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Short communication

Melatonin treatment does not prevent decreases in brain glutathione levels induced by sleep deprivation

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Abstract

Recent findings from this laboratory revealed that sleep deprivation reduces total glutathione (GSH) levels in hypothalamus, suggesting an increased vulnerability to oxidative damage. Since melatonin has been shown to prevent oxidative damage in other experimental situations, the present study tested the effects of exogenous melatonin on sleep deprivation-induced GSH decreases. Rats were deprived of sleep for 96 h on small platforms, and melatonin (10 mg/kg body weight; i.p.) or vehicle was given twice a day. Hypothalamic GSH levels were significantly reduced in sleep-deprived groups, irrespective of melatonin treatment. Indeed, unexpectedly, melatonin treatment resulted in lower hypothalamic GSH levels in all groups, including cage controls. These results confirm that sleep deprivation reduces hypothalamic GSH and further indicate that melatonin treatment not only is ineffective in reversing this effect but may actually potentiate it.

Keywords: Glutathione; Antioxidant; Sleep deprivation; Melatonin; Hypothalamus

1. Introduction

Glutathione (L-γ-glutamil-L-cysteinyl-glycine; GSH) is a tripeptide found in virtually all cells which, in addition to a variety of other functions (Cooper and Kristal, 1997), plays a central role in protecting cells against damage produced by free radicals (Halliwell and Gutteridge, 1989). Reductions in glutathione levels are commonly used as a measure of oxidative stress, defined as an imbalance in the normal equilibrium between formation of oxygen reactive species and antioxidant defense mechanisms. We have recently reported that 4 days of sleep deprivation lead to a reduction in total glutathione (GSH) levels in hypothalamus and thalamus (D'Almeida et al., 1998), which suggests that these specific brain regions could be more susceptible to oxidative stress during sleep deprivation. It has been hypothesized that sleep normally involves a process of detoxification at the cellular level (Inoué et al., 1995), and it is possible that sleep may serve an antioxidant function by removing free radicals or oxygen reactive species produced during waking (Reimund, 1994).

The pineal hormone melatonin has been reported to be an effective free radical scavenger and antioxidant. Melatonin is believed to scavenge the highly toxic hydroxyl radical, the peroxynitrite anion, and possibly the peroxyl radical; secondarily, it reportedly scavenges the superoxide anion (and it quenches singlet oxygen) (Reiter, 1998). The neurotoxicity of a number of compounds like kainic acid (Floreani et al., 1997), haloperidol (Post et al., 1998), and the cellular toxicity of hydrogen peroxide (Baldwin and Barrett, 1998) are inhibited by melatonin administration. In some cases, the accompanying decrease in GSH levels are prevented by melatonin administration (Floreani et al., 1997; but see also Baldwin and Barrett, 1998). The ability of exogenous melatonin to counteract GSH decreases in live animal preparations is illustrated in a recent study where a single injection of kainic acid time-dependently decreased GSH levels in amygdala and hippocampus (Floreani et al., 1997). This reduction in GSH levels was counteracted by melatonin administration (2.5 mg/kg i.p.,

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four times) suggesting that melatonin prevents the neurotoxic effects of reactive oxygen species related to kainic acid by maintaining cellular GSH homeostasis (Floreani et al., 1997). These observations prompted us to investigate whether melatonin could prevent the hypothalamic GSH decreases that occur during sleep deprivation.

2. Materials and methods

2.1. Animals and sleep deprivation procedure

Male Wistar rats weighing approximately 300 g were housed at 21–23°C under a light regimen of 12:12 h (lights on at 7 a.m.) with food and water ad libitum. The animals were submitted to sleep deprivation using the classical platform technique (Mendelson et al., 1974). Animals in the target sleep deprivation group were placed on small platforms (6.5 cm diameter) in individual 23 × 23 × 35 cm containers filled with water up to 1 cm below the platform surface. In this condition, animals are aroused from sleep when the characteristic loss of muscle tone that accompanies paradoxical sleep causes the animal to fall off the platform. Another group of animals was placed on large (14 cm diameter) platforms in similar containers; large platforms in principle allow all phases of sleep to occur. Home cage control rats remained in groups of three in wire mesh cages. Sleep deprivation started at 9 a.m. of day 1 and ended with sacrifice at 9 a.m. of day 4.

2.2. Melatonin treatment

Two daily injections of melatonin (10 mg/kg each) or vehicle (2% Tween 80) were given i.p. at 9 a.m. and 2 p.m. during the 4 days of the experiment. A completely balanced design was used, with eight animals in each group: (1) Home cage + vehicle; (2) home cage + melatonin; (3) small platform + vehicle; (4) small platform + melatonin; (5) large platform + vehicle; and (6) large platform + melatonin. The last injection was given at 2 p.m. of day 3. After 96 h of sleep deprivation, all animals were killed by decapitation and brains were rapidly removed. The hypothalamus was dissected, frozen over dry ice and stored at −80°C until biochemical analyses were conducted.

2.3. Glutathione assays

For GSH assays, the hypothalamus was weighed, homogenized with $HClO_4$ — 0.5 M in a dilution of 1:50 (w:v), and centrifuged for 10 min at 3100 × *g*, at 4°C. The acid extracts were used for determination of GSH, using a method based on the formation of a chromophoric product resulting from the reaction of 5,5′-dithiobis-(2-nitrobenzoic acid) and reduced glutathione, followed at 412 nm (Tietze, 1969), using a DU-650 Beckmann spectrophotometer, against a reagent blank with no homogenates. Total GSH content was calculated using a standard with a known concentration of GSH. All chemical reagents were obtained from Sigma (St. Louis, MO). Comparisons among groups were performed by two-way analyses of variance, followed by *t*-tests.

3. Results

GSH levels after sleep deprivation and melatonin administration are shown in Fig. 1. A two-way analysis of variance revealed a significant sleep deprivation main effect ($F(2,30) = 3.2$; $p < 0.05$) and a significant melatonin main effect ($F(1,30) = 8.49$, $p < 0.007$), but no deprivation × melatonin interaction effect ($F(2,30) = 0.11$). Post-hoc *t*-test comparisons indicated that GSH levels in hypothalamus were significant decreased in vehicle-treated animals submitted to sleep deprivation when compared to either cage control or large platform rats ($p < 0.05$ in both cases). Vehicle-treated large platform rats did not differ from similarly treated cage control rats. As illustrated in Fig. 1, melatonin administration tended to reduce GSH levels in all groups irrespective of sleep deprivation condition, as otherwise indicated by the significant melatonin main effect in the analysis of variance. In the large platform group, the difference between vehicle-treated and melatonin-treated groups was statistically significant ($p < 0.05$).

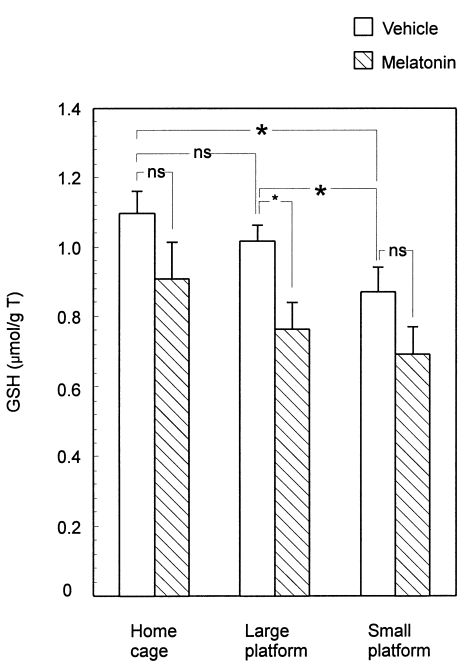

Fig. 1. Hypothalamic levels of GSH after sleep deprivation and melatonin treatment. Error bars represent standard errors of the mean. Melatonin injections (10 mg/kg) were given i.p. twice a day for the duration of the experiment. Asterisks indicate significant differences at the 0.05 level; ns = not significant.

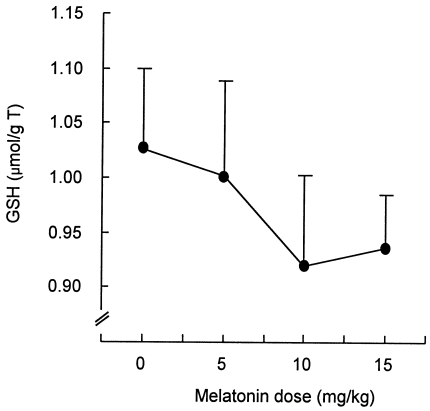

Fig. 2. Effects of different doses of melatonin on hypothalamic GSH levels in normal, non-sleep deprived animals. Each point represents the mean and standard error of eight animals. Each dose was given i.p. twice a day for 4 days, as in the sleep deprivation experiment.

In view of the apparent tendency of melatonin to reduce hypothalamic GSH levels, a separate experiment was conducted where the effects of different doses of melatonin on GSH levels where evaluated under normal sleep–wake cycle conditions. As in the sleep deprivation experiments, melatonin was given i.p. twice a day at 9 a.m. and 2 p.m. for 4 days and rats were sacrificed 19 h later. Results are shown in Fig. 2. Melatonin administration resulted in an apparent tendency towards decreased GSH levels in hypothalamus, but this trend was not statistically significant ($F(3,21) = 0.47,\ p > 0.7$).

4. Discussion

The current results in vehicle-treated rats confirm our recent findings of diminished GSH levels in hypothalamus after 96 h of sleep deprivation using the classical platform technique (D'Almeida et al., 1998). However, contrary to expectation, melatonin treatment did not prevent the sleep deprivation-induced GSH decrease in hypothalamus. Indeed, it appeared to exacerbate this decrease, an effect that may, at least in part, be due to an independent effect of melatonin on hypothalamic GSH.

The mechanisms involved in this unexpected effect are not clear, but other experimental situations have been recently described where neurotoxic effects have been exacerbated by exogenous melatonin treatment. Thus, Gibb et al. (1997) found that melatonin administered in doses of 5 to 25 mg/kg potentiated the neurotoxic effects of methamphetamine on brain tyrosine hydroxylase and tryptophan hydroxylase activity, as well as dopamine and serotonin levels in striatum, hippocampus and cortex. Interestingly, some (but not all) of the brain regional changes in 5-HT-related parameters were also observed when melatonin alone was administered (Gibb et al., 1997). Similarly, Willis and Armstrong (1999) found that slow, constant release of intracerebral melatonin resulted in a significant worsening of motor and feeding impairments induced by 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) in rat models of Parkinson's disease.

It is known that antioxidants can act as pro-oxidants as well as antioxidants, depending on dose and conditions (Li et al., 1995), and there have been suggestions that melatonin can, under certain conditions, act as a pro-oxidant (Marshall et al., 1996). In line with that possibility are observations that 21 days of systemic melatonin administration (5 mg/kg/day; i.p.) reduce GSH levels in striatum of rats (R. Frussa-Filho, personal communication).

It is conceivable that melatonin given at other points in time or via continuous infusion could have produced different results. Nevertheless, the present results indicate that under the dosing regime used here, the antioxidant properties of melatonin were not effective in preserving hypothalamic GSH levels after sleep deprivation. Further, the data suggest that, under these conditions, exogenous melatonin may itself lead to decreases in hypothalamic GSH and may add to the decreases induced by sleep deprivation in this brain region. There is growing interest in melatonin as a medication for insomnia and other sleep disorders. Since decreased GSH is an index of oxidative stress and possible neurotoxicity, our current observations suggest that perhaps caution should be exercised in using melatonin in conditions of sleep deprivation.

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