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Role of Melatonin and/or Vitamin B Complex against Hormonal Changes in Epinephrine-Stressed Rats

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Abstract

The current study aimed to investigate the effect of intramuscular injection of melatonin (MT)(1mg/ kg) and/or vitamin B-complex (Tri-B) (20 mg/kg) on the hormonal changes induced by Epinephrine (Epi) (0.02 mg/kg) in male albino rats. Intramuscular administration of Epi induced significant elevations in adrenocorticotropic hormone (ACTH), corticosterone (CORT), triiodothyronine (T_3) and thyroxine (T_4) levels after the two experimental durations. On the other hand, luetinizing hormone (LH) , testosterone , prolactin (PRL) and growth hormone (GH) levels were decreased under the same conditions. Melatonin treatment seems to constitute a selection therapy, by improving ATCH, CORT, T_3 ,T_4 and GH levels but has no effect on the low levels of LH , testosterone and PRL. Also, the data suggested that Tri-B injection partially improved the different endocrinological changes of Epi**.**

Keywords: Stress, ACTH , CORT, T_3 ,T_4 ,LH , testosterone , PRL ,GH , rats.

1. Introduction

Stress, a familiar aspect of modern life, is a stimulant for some but a concern for many. When an individual experiences stress, the brain triggers the secretion of a cascade of stress hormones regulated by the hypothalamic pituitary adrenal system (HPA) axis (Sapolsky, 2003). Epinephrine causes general physiological changes and plays a central role in the short-term stress reaction, that prepares the body for physical activity (fight or flight response) initiated by the sympathetic nervous system (Aronson, 2000). So, in the present study Epinephrine was used to induce stressed rat as an animal model.

Melatonin (MT) (N-acetyl-5-methoxytryptamine) is a natural substance, structurally simple hormone produced by pinealocytes in the pineal gland. The ability of melatonin (MT) to transduce environmental information into appropriate endocrine signals plays an important role in synchronizing an animal's physiology and behavior with optimal environmental conditions. It has beneficial effects in different trauma cases and probably stress-relieving hormone action (Cuzzocrea *et al.,* 2000). Interactions between MT and other hormones are also important in synchronizing and modulating physiological and behavioral parameters necessary for daily activity (Hadley and Levine, 2007). Melatonin is a powerful antioxidant that can easily cross cell membranes and blood-brain barrier (Hardeland, 2005).

The Tri- B complex (thiamine B1, pyridoxine B6, and cobalamin B12) is essential for the regulation and to correct function of the entire nervous system including brain function. A deficiency in any of the B complex vitamins can lead to feeling stressed, anxious and depressed.It is realized that Tri-B are working best as a team so that benefits of each are enhanced and negative aspects are minimized. They are responsible for helping enzymes to release energy from food, promote proper metabolism, give cells plenty of oxygen and detoxify organs (Bolander, 2006).

The present study aimed to examine the effect of melatonin and /or Tri B supplementation on some hormonal changes in Epi stressed rats. Adrenocorticotropic hormone (ACTH), Corticosterone (CORT), triiodothyronine (T3), thyroxine (T4), luetinizing hormone (LH), testosterone, prolactin (PRL) and growth hormone (GH) were measured

2. Materials and Methods

2.1. Experimental animals

Adult male albino Spargue-Dawley rats (2.5-3 month old, 200-210 gm) were used as experimental animals. Rats were environmentally adapted in the animal holding room for two weeks prior to experiment. Animals were housed in groups in stainless steel cages at room temperature (22-25^{o}C), relative humidity 60-70%, and a photoperiod of

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12h/d. Food (pelleted rat chow) and drinking water were available *ad libitum.*

2.2. Chemicals

Epinephrine, Melatonin and Tri-B (B_1,B_6 and B_{12}) were purchased from Sigma Chemical Co. St Louis, (MO, USA).

2.3. Experimental design

140 male albino rats were divided randomly into 7 groups (I-VII) (20 rats each):

Group I (control group): Animals of this group were injected with saline and served as control during the experimental period.

Group II (Epinephrine group): Animals of this group were injected with an intramuscular Epinephrine dose of 0.02 mg/kg according to Berg *et al*. (1996).

Group III (Melatonin group): Animals of this group were injected with an intramuscular MT dose of 1 mg/kg as described by Mamdouh *et al.* (1996) (negative control group).

Group IV (Tri-B group): Animals of this group were injected with an intramuscular Tri-B dose of 20 mg/kg according to Bolkent *et al*. (2008) (negative control group).

Group V (Epinephrine + MT group): Animals of this group were injected with an intramuscular dose of both Epinephrine and Melatonin by the same previously mentioned doses and manner (in groups II and III).

Group VI (Epinephrine + Tri-B group): Animals of this group were injected with an intramuscular dose of both Epinephrine and Tri-B by the same previously mentioned doses and manner (in groups II and IV).

Group VII (Epinephrine + Melatonin + Tri-B group): Animals of this group were injected intramuscularly with Epinephrine, Melatonin and Tri-B by the same previously mentioned doses and manner (in groups II, III and IV).

The injections were carried out nearly at the end of light period/ onset of dark period. Lights were off at the onset of the injections. Ten rats of each group were sacrificed after 3 hrs of receiving a single dose of saline, Epinephrine, Melatonin, and Tri-B as described above. Another 10 rats were sacrificed after receiving the same doses daily for 10 consecutive days. In all groups, the animals starved for 12 hrs prior to blood collection to ensure homogeneous blood and organ samples. Blood was collected by decapitation of the animal after being lightly anaesthetized after 3 hrs of the injections in the 1^{st} day and 10^{th} day of the experiment**.**

2.4. Hormonal analysis

At the end of the experiment, blood was collected into heparinised tubes, and the plasma was separated by centrifugation for determination of adrenocorticotropic hormone (ACTH) by the method of Odell *et al*. (1989). Corticosterone (CORT) was estimated according to the method of Chiu *et al.* (2003). For the measurement of triiodothyronine (T_3) ,thyroxine (T_4) and luetinizing hormone (LH) the method of Teitz (1995) was applied. For the determination of testosterone and prolactin (PRL) the methods of Wheeler (1995) and Smith and Norman(1990) were used, respectively. Growth hormone (GH) was determined according to Fisher (1977) method.

2.5. Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS/ version 16) software. The significance of the differences between the groups was assessed by one –way ANOVA. All results were considered statistically significant at $P < 0.05$.

3. Results

A significant ($P < 0.05$) increase in plasma level of ACTH ,CORT, T_3 and T_4 after 3 hrs of receiving a single dose and 10 daily consecutive doses of Epi administration compared with the control group (Table, 1). The co-administration of MT and / or Tri-B following Epi offered partial improvement in the levels of these hormones after 3 hrs since there were significant differences between values of these groups and values of both the control and the Epi groups. Significant protective effect observed when MT alone or with Tri-B was administered to the Epi-stressed rats for 10 daily consecutive doses since there was no significant difference between values of these groups and the control group. On the other hand, the administration of Tri-B following Epi offered partial improvement in these hormones.

Plasma LH,testosterone,PRL and GH levels were significantly ($P < 0.05$) decreased after Epi injection for 3 hrs and after 10 days when compared to the control value (Table, 2). The administration of MT and / or Tri-B following Epi partially improved the plasma LH ,testosterone and PRL levels since there were significant differences between values of these groups and values of both control and Epi groups. However, the administration of MT and / or Tri-B following Epi abolished the decrease in GH level since there were no significant difference between values of this group and the control group. The administration of Tri-B following Epi offered a partial improvement in plasma LH, testosterone, PRL and GH levels since there was significant difference between values of this group and values of both the control and the Epi groups (Table 2).

Rats treated with MT (negative control) or Tri-B (negative control) for 3 hrs and after 10 days showed no marked changes in the measured parameters as shown in tables 1and 2.

Table 1. The effect of the intramuscular injection of Epi (0.02 mg/kg) with or without the intramuscular injection of the antidotes MT (1 mg/kg) or Tri-B (20 mg/kg) or both as well as each of the two antidotes alone on the level of ACTH, CORT, T_3 and T_4 in the plasma of male rats after 3 hrs of receiving a single dose and 10 daily consecutive doses.

Treated group	ACTH (pg/ml)		CORT (ng/dl)		T_3 (ng/ml)		***T4*** (µg/dl)	
	3 hrs	10 days	3 hrs	10 days	3 hrs	10 days	3 hrs	10 days
Control	11.81 ± 1.34	11.83 ± 1.34	26.91± 2.11	26.92± 2.11	1.46 ± 0.17	1.47 ± 0.17	3.45 ± 0.22	3.46 ± 0.22
Epinephrine	35.35 ± 2.43*	24.60 ± 2.61*	54.82 ± 3.12*	47.36 ± 3.40*	2. 53 ± 0.24*	3.16 ± 0.22*	6.87± 0.53*	5.19 ± 0.37*
Melatonin	10.89 ± 1.79	10.73 ± 1.00	25.60 ± 1.46	25.71± 1.87	1.21 ± 0.14	1.96 ± 0.20	3.36± 0.25	3.33± 0.31
Tri-B	10.63 ± 1.40	12.10 ± 1.48	25.91 ± 1.70	27.41 ± 1.68	1.29 ± 0.14	1.36 ± 0.21	3.40 ± 0.33	3.33 ± 0.41
Epinephrine + Melatonin	25.26 ± 1.07*#	14.33 ± 1.14#	37.37± 2.2 *#	30.27 ± 2.31#	1.82± 0.13 *#	1.65 ± 0.11 #	4.43 ± 0.42 *#	3.71± 0.41#
Epinephrine + Tri-B	30.20 ± 2.78 *#	19.41 ± 2.48*#♦	42.1± 2.60 *#	37.43± 2.20* # ♦	2. 31 ± 0.21 *#	2.03 ± 0.20 * # ♦	5.14 ± 0.56 *#	4.20 ± 0.31*# ♦
Epinephrine + Melatonin +Tri-B	22.1± 1.15 *#	13.27 ± 1.86 #	39.17± 2.15 *#	29.63 ± 1.99 #	1.94± 0.16 *#	1.41± 0.10 #	4.63± 0.32 *#	4.11± 0.43 #

- Each value corresponds to a mean of 10 animals ± SEM.

- * , # and ♦ are the statistically significant *(P<0.05*) when compared values of all experimental groups.

Table 2. The effect of the intramuscular injection of Epi (0.02 mg/kg) with or without the intramuscular injection of the antidotes MT (1 mg/kg) or Tri-B (20 mg/kg) or both as well as each of the two antidotes alone on the level of LH, testosterone, PRL and GH in the plasma of male rats after 3 hrs of receiving a single dose and 10 daily consecutive doses.

Treated group	**LH (mIU/ml)**		**Testosterone (ng/ml)**		**PRL (ng/ml)**		**GH (ng/ml)**	
	3 hrs	**10 days**	**3 hrs**	**10 days**	**3 hrs**	**10days**	**3 hrs**	**10days**
Control	0.46 ± 0.03	0.47 ± 0.03	0.54 ± 0.031	0.55 ± 0.031	0.43 ± 0.016	0.44 ± 0.016	2.35 ± 0.11	2.36 ± 0.11
Epinephrine	0.15 ± 0.02*	0.10 ± 0.02*	0.26 ± 0.034*	0.16 ± 0.038*	0.28 ± 0.018*	0.20 ± 0.021*	1.10 ± 0.10*	1.22 ± 0.11*
Melatonin	0.40 ± 0.03	0.41 ± 0.01	0.45 ± 0.032	0.49 ± 0.032	0.38± 0.017	0.40 ± 0.023	2.73 ± 0.21	2.60 ± 0.19
Tri-B	0.47 ± 0.04	0.42 ± 0.03	0.56 ± 0.045	0.60 ± 0.041	0.41 ± 0.016	0.40 ± 0.023	2.38 ± 0.13	2.40 ± 0.08
Epinephrine + Melatonin	0.32 ± 0.0 2* #	0.22 ± 0.02* #	0.36 ± 0.034* #	0.31 ± 0.028* #	0.25 ± 0.025* #	0.18 ± 0.014* #	2.13 ± 0.09* #	2.40 ± 0.08 #
Epinephrine + Tri-B	0.20 ± 0.02 * #	0.22 ± 0.02* #	0.26 ± 0.023 * #	0.34 ± 0.032 * #	0.22 ± 0.017 * #	0.25 ± 0.018 * #	1.25 ± 0.08 * #	1.95 ± 0.10* # ♦
Epinephrine + Melatonin +Tri-B	0.22 ± 0.02 * #	0.30± 0.02 * #	0.22 ± 0.027 * #	0.31± 0.031 * #	0.18± 0.027 * #	0.22 ± 0.022 * #	1.81 ± 0.11 * #	2.80 ± 0.09#

- Each value corresponds to a mean of 10 animals ± SEM.

-* # and ♦ are the statistically significant *(P<0.05*) when compared values of all experimental groups.

4. Discussion

Epinephrine had markedly elevated the level of adrenocorticotropic hormone (ACTH). Stress results in the activation of the hypothalamic-pituitary-adrenal (HPA) axis. This response is characterized by the secretion of corticotropin-releasing hormone from hypothalamic nuclei into the hypophyseal portal system, which in turn, stimulates the release of ACTH from the anterior pituitary into the peripheral circulation. Knigge *et al*. (1999) supported this view in their studies using a wide variety of acute stressors. Rats received MT and/or Tri-B following Epi showed a decrease in the elevated ACTH level. Melatonin passes easily through cell membranes; thus each organ, provided it can interpret the melatonin message, can adjust its physiological activity accordingly. A direct communication between the pineal gland and the adrenal gland which produce a number of different hormones that influence virtually all of the major processes in the body (Kanchev *et al.*, 2006). Exogenous melatonin can restrain excitability of hypothalamo-pituitary-adrenal (HPA) axis by decreasing the level of ACTH and corticosterone in plasma, so that melatonin can do some protection in hypoxic-ischemic brain damage (Wang *et al*., 2008). Vitamin B complex has not confirmed its role in modulating ACTH level. There is no complete satisfactory way of assessing the vitamin B efficiency on ACTH level. In spite of the accumulating evidences recording the effect of vitamin B on sympathetic output since it was observed that pyridoxine deficiency leads to increase the sympathetic outflow in rodents (McCarty, 2000).

Corticosterone is a major indicator of stress and is the major stress steroid produced in non-human mammals including rodents. It is synthesized in specific cells of the adrenal glands and produced in response to stimulation of the adrenal cortex by ACTH (Kitaysky *et al*., 2001) **.** MT treatment reduced the level of CORT in stressed rats. Notably, besides lowering basal CORT level, MT also attenuated CORT reactivity to an acute or chronic stressor in rats (Konakchieva *et al.*, 1997). Alonso-Vale *et al.* (2004) suggested that MT has a negative CORT modulator in normal as well as stress situations. This view was strongly supported another in vitro study describing MT functional receptors in adrenal gland cortex and their inhibitory effect on corticotropin -stimulated CORT production (Torres-Farfan *et al*., 2003).Vitamin B_6 is known to down regulate CORT activity in human and to decrease sympathetic nervous system output. It is also effective in decreasing the body's response to cortisol in the different organ systems (Oka *et al.*, 1995).

The elevated levels of T_3 and T_4 in stressed rats may be due to the rapid elevation of serum CORT which may influence the activity of thyroid gland either through a direct effect on the thyroid gland or through the influence exerted on the hypothalamus-pituitary (HP) system, modulating the secretion of thyroid-stimulating hormone (TSH) (Silberman *et al*., 2002).MT and/ or Tri-B following Epi completely improved the elevated T_3 and T_4 levels. Some studies suggested that MT leads to a decrease in thyroid growth and function and have a general inhibitory effect on thyroid hormones (Lewinski, 2002; Baltaci *et al.*, 2004). MT injections led to a reduction in thyroid hormone concentrations in rats (Özturk *et al.*, 2000). Exogenous MT during the day does not affect thyroid function, but if given at night exerts an inhibitor effect (Zwirska-Korczala *et al*., 1991).Similarly, injection of MT in the evening to rats and mouse is reported to affect thyroid hormone synthesis during a 10-day period (Selmaoui *et al.*, 1997). MT administration to rats with thyroid hypertrophy resulted in a decrease in thyroid hormone levels (Mogulkoc and Baltaci, 2002).The co-administration of Tri-B with Epi partially improved the elevated levels of T_3 and T_4. Previous studies indicated that low concentrations of some B-vitamins may coexist with abnormal thyroid function in humans (Apeland *et al*., 2006).It was also found an association between vitamin B_{12} deficiency and autoimmune thyroid disease (Ness-Abramof *et al*., 2006). In animals, vitamin B_6 deficiency causes hypothyroidism of hypothalamic origin (Dakshinamurti *et al*., 1990).

The decreased in LH and testosterone levels may be due to a high concentration of serum ACTH and CORT that increases sensitivity to the negative feedback effects of testosterone and inhibits the testicular function in rats (Rabin *et al*., 1990; Knol, 1991). Both physical and psychological stresses may interfere with the reproductive capacity of several species (Romero and Sapolsky, 1996). Orr and Mann (1992) found that restraint stress decrease the testosterone level without any effect on LH level. Friedl *et al.* (2000) reported that life stresses can cause a reduction in testosterone secretion and conversely, positive emotional state increases testosterone production in man. Rai *et al*. (2004) found a significant fall in serum testosterone level following immobilization stress. In the current work, MT administration failed in modulating the inhibitory effect of Epi on LH and testosterone level since MT has been reported to act as an antigonadotrophic hormone, inhibiting the release of pituitary gonadotrophins and spermatogenesis. The antigonadic effect of MT was also observed in the rats exposed to continuous darkness (Olatunji–Bello and Sofola, 2001). Luboshitzky *et al*. (2000) suggested that long-term melatonin administration does not alter the secretory patterns of reproductive hormones in normal men. The administration of Tri-B in stressed rats showed a partial improvement in the level of LH and testosterone. Vitamin B_{12} is needed to maintain fertility. In addition, vitamin B_6 deficiency results in insufficiency of alteration of the functions of adrenal and pituitary glands, since it is involved in the synthesis of luteinizing hormone, estradiol and testosterone (Manuchair, 1981).

Stress has a paradoxical effect on PRL secretion. It has been proved that the same stress factor may have a stimulatory effect if sustained in the morning and an inhibitory effect if experienced in the afternoon (Goda, 1990). Pituitary prolactin secretion is under a tonic and predominant inhibitory control exerted by the hypothalamus (Fitzgerald and Dinan, 2008). The pituitary PRL is secreted in a manner that reflects the photoperiodic cycle, with high and low concentrations of plasma PRL being concomitant with long and short day lengths, respectively (Adam *et al*., 1992). Suppression of PRL secretion by exogenous MT has been demonstrated for sheep (Poulton *et al*., 1986) and red deer (Milne *et al*., 1990). Ninomiya *et al*. (2001) suggested that exogenous melatonin can affect the spontaneous release of LH and PRL in humans. Vitamin B_6 has been reported as an antilactogenic effect, presumably by suppressing PRL secretion. Delitala *et al.* (1977) mentioned that a single 300 mg intravenous dose of vitamin B_6 produced a significant decrease in PRL in normal healthy subjects. Spiegel *et al.* (1978) hypothesized that B_6 might decrease PRL by increasing conversion of dopa to dopamine, an inhibitor of PRL in the hypothalamus. Harris *et al.* (1978) have demonstrated similar results in rats and attributed it to a direct effect on the pituitary gland. This finding suggested that B_6 inhibits PRL secretion by a direct action on the hypothalamus or pituitary gland. The investigation by Rosenberg *et al*. (1981) may support that pyridoxine hydrochloride partially inhibits prolactin by a mechanism not involving dopamine.

Epi administration induced a significant decrease in the level of GH this observation is in agreement with the reported data about inhibition of GH in rats after a wide range of stressors (Armario *et al*., 1993). The inhibition involves modulation of hypothalamic somatostatin and the stimulation involves direct actions on the pituitary (Andrea and William, 1992). Casanueva *et al*. (1990) found that elevated levels of circulating cortisol have a direct inhibitory effect on growth hormone . Stressed rats that received MT and / or Tri-B showed a significant increase in the level of GH. Ostrowska *et al.* (2001) found that small doses of MT(1 gm orally and 0.4 mg/kg intravenously) have not been shown to affect the 24-hr profile of GH level although larger doses (2 mg) have been shown to have a stimulatory effect on basal GH level. Tri-B supplementation caused a partial improvement in the level of GH. Both pituitary and serum GH levels have been found to be low in rats raised on vitamin B_6-deficient diet (Holman *et al.*, 1995). Delitala *et al.* (1977) have observed an increase in GH response to injection of pyridoxine in adults but a decrease in neonates. Reiter and Root,(1978) also showed the variability of the effect of pyridoxine on serum GH in older children.

In conclusion, the data reported here indicates that stress in the form of Epi injection strongly affected the physiology as shown in the measured parameters. There is growing interest in using exogenous MT as a therapeutic agent for the treatment of a large spectrum of disease and medical conditions. The prominent outcome of this study is that MT is superior to Tri-B in modulating most of the hazardous side effects of Epi. Also, several questions still arise on using Tri-B treatment individually as a hormonal modulator.

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