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), LHRII (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 neurotransmitters 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 al., 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 ovariectomy 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 after 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 radio-immunoassay. 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 significant 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>
<thead>
<tr>
<th></th>
<th>21 Days</th>
<th>30 Days</th>
<th>40 Days</th>
<th>42 Days</th>
<th>45 Days</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>0.96 ± 0.08 (6)</td>
<td>1.15 ± 0.08 (6)**</td>
<td>1.57 ± 0.14 (8)*</td>
<td>2.04 ± 0.19 (8)*</td>
<td>1.61 ± 0.11 (9)*</td>
<td>1.31 ± 0.17 (10)**</td>
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<td>Cerebral Cortex</td>
<td>1.31 ± 0.06 (6)</td>
<td>1.26 ± 0.11 (7)</td>
<td>1.37 ± 0.12 (8)</td>
<td>1.29 ± 0.10 (8)</td>
<td>1.35 ± 0.07 (10)</td>
<td>1.27 ± 0.11 (10)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.32 ± 0.08 (6)</td>
<td>1.23 ± 0.12 (6)</td>
<td>1.48 ± 0.09 (6)***</td>
<td>1.44 ± 0.10 (6)</td>
<td>1.38 ± 0.07 (10)</td>
<td>1.28 ± 0.09 (8)</td>
</tr>
<tr>
<td>Brain stem</td>
<td>1.07 ± 0.14 (6)</td>
<td>0.92 ± 0.13 (8)</td>
<td>1.01 ± 0.13 (8)</td>
<td>1.00 ± 0.10 (8)</td>
<td>1.00 ± 0.07 (10)</td>
<td>0.92 ± 0.15 (6)</td>
</tr>
</tbody>
</table>

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

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.
**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
Fig. 3. Plasma PRL 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 < 0.05
** P <0.02 vs control
* P <0.01
**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 vs control
- * P <0.05
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 glutathione in cerebral cortex, cerebellum and brain stem remained almost the same beginning from day 21 to sexually mature adult rats. However, there is a gradual, but significant, increase in the content of glutathione in the hypothalamus reaching a peak at puberty and decreasing thereafter to the adult levels. Such a unique pubertal peak in glutathione content in the hypothalamus would suggest that glutathione 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 glutathione in hypothalamus prompted the study on the effects of intraventricular glutathione on the release of gonadotropins from the anterior pituitary. Incidentally the release of Prl and GH were also studied following intraventricular glutathione. These studies revealed a significant increase in plasma FSH levels within 5 and 15 min of intraventricular administration of glutathione 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 significant 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 al., 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.