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The circadian and non-circadian effects of vasoactive intestinal peptide on energy use and immune function in C57B mice

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Abstract

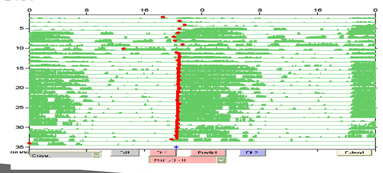
A circadian rhythm is a biological process that has an endogenous rhythm, normally entrained to the 24-hour light dark cycle. Vasoactive intestinal peptide (VIP) has both circadian and non-circadian physiological effects. Using two strains of C57B mice (*Mus musculus*): a transgenic mutant with nonfunctioning VIP, and wildtype strain, we examined the effects of VIP on immune function and energy expenditure. We examined two important measures of energy expenditure: basal metabolic rate (BMR) and daily food intake (FI) and two important measures of immune function: white blood cell (WBC) count and bacterial killing assay in VIP -/- mice, at two different circadian conditions (entrained and free-running) as compared to wildtype controls. Entrained VIP -/- mice had a significantly higher BMR than wildtype mice. The BMR of entrained VIP -/- mice significantly decreased after experiencing the free-running condition. However, after experiencing the free-running condition, VIP -/- mice still had a significantly higher BMR than the wildtype mice. FI differed between the wildtype and entrained VIP-/- mice. However, after experiencing the free-running condition, the difference became more complex. VIP -/- mice had significantly lower total WBC counts as compared to the wildtypes, regardless of circadian condition. Lymphocyte and neutrophil counts did not differ between the entrained VIP -/- and wildtype mice. However, after free-running, VIP-/- mice had higher neutrophil counts and lower lymphocyte counts than the WT mice. VIP KO mice may be experiencing a more disruptive circadian condition when free-running than when entrained, which could be contributing to the reduction in metabolism.

Introduction

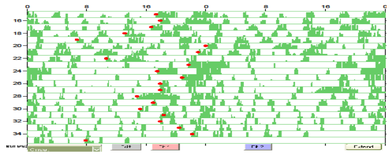
Circadian rhythms are 24 hour periods entrained by environmental cues. In mammals, the SCN is the master circadian pacemaker (or clock) using the 24-hour light/dark cycle as the principal entraining cue. SCN regulates sleep/wake patterns, feeding/fasting behavior, and body temperature rhythms, and SCN also influences immune function, the cardiovascular system and metabolism.
The SCN synchronizes rhythms and behaviors of peripheral tissues to match its own rhythm. Many peripheral tissues such as blood leukocytes, heart, lung, adipose, liver and pancreatic tissues maintain circadian oscillations of their own and are synchronized by the SCN. Without a functioning SCN, peripheral tissues and metabolic processes continue to cycle, but become out of phase with each other and with the external light/dark (LD) cycle. This internal misalignment or circadian desynchronization is likely to have an effect on metabolic processes, including energy expenditure, which could affect immune function.
Our study uses a strain of mutant mice that are transgenic with nonfunctioning vasoactive intestinal peptide (VIP-/-). These mutant mice lack a functional master clock in the brain because the lack of VIP causes a neural disruption within the SCN. VIP plays a key role in communication between individual brain cells. Without VIP, the SCN cannot provide strong, coherent, and rhythmic output signals to tissues of the body. When kept on a standard light/dark cycle (LD, 12:12) (entrained condition), the activity rhythms of VIP-/- mice are similar to those of controls, but in constant conditions (DD, 24) (free-running condition), VIP-/- are arrhythmic or express unstable short periods.
Our study investigated the negative effect of circadian disruption, due to the disturbance of central neural coordination in the SCN, on normal metabolism and immune function in the mammalian system, using a mouse model system. Basal metabolic rate is an estimate of the energy expenditure rate required to maintain minimal tissue and vital organ function. We hypothesized that circadian organization orchestrated by the master clock confers metabolic efficiency to the organism as a whole and that a loss of circadian organization would result in less efficient use of energy resulting in a higher basal metabolic rate in VIP -/- mice as compared to wild type mice. To test this hypothesis, we compared the basal metabolic rate of wild type mice and VIP-/- mice under both entrained and free-running conditions. Additionally, if metabolic rate was higher in VIP -/- mice than in wild type mice, we predict that immune function could be reduced in the VIP -/- mice because less energy is available.

Activity Records

The following is an example of the activity pattern of C57B wildtype mouse when exposed to 24D.



The following is an example of the activity pattern of a per 2:: luc VIP -/- mouse when exposed to 24D (circadian disrupted or arrhythmic).

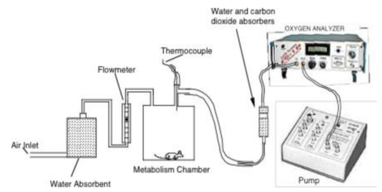


Methods

Experimental Design: Two strains of C57B laboratory mice (*Mus musculus)* were used: a common lab wildtype strain (WT) and a transgenic mutant strain, which lacks functional vasoactive intestinal peptide (VIP -/-). The two strains of mice first experienced normal light conditions (LD, 12:12) for about two weeks. Then, the two strains of mice experienced constant 24 hour darkness with dim red light (DD, 24) (free-running) for two weeks.

Statistical Analyses: Analysis of variance (ANOVA), analysis of covariance (ANCOVA) and repeated measures analyses were performed. Main factors tested: genotype (WT vs. VIP -/-) and circadian cycle (LD vs. DD). The test of effect of circadian cycle is a repeated measures test performed on the same mice. All statistics judged significant at $p < 0.05$.

Respirometry: Basal metabolic rate (BMR) is the rate of metabolism in resting, post absorptive individuals at thermoneutrality. Mice were fasted the previous night and placed in airtight containers at 30°C. During respirometry, mice were kept in a positive pressure, open circuit system, with dried CO_2, free excurrent air then subsampled by a Sable Systems PA-1 paramagnetic oxygen analyzer. Using respirometry (indirect calorimetry), dried incurrent airflow rate and incurrent and excurrent oxygen concentrations were recorded as part of measuring oxygen consumption rate in order to estimate BMR. The lowest oxygen consumption rate obtained in the 3-4 hour testing period was defined as BMR.



Food Intake: The amount of food (in grams per day) that a mouse ate was measured twice a week for all mice. The amount of uneaten food left in the cage was collected and massed. The change in food mass was then calculated and the average daily intake was determined for each animal.

White Blood Cell Counts: Whole blood was collected from each mouse, and mixed with crystal violet (cv) solution and heparin, to create a 1:20 solution of blood to cv. All white blood cells were counted with a Hematocytometer to generate an estimate of total white blood cells per milliliter of whole blood. Additionally blood smears were made on glass slides mixed with methanol and stained with the Hema-3 staining system (similar to a Wright-Giemsa stain). The number of neutrophils, lymphocytes and monocytes were counted for every 100 white blood cells detected. To test for accuracy, each sample was counted by two different counters.

Results

BMR: At LD, after accounting for body mass, the VIP -/- mice had a significantly higher BMR than WT mice ($F_{(1, 12)} = 7.35$; $p=0.019$). After accounting for body mass, the VIP -/- mice had a significantly higher BMR at LD than at DD ($F_{(1, 6)} = 6.64$; $p =0.042$). At DD, after accounting for body mass, the VIP -/- mice had a significantly higher BMR than the WT mice ($F_{(1, 39)} = 13.652$; $p = 0.001$). BMR did not significantly differ between LD and DD in WT mice.

Food Intake: At LD, WT mice food intake is much higher than VIP -/-. At 24D, FI in WT is still higher than FI in VIP -/-, however the difference has decreased.

WBC: At LD, the WT mice had a significantly higher total WBC count than the VIP -/- mice ($F_{(1, 16)} = 14.31$; $p = 0.002$). At DD, the WT mice had a significantly higher total WBC count than the VIP -/- mice ($F_{(1, 19)} = 11.05$; $p = 0.004$). Total WBC count did not significantly differ between LD and DD in WT and VIP -/- mice.

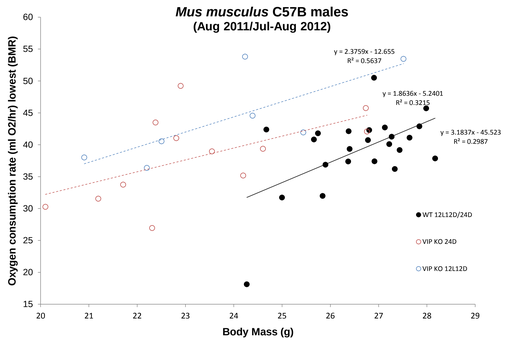


Figure 1. The three lines in this graph represent the oxygen consumption rate (BMR) plotted against body mass in WT and VIP KO -/- at 12L12D and 24D.

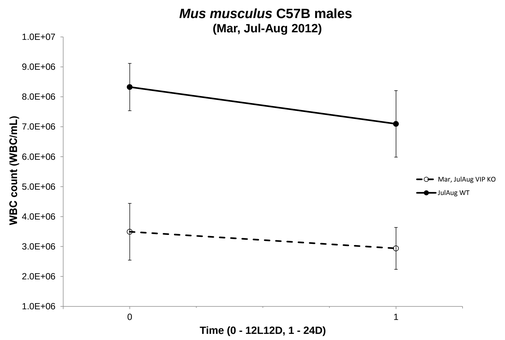


Figure 2. The mean ± SE of total white blood cell count (WBC/mL) of WT and VIP -/- mice at 12L12D and 24D.

N/L Counts: At LD and DD, WT had higher lymphocyte and lower neutrophil counts than VIP-/-. No difference was found in lymphocyte and neutrophil numbers from LD to DD for WT. However, the VIP experienced a decrease in lymphocytes and an increase in neutrophils when moved from LD to DD

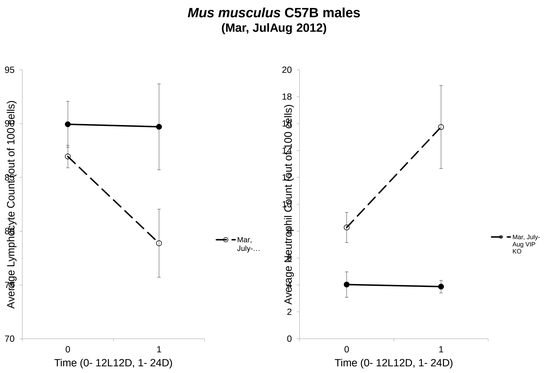


Figure 3. The mean ± SE of Neutrophil and Lymphocyte cell counts (each out of 100 cells) of WT and VIP -/- mice at 12L12D and 24D.

Conclusion

We measured basal metabolic rate (BMR), food intake (FI) and white blood cell counts in both VIP -/- and normal wild type (WT) mice during two kinds of circadian rhythm states. After exposure to entrained condition, VIP -/- mice had a higher BMR compared to WT mice. After exposure to free-running condition, the BMR of the VIP -/- mice was less than when they were entrained but still higher than the WT mice. Our results provide evidence to support the prediction that circadian disruption of a kind should lead to increased metabolic rate due to inefficiency of metabolism in the VIP-/- mouse. When animals experience circadian disruption due to lack of SCN coordination of peripheral body clocks, more energy may be spent to compensate for a lack of cell clock coordination.
Inefficiency requires a higher BMR of the VIP -/- mice as compared to WT mice in order to meet the minimal energy costs required to maintain basic cellular and physiological function. For example, for every "$1" of energy the WT mouse "spends" on maintenance costs, the VIP mutant has to "spend" "$1.10".
Entrained VIP -/- mice are commonly active a short time before lights off as compared to WT mice. VIP -/- mice might as a result eat more during the light period than would WT mice. Forcing the VIP -/- mice to eat at a certain time of day (because of entrainment) may be part of the inefficiency. Inefficiency might result from peripheral clocks in tissues and organs entrained to an abnormal feeding time while the SCN remains entrained to the light/dark cycle. The resulting abnormal phase relationships in the VIP-/- mice might contribute to metabolic inefficiency. In constant (free-running) conditions, the VIP -/- mice feed according to their own variable activity patterns. Under free-running conditions, the SCN and peripheral clocks may be less likely to be forced into abnormal phase relationships.
A previous study compared molecular rhythms in peripheral tissues between WT and VIP-/- mice in both an LD cycle and constant DD conditions. In VIP mutant mice, the molecular rhythms were more disrupted under LD conditions than under DD conditions, suggesting that LD circadian conditions are more disruptive in the VIP-/- mice. Another study found under LD entrainment, short-period heterozygote hamsters experienced disruptive signals from the SCN which resulted in pervasive physiological impairments, which disrupted organ structure and function, producing cardiorenal disease and leading to reduced longevity. However, SCN lesions in the short-period heterozygote hamsters experiencing LD entrainment prevented or reversed circadian related cardiac hypertrophy. Thus, animals with genetically or surgically disrupted clocks appear to be less affected when not entrained. In general, misalignment has been related to physiological and neurological disorders.
The higher BMR but lower food intake in VIP -/- mice compared to WT mice suggests that the VIP -/- mice may have a limit to available energy. When energy is limited, trade-offs in energy use are likely, particularly if energy demands are also high. If more energy is expended due to a disrupted metabolic rhythm, then VIP -/- mice will have less energy to spend on immune function. Therefore, less energy is available to maintain adequate immune function, such total white blood cell levels.
Currently, the link between a normal circadian rhythm and health of an organism is the focus of active research. The interdisciplinary character (i.e., energetics and immunology) of this project only highlights the potential use in helping us understand the possible effects of circadian disruption in humans. In particular, by studying immune function and energy use possible interactions can be revealed that are useful in understanding the consequences of circadian disruption on mammals, including humans.

Acknowledgments

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References

Arble D, Ramsey K, Bass J, Turek F (2010). Circadian Disruption and Metabolic Disease: Findings from Animal Models. Clinical Endocrinal Metab. 24(5): 785-800.
Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, Besing RC, Menaker M, Gewirtz AT, Davidson AJ (2010). Dysregulation of Inflammatory Responses by Chronic Circadian Disruption. The Journal of Immunology. 185: 5796-5805.
Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Tam, J., Lelievre, V., Waschek, J. A. (2003). Disrupted circadian rhythms in VIP- and PHI-deficient mice. Am J Physiol Regul Integr Comp Physiol, 285(5), R939-949.
Davidson AJ, Sellix MT, Daniel J, Yamazaki S, Menaker M, Block GD (2006). Chronic jet lag increases mortality in aged mice. Curr Biol. 16: R914-R916.
Lochmiller, R. L., C. Deerenberg (2000). Trade-Offs in Evolutionary Immunology: Just What Is the Cost of Immunity? Oikos 88 (1): 87-98.
Loh, D. H., J. M. Dragich (2011). Effects of vasoactive intestinal peptide genotype on circadian gene expression in the suprachiasmatic nucleus and peripheral organs." J Biol Rhythms 26(3): 200-209.
Martino, T.A., Oudit, G.Y., Herzenburg, A.M., Tata, N., Koletar, M.M., Kabir, G.M., Belsham, D.D., Backx, P.H., Ralph, M.R., Sole, M.J. (2008). Circadian rhythm disorganization produces profound cardiovascular and renal disease in hamsters. Am J Physiol regul Integr Comp Physiol 294: R1675-R1683.