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Orexin-1-Receptor Blocker, Sb-334867 May Affect Body Weight And Protect Against Hypoglycemia Induced By Paradoxical Sleep Deprivation In Adult Male Rats

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Keywords

- Sleep deprivation
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Abstract

Background: Sleep deprivation (SD) can affect health through its effects on many systems. Orexin is involved in regulation of many physiological functions including sleep. This can give an explanation and a way of protection against some hazards of SD. **Aim**: To test the protective effect of orexin-1 receptor (OX1R) blocker, SB-334867 on changes in food intake, blood glucose level and insulin sensitivity caused by SD.**Method**: 72 adult male rats arranged in 4 equal groups: control group, SD group, SD-OX1R blocked group & SD-DMSO group. The 3 SD groups are subjected to 8 days of paradoxical SD using the modified multiple platform method. The SD-OX1R blocked group was injected intraperitoneally daily with single dose of SB-334867 dissolved in 2 ml DMSO and diluted 1:1000 in saline (3 mg/kg/day). The SD-DMSO group was injected by DMSO alone. Food intake, body weight, blood fasting glucose & insulin levels were assessed and insulin resistance was calculated using HOMA-IR formula.**Results**: The SD and SD-DMSO groups showed loss of weight inspite of increased food intake plus hypoglycemia with increased insulin sensitivity. The SD-OX1R blocked group showed no significant change in food intake but more drop in body weight plus delayed changes in fasting blood glucose and insulin sensitivity. **Conclusion**: SD can affect health through its effect on food intake and induction of hypoglycemia. OX1R blocker, SB-334867 protects against the increase in food intake and delays increased insulin sensitivity and subsequent hypoglycemia. So, orexin most probably is a mechanism by which SD causes these changes.

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INTRODUCTION

Rapid industrialization, technological improvements e.g., computers and smartphones), and lifestyle changes have changed the magnitude of stressors to which our current generation is exposed on a daily basis. These stressors if uncontrolled might translate in the forms of fatigue, irritability, sleep disorders, hair loss, accelerated aging, immune suppression, heart disease, hypertension, depression, obesity, erectile dysfunction (ED), and infertility ***(Pressman et al., 2018)***.

One of these modern stressors is the sleep deprivation (SD). The average sleep duration has declined over the last few decades ***(Youngstedt et al., 2016)*** that represent a major public health issue.

There is increasing studies to demonstrate that SD is associated with increased risk of cardiovascular disease including increased prevalence of hypertension ***(Jeddi et al., 2018)***. In humans, SD is usually associated with increased consumption of excess calories from snacks ***(Vetrivelan et al., 2012)*** that increase the risk of development of obesity ***(Jeddi et al., 2018)***. Night shift workers have a higher risk of diabetes as SD predispose for poor metabolic health by promoting excess caloric intake in response to reduced sleep & food intake at internal biological times when metabolic physiology is not prepared ***(McHill and Wright, 2017)***. These adverse sequelae were linked to sleep disturbances, including insufficient sleep, fragmented sleep, circadian dysregulation even without altering total sleep duration ***(Koren et al., 2015).***

These effects of SD on body functions are still controversy. While some researchers find elevated blood glucose level after SD ***(Zou et al., 2017)*** and increased incidence of diabetes mellitus, others find drop in blood glucose level (***Brianza-Padilla et al., 2015)***. Similar controversy reported while studying other parameters as body weight and food intake.

Also the mechanisms by which SD affects body health and metabolism are still not clearly understood. Adverse effects of SD were explained by increase the levels of catecholamines which inhibit insulin secretion and promotes glycogen breakdown or by lower leptin and higher ghrelin levels that leads to increase in food intake and development of obesity or by increase the 24-h cortisol secretion contributing to a state of greater insulin resistence ***(Koren et al., 2015).***

Orexin, a newly discovered hypothalamic neurotransmitter, is involved in regulation of sleep ***(Kargar et al., 2015)***, food intake, regulation of blood pressure, the neuroendocrine system, body temperature, energy homeostasis & glucose utilization ***(Villano et al., 2017)***.

Orexin has 2 types of receptors: orexin-1 receptors (OX1R) and orexin-2 receptors (OX2R). The OX1R has poor effect on sleep while OX2R has a major role in promoting wakefulness. So Blocking OX1R by SB-334867 does not decrease wakefulness & it is used for the treatment of other conditions, such as substance abuse, withdrawal, obesity and panic disorder ***(Equihua et al., 2013).***

So, the hypothesis of this study is, orexin may play a role in the disturbances that accompany SD that will be reflected on health due to its wide roles in regulation sleep and regulation of different parameters that affected by SD and these

disturbences may be blocked using OX1R blockers without affecting wakefulness in night shift workers to continue their work.

Aim of the work:

- To test the effect of SD on body weight, blood glucose level and insulin sensitivity.
- To evaluate the hypothesis that orexin is involved in the mechanism by which SD produces its adverse effects.
- To test the efficacy of OX1R blocker, SB-334867 as a protective agent against effects of SD on body weight and blood glucose level.

Materials and methods

Animals:

A total of 72 adult male albino rats, aged 10-12 weeks and weighting 200-300 grams obtained from Animal Facility, faculty of science, Sohag University. Rats were housed in Animal Facility, Sohag Faculty of Medicine under a 12 h light/dark cycle (light on at 6:00 am) in the period from 9th of October 2016 to 16th of October 2016. Animals were provided ad libitum & water throughout the study. Experiments were approved by the Ethical Committee of Animal Experiments, Sohag faculty of medicine.

Experimental groups

Animals were randomly divided into four equal groups (n=18):

Group I (G I-the control group):

In this group, each three rats were placed inside a cage (50 x 45 x 30 cm) that contain 7 rectangular platforms specified for standing of rats and filled with sawdust to a level 1 cm below the upper surface of the 7 rectangular plateforms. Rats in this group were allowed to sleep normally. This group was i.p. injected with 2 ml of saline once daily throughout the 8 days of the experiment.

Group II (G II- the sleep deprived group):

In this group, rats were subjected to paradoxical SD for 8 successive days using a modified multiple platform method ***(Brianza-Padilla et al., 2015)***. Each three rats were placed inside a similar cages as G I but water was added to the bottom of the cage instead of the sawdust. The upper surfaces of the platforms were kept 1 cm above the surface of the water. Thus, the rats could move around inside the cage by jumping from one platform to another. Upon reaching the paradoxical phase of sleep, rats experience muscle atonia, which leads them to make contact with, or fall into, the water. At that point, they awaken abruptly and repeat the sleep–wake cycle. This group was injected with 2 ml saline i.p. once daily for 8 successive days as GI.

Group III (G III - the sleep deprived-OX1R blocked group, SD-OX1R blocked group):

In this group, rats were subjected to paradoxical SD as G II but with i.p. injection of SB-334867 (OX1R blocker) dissolved in 2 ml DMSO and diluted 1:1000 in saline in a dose of 3 mg/kg/day once daily for 8 successive days.

Group IV (G IV - the sleep deprived-DMSO group, SD-DMSO group):

In this group, rats were subjected to paradoxical SD as G II but with i.p. injection of 2 ml of DMSO diluted 1:1000 in saline i.p. once daily for 8 successive days.

All rats received daily i.p. injection of saline 4 days before the first day of the experiment to adapt them with the injection procedure.

The food and body weight of rats were weighted using sensitive scale (Denver Instrument

Company, serial No. 0065380. USA). The food consumption was calculated as described by ***(Brianza-Padilla et al., 2015)*** by subtracting the remaining food from the amount of food added and divided on the number of rats and expressed as amount of food intake /day/rat. The amount of spillage of food was considered equal in all animal groups.

Table (1): Time schedule details during the 8 days of the experiment.

Time	Procedure
10 a.m.	Assessment of food consumption by weighting the food remaining in the cages. Removal of food and starting the fasting.
12 p.m.	Removal of water at the bottom of the cages of G II, G III & G IV and drying the cages allowing rats to sleep freely.
4 p.m.	Collection of blood samples for measuring of fasting glucose, fasting insulin levels and determination of insulin resistence. Weighting the Animals. Adding the food after weighting it. Start of SD by filling the cages with water to a level of 1 cm below the upper surfaces of the rectangular platforms in all groups except G I. Injection of rats in G I & G II with saline, rats of G III with OX1R blocker i.p. & rats of G IV with DMSO i.p.

Rats body weight was determined as described by (***She et al., 2014***) a large plastic bowl was placed on the weighting scales, the scales were turned back to 0, the rat was placed gently in the bowl and the weight was recorded.

A blood sample was taken daily at 4:00 p.m. from the lateral tail vein of each rat using 3 ml syringe. The samples were collected in EDTA (20 μL / ml blood) containing tubes. Blood was centrifuged and plasma was separated and stored at −20°C until the time of biochemical analysis as described by ***(Brianza-Padilla et al., 2015)***

The fasting Level of insulin was measured from these blood samples using Insulin ELISA kits (EIA-2935, DRG International, Inc. USA).

Assessment of fasting blood glucose level:

As described by ***(Atal et al., 2016)***; a drop of blood was obtained by tail clip method. Approximately 2 mm of terminal end of rat tail was amputated at the 1st day of experiment then the other samples were obtained by disrupting the scab or clot of the original cut at the end of the tail. A 0.6 µl of blood was aspirated by blood glucose test strip and measured using Safe-Accu blood glucose meter (Changsha Sinocare Inc., China).

Assessment of insulin resistance:

The presence or absence of insulin resistence/sensitivity was assessed using the homeostatic model assessment (HOMA) described by ***(Matthews et al., 1985).*** HOMA-IR is a valid measure to determine insulin-resistance in Wistar rats ***(Antunes et al., 2016)***.

$$HOMA-IR = \frac{\text{Fasting blood glucose } (mg/dl) \times \text{ fasting insulin}(\mu IU/ml)}{405}$$

Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate low insulin sensitivity (insulin resistance) ***(Bonora et al., 2002)***.

Statistical analysis

In this work statistics were done by using prism program, version 7.

Data expressed at mean ± SE (standard error).

Student t-test was used to determine significance between numeric data between two groups.

ANOVA test was used to determine significance between numeric data of different groups.

Probability value (*P*-value) was considered Significant if P < 0.05.

Results

Body weight and Food intake:

Body weight loss was observed during the 8 days of paradoxical SD in all groups subjected to SD. This occurs despite the increase in food consumption except the group which received the OX1R blocker that showed food consumption nearly equal to the control group.

In sleep deprived group (G II) & SD-DMSO group (GIV), body weight showed gradual drop that became statistically significantly at day 8 in G II and at days 7 & 8 in G IV when compared with the control group (P-value = 0.0022 in G II, 0.0280 at day 7 & 0.0005 at day 8 in G IV). This occurs despite the significant increase of food consumption (P-value = 0.0008 in G II & G IV) when compared with the control group.

In the SD-OX1R blocked group (G III), showed gradual drop in body weight during the experiment that became statistically significantly different from those of the control group starting from day 5 and continued to day 8 (P-value = 0.0085 at day 5 & <0.0001 at day 8). There was statistically non-significant difference in body weight when compared with G II. The food consumption was statistically non-significant when compared with G I (P-value = 0.44). While, it was a statistically significant lower when compared with G II (P-value = 0.0032).

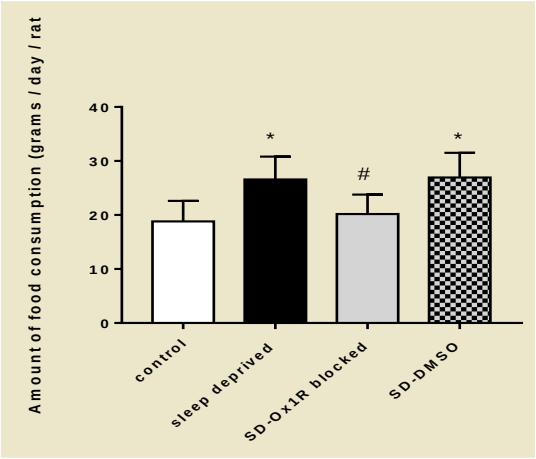

Figure (1): Comparison between mean values of food consumption in different groups throughout the experiment.

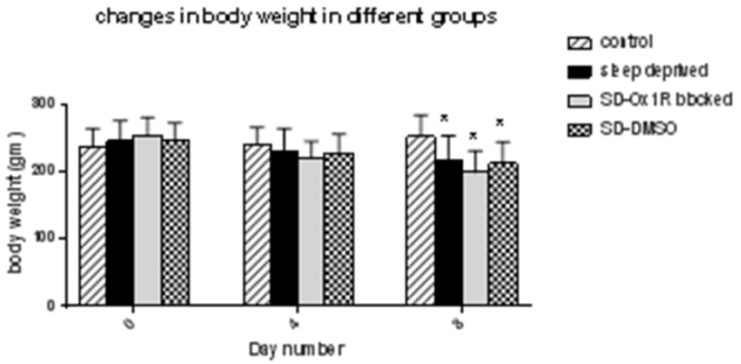

Figure (2): comparison between body weights in different groups at days 0, 4 and 8.

Fasting blood glucose and insulin levels:

In the sleep deprived group (G II) & the SD-DMSO group (G IV), the fasting blood glucose level showed a statistically significant lower values beginning at day 1 (P-value = 0.0002 in G II & 0.0073 in G IV) and continued to day 8 (P-value < 0.0001 in both groups) when compared with the control group. There was a statistically significant drop in the fasting blood insulin level beginning at day 2 (P-value = 0.0047 in G II & < 0.0001 in G IV) and continued to day 8 (P-value < 0.0001 in both groups) when compared with the control group.

In the SD-OX1R blocked group (G III), there was a statistically significant lower fasting blood glucose level beginning at day 6 (P-value = 0.0091) and continued to day 8 (P-value = 0.0002) when compared with the control group. While,

when compared with G II there was a statistically significant higher values in G III than those of G II beginning at day 1 (P-value = 0.0005) and continued to day 8 (P-value = 0.0052). The fasting blood insulin level showed statistically non-significant change during the experiment when compared with the control group but when compared with G II there was a statistically significant higher values in G III than those of G II beginning at day 3 (P-value = 0.0005) and continued to day 8 (P-value < 0.0001).

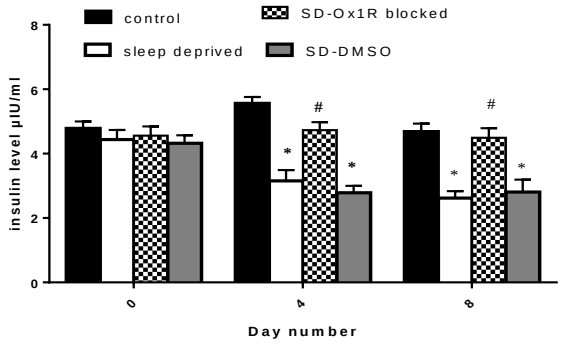

Figure (3): comparison between fasting blood insulin level in different groups at days 0, 4 and 8.

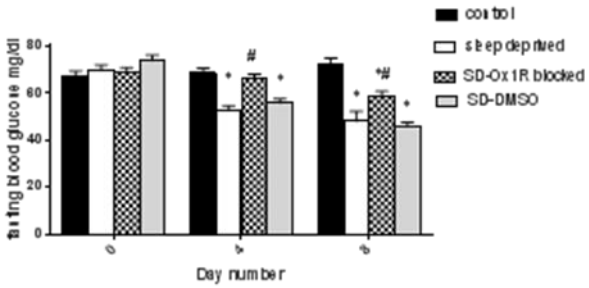

Figure (4): comparison between fasting blood glucose level in different groups at days 0, 4 and 8.

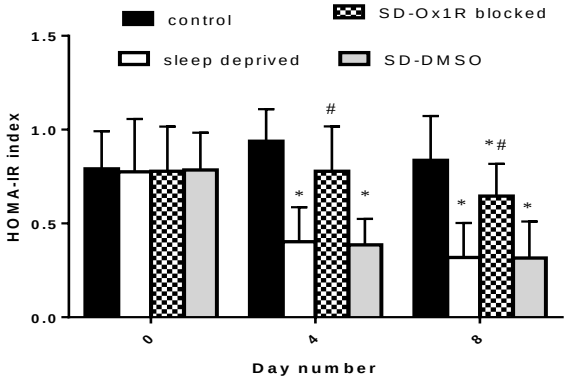

Figure (5): comparison between HOMA-IR index in different groups at days 0, 4 and 8.

Insulin resistance:

In the sleep deprived group (G II) & the SD-DMSO group (G IV), the HOMA-IR index showed a statistically significant lower values when compared with the control group reflecting an increase in insulin sensitivity in G II & G IV beginning at day 1 (P-value = 0.0033 in G II & 0.0315 in G IV) and continued to day 8 (P-value < 0.0001 in both groups).

In the SD-OX1R blocked group (G III), the HOMA-IR index showed a statistically significant lower values when compared with the control group reflecting an increase in insulin sensitivity in G III beginning at day 6 (P-value = 0.0123) and continued to day 8 (P-value = 0.0182). By comparing HOMA-IR index in G III and G II there was a statistically significant higher HOMA-IR index of G III than those of G II beginning at day 1

(P-value = 0.0234) and continued to day 8 (P-value < 0.0001).

Discussion:

Our modern society suffers new kinds of stressors; one of them is the sleep deprivation which has negative effects on different body functions including male health. Nevertheless, the role of orexin in health hazards of SD is not yet evident and the mechanisms by which orexin involved in these hazards and the protective effect of orexin receptor blockers are still debated ***(Tsuneki et al., 2017)***.

To accomplish the goals, an animal model of SD was established and the experiment is formed of control group, sleep deprived group, SD-OX1R blocked group & SD-DMSO group. In this study, Food consumption as well as body weight, fasting blood glucose & insulin levels were measured and the results were recorded in all groups.

Body weight loss was observed during the 8 days of paradoxical SD in all groups subjected to SD. This occurs despite the increase in food consumption except the group which received the OX1R blocker that showed more drop in body weight than the other groups with food consumption nearly equal to the control group.

The loss of body weight can be explained by high metabolic rate and thermogenesis ***(Vetrivelan et al., 2012)*** or by decrease in anabolic hormones as GH & testosterone and increased levels of catabolic hormones as cortisol and noradrenaline (***Brianza-Padilla et al., 2015)***. The increase in food intake can be explained by rise in orexin-A during periods of SD which increases the food intake and this supported by using OX1R blocker in G III in present study in which there was more or less ordinary food consumption. It can be also explained by reduction of leptin secretion or rise in cortisol secretion ***(Koren et al., 2015).*** The more marked drop in body weight in SD-OX1R blocked group may be due to combined effect of increased metabolic rate by SD without increase of food intake by the OX1R blocker.

This is agreed with ***Brianza-Padilla et al., 2015*** who reported increase food consumption during period of SD and weight loss by 20% in sleep deprived group. Also results reported by ***(Mônico-Neto et al., 2015)*** who reported increase in food intake and weight loss in rats subjected to SD using the modified multiple platform method for 96 hours which was high during the first 24 hours of SD, with less weight loss after that period. This is also agreed with ***(Koban et al., 2008)*** that used liquid foods and the effect of food spillage was avoided and reported also an increase in food consumption. Also ***Barf et al., 2010*** who placed the rats on a slowly rotating drum reported no change in food consumption but there was loss of body weight noticed starting from the 2nd day of SD. The different method used for induction of SD may affect the feeding pattern of rats. Most of studies that used less than 5 days of SD didn't find significant change in food consumption ***(Brianza-Padilla et al., 2015)*** but when SD extended beyond 6 days, increased food consumption is readily observed ***(Koban et al., 2008).***

The results of blocking OX1R in present study was agreed with results reported by ***(Li et al., 2016)*** who reported that microinjection of orexin into the arcuate nucleus increases food intake and blocking the OX1R reduces the food intake. also results reported by ***(Piccoli et al., 2012)*** in an animal model of binge eating, they reported that blocking OX1R selectively reduces

binge eating for highly palatable food, which suggests OX1R blockers may be used as a pharmacological treatment for compulsive eating disorders.

These effects contradict with results of ***Vetrivelan et al., 2012*** who reported no change in food intake and weight gain continued but in decelerated manner this can be explained by the prolonged period of SD which lasts for 2 months that may allow some compensatory changes and homeostatic regulation beside the different way of induction of SD that was by surgical lesion of the sleep center in hypothalamus VLPO

Some studies reported an increase in body weight after SD whether in humans or in rats. ***(Parrish and Teske, 2017)*** reported that rats gained more weight during acute SD induced by noise exposure that can be explained by the different method used for induction of SD. In humans, chronic partial SD has been associated with an increased risk for obesity. This apparent different response may be due to a species difference. Several additional factors may contribute as in human SD is almost voluntary ***(Vetrivelan et al., 2012).***

The current study showed an increase in insulin sensitivity after the 1st day of SD that is delayed by the use of OX1R blocker to the 6th day. This increase in insulin sensitivity can be explained by weight loss that noticed in these groups or by the high orexin-A level in G II & G IV during periods of SD which increase glucose uptake and promote insulin induced glucose uptake and glycogen synthesis in skeletal muscle ***(Shiuchi et al., 2009***). High orexin levels also leads to increased physical activity and increased non-exercise thermogenesis and, consequently, glucose utilization ***(Barf and Scheurink, 2011)***.

The finding of effects of SD on fasting insulin and glucose are agreed by findings reported by to (***Brianza-Padilla et al., 2015)*** who reported a significant decrease in insulin level after 24 h of Paradoxical SD and this drop in insulin level continued to the 8 days after SD and a drop in fasting blood glucose level which is noticed after 4 days of paradoxical SD. ***(Barf et al., 2010)*** also reported a decrease in insulin level after 8 days of SD for 20 hours daily by placing them on slowly rotating drums or by sleep restriction for 10 hours daily. Also, according to ***(Barf and Scheurink, 2011)*** who reported lower fasting glucose and lower insulin level in sleep deprived rats.

Findings related to role of orexin in regulation of glucose and insulin levels are agreed by ***(Skrzypski et al., 2016)*** who reported that inhibition of orexin production or orexin receptors deficiency may contribute to obesity as well as insulin resistance in rodents and humans. ***(Funato et al., 2009)*** reported that orexin overexpression protects against insulin resistance.

Results of the current study contradicts with results reported by ***(Zou et al., 2017)*** who reported higher blood glucose level and an increase in insulin resistance in sleep deprived rats by exposure to environmental noise. Also results reported by ***Vetrivelan et al., 2012*** who reported a drop in fasting blood glucose level but with no change in insulin level after sleep deprivation by damaging the VLPO nucleus in hypothalamus. ***She et al., 2014*** reported that fasting insulin level of sleep restricted group was higher than control group after SD by placing them in slowly rotating cages 20 hours daily for 8 days. This can be

explained by the different method used for induction of SD.

In conclusion: SD even leads to increase in food intake however leads to loss of weight and drop in fasting blood glucose level and increase in insulin sensitivity. This increase in food intake may lead to - in some individuals when the food is available - increased calories intake and predispose to obesity and its adverse effects on health. The drop in blood glucose level and the increase in insulin sensitivity can affect negatively the performance of night shift workers. OX1R blockers delays the SD induced increase of food intake, hypoglycaemia and the increase of insulin sensitivity.

Recommendation: OX1R blockers prevents the increase of food intake and help reduction of body weight so it is ideal in obese persons who subjected daily to SD due to night shifts to decrease the excess calorie intake and decrease risk of obesity or even help to get rid of overweight. Even if the food isn't available, OX1R blockers can give a sensation of satisfaction and decrease the desire for food intake without bad effects of hypoglycemia so the night shift worker can continue their work efficiently. Further investigations are needed to explore the effect of OX1R blockers on metabolic state.

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