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Leptin promotes rapid dynamic changes in hippocampal dendritic morphology

Dervla O'Malley, Neil MacDonald, Sarah Mizielinska, Christopher N. Connolly, Andrew J. Irving, and Jenni Harvey*

Neurosciences Institute, Division of Pathology and Neuroscience, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY, UK

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Recent studies have implicated the hormone leptin in synaptic plasticity associated with neuronal development and learning and memory. Indeed, leptin facilitates hippocampal long-term potentiation and leptin-insensitive rodents display impaired hippocampal synaptic plasticity suggesting a role for endogenous leptin. Structural changes are also thought to underlie activity-dependent synaptic plasticity and this may be regulated by specific growth factors. As leptin is reported to have neurotrophic actions, we have examined the effects of leptin on the morphology and filopodial outgrowth in hippocampal neurons. Here, we demonstrate that leptin rapidly enhances the motility and density of dendritic filopodia and subsequently increases the density of hippocampal synapses. This process is dependent on the synaptic activation of NR2A-containing NMDA receptors and is mediated by the MAPK (ERK) signaling pathway. As dendritic morphogenesis is associated with activity-dependent changes in synaptic strength, the rapid structural remodeling of dendrites by leptin has important implications for its role in regulating hippocampal synaptic plasticity and neuronal development.

Keywords: Leptin; Dendritic filopodia; Synaptic plasticity; MAPK; Hippocampus; Real-time imaging

Introduction

It is well documented that leptin regulates energy homeostasis via its actions on specific hypothalamic nuclei (Jacob et al., 1997). Hypothalamic leptin receptors also play an important role in controlling thermogenesis, neuroendocrine function and bone formation (Freidman and Halaas, 1998; Elmquist et al., 1998b; Karsenty, 2001). However, leptin receptors are widely expressed in many brain regions including the hippocampus, cerebellum, brain stem and amygdala (Hakansson et al., 1998; Elmquist et al., 1998a; Shanley et al., 2002), suggesting that leptin plays a role in diverse CNS functions. Recent studies have implicated leptin in associative learning and memory as leptin-insensitive (db/db mice and fa/fa rats) rodents display impairments in hippocampal long-term potentiation (LTP) and long-term depression (LTD), as well as deficits in spatial memory tasks (Li et al., 2002). Direct administration of leptin into the hippocampus enhances LTP in vivo (Wayner et al., 2004). At the cellular level, leptin converts hippocampal short lasting potentiation (STP) into LTP; an action likely to reflect enhanced NMDA receptor function (Shanley et al., 2001). Leptin also contributes to synaptic plasticity changes in the hypothalamus as the efficacy of inhibitory and excitatory synaptic transmission is altered in leptin-deficient ob/ob mice (Pinto et al., 2004). During neuronal development, leptin also plays a pivotal role: leptin receptors are expressed at high levels in neonatal rodent brains (Morash et al., 2001), and reductions in brain weight and protein content are evident in leptin-deficient or -insensitive rodents (Ahima et al., 1999). More recent studies indicate that leptin participates in development of the hypothalamus as specific projection pathways are disrupted in leptin-deficient ob/ob mice (Bouret et al., 2004).

Several lines of evidence indicate that structural, as well as biochemical changes underlie activity-dependent synaptic plasticity in the brain. Indeed changes in the morphology and/or density of dendritic spines contribute to enhanced synaptic efficacy following hippocampal LTP (Yuste and Bonhoeffer, 2001). Moreover, neurotrophic factors, like brain-derived neurotrophic factor (BDNF), further refine synaptic connections during activity-dependent synaptic plasticity (Tyler et al., 2002; Schinder and Poo, 2000). Indeed, BDNF, via a MAPK-dependent process, promotes changes in the morphology and density of dendritic spines in CA1 pyramidal neurons (Tyler and Pozzo-Miller, 2003), which may contribute to this refining process. As leptin has neurotrophic actions in the hypothalamus (Bouret et al., 2004), it is possible that changes in dendritic morphology may also contribute to the changes in hippocampal synaptic efficacy induced by leptin. In this study, we present the first compelling evidence that leptin rapidly increases the density and motility of dendritic filopodia in hippocampal neurons. Moreover, this effect is associated with the formation of new synaptic connections as leptin rapidly increased

* Corresponding author.
E-mail address: j.z.harvey@dundee.ac.uk (J. Harvey).
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the number of hippocampal synapses. These findings have important implications for the role of this hormone in hippocampal synaptic plasticity and neuronal development.

Results

Leptin increases the number of dendritic filopodia in hippocampal neurons

It is well documented that dendritic filopodia are highly motile structures and the motility and/or number of filopodia can be influenced by a range of extrinsic factors, such as neurotrophins (Munno and Syed, 2003). In order to examine if leptin alters the density of filopodia, we compared the number of filopodia extending from dendrites of cultured hippocampal neurons (6–12 DIC) under control conditions or following exposure to leptin (50 nM). Filopodia were identified in fixed, permeabilized cells using Alexa 488-phalloidin labeling. Dendritic processes were identified by their morphology and MAP2 staining. In the absence of leptin, the density of filopodia was generally low, with on average between 2 and 9 filopodia (mean 2.58±0.14; $n=129$) detected on 50 μm segments of randomly selected proximal dendritic processes. However, in neurons treated with leptin (50 nM for 30 min) the filopodial density markedly increased (309±34% of control; mean density of 8.00±0.45; $n=23$; $P<0.001$), suggesting that leptin promotes outgrowth or stabilization of filopodia from dendritic processes. In contrast, exposure to the inactive boiled leptin peptide (30 min) had no effect on the number of dendritic filopodia such that the mean density of filopodia in control conditions and following exposure to the inactive peptide were 3.19±0.21 and 3.04±0.16, respectively ($n=29$; $P>0.05$). In order to demonstrate that the changes in dendritic morphology induced by leptin were attributable to activation of leptin receptors, the effects of leptin were also assessed in neurons transfected with leptin receptor siRNA to reduce leptin receptor expression. In order to aid visual identification of siRNA-targeted cells, neurons were also co-transfected with EGFP cDNA. Under control conditions (normal leptin receptor expression), application of leptin (50 nM; 30 min) resulted in a marked increase in the density of dendritic filopodia (mean density of 4.97±0.25; $n=29$; $P<0.05$) relative to control (mean density of 1.58±0.18; $n=32$). In contrast, in neurons with reduced leptin receptor expression, the ability of leptin (50 nM; 30 min) to alter the density of dendritic filopodia was significantly reduced such that the mean density of filopodia was 1.37±0.15 ($n=36$) and 1.48±0.13 ($n=36$; $P>0.05$) in the absence and presence of leptin, respectively. These data suggest that the alterations in dendritic morphology induced by leptin are mediated by activation of leptin receptors as genetic knockdown of leptin receptor expression in hippocampal neurons markedly attenuated the effects of this hormone.

In order to determine the temporal profile of these leptin-dependent events, we compared the effect of leptin (50 nM) over a range of time courses (10 min to 18 h). The effect of leptin (50 nM) occurred rapidly as a significant increase in filopodial density (to 206±14% of control; mean density of 5.45±0.36; $n=10$; $P<0.01$) was evident after 10 min incubation with leptin, with a peak increase (to 544±95% of control; mean density of 13.8±2.3; $n=12$; $P<0.01$) detected after 3 h exposure (Fig. 1D). Furthermore, increases in the density of filopodia (267±19% of control; mean density of 6.89±0.42; $n=12$; $P<0.001$) were observed after 18 h exposure to leptin, suggesting that this action of leptin does not readily desensitize (Fig. 1D). However, the morphology of the filopodia was altered at this time point, being lengthened, with actin-rich growth cones apparent at their leading edges (not illustrated). This suggests that the effects of leptin on filopodial density lead to the formation of new processes.

Leptin induces actin reorganization in hippocampal neurons

It is well established that the actin cytoskeleton plays a pivotal role in the morphological changes that occur in dendritic filopodia and spines during development and synaptic plasticity (Matus, 2000; Smart and Halpain, 2000). In addition, we have shown that leptin has the ability to promote reorganization of actin filaments in hippocampal neurons (O'Malley et al., 2005). Thus, in order to determine if the leptin-induced formation of new filopodia was accompanied by changes in the actin cytoskeletal architecture, the intensity of Alexa-phalloidin staining in proximal dendrites was examined. In control hippocampal neurons (no leptin), Alexa-phalloidin staining was associated with the plasma membrane and actin rich structures such as filopodia and synapses. Exposure to leptin (50 nM; 30 min) induced a marked reorganization of the actin cytoskeleton such that the intensity of Alexa-phalloidin staining in proximal dendrites was significantly reduced (to 67±8.4% of control; $n=25$; $P<0.05$), and this effect of leptin was accompanied by the appearance of actin-enriched dendritic filopodia (Fig. 1E). Thus, these data suggest that leptin promotes redistribution of actin from the dendritic shaft to dendritic filopodia.

Leptin enhances actin-based motility of filopodial extensions

In order to visualize actin-based structures in living neurones, we transfected cultured hippocampal neurons (up to 12 DIC) with a cytosolic EGFP construct using the cationic lipid, Lipofectamine 2000. In transfected neurons, filopodia were associated with dendrites arising from principle neurons in the culture (pyramidal-shaped, with 2–3 main dendritic branches), and the average number of filopodia (extending from 50 μm proximal regions of randomly selected dendrites) was 7.14±0.58 ($n=34$ dendrites),

Fig. 1. Leptin increases the density of dendritic filopodia in hippocampal neurons. (A) Confocal images of actin staining in hippocampal neurons (6–8 DIC) labeled with Alexa 488-conjugated phalloidin. (Ai) Control neurons display relatively few actin-rich protrusions or growth cones. Exposure of neurons to leptin (50 nM) for 30 min stimulated an increase in the number of filopodia (ii) and growth cones (iii) extending from processes (indicated by arrows). (B) Confocal images of hippocampal neurons (9DIC) dual labeled with Alexa 488-conjugated phalloidin (green) and the somatodendritic marker, MAP2 (red). Leptin (50 nM; 30 min) increased the number of filopodia protruding from dendritic (MAP2-positive) processes (ii) compared to control (i). (C) Histogram illustrating the pooled data of the mean number of dendritic filopodia in control and leptin treated neurons. Leptin stimulates circa a 3-fold increase in the density of filopodia. (D) Histogram illustrating the pooled data of the mean number of dendritic filopodia after 10 min, 20 min, 30 min, 3 h and 18 h exposures to leptin (50 nM). The leptin-induced increase in filopodial density is apparent after only 10 min exposure to leptin and reaches a peak after 3 h. (E) Leptin-induced changes in dendritic morphology are associated with reorganization of the actin cytoskeleton. Histogram illustrating the pooled data of the intensity of Alexa 488-conjugated phalloidin staining in control and leptin-treated neurons. (F) Representative confocal images (i–iii) of leptin receptor (ObR; i) and synapsin-1 (ii) immunoreactivity in 9-day-old hippocampal cultures. The merged image (iii) shows that leptin receptor labeling is highly localized to synapses.