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NMDA receptor subunit composition determines the polarity of leptin-induced synaptic plasticity

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ABSTRACT

Leptin is a hormone that crosses the blood-brain barrier and regulates numerous CNS functions. The hippocampus in particular is an important site for leptin action. Indeed, leptin markedly influences excitatory synaptic transmission and synaptic plasticity in this brain region. Recent studies indicate that leptin modulation of hippocampal excitatory synaptic transmission is age-dependent however the cellular basis for this is unclear. Here we show that early in development leptin evokes a transient (P11–18) or persistent (P5–8) depression of synaptic transmission, whereas leptin evokes a long lasting increase (LTP) in synaptic strength in adulthood. The synaptic depressions induced by leptin required activation of NMDA receptor GluN2B subunits and the ERK signalling cascade. Conversely, leptin-induced LTP in adult was mediated by GluN2A subunits and involved PI 3-kinase dependent signalling. In addition, low-frequency stimulus (LFS)-evoked LTD occluded the persistent effects of leptin at P5–8 and vice versa. Similarly, synaptically-induced LTP occluded the persistent increase in synaptic transmission induced by leptin, indicating that similar expression mechanisms underlie leptin-induced LTD and LFS-induced LTD at P5–8, and leptin-induced LTP and HFS-induced LTP in adult. These findings have important implications for the role of leptin in hippocampal synaptic function during early neuronal development and in aging.

1. Introduction

Synaptic plasticity is one of the most widely studied phenomena of the mammalian central nervous system (CNS). There is compelling evidence that activity-dependent forms of synaptic plasticity such as hippocampal long-term potentiation (LTP) and long-term depression (LTD) are cellular correlates for information storage within the CNS (Bliss and Collingridge, 1993). However, recent studies have focused on the modulation of synaptic strength by various endogenous hormonal systems, such as insulin (Huang et al., 2004; Man et al., 2000, 2003; van der Heide et al., 2005), estrogens (McEwen, 2002) and leptin (Harvey et al., 2006). Leptin is a 167 kDa protein that circulates in the plasma at concentrations relative to body fat. Extensive research has identified a role for leptin in regulating satiety and energy homeostasis via its hypothalamic actions (Spiegelman and Flier, 2001). However the effects of leptin are not restricted to the hypothalamus. Indeed, recent evidence indicates that leptin plays an important role in modulating many neuronal functions including hippocampal synaptic plasticity and glutamate receptor trafficking (Durakoglugil et al., 2005; Irving et al., 2006; Moult et al., 2010, 2009; Shanley et al., 2001). Moreover, it has been shown that leptin-insensitive rodents display impaired hippocampal LTP, LTD and spatial memory (Li et al., 2002), and administration of leptin into rodent hippocampus improves memory processing (Wayner et al., 2004). In addition, leptin has the ability to facilitate hippocampal LTP (Shanley et al., 2001) and promote the induction of a novel form of *de novo* hippocampal LTD (Durakoglugil et al., 2005). Recent evidence indicates that leptin promotes an increase in the synaptic expression of GluA2-lacking AMPA receptors in adult hippocampal slices resulting in a persistent increase in the efficacy of excitatory synaptic transmission (Moult et al., 2010). In contrast to its effects in adult, leptin evokes a transient depression of excitatory synaptic transmission in juvenile hippocampus (Shanley et al., 2001; Xu et al., 2008), suggesting that leptin modulates excitatory synaptic transmission in an age-dependent manner. In order to determine the cellular basis for this age-dependence, we have systematically examined the effects of leptin on excitatory synaptic transmission in hippocampal slices at four distinct ages. Here we show that the direction of synaptic modulation by leptin is age-dependent such that at early stages of postnatal development leptin results in either a transient (P11–18) or persistent (P5–8) depression of synaptic transmission, whereas in adult hippocampus (12–16 week and 12–14 month) leptin evokes a long lasting increase in excitatory synaptic strength.

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Although there are distinct age-dependent differences in the polarity and duration of synaptic modulation induced by leptin, the effects of leptin required NMDA receptor activation at all ages examined. Moreover, the ability of leptin to alter excitatory synaptic transmission displayed subunit-specific NMDA receptor dependence such that at early stages of postnatal development the effects of leptin required the activation of GluN2B NMDA subunits, whereas in adult hippocampus the leptin-driven persistent increase in synaptic strength was mediated by GluN2A subunits. In addition, divergent signalling pathways were found to mediate the effects of leptin at different ages. Thus, both the long lasting (P5–8) and transient (P11–18) synaptic depressions induced by leptin involved the activation of the ERK signalling cascade, whereas the leptin-driven persistent increase in synaptic strength in adult was mediated by a PI 3-kinase-dependent mechanism. Moreover, occlusion studies show that synaptically induced LTP occludes the persistent synaptic potentiation induced by leptin in adult hippocampus and vice versa, suggesting that HFS-induced LTP and leptin-induced LTP share similar expression mechanisms. Similarly, synaptically induced LTD occludes the persistent depression induced by leptin early in development (P5–8) and vice versa, indicating that analogous expression mechanisms also underlie leptin-induced LTD and LFS-induced LTD. These findings have important implications for the role of leptin in regulating hippocampal synaptic strength.

2. Materials and methods

Hippocampal slices (350 μm) were prepared from P5–8, P11–18, 12–16 week or 12–14 month old, male Sprague–Dawley rats. Animals were killed by cervical dislocation (P5–8, P11–18 and 12–16 week) or deep anaesthesia with isofluorane followed by decapitation (12–14 month) according to UK (Scientific Procedures Act 1986) legislation. Brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF; bubbled with 95% O_2 and 5% CO_2) containing (in mM): 124 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgSO_4$, and 10 D-glucose. Once prepared, parasagittal slices (350 μm) were allowed to recover at room temperature in oxygenated aCSF for at least 1 h before use. Slices were transferred to a submerged chamber maintained at 30–31 °C and perfused with aCSF containing the $GABA_A$ receptor antagonist, picrotoxin (50 μM), at a rate of 2–3 ml min^{-1}. Blind whole-cell patch clamp recordings from stratum pyramidale of area CA1 were obtained using electrodes (4–7 MΩ) containing (in mM): 130 Cs^+ methanesulphonate, 5 NaCl, 1 $CaCl_2$, 5 HEPES, 1 EGTA, 5 Mg-ATP, 0.5 Na-GTP, and 5 QX-314, pH 7.3. Cells were voltage clamped at −60 mV. The Schaffer collateral-commissural pathway was stimulated at 0.033 Hz, using a stimulus intensity that evoked peak EPSC amplitudes of ~50% of the maximum. Synaptic currents were low pass filtered at 2 kHz and digitally sampled at 10 kHz. The mean series resistance for all cells was 24 ± 4 MΩ ($n = 308$).

To isolate the NMDA receptor-mediated component of the EPSC, NBQX (5 μM) and picrotoxin (50 μM) were included in the bath solution and the cell was voltage clamped at −30 mV. The weighted decay time constant of NMDA receptor-mediated EPSCs was measured offline, from 2 min averages (4 trials per average). The weighted time constant of the synaptic current was calculated by fitting the decay of the synaptic current with 2 exponentials ($A1e^{-t/\tau1} + A2e^{-t/\tau2}$) as a bi-exponential curve provides a better fit than a single exponential. The value of the weighted time constant (τ_w) was calculated using the expression:

$$\tau_w = \tau_1 * A1/(A1 + A2) + \tau_2 * A2/(A1 + A2).$$

The values for τ_1 and τ_2 were consistent across all cells recorded within each age group.

The paired pulse ratio (PPR) was calculated as ratio of amplitude of second EPSC to first EPSC. To ensure measurement of second EPSC was not contaminated, the residual component of first EPSC was removed (Moult et al., 2010). The coefficient of variation (CV) was calculated as before (Moult et al., 2010). Briefly, the mean and standard deviation (SD) were calculated for EPSC amplitudes recorded during successive 5 min epochs (SD_{EPSC} and $mean_{EPSC}$). The SD of background noise was calculated for each 5 min epoch using a period immediately before electrical stimulation (SDnoise). The CV for each 5 min epoch was calculated as $(SD_{EPSC} - SD_{noise})/mean_{EPSC}$.

Using the same recording setup as above standard extracellular recording techniques were also used to monitor evoked field responses from stratum radiatum. The slope of the evoked field excitatory postsynaptic potentials (fEPSPs) was measured and expressed relative to the pre-conditioning baseline. Baseline responses were set to approximately 50% of the maximal response. Data were monitored online and analysed off-line using the WINltp program (Anderson and Collingridge, 2007). The degree of long term potentiation or depression was calculated 30–35 min after addition of leptin and expressed as a percentage of baseline ± SEM. In order to quantify the magnitude of transient effects, the peak effect over a 5 min period was calculated and expressed as a percentage of baseline ± SEM. All data are expressed as means ± SEM, and statistical analyses were performed using paired *t* test (two-tailed; 95% confidence interval) for comparison of means within subject or two-way ANOVA with Tukey *post hoc* test for comparisons between multiple groups. $P < 0.05$ was considered significant.

3. Results

3.1. Leptin modulates excitatory synaptic transmission in an age-dependent manner

We have recently shown that leptin results in a persistent increase in excitatory synaptic transmission in adult hippocampal slices (12–16 week old; Moult et al., 2010). In contrast, we and others have demonstrated that leptin transiently depresses excitatory synaptic transmission in juvenile hippocampus (Shanley et al., 2001; Xu et al., 2008). In order to determine the cellular basis for the bi-directional modulation of synaptic transmission by leptin, we examined the effects of leptin (25 nM) in hippocampal slices at four different ages; P5–8, P11–18, 12–16 week and 12–14 month old (Fig. 1A–D). In agreement with previous studies (Shanley et al., 2001; Xu et al., 2008), application of leptin (25 nM; 15 min) to slices at P11–18, resulted in a rapid depression in EPSC amplitude (to 73 ± 8% of baseline; $n = 15$; $P < 0.05$; Fig. 1Bi), that returned to baseline levels on washout of leptin. In contrast, at P5–8, application of leptin (25 nM; 15 min) induced a robust decrease in EPSC amplitude (to 82 ± 7% of baseline; $n = 14$; $P < 0.05$; Fig. 1Ai) that persisted on leptin washout for the duration of recordings (up to 90 min). In accordance with our previous studies (Moult et al., 2010), addition of leptin (25 nM; 15 min) to adult hippocampal slices (12–16 week) resulted in a rapid increase in EPSC amplitude (to 198 ± 12% of baseline; $n = 13$; $P < 0.001$; Fig. 1Ci) that was sustained for the duration of recordings. Similarly, addition of leptin (25 nM; 15 min) induced a long-lasting increase in EPSC amplitude (to 157 ± 12% of baseline; $n = 14$; $P < 0.01$; Fig. 1Di) in slices from 12–14 month old animals, however, the magnitude of enhancement at this age was significantly less than that observed at 12–16 weeks ($P < 0.05$). Thus our data indicate that leptin bi-directionally regulates synaptic transmission in an age-dependent manner.

Previously, we have shown high levels of leptin receptor expression at both pre- and post-synaptic sites on hippocampal neurons (Shanley et al., 2002). Therefore leptin receptors located at either locus could mediate the age-dependent effects on excitatory synaptic function. In order to identify the locus of leptin's effects, we analysed the paired pulse ratio (PPR; two pulses delivered at an interval of 50 ms) and coefficient of variation (CV) in the above experiments as changes in these parameters classically reflect alterations in release probability (Kullmann, 1994). The ability of leptin to induce the long-lasting depression of synaptic transmission at P5–8 and the transient synaptic depression at P11–18 were not accompanied by any change in PPR or CV ($n = 6$ and $P > 0.5$ for each; Fig. 1Aii and Bii). In addition, the leptin-induced persistent increase in synaptic strength was not associated with any significant change in PPR or CV in slices from either 12–16 week and 12–14 month animals ($n = 6$ and $P > 0.05$ for each; Fig. 1Cii and Dii, respectively) which is in agreement with our previous studies (Moult et al., 2010). Thus these data indicate that the ability of leptin to modulate excitatory synaptic transmission in the developing and adult hippocampus involves a postsynaptic expression mechanism.

3.2. Regulation of synaptic transmission by leptin is dependent upon NMDA receptor activation

We have shown that the leptin-driven increase in synaptic transmission in adult slices (12–16 week) is dependent upon the