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Scientific Research

Stress-Induced Changes in Testosterone Secretion in Male Rats: Role of Oxidative Stress and Modulation by Antioxidants

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Abstract

Seventy adult male albino rats were randomly allotted into 3 main groups: control group (n = 10), acute stress-exposed group (n = 30) and chronic stress-exposed group (n = 30). Each of the stressed groups was subdivided into 3 equal subgroups (n = 10/subgroup, SG): subgroup 1 animals were exposed to immobilization stress, SG2 animals, were given immobilization stress and supplemented with α-tocopherol (vitamin E), SG3 animals were exposed to immobilization stress and supplemented with ascorbic acid (vitamin C). Immobilization stress exposure was applied once for 6 continuous hours in the acute stressed group and was 6 hours daily for 10 consecutive days in the chronic stressed group. In all vitamin supplemented groups, both vitamin E and C were administered orally mixed with the diet in a similar dose of 500 mg/kg diet. This supplementation started 6 weeks prior to the stress exposure and continued throughout the experimental period. At the end of the last immobilization session, sera were harvested from all animals thereafter, animals were sacrificed and the testes were immediately excised and processed for further biochemical investigations. Serum testosterone and luteinizing hormone levels were measured and the activities of antioxidant enzymes [catalase (CAT) & glutathione-s-transferase (GST)] as well as malondialdehyde (MDA) concentrations were determined in sera and testes. Compared to control, the results revealed that acute and chronic immobilization stress caused significant decrease in levels of serum testosterone and luteinizing hormone (LH). Also, significant reductions ($P < 0.01$) were found in the activities of CAT and GST in sera and testes. Contrariwise, there existed a significant ($P < 0.05$) increase in MDA concentrations in serum and testis. Co-administration of vitamin E or C relatively restored ($P < 0.01$) the above parameters. Thus, this study draws a conclusion that immobilization stress of male rats significantly inhibited testosterone secretion and induced oxidative stress which partially mediated this inhibition. It also proved a protective role of vitamin E and C against the oxidative stress-induced down-regulation of testosterone secretion with a better efficacy of vitamin E.

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Keywords

Stress; Rats; Testosterone; LH; Antioxidants; Enzymes

1. Introduction

The vast numbers of studies in both humans and animals confirm the inhibitory role of different stressors in the hormonal function of the testis [1]-[3]. In addition, a variety of mediating mechanisms have been recenthy suggested [3] [4]. However, considerable variations in the response of the hypothalamo-pituitary-gonadal (HPG) axis to stress have been reported. Many stressors decrease LH and consequently, testosterone levels by inhibiting luteinizing hormone-releasing hormone (LHRH) synthesis and release from the hypothalamus [1]. On the other hand, there are stimuli that attenuate testosterone levels without altering LH values in both rodents and humans [5]. Moreover, testosterone level may be increased at initial stages of acute stress with a constant or even decreased LH level [6].

On the other hand, stress exposure has been implicated in the induction of oxidative stress by excessive production of free radicals and reactive oxygen species (ROS), which can cause alterations in both cell membranes and constituents ending by cell mutation or damage [7]-[9].

Testicular membranes are rich in polyunsaturated fatty acids and therefore are susceptible to oxidative stress [10]. In addition, testicular steroidogenic activity is sensitive to free radicals and ROS [11] [12], and a correlation was noted between free radical production and gonadal steroidogenesis [12].

Other studies have drawn increasing attention to the potential for supplementary antioxidants to reduce free radical-induced oxidative stress. Vitamin E and C were shown to be powerful chain—breaking antioxidants that prevent the propagation of free radical reaction and inhibit lipid peroxidation and oxidative damage [13]-[15].

Therefore, present study aimed at examining the role of oxidative stress in mediating the stress-induced changes in testosterone secretion and determining whether the protective effect of the antioxidants (*i.e.* Vitamin E and C), can modulate such changes.

2. Materials and Methods

2.1. Animals

Seventy adult male albino rats of relatively similar age and uniform strain weighing around 150 - 170 gm were housed under controlled environmental conditions. Animals were randomly allotted into seven treatments, allowed free access to rat chow pellets and water. Animals were handled daily for one week acclimation period prior to the experimentation.

2.2. Experimental Design

Rats were divided into the following three main groups;

1) Control group (n = 10) in which animals were under normal managerial condition.

2) Acute stress-exposed group (n = 30) in which animals were subdivided into three subgroups (n = 10/subgroup) as follow;

a) Animals were exposed to immobilization stress once for 6 hours (between 08.00 - 14.00 hours).

b) Animals were exposed to immobilization stress and supplemented with vitamin E (500 mg/kg diet).

c) Animals were exposed to immobilization stress and supplemented with vitamin C (500 mg/kg diet).

3) Chronic stress-exposed group (n = 30) in which animals were subdivided into three subgroups (n = 10/subgroup) as follow;

a) Animals were exposed to immobilization stress for 6 hours daily (between 08.00 - 14.00 hours) on 10 consecutive days.

b) Animals were exposed to immobilization stress and supplemented with vitamin E (500 mg/kg diet).

c) Animals were exposed to immobilization stress supplemented with vitamin C (500 mg/kg diet).

In all vitamin-treated groups, both vitamin E and C were administered orally mixed with the diet in a similar dose of 500 mg/kg diet [16] [17]. This treatment started 6 weeks prior to the stress exposure and continued

throughout the experimental period.

2.3. Stress Procedure

The immobilization stress model used was technically designed according to Lopez-Calderon *et al.* [18]. The immobilization units were of local design and consisted of a flexible wire mesh in which the rat was wrapped with its tail extended, then the edges of the wire mesh were curved from both sides to restrict the rat's movement. In addition, the rat's tail was held in place by springs by which the rat was suspended unsupported. Food and water were not allowed during the stress procedure. At the end of the last immobilization session, blood samples were collected from orbital sinus and allowed to clot at room temperature for an hour, centrifuged (4000 rpm/10min) and sera were harvested. After blood sampling animals were sacrificed and the testes were immediately excised and processed for biochemical investigations.

2.4. Assay Procedures

Testosterone

Serum testosterone concentrations were determined by an enzyme immunoassay technique according to Trachtenberg [19]. The tracer was horse-radish peroxidase and the chromogen was tetramethyl benzedine (TMB). The intra- and interassay of variations were 6.1% and 8.3%, respectively.

2.5. LH

Serum LH concentrations were measured by IRMA technique according to Santner [20]. This procedure is known as a solid-phase immunoradiometric assay designed for the quantitative measurement of LH in serum and plasma. The tracer used is a radio-labeled polyclonal anti-LH using ^{125}I.

2.6. Catalase and Glutathione-S-Transferase

Antioxidant enzymes CAT & GST activities in testes and sera were determined as follow:

Catalase activity was measured by a colorimetric method [21].

Glutathione-s-transferase activity was measured by UV method [22].

Lipid peroxide (malondialdehyde) levels in testes and sera were determined by a colorimetric method according to Ohkawa *et al*. [23].

2.7. Statistical Analyses

Data were analyzed using the student's "t" test for unpaired sample. Results were given as means ±SEM. Probability values (P) less than 0.05 were considered significant [24].

3. Results

As shown in **Table 1**, acute stress caused significant decrease in serum level of testosterone (49.65%, $P < 0.0005$) and LH (31.57%, $P < 0.01$) as compared with the control. Supplementation with either α-tocopherol (vitamin E) or ascorbic acid (vitamin C) significantly increased testosterone and LH levels as compared with the stressed group. However, the values still significantly lower than the control group.

Chronic stress caused significant and more marked reduction in serum levels of testosterone (60%, 35%) and LH (44.08%) as compared with the control. Vitamin E or C supplementation partially reversed the stress-induced reduction in serum testosterone level. However neither of the vitamins significantly altered LH level when compared to the stressed group.

Tables 2 and **3** present data of the effects of immobilization stress on the activity of the antioxidant enzymes; CAT and GST, in sera and testicular tissues of control and vitamin E- or C-treated rats.

Data revealed that acute stress reduced ($P < 0.005$) CAT activity in serum (24.3%) and testis (60.21%) as compared with the control. Vitamin E supplementation significantly increased CAT activity in serum compared to the stressed group, but the values still lower than in the controls. Vitamin E and C supplementation partially reversed the stress induced reduction of CAT activity in both serum and testis.

Data also revealed that acute stress caused a reduction in the activity of GST in serum (19.16%, $P < 0.005$)

Table 1. Effect of immobilization stress on serum testosterone and LH concentrations of control and vitamin E- or vitamin C-treated rats.

Experimental groups			Testosterone (ng/ml)	LH (ng/ml)
Control		Mean ± SEM %change	4.01 ± 0.07	10.64 ± 1.26
Acute stress	stress	Mean ± SEM %change	2.02 ± 0.01^{a***} −49.65	7.28 ± 0.12^{a**} −31.57
	Stress + Vit.E	Mean ± SEM %change	3.10 ± 0.14^{a,b***} −22.69	7.85 ± 0.23^{a,b*} −26.22
	Stress + Vit.C	Mean ± SEM %change	2.65 ± 0.25^{a***,b*} −33.92	7.55 ± 0.01 −29.04
Chronic stress	Stress	Mean ± SEM %change	1.59 ± 0.23^{a***} −60.35	5.95 ± 1.36^{a**} −44.08
	Stress + Vit.E	Mean ± SEM %change	2.68 ± 0.01^{a,c***} −33.17	6.11 ± 0.14^{a**} −42.58
	Stress + Vit.C	Mean ± SEM %change	2.31 ± 0.11^{a***,c**} −42.39	5.76 ± 0.53^{a**} −45.87

The results are given as mean ± SEM for 10 rats. The percentage of change is compared with the control. Means within a category in the same column with different superscripts are significantly different (P < 0.05 = significant*, P < 0.01 = highly significant**, P < 0.005 very highly significant***).

Table 2. Effect of immobilization stress on the activity of Catalase (CAT) in serum and testis of control and vitamin E- or vitamin C-treated rats.

Experimental groups			Catalase (CAT)	
			Serum (U/L)	Testis (U/mg)
Control I		Mean ± SEM %change	334.7 ± 1.42	8.015 ± 0.05
Acute stress	Stress	Mean ± SEM %change	252.93 ± 1.9^{a***} 24.43	3.19± 0.14^{a***} −60.21
	Stress + Vit.E	Mean ± SEM %change	305.68 ± 1.88^{a,b***} −8.67	6.74 ± 0.93^{b**} −15.91
	Stress + Vit.C	Mean ± SEM %change	279.87 ± 2.52^{a,b***} −16.38	5.48 ± o.13^{a,b***} −31.63
Chronic stress	Stress	Mean ± SEM %change	239.38 ± 2.88^{a***} −28.48	2.32 ± 0.106^{a***} −71.05
	Stress + Vit.E	Mean ± SEM %change	300.38 ± 3.03^{a,c***} −10.25	6.24 ± 0.11^{a,c***} −22.15
	Stress + Vit.C	Mean ± SEM %change	266.73 ± 1.68^{a,c***} −20.31	4.95 ± 0.057^{a,c***} −38.24

The results are given as the mean ± SEM for 10 rats. The percentage of change is compared with the control. Means within a category in the same column with different superscripts are significantly different (P < 0.05 = significant*, P < 0.01 = highly significant**, P < 0.005 very highly significant***).

and testis (47.27%, P < 0.005) compared with the control. Vitamin E or C treatment significantly increased GST activity in serum and testis compared to the stressed group, but with values still lower than the control.

Chronic stress resulted in significant (P < 0.005) and more marked reduction in the activities of CAT and GST in serum (28.48% and 33.75% respectively) and testis (71.05% and 54.76% respectively) compared with the control. Vitamin E or C treatment increased (P < 0.005) the activities of both enzymes in serum and testis compared to the stressed group, but with values still lower than the control.

Collectively, in both acute and chronic-stressed groups, vitamin E supplementation was more effective in reversing the stress-induced inhibition of CAT and GST activities in serum and testis.

As shown in **Table 4**, acute stress increased malondialdehyde (MDA) concentration in serum (77.97%, P < 0.005) and testis (65.31%, P < 0.01) compared to the control. Vitamin E supplementation restored MDA concentration in serum and testis to nearly control levels. Vitamin C significantly decreased MDA concentration in

Table 3. Effect of immobilization stress on the activity of Glutathione S-transferase (GST) in serum and testis of control vitamin E- or vitamin C-treated rats.

Experimental groups			Glutathione S-transferase (GST)	
			Serum (U/L)	Testis (U/mg)
Control I		Mean ± SEM %change	483.35 ± 2.35	249.37 ± 0.87
Acute stress	Stress	Mean ± SEM %change	390.75 ± 2.48^{a***} −19.16	131.50 ± 1.58^{a***} −47.27
	Stress + Vit.E	Mean ± SEM %change	441.06 ± 1.92^{a,b***} −8.75	226.06 ± 1.91^{ab***} −9.35
	Stress + Vit.C	Mean ± SEM %change	419.49 ± 1.91^{a,b***} −13.21	205.12 ± 2.07^{a,b***} −17.74
Chronic stress	Stress	Mean ± SEM %change	320.23 ± 2.13^{a***} −33.75	112.81 ± 2.04^{a***} −54.76
	Stress + Vit.E	Mean ± SEM %change	419.60 ± 1.32^{a,c***} −13.19	196.62 ± 2.42^{a,c***} −21.15
	Stress + Vit.C	Mean ± SEM %change	381.69 ± 2.12^{a,c***} −21.03	154.56 ± 1.91^{a,c***} −38.02

The results are given as the mean ± SEM for 10 rats. The percentage of change is compared with the control. Means within a category in the same column with different superscripts are significantly different (P < 0.05 = significant*, P < 0.01 = highly significant**, P < 0.005 very highly significant***).

Table 4. Effect of immobilization stress on Malondialdehyde (MDA) concentration in serum and testis of control and vitamin E- or vitamin C-treated rat.

Experimental groups			Malondialdehyde (MDA)	
			Serum (n mol/mL)	Testis (n mol/g fresh tissue)
Control I		Mean ± SEM %change	49.38 ± 3.9	24.73 ± 2.31
Acute stress	Stress	Mean ± SEM %change	87.88 ± 7.2^{a***} +77.97	40.88 ± 3.50^{a**} +65.31
	Stress + Vit.E	Mean ± SEM %change	55.28 ± 5.6^{b**} +11.95	28.29 ± 1.91^{b**} +14.40
	Stress + Vit.C	Mean ± SEM %change	66.61 ± 6.2^{a,b*} +34.89	31.35 ± 2.11^{a,b*} +26.77
Chronic stress	Stress	Mean ± SEM %change	95.16 ± 7.8^{a***} +92.71	48.55 ± 4.80^{a***} +96.32
	Stress + Vit.E	Mean ± SEM %change	61.81 ± 4.9^{a*,c***} +25.17	33.68 ± 1.95^{a,c**} +33.68
	Stress + Vit.C	Mean ± SEM %change	70.37 ± 6.5^{a**,c*} +42.51	35.93 ± 2.06^{a**,c*} +45.29

The results are given as the mean ± SEM for 10 rats. The percentage of change is compared with the control. Means within a category in the same column with different superscripts are significantly different (P < 0.05 = significant*, P < 0.01 = highly significant**, P < 0.005 very highly significant***).

serum and testis compared to the stressed group; however the values were still higher than the control.

Chronic stress caused significant and more marked increase in MDA concentration in serum (92.71%, P < 0.005) and testis (96.32%, P < 0.005) compared to the control. Vitamin E or C supplementation decreased (P < 0.01) MDA concentration in serum and testis compared to the stressed group, with values still higher than the controls. Vitamin E was more effective than vitamin C in modulating MDA levels in serum and testis.

4. Discussion

The results of the present study revealed that acute and chronic immobilization stress caused significant decrease

in serum testosterone in mole rats. This finding is consistent with number of studies in humans and animals which confirm the inhibitory role of different stressors on the hormonal function of the testis by decreasing the testosterone level in the blood [2] [3].

This study also resulted in a significant stress-induced reduction in serum LH level, which might be responsible for the decline in testosterone concentration. Previous studies indicated that many stressors decrease LH and consequently testosterone levels by inhibiting LHRH synthesis and release from the hypothalamus [1]. Such stress-induced inhibition of the hypothalamic-pituitary-gonad (HPG) axis may be mediated by corticotropin releasing factor (CRF) and endogenous opioids, mainly β-endorphins which are known to be released from the hypothalamus in response to stress [25]. It has been shown that both CRF and β-endorphins can exert their effects on the HPG axis by inhibiting LH-RH release from the hypothalamus [26], inhibiting LH release from the pituitary [27], and inhibiting testosterone synthesis directly in Leydig cells [28], thus decreasing testosterone levels in the blood circulation.

It is assumed that endogenous opioids could be participating in the effects caused by stress on testosterone secretion. The recent study of Retana-Marquez *et al*. [3] indicated that the decrease in testosterone secretion due to stress was attenuated with the opioid antagonist "Naltrexone".

Excessive secretion of glucocorticoids during stress could be another mechanism for the stress-induced decline in testosterone level in this study. Glucocorticoids directly inhibit Leydig cell function through a glucocorticoid receptor-mediated pathway [29]. It has been shown that glucocorticoids inhibit testosterone synthesis by inhibiting some of the enzymes involved in testicular steroidogenesis, such as NADPH-P450 reductase, P450c 17 (17α-hydroxylase and 17, 20-lyase) and 3β-hydroxysteroid dehydrogenase [29].

In addition, excessive exposure to glucocorticoids initiates apoptosis in Leydig cells, potentially contributing to the suppression of testosterone level caused by the decline in steroidogenic capacity [1] [30].

Moreover, deterioration of the blood flow in the testis might contribute to the stress-induced reduction in testosterone level [31]. It is known that stimulation of the sympathetic nerves of the testis or injection of catecholamines causes vasoconstriction and reduces blood flow in the testes in various mammals [31].

Likewise, data of the current study revealed significant reduction in the activity of the antioxidant enzymes; CAT and GST in sera and testes of rats after exposure to immobilization stress. This effect was more pronounced in case of chronic stress.

Both CAT and GST are important scavenger enzymes against free radicals [11] [12]. CAT acts synergistically with superoxide dismutase (SOD) to remove superoxide anions generated by NADPH-oxidase in the cells. They play an important role in decreasing oxidative stress and membrane lipid peroxidation [32]. Also, GST plays important roles in the detoxification of reactive lipid peroxides [12].

The results of the present study also showed an increase in Malondialdehyde (MDA) concentrations in serum and testis of rats after exposure to acute and chronic immobilization stress. This indicates increased lipid peroxidation as MDA results from the breakdown of polyunsaturated fatty acids and considered as one of the manifestations of free radicals-induced cytotoxicity [33] [34].

Thus, the reduction in the activity of CAT and GST as antioxidant enzymes, together with the increase in MDA concentration indicate an increased production of free radicals and induction of oxidative stress in the immobilization stressed rats. This finding supports previous reports which proved that exposure to various stressors leads to oxidative stress and its consecutive structural and functional tissue damage as a result of increased formation of free radicals and reactive oxygen species (ROS) [35] [36]. It is well known that ROS are responsible for damaging almost all cellular macromolecules including membrane polyunsaturated fatty acids, carbohydrates, proteins and DNA, potentially causing impairment of cellular functions [8] [9]. Testicular membranes are rich in polyunsaturated fatty acids and therefore are susceptible to oxidative stress [10]. Testicular steroidogenesis is sensitive to free radicals and ROS and a correlation was noted between free radicals production and gonadal steroidogenesis [12]. In this concern, several lines of evidence have suggested that nitric oxide (NO) free radical mediates the stress-induced downregulation of testicular steroidogenesis [37].

Accordingly, oxidative stress could be considered a direct mechanism that mediated the downregulation of testicular steroidogenesis and reduction of testosterone level in immobilization stressed rats. Impairment of testicular steroidogenesis might coincide with inhibition of the steroidogenic enzyme activity by the generation of large amounts of ROS in testicular tissue [11]. Also the lipid peroxidation meiabolitc; MDA exerts detrimental effects on testicular steroidogenic enzyme activity [10]. Moreover, a confirmatory evidence for the inhibitory effect of oxidative stress on testicular steroidogenic enzyme activity has been provided by Tatjana *et al.* [37]

who reported significant inhibition of testicular 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase/lyase (P450 C17) and NADPH-P450 reductase activities in immobilization stressed-rats. Also, in the study of Manna *et al.* [36] they reported that swimming exercise-induced oxidative stress in rats caused inhibition of the activities of testicular 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase.

The data obtained in the present study exhibited that supplementation with either α-tocopherol or ascorbic acid partially reversed the stress-induced reduction of serum testosterone levels. Likewise, both vitamins also increased CAT and GST activities and significantly decreased MDA concentrations in serum and testis as compared with the stressed group, denoting less production of free radicals and lipid peroxidation. Such alleviation of oxidative stress could explain the partial restoration of testosterone serum levels in the supplemented groups.

The protective effect of α-tocopherol and ascorbic acid may be attributed to their properties as chain-breaking antioxidants that prevent the propagation of free radical reaction and inhibit lipid peroxidation [10] [15]. They also elevate antioxidant enzymes activities [13] [14], and maintain the balance between antioxidants and oxidants in tissues [38] [39]. Besides, their protective effect from oxidative stress also depends on their role in stabilization of membrane structures [40] [41].

Moreover, apart from its antioxidative properties, α-tocopherol has a direct stimulatory effect on enzymes of gonadal steroid biosynthesis, and may also exert some modulatory action on gonadotropin synthesis and secretion [42].

5. Conclusion

In conclusion, immobilization stress generates some metabolic and hormonal disorders in the body. These could be alleviated by administration of vitamin E and C, which exhibited enhancement effects on the body. Further multidisciplinary studies are needed for monitoring various cellular mechanisms regulating coping for the stress process.

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