

Colocalization of orexin a and glutamate immunoreactivity in axon terminals in the tuberomammillary nucleus in rats

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Abstract

The orexins (also known as hypocretins) are peptide neurotransmitters made by hypothalamic neurons that are thought to play an important role in regulating wake–sleep states. One terminal area for orexin neurons is the tuberomammillary nucleus, a histaminergic cell group that is wake-active, but the relationship of the orexinergic terminals to the tuberomammillary neurons has not been examined in detail. We studied the ultrastructure of orexin A-immunoreactive axons and terminals in the tuberomammillary nucleus using pre- and post-embedding electron microscopic protocols. We confirmed an abundant projection of orexin-immunoreactive boutons to both dorsal and ventral divisions of the tuberomammillary nucleus. These terminals made asymmetric synaptic contacts with proximal and intermediate dendrites of tuberomammillary neurons. They contained small, clear synaptic vesicles and up to 30–40 dense core vesicles were seen per terminal in a single section. Both pre- and post-embedding immunostaining revealed that orexin immunoreactivity was localized to the dense core vesicles, which were always at a distance from the synaptic specialization. We also found glutamate immunoreactivity in the small synaptic vesicles which were at the active zone of the synapses of many of the same terminals. Orexinergic afferents to the tuberomammillary neurons contain separate populations of orexinergic and glutamatergic vesicles, suggesting that the release of these neurotransmitters may be differentially regulated.