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Research Article

PLASMA GH, PROLACTIN LEVELS AND BRAIN GABA CONTENT AFTER INTRAVENTRICULAR GLUTATHIONE INJECTION IN OVARIECTOMIZED STEROID PRIMED RATS

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ABSTRACT

Prolactin and Growth Hormone are anterior pituitary hormones having multiple physiological functions and number of neurotransmitters and neuropeptides are involved in their regulation. Plasma GH, prolactin and brain GABA levels were determined at 5 and 15 mins after thirdventricular injection of glutathione at doses of 15 or 30 µg in ovariectomized steroid-primed rats. Intraventricular glutathione significantly increased plasma growth hormone (GH) levels at 5 and 15 min after injection. A 15 µg dose of glutathione significantly decreased prolactin levels at 5 min after injection. A higher dose of glutathione (30 µg) however, significantly elevated plasma prolactin levels at 5 and 15 min after injection. Glutathione at a 30 µg dose caused a significant increase in hypothalamic GABA levels at 5 and 15 min after injection where as the same dose decreased GABA levels in the cerebral cortex. The release of GH and prolactin by higher dose of glutathione may be mediated through enhanced levels of GABA. These studies demonstrate a neuroendocrine role of glutathione.

Keywords: Glutathione – Growth hormone – Prolactin – GABA – Hypothalamus.

INTRODUCTION

Prolactin (PRL) is a anterior pituitary hormone which modulates multiple physiological functions with important implications for reproduction and sexual behavior(1,2). Growth hormone (GH) synthesized by anterior gland is an important regulator of growth and development, body composition and metabolism (3,4,5). A number of central neurotransmitters and neuropeptides are involved in GH and Prolactin regulation. The tripeptide(L-γ glutamyl L-cysteinyl-glycine; GSH) is the most prevalent low molecular weight intracellular thiol, present in virtually all living cells. It's ubiquitous distribution in various tissues of the body including brain suggest that glutathione may have some fundamental role in living cells. Although a number of biochemical functions such as oxidation-reduction, detoxification, transport of amino acids and peptides and protection against peroxidative damage have been assigned to glutathione, its biological role in brain is not fully understood [6, 7, 10, 11,12, 13]. Earlier studies from this laboratory on the regional distribution of glutathione in rat brain at different ages and the effect of intraventricular glutathione on gonadotropin levels indicate a close interaction between glutathione and gonadotrophic hormones (14,15, 20, 21, 22, 23, 24). The present experiments were designed to evaluate the role of glutathione, if any, on prolactin and growth hormone in ovariectomized steroid-primed rats. Since GABA has been shown to have a modulatory role on the secretion of anterior pituitary hormones (9, 16, 18, 19, 28, 29), it is of interest to see whether glutathione has any effect on brain GABA levels in ovariectomized steroid primed rats.

Materials and methods

Animals: Rats were sourced from the university animal house, where the rats were continuously bred and maintained. Mature female rats weighing 180-200 g of Wistar strain were used. They were maintained at 12h dark: 12h light with free access to drinking water and standard rat pellets. Sexually mature rats were ovariectomized under light ether anaesthesia and used for experimentation 3-4 weeks later. Animal care protocols were in accordance with rules and regulations of animal ethics and approved by the animal welfare committee.

Experimental Procedure

Ovariectomized animals were used for intraventricular cannulation. A stainless steel cannula (17 mm in length) was implanted in to the third ventricle of the experimental animals 5-7 days prior to the experiment as described earlier [9, 12, 17, 18, 19]. A mandril 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 mandril. The animals were housed in individual cages. The rats were primed with estradiolbenzoate (50µg, sc) and progesterone (25mg sc) 72 h, before intraventricular injection [18]. Estradiolbenzoate and progesterone were purchased from Sigma Chemical company, USA.

Reduced glutathione (Sigma Chemical Company ,USA) was prepared freshly in 0.9% saline and microinjected in doses of 15 and 30 µg per rat in to the third ventricle in a volume of 2 µl. Controls received an equal volume of saline. The animals were sacrificed by decapitation at 5 and 15 min. after injection. Trunk blood was collected and plasma separated and stored for the later assay of Prl and GH. Brains were quickly removed and the cerebral cortex, cerebellum, brain stem and hypothalami were dissected out and assayed for GABA (13, 17, 27). GABA levels were estimated by paper chromatography according to the procedure of Chandrakala et al (8). The contents were expressed as µ.moles of GABA / g wet wt. Plasma GH and Prl were measured by a double antibody radio immunoassay using kits supplied by NIADDK-NIH, USA and according to the guidelines provided with the kit. All the samples were run in one assay, each in duplicate to avoid inter assay variation.

Statistical Analysis: Experimental data was analysed by Student's *t* test.

Results

Plasma GH and prolactin levels after intraventricular glutathione

Plasma prolactin levels were significantly ($p< 0.01$) decreased at 5 min after intraventricular injection of 15 µg glutathione. In contrast a higher dose of 30 µg of glutathione produced a significant increase in plasma prolactin levels at both 5 min ($p< 0.02$) and at 15 min ($p < 0.05$) (Figure 1). Plasma GH levels increased significantly ($p< 0.05$) only at 15 min after a 15 µg dose whereas 30 µg dose of glutathione produced a significant ($p < 0.01$) increase in GH levels both at 5 min ($p< 0.01$) and at 15 min ($p < 0.001$) after injection (Figure 1).

Brain GABA levels after intraventricular glutathione injection

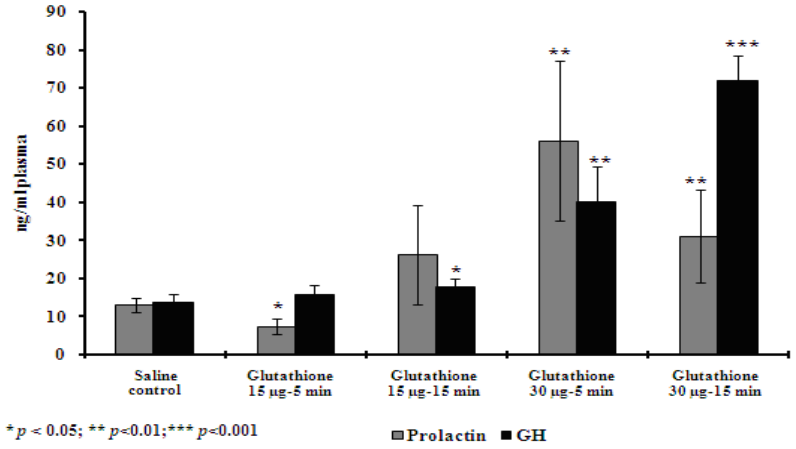
Intraventricular injection of 15 µg glutathione failed to modify hypothalamic GABA concentration. However, a 30 µg dose of glutathione produced a significant ($p < 0.001$) increase in GABA concentration at 5 min and 15 min following injection. There was a significant ($p< 0.02$) decrease in GABA levels in cerebral cortex in these animals after a 30 µg dose. There were no changes in GABA of brain stem and cerebellum. (Table 1).

Table 1: GABA levels in different regions of rat brain after intraventricular glutathione injection

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

Values are µmoles/g wet wt of tissue; Values are mean ± SD n= 6
*** $p < 0.001$ ** $p < 0.02$**

Figure 1: Plasma GH and Prolactin levels after intra-ventricular glutathione injection in ovariectomized steroid primed rats.



Values are ng/ml of plasma; Values are mean ± SD n= 6;
*** $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$**

DISCUSSION

The present results indicate that third ventricular injection of glutathione has a stimulatory effect on GH release. On the other hand, a low dose of glutathione inhibits prolactin release at 5 and 15 min after injection. The content of hypothalamic glutathione was shown to be highest at puberty and glutathione was shown to release the gonadotropins at higher doses which suggest that it may be acting as a general stimulant for the secretion of anterior pituitary hormones (14, 15, 25, 26). It is not clearly understood whether the hypothalamic glutathione is directly 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 the indirect way by regulating other known factors controlling the hypothalamic peptides or other putative neurotransmitters like dopamine and GABA (9, 16).

It is interesting to note that there was no significant alteration in hypothalamic GABA content after the 15 µg dose but it rose significantly with higher dose of glutathione. Such an elevation in the content of GABA may be responsible for the observed changes in prolactin and GH levels. GABA, at higher doses stimulates prolactin release and at lower doses inhibits prolactin release (16, 29). Incubation of hemipituitaries with varying doses of GABA had no effect on GH release (28). The observed effect on GH release may be mediated centrally by lowering hypothalamic somatostatin levels. It is also possible that one of the factors responsible for the significant increase in GH release following the administration of glutathione may be by glutathione transhydrogenase reaction in hypothalamus in that the disulfide peptide hormone; somatostatin may be converted to compound containing sulfhydril groups thus altering its biological activity. However the mechanism in the decrease of GABA levels in cerebral cortex after a 30 µg dose under these experimental cognitions is difficult to explain at the moment.

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