The Effects of Hypo- and Hyperthyroidism on Glucose 6-Phosphate Dehydrogenase Activities in Regions of Rat Brain

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ABSTRACT

The variations of glucose 6-phosphate dehydrogenase (G6PD) activity in different brain regions of normal, hypo- and hyperthyroid rats were investigated. Hypo- and hyperthyrodism were experimentaly induced by administration of methimazol and liothyronine, respectively. In normal rats, midbrain had the minimum (70 mU/mg) and cerebral cortex the maximum (349 mU/mg) G6PD activity. Hyperthyrodism increased the enzyme activities in straitum (72%) and midbrain (144 %). Hypothyrodism also elevated G6PD activities in the two above regioins but to a lesser extent. The enzyme activities were decreased by hypothyrodism in hypothalamus (73%) and cerebellum (45%). Hyperthyrodism, however, increased G6PD activities in cerebellum by (16%) but did not significantly change the enzyme activity in hypothalamus. Neither hypo- nor hyperthyrodism affected cerebral cortex G6PD activity. The data suggest that the changes in G6PD activities of the brain regions may have been occured via different thyroid hormone effect on the regions. *Iran. Biomed. J. 9 (2): 63-66, 2005*

INTRODUCTION

Thyroid hormones regulate a variety of biochemical reactions in virtually all tissues. These hormones are known as important factors in gene regulation in tissues such as brain, liver, muscles and adipose tissue [1]. They are also involved in the control of resting metabolism in rat [2]. Hypothyroidism in developing rat impairs synaptic transmission and has devastating effects on neurological functions that are permanent [3]. In brain, thyroid hormones affect the activities of enzymes such as malate dehydrogenase and hexokinase [4]. In this organ, pentose phosphate pathway shares an important portion of multiple pathways of glucose metabolism [5]. The activity of glucose 6-phosphate dehydrogenase (G6PD), the first enzyme of pentose phosphate pathway, has been affected by epinephrine in human hepatocytes [6] and by adrenal and sex hormones in rat liver [7]. This enzyme provides nicotinamide adenine dinucleotide phosphate (NADPH) that is necessary for many biosynthetic reactions including lipid synthesis. Because bipolar lipids such as phospholipids and sphingolipids are accumulated in the brain regions and NADPH is required for their synthesis, G6PD activity in various brain regions and its hormonal regulation provide valuable information in the development and function of the brain.

Concentrations of thyroid hormones in different regions of the brain have been altered following hypo- and hyperthyroidism [8]. Our preliminary experiments (unpublished data) showed that G6PD is not uniformly distributed through the brain regions. Therefore, the presence of a relationship between G6PD activity and the concentrations of thyroid hormone in brain regions is likely and explains the probable hormonal regulation of this enzyme in the brain areas.

In the present work, the activities of G6PD in various brain areas of normal and experimentally-induced hypo- and hyperthyroidized rats were measured and the possible thyroid hormone-mediated control of the enzyme activity was discussed.

*Corresponding Author; Tel. 09133102965; Fax: (+98-311) 654 0598; E-mail: haghighi@pharm.mui.ac.ir. **Abbreviations:** Glucose 6-phosphate dehydrogenase (G6PD); Nicotinamide adenine dinucleotide phosphate (NADP+); Nicotinamide adenine dinucleotide phosphate (NADPH) (reduced form) .

Table 1. Variations in thyroid hormone concentrations in normal and methimazole- or liothyronine-injected rats.

Injected	T_4 (µg/dl)	T_3 (µg/dl)	TSH (µg/ml)
None (control)	6.65 ± 0.90	0.1090 ± 0.019	1.94 ± 0.28
Methimazole	$2.14 \pm 0.40^*$	$0.0346 \pm 0.005^*$	$9.34 \pm 0.99^*$
Liothyronine	$14.52 \pm 1.42^*$	$0.3780 \pm 0.033^*$	$0.30 \pm 0.03^*$

Rats were injected with methimazol (5 mg/kg) or liothyronine (25 µg/kg) for 10 days and thyroid hormones measured as described in methods. Each value represents mean ± SD of 6 rats. *Signigicantly different from control (*P*<0.05).

MATERIALS AND METHODS

Chemicals. Methimazol and sodium lyothyronine were obtained from Iran Hormone Co. (Iran). NAD^+, glucose 6-phosphate and Trizma were purchased from Sigma (England). All other chemicals were reagent grade.

Animals. Male Wistar rats (180-200 g) were obtained from the Pasteur Institute of Iran (Tehran). The rats were maintained and fed as described before [9]. Hypo- and hyperthyroidism were experimentally induced according to Broedel *et al.* [8] by injecting the rats (in groups of 6) with liothyronine (25 µg/kg) and methimazol (5 mg/kg) daily at 8 AM for 10 days. Control groups received saline during the same periods.

Dissection of brain regions. Each rat was sacrificed by decapitation. The whole brain was taken out and various brain regions dissected according to Glowinsky and Inversen [10]. Cerebral cortex, striatum, midbrain, cerebellum and hypothalamus regions were carefully removed and kept at -25ºC. Each region was homogenized in 15 mM phosphate buffer pH 7.4 and centrifuged at $20,000 \times g$ for 20 minutes. The supernatant was kept at -25ºC for determination of G6PD activity.

Enzyme assay. G6PD activity was measured in a Perkin Elmer spectrophotometer model 551S at 25ºC. The assay mixture was 86 mM Tris-HCl buffer pH 7.4 containing 1.2 mM glucose 6-phosphate, 0.4 mM nicotinamide adenine dinucleotide phosphate ($NADP^+$), 6.9 mM $MgCl_2$ and appropriate amount of the enzyme solution. The increase in the absorbance of NADPH produced was measured at 340 nm to calculate the enzyme activity [6].

Protein determination. Protein concentration was measured by the method of Lowry *et al.* [11].

Hormone determination. The concentrations of T_4, T_3 and TSH were measured by ELISA using commercial kits (Kavoshyar Co., Tehran).

RESULTS

Hypo- and hyperthyroidism induced by methimazole and liothyronine, respectively are shown in Table 1. Significant decrease in T_4! (67%) and T_3 (67%) and increase in TSH (76%) concentrations of methimazole-injected rats are consistent with hypothyroidism. In liothyronine-treated animals also elevation in T_3 (244%) and T_4 (117%) and decline in TSH (89%) levels confirmed hyperthyrodism.

Table 2 shows G6PD activities in the whole brain and various brain regions of normal rats and of those in which hypo-and hyperthyroidism were induced experimentally. In normal rats, the enzyme activity in cerebral cortex (349 mU/mg protein) was significantly greater than in other regions.

Table 2. G6PD activities in brain regions of normal, hypo- and hyperthyroid rats.

Brain region	G6PD activity, mU/mg protein		
	Normal	Hypothyroidism	Hyperthyroidism
Whole brain, Cont.	231 ± 18	-	-
Cerebral cortex	$349 \pm 66^*$	326 ± 37	355 ± 43
Straitum	$113 \pm 18^*$	$137 \pm 21^{**}$	$194 \pm 24^{**}$
Hypothalamus	191 ± 22	$52 \pm 7^{**}$	198 ± 17
Midbrain	$70 \pm 12^*$	$97 \pm 11^{**}$	$171 \pm 20^{**}$
Cerebellum	$196 \pm 11^*$	$108 \pm 16^{**}$	$227 \pm 19^{**}$

Experimental hypo- and hyperthyroidism were induced, brain regions were homogenized and G6PD activity measured as described in the text. Values represent mean ± SD of 6 rats. *, significantly different from whole brain (*P*<0.05). **, significantly different from corresponding regions of normal animals (*P*<0.05).

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Midbrain, however, had the least enzyme activity (70 mU/mg protein) among the regions. Similar enzyme activities were shown in hypothalamus and cerebellum.

Neither hypo- nor hyperthyroidism affected cerebral cortex G6PD activity. Hyperthyroidism increased the enzyme activity in straitum (72%) and midbrain (144%). The enzyme activities in these two regions were also elevated by hypothyroidism but to a lesser extent. G6PD activities were decreased by hypothyroidism in hypothalamus (73%) and cerebellum (45%). Hyperthyroidism, however, increased G6PD activities in cerebellum (16%) but did not significantly change the enzyme activity in hypothalamus. Cerebral cortex G6PD activity was not affected by hypo- or hyper-thyroidism. The presence of neither methimazol nor liothyronine in the assay mixture affected G6PD activity, ruling out the direct effect of these substances on the enzyme activity.

DISCUSSION

The alterations observed in thyroid hormone concentrations following administration of methimazole or liothyronine are consistent with the induction of hypo- and hyperthyrodism [12, 13]. In normal rats, the marked variations in G6PD activities of various brain regions might be attributed to the different cellular structures of the regions. The cerebral cortex of the brain is accumulated with cell bodies of the neurons but mid brain consists mainly the neuron axons [14]. Since the cell bodies are the central locations of enzymes [4], it seems likely that G6PD activity being higher in the cortex and lower in the midbrain than other brain regions. G6PD activities in striatum, hypothalamus and cerebellum are consistent with this hypothesis. These observations are also in agreement with the histochemical studies of the midbrain region [15] and the higher G6PD activity in the olfactory bulb than other brain areas [16].!

Hypo- and hyperthyrodism exerted different effects on G6PD activities of various brain regions. Broedel *et! al*. [8] have demonstrated that the treatment of rats with thyroxine (T_4) increased T_4 concentrations in all brain regions, whereas 3,5,3′-triodothyronine (T_3) levels were reduced in some regions and remained unchanged in others. Other report has shown that thyroid hormones influence the activities of lipogenic enzymes such as malic enzyme and G6PD [17]. The effect of T_3 on malic enzyme has been exerted at the transcriptional levels, but it is unclear whether the effect on G6PD is also nuclear mediated. The alterations were observed in G6PD activities of different brain regions, therefore, it could be associated with the different thyroid hormone concentrations in these regions. It appears that different responses of brain regions to thyroid hormones is related to the different cellular structures of these areas. Because the addition of either methimazole or liothyronine did not affect G6PD activity *in vitro,* it seems that the change in G6PD activities of the brain regions has been occurred via hormone effect through specific receptors. Little change of the enzyme activity, however, in the cerebral cortex might be consistent with the limited receptors for thyroid hormones. T_3-binding protein from rat brain cytosol has been characterized and depended on NADPH, $NADP^+$ and thioredoxin [18]. The present data plus those of the thyroid hormone-mediated control of gene expression and the roles of different thyroid hormone receptor isoforms [1] may lead to a better understanding of the mechanism by which G6PD activity has been affected.

The significant decrease in G6PD activity of hypothalamus in methimazole-injected rats is resulted from low concentrations of thyroid hormones in this area. Hypothalamus is the site for feed-back control of hypothalamus/hypophysis loop of thyroid gland and the synthesis of thyrotropin releasing hormone (TRH) occurs in this site [13]. TRH synthesis is inhibited by thyroid hormones and the pathway is mediated by dopamin and norepirephrine which, in turn, their synthesis require NADPH and NADH [19]. G6PD activity, therefore, plays an important role in this regard.

The mechanism by which G6PD activities increased in striatum or midbrain by both hypo- and hyperthyroidism is unclear. The increased G6PD activities in these regions may lead to fat accumulation and subsequently cellular disorders. This could explain the physiological and behavior changes observed in the animals following methimazole or liothyronine treatments.

In rat brain, glucose is metabolized through glycolysis and pentosephosphate pathway with a ratio of 1.5:1 [5]; G6PD, a key enzyme in the latter pathway, therefore, takes part in the brain glucose metabolism and its activity may be regulated by thyroid hormone. The possible regulation of this enzyme by T_3 has been reported [17]. Developmental hypothyrodism in developing rat has caused functional impairments in synaptic transmission and plasticity in the dentate gyrus of

the adult hippocampus [3]. This may also be attributed to the change in G6PD activity. The association between G6PD activity and brain function impairment is also seen in Alzheimer's disease in which cerebral G6PD activity is increased [20]. Bockmann and Winter [21] have reported that the expression of a TSH reccptor in the CNS indicates that TSH is not only a hormonal messenger for the thyroid gland but also can act directly in the brain. A result of such action may cause alterations in G6PD activity in the brain regions. Further studies are required to clarify the mechanism by which thyroid hormones affect G6PD activity in brain regions.

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