STUDIES ON THE EFFECT OF HYPOPHYSECTOMY ON PROTEIN METHYLASE II OF RAT

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1. Introduction

Protein methylase II (*S*-adenosylmethionine:protein-carboxyl methyltransferase; EC. 2.1.1.24), which methylates (esterifies) free carboxyl groups of substrate protein with *S*-adenosyl-L-methionine as methyl donor, has been studied in several laboratories [1–3]. The prominent feature of the enzymatic reaction is the formation of a protein-methyl ester, which undergoes non-enzymatic hydrolysis in mild alkali medium to yield methanol [2–4]. Thus, the enzyme was initially referred to as the methanol-forming enzyme [5]. That this enzyme is identical to protein methylase II has recently been established by studies comparing the properties of the two enzymes [6] and the unstable nature of the enzymatic product [3,7].

The distribution studies of the enzyme in various rat organs indicate that the enzyme is highly localized in the testis, brain, pituitary gland, erythrocyte and many other organs [3,8–10]. The posterior and anterior lobes of the pituitary gland were found to be particularly rich in methyl acceptor protein(s) [11]. Specifically luteinizing hormone and follicle-stimulating hormone serve as good substrates for protein methylase II in vitro [3].

In a continued effort to elucidate the biological significance of the protein methylase II reaction, we have studied the effect of hypophysectomy on the tissue level of protein methylase II in the testis, since testicular function is under the control of gonadotropins [12] and this organ constitutes one of the richest sources of the protein methylase II activity.

2. Materials and methods

2.1. *Materials*

S-adenosyl-L-(methyl-^{14}C) methionine (Sp. Act. 60 mCi/mmol) was purchased from New England Nuclear Corporation. Bovine γ-globulin was obtained from Sigma Chemical Co. Testosterone propionate was purchased from Nutritional Biochemical Corporation. Other chemicals used were from local sources and of analytical grade.

2.2. *Animals*

Hypophysectomized male rate, weighing between 180 to 200 g were purchased from Charles River Breeding Laboratories, Boston, Mass., and were maintained on a commercial stock diet with 5% sucrose solution as drinking water. Lighting of the room is controlled as a 12-hr alternating light and dark period. Body weight of the animals decreased to about 150 g approximately the 40th day after hypophysectomy. The decrease in weight of the testis following surgery is indicated in the text. Hypophysectomized rats received 3 mg of testosterone propionate in 0.2 ml of olive oil daily subcutaneously starting on the 5th post-operative day. This dose has been shown to cause a maximum increase in testicular weight when administered to previously hypophysectomized rats [17].

2.3. *Preparation of enzyme*

The animals were sacrificed by decapitation and tissues were immediately removed. The tissues were then homogenized in four volumes of cold 0.25 M

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sucrose and the homogenate was centrifuged at 105 000 *g* for 60 min. Both whole homogenate and supernatant fractions were assayed for protein methylase II activity. Testes from 3 to 4 hypophysectomized rats were pooled to obtain sufficient material.

Protein concentration was estimated by the method of Lowry et al. [13] with bovine albumin as standard protein.

2.4. *Assay of protein methylase II*

Protein methylase II activity was assayed at pH 6.0 [2] as described previously. Ten mg of γ-globulin were used as added substrate. Specific activity of the enzyme is defined as picomoles of methyl groups transferred per min per mg protein.

2.5. *Separation of seminiferous tubules from the interstitial tissue*

One gram of rat testis was suspended in cold Ringer solution, and the separation of seminiferous tubules from interstitial tissues were carried out according to the method described by Christensen and Mason [14]. Each component isolated in the Ringer solution was centrifuged, and the supernatant was saved. The packed unbroken pellets were homogenized in about 2 ml of 5 mM sodium phosphate–5 mM EDTA–2.4 mM 2-mercaptoethanol. The protein methylase II activity was assayed in both the supernatant and the homogenized pellet. The total enzyme activity in both the supernatant and the pellet was considered as the enzyme activity in either the tubule or the interstitial component.

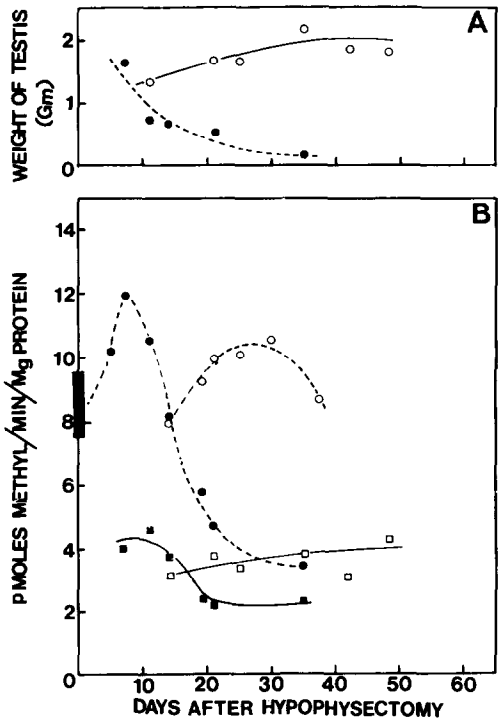

Fig.1. Change of testis weights after hypophysectomy and testosterone treatment: weight of testis is expressed on the basis of per testis. (●——●——●), hypophysectomized; (○——○——○), testosterone injected after hypophysectomy. Effect of hypophysectomy and testosterone-treatment on protein methylase II activity of rat testis: The assay conditions for protein methylase II are as described in the text. (■——■——■), whole homogenate; (●– – –●– – –●); cytosol; (□——□——□), whole homogenate of testosterone injected; (○– – –○– – –○), cytosol of testosterone injected. Specific activities of protein methylase II in normal rat testis are 4–5.2 for whole homogenate and 7.8–9.9 for cytosol.

3. Results and discussion

3.1. *Effect of hypophysectomy on the level of protein methylase-II in rat testis*

The specific activity of protein methylase II in the testis of hypophysectomized rats before and after testosterone treatment is shown in fig.1. The enzyme activity in both whole homogenate and cytosol fraction began to decrease approximately 15 days after the operation. At about the 35th day after hypophysectomy, the enzymatic level reached a minimum plateau of less than half the control value. It is seen in the figure that the specific activity (enzyme activity expressed on the basis of protein content) in the cytosol fraction is much higher than that of whole homogenate. This is due to the fact that protein methylase II is almost exclusively located in the soluble fraction [15]. It is also seen in the figure that the specific activity in the cytosol fraction on the 7th day after hypophysectomy is about 20% higher than the normal control value. At present this initial increase of the enzyme activity later followed by drastic decrease in activity is not understood.

Daily subcutaneous injections of 3 mg testosterone propionate into hypophysectomized rats prevented

Table 1
Effect of hypophysectomy and subsequent testosterone-treatment on protein methylase II* activities in brain and liver of rat

Condition	Brain		Liver	
	Whole homogenate	Cytosol	Whole homogenate	Cytosol
Hypophysectomized	2.21 ± 0.44	8.59 ± 0.96	0.24 ± 0.03	1.3 ± 0.35
Hypophysectomized and treated	2.17 ± 0.22	8.85 ± 1.63	0.17 ± 0.05	0.98 ± 0.19

Detailed experimental procedures and assay conditions are described under methods. The enzyme activities were assayed at the 7, 11, 14, 21, 28, 35 and 42nd day after the operation. The mean values were calculated, because there were no significant changes in the activities at the various days of post-hypophysectomy.

* The enzyme activity is expressed as picomol of methyl transferred/min/mg protein.

the decrease of protein methylase II activity in both whole homogenate and the cytosol fraction. This testosterone treatment also prevented the decrease of the weight of testis occurring after hypophysectomy (fig.1A). It is clear in fig.1A that the relative decrease in testicular weight after hypophysectomy is much greater than that of protein methylase II activity; at about the 35th day following hypophysectomy testicular weight fell to a level about one-sixth that of normal controls. This finding suggests that the decrease in protein methylase II activity in the testis is not merely a function of the decrease in testicular mass. It should be mentioned that the level of the testicular enzyme related to steroid bioconversion are not all related to testicular size [18].

3.2. *Protein methylase II activities in brain and liver of hypophysectomized rats*

Table 1 lists the results on the protein methylase II activities in brain and liver of hypophysectomized rats before and after testosterone-treatment. Daily injections of testosterone propionate to the hypophysectomized rats had no effect on the protein methylase II activity in these tissues. Furthermore, the enzyme activities in both brain and liver of hypophysectomized rats were also compared to those of non-operated and shown to have no significant differences in their activities (not shown in the table). These results indicate that hypophysectomy or subsequent treatment with testosterone does not affect the natural inhibitor of protein methylase II which is present in greatest concentration in the liver [15]. The results listed in table 1 together with those in fig.1 therefore suggest that the change of protein methylase II activity responses to hypophysectomy or testosterone administration is specific only to testis but not to liver or brain under the present experimental conditions.

3.3. *Distribution of protein methylase II in different cellular components of rat testis*

The distribution of protein methylase II activity in different cellular components of rat testis was examined before and after hypophysectomy. The seminiferous tubules and interstitial tissues of rat testis were separated and the protein methylase II activity was determined in each tissue. As shown in table 2, approximately 70% of the total protein methylase II activity was found in the seminiferous tubules and the remaining in the interstitial tissues of normal rat testis. On the other hand, in the testis of rats hypophysectomized 15 days previously, the enzyme activity was practically equally distributed among these two cellular components. The numbers in the table indicate two separate determinations. Although some technical difficulties were encountered during the isolation of cellular components of rat testis of hypophysectomy due to the fragility of the tissues, the duplicate values are remarkably close. Since protein methylase II activity decreased considerably in the first 15 days following hypophysectomy, the result in table 2 suggests a preferential decrease in enzyme activity in the seminiferous tubules by the removal of pituitary gland. This decrease might be as expected, since the rapid tubular shrinkage after hypophysectomy is reported [16].

Table 2
Distribution of protein methylase II in cellular components of rat testis

Preparation	Normal* (%)	Hypophysectomized** (%)
Whole testis	100	100
Tubular	70;77	54;54
Interstitial	30;22	53;54

* Body weight of normal rat is 145 g.
** 15 days post-hypophysectomized rats were used.

Conditions for the enzyme assay were the same as described [2] at pH 6.0. 10 mg of γ-globulin was used as substrate.

The biochemical significance of enzymatic esterification of protein (peptide) molecules is not well understood at present. However, the present report represents a possible explanation for understanding the physiological role of the protein methylase II reaction by the use of testis from hypophysectomized rats. The changes of the enzyme level due to deprivation of gonadotropin (hypophysectomy) and administration of testosterone (fig.1) in rat testis seem to be rather specific, since the enzyme activities in brain or liver do not change under the same experimental conditions (table 1). Further investigations aimed at resolving the mechanism underlying the relationship between gonadotropin and testosterone in the testis in terms of their effect upon the enzyme level, are in progress.

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