

Effect of brief constant darkness and illumination on mitochondrial respiratory control of the pineal gland, Harderian gland, spleen and thymus of adult rat

Efeitos da escuridão e da luminosidade breve e constante no controle da respiração mitocondrial das glândulas pineal, Harderiana, baço e timo

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ABSTRACT

Background and objectives: Constant environmental conditions can lead to changes in the synthesis of melatonin. *In vitro* studies have shown that this hormone modulates the efficiency of mitochondrial respiration. Therefore, this work examined whether the efficiency of mitochondrial respiration changes in rats that have been subjected to constant illumination or darkness for a short period. **Methods:** Rats were randomly distributed in three groups: Control, Constant Illumination (72 hours) and Constant Darkness (72 hours). Upon completion of treatment, rats were sacrificed and mitochondria from the pineal gland, Harderian gland, thymus and spleen were isolated. Subsequently, mitochondrial respiratory control was quantified from the removed tissues in the three experimental groups. **Results:** Our findings show that brief treatments of continued illumination or continued darkness had no significant effect on mitochondrial respiratory control in spleen, thymus or Harderian glands. In contrast, we observed a slight increase in mitochondrial respiratory control in the pineal gland of animals exposed to constant illumination. **Conclusions:** Our results suggest that brief treatment with continuous light or darkness does not have a significant effect on the efficiency of mitochondrial activity in spleen, thymus or Harderian gland. This is probably due to the endogenous circadian rhythms that tightly regulate mitochondrial enzymatic activity in these tissues.

Keywords: Mitochondria; Cell respiration; Pineal gland; Circadian rhythm; Disease models, animal; Rats, Wistar

RESUMO

Introdução e objetivos: Mudanças constantes do meio ambiente podem levar a alterações na síntese de melatonina. Estudos *in vitro* têm evidenciado que o hormônio melatonina atua na modulação da respiração mitocondrial. Este trabalho investigou se a eficiência de

tal respiração é alterada em ratos que foram submetidos à luminosidade ou à escuridão constante por um breve período. **Métodos:** Os ratos foram aleatoriamente distribuídos em três grupos: Controle, Luminosidade Constante (72 horas) e Escuridão Constante (72 horas). Ao término do tratamento, os ratos foram sacrificados e as mitocôndrias das glândulas pineais, Harderiana, timo e baço foram isoladas. Em seguida, o controle da respiração mitocondrial dos tecidos dos quais foram removidos foi quantificado nos três grupos. **Resultados:** Os resultados deste estudo mostraram que tratamentos breves utilizando luminosidade ou escuridão contínua não tiveram nenhum efeito significativo no controle da respiração mitocondrial das glândulas pineais, Harderiana, timo e baço. Em contrapartida, verificou-se um pequeno aumento de controle na respiração mitocondrial na glândula pineal de animais expostos à luminosidade constante. **Conclusões:** Os resultados deste estudo indicam que tratamento breve com luminosidade ou escuridão contínua não exerce efeito na eficiência da atividade mitocondrial das glândulas do baço, timo ou Harderiana, e que tal fato provavelmente se deve aos ritmos circadianos endógenos que exercem um controle rigoroso da atividade enzimática mitocondrial existente nesses tecidos.

Descritores: Mitocôndrias; Respiração celular; Glândula pineal; Ritmo circadiano; Modelos animais de doenças; Ratos Wistar

INTRODUCTION

Mammals possess a physiological system that coordinates all metabolic functions, operates as a pacemaker, is sensitive to light and that regulates circadian rhythms, seasonal cycles and neuroendocrine responses in many species, including humans. This system consists of the retina, the suprachiasmatic nucleus (SCN) and the pineal gland. The retina con-

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veys light information to the SCN through the retinohypothalamic projection, which connects to the pineal gland through an additional pathway ⁽¹⁾.

One of the consequences of the activation of these pathways by light is the circadian modulation of melatonin production by the pineal gland. Melatonin secretion is high at night and low during the day and can be suppressed by a short, continuous pulse of light ⁽²⁾. This has important metabolic consequences because melatonin influences the production of two hormones that play key roles in lipid and carbohydrate metabolism: insulin and cortisol ⁽³⁾. Activity of the pineal gland has also been proposed to influence the endocrine, nervous and immune systems ⁽⁴⁾. *In vitro* studies have shown that melatonin increases the efficiency of oxidative phosphorylation in the liver and brain ⁽⁵⁾. Whereas some of the *in vitro* effects of melatonin on mitochondria have been well characterized, little is known about the *in vivo* consequences of light-regulated changes in melatonin levels. However, changes in the efficiency of mitochondrial respiration (or respiratory control) in the rat brain follow a circadian pattern ⁽⁵⁾.

This study examines the effect of a short period (72 hours) of continuous illumination or darkness on mitochondrial respiration in the pineal and Harderian glands, the spleen and the thymus of rats.

METHODS

Subjects and experimental treatments

Adult male Wistar rats (280–300 g) were used for this study. All animals were housed under controlled temperature (22±1 °C) and had free access to standard food (Purina) and water.

All animal experiments were approved by the ethical committee (Mexico) and were conformed to international guidelines on the ethical use of animals, according to the “NORMA Oficial Mexicana NOM-062-ZOO-1999”.

Rats were divided into three experimental groups, each containing 20 animals. The first group (Control) was housed under a normal 12-hour light/dark cycle, and the second and third groups were housed under either constant illumination or darkness for three days. Illumination for the Control and the second experimental group was provided by Vita-Lite fluorescent lights. At the end of the experimental period, rats were sacrificed by decapitation and the spleen, thymus, pineal and Harderian glands were removed immediately.

To prevent any circadian variability of mitochondrial metabolic activity, treatments for all groups were initiated at the same time (10h). All animals were sacrificed exactly 72 hours after the onset of treatment for harvesting of the tissues of interest.

Mitochondrial isolation

The removed glands and organs were homogenized in SHE medium (250 mM sucrose, 25 mM Hepes (pH 7.5), 1 mM EGTA) using a Potter-Elvehjem homogenizer ⁽⁶⁾. Mitochondrial proteins were quantified by Lowry's method ⁽⁷⁾.

Mitochondrial respiration

Oxygen uptake of mitochondrial suspensions was determined at 30°C using a Clark-type oxygen electrode (Hansatech, UK) in an air saturated media (0.5 ml) containing 125 mM KCl, 20 mM MOPS (pH 7.6), 0.1 mM EGTA, 5 mM KH₂PO₄, 2 mM MgCl₂ and 0.1 mg protein. The rate of mitochondrial respiration in state 3 was determined in the presence of 1 mM ADP and 3 mM succinate. The rate of respiration in state 4 (basal oxygen consumption) was determined in the presence of 3 mM succinate, without the addition of exogenous ADP or after endogenous ADP had been consumed by the mitochondria. The respiratory control index was calculated as the ratio between the rate of oxygen consumption in state 3 and that in state 4.

Statistical analysis

Data are shown as mean±SEM, being analyzed by one-way analysis of variance (ANOVA). The Student-Newman-Keuls test was used to compare the experimental groups with the Control, if appropriate. The level of statistical significance was set at p<0.05.

RESULTS AND DISCUSSION

Figure 1 shows the respiratory control in mitochondria from the spleen, thymus, pineal gland and Harderian gland of control rats and experimental rats exposed to either constant illumination or darkness for 72 hours. A significant, albeit

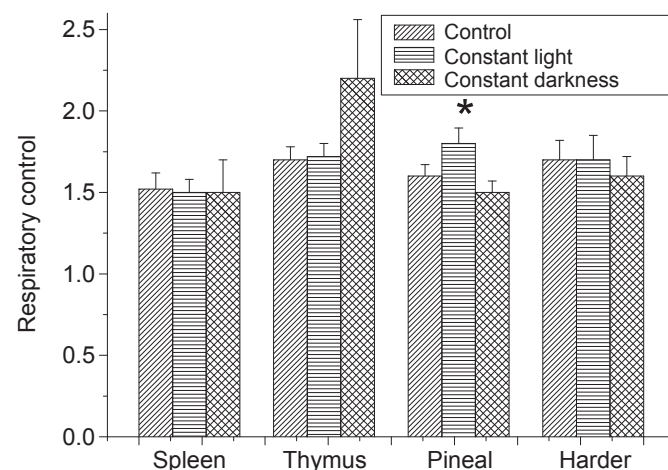


Figure 1: Respiratory control of the indicated organs and glands of rats subjected to brief treatment of continuous illumination or continuous darkness. Respiratory control was measured as indicated in Methods. Asterisks indicate a significant difference from the control group (p<0.05). The bar indicates the mean±SEM.

small, increase in the respiratory control of the pineal gland in the constant illumination group compared with the control and constant darkness groups was found. This means that under relatively brief, constant illumination, there is a slight increase in the efficiency of mitochondrial ATP synthesis. No significant differences were found in the spleen, thymus or Harderian gland for any of the treatments. In the thymus, a trend towards an increase in respiratory control under constant darkness was observed, but this tendency was not statistically significant.

It is well known that light is a dominant signal for entrainment of the circadian system and, in particular, mitochondrial metabolism⁽⁸⁻¹¹⁾. However, our results suggest that oxidative phosphorylation in the mitochondria remains fully functional when rats are subjected to brief (72 hours) constant darkness or illumination. In particular, we did not observe any differences in mitochondrial efficiency in the Harderian gland, thymus or spleen. The only difference observed was an increase in the efficiency of mitochondrial ATP synthesis in the pineal gland in the constant illumination condition.

These results were explained by the presence of endogenous circadian clocks. The free-running period of the rat is longer than 24 hours. Under brief constant environmental conditions (either darkness or illumination), the rat's endogenous circadian clocks can express their endogenous periodicity. Therefore, when rats are submitted to constant environmental conditions for a brief period, this normal rhythmicity is preserved.

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