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The Impact of Both Physical Exhaustion and Disruption of Circadian Rhythm on Blood Coagulation Factors in Rats

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Abstract:
Effects of circadian rhythm and different durations of physical exercise on blood clotting and fibrinolytic systems were measured in 60 male Wistar rats.
Rats were divided into control (n=20) and experimental groups (n=40). Blood samples were collected from rat tail tip at several stages; before and after physical activity in various functional conditions of pineal gland (activation and inhibition). The first group of rats were housed in absolutely light- conditions (inhibition phase) for 10 days ,while the other group was kept in absolutely dark room for 10 days , at ambient temperature 23ºC±2ºC. A standard rat diet and water were supplied ad labium.
Our data showed that no statistically significant variation was found in blood clotting time and thrombin time of samples after a short – physical exercise in the normal subject, but there was a significant shortening in blood clotting time and thrombin time after strenuous exercise.
Physical activity significantly prolonged clotting time in animals with inhibited pineal gland.
Rats with activated pineal gland (dark phase), the clotting time prolonged after both durations (short and long exercise); thrombin generation time shortened.
Conclusion:
It is important that individuals who experience disturbances in their circadian cycle aware of their impact on the function of the pineal gland(i.e., clotting and fibrinolytic system, such as shift workers, jet lag, physical workers and even athletes who train heavily . Furthermore, individuals need also to consider the time of exposure and avoidance of light options which interfere with this system, including time of exercise and the use of melatonin agonists.
Keywords: circadian cycle; blood coagulation; physical activity; Pineal gland

Experimental Protocols:
All experimental protocols were performed in accordance with the guidelines of the American physiological Society and approved by the Animal Ethics Committee in the University.

Introduction

The pineal gland, also called the pineal body, epiphysis cerebri, or the third eye, is a small endocrine gland in the vertebrate brain. It produces melatonin, a hormone that affects the modulation of wake/sleep patterns and photoperiodic (seasonal) functions [1]. It is located near the center of the brain between the two hemispheres, and is tucked in a groove where the two rounded thalamic bodies join . Some evolutionary biologists believe that the vertebrate pineal cells share a common evolutionary ancestor with retinal cells [2]. The pineal gland is associated with the sixth chakra whose awakening is linked to prophecy and increased psychic awareness as consciousness ascends [3]. It has attracted scientists' attention from the ancient times, but the present epiphysiolgy, in fact, started after revealing its hormone, melatonin, in 1960 [4].

Studies on the role of epiphysis in regulating the metabolic-vegetative functions started during the past 25 years. These studies had firmly determined that the epiphysis is one of the main neuro-hormone transformations . The basic hormone of epiphysis, melatonin, is synthesized from serotonin under the influence of the specific enzyme hydroxyindol-o-methyltransferase [5]. At daytime and under the influence of light melatonin function from the epiphysis is inhibited 10 times its usual level during night. By the interchange of

activation and inhibition due to light and darkness epiphysis function is also alternated [6]. This process plays a significant role in the daily rhythm of the physiological process, so called circadian rhythm.

The circadian rhythm is a 24-hour cycle that regulates many biological processes in living creatures. It helps in regulating body functions in an orderly fashion. Disruptions of this rhythm have negative impact, which results in fatigue, disorientation, insomnia as well as other symptoms [7].

Although aerobic exercise increase heart rate during exercising, it can be beneficial to the circadian rhythm as it causes heart rate to slow down during other parts of day. This decrease in heart rate is most prominent in the morning [8]. The pacemaker cells of the heart signal from light sources that set off a cascade of molecular effects [9]. Regular participation in sports reduces the risk of developing blood clots by 39 percent in women and 22 percent in men [10].

The production of melatonin by the pineal gland is inhibited by light to the retina and permitted by darkness. Its onset each evening is called the dim-light melatonin onset (DOLMO). And the duration of melatonin secretion each day is directly proportional to the length of night. It is the blue light, around 460 to 480 nm, which suppresses melatonin with increased light intensity and length of exposure [11]. Light containing only wavelength greater than 530 nm does not suppress melatonin in bright - light conditions [12].

Light exposure to the retina is relayed to the suprachiasmatic nucleus [13], which controls the daily cycle in most components of the paracrine and endocrine systems [14,15]. Infants' melatonin levels become regular in about the third month after birth with the highest levels measured between midnight and 08:00 in the morning [16]. In humans 90% of melatonin is cleared in a single passage through the liver, a small amount is excreted in the urine, [17] and a small amount is found in the saliva. Human melatonin production decreases as a person ages [18]. As children become teenagers, the nightly schedule of melatonin release starts to delay, leading to later sleeping and waking times [19].

There are serious potential consequences to problems in the circadian rhythm that are so physically potent- amongst them are disturbances in human blood clotting factors. Investigating epiphysis role in regulating functional condition of blood coagulation and its value in the circadian rhythm can be beneficial to chronophysiology and homeostasis. Exogenously administrated melatonin reduces skin oxidant damage and normalizes the activated blood coagulation induced by thermal trauma [20]. It is known that physical activity induces modification in blood homeostasis and leads to an activation of blood coagulation and fibrinolysis [21].

Physical stress has been associated with the activation of blood cell [22]. Strenuous exercise leads to shortening of the activated partial thromboplastin time resulting in an increase of thrombin generation markers [23]. Exhaustive exercise alters blood coagulation and fibrinolysis [24]. Although blood coagulation alterations have been indicated in literature, the extent of these changes and how they influence normal human coagulation status is an area of limited knowledge. In fact, the assessment of the dimensions and importance of these changes is difficult in humans as they might put human life in danger. Long – term physical exercise promotes changes in coagulation and fibrinolysis of blood.

Few studies exist on the relationship between the clotting times and exercise. There is also limited knowledge on the impact of combining the effect of disturbances in the circadian rhythm and physical activity on blood clotting factors. This study investigates the impact of both physical exhaustion and disruption of the circadian rhythm on blood coagulation factors in rats.

1. Materials and Methods

Animals

Adult male Wistar rats (n=60) by the living mass of 150 to 200 grams, were distributed among groups according to the balanced Latin- square block design (based on body weight). Room temperature was kept constant at20ºC - 22º C with 10 days light – dark conditions as follows:

First group: control group

Second group: animals were kept in absolute darkness for ten days

Third group: animals were kept in absolute light for ten days.

Blood collection method

The tail of the animal was warmed for 1 min in water with temperature of 40º C. The tail was dried and cut at the tip with a sterile razor blade. Animals were anaesthetized with chloral hydrate (4% solution, 7 ml/kg) prior to blood withdrawal. Arterial blood was then collected by needle aspiration from the iliac bifurcation, which provides an abundant blood sample free of hemolysis. The blood sample was immediately emptied into a plastic tube containing 0.11 mL of sodium citrate at a ratio of 1:10 anticoagulant blood, gently mixed and centrifuged at 2500 gm and temperature of 4º C for 10 min. Plasma was separated and maintained in ice bath throughout its processing.

2. Procedure

The tests were held in the following series of experiments:

First series: blood coagulation factors in intact animals (i.e., with no intervention) before and after physical loading.

Second series: blood coagulation factors in animals with activated pineal gland (i.e., animals which were kept in absolutely dark area for 10 days) before and after physical activity.

Third series: investigation of changing substances and activity of coagulation factors in animals before and after physical loading in animals with inhibited pineal gland (i.e., animals which were kept in lighting phase within 10 days of light.

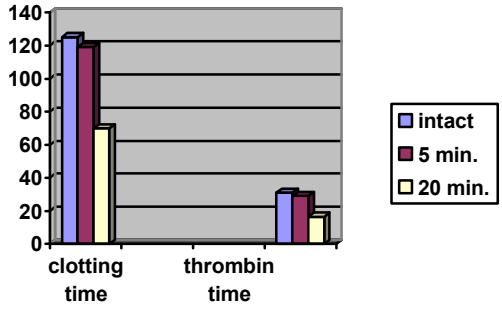
The time of physical loading was determined as short time physical load for 5 minutes, and long time physical load for 20 minutes. Swimming in a swimming pool was used as the physical activity for animals in this study.

3. Results

The period of clotting time in intact animals was 125±3.86 seconds (Table 1). For animals which went through 5-minute physical activity, there was no statistically significant variation in blood clotting time of samples drawn. However, there was a shortening in the blood clotting time for prolonged physical activity; the mean time was 70±3.0 (Table 1). No change in thrombin generation rate after a short exercise, whereas seven of ten animals showed acceleration in thrombin generation from mean time after heavy exercise.

Table 1. Blood clotting time and thrombin generation time after both short and long physical activities in the first group of animals.

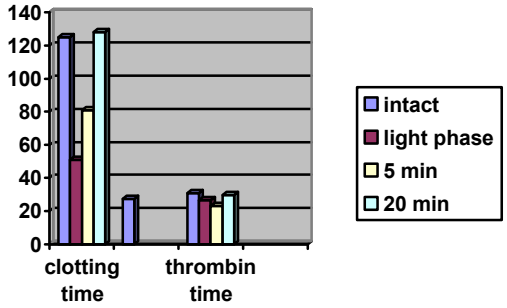
Test	Before physical Activity	After physical Activity	
		Short (5 min)	long (20 min)
C.T (sec)	125±3.86	119±3.86	70±3.0
T.T (sec)	31.1±0.5	29±1.0	16.5



As shown in table 2 , the clotting time in animals with inhibited pineal gland (lightning phase) is sharply shortened to 51 seconds, but after the short physical load, the clotting time is prolonged to 81 seconds, and sharply prolonged to its normal value as in intact subjects after exhaustive physical activity 128.7 sec. where as the thrombin generation time in animals with inhibited pineal gland is shortens markedly only after short physical activity.

Table 2. Blood clotting time and thrombin time changes after physical activity in animals with inhibited pineal (lighting phase).

			After physical activity	
Test	before physical activity	(5 min)	(20 min)	Nº
Blood clotting time(s)	51.17	81.2	128.7	10
Thrombin time (s)	26.7	23.3	29,6	10

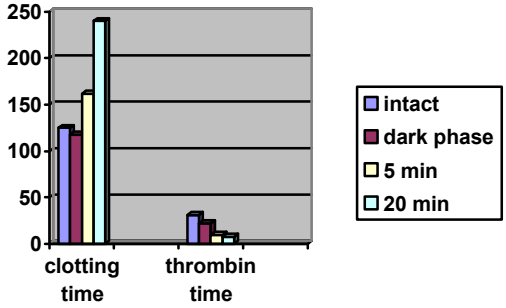


Alternations of blood coagulation factors in animals with activated pineal gland (dark phase) before and after physical activity are shown in table 3.

After the physical load as in momentary, as prolonged, the blood coagulation time lengthens and reaches to 240 second. The thrombin time shortens in animals with activated pineal gland, and this kind of shortening is accompanied also after both physical activities

Table 3. Blood clotting and thrombin time changes after physical load in animals with activated pineal gland (dark phase).

Test	before physical Act.	After physical Act.		Nº
Sec.	In activated pineal g.	(5 min)	(20 min)	
C.T	118	162.4	240	10
T.T	21.92	9.81	7.90	10



4. Discussion

Whole blood clotting time and thrombin time were measured before and after physical exercise with different intensities and duration, using 60 rats as subjects. The exercises consisted of a 5 minute swimming in water pool as a short time exercise, and 20 minute swimming to near exhaustion.

Blood clotting time was measured in control animals without physical activity. Hence, ranges considered normal for rats are not comparable to human references. However, it was possible to demonstrate that clotting and bleeding times are longer in humans than in rats [25]. The clotting time was 125±3.86 seconds with minimal-maximal values 113 -136 seconds. Neither was there any significant change in clotting and thrombin times after momentary physical exercises. However, seven subjects experienced an acceleration of thrombin generation time and clotting time after a heavy exercise, table 1a and 1b.

It seems that a swimming causes an activation of the clotting factors by increasing fibrinolytic activity. Hilberge and et.al showed that the maximal short time exercise does not lead to a relevant activation of blood coagulation in healthy young subjects. It is only slightly altered within the normal range. In this study immediately after a heavy exercise, a shortening of PTT was seen.

The effect of muscular exercise on blood coagulation has been the subject of several investigations in both human and laboratory animals, results indicated that coagulation accelerated immediately after muscular exercise. According to the results of Riberrio and et al., exhaustive exercise in adults decreases thromboplastin time

In animals with inhibited pineal gland observed shortening of thrombin time. As it marked in literature, the circadian cycle of physiological processes is regulated by pineal gland. In day times the activity of hydroxyindol-o-methyltransferase is inhibited approximately 10 times and caused the synthesis of melatonin.

After the physical loading, the thrombin generation time shortens more sharply after short physical exercise, but prolonging physical exercise inconsiderably increases it.

The clotting time in this group of animals was shortened before any physical activity, whereas the clotting time delayed after different durations of physical activities. These changes are seen in table 2.

In dark - phase with the increasing of pineal activity, the blood coagulation time sharply raises. After both physical activities, the blood coagulation time lengthens to reach 240 sec.

All the above stated gives a ground to consider that the intense influence of physical activities is connected with neuro-hormonal regulation of hemostasis.

In activated pineal gland function, the physical activity causes the thrombin time to shorten to 47% .

Long physical activity causes more sharp effects on both groups of animals with various conditions of pineal gland.

5. Conclusion

Conclusively, the results of this study indicate that the pineal gland, melatonin and physical activity play an important role on haemostasis. However further studies are needed to determine the effects of circadian rhythm and physical exercise on blood coagulation cascades.

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