Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Continuous Darkness Induces Changes in the Urinary Space of the Rat's Kidney

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Abstract

Background Melatonin known as "the light of night" secreted from the pineal gland and implicated in a number of cyclical bodily performance and circadian rhythmic activities in humans. The kidney is the main organ regulating water-electrolyte homeostasis in the body adjusted to a daily rhythm.

Objective To study the effect of rising periods of continuous darkness onthe urinary space of adult male rat's kidney.

Methods Eight groups of adult Wister albino rats, each of 4 rats were kept in absolute 24 hours darkness for successively rising 4 periods. Group II, III, IV and V were situated in continuous darkness for two, four, six and eight weeks in succession. Group I^a, Group I^b, Group I^c and Group I^d were the control groups for group II, III, IV and V, respectively. All rats were dissected under general anesthesia at the end of experiment and the right kidney was removed, weighed and arranged for morphometric as well as anatomical and histological studies.

Result No imperative structural effects were noticed with the short and medium periods, but with long periods significant effects were noticed, which were in ratio to the length of darkness.

Conclusion The continuous darkness has structural influence on the glomerular urinary space in the kidney of adult male rats according to the length of exposure.

Key words Darkness, melatonin, kidney and urinary space.

Introduction

All organisms are subjected to the Earth's rotation around the sun with its periodicity of night darkness and day light, with cyclic changes in the length of the daily light and dark and, also, with seasons and climatic changes. Hence, a range of biological activities oscillate inside the organism including: blood and fluid homeostasis, physiological functions, biochemical factors and even behavior. If an event within the biological system returns at roughly regular intervals, it is known as a biological rhythm. These rhythms affect a range of activities, such as the migration behavior (birds, fish), sleep-wake cycle, seasonal change in weight, reproductive cycles etc. The prime rhythms in life are daily rhythms e.g., heart and respiratory rate, blood pressure, rest-activity, body temperature, synthesis and secretion of many hormones, renal plasma flow, glomerular filtration rate, electrolyte concentrations in urine . . etc. Revision on chronobiological side of physiological functions had been carried out essentially on humans and laboratory animals [1-4].

Melatonin is called "the light of night", since it is secreted from the pineal gland mostly at night. It is concerned with the circadian rhythm and in a number of so many cyclical activities in humans. Melatonin is completely involved in signalling the time of day and time of year to all physical

tissues, so, it is considered to be the body's chronological pacemaker [5-7].

Quiroz *et al* had recognized that; melatonin administration ameliorates the course of chronic renal failure, in rats having renal mass reduction. But they suggested that further studies are necessary to describe the potential usefulness of this treatment in the other animal models and in the patients with chronic renal disease [8].

Kidney is the main organ, which regulates the water-electrolyte homeostasis in the body. It is responsible for uphold the total volume of water and its distribution in exacting water spaces, for the electrolyte contents of systemic fluids and also for preserving the acid-base balance. These functions are done, normally, by the plasma filtration process in the renal glomeruli, and the filtrate enters, soon, after passing through the filtration slits, to Bowman's space or known as the urinary space, or the capsular space, which presents just between the visceral and parietal layers of Bowman's capsule. The practice of filtration is regulated to what is called an activity-rest rhythm. These diurnal changes are manipulated by a twenty four hour cycle of activity, of the hormones involved in the regulation of the renal activity. Characteristic of the prospect knowledge of these rhythms is precious for clinical, pharmacological and practical purposes, as well as studies on their physical act [1,8-10].

Objective: To study the effect of rising periods of continuous darkness on the urinary space of adult male rat's kidney.

Methods

This project was achieved in the course of the period from the morning of 4th of March, till the morning of 29th of April of 2013. Thirty two of male Wister albino rats, of ten weeks age were acquired. They were placed in a controlled animal room, specialized for the post graduated researches, at the Department of Anatomy, Histology and Embryology of College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq. These rats were divided into eight groups, each hold four rats. Group I^a, group I^b, group $I^c$and group I^d were the control of group II, III, IV, and V, respectively. The entire of the 4 control groups were put on controlled 12:12 light – dark cycle.

Group II, III, IV, and V were kept in more or less exactly uninterrupted darkness, for a period of 2, 4, 6 and 8 weeks correspondingly. All of the 8 groups were set in a restricted room temperature of 22 ± 2°C, and were located separately in wire meshed stainless steel special cages, fed prohibited food and the tap water was offered for drinking *ad libitum*.

At end the last day of 1st couple of weeks, rats of group II with their control rats (GroupI^a),were dissected with the use of diethyl ether anesthesia. The whole right kidney was in use, fixed in Boun's solution right away and processed through for histopathological study by aid of light microscopy, using serial paraffin sections of 4-6 µm thickness, then, stained with haematoxylin and eosin [11-12]. At the equivalent approach the rats of group III were dissected, just at the end of the 4th weeks also with its control group (Group I^b). The rats of group IV with its own control rats (Group I^c) were managed at the equal way, of the previous groups, at end of the 6th week, and rats belong to group V with their own control group (Group I^d), were maneuvered, at the end of the 8th week.

Anatomical, histopathological as well as biochemical examinations were prepared. The morphometric measurement of the urinary spaces were made by a light microscope, with digital camera, to select the images of the studied section, then, the images of the prepared slides were managed and analyzed, by means of the computerized program named Image J, so that the urinary space width was estimated for any proposed diminution [13]. Morphological description, also, were done to evaluate any probable histopathological changes, might be come to mind. Biochemical analysis was done, as well, by measuring some selected electrolytes and urea level in the serum, to assess the degree of physiological disturbance, if present, in the renal filtration.

Biostatical analysis was prepared to assess the significance of results by analysis of variance, using student-t-test [14].

Results

The data collected from the revision of every variable group, whether for the histometrical, biochemical or morphological examination might be highlighted as the followings:

In group II: the renal tissues samples, showed no any clear deformity, when they were studied, by the light microscope, whenever they were compared (as usually carried out in any research) with its own control group (Fig. 1 & 2 and table 1).

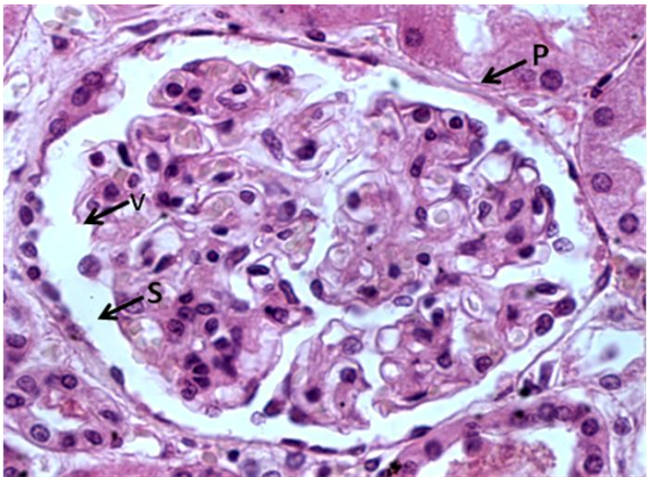

Fig. 1. Renal corpuscle of control adult male rat, P: parietal layer, V: visceral layer & S: urinary space. X400, H & E.

In group III: In the same slide; the renal tissue assessment revealed some fields with a significant decrease in the average width of the Bowman's space of the renal corpuscle, whilst other fields illustrated more or less the same picture as that seen at the examination of its control, apart from some scattered dilated blood vessels (Table 1).

In group IV: The renal tissue study naked the significant decrease in the average width of the Bowman's space of the renal corpuscle, plus, on some corpuscles this space was diminished, to an extent, that it was hardly distinguished, with more dilated blood vessels seen (Fig. 3 and table 1).

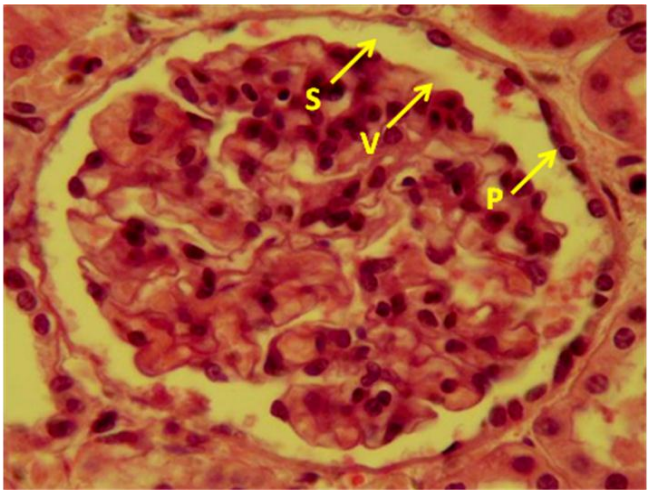

Fig. 2. Renal corpuscle of adult male rat of group II: No obvious changes as that of the control. P: parietal layer, V: visceral layer & S: urinary space. X400, H & E.

Table 1. The effect of continuous darkness on average width ofthe corpuscular urinary space in male rat.

Time of continuous darkness	% Decrease in urinary space of renal corpuscle
Two weeks	0.66±1.43
Four weeks	32.44±15.20*
Six weeks	61.12±18.1**
Eight weeks	94.89±12.7***

* = *P*< 0.01), ** = *P*< 0.005, *** = *P*< 0.001.

In group V: The renal corpuscles were viewed with even more shrink in the average width of the Bowman's space, than that of previous group, and on most of the corpuscles this space was diminished, to a point, that it was approximately obliterated (Fig. 4 and table 1). Dilated blood vessels seemed to be greatly more abundant in this group, with many areas of local hemorrhage.

Biochemical changes were shown in Table 2. Which illustrated non-significant effects, on short dark periods, whereas, it has injurious outcome with the longer periods of darkness.

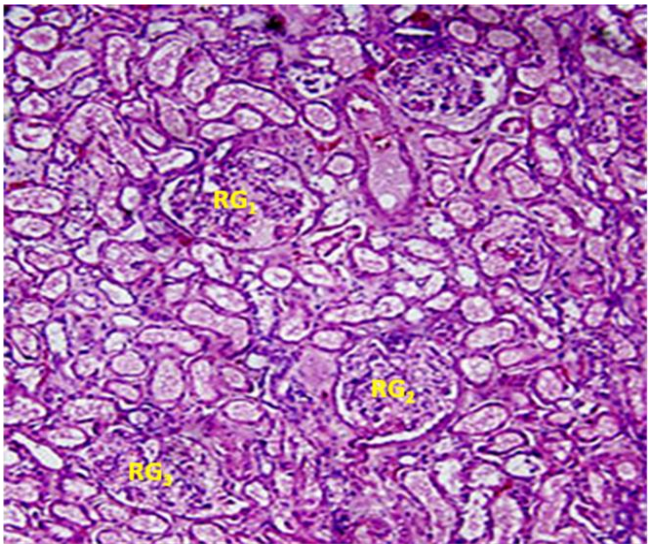

Fig. 3. Renal tissue of adult male rat of group IV showing gloeruli: partial obliteration (RG1); normal width (RG2); complete space obliteration (RG3): X 100, H & E.

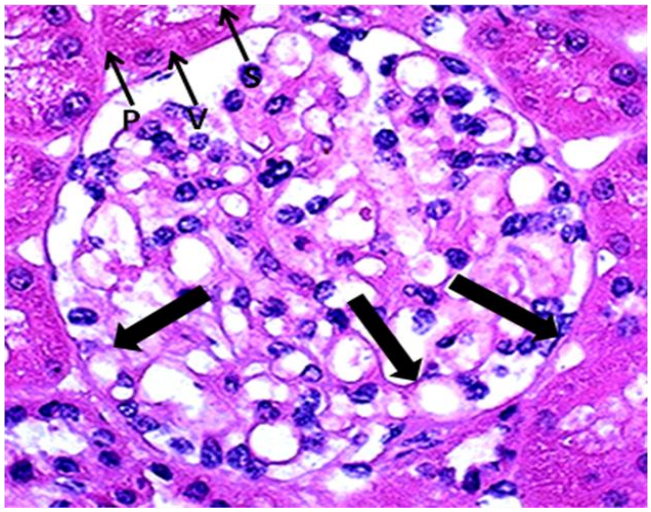

Fig. 4. Renal corpuscle of adult male rat of group V: P = parietal layer, V = visceral layer & S = urinary space. Urinary space was almost obliterated (Black arrows). X400, H & E.

Discussion

Melatonin is the well celebrated pineal neuro-hormone that released to the circulation, primarily at night. It is made from the amino acid, tryptophan, via the serotonin within the pineal gland [5-7]. Even though, many decades of investigations concerning its role on the tissue histology, physiology, and biochemistry had been performed, but, it is still a context of vast interest to investigate more and more its function, hence, it was selected to be involved at this study [15-17].

The kidney was chosen in the instant study because, indeed, it is one of the most vital bodily organs. It has a former role in the all of body homeostasis, controlling the electrolyte concentrations, acid-base balance, extracellular fluid volume, as well as it plays a crucial role in the blood pressure regulation. It eliminates a wide variety of waste products. It secretes a group of hormones, such as erythropoietin. It also secretes the enzyme rennin and Calcitriol which is activated form of vitamin D. All of the variety of kidney's functions are finished, simply, by the mechanisms of re-absorption, filtration and secretion [18-22], hence the filtration site was tried to be investigated here.

The anatomical, histological and biochemical changes seen in the ongoing study, which were induced by putting in continuous darkness, might be discussed by the fact; that melatonin is able, relatively, to reach all bodily tissues and cells, inducing its action, via the meticulous melatonin receptors, which present, nearly, in all of the body tissues [23-26]. These changes were in ratio with the length time exposure to darkness; because melatonin has a dose – dependent natural achievement [27-29], so no factual injurious effect was occurred, to the group put on 2 weeks of continuous darkness, still with the longer dark exposure; there was an apparent renal harm. These consequences could be confer by the fact that; melatonin is known to have no harmful effect, on little doses, whereas, it has injurious outcome, whenever it is supplied on prominent levels [17,27-29].

The time-route of 30 days, for rats, is regarded as a long period of treatment, according to Peltier *et al.* [30]. Hence, it caused no any detectible harmful histological nor functional effects on short period of darkness, and those findings were equivalent with the findings of Hoyos *et al* [31], when he planned that; melatonin, in its normal pharmacological level, do not affect the serum biochemical parameters

including overall serum proteins, creatinin and urea. Thus, in the ongoing study, it did not affect the renal function; yet, it slightly decreased the uric acid, which was regarded as a pleasant effect for sure.

When group II and III were assessed, the products were quite different, and significant histological changes were noticed on corpuscle space, presented by the obvious narrowing of the corpuscular urinary space with concomitant biochemical changes as well.

Table 2. The effect of continuous darkness on some renal biochemical profiles of male rat

Time of continuous darkness	Sugar mg/100ml	Urea mg/100ml	Ceatinine mg/100ml	K MEq/L	TSP g/100ml	Albumin g/100ml
Control Group I[a]	87.9± 0.9	23.8±1.3	0.33±0.04	4.1±0.1	7.4±0.6	3.22±0.1
2 wk	89.1±1.4	22.9±1.9	0.32±0.07	4.0±0.3	7.5±0.8	3.21±0.9
Control Group I[b]	88.8±1.9	22.6±2.1	0.35±0.06	3.9±0.7	7.5±0.02	3.31±0.2
4 wk	90.3±2.1	24.7±2.3	0.47±0.09*	4.6±0.9	7.3±0.5	3.25±0.8
Control Group I[c]	89.1±2.4	23.6±2.8	0.36±0.1	4.0±0.7	7.5±0.4	3.20±0.6
6 wk	112.8±1.7*	41.9±1.8*	0.89±0.05**	4.9±0.6*	6.7±0.3*	2.93±0.2**
Control Group I[d]	88.3±2.2	22.9±2.6	0.32±0.3	4.1±0.5	7.3±0.6	3.24±0.6
8 wk	119.8±1.6**	67.9±1.6**	1.23±0.8**	5.8±0.4***	6.1±0.2**	2.07±0.4***

* = $P< 0.04$, ** = $P< 0.03$, *** = $P< 0.001$).

Those unwanted changes whether histological or functional might be caused by the harmful damaging effect of melatonin wherever it is used in a large dosage [27-29], because melatonin can reach almost all body tissues and cells, applying its action through its receptors [23-26], so it perhaps had damaged any of the corpuscular constituents such as the endothelium of the glomerular capillaries, glomerular basement membrane, mesangealcomponants or the podocytes; leading to resultant glomerular filtration abnormalities, since a damage to any of these components whether functional or structural could contribute to the filtration disturbance [18,19,21]. Although the study of the blood vessels is not our main goal in the instant research, but it was observed there for interest; the dilatation of blood vessels, could be attributed to the fact which documented the melatonin, to have a vasodilation action [32-34]. Uncertainties and doubts seemed to be still surrounding the role of melatonin, in human biology and pathophysiology and future researches might be needed.

In conclusion: The continuous darkness has structural as well as functional influence on the glomerular urinary space in the kidney of adult male rats in accordance with the length of darkness exposure.

Acknowledgement

Great thanks to my supervisors for their significant advice and encouragement.

Conflict of Interest

The author declares no conflict of interest.

Funding

No specific grant from any funding agency in the public, commercial or not-for-profit sector.

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Received 3rd Feb. 2013: Accepted 19th Feb. 19th Feb. 2014