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Full Length Research Paper

Effects of physical activity on some of hemostatic parameters changes in epiphysectomized rats

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This study was designed to determine the effects of physical activity on thrombin time and coagulation time in epiphysectomized rats. Male Wistar rats (n = 60) weighing 90 to 100 g were divided into 2 groups; control group (without epiphysectomy) and experimental group (epiphysectomy). In each group, animals were divided into three subgroups: control (without physical activity), short-time (5 min physical activity) and long-time (with 20 min physical activity). Blood samples were collected from rat tail tip in several stages as before and after epiphysectomy and physical activity from experimental group, to determine coagulation time. Exercise programs were performed including; swimming on water pool until 5 min (short-time) and 20 min (long-time) in experimental and control groups. Autopsy was done on all the rats. Thrombin time was measured for each tissue. Our data showed that physical activity significantly decreased thrombin time on tissues of rats as compared to baseline values in control group (P < 0.001). In contrast, physical activity significantly increased thrombin time on different tissues in epiphysectomy rats (P < 0.001). Also, our results showed that in epiphysectomized rats after long-time physical activity, coagulation time increased but after short-time it decreased. In conclusion, these results suggest that there is a functional relationship between the pineal gland and the exercise on changes of hemostatic parameters in blood via its hormone melatonin.

Key words: Thrombin time, coagulation time, physical activity, epiphysectomized rats.

INTRODUCTION

It is well known that melatonin plays an important role in the control of several physiological processes. A number of investigations have suggested that there is a functional relationship between the pineal gland, via its hormone melatonin and the coagulation system (Tunali et al., 2005). Recently, a report showed that the chrono-biological patterns should be considered to analyze activity levels of coagulation factors (Pinotti et al., 2005). It was shown that thrombin is the primary activator of platelets at the site of thrombus formation and a major driving force in thrombus growth (Chesebrom et al., 1995). In addition, the hemostatic system is involved not only in the maintenance of the liquid state of the blood, vascular wall resistance and the arrest of bleeding from injured vessels, but also in the regulation of hemodynamics and vascular permeability (Tikhomirova et al., 2007). Several studies have shown that strenuous exercise leads to a shortening of the activated partial thromboplastin time, and results in an increase of thrombin generation markers (Hilberg et al., 2005).

It has been demonstrated that exhaustive exercise alters blood coagulation and fibrinolysis (Hilberg et al., 2003). It has been reported that exercise induced a significant increase in factor VIII activity with a significant shortening of activated partial thromboplastin time (EL-sayed et al., 2000). In addition, blood haemostasis is a complex interaction among platelets, coagulation and fibrinolysis. According to previous studies, the intensity of acute exercise is a critical factor affecting blood platelet function (Wang et al., 1997). Various studies identified increased platelet counts of 18 to 80% immediately after treatmill or bicycle exercising (EL-sayed et al., 2005). An

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Abbreviations: CT, Coagulation time; **TT,** thrombin time; **EP,** epiphysectomy; **NEP,** without epiphysectomy; **PHA,** physical activity; **NPHA,** without physical activity.

interesting possibility is that thrombin is involved in the platelet activation induced by strenuous exercise. Exercise also enhances blood coagulation and fibrinolysis, as evidenced by elevated plasma levels of prothrombin fragment and tissue plasminogen activation (Nailin et al., 2007). The increase in clotting and fibrinolytic activity due to exercise has been widely documented in humans, both for maximal and near maximal effects, the increased fibrinolytic activity appears to counter balance the exercise-induced increase in coagulability (Piccione et al., 2005). Few investigations exist on the relationship between the pineal gland, exercise and changes of coagulation time and thrombin time. Thus, the objective of this investigation was to examine the effects of physical activity on the changes of coagulation and thrombin time in epiphysectomized rats and compare it to those without epiphysectomy rats.

MATERIALS AND METHODS

Animal care and selection

Male Wistar rats (n = 60), weighing about 90 to 100 g were used in these experiments. The animals were housed at an ambient temperature of 25 ± 2°C under a 12 h/12 h light-dark cycle and acclimated to these conditions for 10 days before they were used in the experiments. All rats had free access to standard feed of rat and water *ad libitum*.

Experimental design and animal grouping

Animals were divided into two groups of 30 rats in each group. Control group (without epiphysectomized) and experimental group (epiphysectomized). In each group, rats were divided into three subgroups of ten: control (without physical activity), short-time (5 min physical activity) and long-time (with 20 min physical activity. Blood samples were collected from rat tail tip in several stages as before epiphysectomy, after epiphysectomy, after short-time and after long-time physical activity from experimental group to determine the coagulation time (Margolis, 1958).

Surgical procedure and epiphysectomy

Animals were deeply anaesthetized during all surgical procedures, with Ketamin 50 mg/kg body weight (BW) and Xylazine 10 mg/kg BW, by intraperiton injection and were submitted for surgery, according to Hoffman and Reiter (1965). In brief, the anesthetized rats were placed in a stereotaxic apparatus for small animals and a sagittal opening was on the scalp. The skin and muscles were pushed aside in order to expose the lambda suture. By means of a circular drill, a disc-shaped perforation was done around the lambda and the disc-shaped piece of bone was delicately removed. Thereafter, the pineal gland (which is located just below the posterior venous sinus confluence) was pulled out with fine forceps. After a brief period of haemostasis, the skull was closed by returning the disc-shaped bone, and the scalp was sutured with cotton threads.

Determination of thrombin time in tissues

Ten days after epiphysectomy in experimental and control groups, exercise programs performed included; swimming on water pool until 5 min (short-time) and 20 min (long-time). Rats were killed and then, autopsy was done on all the animals. Blood, liver, spleen and heart muscle tissues were isolated. After isolation of the mentioned tissues (liver, spleen and heart muscle), they were weighed on calibrated and accurate scale of 500 mg. They were detached from each tissue and were crushed on special mortar, after which the detached tissue was mixed with five milliliter physiological serum solution (NaCl). After complete crushing of each tissue, samples of prepared solution tissues and blood were poured on natrium oxalated test tube. Then, these test tubes were centrifuged with a 1500 round for twenty minutes. Thrombin time was measured for each tissue after which plasma was prepared from the mentioned tissue and documented (Flanders et al., 2003).

Statistical analysis

All results were expressed as mean ± SD, with the range in parentheses. An unpaired Student's t-test was used to analyze all the parameters (Statistical Software, Stat Soft). Statistical significance was attained at $p < 0.05$.

RESULTS

The changes of thrombin time responses to physical activity (short-time, 5 min) are presented in Table 1. Our results showed that thrombin time (TT) on different tissues decreased significantly in physical activity (PHA) group as compared to without physical activity (NPHA) group ($p < 0.001$). Our data clearly demonstrated that the greatest decrease on TT was observed in liver tissue. Also, our data showed that long-time (20 min) PHA significantly decreased TT on different tissues in PHA group as compared to NPHA group ($p < 0.001$) and greater decrease was observed in liver tissue (Table 2). In addition, as shown in Table 3, TT on different tissues significantly increased in epiphysectomy (EP) rats with short-time physical activity as compared to without epiphysectomy (NEP) rats ($p < 0.001$). Also, our results showed that long-time PHA significantly increased TT on different tissues in EP rats as compared to NEP rats ($p < 0.001$) (Table 4). According to the data of Table 5, coagulation time (CT) significantly decreased in EP rats after short-time physical activity as compared to control group (NEP with short-time physical activity) ($p < 0.001$). In contrast, after long-time physical activity, CT significantly increased in EP rats as compared to control group (NEP with long-time physical activity) ($p < 0.001$).

DISCUSSION

The results obtained in the present investigation suggest that physical activity significantly decreased TT and long-time physical activity had a more decreasing effect on TT than short-time physical activity (Tables 1 and 2). It seems that swimming caused activation of the clotting system by increasing fibrinolytic activity (Lins et al., 2003). It is well understood that physical activity evokes

Table 1. Effect of physical activity (short-time) on TT (second) in different tissues in control group (NEP) in male rats.

Tissue	Group		p
	NPHA (n = 10)	PHA (n = 10)	
	Mean ±SD	Mean ±SD	
Blood	29.5 ± 1	21.3 ± 0.59	<0.001
Liver	41.0 ± 1.6	16.2 ± 0.52	<0.001
Heart muscle	20.0 ± 0.7	17.0 ± 0.6	<0.01
Spleen	21.1 ± 0.9	19.5 ± 0.41	<0.05
Total	27.9 ± 0.92	18.5 ± 0.38	<0.001

Data are presented as mean ± SD; TT, thrombin time; PHA, physical activity; NPHA, without physical activity; NEP, without epiphysectomy.

Table 2. Effect of physical activity (long-time) on TT (second) in different tissues in control group (NEP) in male rats.

Tissue	Group		p
	NPHA (n = 10)	PHA (n = 10)	
	Mean ± SD	Mean ± SD	
Blood	29.5 ± 1.23	17.6 ± 0.77	<0.001
Liver	41.0 ± 1.6	7.0 ± 0.4	<0.0001
Heart muscle	20.0 ± 0.6	12.0 ± 0.8	<0.001
Spleen	21.1 ± 0.9	6.0 ± 0.38	<0.001
Total	27.9 ± 0.92	10.65 ± 0.28	<0.001

Data are presented as mean ± SD; TT, thrombin time; NEP, without epiphysectomy; PHA, physical activity; NPHA, without physical activity.

Table 3. Effect of physical activity (short-time) on TT (second) in different tissues in experimental group (EP) in male rats.

Tissue	Group		p
	NPHA (n = 10)	PHA (n = 10)	
	Mean± SD	Mean± SD	
Blood	21.3±0.59	30.2±0.33	<0.001
Liver	16.2±0.52	20.7±0.32	<0.01
Heart muscle	17.0±0.8	20.0±0.4	<0.05
Spleen	19.5±0.41	29.9±0.41	<0.001
Total	18.5±0.38	25.2±0.27	<0.01

Data are presented as mean ± SD; TT, thrombin time; EP, epiphysectomy; NEP, without epiphysectomy; PHA, physical activity; NPHA, without physical activity.

multiple effects on blood haemostasis via reduced inflammation and coagulation, which leads to reduced mortality. Also, a recent study showed that anaerobic exercise accelerates blood coagulation and activates blood fibrinolytic activity (Ali and Hanachi, 2011). In this regard, if the stimulus responsible for exercise induced increase in plasma von Willebrand factor (vWF) and coagulation factor VIII (FIII), the content seems to be mediated by b-adrenergic receptors through a nitric oxide-dependent mechanism, where the haemostastic system could be conditioned by endothelial function and be modified during the aging process (Jilma et al., 1997). Also, based on one marathon study, despite increased levels of B-thromboglobulin, platelet aggregation was found to decrease after exercise (Rock et al., 1997). Platelet activation during exercise may be related to

Table 4. Effect of physical activity (long-time) on TT (second) in different tissues in experimental group (EP) in male rats.

Tissue	Group		p
	NPHA (n = 10)	PHA (n = 10)	
	Mean ± SD	Mean ± SD	
Blood	17.6 ± 0.77	75.3 ± 0.77	<0.001
Liver	7.0 ± 0.8	20.0 ± 0.5	<0.001
Heart muscle	12.0 ± 0.6	38.0 ± 0.7	<0.001
Spleen	6.0 ± 0.38	59.9 ± 0.84	<0.001
Total	10.65 ± 0.28	48.3 ± 0.95	<0.001

Data are presented as mean ± SD; TT, thrombin time; EP, epiphysectomy; NEP, without epiphysectomy; PHA, physical activity; NPHA, without physical activity.

Table 5. Effects of physical activity (short and long time) on CT (second) in EP and NEP rats after short and long time physical activity.

Group PHA	NEP (n = 20)	EP (n = 20)	p
	Mean ± SD	Mean ± SD	
Short-time	296.9 ± 8.57	240.6 ± 1.0	<0.001
Long-time	100.0 ± 0.4	323.6 ± 0.99	<0.001

Data are presented as mean± SD; CT, coagulation time; EP, epiphysectomy; NEP, without epiphysectomy.

shear stress causing endothelial damage, increase in plasma thrombin generation, catecholamines and mobile-zation of more active platelets from the reticuloendothelial system (Rocker et al., 1986). Based on the results presented in this study, it was observed that TT significantly increased on different tissues in EP rats after short and long times physical activity (Tables 3 and 4). Also, our data showed that CT significantly decreased in EP rats after physical activity (short-time), but CT significantly increased after long-time physical activity in EP rats (Table 5). It was reported that exogenously administered melatonin reduced the skin oxidant damage and normalized activated blood coagulation induced by thermal trauma (Tunali et al., 2005). A dose-response relationship has been demonstrated between the plasma concentration of melatonin and coagulation activity [Wirtz et al., 2008). Also, it was demonstrated that exogenously administered melatonin normalizes the activated blood coagulation (Cardinali et al., 1993). Therefore, the result of the present study shows the role of pineal gland on the changes of thrombin time and coagulation time. Interestingly, our data showed that long-time physical activity caused CT increased in EP rats, but short time physical activity increased it. One of the possible explanations for this is that physical activity effects may be more effective than pineal gland effect on the change of coagulation time. Researches have shown that exhaustive exercise alters blood coagulation and fibrinolysis (Ferguson et al., 1987). In this regard, after strenuous short-term exercise in male subjects, varying fitness was observed as signs of increased blood coagulation and fibrinolysis by measuring global tests, factor VII, tPA, and fibrin split products, such as D-dimer and fibrin monomers (Gunga et al ., 2002).

Conclusion

Conclusively, the results of this study indicate that physical activity could decrease thrombin time. Also, TT increased in EP rats. Interestingly, our data showed that in EP rats after long-time physical activity CT increased but after short-time decreased. These data clearly indic-ate that pineal gland and melatonin, similar to physical activity play important role on haemostasis. However, further studies are needed to determine possible mechanisms of action, physical activity and pineal gland on changes of coagulation and thrombin system.

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