Effect of Intraventricular Luteinizing Hormone Releasing Hormone and Somatostatin on Total Sulfhydryl Groups Content of Rat Brain

K. Vali Pasha*

Department of Biochemistry, Yogi Vemana University, Kadapa - 516 003, Andhra Pradesh, India.

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ABSTRACT

Synergistic functional role have been observed in the case of hypothalamic releasing hormones. Glutathione was also shown to interact with the neuroendocrine system. Intraventricular injection of leuteinizing hormone releasing hormone (LHRH) and somatostatin produced no significant changes in the content of total sulfhydryl groups in cerebellum and brain stem with the doses employed in the present study while a significant increase in the content of total sulfhydryl groups of hypothalamus and cerebral cortex was observed with 1 µg LHRH and 0.5 µg somatostatin. These studies demonstrate a neuroendocrine role of thiols.

Key words: Leuteinizing hormone releasing hormone, Somatostatin, Total sulfhydryl groups, Glutathione.

1. INTRODUCTION

Leuteinizing hormone releasing hormone (LHRH) is one of the hypothalamic factors, which promote gonadotropin release from the anterior pituitary. Somatostatin is a hypothalamic peptide having dramatic inhibitory effect on the release of growth hormone (GH) by the pituitary [1-3]. Glutathione has been found to stimulate gonadotropin and GH release and glutathione was found to be very high at puberty. Apart from a number of biochemical functions such as oxidation-reduction, detoxification, protection against free radicals glutathione was also shown to interact with the neuroendocrine system [4-10]. Most of the anterior pituitary hormones and a variety of neuropeptides including oxytocin and vasopressin were shown to be present in cerebrospinal fluid [11,12]. A number of hypothalamic releasing peptides were found to have synergistic effects and it is probable that such an effect may exist between LHRH, somatostatin and glutathione. Thiol involvement in the regulation of a broad spectrum of biological processes has been clearly demonstrated. The influence of thiols and thiol-active agents is exerted on protein synthesis, neurotransmitter activity, receptor binding, hormone structure and activity [13,14]. Thiols may influence hormone release from isolated secretory granules at multiple sites and thiol sensitive granule membrane proteins [15]. An attempt has been made to evaluate the changes in total sulfhydryl groups levels in different regions of the rat brain including hypothalamus after intra-ventricular injection of LHRH and somatostatin.

2. MATERIALS AND METHODS

LHRH, Somatostatin, DTNB were purchased from Sigma Chemical Company, St. Louis, USA. All other chemicals used were of analytical grade.

2.1. Animals

Female rats of Wistar strain maintained under controlled conditions of light (12 h light: 12 h dark) with free access to drinking water were used in the study. All animal experiments were performed in accordance with rules and regulations of animal ethics and the international guidelines for handling of laboratory animals.

2.2. Experimental Procedure

Mature female rats weighing 180-200 g of Wistar strain were used for intraventricular (IVT) cannulation. A stainless steel cannula (17 mm in length) was implanted into the third ventricle of the experimental animals 5-7 days prior to the experiment as described earlier [7,16]. A mandrill prevented the obstruction of the cannula. The cannula was considered to be located in the third ventricle if cerebrospinal fluid flowed continuously upon removal of the mandrill. The animals were housed in individual cages. LHRH, Somatostatin (Sigma Chemical Company, USA) were prepared freshly in 0.9% saline. Somatostatin at a dose of 0.5 and 1.0 µg and LHRH at a dose of 0.1 µg, 0.5 µg and 1.0 µg were separately microinjected into the third ventricle in a volume of 2 µl. Controls received an equal volume of saline. The animals were sacrificed by decapitation at 10 and 30 min after injection. Brains were quickly

*Corresponding Author:
E-mail: kotwal4pasha@yahoo.co.in
removed and the cerebral cortex, cerebellum, brain stem and hypothalami were dissected out as per the procedure of Sadasivudu and Lajtha [17]. Hypothalami were dissected out as a single block as described by Vijayan [18]. Total sulfhydryl groups were estimated according to the procedure of Sedlak and Lindsay as described by Vali Pasha and Sadasivudu [19,20].

2.4. Statistical Analysis
Experimental data were analyzed by Student’s t-test.

3. RESULTS
3.1. Total Sulfhydryl Groups After IVT LHRH
IVT injection of 0.1 or 0.5 µg dose of LHRH could not produce any significant change in total sulfhydryl 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 a significant increase in total sulfhydryl groups of cerebral cortex and hypothalamus at 30 min after injection (Table 1).

3.2. Total Sulfhydryl Groups after IVT Somatostatin
IVT injection of 0.5 µg dose of somatostatin caused significantly increase in total sulfhydryl groups of only hypothalamus and cerebral cortex at 30 min after injection. There was no change in the total sulfhydryl groups of cerebellum and brain stem (Table 2).

4. DISCUSSION
Although there are no significant changes in the content of total sulfhydryl groups in cerebellum and brain stem with the doses of LHRH and somatostatin employed in the present study a significant increase in the content of total sulfhydryl groups of hypothalamus and cerebral cortex was observed with 1 µg LHRH and 0.5 µg somatostatin. The observed increase in the total sulfhydryl compounds in the presence of decreased glutathione content reported in earlier studies indicate an increase in the content of protein sulfhydryls. Such an increase in the in sulfhydryls suggesting the breakdown of disulfide bonds in the proteins and peptides. Although the rise in the content of total sulfhydryl groups in different brain regions might be occurring as a result of metabolic alterations within the cells, the rise in total sulfhydryl groups in the absence of any change in γ-glutamyltranspeptidase activity may be occurring as a result of stimulation of transhydrogenase activity involving the glutathione and the number of disulfide peptide hormones in hypothalamus. This would further

### Table 1: Total sulfhydryl groups levels in adult female rat brain after IVT injection of LHRH.

<table>
<thead>
<tr>
<th>Control</th>
<th>LHRH 0.1 µg</th>
<th>LHRH 0.5 µg</th>
<th>LHRH 1.0 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>30 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>9.44±1.37 (5)</td>
<td>9.55±0.97 (6)</td>
<td>9.61±0.43 (5)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>11.07±2.29 (5)</td>
<td>12.24±1.45 (6)</td>
<td>11.91±0.95 (5)</td>
</tr>
<tr>
<td>Brain stem</td>
<td>9.46±2.11 (5)</td>
<td>9.68±1.31 (6)</td>
<td>11.83±1.00 (5)</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>12.67±4.20 (5)</td>
<td>11.88±2.07 (6)</td>
<td>11.81±1.60 (5)</td>
</tr>
</tbody>
</table>

Values are µmoles/g. wet wt. tissue, values are means±standard deviation of number of experiments given in parenthesis, *This value at a p<0.02 and **p<0.05 are significantly different from those of control group, LHRH=Luteinizing hormone releasing hormone, IVT: Intraventricular

### Table 2: Total sulfhydryl groups levels in adult female rat brain after IVT injection of somatostatin.

<table>
<thead>
<tr>
<th>Saline control</th>
<th>Somatostatin 0.5 µg</th>
<th>Somatostatin 1.0 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebral cortex</td>
<td>Cerebellum</td>
</tr>
<tr>
<td></td>
<td>9.44±1.37 (5)</td>
<td>11.07±2.29 (5)</td>
</tr>
<tr>
<td>0.5 µg</td>
<td>10 min</td>
<td>11.19±0.74 (5)</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>12.14±0.41* (5)</td>
</tr>
<tr>
<td>1.0 µg</td>
<td>10 min</td>
<td>9.88±0.35 (5)</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>10.53±0.66 (5)</td>
</tr>
</tbody>
</table>

Values are µmoles/g. wet wt. tissue, values are means±standard deviation of number of experiments given in parenthesis, *This value at a p<0.01 and **p<0.02 are significantly different from those of control group, IVT: Intraventricular
lend support to the contention that glutathione may be involved under these circumstances in the inactivation of disulfide peptide hormones locally through transhydrogenation mechanism. The reported formation of γ-glutamyl derivatives such as γ-glutamyl dopamine in hypothalamus under the influence of LHRH and somatostatin may carry functional significance as dopamine is known to stimulate the release of a number of trophic hormones from the pituitary. It is probable that under the experimental conditions the pathways of utilization of glutathione may be 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 with consequent changes in biological activity.

5. REFERENCES

*Bibliographical Sketch*

Dr. K. Vali Pasha has done his M.Sc and Ph.D from University of Hyderabad, Hyderabad in the years of 1982 and 1988 respectively. His major areas of research are neurobiochemistry, clinical biochemistry, herbal drugs, antioxidants. He was a NIH post-doctoral fellow at University of Connecticut, USA. He was a faculty member at Jamia Hamdard University, New Delhi and later moved to Nizam Institute of Medical Science, Hyderabad where he worked as Asst. Professor and Associate Professor. At present he is a Professor of Biochemistry and Dean of Faculty of Science in Yogi Vemana University, Kadapa, Andhra Pradesh, India.