STUDIES ON THE INTERACTION BETWEEN GLUTATHIONE AND HYPOTHALAMIC PEPTIDE HORMONES IN THE RAT BRAIN

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BY

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This is to certify that **I, K. Vali Pasha** have carried out the research embodied in the present thesis under the guidance of **Prof. E. Vijayan** for the full period prescribed under Ph.D Ordinances of the University.

] declare to the best of my knowledge that no part of this thesis was earlier submitted for the award of research degree of any University.

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Prof. K. Subba Rao 13/5/88
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TO MY PARENTS

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K. VALI PASHA

CONTENTS

ABBREVIATIONS

DNA	deoxyribonucleic acid
GSH	reduced glutathione
GSSG	oxidised glutathione
ACTH	adreno cortico trophic hormone
CRF	corticotropin releasing factor
LHRH	lueteinizing hormone releasing hormone
SRIF	Somatostatin
TRH	thyrotropin releasing hormone
GRF	growth hormone releasing factor
PIF	prolactin inhibiting factor
GABA	gamma amino butyric acid
LH	lueteinizing hormone
FSH	follicle stimulating hormone
Prl	prolactin
GH	growth hormone
OVX	ovariectomized
1VT	intraventricular
CSF	cerebro spinal fluid
MBH	medial basal hypothalarnus
POA	preoptic area
ME	median eminence
EDTA	ethylene diamine tetraacetic acid

DTNB	5,5'-dithiobis-2-nitrobenzoic acid
TCA	trichloroacetic acid
BSA	bovine serum albumin
R1A	radioimmunoassay
h	hours
min	minutes
sc	subcutaneous
ip	intraperitoneal
CNS	central nervous system
CCK	cholecystokinin
ARGG	anti-rabbit gamma globulin
B	bound
ng	nanograms
µmoles	micromoles
wt	weight
cAMP	3',5'-cyclic adenosine monophosphate
cGMP	3'5'-cyclic guanosine monophosphate

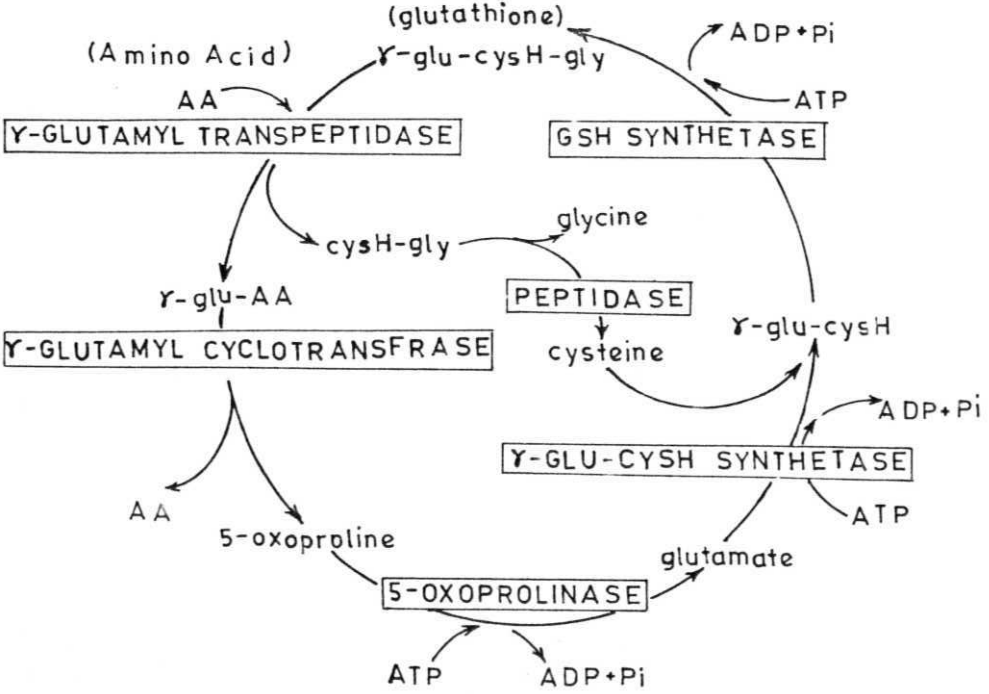
CHAPTER I

GENERAL INTRODUCTION AND REVIEW OF LITERATURE

The tripeptide gJutathione, (L-γ-glutamyl-L-cysteinylglycine ; GSH) an almost universal constituent of functioning biological systems, was discovered in 1888 by deRay-Pailhade (deRay-Pailhade, 1888). The structure of glutathione was proved by synthesis in 1935 (Harrington and Mead, 1935). It is probably the most prevalent low molecular weight intracellular thiol found virtually in all cells in appreciable concentrations ranging from 0.4 mM-12 mM. The concentration of this peptide in extracellular fluids such as blood plasma is very low. Its ubiquitous distribution suggests that glutathione may have some fundamental role in living cells. There is evidence that in the course of evolution glutathione has been adopted by various cells **for** specialized functions. This peptide now known to function directly or indirectly in many important biological phenomena including synthesis of proteins and DNA, transport, enzyme activity, metabolism and protection of cells. Recent studies have begun to delineate its metabolism and biological function (Meister and Tate, 1976 ; Meister, 1981 ; Arias and Jakoby, 1976 ; Meister and Anderson, 1983).

METABOLISM OF GLUTATHIONE : THE Y-GLUTAMYL CYCLE

Studies in Alton Meister's laboratory lead to the formulation **of the** γ-glutamyl cycle by which the synthesis and degradation of glutathione takes place (Orlowski and Meister, 1970). The cycle functions in vivo and it may be one of the systems that mediates amino acid transport. Glutathione is synthesized intracellularly by successive actions of γ-glutamylcysteine synthetase and glutathione synthetase. γ-glutamylcysteine synthetase is feedback inhibited by glutathione. Utilization of glutathione is initiated



The γ-glutamyl cycle

by γ-glutamyl transpeptidase, a membrane-bound enzyme that catalyses the transfer of the γ-glutamyl moiety to amino acid acceptors to form γ-glutamyl amino acids. The γ-glutamyl amino acids formed by transpeptidase reactions are transported in to cells. The intracellular enzyme γ-glutamyl cyclotransferase converts γ-glutamyl amino acids in to corresponding amino acids and 5-oxoproline. 5-oxoproline is converted by 5-oxoprolinase to glutamate, a reaction which is ATP dependent. The cysteinylglycine formed in the transpeptidase reaction is split by dipeptidase to cysteine and glycine. Thus a series of six enzyme catalysed reactions account for the synthesis and degradation of glutathione (Meister and Anderson, 1983).

TRANSPORT OF GLUTATHIONE

The finding of γ-glutamyl transpeptidase on one side of the membrane and of its substrate (GSH) on the other side and the finding that glutathione accumulates in extracellular fluids and plasma in a patient with γ-glutamyl transpeptidase deficiency lead to the suggestion that transport of intracellular glutathione to membrane-bound γ-glutamyl transpeptidase is a discrete step in Y-glutamylcyclc (Griffith and Meister, 1979a ; Schulman et *ah*, 1975). Transport of glutathione has been observed in perfused isolated liver preparations (Bartoli and Sies, 1978) in human lymphoid cells (Griffith et al., 1979) and fibroblasts grown in culture (Bannai and Tsukeda, 1979). Kidney uses glutathione that is present in plasma (Griffth and Meister, 1979a, 1979b). There is normally an appreciable flow of glutathione from liver to plasma and those cells that have transpeptidase activity utilize plasma glutathione. The major organs involved in this inter organ

circulation of glutathione are the liver and kidney but other organs may also participate (Anderson **et al.,** 1980).

Transport of glutathione out of cells is a property of many cells (Meister et al., 1980 ; Meister, 1981) which functions in **the** transport of cysteine sulfur between cells. It will also protect **the** cell membrane against oxidative damage by maintaining essential SH groups. This transport of glutathione may provide reducing compounds in the immediate environment of the cell.

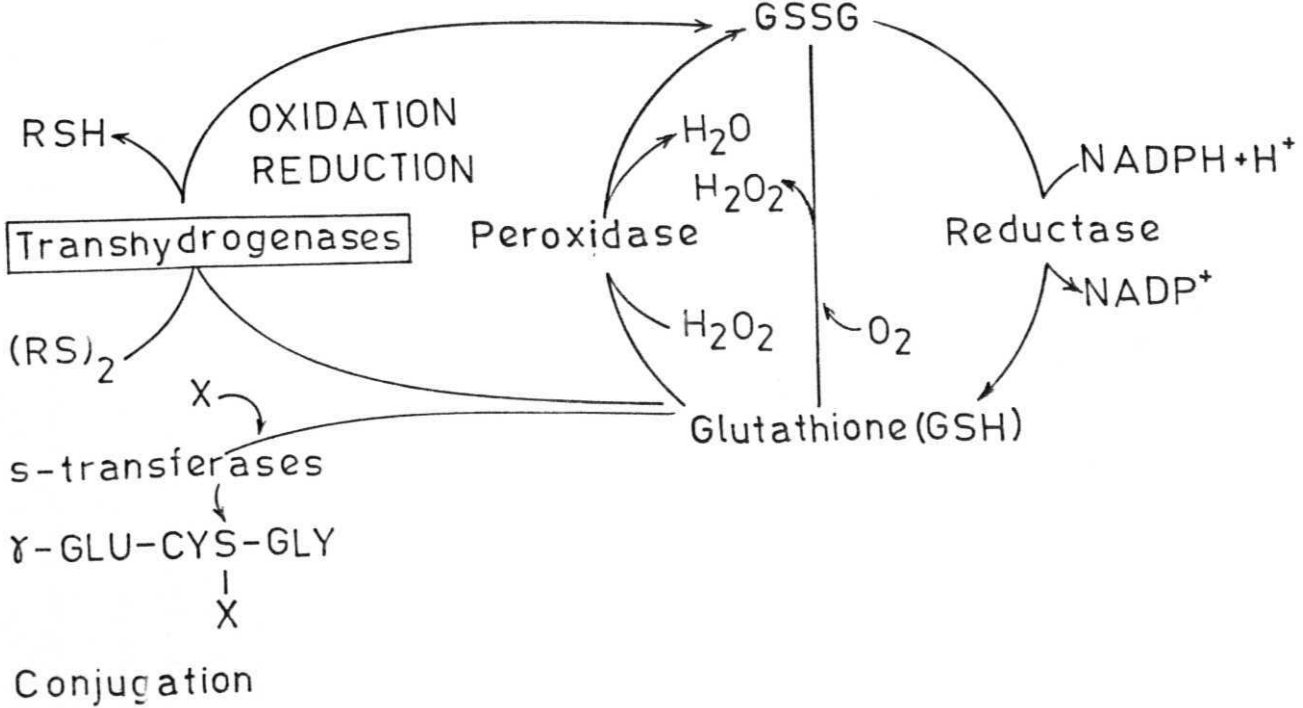
GAMMA - GLUTAMYL TRANSPEPTIDASE

Gamma-glutamyl transpeptidase, an enzyme of major importance in glutathione metabolism, initiates glutathione degradation (Meister et al., 1981 ; Tate and Meister, 1981). It can catalyse three types of reactions (a) transpeptidation, in which the γ-glutamyl moiety is transferred to an acceptor, (b) autotranspeptidation, in which **the** γ-glutamyl moiety is transferred to glutathione to form γ-glutamyl glutathione and (c) hydrolysis, in which the γ-glutamyl moiety is transferred to water. Glutathione (GSH), oxidized glutathione (GSSG), S-substituted glutathione and other Y-glutamyl compounds are substrates. The L-isomcrs of cystine, methionine and other amino acids as well as many dipeptides especially aminoacylglycines are good acceptors. Highly purified enzyme preparations have been obtained from kidney and from other tissues. Kidney exhibits the highest activity followed by pancreas, epididymis, seminal vesicle, jejunal epithelial cells, liver and spleen (Tate and Meister, 1981). The enzyme has a donor site that interacts with both L-and D-γ-glutamyl compounds. The acceptor

site consists of subsites for the cysteinyl and glycine moieties of cysteinyJ-glycine. Kinetic studies are consistent with a ping pong mechanism involving γ-glutamyl enzyme (Meister and Tate, 1976). The enzyme purified preparations are glycoproteins and exhibit heterogeneity associated with the presence of isozymes containing different amounts of sialic acid (Meister et al., 1981 ; Tate and Meister, 1981 ; Hughey et ah, 1979). The proteinase solubilized rat kidney enzyme (M 68,000) consists of two subunits (M 46,000, 22,000). The M 22,000 subunit contains the active site residues involved in the formation of Y-glutamyl cysteine intermediate. Although there is little species variation in size of the light subunit the transpeptidases fall into two groups with **respect** to the heavy subunit : (M 46,000 - 50,000) rat and rabbit ; M 60,000 (bovine, sheep, hog and human). The enzyme is inhibited by L-serine plus borate, owing to the formation of tetrahedral borate complex, a transition state analog (Tatc and **Meister,** 1978). Other inhibitors include Y-glutamyl hydrazones of α-ketoacids (Tate and Meister, 1974) and various γ-glutamyl phenylhydrazides (Griffith and Meister, 1979a). The L-and D-isomers of γ-glutamyl-(O-carboxy)phenylhydrazide are effective competitive inhibitors (Griffith and Meister, 1979a ; Minato, 1979). Glutamine antagonists, such as 6-diazo-5-oxo-L-norleucine, L-azaserine and L-(αS, 5S)-α-amino-3-chloro-4, 5-dihydro-5-isoxazole acetic acid (AT-125) are effective irreversible inhibitors of the enzyme and serve as affinity labels of the Y-glutamyl site (Allen et ah, 1980 ; Reed et al., 1980 ; Tate and Meister, 1977, 1981 ; Meister et al., 1980 ; Griffith and Meister, 1980).

FUNCTIONS OF GLUTATHIONE

Glutathione functions to form or maintain protein thiol groups



Other metabolic pathways of glutathione

which may be required for catalysis and involved in protein assembly and degradation. It provides reducing capacity to various reactions eg., formation of deoxyribonucleotides by ribonucleotide reductase (Holmgren, 1981). Intermediates such as O_2^- and H_2O_2 are formed extensively in biological systems and these produce reactive oxygen species that can lead to organic peroxide formation (Chance et a]., 1979). Glutathione protects proteins and cell membranes against these peroxides and free radicals. Glutathione peroxidase catalyses the reaction of glutathione with H_2O_2 and organic peroxides to yield oxidized glutathione (GSSG). The reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) is carried out by a widely distributed flavoprotein, glutathione reductase (Flohe, 1979). Glutathione would probably help in the prevention of peroxidative damage to cell membranes and lipids which may occur in the post anoxic period (Vali Pasha and Sadasivudu, 1984).

Glutathione reacts with a large number of foreign compounds leading to the formation of glutathione conjugates, a process mediated by glutathione-S-transferases (Boyland and Chasseaud, 1969 ; Jakoby, 1980, 1981). Such a process may lead to detoxification of drugs and peroxides (Saneto et ah, 1982 ; Chasseaud, 1979). Glutathione derivatives are also formed with endogenous metabolites. For eg., Estradiol-17β conjugates with glutathione in rat liver preparations and in vivo (Kuss, 1969, 1971 ; Jellinck et ah, 1967 ; Elce and Harris, 1971). There are also reports on the conjugation of glutathione with prostaglandins (Cagen et al., 1976 ; Cagen and Pisano, 1979 ; Chaudhari et ah, 1978) and Leukotrienes (Orning et al., 1980 ; Hammarstrom et al., 1980) which may have important physiological functions.

Glutathione may be involved in peptide chain separation breaking

disulfide bonds, a reaction catalyzed by glutathione transhydrogenases (**Varandhani** and Naiz, 1970, 1976 ; Varandani, 1973, 1974). Such a process may be involved in the inactivation or generation of active neuropeptides. Although glutathione is present in various cells it has been adapted for specialized functions by tissues. However the role of glutathione in case of central nervous system is much less understood. It is probable that glutathione may be involved in a variety of functions by this tissue. The glutathione levels of blood and tissues are influenced by adrenal, thyroid, pituitary and pancreatic hormones. Within the recent years glutathione is implicated in release of hormones (prolactin and growth hormone) from isolated pituitary secretory granules and can cause granule dissolution (Lorenson and Jacobs, 1982). Glutathione levels were seem to decrease with age **in** different organs studied (Hazelton and Lang, 1980).

HYPOTHALAMIC RELEASING FACTORS

The demonstration that nerve fibres secrete chemical agents was a major advance in ncurophysiology. Professor G.W. Harris is perhaps the first scientist to focus attention on the role of hypothalamus in control of the pituitary (Harris, 1948). Harris carried out pioneering experiments pointing to the neurohumoral control of the pituitary via the hypophyseal portal system of veins (Harris, 1950). In the early 50's the first evidence for peptidergic control of anterior pituitary secretion emerged with the finding that commercial extracts containing vasopressin and even synthetic vasopressin could release ACTH in animals in which the ubiquitous stress response was blocked by median eminence lesions (McCann and Brobeck, 1954) or by pharmacological treatments which would inhibit the stress res-

ponse (Martini, 1966). It was also observed that neurohypophyseal extracts would release ACTH from pituitaries incubated in vitro but only in the presence of norcpinephrine (Saffran and Schally, 1955). Saffran et al., (1955) claimed separation of a substance different from vasopressin by chromatography of posterior pituitary fractions which would release ACTH in vitro and named the putative neurohormone corticotropin releasing factor (CRF). The search for other putative releasing factors to control the release of other pituitary hormones was intensified. As a result, LH releasing hormone (LHRH) was soon observed which would deplete ovarian ascorbic acid on systemic administration and evoke ovulation following its injection directly into the pituitary (McCann et al., 1960 ; Harris, 1961). Soon the other factors thyrotropin releasing hormone (TRH), growth hormone releasing factor (GRF), prolactin inhibiting factor (PIF) were discovered (McCann et ah, 1974). An inhibitor was also discovered which selectively suppressed growth hormone release (Krulich et al., 1972). An intensive attempt to purify, isolate and determine the structure of these new peptides was undertaken in several laboratories. As a result of these herculean efforts in elucidating the structures of TRH and LHRH (Brazeau et al., 1973) Drs. Schally and Guillemin shared the Nobel Prize in 1977. Susan Leeman and coworkers while trying to purify CRF, took advantage of the sialogogic and blood pressure lowering actions of some of their fractions to purify, characterize and synthesize two important neuropeptides, substance P and neurotensin respectively (Chang and Leeman 1970 ; Carraway and Leeman, 1973).

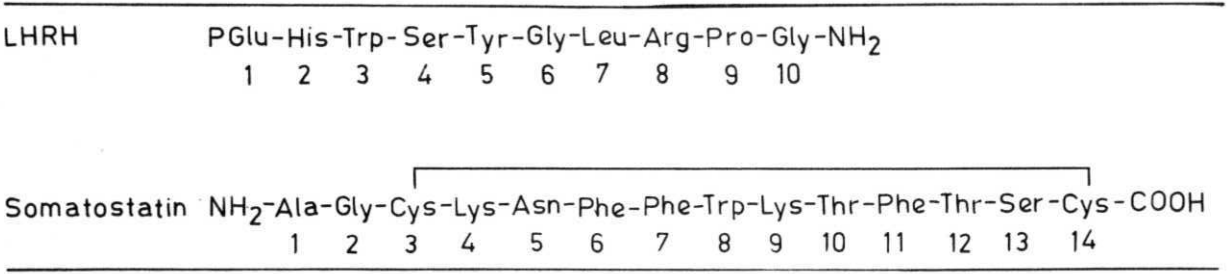
NEUROTRANSMITTERS IN THE CONTROL OF ANTERIOR PITUITARY FUNCTION

With the realization that the neurohumoral control of the pituitary

Neurotransmitters	Acetylcholine, norepinephrine, cpinephrine, Dopamine, 5-Hydroxytryptamine, GABA, **glutamic** acid, aspartic acid.
Hypothalamic Releasing Hormones/Peptides	TRH, GnRH, SRIF, **CRF, GRF,** Vasopressin, Oxytocin, Enkephalins.
Pituitary peptides	ACTH, 3-endorphin, α-MSH, Prolactin, Growth hormone.
Gastrointestinal peptides	VIP, CCK, Gastrin, substance P, Neurotensin, insulin, glucagon, Bombesin, secretin, motilin.
Others	Angiotensin II, bradykinin, **Carnosine,** sleep peptide(s), calcitonin, neuropeptide Y.

Table I. Categories of ncurotransmitters and peptides present **in** the mammalian Central Nervous System.

itself was primarily peptidergic attention was refocussed on factors affecting the release of various hypophysiotropic factors. Using histofluourescence methods for catecholamines the existence of the tuberoinfundibular dopaminergic tract (Fuxe, 1964) and of the projections from the brain stem of noradrenergic (Ungerstedt, 1971) and adrenergic axons (Hokfelt et al., 1974) into various sites within the hypothalamus was demonstrated. Birge et ah, (1969) and Macleod (1969) first demonstrated that dopamine acts directly on the pituitary to supress prolactin release. High affinity dopamine receptors have been found on the gland (Cheung and Weiner, 1976). Considerable evidence still suggests the existence of a peptidic prolactin inhibiting factor as well (McCann et al., 1974). The existence of high affinity muscarinic cholinergic receptors on the gland has been demonstrated and indeed acetylcholine can increase growth hormone and depress prolactin release from pituitary cells in vitro (Mukherjee et al., 1980). Recently high affinity GABA receptors have also been found in the anterior lobe raising the possibility that GABA may have important actions directly on the gland (Grandison and Guidotti, 1979). High concentrations of GABA were shown to inhibit prolactin release by pituitaries incubated in vitro (Schally et ah, 1977). There may be a number of small molecular weight transmitters which directly alter pituitary hormone release. The list may include not only dopamine and GABA but acetylcholine, histamine, serotonin, norepinephrine and epinephrine (McCann et al., 1979). It is now known that there may be as many as 30-40 neurotransmitters and peptides (Table-I) present in the mammalian central nervous system.



Structure of LHRH and Somatostatin

LUTEINIZING HORMONE RELEASING HORMONE (LHRH)

LH releasing activity was first found in hypothalamic extract utilizing the ovarian ascorbic acid depletion assay of Parlow to measure LH released following injection of acid extracts of stalk median eminence (McCann et al., 1960). The active component was rapidly purified and its structure was determined in Schally's laboratory by Matsuo and confirmed by synthesis (Matsuo et al., 1971).

LOCALIZATION OF LHRH

LHRH cell bodies are localized in the septal-preoptic and anterior hypothalamic areas and the axons of these neurons project particularly to the external layer of the anterior median eminence by passing caudally along a predominantly mediobasal course. The axons also project to the organum vasculosum lamina terminalis (OVLT) and to more caudal structures (Barry et al., 1974). LHRH axons have also been found in the brain stem regions thought to be involved in mating behaviour (Samson et al., 1980). Recent studies show a distribution of LHRH like peptides outside the nervous system particularly in reproductive organs like testicular lymph (Sharpe, 1980), interstitial cells of the testis (Taurkelson et **ah,** 1981) placenta (Khodr and Siler-Khodr, 1980) and Milk (Koch and Baram, 1977).

ACTIONS OF LHRH ON THE PITUITARY

LHRH acts on the pituitary to promote rapid release of LH and to a lesser extent FSH from the gland. Pulse injection of LHRH may lead to only LH release however, prolonged exposure to the peptide leads to

FSH release (McCann, 1974). The FSH releasing potency is often of the order of 20% of the LH releasing potency ; however, the FSH releasing potency varies depending on the hormonal state of the animal and the frequency of LHRH injections. Responsiveness to LHRH is quite high in the castrate (Ajika et ah, 1972). Responsiveness to LHRH declines rapidly in rat, man and other species following administration of estrogen to the castrate female and this is followed by augmented responsiveness to the peptide (Libertun et *ah*, 1974 ; Fink, 1979). Testosterone has an inhibitory action supressing responsiveness (Fink, 1979). There is clear evidence that there is increased LHRH release from the hypothalamus at the time of preovulatory LH surge in rat (Fink, 1979). The evidence for this phenomenon in primates is suggestive (Neill, 1980).

PUTATIVE SYNAPTIC TRANSMITTERS INVOLVED IN THE CONTROL OF LHRH RELEASE

The LHRH neurons are in potential synaptic contact with a host of other putative transmitters, monaminergic and peptidergic. There is an overlap of the LHRH terminals in the median eminence with those containing dopamine, somatostatin and TRH. The median eminence also contains terminals which presumably contain GAB A, histamine, serotonin and norepinephrine (Elde and Hokfelt, 1979). Studies with basal hypothalamus incubations with dopamine showed that dopamine cause release of LHRH (Schneider and McCann, 1969). Fuxe and Hokfelt (1969) on the other hand showed that dopamine has an inhibitory role in control of LHRH release. In vivo studies with intraventricular injection of dopamine clearly showed a stimulatory role for LH release (Schneider and McCann, 1970). Vijayan and McCann

(1978c) demonstrated both stimulatory and inhibitory effects of dopamine. High doses of dopamine was shown to inhibit LH release in rats (Gnodde and Schuiling, 1976). However, Negro-Vilar et ah, with in vitro median eminence preparations showed that dopamine had a stimulatory effect which was suppressed by the dopamine receptor blocker, pimozide (Negro-Villar et al., 1979). Norepinephrine and epinephrine were shown to release LHRH after their intraventricular injection in to ovariectomized steroid primed rat (Vijayan and McCann, 1978c). Recent findings indicate there is an increased turnover of norepinephrine in regions involved in control of LH release at the time of preovulatory LH surge and that this preceded by an increased turnover of dopamine suggesting that both dopamine and norepinephrine may be involved in the preovulatory release of LHRH (Rance et al., 1981). Serotonin may have a inhibitory action in the castrate (Schneider and McCann, 1970) but serotonergic tone may be necessary for preovulatory LH release (Hery et al., 1976). GABA clearly acts to stimulate LH release however, since result with the GABA blocker bicuculine were equivocal the physiological significance of GABA in LH release remains to be established (McCann et al., 1981). Histamine can stimulate LH release ; however, since the dose required is large it is quite possible that this may not have a physiological significance. A possible role for a acetylcholine in generation of the pulsatile release is also evidenced by the ability of acetylcholine to increase LH release in castrates and of the muscarinic blocker atropine to suppress LH release in the castrate (Fiorindo et al., 1974 ; Vijayan and McCann, 1980). The factors responsible for the initial appearance of LHRH are not well understood. However it is known that

radioimmunoassayable LHRH content oi infantile rats are distinctly lower than those of immature or older rats (Araki et al., 1975 ; Lumpkin et al., 1980).

SOMATOSTATIN

While screening fractions obtained in purificauon of growth hormone releasing factor (GRF) by Sephadex G-25 gel filtration, fractions were found which inhibited GH release by pituitaries incubated in vitro. The inhibitory factor named GH-inhibiting factor (GIF) was localized to various sites within **the** hypothalamus (Krulich et al., 1972). Brazeau et al., (1973) later isolated, characterized and synthesized a tetradecapeptide with selective GH release inhibiting action which was renamed as somatostatin.

DISTRIBUTION OF SOMATOSTATIN

Hypothalamus contains the highest concentration of somatostatin (Krulich et al., 1972). Somatostatin is also widely distributed in the lirnbic system in the amygdala, hippocampus, nucleus accumbens and olfactory tuburcle (Elde, 1979). Immunoreactive fibres have been localized to the caudate nucleus, cerebral cortex, substantia gelatinosa of spinal trigeminal nucleus and also in the dorsal horn of the spinal cord. Somatostatin is also localized in the retina (Shapiro et al., 1979) and throughout the gastrointestinaltract (Brazeau et al., 1973). D-cells of pancreas also contain somatostatin which inhibits insulin and glucagon release (Elde, 1979 ; Koerker et **ah,** 1974).

ACTIONS OF SOMATOSTATIN ON THE PITUITARY

Somatostatin has a dramatic inhibitory effect on the release of GH by the adenohypophysis (Krulich et al., 1972). TSH release is also subs-

tantially inhibited by somatostatin (HaJJ **et** al., 1978). It is possible **that** given in sufficient dosage the inhibitor could block release of all the pituitary hormones. In rat somatostatin blocks induction of GH release by pentobarbital anaesthesia, electrical stimulation of the ventromcdial and dorsolateral amygdala and episodic GH surges (Tannerbaum et ah, 1978). The effects of somatostatin on GH levels are **short** lived because of the rapid decrease of hormone from the circulation.

REGULATION OF THE RELEASE OF SOMATOSTATIN FROM HYPOTHALAMUS

Low doses of dopamine released somatostatin from the median eminence and **the** effects were blocked by **the** dopamine receptor bJocker **pimozide** whereas higher doses of norepinephrine were stimulatory and these effects were blocked by the alpha receptor blocker phentolamine (Negro-Vilar et ah, 1978a).GABA inhibited somatostatin release from **the** hypothalamic cells in culture and the action was blocked by **bicuculine** (Gamse et **ah,** 1980). Opiod peptides may inhibit the release of somatostatin (Drouva et **ah,** 1981). Somatostatin concentrations increased after intraventricular injections of dopamine, norepinephrine or acetylcholine but was unaffected by serotonin **(Chihara** et al., 1979). Increased somatostatin has been detected in hypophyseal portal blood of **the** rat following lateral ventricular injection of neurotensin (Abe et al., 1981). Somatostatin may also act via an ultra short loop feedback to suppress its own release since intraventricular injection of the peptide led to a paradoxical rise in plasma **GH** (Lumpkin et ah, 1981).

ACTIONS OF SOMATOSTATIN ON THE NERVOUS SYSTEM

The wide spread distribution of the peptide within the central nervous system certainly speaks for CNS actions. It was first shown that somatostatin will antagonize strychnine convulsions and enhance the duration of barbiturate anaesthesia (Brown and Vale., 1975). It also has actions in a number of other behavioral tests (Kastin et al., 1978). Intraventricular injection of the peptide inhibited both feeding and drinking behaviour suggesting possible inhibitory roles in the control of these vegetative functions which appear reasonable since the peptide is localized in the vicinity of **ventromediaJ** and lateral hypothalarnic areas known to be involved in these behaviour (Vijayan and McCann, 1977). Somatostatin has been shown to have inhibitory effects on firing of central neurons. However, increase firing has been found recently **in** a number of loci **in** the rat cortex (Phillis and Kirkpatrick., 1980). Somatostatin inhibits release of acetylcholine in myenteric plexus (Guillemin, 1976) and also inhibits release of labelled norepinephrine from slices of hypothalamus but not cortex (Gothert, 1980).

SCOPE OF THE PRESENT INVESTIGATION

From the foregoing review of literature it is evident that there is very scanty information regarding the biological role of this tripeptide in brain. A number of peptide releasing and/or inhibiting hormones ranging from 3-39 amino acids have been characterized from the brain and found to be causing either the release or inhibition of anterior pituitary hormones. Some of these peptides were shown to be present in tissues other than brain and pituitary. The biological role of glutathione, an ubiquitous tripeptide,

present in the central nervous system has not been investigated in any detail either in the brain or in the function of the hypothalamo-hypophyseal axis. It is therefore thought that

1. a study regarding its distribution and the levels in different brain regions and at varying ages would throw light on its biological function, if any in the brain.

2. a study of the effects of intraventricular administration on anterior pituitary hormone release would provide information regarding its action, if any, on the hypothalamo-hypophyseal axis.

3. the observed pubertal increase in the content of hypothalamic glutathione prompted a study on its **interaction** with gonadotrophic hormones and the established gonadotropin releasing hormone, LHRH.

4. furthermore it was thought that a study on the release of other pituitary hormones other than gonadotropins may provide information regarding the biological role of glutathione.

5. to understand the interaction between glutathione in hypothalamus with **LHRH** and with gonadotropins the effects of intraventricular administration of **LHRH** and intracerebral administration of FSH on the glutathione levels in hypothalamus was also made.

6. Hypothalamic concentration of GABA which is known to be involved in the release of anterior pituitary hormones was evaluated after intraventricular glutathione injection.

7. As glutathione is metabolically involved in the formation of γ-glutamyl derivatives by γ-glutamyl transpeptidase the activity of this enzyme was studied following the administration of LHRH and somatostatin, which is a potent inhibitor of release of GH and which contain disulfide bond and also following administration of FSH. Such a study would indicate the interaction between these hormones and glutathione at the cellular level.

CHAPTER II

MATERIALS AND METHODS

ANIMALS

All the experiments were performed in colony bred rats, derived from Wistar strain, raised in our animal facility. Adult, ovariectomized (OVX), OVX-estrogen, progresterone primed and immature female rats of age 21, 30, 40 days old, pubertal rats (42 days oid) and postpubertal rats (43 days old) were used. They were fed standard rat pellet (Lipton India Ltd., Bangalore, India) ad libitum with free access to drinking water. Rats were housed in an air conditioned room (25 ± 2°C) and were maintained under controlled conditions of light (12 hrs light : 12 hrs dark).

SURGICAL PROCEDURES

a. Ovariectomy

Adult female rats weighing 180 - 200 g were bilaterally overiecto-mized under light ether anaesthesia using semi-sterile conditions. The animals were used 3 - 4 weeks after ovariectomy.

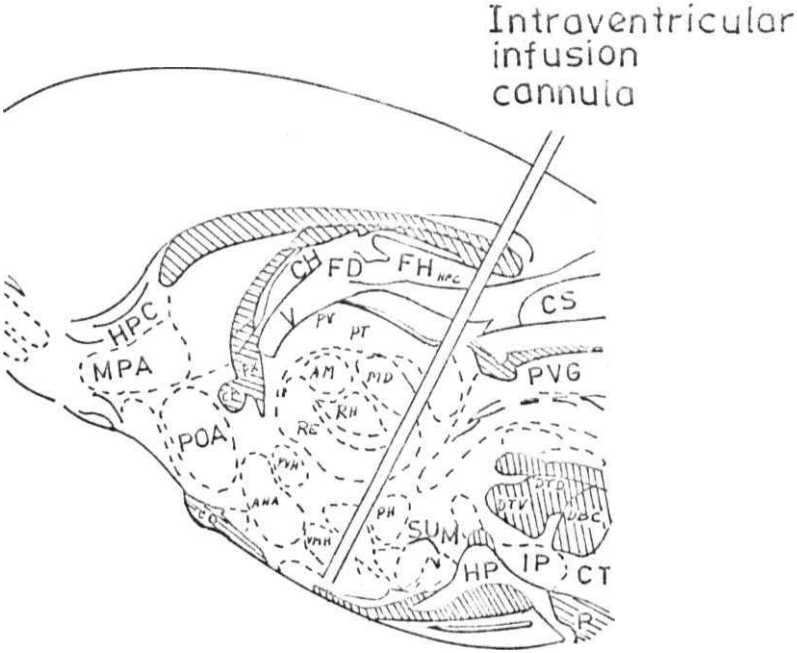
b. Implanation of third ventricular (IVT) cannula

Cannulae were prepared from 23 guage stainless steel tubing (Small Parts Inc. Miami, USA). Each cannula was 17 mm in length and had a flat tip with a beveled edge and was provided with a mandril to prevent its obstruction. Cannulation was performed following the procedure described by Antunes-Rodrigues and McCann (1970) and Vijayan and McCann (1978a & b). The animals were anaesthetized with nembutal (Abbot Labs, USA 40 mg/kg, ip) and the rat's head was fixed on a David-Kopf rat stereotaxic instrument. The following coordinates were used for the third ventricle

anterior-posterior = 1.3 mm behind bregma, lateral = just on the midline (above the superior longitudinal sinus) and vertical = 0.3 mm above the base of the skull. The cannula was mounted in the stereotaxic instrument with the aid of a stainless steel wire fitting exactly its inside diameter which served as the cannula guide. Two small holes were drilled in the adjacent parietal and frontal bones. Brass microscrews were screwed firmly into the holes to serve as an anchor for the dental cement. The cannula was introduced through a hole over the superior longitudinal sinus after the sinus was pulled gently to the left side with a hypodermic needle. This procedure prevented rupture of the sinus and consequent hemorrhage. The cannula was lowered to the skull base and then raised 0.3 mm. Its location in the 3rd ventricle was confirmed when cerebrospinal fluid (CSF) flowed continuously from the cannula. A small amount of dental cement was placed around the cannula and screws. Finally, after removing the guider mounted in the stereotaxic instrument an additional amount of cement was added around the cannula to cover the screws completely. A small amount of neosporin ointment was mixed along with the dental cement to prevent bacterial infection. The cannula was now firmly fixed into position. The skin was left unsutured and the animals were returned to their own individual cages until the day of the experiment. The mandril was removed every other day rinsed in isotonic saline and returned to its original position so as to maintain the cannula clean and to get the animal acquainted with the experimental procedure to be followed (Mistry and Vijayan, 1987).

The intraventricular cannulated rats were primed with estradiol benzoate (50 μg SC) and progesterone (25 mg SC) 72 hours before intraventri-

Fig. 1. Diagram showing intravcntricular injection cannuJa. A 28 guage cannuJa was placed inside the chronically impJanted 23 guage guide tube at time of injection.


Intraventricular
infusion
cannula
CH
FD
FH
CS
HPC
PV
PT
MPA
PVG
POA
SUM
HP
IP
CT

cular injection (Vijayan and McCann, 1978b).

c. **Intraventricular Injection**

Five to seven days after the cannulation, third ventricular injection of the peptide and or control medium was performed according to the following procedure. The mandril was removed and the inner cannula of the same length was introduced. The cannula was connected by a **polyethylene** tube to a 10 µl Hamilton microsyringe filled with freshly prepared test material to be injected. After 60 seconds with the inner cannula in its position in the animal's brain the test solution was injected slowly in a volume of 2 µl while the animal was freely moving around. The inner cannula was removed approximately 60 seconds later so that the entire test substance reached the third ventricle.

Rats were killed by instant decapitation at different time intervals following injection of test substance and/or control medium. Trunk blood was collected into heparinized tubes and plasma was separated by centrifugation at 4°C and stored frozen. The frozen plasma samples were later assayed for hormones by radioimmunoassay. Brains were rapidly removed, cerebral cortex, cerebellum and brain stem were quickly dissected out as per the procedure of Sadasivudu and Lajtha (1970). Hypothalami were dissected out as a single block which included the proptic area and was limited laterally by the hypothalamic fissures and posteriorly by the mamillary bodies. The upper section of the block was cut 3-4 mm **from** the basal surface of the hypothalamus. Thus the hypothalamus included medial basal hypothalamus (MBH), preoptic area (POA) and median eminence (Vijayan, 1974).

CHEMICALS

Luteinizing hormone releasing hormone (LHRH), somatostatin (SRIF), Gamma-glutamyl para-nitroanilide, glycylglycine, , 5-5'-dithiobis-2-nitro-benzoic acid (DTNB), glutamic acid, gamma aminobutyricacid (GABA), scphadex G-75, sodium azide, chloramine-T, heparin, bovine serum albumin were purchased from Sigma Chemical Company, St. Louis, USA. Kits for RIA of LH, FSH and Prl were obtained from the National **Pituitary** Agency, National Institute of Arthritis Metabolism and Digestive diseases (NIAMDD), Bethesda, USA. Ninhydrin was purchased from Fluka, Switzerland. All other chemical used were of analytical grade.

ESTIMATION OF GAMMA GLUTAMYL TRANSPEPTIDASE ACTIVITY

The activity of gamma glutarnyl transpeptidase was estimated according to the method of Tate and Meister (1974) as described by Vali Pasha and Sadasivudu (1984). The assay mixture (2.0 ml) consisted of 80 micromoles of Tris-Hcl buffer (pH 9.0), 150 micromoles of NaCl, 40 micromoles of glycylglycine, 5 micromoles of gamma-glutamyl para-nitroanilide and 0.4 ml 2% homogenate (in 0.25 M sucrose). After 30 min of incubation at 37°C **the** reaction was terminated by the addition of 2 ml of 10% acetic acid. A control was set up having all the above ingradients except that the homogenate was added after the addition of 10% acetic acid. After centrifugation at 5,000 rpm for 20 min the absorbance of clear supernatant was read at 410 nm. The enzyme activity was expressed as micromoles of paranitroaniline liberated per gram weight of tissue per hour with reference to a standard graph plotted using different concentrations of paranitroaniline and treating it with acetic acid.

ESTIMATION OF SULFHYDRIL GROUPS IN THE TISSUE

The totaJ sulfhydril groups and non-protein sulfhydril groups were estimated according to the procedure of Sedlak and Lindsay (1968). A known weight of the tissue was homogenized in 5.5 ml of cold EDTA (0.02 M) using a potter-Elvehjem homogenizer with a teflon pestle.

ESTIMATION OF TOTAL SULFHYDRIL GROUPS

0.5 ml of the above homogenate was mixed with 1.5 ml of 0.2 M tris buffer (pH 8.2). The mixture was then treated with 0.1 ml 5-5'-dithiobis-2-nitrobenzoic acid (DTNB, 0.01 M). 7.9 ml of absolute **methanol** was then added to make up the volume to 10 ml and centrifuged for 15 minutes at 3,000 rpm. The absorbance in the supernatant was read at 412 nm against a reagent blank having 0.5 ml of EDTA (0.02 M) in place of homogenate and treated as **in** the case of homogenate described above.

DETERMINATION OF REDUCED GLUTATHIONE (Non-protein sulihydril groups)

5 ml of the above EDTA hornogenate was mixed with 4 ml of distilled water followed by the addition of 1 ml of 50% TCA after shaking intermittently for 10 min using a vortex mixer. The solution was centrifuged at 3,000 rpm for 15 min. 2 ml of the supernatant was mixed with 4 ml of 0.4 M tris buffer (pH 8.9) followed by the addition of 0.1 ml DTNB (0.01 M). The absorbance was measured at 412 nm within 5 min against a blank containing 2 ml of EDTA (0.02 M) in place of TCA supernatant and mixed with 4 ml of 0.4 M tris buffer (pH 8.9) and 0.1 ml of DTNB (0.01 M). The

concentration of sulfhydril compound was determined using the molar extinction coefficient of 13,100.

ESTIMATION OF GLUTAMATE AND GABA

Glutamate and GABA contents were estimated by paper chromatography as described by Chandrakala et al., (1987). Immediately after decapitation, the tissues were separated and weighed. The tissue was homogenized in .5 ml of ice coJd 80% ethyl alcohol and centrifuged at 8,000 rpm at 0°C for 20 min. The clear supernatant was evaporated to dryness at 70°C-80°C and the residue was dissolved in 200 µl of distilled water. The amino acid content was determined by paper chromatography using butanol : acetic acid : Water (65 : 15 : 25) as the solvent and multiple developments as given Sadasivudu and Lajtha (1970). Color was developed by ninhydrin (0.25% in acetone with pyridine). The amino acids were eluted in 3 ml of 75% alcohol (with 0.005% $CuSo_4$) and the color was measured at 515 nm. Amino acid content was expressed as µmoles of amino acid/gram wet weight of tissue.

ASSAY OF HORMONES

Plasma levels of LH, FSH and Prl were measured by radioimmunoassay (RIA) using a double antibody procedure as standardised in our laboratory (Babu, 1982). Radioimmunoassay kits for rat LH, FSH and Prl were obtained from the NIAMDD-NIH pituitary hormone distribution programme. Radioimmunoassay was performed according to the guidelines provided with the kit for each hormone.

RIA OF **LH**

The LH kit consisted of

1. Rat luteinizing hormone antigen, highly purified for iodination NIAMDD - rLH - 15.
2. Rat luteinizing hormone antiserum (rabbit) NIAMDD-antiserum (rabbit) NlAMDD-anti-r-LH-S-6.
3. Rat luteinizing hormone reference preparation (NIAMDD-r-LH-RP-1).

PREPARATION OF THE GEL

Five grams of sephadex G-75, was added to 100 ml of phosphosaline buffer (PBS) (0.01 M PO_4, 0.05 M NaCl, 0.1% sodium a/.ide, pH 7.6) and stirred for 30 min using a magnetic stirrer. It was (a) kept in a boiling water bath for .5 h (b) allowed to stand 72 h at room temperature (c) stored in a refrigerator upto 4 weeks (d) placed at room temperature for *2k* h before use.

PREPARATION *OF* THE COLUMN

1. Ten ml of pipettes were used. The mouthpiece was cut off.
2. Tubes were scrupulously cleaned with chromic acid, hot water, tap water and double distilled water and dried.
3. A three way stopcock (Pharmaseal, Puerto Rico, USA) was attached to the tube by a 4 cm long latex tubing. Glass wool was placed in the tip of the tube.

4. The tube was washed twice with phosphosaline buffer and filled upto the 7 ml mark.

5. The gel was continuously stirred using a magnetic stirrer to keep the suspension homogenous.

6. The gel was pipetted from the bottom of the flask as a well mixed slurry. When settling was under way, the outlet was opened and allowed to run freely. The slurry was continuously added as needed. The top was never allowed to settle **before** adding more slurry. The column was filled to a height of 15-20 cm. About 2 ml of buffer (PBS) was left at the top of the column. On the day **of** iodination (maximum 4 h before use) the column was equilibrated with 1 ml of *2%* bovine serum albumin (BSA) in PBS and then washed with PBS. After a single use the column was discarded.

IODINATION OF RAT LH

Reagents

1. Iodine, carrier free, as sodium iodide with specific activity of 400 mCi/ml suitable for iodination of protein.

2. 0.5 M sodium phosphate buffer, pH 7.6.

3. Chloramine-T (5 mg/10 ml of 0.05 M PO_4, pH 7.6 buffer).

4. Sodium metabisulfite ($Na_2S_2O_5$) 25 mg/10 ml of 0.05 M PO_4, pH 7.6 buffer.

Chloramine-T and sodium metabisulfite were prepared freshly just prior to use.

1 mCi of I was added to a small disposable glass vial used as the reaction vessel. 25 µl of 0.5 M PO **buffer** pH 7.6 was added. 2 µg of NIAMDD-r-LH-1-5 **in** 20 µl of PO buffer was added next. 10 µl of chloramine T was then added. The vial was then agitated for 50 seconds **after** which 25 µl f sodium metabisulfite was added. The entire reaction **mixture** was applied to the sephadex G-75 column. The column was then eluted with phosphosaline (0.01 PO. 0.15 M NaCl buffer, pH 7.6). Fractions of 0.5 ml were collected in test tubes containing 50 µl of 2% BSA in PBS buffer. These fractions were counted in a Packard autogamma scintillation spectrometer. Two peaks of radioactivity were detected. The first peak began at tubes 3-4 and trailed off by tube 6. A second peak containing free I began at about tube 7. The iodinated rat LH was contained in the first peak (tubes 4-5). The fraction high on the trailing shoulder of this peak (tubes 4 and 5) contained the most immunoreactive and least damaged rat LH. This fraction was added to buffer in order to give 10,000 cpm per 100 µl, and stored at -20°C until use.

DOUBLE ANTIBODY RIA PROCEDURE

The following steps were performed in sequence for the assay of plasma LH.

1. 10 X 75 mm disposable test tubes were used.
2. Buffer (1% BSA in 0.01 M PO_4, 0.15 M NaCI, 0.1% sodium azide, pH 7.6) was added to each tube in sufficient quantity to produce a final volume of *0.7* ml.

3. (a) 25. µl plasma to be assayed was added or

(b) The reference preparation (NIAMDD-r-LH-RP-1) was dissolved in 1% BSA in phosphosaline and added in doses ranging from 1,000 ng to 1 ng per tube, in sufficient detail (1,000, 500, 250, 100, 50, 25, 10, 5, 2.5 and 1 ng) so that the entire curve can be constructed **graphically.**

4. Iodinated rat LH was added such that approximately 10,000 cpm were contained in 100 µl of 0.1% BSA-phosphosaline buffer.

5. 200 µl of the antiserum (NIAMDD-r-LH-S-6) in a final dilution of 1 : 40,000 in 3% normal rabbit serum **(NRS)** - 0.05 M EDTA-**PBS** was added (at these dilutions, the antiserum was observed to bind 25% of the labelled rat LH ($B - \frac{Z}{TC} \times 100$, see below).

6. In some tubes 200 µl buffer and 200 µl 3% NRS-EDTA-PBS and 100 µl label were added to serve as background.

7. In a few tubes 200 µl buffer and 100 µl label and 200 µl antiserum were added to serve as zero (100% binding Z).

8. In 2 or 3 tubes 100 µl label was taken to get the total counts (TC).

9. Tubes were agitated on vortex mixer.

10. Tubes were incubated for 24 h at room temperature.

11. At the end of this period, 200 µl of goat anti rabbit gamma globulin (ARGG) was added to precipitate maximally the antibody bound labelled rat LH.

12. Tubes were agitated on a vortex mixer.

13. Tubes were again incubated for *2k* h at room temperature.

14. At the end of this incubation period all tubes were centrifuged at 1,000 g for 20 min in a refrigerated centrifuge. The supernatant was discarded and the precipitate was counted in a gamma spectrometer.

15. The unknown samples were compared to the percentage of counts precipated with the rat LH reference preparation, NIAMDD-r-LH-RP-1. A curve was constructed on semilogarithmic paper, and the unknown was read directly from the curve obtained **with** LH-RP-1. Results are expressed as nanograms (ng) of **rat** LH-S-1 per ml of plasma.

R1A OF FSH

The following were provided with the FSH kit.

1. Rat FSH antigen NIAMDD-r-FSH-1-5, highly purified for iodination.

2. Rat FSH antiserum (Rabbit) NIAMDD-Anti-r-FSH-S-1 1.

3. Rat FSH reference preparation NIAMDD-r-FSH-RP-1 (Biological potency - 150 X NIH-FSH-S1) (HCG augmentation assay).

IODINATION OF RAT FSH

Iodination was performed as for LH except that 10 mg/ml of chloramine T was used. Double antibody R1A procedures : Procedures was same as LH except reference preparation (FSH-RP-1) was dissolved in 1%

BSA phosphosaline in doses ranging **from** 2,000 ng to 10 ng (2,000, 1,000, 500, 250, 100, 50, 25, 10 ng). FSH **antiserum** was used at a dilution of 1 : 2 500.

R1A OF **Prl**

The RIA kit for Prl consisted of

1. Rat prolactin antigen NIAMDD-r-Prl-I-5, highly purified lor iodination.
2. Rat prolactin antiserum (rabbit) **NIAMDD-anti-r-Prl-S-8.**
3. **Rat** Prl reference preparation NIAMDD-r-Prl-RP-2. (Biological potency = 30 iu/mg (pigeon local crop sac assay of Nicoll).

1ODINATION

As for **LH.**

DOUBLE ANTIBODY PROCEDURE

As per **LH** and FSH except the reference preparation was diluted in PBS in a range of 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100 and 200 ng and antiserum was diluted so as to get a final dilution of 1 : 12,500.

As far as possible samples from a particular experiment were run in one assay, each in duplicate, to avoid interassay variation. In our laboratory the sensitivities ofthe assay were 5 ng LH, 10 ng FSH and 0.25 ng PrJ. The inter and interassay co-efficients of variation were 10 and 6% for LH, 9 and 5% for FSH and 10.4 and 5.5% for Prl respectively.

Samples for GH were assayed at department oi physiology, University of Texas Health Science Centre at Dallas, Dallas, Texas.

STATISTICAL EVALUATION

Statistical evaluation of the data was done using the Student's 't' test.

CHAPTER HI

GLUTATHIONE LEVELS IN AGING RAT BRAIN AND THE ROLE OF INTRAVENTRICULAR GLUTATHIONE ON PITUITARY HORMONE RELEASE IN VIVO

INTRODUCTION

It is well established that a number of small peptides having amino acids starting from 3 to 39 have been found in different regions of the brain and in higher amounts particularly in hypothalamus (McCann et al., 1974 ; Vijayan, 1985). To quote a few they **are** TRH (tripeptide). enkephalins (pentapeptide), LHRH (decapeptide), SRIF (tetradecapeptide) and ACTH (39 amino acids). These peptides act as neurohormones, neuromodulators as well as neurotransmitter substances. In hypothalamus a number of these peptides have been shown to be released under different physiological stimuli effecting the secretion of anterior pituitary hormones thereby regulating a number of biological processes such as growth, reproduction and general tissue metabolic activity (McCann et al., 1981). Glutathione which is a tripeptide has an ubiquitous distribution in various tissue of the body (Chapter I). Although a number of biochemical functions such as oxidation-reduction, detoxification, transport of amino acids and peptides and protection against peroxidative damages have been assigned to glutathione (Chance et al., 1979 ; Jakoby, 1981 ; Meister and Anderson, 1983). However,its biological role in the brain is not fully understood. It is possible that being a peptide, glutathione may have functional role in line with other peptidergic neurotrnsmitters present in the brain as mentioned above besides metabolic functions. The present study was an attempt to determine the glutathione levels in different regions of the brain at different ages and the possible role of this peptide, if any, in anterior pituitary hormone release in ovariectomized steroid primed rats. In recent years, GABA has been shown to have a modulatory role on the secretion of anterior pituitary hormones

through its action on hypothalamic neurons besides its well established inhibitory role at various synapses in the brain (Vijayan and McCann, 1978a, 1978b ; Lamberts et ah, 1983 ; McCann et al., 1984). Hence it was also of interest to see whether glutathione has any effect on GABA levels in hypothalamus, cerebral cortex, cerebellum and brain stem.

EXPERIMENTAL PROCEDURE

Twenty one, 30, 40, 42, 45 days old and adult female rats were sacrificed by decapitation and the brains were quickly removed and assayed for glutathione in different regions of the brain as described in Chapter II.

Adult female rats were bilaterally ovariectomized under light ether anaesthesia. Three - four weeks after overiectomy stainless steel cannulae were implanted into the third ventricle as described in Chapter II. The rats were primed with estradiol benzoate (50 μg SC) and progesterone (25 mg SC) 72 h before use (Vijayan and McCann, 1978b). Reduced glutathione (GSH) was prepared fresh in 0.9% saline and microinjected in doses of 15 and 30 μg in to the third ventricle in a volume of 2 μl. The animals were sacrificed by decapitation at 5 and 15 min alter injection and trunk blood was collected. Plasma was separated under centrifugation at 4°C and stored frozen for the later assay of LH, FSH, Prl and GH by radioimmunoassay. Brains were quickly removed and different regions were separated for assay of GABA as described in Chapter II.

RESULTS

Glutathione levels in different ages of rat brain

Glutathione levels in different regions of the brain from rats

of different ages are given in Table I. Hypothalamic glutathione levels were significantly higher in 30, 40, 42, 45 days old and adults when compared to 21 days old rat brain. However, there is no significant changes in glutathione levels in other brain regions studied except in cerebellum where glutathione levels are significantly higher in 40 days old rats. The pattern of distribution was similar to that reported earlier (Vali Pasha and Sadasivudu, 1984).

Plasma hormone levels after intraventricular glutathione administration

Intraventricular injection of 15 μg dose of glutathione produced significant decrease in plasma LH at 5 and 15 min after injection. Injection of 15 or 30 μg glutathione produced significant increase in plasma FSH levels at 5 and 15 min. Lower dose of 15 μg glutathione given intraventricularly caused signficant decrease in plasma Prl at 5 min after injection. However, the higher dose of glutathione produced significant increase in plasma Prl levels at 5 and 15 min. Plasma GH levels increased significantly only at 15 min after a 15 μg dose of glutathione whereas 30 μg dose produced significant increase in plasma GH both at 5 min after injection (Fig. **1** to 4).

Brain GABA levels after intraventricular glutathione injection

Glutathione at a dose of 30 μg administered intraventricularly evoked a significant increase in hypothalamic GABA concentration at 5 min after injection. On the contrary the same dose decreased significantly GABA concentration in cerebral cortex. There were no changes in GABA levels in brain stem and cerebellum (Table II).

TABLE I

GLUTATHIONE CONTENTS OF DIFFERENT REGIONS OF RAT BRAIN AT DIFFERENT AGES

	21 Days	30 Days	40 Days	42 Days	45 Days	Adult
Hypothalamus	0.96 ± 0.08 (6)	1.15 ± 0.08 (6)**	1.57 ± 0.14 (8)*	2.04 ± 0.19 (8)*	1.61 ± 0.11 (9)*	1.31 ± 0.17 (10)**
Cerebral Cortex	1.31 ± 0.06 (6)	1.26 ± 0.11 (7)	1.37 ± 0.12 (8)	1.29 ± 0.10 (8)	1.35 ± 0.07 (10)	1.27 ± 0.11 (10)
Cerebellum	1.32 ± 0.08 (6)	1.23 ± 0.12 (6)	1.48 ± 0.09 (6)***	1.44 ± 0.10 (6)	1.38 ± 0.07 (10)	1.28 ± 0.09 (8)
Brain stem	1.07 ± 0.14 (6)	0.92 ± 0.13 (8)	1.01 ± 0.13 (8)	1.00 ± 0.10 (8)	1.00 ± 0.07 (10)	0.92 ± 0.15 (6)

Values are μmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001, ** these values at a P value of < 0.01 and *** this value at a P value of < 0.02 are significantly different when compared to 21 days old rat brain.

TABLE II

GABA LEVELS IN DIFFERENT REGIONS OF
OVARIECTOMIZED STEROID PRIMED RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF GLUTATHION

			Hypothalamus	Cerebral Cortex	Cerebellum	Brain stem
Saline Control			2.14 ± 0.34 (6)	1.85 ± 0.28 (5)	2.04 ± 0.28 (5)	1.00 ± 0.14 (5)
Glutathione	15 µg	5 min	2.34 ± 0.29 (6)	1.72 ± 0.22 (5)	2.21 ± 0.16 (5)	1.11 ± 0.25 (5)
		15 min	1.77 ± 0.28 (6)	1.97 ± 0.44 (5)	2.29 ± 0.28 (5)	1.02 ± 0.10 (5)
	30 µg	5 min	3.47 ± 0.22 (6)*	1.35 ± 0.07 (5)**	1.97 ± 0.24 (5)	1.10 ± 0.17 (5)
		15 min	3.44 ± 0.22 (6)*	1.34 ± 0.08 (5)**	1.96 ± 0.13 (5)	1.11 ± 0.12 (5)

Values are µmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001 and ** these values at a P value of < 0.02 are significantly different from those of control group.

Fig. 1. Plasma LH levels after intraventricular injection of 15 or 30 μg glutathione in ovariectomized steroid primed rats at 5 and 15 min after injection. In this figure, numbers at the base of each column indicate the number of animals in each group. Vertical lines above and/or below the mean represent mean ± S.D.

* $P < 0.01$
** $P < 0.02$ vs control.

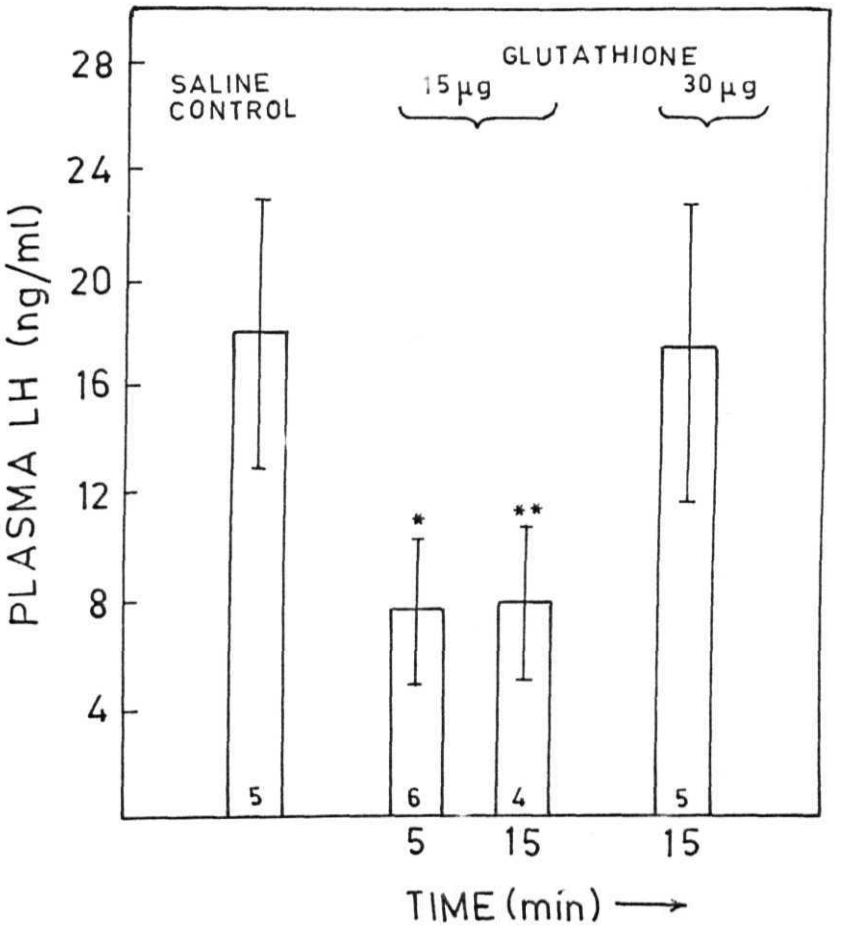

28
24
20
16
12
8
4
PLASMA LH (ng/ml)
SALINE CONTROL
GLUTATHIONE
15 µg
30 µg
*
**
5
6
4
5
5
15
15
TIME (min) ⟶

Fig. 2. Plasma FSH levels after intraventricular injection of 15 or 30 μg glutathione in ovariectomized steroid primed rats at 5 and 15 min after injection. In this figure, numbers at the base of each column indicate **the** number of animals in each group. Vertical lines above and/or below the mean represent mean ± S.D.

** P < 0.01
• P <0.001 | vs control

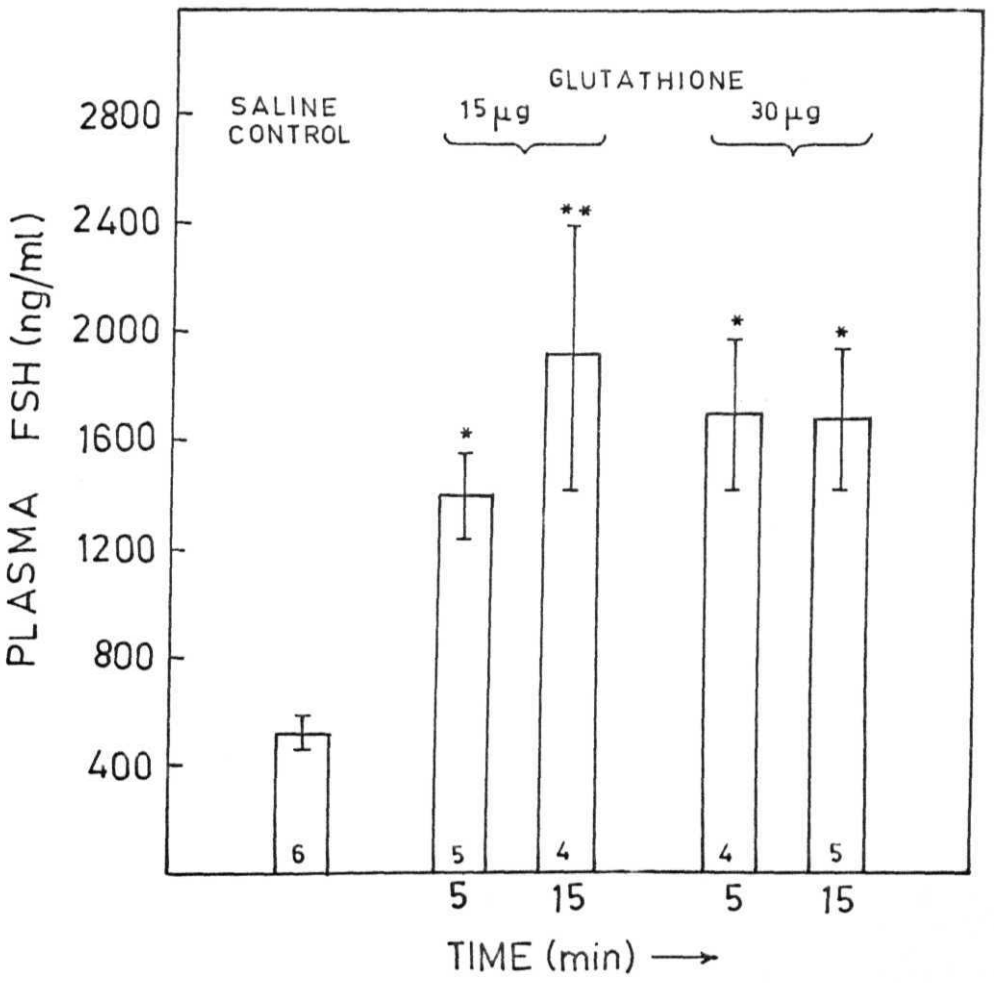

GLUTATHIONE
SALINE CONTROL
15 µg
30 µg
2800
2400
2000
1600
1200
800
400
PLASMA FSH (ng/ml)
**
*
*
*
6
5
4
4
5
5
15
5
15
TIME (min) ⟶

Fig. 3. Plasma Prl levels after intraventricular injection of 15 or 30 μg glutathione in ovariectomized steroid primed rats at 5 and 15 rnin after injection. In this figure, numbers at the base of each column indicate the number of animals in each group. Vertical lines above and/or below the mean represent mean ± S.D.

*** P < 0.05
** P <0.02 vs control
* P <0.0J

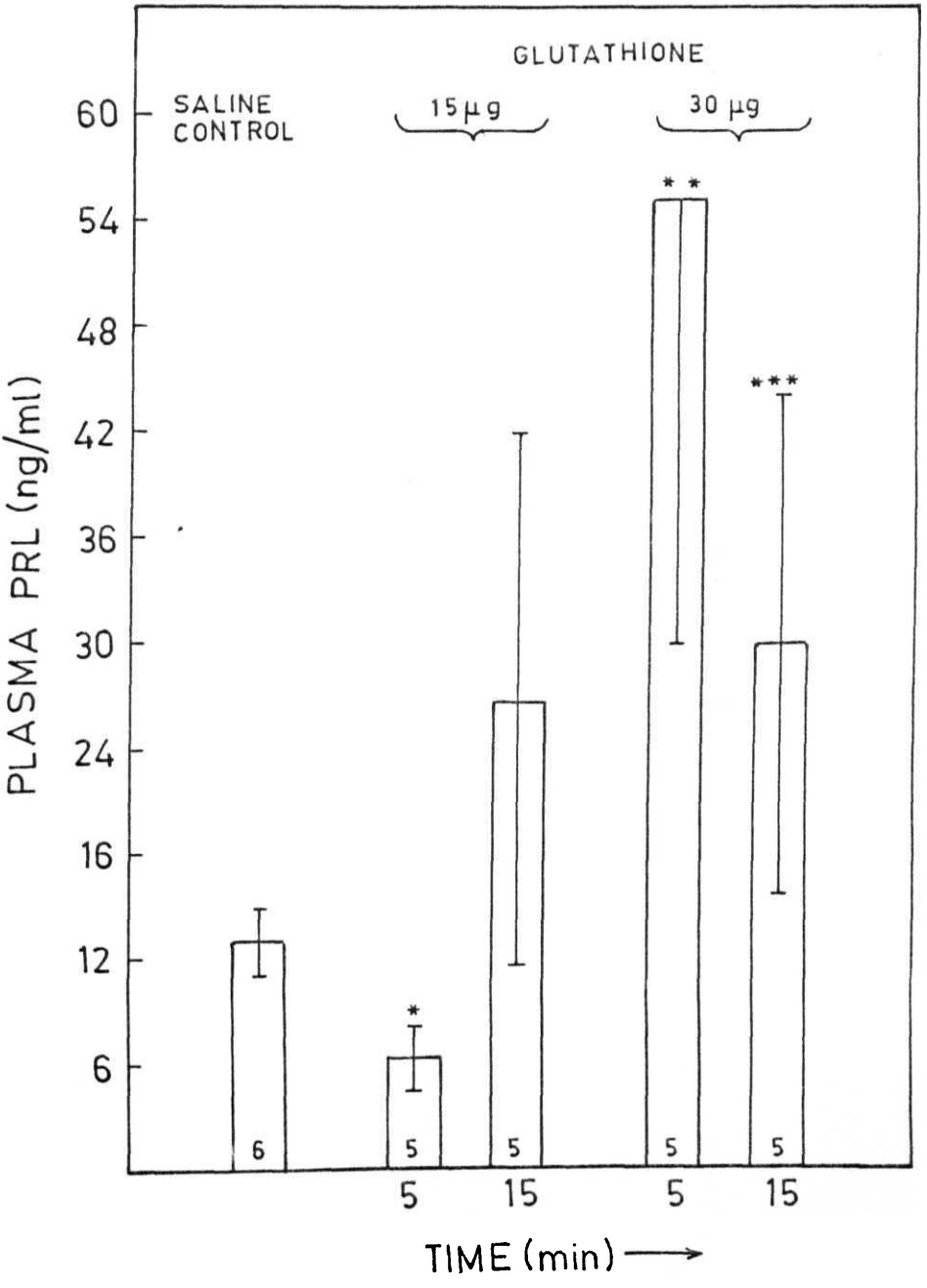
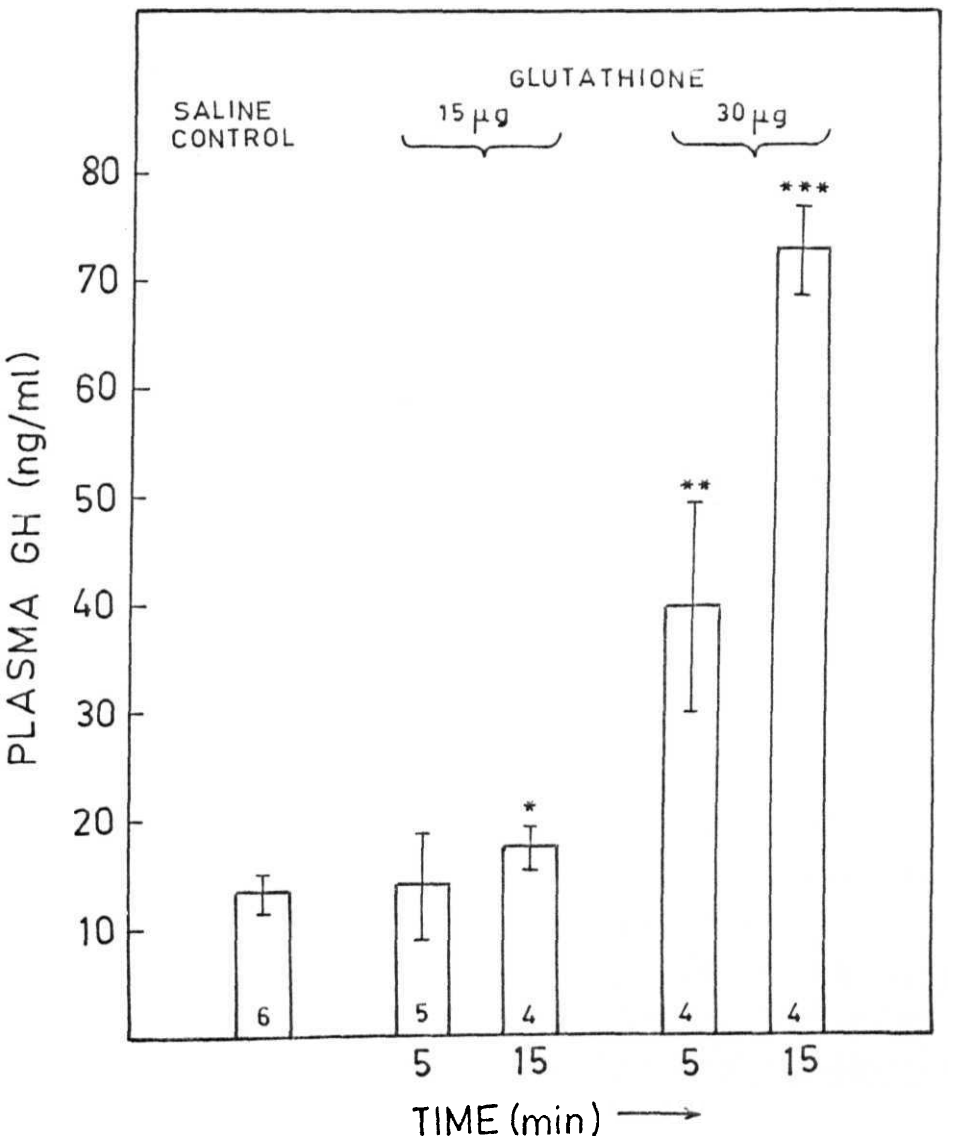

GLUTATHIONE
SALINE CONTROL
15 µg
30 µg
PLASMA PRL (ng/ml)
60
54
48
42
36
30
24
16
12
6
* *
***
*
6
5
5
5
5
5
15
5
15
TIME (min) ⟶

Fig. 4. Plasma GH levels after intraventricular injection of 15 or 30 μg glutathione in ovariectomized steroid primed rats at 5 and 15 min after injection. **In this** figure, numbers at the base of each column indicate **the** number of animals in each group. Vertical lines above and/or below the mean represent mean ± S.D.

••• P < 0.001
** P < 0.01
* P <0.05

vs control


GLUTATHIONE
SALINE CONTROL
15 µg
30 µg
PLASMA GH (ng/ml)
80
70
60
50
40
30
20
10
***
**
*
6
5
4
4
4
5
15
5
15
TIME (min)

DISCUSSION

Although the distribution of glutathione in different regions of the brain appears to be uniform, it is interesting to note that the content of it is significantly higher in the hypothalamus and the least in brain stem. In the adult rat brain such a distribution would point out a functional **role for** this compound in the hypothalamus.

The content of giutathione in cerebral cortex, cerebellum and brain stem remained almost the **same** begining from day 21 to sexually mature adult rats. However, there is a gradual, but signficant, increase in the content of giutathione in the hypothalamus reaching a peak at puberty and decreasing thereafter to the adult levels. Such a unique pubertal peak in giutathione content in the hypothalamus would suggest that giutathione may have a role in the regulation of onset of puberty. Changes in glutathione levels in the hypothalamus which controls the secretion of the gonadotropins and prolactin from anterior pituitary indicate a possible role for this peptide in neuroendocrine processes controlling reproduction.

The pubertal peak observed on **the** content of giutathione in hypothalamus prompted the study on the effects of intraventricular giutathione on the release of gonadotropins from the anterior pituitary. Incidentally **the** release of Prl and GH were also studied following intraventricular giutathione. These studies revealed**.** a significant increase in plasma **FSH** levels within 5 and 15 min of intraventricular administration of giutathione in ovariectomized steroid primed rats. This would suggest a stimulatory **role** for glutathione in gonadotropin secretion. Although administration of 15

ug glutathione significantly decreased plasma LH levels, administration of 30 µg dose of glutathione did not cause any significant change in LH levels. Such an effect of glutathione on gonadotropin levels together with the observation of pubertal peak in glutathione content in hypothalamus would provide evidence for the interaction of glutathione with the hypothalamic-pituitary axis. Although glutathione produced signficant increase in both GH and prolactin levels the increase in FSH levels produced by similar dose of glutathione was nearly four fold than that of other hormones. This selective increase in FSH would tempt to speculate **that** glutathione may be acting as FSH-releasing peptide in the hypothalamus. Existence of a specific FSH-releasing factor in the hypothalamus has been speculated (Lumpkin et ah, 1980 ; McCann et al., 1983). However, with higher doses of glutathione. a very significant rise in GH and also prolactin was observed which suggests that glutathione may be acting as a general stimulus for secretion of anterior pituitary hormones. It is not clearly understood whether the hypothalamic glutathione is acting on the anterior pituitary since no studies on the content of glutathione in the hypophyseal portal system are available. It is possible that glutathione may be acting in an indirect way by regulating other known factors controlling the hypothalamic-pituitary axis such as hypothalamic peptides or other putative neurotransmitters like dopamine and GABA.

It is interesting to note that a very significant **rise** in GABA content in the hypothalamus with a higher dose of glutathione. Such an elevation in the content of GABA may be responsible for the release of FSH as earlier studies by other workers with intraventricular GABA showed an increase

in the suprachiasmatic-preoptic region content of LHRH (McCann et al., 1984). This effect of glutathione on GABA levels seem to be more specific to hypothalamus since intraventricular glutathione did not bring about any significant changes in GABA content in any other brain regions studied except in cerebellum where a significant decrease was observed. The functional significance of such reduction in GABA content in the cerebellum is not clearly understood.

CHAPTER IV

GLUTATHIONE, GAMMA-GLUTAMYL TRANSPEPT1DASE, TOTAL SULFHYDRIL GROUPS, GLUTAMATE AND GABA LEVELS AFTER INTRACEREBRAL INJECTION OF FSH IN PUBERTAL AND POST PUBERTAL RAT BRAIN

INTRODUCTION

Most of the anterior pituitary hormones and a variety of neuropeptides including oxytocin and vasopressin were shown to be present in cerebrospinal fluid (Rodriguez, 1976 ; Ganong, 1980 ; **Kendall** and Orwoll, 1980 ; Login and MacLeod, 1977). Intraventricular administration of glutathione has been found to release selectively more of FSH (see Chapter III). Hypothalamic glutathione level were found to be very high at puberty (see Chapter III). These results indicate a possible interaction between glutathione and FSH. Although the extrapituitary localization of FSH in the brain is not known, it is possible that FSH secreted from pituitary may have effects on the behaviour apart from its role in gonadal function. An attempt has been made to evaluate the changes in glutathione levels in different regions of the brain including hypothalamus after intracerebral injection of FSH in pubertal and post-pubertal period. Furthermore, as the major proportion of synaptic function is mediated by the two important neuroactive amino acids namely GABA and glutamate, the levels of these two amino acids have also been studied to know whether FSH would effect the synaptic function besides the metabolism in brain.

EXPERIMENTAL PROCEDURE

Rats attain puberty around 42 days in our animal facility. Female pubertal rats (42 days old) and immediate post pubertal rats (45 days old) were given intracerebral injection of FSH at a dose of 10 μg in a volume of 10 μl and the animals were sacrificed by decapitation after 30 min of injection. Brains were quickly removed and the different regions of the

brain were separated and assayed for glutathione, γ-glutamyl transpeptidase, total sulfhydril groups, glutamate and GABA as described in Chapter **II.**

RESULTS

FSH, given intracerebralJy at a dose of 10 ug produced a significant decrease in hypothalamic glutathione levels in pubertal rats (42 days old) whereas there was no significant change in glutathione levels in the hypothalamus of post pubertal rats (45 days old). However, there is no significant change in the glutathione levels of cerebral cortex, cerebellum and brain stem in pubertal and post pubertal rats. With the exception of cerebellum of pubertal rat brain and brain stem of post pubertal rat brain intracerebral injection of FSH induced a significant increase in total sulfhydril groups of Hypothalamus, cerebral cortex, cerebellum and brain stem of pubertal and post pubertal rats. FSH given intracerebrally produced a significant increase in γ-glutamyl transpeptidase of cerebral cortex in case of post pubertal rats and cerebral cortex and cerebellum in case of pubertal rats (Table I and Table III). Glutamate levels were significantly elevated in cerebral cortex, cerebellum and brain stern after intracerebral FSH in pubertal rat brain. However, in case of post pubertal rats, only cerebral cortex glutamate levels were increased significantly. Intracerebral FSH brought about significant increase in GABA levels of brain stem of pubertal rat brain and cerebral cortex GABA levels of post pubertal rat brain (Table II and Table IV).

DISCUSSION

The significant increase in the content of glutathione in hypothalamus

TABLE I

EFFECT OF INTRACEREBRAL FSH ON GLUTATHIONE, γ-GLUTAMYL TRANSPEPTIDASE AND TOTAL SULFHYDRIL GROUPS OF 42 DAYS OLD FEMALE RAT BRAIN AT 30 MIN AFTER INJECTION

	Glutathione[I]		Total Sulfhydril Groups[II]		γ-glutamyltranspeptidase[III]	
	Saline	FSH	Saline	FSH	Saline	FSH
Cerebral Cortex	1.23 ± 0.08 (7)	1.22 ± 0.05 (7)	7.92 ± 0.31 (5)	8.81 ± 0.16 (6)*	38.33 ± 1.88 (6)	44.66 ± 5.86 (6)**
Cerebellum	1.45 ± 0.09 (7)	1.43 ± 0.07 (7)	11.98 ± 0.56 (5)	12.50 ± 0.36 (6)	33.25 ± 3.38 (6)	38.29 ± 2.05 (6)**
Brain stem	1.06 ± 0.09 (7)	1.08 ± 0.13 (6)	13.16 ± 0.05 (5)	14.12 ± 0.47 (6)*	46.04 ± 5.56 (6)	49.12 ± 5.27 (6)
Hypothalamus	2.20 ± 0.19 (7)	1.50 ± 0.22 (7)***	19.04 ± 0.39 (5)	21.07 ± 1.15 (6)*	52.29 ± 1.46 (6)	53.95 ± 2.89 (6)

I, II - Values are μmoles/gm wet wt. tissue.

III - Values are μmoles p-nitroaniline released/gm wet wt. tissue/hr.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.01, ** these values at a P value of < 0.05 and *** this value at a P value of < 0.001 are significantly different from those of control group.

TABLE II

EFFECT OF INTRACEREBRAL FSH ON GLUTAMATE AND GABA LEVELS IN 42 DAYS OLD FEMALE RAT BRAIN AT 30 MIN AFTER INJECTION

	Glutamate		GABA	
	Saline	FSH	Saline	FSH
Cerebral Cortex	4.00 0.2(5)	5.28 0.67 (6)*	1.08 0.07 (5)	1.28 0.20 (6)
Cerebellum	6.66 ± 0.34 (5)	7.89 ± 0.57 (6)*	1.58 ± 0.06 (5)	1.59 ± 0.09 (6)
Brain stem	4.05 ± 0.47 (5)	5.75 ± 0.73 (6)*	1.30 ± 0.06 (5)	1.55 ± 0.05 (6)**

Values are μmoles/gm wet wt. of tissue.

Values are means + S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.01 and ** this value at a P value of < 0.02 are significantly different from those of control group.

TABLE III

EFFECT OF INTRACEREBRAL FSH ON GLUTATHIONE, γ-GLUTAMYL TRANSPEPTIDASE AND TOTAL SULFHYDRIL GROUPS OF 45 DAYS OLD FEMALE RAT BRAIN AT 30 MIN AFTER INJECTION

	Glutathione[I]		Total Sulfhydril Groups[II]		γ-glutamyltranspeptidase[III]	
	Saline	FSH	Saline	FSH	Saline	FSH
Cerebral Cortex	1.21 ± 0.10 (7)	1.27 ± 0.07 (7)	6.48 ± 0.21 (5)	8.36 ± 0.37 (6)**	33.45 ± 4.19 (6)	40.25 ± 1.73 (6)*
Cerebellum	1.38 ± 0.09 (7)	1.36 ± 0.08 (7)	9.92 ± 0.70 (5)	11.56 ± 0.90 (6)	37.29 ± 2.28 (6)	39.41 ± 3.71 (6)
Brain stem	0.94 ± 0.11 (7)	0.93 ± 0.08 (7)	9.26 ± 0.30 (5)	9.59 ± 0.41 (6)	51.66 ± 3.19 (6)	53.00 ± 2.72 (6)
Hypothalamus	1.70 ± 0.15 (7)	1.69 ± 0.19 (7)	16.17 ± 0.42 (5)	18.80 ± 1.60 (6)*	49.79 ± 2.89 (6)	50.12 ± 4.12 (6)

I, II - Values are μmoles/gm wet wt. tissue.

III - Values are μmoles p-nitroaniline released/gm wet wt. tissue/hr.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.02 and ** this value at a P value of < 0.001 are significantly different from those of control group.

TABLE IV

EFFECT OF INTRACEREBRAL FSH ON GLUTAMATE AND GABA LEVELS IN 45 DAYS OLD FEMALE RAT BRAIN AT 30 MIN AFTER INJECTION

	Glutamate		GABA	
	Saline	FSH	Saline	FSH
Cerebral Cortex	4.42 ± 0.47(5)	5.57± 0.40(6)*	1.09 ± 0.20 (5)	1.68 ± 0.12(6)*
Cerebellum	7.43 ± 0.33 (5)	7.98 ± 1.24 (6)	1.67 ± 0.08 (5)	1.60 ± 0.29 (6)
Brain stem	5.48 ± 0.74 **(5)**	5.31 ± 0.77 **(6)**	1.42 *t* 0.06 **(5)**	1.38 ± 0.21 **(6)**

Values are μmoles/gm wet wt. of tissue.

Values are means + S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.01 are significantly different from those of control group.

at puberty without any change in the same in the immediate post pubertal period following intracerebral injection of FSH indicate that **the** action of FSH in this regard on the glutathione content in hypothalamus is not direct but probably mediated by other mechanisms such as involvement of neurotransmitters. The universal rise in the glutamate in cerebral cortex, cerebellum and brain stem along with a lone increase in GABA in brain stem would suggest that FSH administration through intracerebral route brought about generalized changes in metabolism of brain promoting either increased formation of glutamate or decreased utilization of glutamate. Since both glutamate and GABA showed an increase in brain stem under these experimental conditions the effects of both glutamate and GABA on reticular activating system located inbrain stem get neutralized. However, the increased content of glutamate in cerebral cortex and cerebellum would facilitate a state of neuronal excitation in these regions with effects on the subcortical brain regions including hypothalamus. During the onset of puberty a number of stimuli from different parts of the brain impinge on hypothalamic neurons causing the release of hypothalamic peptides. It is tempting to speculate that the specific decrease in glutathione in hypothalamus might have been through glutamatergic pathway. However, the mechanisms involved in the changes in the content of glutamate and GABA by intracerebral FSH are not well understood. Although the rise in the content of total sulfhydril groups in different brain regions might be occuring as a result of metabolic alterations within the cells, the rise in total sulfhydril groups in the absence of any change in γ-glutamyl transpeptidase activity may be occuring as a result of stimulation of transhydrogenase activity involving

the glutathione and the number of disulfide peptide hormones in hypothalamus. The increase in γ-glutamyl transpeptidase activity in cerebral cortex and cerebellum may be attributed to the local glial reaction and irritation caused by intracerebral route of administration of FSH. Irrespective of the nature of mechanisms operating the present data is suggestive of an interaction between FSH and glutathione in the CNS.

CHAPTER V

GLUTATHIONE, GAMMA-GLUTAMYL TRANSPEPTIDASE, TOTAL SULFHYDRIL GROUPS, GLUTAMATE AND GABA LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF LHRH

INTRODUCTION

LHRH is one of the first hypothalamic factors described which promote gonadotropin release from the anterior pituitary and cause ovulation (McCann et al., 1960). LHRH has been localized mainly **in** the hypothalamus and brain stem (Barry **et** al., 1974 ; Samson et al., 1980). LHRH like immuno-reactivity **is** also been shown in placenta, interstitial cells of **testis** and also in milk (Koch and Baram, 1977 ; Khodr and Siler-Khodr, 1980 ; Turkelson et al., 1981). LHRH localized in the brain stem has been suggested to be involved in mating behaviour (Moss and McCann, 1973). LHRH has been thought to be exerting its effect on the pituitary through cAMP and **cGMP** (Naor et al., 1978 ; Snyder et al., 1980). Glutathione has been found to release more of FSH as described in Chapter III. Hypothalamic glutathione levels has been found to be very high at puberty. A number of hypothalamic releasing peptides were found to have synergistic effects in **the** release of target hormones from the anterior pituitary. It is probable that such an effect may exist between LHRH and glutathione. In order to understand this the present study has been undertaken regarding the glutathione content in hypothalamus and brain stem where LHRH is localized in abundance. Furthermore, to establish such an effect specifically localized to brain stem, hypothalamus only, glutathione content was estimated in the major regions of brain such as cerebral cortex and cerebellum. In the brain it is well established that a greater proportion of the synaptic function is mediated by neuroactive amino acids such as glutamic acid and GABA while glutamic acid is neuroexcitatory and GABA is generally found to be neuro-inhibitory. Besides these functions, these amino acids are also involved in general metabolic reactions by the brain. Furthermore specific to the

problem under investigation GABA is known to be facilitating LH release (Vijayan and McCann, 1978a). In order to clearly understand the interaction between LHRH and GABA a study has been made on the content of GABA in hypothalamus, brain stem, cerebral cortex and cerebellum. The study has been further extended and content of glutamic acid which is metabolically closely related to GABA has been made in the different regions of the brain. Such a study would be helpful in understanding a generalized role of LHRH in the brain.

EXPERIMENTAL PROCEDURE

Implantation of stainless steel cannula in to the third ventricle was carried out as described in Chapter **II.** LHRH was prepared freshly in 0.9% saline. 0.1 μg, 0.5 μg and 1 μg dose of LHRH was microinjected in to the third ventricle in a volume of 2 μl using a 10 μl Hamilton microsyringe as described in Chapter II. Controls received equal volume of saline. 10 and 30 min after injection the animals were decapitated, brains were removed and processed for glutathione, γ-glutamyl transpeptidase, total sulfhydril groups, glutamate and GABA as described in Chapter **II.**

RESULTS

Glutathione levels in the brain after IVT LHRH

Intraventricular injection of 0.1 μg LHRH produced a significant increase in hypothalamic glutathione at 10 and 30 min following administration (Table I). The same dose of LHRH produced significant increase **only** at 10 min in the glutathione levels of cerebral cortex. Intraventricular

injection of 0.5 μg LHRH however caused significant reduction in glutathione levels in the hypothalamus, cerebral cortex and cerebellum at 10 and 30 min following injection but not in brain stem. A higher dose of 1 μg LHRH also produced significant reduction in glutathione levels in all the regions of the brain studied except in brain stem where there was no significant change in glutathione levels at 30 min (Table 1).

Gamma-glutamyl transpeptidase activity after **IVT LHRH**

Intraventricular injection of 0.1 μg LHRH produced a significant increase in γ-glutamyl transpeptidasc activity in hypothalamus and cerebral cortex at 10 and 30 min following injection. However, the same dose produced a significant increase in γ-glutamyl transpeptidase activity only in the brain stem after 30 rnin. Third ventricular injection of 0.5 μg LHRH significantly elevated γ-glutamyl transpeptidase in hypothalamus and brain stem at 10 and 30 min. The same dose of LHRH elicited a significant increase in cerebral cortex and cerebellum γ-glutamyl transpeptidase activity at 30 min. LHRH at 1 μg dose also caused a significant increase in γ-glutamyl transpeptidase activity in hypothalamus and cerebellum at 10 min. However, the same dose produced a significant increase in γ-glutamyl transpeptidase activity in all the regions studied i.e., hypothalamus, cerebral cortex, cerebellum and brain stem at 30 min (Table 11).

Glutamate levels after IVT LHRH

0.1 μg LHRH produced significant increase in cerebellum and brain stem glutamate levels at 10 and 30 min following injection. Significant

increase in cerebral cortex glutamate is noticed only at 10 nun. 0.5 µg intraventricular LHRH produced significant increase in glutamate levels only in hypothalamus and brain stem at 30 min. Higher dose of 1 µg LHRH, produced a significant increase in glutamate levels in hypothalamus and cerebral cortex at 10 min. However, the same dose of LHRH produced significant increase in cerebral cortex, cerebellum and hypothalamic glutamate levels at 30 min (Table IV).

GAB A levels after IVT LHRH

GABA levels in cerebral cortex, cerebellum and brain stem increased significantly at 10 min following injection. However, the same dose produced **significant** increase in brain stem GABA levels only at 30 min. 0.5 µg dose of intraventricular LHRH brought about significant increase in hypothalamic GABA at 10 and 30 **min** respectively. However, there was a significant decrease in GABA levels of cerebellum and brain stem at 30 min. Third ventricular injection of 1 µg LHRH produced significant increase in hypothalamic GABA levels at 10 and 30 min (Table III).

Total sulfhydril groups after IVT LHRH

Intraventricular injection of 0.1 or 0.5 µg dose of LHRH could not produce any significant change in total sulfhydril groups in any of the brain regions studied i.e., hypothalamus, cerebral cortex, cerebellum and brain stem at 10 and 30 min after administration. However, a higher dose of 1 µg LHRH produced significant increase in total **sulfhydril** groups of cerebral cortex and hypothalamus **at** 30 min after injection (Table V).

TABLE I

GLUTATHIONE LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF LHRH

	CONTROL	LHRH 0.1 μg		LHRH 0.5 μg		LHRH 1 μg	
		10 min	30 min	10 min	30 min	10 min	30 min
Cerebral Cortex	1.45 ± 0.04 (6)	1.61 ± 0.07** (6)	1.50 ± 0.04 (6)	1.26 ± 0.05* (6)	1.06 ± 0.12* (5)	1.12 ± 0.09* (6)	1.18 ± 0.06* (5)
Cerebellum	1.67 ± 0.15 (6)	1.89 ± 0.29 (6)	1.75 ± 0.15 (6)	1.29 ± 0.06** (6)	1.17 ± 0.12* (5)	1.15 ± 0.10* (6)	1.34 ± 0.07** (5)
Brain stem	1.14 ± 0.28 (6)	1.53 ± 0.36 (6)	1.34 ± 0.18 (6)	0.88 ± 0.15 (6)	0.83 ± 0.08 (5)	0.67 ± 0.10**** (6)	1.02 ± 0.02 (5)
Hypothalamus	1.77 ± 0.19 (6)	2.18 ± 0.34*** (6)	2.32 ± 0.47*** (6)	1.17 ± 0.07* (6)	1.23 ± 0.13** (5)	0.83 ± 0.15* (6)	1.25 ± 0.14** (5)

Values are μmoles/gm wet wt. tissue.

Values are means±S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001, ** these values at a P value of < 0.01, *** these values at a P value of < 0.05 and **** this value at a P value of < 0.02 are significantly different from those of control group.

TABLE II

GAMMA GLUTAMYL TRANSPEPTIDASE ACTIVITY IN
ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF LHRH

	CONTROL	LHRH 0.1 μg		LHRH 0.5 μg		LHRH 1 μg	
		10 min	30 min	10 min	30 min	10 min	30 min
Cerebral Cortex	39.47 ± 4.04 (12)	43.95 ± 0.94** (6)	48.95 ± 3.48* (6)	40.83 ± 4.65 (6)	46.87 ± 1.53* (6)	39.79 ± 3.29 (6)	45.50 ± 2.91** (6)
Cerebellum	48.75 ± 2.40 (8)	46.66 ± 2.81 (6)	48.33 ± 7.91 (6)	50.00 ± 1.58 (6)	57.08 ± 5.57*** (6)	43.75 ± 2.85*** (6)	54.58 ± 3.67*** (6)
Brain stem	60.00 ± 2.91 (12)	58.54 ± 2.15 (6)	66.04 ± 5.99**** (6)	64.79 ± 3.29*** (6)	67.91 ± 3.22* (6)	59.37 ± 1.31 (6)	66.25 ± 2.09* (6)
Hypothalamus	51.45 ± 3.19 (12)	56.66 ± 2.81** (6)	58.12 ± 4.52*** (6)	57.91 ± 1.70* (6)	75.20 ± 2.50* (6)	60.00 ± 3.35* (6)	65.20 ± 1.46* (6)

Values are μmoles p-nitroaniline released/gm wt. tissue/hr.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.01, ** these values at a P value of < 0.01, *** these values at a P value of < 0.02 and **** this value at P value of < 0.05 are significantly different from those of control group.

TABLE III

GABA LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF LHRH

	CONTROL	LHRH 0.1 μg		LHRH 0.5 μg		LHRH 1 μg	
		10 min	30 min	10 min	30 min	10 min	30 min
Cerebral Cortex	1.10 ± 0.28 (6)	1.88 ± 0.31** (5)	1.32 ± 0.14 (5)	1.11 ± 0.12 (6)	1.09 ± 0.03 (5)	0.75 ± 0.11 (6)	1.32 ± 0.04 (5)
Cerebellum	1.04 ± 0.21 (6)	2.00 ± 0.28* (5)	1.37 ± 0.25 (5)	0.95 ± 0.19 (6)	0.72 ± 0.06**** (5)	0.87 ± 0.24 (6)	1.04 ± 0.09 (5)
Brain stem	0.98 ± 0.14 (6)	1.82 ± 0.36** (5)	1.78 ± 0.36** (5)	0.81 ± 0.13 (6)	0.57 ± 0.04* (5)	0.94 ± 0.14 (6)	0.93 ± 0.05 (5)
Hypothalamus	2.52 ± 0.07 (6)	2.23 ± 0.29 (5)	2.62 ± 0.13 (5)	2.76 ± 0.15*** (6)	3.87 ± 0.18* (5)	4.36 ± 0.10* (6)	4.45 ± 0.20* (5)

Values are μmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001, ** these values at a P value of < 0.01, *** this value at a P value of < 0.05 and **** this value at a P value of < 0.02 are significantly different from those of control group.

TABLE IV

GLUTAMATE LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF LHRH

	CONTROL	LHRH 0.1 μg		LHRH 0.5 μg		LHRH 1 μg	
		10 min	30 min	10 min	30 min	10 min	30 min
Cerebral Cortex	4.86 ± 0.57 (6)	5.96 ± 0.71*** (5)	5.86 ± 1.30 (5)	5.57 ± 0.59 (6)	4.31 ± 0.15 (5)	3.54 ± 0.78*** (6)	4.02 ± 0.20*** (5)
Cerebellum	4.54 ± 0.83 (6)	6.63 ± 0.79** (5)	6.49 ± 1.50*** (5)	5.52 ± 1.26 (6)	4.04 ± 0.16 (5)	5.64 ± 0.66 (6)	5.79 ± 0.45*** (5)
Brain stem	3.66 ± 0.43 (6)	4.90 ± 0.60**** (5)	5.45 ± 0.34* (5)	3.69 ± 0.37 (6)	2.30 ± 0.18* (5)	3.39 ± 0.52 (6)	3.93 ± 0.24 (5)
Hypothalamus	4.91 ± 0.52 (6)	4.44 ± 0.63 (5)	4.79 ± 0.19 (5)	4.81 ± 0.38 (6)	6.06 ± 0.22** (5)	8.84 ± 0.25* (6)	8.72 ± 0.35* (5)

Values are μmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001, ** these values at a P value of < 0.01, *** this value at a P value of < 0.05 and **** this value at a P value of < 0.02 are significantly different from those of control group.

TABLE V

TOTAL SULFHYDRIL GROUPS

LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF LHRH

	CONTROL	LHRH 0.1 μg		LHRH 0.5 μg		LHRH 1 μg	
		10 min	30 min	10 min	30 min	10 min	30 min
Cerebral Cortex	9.44 ± 1.37 (5)	9.55 ± 0.48 (6)	9.11 ± 0.97 (6)	9.61 ± 0.43 (5)	9.54 ± 0.14 (5)	9.15 ± 0.33 (5)	12.17 ± 1.17* (5)
Cerebellum	11.07 ± 2.29 (5)	12.24 ± 1.45 (6)	11.37 ± 2.46 (6)	11.91 ± 0.95 (5)	11.58 ± 0.23 (5)	10.63 ± 0.31 (5)	12.29 ± 0.59 (5)
Brain stem	9.46 ± 2.11 (5)	9.68 ± 1.31 (6)	9.05 ± 0.94 (6)	11.83 ± 1.00 (5)	10.43 ± 0.53 (5)	9.94 ± 0.39 (5)	11.77 ± 0.61 (5)
Hypothalamus	12.67 ± 4.20 (5)	11.88 ± 2.07 (6)	12.54 ± 1.83 (6)	11.81 ± 1.60 (5)	11.81 ± 0.34 (5)	14.68 ± 0.66 (5)	20.41 ± 4.95** (5)

Values are μmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* This value at a P value of < 0.02 and ** this value at a P value of < 0.05 are significantly different from those of control group.

DISCUSSION

The studies on the intraventricular administration of glutathione clearly indicates a selective stimulatory effect of the peptide on FSH release thereby implicating it in the release of gonadotropin from the anterior pituitary. However, LHRH is known to be the most potent gonadotropin releasing hormone. The significant increase in the activity of γ-glutamyl transpeptidase in the hypothalamus irrespective of the dose employed and at the two time intervals studied indicate that LHRH may have an effect on the hypothalamic content of glutathione. Under these circumstances the changes in contents of glutathione in hypothalamus, however, appear to be variable (Table I). With higher doses of LHRH the glutathione content has decreased in parallel with a rise in γ-glutamyl transpeptidase activity while the glutathione content has significantly increased both at 10 and 30 min following the administration of 0.1 μg LHRH. The increase in γ-glutamyl transpeptidase activity may facilitate increased transport of amino acids or peptides, although as mentioned earlier this function of glutathione has not been clearly understood in the brain. The reported formation of Y-glutamyl derivatives such as γ-glutamyl dopamine may be through this mechanism and increased formation of γ-glutamyl dopamine in hypothalamus under the influence of LHRH may carry functional significance as dopamine is known to stimulate the release of a number of trophic hormones from the pituitary (Tsuji et **ah,** 1977 ; McCann et ah, 1979 ; Vijayan, 1985 ; Ichinose et al., 1987). It is intriguing that the increase in γ-glutamyl transpeptidase activity is observed in all the other three brain regions studied. **The** functional significance of such an increase in these regions is not **under-**

stood and much less regarding the mechanism of increased γ-glutamyl transpeptidase activity by LHRH. It is probable that LHRH may be exerting a direct effect. The utilization of glutathione in the presence of increased γ-glutamyl transpeptidase activity is always expected and the decrease in the content of glutathione which is considerable in brain stem and hypothalamus with higher dose of LHRH. However, the changes in the content of glutathione can not be solely attributed to changes in γ-glutamyl transpeptidase as glutathione is utilized in the protective function against lipid peroxidation, in the formation of glutathione S-adducts, in oxidation-reduction reactions and in transhydrogenation reactions. It is probable that under the experimental conditions and following the administration of LHRH the pathways of utilization of glutathione may be through γ-glutamyl transpeptidase or through transhydrogenase. Glutathione utilization through transhydrogenase reaction is plausible as a number of disulfide peptides such as vasopressin, oxytocin and insulin have been shown to be inactivated through this process. Utilization of glutathione in hypothalamus by this mechanism may be relevant since a number of peptide hormones such as vasopressin, oxytocin and somatostatin containing disulfide bonds may undergo inactivation.

Sinct LHRH and glutathione appear to be sharing a common function as regards the release of gonadotropins it is tempting to speculate that administration of very low doses of LHRH brings about a rise in content of glutathione in hypothalamus as it would facilitate release of gonadotropins although the dose of LHRH employed may be physiologically ineffective. However, LHRH in the physiological range and above appear to be diverting

the glutathione in the tissues into other pathways since the amount of LHRH is sufficient and even more in the release of gonadotropins. Synergistic functional role have been observed in the case of hypothalamic releasing hormones such as vasopressin and CRF.

The role of GABA on hypothalamic-pituitary axis have been studied and majority of the results are in agreement regarding the release of anterior pituitary hormones such as ACTH, Prl, LH and GH (Vijayan and McCann, 1978a and 1978b ; Lamberts et al., 1983 ; McCann et ah, 1984). Besides it's role in pituitary hormone release the role of GABA in central nervous system is well established as neuroinhibitory transmitter. The significant increase in GABA following the intraventricular administration of higher doses of LHRH is suggestive of a synergistic role for both LHRH and GABA. However, the mechanism involved in the rise in GABA levels under these circumstances is not properly understood. Although the increase in the levels of GABA in the hypothalamus may be helpful in the release of gonadotropins. It is interesting to know that LHRH in very low doses seem to be causing a rise in the levels of GABA in brain stem, cerebral cortex and cerebellum. Such a rise in the content of GABA in brain stem may negatively influence the reticular activating system. However, when LHRH was administered in doses nearing physiological range the decrease in the content of GABA in brain stem observed may have relevance to the mating behavioral effects of LHRH.

The nearly two fold increase in the content of glutamate in hypothalamus following intraventricular administration of a high dose of LHRH

suggest increased formation of glutamate implying increased metabolic activity in hypothalamus under these experimental conditions. The observed decrease in the content of glutamate in cerebral cortex under these experimental conditions may also have a metabolic significance rather than functional implying absence of cerebral neuronal excitation. The significance in the decrease in glutamate along with GABA in brain stem following 0.5 μg LHRH is not clear.

Although there are no significant changes in the content of total sulfhydril groups in cerebellum and brain stem with the doses of LHRH employed in the present study a significant increase in the content of total sulfhydril groups of hypothalamus and cerebral cortex was observed with 1 ug LHRH. Such an increase in the total sulfhydril compounds in the presence of decreased glutathione content indicate an increase in the content of protein sulfhydrils suggesting the breakdown of disulfide bonds in the proteins and peptides. This would further lend support to the contention that glutathione may be involved under these circumstances in the inactivation of disulfide pcptide hormones locally through transhydrogenation mechanism as discussed above.

CHAPTER VI

GLUTATHIONE, GAMMA-GLUTAMYL TRANSPEPTIDASE, TOTAL SULFHYDRIL GROUPS, GLUTAMATE AND GABA LEVELS IN ADULT FEMALE RAT BRAIN FOLLOWING INTRAVENTRICULAR SOMATOSTAT1N

INTRODUCTION

The tatradecapeptide, somatostatin is a hypothalamic factor having a dramatic inhibitory effect on **the** release of growth hormone (GH) by the pituitary somatotrophs (Brazeau et ah, 1973). Somatostatin has been localized by electron microscopic immunohistochemistry to secretory granules of neurons located within the hypothalamus (Krulich et al., 1972). Furthermore, somatostatin has a widespread distribution **in the** central nervous system and is also found to be localized to D-cells of **the** gut and endocrine pancreas (Brazeau et al., 1973 ; Koerker et al., 1974). At the electron microscopic level somatostatin immunoreactivity has been demonstrated **in** synaptosomes (Elde, 1979). Somatostatin has been shown to inhibit cAMP formation and also calcium mobilization (Vale **et** al., 1972 ; Leitner **et** al., 1980 ; Kraicer and Spence, 1981). Glutathione has been found to be stimulatory to growth hormone release and **hypothalamic** glutathione levels is high at puberty (Chapter III). Since somatostatin inhibits GH release and glutathionc was shown to stimulate GH release, it is probable that there may be an interaction between somatostatin systems and glutathionc in the brain. In order to explain this the present study has been undertaken to evaluate the glutathione content in hypothalamus, cerebral cortex, **cerebellum** and brain stern after intraventricular somatostatin. In the brain it is well known that a greater proportion of the synaptic function is mediated by neuroexcitatory and neuroinhibitory amino acids like glutamate and GABA respectively. Although GABA was shown to inhibit somatostatin release from hypothalamic cells in culture (Gamse et **ah,** 1980) the effect of somatostatin on the content of GABA in the brain regions has not been established. A study, therefore,

has been made on the content of GAB A in the hypothalamus, cerebral cortex, cerebellum and brain stem after the intraventricular injection of somatostatin. The study has been further extended and **the** content of glutamic acid which is metabolically closely related to GABA has also been made in different regions of the brain. Such a type of study may be helpful in understanding the functional interactions between somatostatin and glutathione and the two major neuroactive amino acids, glutamic acid and GABA.

EXPERIMENTAL PROCEDURES

Implantation of stainless steel cannula in the third ventricle was carried out as described in Chapter II. Somatostatin was prepared fresh **in** 0.9% saline. 0.5 μg and 1 μg dose of somatostatin was microinjected into the third ventricle in a volume of 2 μl using a 10 μl Hamilton microsyringe as described in chapter **II.** The animals **were** sacrificed by decapitation at 10 and 30 min after injection**.** The brains were quickly removed and different regions of the brain was assayed for glutathione, γ-glutamyl transpeptidase, totalsulihydril groups, glutamate and GABA as described in the materials and methods (Chapter 11).

RESULTS

Brain glutathione levels following 1VT somatostatin

Intraventricular injection of 0.5 μg and 1 μg doses of somatostatin significantly decreased the glutathione levels in hypothalamus, cerebral cortex, cerebellum and brain stem at 10 and 30 min (Table-1).

γ-glutamyl transpeptidase activity after **IVT** somatostatin

Third ventricular injection of 0.5 μg and 1 μg dose of somatostatin produced significant increase in γ-glutamyl transpeptidase activity of cerebral cortex, brain stem and hypothalamus at 10 and 30 min. However, in case of cerebellum significant increase in γ-glutamyl transpeptidase activity was seen only at 30 min after the 0.5 μg dose and at 10 min after 1 μg dose of somatostatin (Table-II)

Glutamate levels after **IVT** somatostatin

Intraventricular administration of 1 μg somatostatin, produced significant increase in glutamate levels of cerebral cortex and cerebellum at 30 min after injection. Significant increase in hypothalamic glutamate levels occured at 10 min after 0.5 μg dose while 1 μg dose significantly elevated glutamate levels at 30 min (Table III)

GABA levels after IVT somatostatin

Third ventricular injection of 1 μg somatostatin produced significant decrease in hypothalamic GABA levels at 10 and 30 min following injection. However, 0.5 μg dose caused significant decrease in brain stern GABA levels at 10 min (Table IV).

Total sulfhydril groups after IVT somatostatin

Intraventricular injection of 0.5 μg dose of somatostatin caused significant increase in total sulfhydril groups of only hypothalamus and cerebral cortex at 30 min after injection. There was no change in the total

TABLE I

GLUTATHIONE LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF SOMATOSTATIN

			Cerebral Cortex	Cerebellum	Brainstem	Hypothalamus
Saline Control			1.45 ± 0.04 (6)	1.67 ± 0.15 (6)	1.14 ± 0.28 (6)	1.77 ± 0.19 (6)
Somatostatin	0.5 µg	10 min	1.04 ± 0.12(6)*	1.05 ± 0.13 (6)*	0.60 ± 0.09 (6)**	1.33 ± 0.19 (6)**
		30 min	1.20 ± 0.09 (5)**	1.30 ± 0.08 (5)**	0.75 ± 0.11 (5)****	1.06 ± 0.10 (5)*
	1 µg	10 min	1.30 ± 0.08 (6)**	1.15 ± 0.15 (6)*	0.64 ± 0.08 (6)**	0.95 ± 0.18 (6)*
		30 min	1.33 ± 0.09 (6)***	1.28 ± 0.08 (6)**	0.78 ± 0.08 (6)***	1.21 ± 0.14 (6)**

Values are µmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001, ** these values at a P value of < 0.01, *** these values at a P value of < 0.05 and **** this value at a P value of < 0.02 are significantly different from those of control group.

TABLE II

GAMMA GLUTAMYL TRANSPEPTIDASE ACTIVITY IN
ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF SOMATOSTATIN

			Cerebral Cortex	Cerebellum	Brainstem	Hypothalamus
Saline Control			39.47 ± 4.04 (12)	48.75 ± 2.40 (8)	60.00 ± 2.91 (12)	51.45 ± 3.19 (12)
Somatostatin	0.5 μg	10 min	44.37 ± 2.70 (6)***	49.79 ± 2.29 (6)	62.91 ± 1.02 (6)**	61.87 ± 2.93 (6)*
		30 min	56.45 ± 4.70 (6)*	75.00 ± 1.93 (6)*	66.66 ± 1.02 (6)*	63.83 ± 1.74 (6)*
	1 μg	10 min	50.83 ± 3.41 (6)*	60.41 ± 2.70 (6)*	72.29 ± 3.98 (6)*	72.91 ± 4.51 (6)*
		30 min	50.40 ± 5.34 (6)****	50.41 ± 1.29 (6)	63.12 ± 1.72 (6)**	58.54 ± 6.95 (6)***

Values are μmoles p-nitroaniline released/gm wt. tissue/hr.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001, ** these values at a P value of < 0.02, *** these values at a P value of < 0.05 and **** this value at P value of < 0.01 are significantly different from those of control group.

TABLE III

GLUTAMATE LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF SOMATOSTATIN

			Cerebral Cortex	Cerebellum	Brainstem	Hypothalamus
Saline Control			4.86 ± 0.57 (6)	4.54 ± 0.83 (6)	3.66 ± 0.43 (6)	4.91 ± 0.52 (6)
Somatostatin	0.5 μg	10 min	4.84 ± 0.89 (6)	5.22 ± 0.60 (6)	3.36 ± 0.37 (6)	6.62 ± 1.57 (6)*
		30 min	4.19 ± 0.79 (6)	4.94 ± 0.51 (6)	3.34 ± 0.45 (6)	5.43 ± 0.92 (6)
	1 μg	10 min	4.65 ± 0.33 (6)	4.72 ± 0.38 (5)	3.37 ± 0.37 (6)	4.90 ± 0.59 (6)
		30 min	5.70 ± 0.23 (5)*	5.71 ± 0.16 (5)*	3.81 ± 0.36 (5)	5.94 ± 0.51 (5)*

Values are μmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* These value at a P value of <0.05 are significantly different from those of control group.

TABLE IV

GABA LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF SOMATOSTATIN

			Cerebral Cortex	Cerebellum	Brainstem	Hypothalamus
Saline Control			1.01 ± 0.28 (6)	1.04 ± 0.21 (6)	0.98 ± 0.14 (6)	2.52 ± 0.07 (6)
Somatostatin	0.5 µg	10 min	1.22 ± 0.13 (6)	1.12 ± 0.04 (6)	0.79 ± 0.03 (6)**	2.34 ± 0.44 (6)
		30 min	0.96 ± 0.16 (6)	1.24 ± 0.12 (6)	1.18 ± 0.20 (6)	2.69 ± 0.47 (6)
	1 µg	10 min	1.20 ± 0.10 (6)	0.98 ± 0.11 (5)	0.99 ± 0.15 (6)	1.89 ± 0.24 (6)*
		30 min	1.11 ± 0.08 (5)	0.84 ± 0.09 (5)	0.96 ± 0.04 (5)	2.23 ± 0.05 (5)*

Values are µmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* These values at a P value of < 0.001 and ** This value at a P value of < 0.02 are significantly different from those of control group.

TABLE V

TOTAL SULFHYDRIL GROUPS
LEVELS IN ADULT FEMALE RAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF SOMATOSTATIN

			Cerebral Cortex	Cerebellum	Brainstem	Hypothalamus
Saline Control			9.44 ± 1.37 (5)	11.07 ± 2.29 (5)	9.46 ± 2.11 (5)	12.67 ± 4.20 (5)
Somatostatin	0.5 µg	10 min	11.19 ± 0.74 (5)	11.00 ± 0.40 (5)	9.92 ± 0.17 (5)	15.98 ± 0.33 (5)
		30 min	12.14 ± 0.41 (5)*	12.77 ± 0.41 (5)	10.56 ± 0.37 (5)	21.09 ± 2.76 (5)**
	1 µg	10 min	9.88 ± 0.35 (5)	10.98 ± 0.18 (5)	9.46 ± 0.35 (5)	8.38 ± 0.58 (5)
		30 min	10.53 ± 0.66 (5)	11.03 ± 0.22 (5)	8.50 ± 0.44 (5)	9.58 ± 0.54 (5)

Values are µmoles/gm wet wt. tissue.

Values are means ± S.D. of number of experiments given in parentheses.

* This value at a P value of < 0.01 and ** This value at a P value of < 0.02 are significantly different from those of control group.

sulfhydril groups of cerebellum and brain stem (Table V).

DISCUSSION

Significant increase in the activity of γ-glutamyl transpeptidase in hypothalamus, cerebral cortex and brain stem, irrespective of the doses employed and at the two time intervals studied, indicate that somatostatin may exert regulatory control on glutathione content in these regions. With the doses of somatostatin employed in the present study the decrease in glutathione content parallel with a rise in γ-glutamyl transpeptidase activity. The increase in γ-glutamyl transpeptidase activity may facilitate increased transport of amino acids or peptides although as mentioned earlier this function of glutathione has not been clearly established in brain. The reported formation of γ-glutamyl dopamine may be through this mechanism and increased formation of γ-glutamyl dopamine in hypothalamus is possible under the influence of somatostatin and may have a functional significance since dopamine was shown to stimulate GH release (Tsuji et al., 1977 ; Vijayan, 1985 ; Martini et al., 1978 ; Ichinose et al., 1987). Intraventricular administration of glutathione clearly indicated a stimulatory role for glutathione on GH release. However, somatostatin was shown selectively inhibit GH release from somatotrophs (Brazeau, et al., 1973). Although the mechanism involved in the elevation of γ-glutamyl transpeptidase activity is not known it is probable that somatostatin may have a direct effect on γ-glutamyl transpeptidase. The utilization of glutathione in the presence of increased γ-glutamyl transpeptidase activity is expected. However, the changes in glutathione content can not entirely be attributed to change in γ-glutamyl transpeptidase activity as glutathione can also be utilised in a number of

other metabolic reactions such as oxidation-reduction reactions and trans-hydrogenation reactions. This probability appears to be more as the decrease in the content of glutathione is much more than could be accounted by the degree in the rise in γ-glutamyl transpeptidase activity. Glutathione may be significantly utilised in transhydrogenation of peptides containing disulfides. Utilization of glutathione by this mechanism in hypothalamus may be relevant as number of peptide hormones such as vasoprcssin, oxytocin and somatostatin containing disulfide bonds may undergo such a metabolic change with consequent changes in biological activity.

The involvement of GABA in the release of ACTH, Prl, LH and GH from the anterior pituitary is clearly established (McCann et al., 1979 ; McCann et ah, 1984 ; Vijayan, 1985). The finding that intraventricular injection of somatostatin significantly elevated GABA levels indicate that the action of somatostatin on GH release is probably mediated through GABA. It is probable that somatostatin may be acting on pituitary directly and also at the same time probably inhibiting the factors responsible for the release of GH thus exerting its effect at more than one site.

The significant increase in glut am ate levels of cerebral cortex, cerebellum and hypothalamus may be of metabollic significance rather than functional. The significant increase in total sulfhydril groups of hypothalamus and cerebral cortex at 30 min and the mechanism and functional role has been discussed in Chapter V.

CHAPTER VII

GENERAL DISCUSSION AND CONCLUSION

It is well established that brain controls the release of hormones from the anterior pituitary by hypothalamic releasing and/or inhibitory factors. The hypothalamic releasing factors are mostly peptides having amino acids starting from 3-39. For eg., TRH (tripeptide), enkephalins (pentapeptide), cholecystokinin (octapeptide), LHRH (decapeptide), somatostatin (tetradecapeptide)and ACTH (39 amino acids). The extrapituitary localization of pituitary hormones is also known. Some of the hypothalamic peptide hormones such as somatostatin, TRH, CCK, LHRH have also been foundin tissues other than the nervous system (Brazeau et al., 1973 ; Elde, 1979 ; Sharpe, 1980 ; Khodr and Siler-Khodr, 1980 ; Turkelson, 1981). The tripeptided glutathione is ubiquitously localized in different tissues of the body and is present in brain (Meister and Anderson, 1983). Although metabolic functions of glutathione are known, its biological role as an antioxidant and as a substance in mopping the effects of free radicals is well established in many tissues (Flohe, 1979 ; Chance et al., 1979). Glutathione may particularly be helpful in preventing damage caused in the postanoxic period (Vali Pasha and Sadasivudu, 1984). However, not many studies are available regarding its biological role in central nervous system although it is the most simple tripeptide present in various tissues including the brain. The distribution of glutatione in different regions of the rat brain starting from 21 days to the adult period though found to be uniform, the glutathione content is significantly higher in hypothalamus reaching a peak at puberty. The striking increase in the glutathione levels at puberty suggest a possible involvement of glutathione in the events occurring during the onset of puberty. It is very interesting to note that intraventricular administration of glutathione stimulate the release of anterior pituitary hormones in appreciable

amounts. Glutathione at lower doses caused selective release of FSH when compared with the release of other pituitary hormones. The nearly four fold increase in FSH indicate that the tripeptide may have a role in FSH release. However, glutathione when injected in large doses cause release of other pituitary hormones as well. It seems to be highly potent in inducing GH release when given in higher doses. These results indicate that glutathione may be an important peptide, at least, in the release of FSH although both LH and FSH are controlled specifically by a single releasing hormone LHRH. The LH releasing potency of LHRH is more when compared to FSH (McCann, 1974 ; Ojeda, 1980). Although presence of a specific hypothalamic factor controlling FSH release has been suspected its isolation and chemical identity has not been successful (Lumpkin et ah, 1980 ; McCann et al., 1983). LHRH and somatostatin given intraventricularly decrease glutathione levels in parallel with a rise in γ-glutamyl transpeptidase activity. The formation of γ-glutamyl dopamine by γ-glutamyl transpeptidase has already been reported (Tsuji et ah, 1977 ; Ichinose et ah, 1987). It is possible that the increased formation of γ-glutamyl dopamine in the hypothalamus under the influence of these hypothalamic releasing factors and may have a functional significance as dopamine has been shown to release anterior pituitary hormones (Vijayan and McCann, 1978c, Negro-Vilar et al., 1978b). Glutathione can also be utilized by other routes other than γ-glutamyl transpeptidase. The utilization of glutathione by transhydrogenase reaction in hypothalamus may have functional significance in that the disulfide peptide hormones such as vasopressin, oxytocin and somatostatin may be converted to compounds containing sulfhydril groups thus altering their biological activity.

The role of FSH in gonadal function is well established. However, its extra pituitary localization particularly in brain is not known and much less about its role in behaviour. Intracerebral injection of FSH, curiously produced a decrease in hypothalamic glutathione content and changes in the content of glutamate and GAB A the two potent neuroactive amino acids subserving a major proportion of synaptic function, during the onset of puberty without any significant change in post pubertal period. Such a role is suggestive of a functional and metabolic role for FSH in brain although the mechanism involved is not known. The decrease in the content of glutathione at puberty without any significant change in postpubertal period may be due to the involvement of glutathione in breaking down the disulfide bonds in the peptide hormones by transhydrogenase reaction. The increase in the total sulfhydril groups content in hypothalamus under these experimental conditions lend support to such a contention.

Glutamate in brain has both a metabolic and functional role, about 60% of glucose carbon is known to be utilized for the formation of amino acids and in particular glutamate. Glutamate is one of the amino acids known to maintain functional integrety of the brain in the absence of glucose. Neuropharmacological studies indicate that glutamate is a neuro-excitatory substance. Furthermore, there is a close metabolic interrelation-ship between glutathione and glutamate in that the former may be a source for the latter and the latter may be involved in the synthesis of the former. Glutamate is also the precursor for GABA, a powerful neuroinhibitory substance which is also implicated in the activity of hypothalamic-hypophyseal axis (Vijayan and McCann, 1978a, 1978b ; Negro-Vilar et al., 1980 ; Lamberts

et ah, 1983 ; McCann et al., 1984). The significant increase in GABA levels in hypothalamus after the higher dose of glutathione administration would suggest that glutathione may be acting through GABA since earlier studies have clearly demonstrated that GABA in smaller doses is having a role in the release of LH and at higher doses released prolactin (Negro-Vilar et al., 1980). The significant increase in the levels of hypothalarnic GABA observed after intraventricular injection of LHRH and after intraventricular glutathione administration suggest a functional interaction between LHRH, glutathione and GABA. One of the factors responsible for the significant increase in GH release following the adminstration of glutathione may be by the biological alteration of somatostatin through transhydrogenation by glutathione. The significant decrease in **the** content of glutathionc following the administration of somatostatin provide proof to such a contention.

From the foregoing experimental observations and discussion it may be concluded :

1. that the pubertal spurt in the hypothalamic glutathione content and increased secretion of FSH following the intraventricular administration of glutathione together with the observation that the content of glutathione decreased following intraventricular LHRH and intracerebral FSH indicate a close interaction between glutathione, LHRH and the gonadotropic hormone FSH.

2. that GABA may be mediating the actions of glutathione and LHRH in hypothalamus since a significant increase in GABA was observed following intraventricular administration of glutathione **and LHRH.**

3. that glutathione may also be involved in the release of other anterior pituitary hormones such as prolactin and GH when administered in higher amounts.

4 that the release of Prl and GH by glutathione may be related to increased GABA in hypothalamus and probably by metabolic alteration of the disulfide peptide hormone somatostatin by transhydrogenation since under these conditions there is an increase in the total sulfhydril groups.

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