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Orexin A induces bidirectional modulation of synaptic plasticity: Inhibiting long-term potentiation and preventing depotentiation

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ABSTRACT

The orexin system consists of two peptides, orexin A and B and two receptors, OX_1R and OX_2R. It is implicated in learning and memory regulation while controversy remains on its role in modulating hippocampal synaptic plasticity *in vivo* and *in vitro*. Here, we investigated effects of orexin A on two forms of synaptic plasticity, long-term potentiation (LTP) and depotentiation of field excitatory post-synaptic potentials (fEPSPs), at the Schaffer Collateral-CA1 synapse of mouse hippocampal slices. Orexin A (≧30 nM) attenuated LTP induced by theta burst stimulation (TBS) in a manner antagonized by an OX_1R (SB-334867), but not OX_2R (EMPA), antagonist. Conversely, at 1 pM, co-application of orexin A prevented the induction of depotentiation induced by low frequency stimulation (LFS), i.e. restoring LTP. This re-potentiation effect of sub-nanomolar orexin A occurred at LFS of 1 Hz, but not 2 Hz, and with LTP induced by either TBS or tetanic stimulation. It was significantly antagonized by SB-334867, EMPA and TCS-1102, selective OX_1R, OX_2R and dual OXR antagonists, respectively, and prevented by D609, SQ22536 and H89, inhibitors of phospholipase C (PLC), adenylyl cyclase (AC) and protein kinase A (PKA), respectively. LFS-induced depotentiation was antagonized by blockers of NMDA, A_1-adenosine and type 1/5 metabotropic glutamate (mGlu1/5) receptors, respectively. However, orexin A (1 pM) did not affect chemical-induced depotentiation by agonists of these receptors. These results suggest that orexin A bidirectionally modulates hippocampal CA1 synaptic plasticity, inhibiting LTP via OX_1Rs at moderate concentrations while inducing re-potentiation via OX_1Rs and OX_2Rs, possibly through PLC and AC-PKA signaling at sub-nanomolar concentrations.

1. Introduction

In the hippocampus, several forms of synaptic plasticity, including long-term potentiation (LTP), long term depression (LTD) and depotentiation, of excitatory synaptic transmission have been intensively studied. LTP can be induced by high frequency stimulation (HFS) with either tetanic stimulation (TS) or theta bust stimulation (TBS) protocols, and is believed to be a cellular correlate of learning and memory (Bliss and Collingridge, 1993). Conversely, depotentiation can be induced if low frequency stimulation (LFS) (Huang et al., 2001) or certain pharmacological interventions (Huang et al., 1999; Zho et al., 2002) are applied within minutes after HFS. Depotentiation is considered to be a kind of LTD (Collingridge et al., 2010) and usually can be induced in adult animals (Wagner and Alger, 1996), unlike LTD that was commonly induced at neonatal ages (Zhou and Poo, 2004). Depotentiation is considered to be a form of synaptic plasticity that might prevent

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saturation of synaptic potentiation and increases the flexibility and storage capacity of neuronal circuits (Huang et al., 2001).

Many neuropeptides have been reported to affect hippocampal synaptic plasticity and learning and memory tasks, including orexins. The orexin system consists of two neuropeptides, orexin A and orexin B (Sakurai et al., 1998), also named hypocretin 1 and hypocretin 2 (de Lecea et al., 1998), and a pair of G-protein coupled receptors, the OX_1 and OX_2 receptor (OX_1R and OX_2R) (Kukkonen and Leonard, 2014). Activation of both OX_1Rs and OX_2Rs, coupling to Gq, Gs or Gi/o (Kukkonen and Leonard, 2014), elevates intracellular Ca^{2+}, activates PLC (Lund et al., 2000) via Gq, or modulates protein kinase A (PKA) via regulation of adenylyl cyclase (AC) by Gs or Gi/o (Karteris et al., 2005). Orexin-containing neurons are distributed only in the perifornical area and lateral hypothalamus, but send projections widely throughout the brain (Peyron et al., 1998), including the hippocampus. The hippocampus is a brain region central to learning and memory and is enriched with OX_1Rs and OX_2Rs (Cluderay et al., 2002; Selbach et al., 2004; Trivedi et al., 1998).

The orexin system has been implicated in several normal physiological functions as well as pathological conditions (Leonard and Kukkonen, 2014), such as sleep, metabolic homeostasis, reward (Li et al., 2014) and pain (Chiou et al., 2010; Ho et al., 2011). However, the role of orexins in regulating learning and memory has been less well studied with conflicting results. When given by *i.c.v.* injection, orexin A (0.14–0.56 nmol) facilitated the performance of active and passive avoidance learning tasks in rats (Jaeger et al., 2002; Telegdy and Adamik, 2002). Conversely, Aou et al. (2003) showed that *i.c.v.* orexin A at higher doses (1.5–3 nmol) impaired spatial memory in rats performing the Morris water maze task. Microinjection of SB-334867, an OX_1R antagonist, into the hippocampal CA1 or dentate gyrus (DG) region impaired the performance of rats in passive avoidance (Akbari et al., 2008) and Morris water maze (Akbari et al., 2006, 2007) tests, suggesting endogenous orexins positively modulate the performance of learning tasks via OX_1Rs. However, Dietrich and Jenck, 2010 recently reported that almorexant, a dual orexin receptor antagonist, administered orally had no effect on spatial and avoidance learning in rats.

Two *in vivo* electrophysiological studies suggest that orexins positively regulate hippocampal synaptic plasticity. When injected into the hippocampal DG region, orexin A (0.3–0.9 nmol) enhanced electrical stimulation-induced LTP of population excitatory postsynaptic potentials (EPSPs) in a manner antagonized by SB-334867 in anesthetized rats (Wayner et al., 2004). Intra-DG injection of SB-334867 decreased LTP in freely moving rats (Akbari et al., 2011). In hippocampal slice studies *in vitro*, Selbach et al. (2004) also reported that orexin A *per se* induced LTP of EPSPs at hippocampal Schaffer collateral-CA1 synapses in mice. However, Aou et al. (2003) found that orexin A inhibited the LTP induced by HFS *in vitro* at the same synapse in rats.

The above conflicting findings could be attributed to the difference between species (rats *v.s.* mice), doses/concentrations of orexin A, hippocampal regions (CA1 *v.s.* DG) or approaches (*in vivo v.s. in vitro*) among studies. To clarify the role of orexins on synaptic plasticity in the same *in vitro* system, we examined effects of orexin A on two forms of synaptic plasticity, LTP and depotentiation, at the Schaffer collateral-CA1 synapse in adult mouse hippocampal slices and elucidated the OXR subtype(s) involved. Surprisingly, we found that orexin A exerted bidirectional effects on LTP and depotentiation; inhibiting LTP at the concentrations used in previous studies (30–300 nM) but preventing depotentiation, i.e. restoring LTP or inducing re-potentiation, at sub-nanomolar concentrations. The latter effect induced by physiological concentrations of orexin A may have significant functional roles in regulating learning and memory. We further characterized the induction conditions and possible underline mechanism(s) of this re-potentiation.

2. Materials and methods

All animal experiments were approved by the Institutional Animal Care and Use Committee of College of Medicine, National Taiwan University. All efforts were made to minimize the number of animals used.

2.1. Brain slices preparation

Hippocampal slices were dissected from adult male C57BL/6JNarl mice (8–12 weeks) as described previously (Hwang et al., 2010) with modifications. Briefly, after decapitation, coronal hippocampal slices (300 μm) were dissected and equilibrated in an artificial cerebral spinal fluid (aCSF) at room temperature for at least 1.5 h before recording. The aCSF consisted of (mM): NaCl 117, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11, and was oxygenated with 95% O_2/5% CO_2 (pH = 7.4).

2.2. Electrophysiological recordings

Extracellular electrophysiological recordings of field excitatory postsynaptic potentials (fEPSPs) at Schaffer collateral-CA1 synapses in mouse hippocampal slices were performed with an MED64 multichannel recording system (Alpha MED sciences Co., Ltd., Tokyo, Japan) with a data acquisition and analysis program (MED64 Conductor ver. 3.1, Alpha MED sciences Co., Ltd.) as described in a previous report (Oka et al., 1999). A hippocampal slice was gently placed on the center of a 0.1% polyethyleneimine-coated Multi-Electrode Dish probe (MED-P515A; Alpha MED sciences Co., Ltd.). Recordings were performed at room temperature with the MED probe, which was capped and connected with a perfusion system containing oxygenated fresh aCSF (95% O_2/5% CO_2, pH = 7.4) at a rate of 0.8–1.0 ml/min.

The fEPSPs were evoked by the stimulating point electrode, which was selected from one of 64 point electrodes based on its location on the Schaffer collateral fiber path in the CA1 region. The sharpest and largest fEPSP detected from one of the other 63 electrodes in the apical dendritic field (the stratum radiatum) of the CA1 region in each hippocampal slice was selected. The stimulation was given at 0.03 Hz by biphasic constant current pulses (0.1 ms) and the intensity (0.1–10 μA) was adjusted to evoke a fEPSP with an amplitude at 40–50% of the maximal amplitude. Signals were filtered through a 0.1–10 kHz band pass filter and sampled at 20 kHz. The slope of each fEPSP is calculated and displayed by the Conductor® software. Using this extracellular recording system, which is equipped with low-impedance planar multiple electrodes, both the noise level (~0.08 mV) and fEPSP signals (0.4–0.6 mV) are smaller than the signals recorded with the conventional extracellular recording system while retaining a high signal to noise ratio (Oka et al., 1999).

2.2.1. LTP

LTP of fEPSPs at Schaffer collateral-CA1 synapses of hippocampal slices was induced by HFS either via TBS that consists of 3 trains of ten bursts separated by 200 ms with each burst consisting of four pulses at 100 Hz (Hwang et al., 2010) or via TS at 100 Hz for 1 s. In each slice, fEPSPs were monitored for at least 30 min until stable fEPSPs were obtained. The average slope of fEPSPs recorded for 10 min before stimulation was taken as the baseline of fEPSP slope. The magnitude of LTP was measured by the average slope of 20 fEPSPs recorded 50–60 min after TBS or TS and expressed as % of baseline fEPSP slope.