Effects of Administration of Alpha-melanocyte Stimulating Hormone (α-MSH) on the Content of Corticosterone in Some Tissues of Alloxan-induced Diabetic Rats

Kamal Mohmoud Saleh Mansi
Department of Biological Sciences, Al al-Bayt, University Al-Mafraq-Jordan

Abstract: This study was designed to evaluate the role of alpha-melanocyte stimulating hormone (α-MSH) on the content of corticosterone in some tissues of alloxan induced diabetic rats. Forty male white rats were divided into four experimental groups: control, diabetic, α-MSH-treated and α-MSH-treated diabetic. At the end of the experimental period (3 weeks), animals in all four groups were fasted for 12 h and blood samples were taken for the determination of plasma insulin, corticosterone, ACTH, glucose levels and concentration of corticosterone in various tissues of diabetic rats as kidney, hypothalamus, liver, adrenal gland, cerebral, hemispheres and thyroid gland. It was found that at the end of the experiment the insulin concentration in blood progress in decreasing reaching the value (0.68±0.07 mU L⁻¹) on the 10th day after introducing of alloxan in comparison with the control (1.84±0.42 mU L⁻¹), blood glucose level increased and reached (11.52±1.34 mole L⁻¹) and still higher than control and other groups and the plasma concentrations of ACTH (158.53±6.45 pmol L⁻¹) in diabetic group and (84.53±3.94 pmol L⁻¹) in α-MSH-treated diabetic compared with the control (63.84±5.52 pmol L⁻¹) and α-MSH-treated (58.36±4.81 pmol L⁻¹). Corticosterone level was increased in diabetic group (7.16±1.54 ng mL⁻¹) and in α-MSH-treated diabetic (9.64±1.68 ng mL⁻¹) compared with the control (1.64±0.342 ng mL⁻¹) and α-MSH-treated (2.87±0.418 ng mL⁻¹). After the analysis and measuring the content of corticosterone in various tissues of diabetic rats it was found that alloxan decreased the corticosterone level in all selected tissues and after the injection of α-MSH the content of corticosterone increased in tissues compared with diabetic group and still lower than control and α-MSH-treated. It is concluded that exogenous introduction of (α-MSH) in alloxan-induced diabetic rats increased the concentration of the total plasma level of corticosterone, at the same time decreased the content of corticosterone occurs adrenal gland, hemispheres and increased it in hypothalamus, kidney, liver and thyroid gland.

Keywords: Alloxan, diabetic mellitus, α-MSH, corticosterone, ACTH, HPA, hypothalamus

INTRODUCTION

Diabetes Mellitus DM represents a heterogeneous group of disorders that have hyperglycemia as a common feature (Tich and Mc Devit, 1996; Bell and Polonsky, 2001). Although diabetes has long been considered a disease of minor significance, it is considered now as one of the main threats to human health in 21st century. Great changes in the human environment, behavior and lifestyle resulted in the raising rates of diabetes (Zimmet et al., 2001). DM is consequence of defects in secretion, insulin action or both, which is translated into abnormalities of carbohydrate, fat and protein metabolism resulting in hyperglycemia (Klip et al., 1992; Taskinen et al., 1996). Symptoms of chronic hyperglycemia include polypro, polydipsia and olyphagia as well as weight loss. Although varying among patients, long-term complications of diabetes can also include changes in arteries (Atherosclerosis), basement membranes of small vessels (microangiopathy), kidneys (nephropathy), retina (retinopathy) and nerves (neuropathy) (Vlassara et al., 1984; Yabe-Nishimura, 1998;
Brownle, 2001). DM leads to many complications, such as increasing the risk of developing arterial disease by two to six folds (Saks, 1997). Diabetes Mellitus (DM), as chronic stress activates the Hypothalamo-Pituitary-Adrenocortical (HPA) axis and related to the disturbances in the (HPA) axis, it has been suggested that there is an association between plasma levels of hormones (ACTH), corticosterone and diabetes (Cameron et al., 1984). Hyperactivation of the HPA axis of patients with diabetes mellitus has been reported previously, especially when poor glycemic control and ketosis are present (Roy et al., 1990; Coiro et al., 1995). Both type 1 and type 2 diabetic patients have been characterized with elevated circulating cortisol levels along with increased 24 h urinary free cortisol levels (Roy et al., 1993). Moreover, diabetic patients have been shown to have disrupted circadian patterns of cortisol secretion, with elevated cortisol levels, during trough and normal or slightly elevated values during peak secretion (Cameron et al., 1984). Studies have revealed that increases in HPA activity in diabetic patients may be attributable to altered control of ACTH release from corticotrophs, as well as direct actions of CRH at the adrenal gland to release Cortisol independently of pituitary ACTH release (Fehm et al., 1988). In addition, both type 1 and type 2 diabetic patients exhibit greater incidences of no suppression of pituitary-adrenal activity, after glucocorticoid administration, compared with no diabetic individuals (Hudson et al., 1989). This study suggests that hyperactivation of the HPA axis in diabetic patients may be attributable, in part, to decreased glucocorticoid-negative feedback sensitivity. However, the precise mechanism remains to be determined. Molecular regulation of the HPA axis has not been studied in humans. Increases in plasma glucocorticoid levels are beneficial, during times of stress, to aid in the mobilization of glucose stores from the liver and FFA from adipocytes, as well as to suppress further activity of the HPA axis. However, chronic exposure to elevated glucocorticoid levels is harmful (De Kloet, 1984; Kadoke, 1988; Doyle et al., 1993; Sapolsky, 1996). Glucocorticoids inhibit glucose uptake in adipocytes and fibroblasts, decrease local cerebral glucose utilization and inhibit glucose uptake in hippocampal neurons in vitro. Prolonged exposure of hippocampal neurons to elevated glucocorticoid levels can lead to neurodegeneration or suppressed neurogenesis in the hippocampus, particularly in CA3 pyramidal neurons (Reagan et al., 1999). This may have important implications in neuropathologies associated with diabetes, especially in the areas of learning and memory and cognitive dysfunction (McCall, 1992). Corticosterone (C21H30O4, also called 11β, 21-Dihydroxy-4-pregnen-3, 20-dione, 11β, 21-Dihydroxyprogesterone, Kendall's Compound B, 4-Pregnene-11β, 21-diol-3, 20-dione, Reischstein's Substance H) is the primary glucocorticoid in rats, whereas, cortisol is the primary glucocorticoid in humans. Corticosterone resembles cortisol in structure. Corticosterone helps regulate the conversion of amino acids into carbohydrates and glycogen by the liver and helps stimulate glycogen formation in the tissues. The adrenal gland cortex produces corticosterone. Corticosterone is derived from cholesterol through a series of enzymatically-mediated steps and is produced in response to stimulation by adrenocorticotropic hormone (ACTH). High levels of corticosterone inhibit ACTH secretion and a feedback loop is maintained via the hypothalamus, pituitary and adrenal gland. Stress leads to the release of corticosterone and may help protect the brain from damage due to elevated epinephrine levels. Corticosterone may also be involved in the learning/memory process (De Kloet, 1984). Corticosterone production has an ACTH-dependent circadian rhythm with peak levels in the latter portion of the day in Wistar rats (Solberg et al., 2001).

Alpha-melanocyte-stimulating hormone (Alpha-MSH) is a tridecapeptide that was originally characterized as a neuropeptide derived from a pituitary α-MSH is hormone derived by post-transnational processing from POMC and involved in stress and background adaptation. Alpha-MSH is synthesized from pro-opiomelanocortin (POMC) by the action of specific prohormone convertases that cleave into α-MSH, ACTH and Beta-endorphin. Alpha-MSH seems to be modulated by the release of two hormones within the hypothalamus: melanocyte stimulating hormone releasing factor (MSHRF) and Melanocyte Stimulating Hormone Release-Inhibiting Factor (MSH-RIF). More studies suggest that α-MSH, decrease body fat in humans (Horst et al., 2001). As a potential neurotransmitter (Leiba et al., 1990) regulate energy balance, appetite control, as well as glucose transport in rat adipocytes. It was established that the quantity of the corticosterone in the tissues is
significant greater than the free hormone in blood and that various tissues (hypothalamus, liver, heart, kidney etc.) capture the corticosterone from peripheral blood in different ways (Robu, 1982). The rate of hepatic inactivation of glucocorticoids is depressed in liver disease and during stresses (Ganong-William, 1993). Hypoglycemia is one of the form of stress appear range influencing the state of (HPA) axis (Schwartz et al., 1997; Tsigo et al., 1993) caused alterations in corticosterone content of various tissues (Henley and Bellush, 1992; Musabayana et al., 1995). Few studies have examined the correlation between melanocyte stimulating hormone and the activity of (HPA) axis. The conclusions of those works suggest that corticosterone response not significantly altered under MSH effect in normal conditions (Jessop et al., 1994). Present study was designed to evaluate the role of alpha-melanocyte stimulating hormone (α-MSH) on dynamics of corticosterone content during diabetic rats.

MATERIALS AND METHODS

Animals

White laboratory male rats (150-200 g) of both sexes (male and female) six to eight weeks of age were housed in cages under standard laboratory conditions for at least 1 week before starting the experiments. Forty white male rats were divided equally into four experimental groups (control, diabetic, α-MSH treated and α-MSH treated diabetic). The control group was injected with physiological saline. The second group was made diabetic by intraperitoneal injections of 10% alloxan (Sigma Firm, USA) dissolved in physiological saline to induce diabetes at dose of 20 mg per 100 g of mass. The diabetic group-α-MSH-treated group was injected intramuscularly with α-MSH (Sigma Formula) 2 mg 100 g of body mass daily for three weeks after the induction of diabetes. For obtaining of the dynamics in the changes the experiment continued for 10 days, taking into consideration the seasonal and circadian changes of hormonal concentrations in blood and tissues, the investigations were carried out in winter and spring (Giangula et al., 2000) since it is well known that at this time the rats possess a more stable content of corticosterone.

Metabolite Determination

Plasma glucose concentrations were determined by an enzymatic-colorimetric assay from Weiner Lab. Circulating Insulin, corticosteron, ACTH concentrations and corticosterone in tissues of hypothalamus, thyroid gland, cerebral hemisphere, liver, kidney and adrenal gland were measured by a previously validated immunoradiometric assay.

RESULTS

Effects of Alloxan

It was found that at the end of the experiment the insulin concentration in blood progress in decreasing reaching the value (0.68±0.07 mU L⁻¹) (Table 1) on the 10th day after

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>α-MSH-treated</th>
<th>Diabetic</th>
<th>α-MSH-treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>186±9.88</td>
<td>198±10.88</td>
<td>178±13.0</td>
<td>177±6±6.3</td>
</tr>
<tr>
<td>Water drank (mL day⁻¹)</td>
<td>9.4±1.64</td>
<td>9.6±0.65</td>
<td>23.3±4.40</td>
<td>15.3±3.01</td>
</tr>
<tr>
<td>Urine excreted mL day⁻¹</td>
<td>4.8±0.42</td>
<td>5.6±0.83</td>
<td>18.3±1.26</td>
<td>12.4±0.42</td>
</tr>
<tr>
<td>Protein in urine</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ktrole in urine</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Glucose in urine</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Blood glucose (mmol L⁻¹)</td>
<td>5.7±0.28</td>
<td>5.9±0.36</td>
<td>11.8±3.25</td>
<td>10.4±1.23</td>
</tr>
</tbody>
</table>

*α-MSH-treated rats—Component is absent; + : Component is present in small amounts; +++: Component exists in large amount; ++++: Component exists in the largest level.
Table 2: Plasma hormone concentrations (insulin mU L⁻¹, corticosterone ng mL⁻¹, ACTH pmol L⁻¹) of normal control, α-MSH-treated, diabetic and α-MSH-treated diabetic rats

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control</th>
<th>α-MSH-treated</th>
<th>Diabetic</th>
<th>α-MSH-treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>1.84±0.423</td>
<td>1.92±0.36</td>
<td>0.68±0.07</td>
<td>0.94±0.47</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>1.64±0.3420</td>
<td>2.87±0.418</td>
<td>7.16±1.5</td>
<td>9.67±1.68</td>
</tr>
<tr>
<td>ACTH</td>
<td>63.84±5.5200</td>
<td>58.36±4.810</td>
<td>158±8.41</td>
<td>84.53±3.90</td>
</tr>
</tbody>
</table>

Table 3: Content of corticosterone in tissues (μmol kg⁻¹) of normal control, α-MSH-treated, diabetic and α-MSH-treated diabetic rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>α-MSH-treated</th>
<th>Diabetic</th>
<th>α-MSH-treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral hemisphere</td>
<td>2.78±0.49</td>
<td>2.11±0.26</td>
<td>0.35±0.07</td>
<td>1.63±0.20</td>
</tr>
<tr>
<td>Adrenal gland (μmol g⁻¹)</td>
<td>3.37±0.44</td>
<td>2.79±0.53</td>
<td>0.38±0.03</td>
<td>1.18±0.04</td>
</tr>
<tr>
<td>Liver (μmol g⁻¹)</td>
<td>2.16±0.24</td>
<td>2.51±0.29</td>
<td>0.49±0.05</td>
<td>1.66±0.04</td>
</tr>
<tr>
<td>Thyroid gland (μmol g⁻¹)</td>
<td>1.62±0.28</td>
<td>1.96±0.18</td>
<td>0.24±0.05</td>
<td>0.59±0.05</td>
</tr>
<tr>
<td>Hypothalamus (μmol g⁻¹)</td>
<td>1.57±0.04</td>
<td>2.51±0.29</td>
<td>0.36±0.03</td>
<td>0.96±0.18</td>
</tr>
<tr>
<td>Kidney (μmol g⁻¹)</td>
<td>1.86±0.17</td>
<td>2.54±0.38</td>
<td>0.46±0.09</td>
<td>1.35±0.15</td>
</tr>
</tbody>
</table>

Introducing of alloxan in comparison with the control (1.84±0.423 mU L⁻¹) (Table 1), blood glucose level increased and reached (11.52±1.34 mmol L⁻¹) and still higher than control and other groups and the plasma concentration of ACTH (158.53±6.45 pmol L⁻¹) in diabetic group comparison with the control (63.84±5.52 pmol L⁻¹) (Table 2). During the experimental period we studied the symptomatic complex of features in development of DM in rats after administration of alloxan, such as changes in appearance of an animal, the body weight and volume of water drunk, volume of urine excreted and determination of protein, ketones and serum glucose (Table 1) and the animals showed the following symptoms Polydipsia, polyuria, weight loss, weakness and dehydration. Corticosterone level was increased in diabetic group (7.16±1.54 ng mL⁻¹) compared with the control (1.64±0.342 ng mL⁻¹). (Table 2). After the analysis and measuring the content of corticosterone in various tissues of diabetic rats it was found that alloxan decreased the corticosterone level in all selected tissues (Table 3).

Effects of Alpha-MSH Intramuscular injection of 2 mg 100 g of body mass daily of alpha-melanocyte stimulating hormone (alpha-MSH) eased plasma levels of insulin to increase (1.92±0.36 mU L⁻¹) in comparison with the control (1.84±0.423 mU L⁻¹) and still lower than α-MSH treated diabetic (0.94±0.47 mU L⁻¹). After MSH administration certain decreasing of the plasma concentrations of ACTH (58.36±4.81 pmol L⁻¹) compared with the control (63.84±5.52 pmol L⁻¹) and with α-MSH-treated diabetic (84±3.94 pmol L⁻¹). Corticosterone level was increased in diabetic group (7.16±1.54 ng mL⁻¹) and in α-MSH-treated diabetic (9.67±1.68 ng mL⁻¹) compared with the control group (1.64±0.342 ng mL⁻¹) and α-MSH-treated group (2.87±0.418 ng mL⁻¹). The data obtained show that exogenous introduction of MSH at the background of the alloxan-induced diabetes contributes to changing in the content of corticosterone in selected tissues. It is increased in the hypothalamus, liver, kidney and thyroid gland, at the same time decreasing occurs adrenal gland and hemisphere (Table 3).

**DISCUSSION**

Alloxan induces damage and death of pancreatic islet-cells in several experimental animal models, thus causing diabetes mellitus and decreasing the secretion of insulin. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species, Alloxan and the product of its reduction, dial urine acid; establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytotoxic calcium concentration causes rapid destruction of β-cells. Intramuscularly injection of alpha-melanocyte stimulating hormone (alpha-MSH) eased plasma levels of insulin to increase (1.92±0.36 mU L⁻¹) but increased insulin resistant (cells in the body are not using the insulin produced by pancreas well enough, while the beta cells in the pancreas are producing adequate levels of insulin), plasma levels of glucose were not significant decreased by alpha-MSH in fed
rats (5.9±0.36 mmol L⁻¹). It is possible, that the effects were mediated by both a central nervous action and a direct action on the endocrine pancreas. It was suggested that administration of α-MSH to mice or rabbits increased plasma levels of glucagon, free fatty acids and increased resistance to insulin and directly affected blood sugar levels, therefore, α-MSH may be a factor in the development of Type 2 Diabetes. Another finding is that obese people with high levels of the hormone α-MSH may be more likely to be diabetic than obese people with low levels of the hormone α-MSH and it was suggested that the mice didn’t induce any glucagon in the absence of α-MSH and will be hypersensitive to insulin. It has been suggested that Melanocortin 5 receptor was detected in rat adipocytes by RT-PCR. NLε4, D-Phe7-α-MSH did not affect the basal glucose transport but reduced the insulin-stimulated glucose transport in adipocytes in a dose-dependent manner. About 45% of inhibition was observed with 1 nM NLε4, D-Phe7-α-MSH under insulin stimulation. NLε4, D-Phe7-α-MSH did not affect the insulin-stimulated translocation of GLUT-1 from the intracellular pool to the plasma membrane (Araki-Sasaki et al., 2002) and some results suggest that the beta-endorphin-induced hyperglycemia was caused, at least in part, by a peripheral inhibition of insulin release and a central stimulation on glucoregulation.

The Relationship Between Hypothalamus-pituitary-adrenal Axis and Diabetes Mellitus

Diabetes is associated with increased basal hypothalamo-pituitary-adrenal (HPA) activity and impaired stress responsiveness. Previously, we demonstrated that the HPA response to hyperglycemia is significantly impaired in diabetic rats. Plasma ACTH and corticosterone concentrations were significantly (p<0.05) higher in uncontrolled diabetic rats compared with normal and α-MSH-treated diabetic rats. Animals (Shapiro et al., 1991; Schnitzler et al., 1993; De Nicola et al., 1976; Chan et al., 2001) have reported that diabetes mellitus represents a sustained stimulus to the HPA axis during the nadir of circadian activity. Although hyperactivation of pituitary-adrenal function has been demonstrated in both diabetic humans and animals, the underlying mechanisms responsible for these alterations are still unclear we have demonstrated that HPA dysregulation in early diabetes may be mediated at least in part, by an increase in central drive at and/or above the level of the hypothalamic paraventricular nucleus. We now provide evidence that HPA hyperdrive in diabetes is partially mediated by decreased glucocorticoid negative feedback sensitivity (as demonstrated by dexamethasone nonsuppression) and that impaired responsiveness of the diabetic HPA axis to stress, may be due to decreased pituitary and adrenal sensitivity. This latter point is evidenced by decreased responses to both CRH and ACTH challenge in diabetic animals. Despite significantly elevated basal plasma ACTH and corticosterone concentrations, the pituitary-adrenal response of diabetic rats to restraint stress was greatly diminished in comparison to control and treated diabetic animals. In a previous study, using the STZ-diabetic rat model, impaired responsiveness to a CRH challenge was correlated with a decreased number of CRH receptors in the anterior pituitary and it was suggested that this resulted from the hypersecretion of hypothalamic CRH (Scribner et al., 2001).

The results of analysis showed that the MSH treatment might increase the plasma corticosterone from (7.16±1.54 ng mL⁻¹) in diabetic group to (9.64±1.68 ng mL⁻¹) in α-MSH-treated diabetic group and still higher than control (1.64±0.342 ng mL⁻¹), serum adrenocorticotropic hormone (ACTH) increases in diabetic group (158.36±4.81 pmol L⁻¹) compared to control group (63.84±5.52 pmol L⁻¹) and still lower in α-MSH-treated (58.36±4.81 pmol L⁻¹) and in group α-MSH-treated diabetic (84.53±3.94 pmol L⁻¹) compared to diabetic group. This might be explained as the hypoglycemic effect of α-MSH-related with increasing the level of corticosterone and Adrenocorticotropic hormone (ACTH) dependent at the relation between insulin and these hormones. Normalization of pituitary-adrenal activity with α-MSH treatment seems to involve complex changes in the HPA axis, primarily through increased GR mRNA in the pars distalis, with normalization of the dramatically elevated hippocampal MR and hypothalamic CRH mRNA levels. In this study, significantly higher circulating plasma concentrations of both ACTH and corticosterone were observed in diabetic rats in the morning. Together, the endocrine data confirms hyperactivation of the HPA axis at the level of the pituitary and adrenal cortex under conditions of uncontrolled diabetes mellitus. More significantly, normalization
of plasma insulin and glycemia, with \( \alpha \)-MSH treatment, restored basal ACTH and corticosterone concentrations to control levels. These results indicate that glucocorticoid negative feedback sensitivity is decreased in the early stages of alloxan-diabetes and that sensitivity are restored with \( \alpha \)-MSH treatment. In conclusion, results indicate that hyperactivation of the HPA axis in early alloxan-diabetes is likely caused by both an increase in central drive and a decrease in glucocorticoid negative feedback sensitivity. Whereas impaired responsiveness to stress in alloxan induced diabetic rats likely involves a decrease in sensitivity of the pituitary corticotrophin and adrenal cortex to CRH and ACTH, respectively. More importantly, normalization of pituitary-adrenal activity in induced-diabetic rats with \( \alpha \)-MSH therapy can be attributed, in part, to restoration of insulin concentrations or pituitary-adrenal function. These impairments in HPA function in diabetes may contribute to cognitive dysfunction (Reagan et al., 1999). Decreased counterregulation to hypoglycemia (Cryer and Gerich, 1997) and an impaired ability to respond to stress. The results for studying of the concentration of corticosterone in the peripheral blood, during alloxan-induced diabetes with introduction of the MSH, it ought to be noted that alloxan induced diabetes negatively influences on organism and in the cases when at these backgrounds MSH was introduced then relieves an organism of unfavorable effects to a certain in the adrenal cortex varies mainly within the initial values. The results of the investigation also showed that repeated introducing of MSH into rats substantially influences the concentration of corticosterone in the peripheral blood and its concentration in tissues of animal organism such as hypothalamus, liver, kidneys and the adrenal tissue itself. The certain dependence injection of the islets of pancreas, ratio between the processes of the glucocorticosteroid synthesis, velocity of hormonal secretion, a degree of their co-optation by receptors of the peripheral tissues, intensity of their metabolism and velocity of removing of the hormonal metabolites from an organism. The consideration of the results obtained established that exogenous introduction of MSH preparation exerted a favorable effect on an on an organism under the extremely hard conditions on the alloxan-induced diabetes. Thus on the base of the data in literature which investigated the problems of the shift of MSH concentrations under various stress forms as well as on the base of the experimental results the role of a adaptive significance which MSH plays in the warm blood animals and humans can be definitely formulated.

REFERENCES


