Physiological significance of serotonergic inhibitory input to orexin/hypocretin neurons on the regulation of sleep/wakefulness

Orexin/hypocretin is a neuropeptide produced in the specific type of neurons in the lateral hypothalamic area. Orexin/hypocretin-producing neurons (orexin neurons) are implicated in the maintenance of arousal. Previous studies reveal that serotonergic neurons in the raphe nucleus play an important role in the regulation of sleep/wakefulness. Serotonergic neurons in the raphe nucleus are densely innervated by orexin neurons. These serotonergic neurons express both orexin receptors, OX1R and OX2R, and are activated by orexin. On the other hand, orexin neurons are innervated by serotonergic neurons in the raphe nucleus, and are inhibited by serotonin through the serotonin 1A receptor (Htr1a). These results suggest that serotonergic inputs form a negative feedback circuit. However, its physiological role has not been completely understood.

To reveal this, *Htr1a* mRNA transcription is controlled using Tet-Off system in the orexin neurons. In situ hybridization confirmed that Htr1a mRNA expression is reversibly controlled in the orexin neurons by applying or removing doxycycline (DOX) from chow. To reveal the effect of *Htr1a* mRNA over expression in orexin neurons, orexin neurons were patch clamped using brain slice preparations. Serotonin hyperpolarized orexin neurons by opening G protein coupled inward rectifier potassium channel in the downstream of the Htr1a. Electrophysiological analysis of orexin neurons revealed that concentration-dependency of serotonin-induced hyperpolarization was not altered in the *Htr1a* over expressed mice. However, inhibitory effect of serotonin was approximately 2-hold prolonged in the orexin neurons in *Htr1a* over expressed mice compared with control mice. These results suggest that these mice allow us to study serotonergic inhibitory input to orexin neurons in vivo.

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