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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Effects of Melanocyte-stimulating Hormone on Serum Levels of Thyroid Hormones in Short-term Alloxan-induced Diabetic Rats

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Abstract: The changes induced by MSH after short-term alloxan -induced diabetes mellitus on both male and female Sprague Dawley rat's serum T₄ and T₃ were evaluated. Serum hormones were determined by specific radioimmunoassays and short-term diabetes mellitus induction was carried out by daily injection of 20 mg alloxan solution/100 g body weight intraperitoneally for 10 days. Mean serum T₄ was significantly decreased to 11.044±2.588 nmol L⁻¹ and serum T₃ to 0.132±0.0137 nmol L⁻¹ in diabetic rats. After 10 days with daily injection of alpha-MSH at a dose of 2 µg/100 g body weight, serum T₄ increased significantly up to 82.557±25.815 nmol L⁻¹ and serum T₃ increased up to : 1.027±0.311 nmol L⁻¹. The results can be explained by the role of the Melanocyte Stimulating Hormone in the peripheral regulation of thyroid function and that it may act as a mediator of hyperglycemia effects on the HPT axis.

Key words: MSH, thyroxin, T₄, triiodothyronine, T₃, alloxan induced-diabetic rats, type 2 diabetes mellitus

INTRODUCTION

It has been shown that diabetes mellitus complications are associated with many endocrine diseases such as gonadal dysfunction (Yaman *et al.*, 2006; Cho *et al.*, 2006; Ebrahim *et al.*, 2006; Ortiz Nunez *et al.*, 2005), hypercortisolism, Cushing syndrome (Radahamadi *et al.*, 2006; Liu *et al.*, 2005; Chiodini *et al.*, 2005) and a depression of the thyroid axis (Moriyama *et al.*, 2006). Thus, triiodothyronine (T₃), thyroxin (T₄), Thyroid Stimulating Hormone (TSH) and hypothalamic Thyrotropin Releasing Hormone (TRH) are reduced in humans and rodents during diabetes mellitus (Katovich *et al.*, 1993). However, little attention is paid to the diagnosis of thyroid diseases in diabetics. Vondra *et al.* (2005) reported that prevalence of thyroid diseases in diabetic patients is 2-3 times higher than in nondiabetic subjects. Chubb *et al.* (2005) noted that there are interactions among thyroid dysfunction, insulin sensitivity and type 2 diabetes. Martin *et al.* (2006) and Lechan and Fekete (2006) provided compelling evidence for the interaction between the Hypothalamic-pituitary-thyroid (HPT) axis and the melanocortin system and reported that Melanocyte-stimulating hormone has a stimulatory effect on the HPT axis.

Melanocortins have a multitude of actions including modulating disease pathologies such as obesity (Gettling,

2006) and diabetes mellitus (Abu-Samak *et al.*, 2006). Since MSH appears to increase sensitivity to insulin (Costa *et al.*, 2006) and has stimulatory effect on HPT axis, it is hypothesized that MSH may be a potential mediator of the HPT axis during the early stages of diabetes. The purpose of this research is to investigate whether alpha-MSH altered the thyroid hormone T₄, T₃ levels and thus play a modulatory role in diabetic rats.

MATERIALS and METHODS

Animals: Sixty eight male and female Sprague Dawley white rats, were housed in the laboratories of Medical Technology Department, Applied Science University at least 10 days before the experiments. The animal facility was kept at a room temperature of 21 °C. Food and water were available for the animals all the time and without any restrictions. The weights of rats were taken on the day of the experiment and only those weighing 200-250 g were used in this study. The animals were divided into four groups, each injected intraperitoneally (i.p.) with one of the following preparations: C group : Control rats injected with 1 mL of normal saline daily for 10 days. DM group : Alloxan-induced diabetic rats were given intraperitoneally (i.p.) a daily injection of 20 mg alloxan solution/100 g of body weight (Sigma Firm, USA) for 10 days. MSH group: Rats injected (i.p.) daily with melanocyte stimulating

hormone (Sigma Firm, USA) at a dose of 2 $\mu\text{g}/100\text{ g}$ of body weight for 10 days and DM/MSH: Alloxan-induced diabetic rats injected (i.p.) with both alloxan (20 mg/100 of body weight) and MSH 2 $\mu\text{g}/100\text{ g}$ of body weight) daily for the 10 days.

The seasonal and circadian changes of hormonal concentrations in blood and the tissues. The investigations were carried out, in winter and spring of 2005-2006 were taking into consideration since it is well known that at this time the rats possess a more stable content of hormones. At the end of the of the experiment, all rats were fasted for 12 h before they were sacrificed and blood collected.

Radioimmunoassay: The concentration of insulin, thyroxine and triiodothyronine was measured in serum by radioimmunoassay using radioimmunoassay kit supplied by the (Cea-Jre-Sorin Firm, France).

Serum glucose: Concentrations were measured by the glucose oxidase method using a spectrophotometer (Cecil ce 1010 England).

Statistical analysis: Data were expressed as means \pm SE and were analyzed with a two-way ANOVA followed by

LSD multiple comparison test, using Statistica Software (OK, USA). Differences were considered significant at $p < 0.05$.

RESULTS

All short-term DM, MSH and DM/MSH animals had a slight body weight loss during the experimental period in compression with control rats (Table 1). Plasma glucose levels were significantly increased in all DM groups, being 2 folds higher than in the controls. (C: $95.4 \pm 4.5\text{ mg dL}^{-1}$, DM: $199 \pm 12.3\text{ mg dL}^{-1}$, $p < 0.01$) (Fig. 1). After 10 days of alloxan DM induction, serum insulin was significantly reduced in DM rats, (C: $2.06 \pm 0.32\text{ }\mu\text{IU L}^{-1}$, DM: $0.65 \pm 0.06\text{ }\mu\text{IU L}^{-1}$, $p < 0.01$).

Table 1: Changes of body weights (g) in normal and diabetic rat groups under effect of Melanocyte Stimulating Hormone 2 $\mu\text{g}/100\text{ g}$ of body weight

Rat body weight (g)	Control	MSH	DM	DM/MSH
Initial body weight:	202.5 \pm 8.24	236.5 \pm 11.2	226.4 \pm 13.3	192.66 \pm 7.66
weight: (1st day				
body weight:	209.3 \pm 9.73	237.0 \pm 9.6	220.7 \pm 12.84	196.66 \pm 9.38
(3rd) day				
Final body weight:	216.5 \pm 9.88	228.5 \pm 0.8	208.9 \pm 13.1	177.6 \pm 6.28
(10th) day				

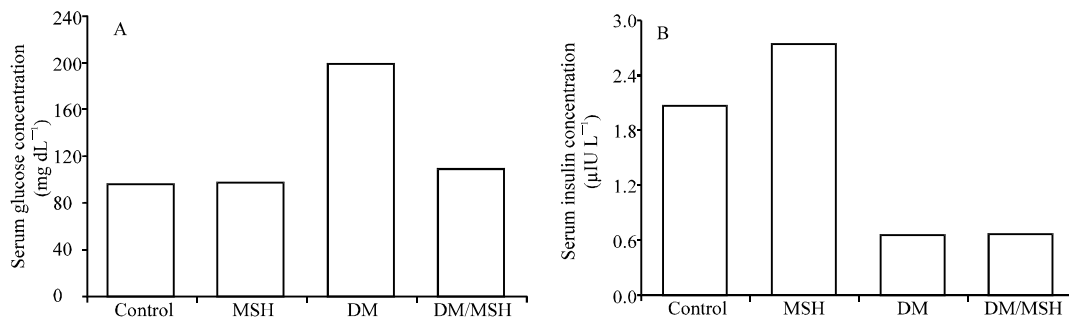


Fig. 1: The effects of α -MSH (2 $\mu\text{g}/100\text{ g}$ of body weight), on serum levels of (A) glucose and (B) insulin after short-term (10 days) of alloxan-induced diabetes

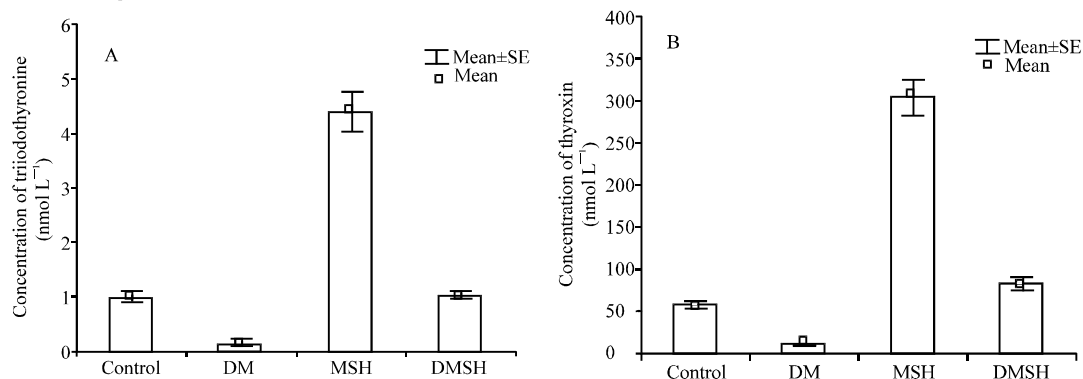


Fig. 2: The effects of α -MSH 2 $\mu\text{g}/100\text{ g}$ body weight on serum levels of Triiodothyronine (T_3) and Thyroxine (T_4) in short-term (10 days) alloxan-induced diabetes

Although there was no significant difference in serum insulin levels between DM and DM/ MSH injected rats in short-term DM, (DM: $0.65 \pm 0.06 \mu\text{IU L}^{-1}$, MSH/DM: $0.66 \pm 0.10 \mu\text{IU L}^{-1}$) serum glucose levels were significantly decreased in DM/MSH rats to 108.72 ± 11.7 , $p < 0.01$) in comparison with DM rats ($199 \pm 12.3 \text{ mg dL}^{-1}$) (Fig. 1).

At the final day of experiments, serum T_3 and T_4 were significantly decreased in DM rats (T_3 , C: $0.964 \pm 0.229 \text{ nmol L}^{-1}$ DM: $0.132 \pm 0.0137 \text{ nmol L}^{-1}$, $p = 0.0489$), (T_4 , C: $57.416 \pm 10.894 \text{ nmol L}^{-1}$, DM: $11.044 \pm 2.588 \text{ nmol L}^{-1}$, $p = 0.0423$) MSH administration increased both Serum T_3 and T_4 in DM/MSH groups (T_3 , $1.027 \pm 0.311 \text{ nmol L}^{-1}$) and (T_4 $82.557 \pm 25.815 \text{ nmol L}^{-1}$) (Fig. 2).

DISCUSSION

The present findings indicate a modulatory role for MSH during endocrine system disturbances. Although the causes of thyroid dysfunction in DM are still unknown, it has been shown that the metabolic alterations caused by DM, or the lack of insulin itself, can directly affect some aspects of thyroid function (Fekete *et al.*, 2006). The use of multi-low doses of alloxan and short-term DM model in this study to show that low doses of alloxan were effective in inducing DM as shown by early stages of DM type 2. Serum T_3 and T_4 were decreased in the short and long-term DM rats, as reported by others (Baydas *et al.*, 2002; Szkudelski *et al.*, 2003), this indicates a change in the factors that determine the decreased thyroid hormones secretion during the initial period of insulinopenia. Nascimento-Saba *et al.* (1997) showed that the changes produced by short-term insulinopenia cannot be explained by a diminished TSH stimulation of the thyroid gland in diabetic mice.

It has been demonstrated that the melanocortin system interacts with the hypothalamo-pituitary-thyroid (HPT) axis (Martin *et al.*, 2006) and the stimulatory effects of MSH on (HPT) axis is known (Fekete *et al.*, 2000). MSH increases the influence of thyroid hormones on metabolism and hence, energy expenditure. This may explain the decrease in body weight after 10 days which was similar to that reported by other investigators studying periods ranging from 6 to 15 days after DM induction (Szkudelski *et al.*, 2003; Nascimento-Saba *et al.*, 1997). Therefore, an interaction between the HPT axis and the melanocortin system would allow control of both sides of the energy balance equation, by the regulation of both energy input and energy expenditure.

Fekete *et al.* (2006) noted that alpha-MSH also contribute to the mechanism by which leptin administration restores thyroid hormone levels to normal in fasted animals. Recent results have shown that alpha-MSH is the complement of leptin in the endocrine circuit (Costa *et al.*, 2006), regulate body weight, food intake and metabolic rate (Seeley *et al.*, 2005) and decrease body weight, by decreasing body fat (Fehm *et al.*, 2001).

MSH analogs have also been shown to affect blood glucose levels in some mouse models of obesity and improve insulin sensitivity according to Costa *et al.* (2006) and reduces blood sugar as noted by Abu-Samak *et al.* (2006), Savontaus *et al.* (2004). These reports support our findings and may explain part of peripheral mechanism by which MSH raised thyroid hormones levels in diabetic rats. Therefore, present results suggest a role of the Melanocyte stimulating hormone in the peripheral regulation of thyroid function and provide evidence that it may act as a mediator of hyperglycemia effects on the HPT axis.

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