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CHALLENGES TO OREXINS IN THE MAINTENANCE OF HOMEOSTASIS – THEIR PARTICIPATION IN WAKE-SLEEP CYCLE AND MOTOR ACTIVITY

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Orexin A and B (hypocretins -1 and -2) are hypothalamic neuropeptides derived from a proteolytic process from the same 130-amino acid precursor prepro-orexins (de Lecea et al., 1998; Sakurai et al., 1998). Initially they were associated with feeding behavior and, subsequently, to sleep-wake regulation and the pathophysiology of narcolepsy (Lin et al., 1999). Their normal function is still not fully understood, although a number of studies have pointed to a role in energy homeostasis and in promoting or maintaining wakefulness, thus suggesting that orexin neurons may provide an integrative link between peripheral metabolism and central regulation of behaviors required for an adaptive response to homeostatic challenges (Sakurai, 2002).

We performed a series of experiments in order to evaluate the response of the orexinergic system in rats to several homeostatic challenges, i.e., sleep deprivation (Pedrazzoli et al., 2004; Martins et al., 2004), physical exercise, cold stress, and short term immobilization, among others (Martins et al., 2004). Since the orexin system presents circadian variation, some of our observations concern two different periods, ZT-0 (Zeitgeber 0 – hour that lights turn on), and ZT-8 (Zeitgeber 8 – four hours before lights turn off).

Orexin A levels in cerebrospinal fluid (CSF) were measured after 6 or 96 h of sleep deprivation by the platform technique and following 24 h of sleep rebound. Sleep deprivation was found to increase CSF orexin A collected at ZT-8 but not at ZT-0. These results indicate that sleep deprivation increases CSF orexin A. The degree of increase is also dependent on the length of the sleep deprivation. Orexin A levels were also found to decrease after sleep recovery. Interestingly, however, this effect was only evident at the end of the inactive period (ZT-8), at the time when orexin tone is normally low. At ZT-0, neither sleep deprivation nor 24-h sleep rebound significantly changed orexin levels.

When analyzing the effects of forced activity and homeostatic challenges on orexin A levels in CSF of rats, we found a significant decrease in these levels after long-term immobilization at ZT-0 and an increase after short-term forced swimming (Martins et al., 2004) during the light phase. Nevertheless, no effects were observed after short-term immobilization, total sleep deprivation or exposure to cold neither during the light phase (ZT-8) nor when challenges were performed at ZT-0, irrespective of duration. In conclusion, orexins neurons are activated by forced activity, orexin A levels increasing in rat CSF when activity was performed during the rest phase. In addition, these effects apparently are not stress-related mechanisms, since other stress procedures have no effect, and a decrease in CSF orexin A levels is observed after long-term immobilization at the end of the active phase of rats (Martins et al., 2004).

Concerning the effects of sleep deprivation and sleep recovery on the expression of orexin 1 (OX_1R) and orexin 2 (OX_2R) receptors throughout the brain, we studied three groups of rats: control group (CT group, which was allowed to sleep), 96 hr of sleep

deprivation (SD group) and sleep deprivation followed by 24 hr of sleep recovery (RB group) (D'Almeida et al., 2004). Relative to the CT group, prepro-orexin mRNA showed a non-significant increase in the SD group, but a pronounced and significant increase in the RB group (+88%, $p < 0.007$). Similarly, sleep deprivation produced no effects on OX_1 or OX_2 mRNA levels. However, in the RB group OX_1R mRNA levels increased significantly, compared to either control or SD groups, in 37 of 92 brain regions analyzed, with particularly strong effects in the amygdala and hypothalamus. Changes in OX_2R mRNA levels were also seen only in the sleep RB group, but they were fewer in number (10 out of 86 regions), tended towards the decreased rather than increased expression, and were predominantly confined to cerebral cortical areas. These observations indicate that while prolonged wakefulness does not affect mRNA levels of orexin nor its receptors, factors associated with sleep recovery, possibly the compensatory hypersomnia itself and/or increased motor activity, have strong effects on the orexin system at the mRNA expression level. They further indicate that these factors affect OX_1 and OX_2 receptors in opposite ways, and that the former are more vulnerable to these effects than the latter (D'Almeida et al., 2004).

Taken together our results suggest that orexinergic system activation is related to wakefulness and motor activity and this process is dependent on the period this activity occurs.

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