., Szwarcfarb, B., Otero Losada, M.E. and Moguilevsky, J.A. 1992. Effects of ovarian steroids on the gonadotropin response to N-methyl-D-aspartate and on hypothalamic excitatory amino acid levels during sexual maturation in female rats. Endocrinology. 130: 1365-1370.

Carlson, H.E. and Ippoliti, A.F. 1977. Cimetidine, an H_2 –antihistamine, stimulates prolactin secretion in man. J. Clin Endocrinol. Metab. 45: 367-370.

Caron, M.G., Amliky, N. and Kilpatrick, B.F. 1986. D2-dopamine receptors: biochemical characterization. In: Ganong, W.F., Martini, L., eds. Frontiers in Neuroendocrinolgy, vol. 6. New York: Raven Press; 205-224

Chang, W.J., Barb, C.R., Krealing, R.R., Rampacek, G.B. and Asanovich, K.M. 1993. N-methyl-D, L-Aspartate modulation of pituitary hormone secretion in the pig: role of opioid peptides. Dom. Anim. Endocrinol. 10(4): 305-313.

Chapman, A.G. 1992. Effect of NMDA antagonists and non-NMDA antagonists in experimental models of epilepsy. In: Simon, R.P. (eds). Excitatory Amino Acids. Vol. 9, New York: Thieme Medical Publishers. 165-272.

Chappel, S. and Selker, F. 1979. Relation between the secretion of FSH during the periovylatory period and ovulation during the next cycle. Biol. Reprod. 21: 347-352.

Charreau, E.H., Attramadal, A., Torjesen, P.A., Purvis, K., Calandra, R. and Hansson, V. 1977. Prolactin binding in rat testis: Specific receptors in interstitial cells. Mol. Cell. Endocrinol. 6: 303-307.

Chen, C. L. and Meites, J. 1972. Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. Neuroendocrinology. 9: 304-308.

Chen, C.L. and Meites, J. 1970. Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. Endocrinology. 86: 503-505.

Clark, W.C. and Bern, H.A. 1980. Comparative endocrinology of prolactin. Hormonal Proteins and Peptides 8: 105-197.

Clemens, J.A., Smalstig, E.B. and Sawyer, B.D. 1974. Antipsychotic drugs stimulate prolactin release. Psychopharmacologia. 40: 123-127.

Clemens, J.A. and Shaar, C.J. 1980. Control of prolactin secretion in mammals. Fed. Proc. Fed. Am. Soc. Exp. Biol. 39: 2588-2592.

Clemens, J.A., Rousch, M.E. and Fuller, R.W. 1978. Evidence that serotonergic neurons stimulate secretion of prolactin releasing factors. Life. Sci. 22: 2909-2913.

Clemens, J.A., Smalstig, E.B. and Sawyer, B.D. 1974. Antipsychotic drug stimulate prolactin release. Psychopharmacologia. 40: 123.

Cocchi, D., Santagostino, A., Gil-Ad, I., Ferri, S. and Muller, E.E. 1977. Leu-enkaphalin-stimulated growth hormone and prolactin release in the rat: comparison with the effect of morphine. Life Sci. 15; 20(12): 2041-2045.

Cooke, N.E., Coit, D., Shine, J., Baxter, J.D. and Martial, J.A. 1981. Human prolactin: cDNA structural analysis and evolutionary comparisons. J Biol Chem. 256: 4007-4016.

Coppola, J.A. 1986. The apparent involvement of the sympathetic nervous system in the gonadotropin secretion of female rats. J. Reprod. Fertil. 4 [suppl]:35-45.

Cotman, C. and Iverson, L. 1987. Excitatory amino acids in the brain- Focus on NMDA receptors. Trends Neurosci. 10: 263-272.

Cotman, C., Bridges, R., Tawbe, J., Clark, A.S., Geddes, J. and Monaghan, D. 1989. The role of the NMDA receptor in central nervous system plasticity and pathology. J. NIH. Res. 1: 65-74.

Cotman, C., Geddes, J., Bridges, R. and Monagham, D. 1989. NMDA receptors and Alzheimer's disease. Neurobiol. Aging. 10: 603-605.

Cramer, O.M. Parker,C.R. and Porter, J.C. 1979. Estrogen inhibition of dopamine release into hypophyseal portal blood. Endocrinology. 104: 419-422.

Creese, I.R., Schneider, P. and Synder, S.H. 1977. H3- spiroperidol labels dopamine receptors in pituitary and brain. Eur. J. Pharmacol. 46: 377-381.

Crisp, T.M. 1977. Hormone requirements for early maintenance of rat granulosa cell culture. Endocrinology. 101: 1286-1297.

Cusan, L., Dupont, A., Kledzik, G.S., Labrie, F., Coy, H.D. and Schally, A.V. 1977. Potent prolactin and growth hormone releasing activity of more analogues of met-enkephalin. Nature. 268(5620): 544-547.

D' Aniello, G., Tolino, A., D' Aniello, A., Errico, F., Fisher, G.H. and Maddalena Di Fiore, M. 2000. The role of D-Aspartic Acid and N-methyl-D-Aspartic Acid in the regulation of prolactin release. Endocrinology. 141: 3862-3870.

Daftary, S.S., Boudaba, C., Szabo, K. and Tasker, J.G. 1998. Noradrenergic excitation of magnocellular neurons in the rat hypothalamic paraventricular nucleus via intranuclear glutamatergic circuits. J. Neurosci. 18: 10619-10628.

De Paolo, L.V. and Barraclough, C.A. 1979. Interaction of estradiol and progersterone on pituitary gonadotropin secretion: possible sites and mechanisms of action. Biol Reprod. 20: 1173-1185.

De Villiers, A.S., Russell, V.A., Sagvolden, T., Searson, A., Jaffer, A. and Taljaard, J.J. 1995. α_2-Adrenoceptor-mediated inhibition of [^{3}H] dopamine release from nucleus accumbens slices and monoamine levels in a rat model for attention-deficit hyperactivity disorder. Neurochem Res. 20: 427-433.

De Vlaming, V.L. 1979. Action of prolactin among vertebrates. In: Barrington, E.J.W., eds. Hormones and evolution. New York: Academic Press; 561-642.

Dent, J.N. 1975. Integumentary effects of prolactin in the lower vertebrates. Am. Zool. 15:923-935.

Dodge, J.C. and Badura, L.L. 2001. Norepinephrine dialysate levels in the hypothalamic paraventricular nucleus: Influence on photoperiod-driven prolactin levels in the female Siberian hamster. Neuroendocrinology. 73: 102-110.

Dodge, J.C. and Badura, L.L. 2002. Infusion of alpha-2-adrenergic agents into the paraventricular and arcuate nuclei of the hypothalamus in the Siberian Hamster: Opposing effects on basal prolactin. Neuroendocrinology. 75: 175-184.

Domae, M., Yamada, K., Hanabusa, Y. and Furukawa, T. 1992. Inhibitory effects of endothelin-1 and endothelin-3 on prolactin release: possible invovement of endogeneous endothelin isopeptide in the rat anterior pituitary. Life Sci. 50: 715-722.

Donoso, A.O., Bishop, W. and McCann, S.M. 1973. The effect of drug, which modify catecholamine synthesis on serum prolactin in rats with median eminence lesions. Proc. Soc. Exp. Biol. Med. 143(2): 360-363.

Donoso, A.O., Bishop, W., Fawcett, C.P., Krulich, L. and McCann, S.M. 1971. Effects of drugs that modify brain monoamine concentrations on plasma gonadotropin and prolactin levels in the rat. Endocrinology, 89(3): 774-784.

Dotti, C. and Teleisnik, S. 1982. Inhibition of release of LH and ovulation by activation of the noradrenergic system. Effect of interrupting the ascending pathways. Brain Res. 249: 281-290.

Eidelberg, E. 1976. Possible actions of opiates upon synapses. Prog. Neurobiol. 3(2): 81-102.

Enjalbert, A., Ruberg, M., Arancibia, S., Fiore, L., Priam, M. and Kordon, C. 1979. Independent inhibition of prolactin secretion by dopamine and gamma-aminobutyric acid *in vivo*. Endocrinology. 105: 823-826.

Enroth, P., Fuxe, K., Gustaffason, J.A., Hockfelt, T., Lofstrom, A., Skett, P. and Agnati, L. 1977. The effect of nicotine on central catecholamine neurons and gonadotropin secretion. III. Studies on prepubertal female rats treated with pregnant mare serum gonadotropin. Med. Biol. 55(3): 167-176.

Erecinska, M. and Silver, I. 1990. Metabolism and role of glutamate in mammalian brain. Prog. Neurobiol. 35: 245-296.

Eskay, R. L., Oliver, C., Ben-Jonathen, N. and Porter, J.C. 1975. Hypothalamic hormones in portal and systemic blood. In: Motta, M., Crosighani, P. G., Martini, L., eds. Hypothalamic hormones: Chemistry, physiology, pharmacology and clinical uses. New York: Academic Press. 125-137.

Estienne, M.J., Schillo, K.K., Hileman, S.M., Green, M.A. and Hayes, S.H. 1989. N-methyl-D,L-aspartate stimulates growth hormone but not luteinizing hormone secretion in the sheep. Life Sci. 44: 1527-1533.

Everett, J.W. 1954. Luteotropic function of autografts of the rat hypophysis. Endocrinology. 54: 685-690.

Fenske, M. and Wuttke, W., 1976. Effects of intraventricular 6-hydroxydopamine injections on serum prolactin and LH levels: absence of stress induced pituitary prolactin release. Brain. Res. 104(1): 63-70.

Ferland, L., Fuxe, K., Eneroth, P. Gustafsson, A. 1977. Effects of methionine-enkephalin on prolactin release and catecholamine lavels and turnover in the median eminence. Eur. J. Pharmacol. 43: 89-90.

Fink, G., Koch, Y. and Ben Aroya, N. 1982. Release of thyrotropin releasing hormone into hypophysial portal blood is high relative to other neuropeptides ans may be related to prolactin secretion. Brain Res. 234: 186-189.

Fish, H.R., Chernow, B. and O'Brain, J.T. 1986. Endocrine and neurophysiologic responses of the pituitary to insuline-induced hypoglycemia: A review, Metabolism. 35: 763-780.

Fitzsimmons, M.D., Olschowka, J.A., Wiegend, S.J. and Hoffman, G.E. 1992. Interaction of opioid peptide-containing terminals with dopaminergic perikarya in the rat hypothalamus. Brain Res. 581: 10-18.

Fleckenstein, A.E., Lookingland, K.J. and Moore, K.E. 1992. Evidence that histamine induced prolactin secretion is not mediated by an inhibition of tuberoinfundibular dopaminergic neurons. Life Sci. 51: 741-746.

Fonnum, F. 1984. Glutamate: A neurotranmitter in mammalian brain. J. Neurochem. 42: 1-11.

Frantz, A.G., Kleinberg, D.L.and Neol, G.L. 1972. Studies on prolactin in man. Rec. Prog. Horm. Res. 28: 527-590.

Freeman, M.E., Kanyicska, B., Lerant, A. and Nagy, G. 2000. Prolactin: structure, function and regulation of secretion. Physiol.Rev. 80: 1523-1631.

Fujii, T., Hoover, D.J. and Channing, C.P. 1983. Changes in inhibin activity and progesterone, oestrogen and androstenedion concentrations, in rat follicular fluid throughout the oestrous cycle. J. Reprod. Fertil. 69: 307-314.

Furth, J. and Clifton, K. H. 1966. Experimental pituitary tumors. In: The Pituitary Gland. Vol. 2. Harris, G. W and Donoyan, B. eds. Butterworth, London. 460.

Fuxe, K. 1965. The distribution of monoamine terminals in central nervous system. Acta. Physiol. Scand. 274[suppl]: 39-85.

Gallo, R.V., Rabii, J. and Moberg, G.P. 1975. Effects of methysergide, a blocker of serotonin receptors on plasma prolactin levels in lactating and overiectomized rats. Endocrinology. 97: 1096-1105.

Garber, A.J., Cryer, P.E., Santiago, J.V., Haymond, M.W., Pagliara, A.S. and Kipnis, D.M. 1976. The role of adrenergic mechanisms in the substrate and hormonal response to insulin-induced hypoglycemia in man. J.Clin.Invest. 58: 7-15.

Gay, V.L. and Plant, T.M. 1987. N-methyl-D-aspartate elicits hypothalamic gonadotropin-releasing hormone release in prepubertal male rhesus monkeys (*Macaca mulatta*). Endocrinology. 120: 2289-2296.

Gay, V.L., Midgley, A.R., Jr. and Niswender, G.D. 1970. Patterns of gonadotropin secretion associated with ovulation. Fed. Proc. 29: 1880-1887.

George, S.R., Zastwny, R.L., Briones-Urbina, R., Chang, R., Nguyen, T., Heiber, M., Kouvelas, A., Chan, A.S. and O'Dowd, B.F. Distinct distributions of mu, delta and kappa opioid receptors mRNA in rat brain. Biochem. Biophys. Res. Commun. 205: 1438-1444.

Gershengorn, M.C. 1982. Thyrotropin-releasing hormone: A review of the mechanisms of acute stimulation of pituitary hormone release. Mol. Cell. Biochem. 45(3): 163-179.

Gessa, G.L. and Tagliamonte, A. 1975. Effect of methadone and dextromoramide on dopamine metabolism: comparison with haloperidol and amphetamine. Neuropharmacology. 14(12): 913-920.

Gibbs, D.M. and Neill, J.D. 1978. Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion *in vivo*. Endocrinology. 102(6): 1895-1900

Gibbs, D.M., Plotsky, P.M., de-Greef, W.J. and Neill, D.J. 1979. Effects of histamine and acetycholine on hypophysial stalk plasma dopamine and peripheral plasma prolactin levels. Life Sci. 24: 2063-2070.

Gold, M.S., Donabedian, R.K. and Redmond, D.E. 1979. Further evidence for the α_2-adrenergic receptor-mediated inhibition of prolactin secretion: The effect of yohimbine. Psychoneuroendocrinology. 3: 253-260.

Gold, M.S., Donabedian, R.K., Dillard, M., Slobetz, W.F., Riordan, C.E. and Kleber, H.D. 1977. Antipsychotic effect of opiate agonists. Lancet. 2: 398-399.

Gold, M.S., Redmond, D.E. Jr. and Donabedian, R.K. 1978. Increase in serum prolactin by exogenous and endogenous opiates: Evidence for anti-dopamine and antipsychotic effect. Am. J. Psychiatry. 135: 1415-1416.

Golder, M.P., Boyns, A.R., Harper, M.E. and Griffiths, K. 1972. An effect of prolactin on prostatic adenylate cyclase activity. Biochem. J. 128: 725-727.

Goldsmith, P.C., Cronin, M.J. and Weiner, R.I. 1979. Dopamine receptors sites in the anterior pituitary . J. Histochem. Cytochem. 27: 1205-1207.

Gonzalez-Villapando, V., Szabo, M. and Frohman, L.A. 1980. Central nervous system-mediated stimulation of prolactin secretion by cimetidine, a histamine H_2 –receptors antagonist: Impaired responsivness in patients with prolactin secreting tumors and idiopathic hyperprolactinemia. J. Clin. Endocrinol. Metab. 51: 1417-1424.

Goodman, R.R., Adler, B.A. and Pasternak, G.W. 1988. Regional distribution of opiate receptors: in Pasternak G.W. (eds): The opiate Receptors. Clifton, Humana Press. pp 197-230.

Gottschall, P.E., Sarker, D.K. and Meites, J. 1986. Persistance of low hypothalamic dopaminergic activity after removal of chronic estrogen treatment. Proc. Soc. Exp. Biol. Med. 181: 78-86.

Goudreau, J.L., Lindley, S.E., Lookingland, K.J. and Moore, K.E. 1992. Evidence that hypothalamic periventricular dopamine neurons innervate the intermediate lobe of the rat pituitary. Neuroendocrinolgy. 56: 100-105.

Grandison, L. and Guidotti, A. 1979. Gamma- aminobutyric acid receptor function in rat anterior pituitary: evidence for control of prolactin release. Endocrinology. 105: 754-759.

Grandison, L. and Meites, J. 1976. Evidence for adrenergic mediation of and cholinergic inhibition of prolactin release. Endocrinology. 99: 775-779.

Grandison, L. Cavagnini, F., Schmid, R., Invitti, C. and Guidotti, A. 1982. Gamma-aminobutyric acid benzodiazepine-binding sites in human anterior pituitary tissue. J. Cin. Endocrinol. Metab. 54: 597-601.

Greenamyre, J.T. and Young, A.B. 1989. Excitatory amino acids and Alzheimer's disease. Neurobiol. Aging. 10: 593-602.

Greenamyre, J.T., Klockgether, T., Trishi, L., Zhang, Z., Kurlan, R. and Gash, D.M. 1992. Glutamate receptor antagonism as a novel therapeutic approach in Parkinson's disease. In: Simon, R.P. (eds). Excitatory Amino Acids. Vol. 9. New York Thieme Medical Publishers. 195-198.

Grosvenor, C.E. and Mena, F. 1980. Evidence that thyrotropin-releasing hormone and a hypothalamic prolactin –releasing factor may function in the release of prolactin in the lactating rat. Endocrinology. 107: 863-868.

Grosvenor, C.E., Mena, F. and Whitworth, N.S. 1980. Evidence that the dopaminergic PRL-inhibiting factor mechanism regulates only the depletion-transformation phase and not the release phase of PRL secretion during suckling in the rat. Endocrinology. 106: 481-485.

Gudelsky, G.A. and Porter, J.C. 1979. Morphine and opioid peptide –induced inhibition of the release of dopamine from tuberoinfundibular neurons. Life Sci. 25: 1697-1702.

Gudelsky, G.A. and Porter, J.C. 1980. Release of dopamine from tuberoinfundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. Endocrinology. 106: 526-529.

Hafiz, A.A., Lloyd, C.W. and Bartke, A. 1972. The role of prolactin in the regulation of testis function: The effects of prolactin and luteinizing hormone on the plasma levels of testosterone in hypophysectomized rats. J. Endocrinol. 52: 327-332.

Hanson, J.J. and Krogsgaard-Larson, P. 1990. Structural, conformational and stereochemical requirements of central excitatory amino acid receptors. Med. Rev. 10: 55-94.

Havel, P.J., Mundinger, G.J. and Taborsky, Jr. 1996. Pancreatic sympathetic nerves contribute to increased glucagons secretion during severe hypoglycemia in dogs. Am. J. Physiol. 270: E20-E26.

Headley, P. and Grillner, S. 1990. Excitatory amino acids and synaptic transmission: The evidence for a physiological function. Trends Pharmacol. Sci. 11: 205-211.

Hevener, A.L., Bergman, R.N. and Donovan, C.M. 2000. Portal vein afferents and critical for the sympathadrenal response to hypoglycemia. Diabetes. 49: 8-12.

Hinkel, P. M. and Tashjian, A. J. 1975. Receptors for thyrotropin-releaseing hormone in prolactin producing rat pituitary cells in culture. J. Biol. Chem. 248: 6180-6186.

Hodson, C. 1982. Prolactin. In: Hand Book of Endocrinology. Gass, G.H. and Kaplan, H.M. (eds). CRC Press, Florida.

Hokfelt, T. and Fuxe, K. 1972. On the morphology and neuroendocrine role of the hypothalamiccatecholamine neurons. In: Brain-Endocrine Interaction Median Eminence: Structure and Function. International Symposium Munich 181-223.

Honma, K. and Wuttke, W. Norepinephrine and dopamine turnover rates in the medial preoptic area and the mediobasal hypothalamus of the rat brain after various endocrinological manipulations. Endocrinology. 106: 1848-1853.

Horvath, T.L., Naftolin, F. and Leranth, C. 1992. β-Endorphin innervation of dopamine neurons in the rat hypothalamus: A light and electron microscopic double immunostaining study. Endocrinology. 131(3): 1547-1555.

Hostetter, N.W. and Piacsek, B.E. 1977. The effect of prolactin deficiency during sexual maturation in the male rat. Biol. Reprod. 17: 574-577.

Hourani, H., Lacy, B., Eltayeb, K. and Abumrad, N.N. 1992. The role of the central nervous system in modulating glucose and protein metabolism during insulin-induced hypoglycemia. Brain Res. 587: 276-284.

Janik, J., Callahan, P. and Rabii, J. 1992. The role of the μ_1 opiate receptor subtype in the regulation of PRL and growth hormone secretion by β-endorphin in female rats: Studies with naloxonazine. J. Neuroendocrinol. 4: 701-708.

Jaworski-Parman, R., Callahan, P. and Janik, J. 1997. Immunoneutralization of β-endorphin blocks prolactin release during suckling without affecting tuberoinfundibular dopaminergic neural activity. Life Sci. 61: 1301-1311.

Johnston, C. and Negro-Vilar, A. 1986. Maturation of the prolactin and proopiomelanocortin-derived peptide responses to either stress and morphine neurochemical analysis. Endocrinology. 118: 797-804.

Jorgenson, H., Knigge, U., Kjaer, A. and Warberg, J. 1996. Interaction of histaminergic and serotonergic neurons in hypothalamic regulation of prolactin and ACTH secretion. Neuroendocrinology. 64: 329-336.

Kamberi, L.A., Mical, R.S. and Porter, J.C. 1971. Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology. 88: 1294-1299.

Kanematsu,S., Hillard, J. and Sawyer, C.H. 1963. Effect of reserpine on pituitary prolactin content and its hypothalamic site of action in the rabbit. Acta Endocrinol. 44: 467-474.

Kato, Y., Iwaski, Y., Iwaski, J., Abe, H., Yanaihara, N. and Imura, H. 1978. Prolactin release by vasoactive intestinal peptide in rats. Endocrinology. 103: 554-558.

Kato, Y., Matsushita, N., Onta, H., Tojo, K., Shimatsu, A. and Imura, H. 1985. Regulation of prolactin secretion. In: Imura, H., eds. The pituitary gland. New York: Raven Press. 261-278.

Kehoe, L., Parman, R., Janik, J. and Callahan, P. 1993. Opiate receptor subtype involvement in the stimulation of PRL release by β-endorphin in female rats. Neuroendocrinolgy. 57: 875-883.

Khachaturian, H., Lewis, M.E. and Watson, S.J. 1983. Enkephalin systems in diencephalon and brain-stem of rat. J.Comp. Neurol. 220: 310-320.

Kjaer, A. Knigge, U., Olsen, L., Vilhard, H. and Warberg, J. 1991. Mediation of the stress –induced prolactin release by hypothalamic histaminergic neurons and the possible involvement of vasopressin in this response. Endocrinology. 128: 103-110.

Kjaer, A. Knigge, U., Vilhard, H. and Warberg, J. 1993. Involvement of vasopressin in histamine and stress induced prolactin release: Permissive, mediating or potentiating role? Neuroendocrinology. 57: 314-321.

Kleber, H.D. and Gold, M.S. 1978. Use of psychotropic drugs treatment of methadone maintained narcotic addicts. Ann. Acad. Sci. 311: 81-98.

Kledzik, G.S., Marshall, S., Campbell, G.A., Gelato, M. and Meites, J. 1976. Effects of castration, testosterone, estradiol and prolactin on specific prolactin-binding activity in ventral prostate of male rats. Endocrinology. 98: 373-379.

Klochgetter, T. 1992. Excitatory amino acids and basal ganglia: Implications for Parkinson's disease. In: Simon, R.P. (eds). Excitatory Amino Acids. Vol. 9. New York Thieme Medical Publishers. 183-188.

Knigge, U., Dejgaard, A., Wollesen, F., Thuesen, B. and Christiansen, P.M. 1982. Histamine regulation of prolactin secretion through H_1 and H_2 –receptors. J.Clin. Endocrinol. Metab. 55: 118-122.

Knigge, U., Matzen, S. and Warberg, J. 1988. Histaminergic regulation of prolactin secretion: Involvement of tuberoinfundibular dopaminergic neurons. Neuroendocrinology. 48: 167-173.

Knigge, U.,Sleimann, I., Matzen, S. and Warberg, J. 1988. Histaminergic regulation of prolactin secretion: Involvement of serotonergic neurons. Neuroendocrinology. 48: 527-533.

Kordon, C., Blake, C.A., Terkel, J. and Sawyer, C.H. 1974. Participation of serotonin containing neurons in the suckling induced rise in plasma prolactin levels in lactating rats. Neuroendocrinology. 13: 213-223.

Kurose, H. and Lefkowitz, R.J. 1994. Differential desensitization and phophorylation of three cloned and transfected α_2-adrenergic receptor subtype. J. Biol. Chem. 269: 100093-10099.

Labrie, F., Ferland, L., Dipaolo, T. and Villeux, R. 1980, Modulation of prolactin secretion by sex steroids and thyroid hormones: in Central and Peripheral Regulation of Prolactin Function, Macload, R.M. and Seapagnini, U. (eds). Raven Press, New York: 97.

Lal, H. 1975. Narcotic dependence, narcotic action and dopamine receptors. Life Sci. 17(4): 483-495.

Lang, C.H. and Ajmal, M. 1995. Metabolic, hormonal and hemodynamic changes induced by metabotropic excitatory amino acid agonist (1S,3R)-ACPD. Am. J. Physiol. 268: R1026-R1033.

Larson, L.I., Fahrenkrug, J., Schaffalistsky de Muckadell, O.B., Sundler, F., Hakanson, R. and Rehfeld, J.F. 1976. Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral nerves. Proc. Natl. Acad. Sci. USA. 73: 3197-3200.

Lawson, D.M. and Gala, R.R. 1975. The influence of adrenergic, dopaminergic, cholinergic and serotonergic drugs on plasma prolactin levels in overiectomized estrogen treated rats. Endocrinology. 96: 313.

Lee, V.W.K. 1983. PMSG treated immature female rat- a model system for studying control of inhibin secretion. In: Factors Regulating Ovarian Function. Edited by G.S. Greenwald and P.F. Terranova. 157-161. Raven Press, New York.

Lee, W., Abbud, R., Hoffman, G.E. and Smith, M.S. 1993. Effects of N-methyl-D-aspartate receptor activation on cFos expression in luteinizing hormone-releasing hormone neurons in female rats, Endocrinology. 133: 2248-2254.

Leibowitz, S.F., Jhanwar-Uniyal, M. Dvorkin, B. and Makman, M.H. 1982. Distribution of α-adrenergic, β-adrenergic and dopaminergic receptors in the discrete hypothalamic area of rat. Brain Res. 233: 97-114.

Lein, E.L., Morrison, A., Kassarich, J. and Sullivan, D. 1986. α_2-Adrenergic control of prolactin release. Neuroendocrinology. 44: 184-189.

Leong, D.A., Frawley, S.L., Neill, J.D., 1983. Neuroendocrine control of prolactin. Annu Rev Physiol 45: 109-127.

Libertun, C. and McCann, S.M. 1976. The possible role of histamine in the control of prolactin and gonadotropin release. Neuroendocrinology. 20(2): 110-120.

Lien, E. L., Fenichel, R.L., Garsky, V., Sarantakis, D. and Grant, N. 1976. Enkephalin stimulated prolactin release. Life Sci. 19(6): 837-840.

Login, S.S. 1990. Direct stimulation of pituitary prolactin release by glutamate. Life Sci. 47: 2269-2275.

Loose, M.D., Ronnekleiv, O.K. and Kelly, M.J. 1991. Neurons in rat arcuate nucleus are hyperpolarized by $GABA_B$ and μ-opioid receptor antagonists: Evidence for convergence at a ligand-gated potassium conductance. Neuroendocrinology. 54: 537-544.

Lopez-Sanudo, S. and Arilla, E. 1994. Somatostatin receptors coupled to the inhibition of adenylyl cyclase in the rat frontoparietal cortex are modulated by α_2-adrenoceptors. Brain Res. 25: 143-146.

Luderer, U., Strobl, F., Levine, J. and Schwartz. N. 1993. Differential gonadotropin responses to N-methyl-D-aspartate (NMDA) in metestrous, proestrous and ovariectomized rats. Biol. Reprod. 48: 857-866.

MacLeod, R.M., 1976. Regulation of prolactin secretion. In: Martini L, Ganong, W.F., eds. Frontiers in neuroendocrinology, vol 4. New York: Raven Press, 169-194.

Mansour, A., Fox, C.A., Akil, H. and Watson, S.J. 1995. Opioid-receptor mRNA expression in the rat CNS: Anatomical and functional implications. Trends Neural. Sci. 19: 22-29.

Martin, T. F. J. and Tashjian, A. H. Jr. 1977. Cell culture studies of thyrotropin-releasing hormone action. In: Litwack, G. ed. Biochemical actions of hormones, vol. 4. New York: Academic Press. 270-275.

Matsushita, N., Kato, Y., Shimatsu, A., Katakami, H., Hanaihara, N. and Imura, H. 1983. Effects of VIP, TRH, GABA and dopamine on prolactin release from superfused rat anterior pituitary cells. Life Sci. 32: 1263-1288.

Matthews, M.J., Benson, B. and Richardson, D.I. 1978. Partial maintenance of testes and accessory organs in blinded hamsters by homoplastic anterior pituitary grafts or exogenous prolactin. Life Sci. 23: 1131-1138.

Mayer, M.L. and Westbrook, G.L. 1987. The physiology of excitatory amino acids in the vertebrate central nervous system. Prog. Neurobiol. 28: 197-276.

Mayer, M.L. and Westbrook, G.L. 1987. The physiology of excitatory amino acids in the vertebrate central nervous system. Progress in Neurobiology. 28: 197-276.

McCann, S.M. and Friedman, H.M. 1960. The effect of hypothalamic lesions on the secretion of luteotrophin. Endoctinology. 67: 597-608.

McDonald, J. and Johnson, M. 1990. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. Brain Res.Rev. 15: 41-70.

McGeer, E.G. and McGeer, P.L. 1976. Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamate and kainic acids. Nature. 517-519.

McGuire, J.L. and Lisk, R.D. 1971. Estrogen receptors and their relation to reproductive physiology: in The Sex Steroids, McKerns, K.W. (eds): Meredith, New York. 53.

McNeilly, A.S., Sharpe, R.M., Davidson, D.W. and Fraser, H.M. 1978. Inhibition of gonadotropin secretion by induced hyperprolactinaemia in male rat. Endocrinol. 79: 59-68.

Meites, J. 1977. Evaluation of research on control of prolactin secretion. In: Comaparative Endocrinology of Prolactin, Dellmann, H., D., Johnson, J. A. and Klanchko, D. M. Eds., Plenum Press, New York. 135:

Meites, J., Nicoll, C.S. and Talwalker, P.K.,1963. The central nervous system and the secretion and release of prolactine. In: Nalbandov AV ed. Advances in neuroendocrinology. Urbana: University of Illinois Press. 238-288.

Melis, B.G., Paoletti, A.M., Mais, V., Mastrapasqua, N.M., Strigini, F., Fruzzetti, F., Guarnieri, G., Gambacciani, M. and Fioretti, P. 1982. The effects of the GABAergic

drug, sodium valporate, on prolactin secretion in normal and hyperprolactinemic subjects. J. Endocrinol. Metab. 54: 485-489.

Melis, G.B., Fruzzetti, F., Paoletti, M., Mais, V.,Kemeny, A. Strigini, F.,Boldrini, A. and Fioretti, P. 1984. Pharmacologic activation of γ-aminobutyric acid system blunts prolactin response to mechanical breast stimulation in puerperal women. J. Endocrinol. Metab. 58(1): 201-205.

Meltzer, H.Y., Fang, V.S., Fessler, R., Simohovic, M. and Stanisic, D. 1977. In: Hanin, I. and Usdin, E. (eds) Animal Models in Psychiatry and Neurology, Oxford Press, p: 443.

Meltzer, H.Y., Simonovic, M., and Gudelsky, G.A. 1982. Effect of yohimbine on rat prolactin release. J. Pharmacol. Exp. Ther. 224: 21-27.

Mezey, E., Kiss, J.Z., Mueller, G.P., Eskay, R., O'Donohue, T.L. and Palkovits, M. 1985. Distribution of the pro-opiomelanocortin derived peptides, adrenocorticotrope hormone, α-melanocyte-stimulating hormone and β-endorphin (ACTH, α-MSH, β-END) in the rat hypothalamus. Brain Res. 328: 341-347.

Minneman, K.P. and Iverson, L.L. 1977. Morphine selectively blocks dopamine-stimulated cyclic AMP formation in rat neostriatal slices. [Proceedings] Br. J. Pharmacol. 59(3): 480-481.

Molina, P.E., Tepper, P.G., Yousef, K.A., Abumrad, N.N. and Lang, C.H. 1994. Central NMDA enhances hepatic glucose output and noninsulin-mediated glucose uptake by a non-adrenergic mechanism. Brain Res. 634: 41-48.

Molina, P.E., Williams, P. and Abumrad, N.N. 1997. Histaminergic contribution to the metabolic effects of neuroglucopenia. Am. J. Physiol. 272: R1918-R1924.

Monagham, D., Bridges, R. and Cotman, C. 1989. The excitatory amino acid recptors: Their classes, pharmacology and distinct properties in the function of the central nervous system. Annu. Rev. Pharmacol. Toxicol. 29: 365-402.

Moore, K.E. and Demarest, K.T. 1982. Tuberinfundibular and tuberohypophyseal dopaminergic neurons; in Ganong W.F. and Martini, L. (eds): Frontiers in Neuroendocrinology. New York, Ravan Press, vol 7, pp 161-189.

Moore, K.E. and Lookingland, K.J. 1995. Dopaminergic neural systems in the hypothalamus; in Bloom FE, Kupfer, D.J.(eds): Psychopharmacology: The Fifth Generation of Progress. New York, Raven Press, pp 245-256.

Morel, G. and Pelletier, G. 1986. Endorphinic neurons are contacting the tuberoinfundibular dopaminergic neurons in the rat brains. Peptides. 7: 1197-1198.

Mueller, G.P., Twohy, C.P., Chen, H.T., Advis, J.P. and Meites, J. 1976. Effects of L-tryptophan and restraining stress on hypothalamic and brain serotonin turnover and pituitary FSH and prolactin release in rats. Life Sci. 18: 715-724.

Mulder, E., Peters, M.J., van Beurden, W.M.O., Galdieri, M., Rommerts, F.F.G., Janszen, F.H.A. and van der Molen, H.J. 1976. Androgen receptors in isolated cell preparations obtained from rat testicular tissue. J. Endocrinol. 70(2): 331-332.

Mueller, G .P., Chen, H. T., Dibbet, J. A. Chen, H, J. and Meites, J. 1974. Effects of warm and cold temperatures on release of TSH, GH and prolactin in rats. Proc. Soc. Exp. Biol. Med. 147(3): 698-700.

Murai, I. and Ben-Jonathan, N. 1986. Chronic posterior pituitary lobectomy: Prolonged elevation of plasma prolactin and interruption of cyclicity. Neuroendocrinology. 43: 453-458.

Murai, I. and Ben-Jonathan, N. 1990. Acute stimulation of prolactin release by estradiol: mediation by posterior pituitary. Endocrinology. 126: 3179-3184.

Murai, I., Garria, P.A. and Ben-Jonathan, N.1989. Time-dependent increase in plasma prolactin after pituitary stalk section: Role of posterior pituitary dopamine. Endocrinology. 124: 2343-2349.

Murer, R.A. 1982. Regulation of prolactin gene expression, In Conn, P.M. (eds). Cellular Regulation of Secretion and Release. Academic Press, New York, p 267.

Nagy, G.M., Arendt, A., Banky, Z. and Halasz, B. 1992. Dehydration attenuated plasma prolactin response to suckling through a dopaminergic mechanism. Endocrinology. 130: 819-824.

Nagy, G.M., DeMaria, J.E. and Freeman, M.E. 1998. Changes in the local metabolism of dopamine in the anterior and neural lobes but not in the intermediate lobe of the pituitary gland during nursing. Brain Res. 790: 315-317.

Nakanishi, N. 1992. Molecular diversity of glutamate receptors and implications for brain function. Science. 258: 597-603.

Negro-Vilar, A., Saad, W.A. and McCann, S.M. 1977. Evidence for a role of prolactin in prostrate and seminal vesicle growth in immature male rats. Endocrinology. 100: 729-787.

Neilly, J.D. and Nagy, G.M. 1994. Prolactin secretion and its control; in Knobil, E., Neill, J.D. (eds): The Physiology of Reproduction. New York, Raven Press, pp 1833-1860.

Nicoll, C.S. 1974. Endocrinology: physiological actions of prolactin. In: Knobil, E., Sawyer, W.H., (eds). Handbook of Physiology, vol. 4, sect., 7. Washington, D.C.: American Physiological Society; 253-292.

Nicoll, C.S. 1980. Ontogeny and evolution of prolactin function. Fed. Proc. Fed. Am. Soc. Exp. Biol. 39: 2563-2566.

Nicoll, C.S. and Bern, H. A. 1971. On the actions of prolactin among the vertebrates: Is there a common denominator, in Lactogenic Hormones. Wolstenholm, G.E.W.and Knight, J., Eds., Churchill Livingstone, London. 299.

Nicoll, C.S., Tarpey, J.F., Mayer, G.L. and Russell, S.M. 1986. Similarities and differences among prolactins and growth hormones and their receptors. Am. Zool. 26: 965-985.

Nowak, L., Bregestovski, P., Ascher, P., Herbest, A. and Prochiantz, A. 1984. Magnesium gates glutamate-activated channels in mouse central neurons. Nature. 307: 462-465.

Olney, J.W. and Price, T.M. 1980. Neuroendocrine interactions of excitatory and inhibitory amino acids. Brain Res. Bull. 5 Suppl. 2: 361-368.

Paramore, D.S., Fanelli, C.G., Shah, S.D. and Cryer, P.E. 1999. Hypoglycemia per se stimulates sympathetic neural as well as adrenomedullary activity, but unlike the adrenomedullary response, the forearm sympatheticneural response is not reduced after recent hypoglycemia. Diabetes. 48: 1429-1436.

Parker, S. and Crowley, W.R. 1993. Stimulation of oxytocin release in the lactating rat by central excitatory amino acid mechanisms: Evidence for specific involvement of R, S-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid sensitive glutamate receptors. Endocrinology. 133: 2847-2854.

Pasteels, J.L. 1963. Researches morphologiques et experimentales sur la secretion de prolactin. Arch. Biol. 74: 439-553.

Pasteels, J.L., 1961. Secretion de prolactine par l' hypophyse en culture tissues. C.R. Acad. Sci. Ser. D. 253: 2140-2142.

Pasteels, J.L., Gausset, P., Danguy, A. and Ectors, F. 1972. Immunofluorescent studies on prolactin and the pituitary. In: Prolactin and Carcinogenesis.(Boyns, A.R. and Griffiths, K. eds.).

Pedron, N., Gonzalez-Unzaga, M., Galvan, R.E. and Fonseca, M.E. 1998. Effects of naloxone on serum prolactin levels in adult male rabbits. Life Sci. 63 (6): 485-488.

Pelletier, G., Leclerc, R. Puviani, R. and Polak, J.M. 1981. Electron immunocytochemistry in vasoactive intestinal peptide (VIP) in the rat brain. Brain Res. 210: 356-360.

Peters, L.L., Hoefer, M.T. and Ben-Jonathan, N. 1981. The posterior pituitary: Regulation of anterior pituitary prolactin secretion. Science. 213: 659-661.

Petralia, R.S., Yokotani, N. and Wenthold, R.J. 1994. Light and electron microscope distribution of the NMDA receptors subunit NMDAR1 in rat nervous system using a selective anti-peptide antibody. J. Neurosci. 14: 667-696

Pickford, G.E., Griffith, J., Torretti, E., Hendler, E. and Epstein, F.H. 1970. Bronchial reduction and renal stimulation of (Na^+ K^+) ATPase by prolactin in hypophysectomized killifish in freshwater. Nature. 228(269): 278-379.

Pinilla, L., Gonzales, D., Tena-Sempere, M., Aguilar, R. and Aguilar, E. 1996. Effects of N-methyl-D-aspartate and kainic acid on prolactin secretion in prepubertal female rats. Eur. J. Endocrinol. 135: 464-468.

Pinilla, L., Tena-Sempere, M., Aguilar, E. 1995. The role of excitatory amino acid pathways in the control of pituitary function in neonatally oestrogenized male rats. J. Endocrinol. 147: 51-57.

Pinilla, L., Tena-Sempere, M., Aguilar, R. and Aguilar, E. 1998. Effects of N-methyl-D-aspartic acid and kainic acid on prolactin secretion in hyper-and hypoprolactinaemic conditions. Eur. J. Endocrinol. 138: 460-466.

Plotsky, N. and Neilly, J.D. 1982. The decrease in hypothalamic dopamine secretion induced by suckling: Comparison of voltametric and radioisotopic methods of measurement. Endocrinology. 1982. 110: 691-696.

Plotsky, P.M., Gibbs, D.M. Neill, J.D. 1978. Liquid chromatographic-electrochemical measurement of dopamine in hypophysial stalk blood of rats. Endocrinolgy. 102: 1887-1894.

Pohl, C.R., Lee, L.R. and Smith, M.S. 1989. Qualitative changes in luteinizing hormone and prolactin responses to N-methyl-aspartic acid during lactation in the rat. Endocrinology. 124: 1905-1911.

Racagni, G., Apud, J.A., Locatelli, V., Cocchi, D., Nistico, G., di Giorgio, R.M. and Muller, E.E. 1979. GABA of CNS origin in the rat anterior pituitary inhibits prolactin secretion. Nature. 281: 575-578.

Radosevich, P.M., Lacy, D.B., Brown, L.L., Williams, P.E. and Abumrad, N.N. 1988. Effects of insulin-induced hypoglycemia om plasma and cerebrospinal fluid levels of ir-beta-endorphin, ACTH, cortisol, norepinephrine, insulin and glucose in the conscious dog. Brain Res. 458: 325-338.

Raiteri, M., Maura, G. and Versace, P. 1983. Functional evidence for two stereochemically different α_2-adrenoceptors regulating norepinephrine and serotonin release. J. Pharmacol. Exp. Ther. 224: 679-684.

Ravault, J.P., Courot, M., Groom, G.V., Wilson, D.W. and Gow, J.G. 1978. Prolactin and testosterone levels in the plasma of fertile and infertile men. J. Endocrinol. 76: 171-172.

Raymond, V., Beaulieu, M., Labrie, F., and Boissier, J. 1978. Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. Science. 200(4346): 1173-1175.

Reiter, R.J. and Johnson, L.Y. 1974. Depressant action of the pineal gland on the pituitary luteinizing hormone and prolactin in male hamsters. Horm. Res. 5: 311-320.

Reynolds, I., Murphy, S. and Miller, R.J. 1987. ^{3}H-labeled MK-801 binding to the excitatory amino acid receptor complex from rat brain is enhanced by glycine. Proc. Natl. Acad. Sci. USA. 84: 7744-7748.

Riddle, O. 1963. Prolactin in vertebrate function and organization. J. Natl. Cancer Inst. 31: 1039-1110.

Rivier, C., Vale, W., Liang, N., Brown, M. and Guillemin, R. 1977. Stimulation vivo of the secretion of prolactin and growth hormone by β-endorphin. Endocrinology. 100(1): 238-241.

Rondeel, J.M.M., de Greef, W.J., Visser, T.J. and Voogt, J.L. 1988. Effect of suckling on the in vivo release of thyrotropin-releasing hormone, dopamine and adrenaline in the lactating rat. Neuroendocrinology. 48: 93-96.

Rothchild, I. 1981. The regulation of the mammalian corpus luteum. Rec. Prog. Horm. Res. 37: 183-298.

Ruberg, M., Rotsztejn, W.H., Arancibia, S. Besson, J. and Enjalbert, A. 1978. Stimulation of prolactin release by vasoactive intestinal peptide. Eur. J. Pharmacol. 51: 319-320.

Said, S.I. and Mutt, V. 1991. Polypeptide with broad biological activity in the porcine small intestine. Science. 169: 1217-1218.

Said, S.I. and Porter, J.C. 1979. Vasoactive intestinal polypeptide: release into hypophyseal portal blood. Life. Sci. 24: 227-230.

Saitoh, Y., Silverman, A. and Gibson, M. 1991. Norepinephrine neurons in mouse locus coeruleus express c-fos protein after N-methyl-D, L-aspartic acid (NMDA) treatment: Relation to LH release. Brain Res. 561: 11-19.

Sasame, H.A., Perez-Cruet, J., DiChiara, G., Tagliamonte, A. Tagliamonte, P. and Gessa, G.L. 1972. Evidence that methadone blocks dopamine receptors in the brain. J. Neurochem. 19(8): 1953-1957.

Samson, W. K. and Skala, K. D. 1992. Comparison of the pituitary effects of the mammalian endothelines: vasoactive intestinal contractors (endothelin-β , rat endothelin-2) is a potent inhibitor of prolactin secretion. Endocrinology. 130: 2964-2970.

Samuels, M.H., Kleinschmidt-Demasters, B., Lillehei,K. and Ridgway, E.C. 1991. Pulsatile prolactin secretion in hyperprolactinemia due to presumed pituitary stalk interruption. J. Clin. Endocrinol. Metab. 73: 1289-1293.

Sassin, J.F. Frantz, A.G., Kapen, S. and Weitzman, E.D. 1973. The nocturnal rise of human prolactin is dependent on sleep. J. Clin. Endocrinol. Metab. 37: 436-440.

Sauder, S.E., Case, G.D., Hopwood, N.J. 1984. The effect of opiate antagonistism on gonadotropin secretion in children and in women with hypothalamic amenorrhea. Pediatr. Res. 18: 322-328.

Schally, A.V., Bowers, C.Y., Redding, T.W. and Barrett, J.F. 1966. Isolation of thyrotropin releasing factor (TRF) from porcine hypothalamus. Biochem. Biophys. Res. Commun. 25: 165-169.

Schally, A.V., Redding, T.W., Arimura, A., Dupont, A. and Linthicum, G.L. 1977. Isolation of gamma-amino butyric acid from pig hypothalami and demonstration of its prolactin release-inhibiting (PIF) activity *in vivo* and *in vitro*. Endocrinology. 100: 681-691.

Selmanoff, M. and Gregerson, K.A. 1986. Suckling-induced prolactin release is suppressed by naloxone and stimulated by beta-endorphin. Neuroendocrinology. 42: 255-259.

Seltzer, A.M. and Donoso, A.O. 1986. Histamine induced prolactin release and activity of tuberoinfundibular dopaminergic neurons in male rats. J. Neural. Trans. 65: 115-123.

Shaar, C.J., Clements, J.A. and Dininger, N.B. 1979. Effect of vasoactive intenstinal polypeptide on prolactin release in vitro. Life Sci. 25(24-25): 2071-2074.

Shaar, C.J., Frederickson, C.A., Dininger, N.B. and Jackson, L. 1977. Enkephalin analogues and naloxone modulate th release of growth hormone and prolactin – evidence for regulation by an endogenous opioid peptide in brain. Life Sci. 21(6): 853-860.

Shaar, C.R. and Clemens, J.A. 1980. The effects of opiate agonists on growth hormone and prolactin release in rats. Fed. Proc. Fed. Am. Soc. Exp. Biol. 39(8): 2539-2543.

Shimatsu, A., Kato, Y., Inoue, T.,Christofides, N.D, Bloom, S.R., and Imura, H. 1983. Peptide histidine isoleucine and vasoactive intestinal polypeptide-like immunoreactivity coexist in rat hypophysial portal blood. Neurosci. Lett. 43: 259-262.

Shimatsu, A., Kato, Y., Matsushita, N., Katakami, H., Yanaihara, N. and Imura, H. 1981. Immunoreactive vasoactive intestinal polypeptide in rat hypophysial portal blood. 108: 395-398.

Shimatsu, A., Kato, Y., Matsushita, N., Katakami, H., Yanaihara, N. and Imura, H. 1982. Stimulation by serotonin of vasoactive intestinal polypeptide release into rat hypophysial portal blood. Endocrinology. 111: 338-340.

Shin, S.H. 1979. Estradiol generates pulses of prolactin secretion in castrated male rats. Neuroendocrinology. 1979: 29: 270-275.

Shin, S.H. 1980. Physiological evidence for the existence of prolactin-releasing factor: Stress-induced prolactin secretion is not linked to dopamine receptors. Neuroendocrinology. 25: 1829-1836.

Shin, S.H. Papas, S. and Obansawin, M.C. 1987. Current status of the rat prolactin-releasing factor. J. Physiol. Pharmacol. 65: 2036-2043.

Shin, S.H. and Reifel, C.S. 1981. Adenohypophysis has an inherent property for pulsatile prolactin secretion. Neuroendocrinology. 32: 139-144.

Shome, B. and Parlow A.R., 1977. Human pituitary prolactin (hPRL): The entire linear amino acid sequence. J.Clin Endocrinol Metab. 45; 1112-1115.

Siegel, H.I., Bast, J.D. and Greenwald, G.S. 1976. The effects of Phentobarbital and gonadal steroids on periovulatory serum levels of luteinizing hormone and follicle stimulating hormone in hamster. Endocrinology. 98: 48-55.

Snyder, S.H. 1974. Drugs, neurotransmitters and psychosis. Psychopharmacol. Bull.10(4): 4-5.

Strobl, F.J., Luderer, U., Beseck, L. and Stoffel, W.H. 1993. Differential gonadotrpin responses to N-methyl-D,L-aspartate in intact and castrated male rats. Biol. Reprod. 48: 867-873.

Subramanian, M.G. and Gala, R.R. 1976. The influence of cholinergic, adrenergic and serotonergic drugs on the afternoon surge of plasma prolactin in ovariectomized estrogen-treated rats. Endocrinology. 98: 842-848.

Swanson, L. and Morgenson, G. 1981. Neural mechanisms for the functional coupling of autonomic, endocrine and somatomotor responses and adaptive behavior. Brain Res. Rev. 31: 1-34.

Swanson, L., Sawchenko, P. and Lind, R. 1986. Regulation of multiple peptides in CRF paravocellular neurosecretory neurons: Implications for the stress response. Brain Res. 68: 169-190.

Talwalker, P.K., Ratner, A. and Meites, J. 1963. *In vitro* inhibition of pituitary prolactin synthesis and release by hypothalamus extract. Am. J. Physiol. 205: 213-218.

Tappaz, M. L., Brownstein, M.J. and Kopin, I. J. 1977. Glutamate decarboxylase (GAD)_ and Gamma- aminobutyric acid (GABA) in discrete nuclei of hypothalamus and substantia nigra. Brain Res. 125: 109-121.

Tashjian, A., Barowsky, N. and Jensen, D. 1971. Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cell in culture. Biochem. Biophys. Res. Commun. 43: 516-523.

Thomas, J.A. and Keenan, E.J. 1976. Prolactin influences upon androgen action in male accessory sex organs. Singhal, R.L., Thomas, J.A.,eds. Cellular mechanism modulating gonadal hormone action. Baltimore: University Park Press; 425-470.

Thorner, M.O. ans Besser, G.M. 1978. Bromocriptine treatment of hyperprolactinaemic hypogonadism. Acta. Endocrinol. 88: Suppl. 216: 131-146.

Tolis, G., Hickey, J. and Guyda, H. 1975. Effects of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in man. J. Clin. Endocrinol. Metab. 41(5): 827-832.

Unterwald, E.M., Knapp, C. and Zukin, R.S. 1991. Neuroanatomical localization of κ_1 and κ_2 opioid receptors in rat and guinea pig brain. Brain Res. 562: 57-62.

Van Cauter, E., L'Hermite, M., and Copinschi, G. 1981. Quantitative analysis of spontaneous variations of plasma prolactin in normal man. Am. J. Physiol. 241: E355-E363.

Van Loon, G.R., Ho, D. and Kim, C. 1980. Beta-endorphin-induced decrease in hypothalamic dopamine turnover. Endocrinology. 106: 76-80.

Van Vugt, D.A. and Meites, J. 1980. Influence of endogenous opiates on anterior pituitary function. Fed. Proc. Fed. Am. Soc. Exp. Biol. 39(8): 2533-2538.

Van Vugt, D.A., Bruni, J.F. and Meites, J. 1978. Naloxone inhibition of stress-induced increase in prolactin secretion. Life Sci. 22(1): 85-89.

Vecsernyes, M., Krempels, K., Toth, B.E., Julesz, J., Makara, G.B. and Nagy, G.M. 1997. Effect of posterior pituitary denervation on prolactin and α-melanocyte-stimulating hormone secretion of lactating rats. 43: 313-319.

Veldhuis, J.D. and Hammond, J.M. 1980. Oestrogens regulate divergent effects of prolactin in the ovary. Nature. 284: 262-264.

Veldhuis, J.D. and Johnson, M.L. 1988. Operating characteristics of the hypothalmo-pituitary-gonadal axis in men: Circadian, ultradian and pulsatile release of prolactin and its temporal coupling with luteinizing hormone. J. Clin. Endocrinol. Metab. 67: 116-123.

Vekemans, M. and Robyn, C. 1975. The influence of exogeneous estrogen on the circadian periodicity of circulating prolactin in women. J. Clin. Endocrinol. Metab. 40(5): 886-889.

Vijayan, E. and McCann, S.M. 1978. Re-evaluation of the role of catecholamines in the control of gonadotropin and prolactin release. Neuroendocrinology. 25(3): 150-165.

Vijayan, E. Samson, W.K., Said, S.I. and McCann, S.M. 1979. Vasoactive intestinal peptide: evidence for a hypothalamic site of action to release growth hormone, luteinzing hormone and prolactin in conscious ovariectomized rats. Endocrinology. 104: 53-57.

Vincent, S. R., Hokfelt, T. and Wu, J. Y. 1982. GABA neuron systems in hypothalamus and the pituitary gland: immunohistochemical demonstration using antibodies against glutamate decarboxylase. Neuroendocrinology. 34: 117-125.

Voogt, L. and Carr, L, A. 1975. Potentiation of suckling induced release of prolactin by inhibition of brain catecholamine synthesis. Endocrinology. 97(4): 891-897.

Wagner, E.J., Moore, K.E. and Lookingland, K.J. 1993. Sexual differences in N-methyl-D-aspartate receptor-mediated regulation of tuberoinfundibular dopaminergic neurons in rat. Brain Res. 611: 139-146.

Wamil, A.W. and McLean, M.J. 1992. Use-, Concentration- and voltage-dependent limitation by MK-801 of action potential firing frequency in mouse central neurons in cell cultures. J. Pharmacol. Exp. Ther. 260: 376-383.

Watkins, J.C. and Evans, R.H. 1981. Excitatory amino acid transmitters. Annu. Rev.Pharmacol.Toxicol. 21: 165-204.

Watts, A.G., Sheward, W.J., Wale, D. and Fink, G. 1989. The effects of knife cuts in the subparaventricular zone of the female rat hypothalamus on oestrogen-induced diurnal surges of plasma prolactin and LH and circadian wheel-running activity. J. Endocrinol. 122: 593-604.

Weiner, R.J., Shryne, J.E., Gorski, R.A. and Sawyer, C.H. 1972. Changes in the catecholamine content of the rat hypothalamus following deafferentation. Endocrinology. 90: 867-873.

Welschen, R., Hermans, W.P. and de Jong, F.H. 1980. Possible involvement of inhibin in the interrelationship between numbers of antral follicles and peripheral FSH concentrations in female rats. J. Reprod. Fertil. 60: 485-493.

Wieloch, T. 1985. Hypoglycemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. Science. 230: 681-683.

Wilson, R. and Knobil, E. 1983. Acute effects of N-methyl-D-aspartate on the release of pituitary gonadotropins and prolactin in the female rhesus monkeys. Brain Res. 248: 177-179.

Wise, P.M., Rance, N. and Barraclough, C.A. 1981. Effects of estradiol and progesterone on catecholamine turnover rates in discrete hypothalamic regions in ovariectomized rats. Endocrinology. 108: 2186-2193.

Yang, A.B., Dure, L.S. and Penney, J.B. 1992. Excitatory amino acids in Huntington's disease. In: Simon, R.P. (eds). Excitatory Amino Acids. Vol. 9. New York Thieme Medical Publishers. 217-222.

Yavich, L., Lappalainen, R., Sirvio, J., Haapalina, A. and McDonald, E. 1997. α_2-Adrenergic control of dopamine overflow and metabolism in mouse striatum. Eur. J. Pharmacol. 339: 113-119.

Yen, S.S.C., Ehara, Y. and Siler, T.M. 1974. Augmentation of prolactin secretion by estrogen in hypogonadal women. J. Clin. Invest. 53: 650-655.

Yousef, K.A., Tepper, P.G., Molina, P.E., Abumrad, N.N. and Lang, C.H. 1994. Differential regulation of stress hormone response and glucose metabolism by NMDA and kainite. Brain Res. 634: 131-140.

Zamir, N., Palkovits, M. and Brownstein, M. 1985. Distribution of immunoreactive met-enkephaliarg^6-gly^7-leu^9 and leu-enkephalin in discrete regions of rat brain. Brain Res. 326: 1-8.

Zimmerman, E.A., Defendini, R. and Frantz, A. G. 1974. Prolactin and growth hormone in patients with pituitary adenomas: A correlative study of hormone in tumor and plasma by immunoperoxidase technique and radioimmunoassay. J. Clin. Endocrinol. Metab. 38; 577.

Zipf, W.B., Payne, A.H. and Kelch, R.P. 1978. Prolactin, growth hormone and luteinizing hormone in the maintenance of testicular luteinizing hormone receptors. Endocrinology. 103: 595-600.