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Research Report

Oleic acid synthesized by stearoyl-CoA desaturase (SCD-1) in the lateral periventricular zone of the developing rat brain mediates neuronal growth, migration and the arrangement of prospective synapses

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ABSTRACT

Our previous work has shown that oleic acid synthesized by astrocytes in response to serum albumin behaves as a neurotrophic factor in neurons, upregulating the expression of GAP-43 and MAP-2 proteins, which are respectively markers of axonal and dendrite growth. In addition, oleic acid promoted neuron migration and aggregation, resulting in clusters of neurons connected each other by the newly formed neurites. In this work we show that the presence of albumin or albumin plus oleic acid increases neuron migration in cultured explants of the lateral periventricular zone, resulting in an increase in the number of GAP-43-positive neurons leaving the explant. Upon silencing stearoyl-CoA desaturase-1 (SCD-1), a key enzyme in oleic acid synthesis by RNA of interference mostly prevented the effect of albumin but not that of albumin plus oleic acid, suggesting that the oleic acid synthesized due to the effect of albumin would be responsible for the increase in neuron migration. Oleic acid increased doublecortin (DCX) expression in cultured neurons, explants and organotypic slices, suggesting that DCX may mediate in the effect of oleic acid on neuron migration. The effect of oleic acid on neuron migration may be destined for the formation of synapses because the presence of oleic acid increased the expression of synaptotagmin and that of postsynaptic density protein (PDS-95), respectively markers of

Abbreviations: DAPI, 4',6-diamidine-2'-phenylindole, DIV, days *in vitro*; DCX, doublecourtin; GAP-43, growth-associated protein 43; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NT-siRNA, non-targeting small interfering RNA; PBS, phosphate-buffered saline; siRNA, small interfering RNA; SCD-1, stearoyl-CoA desaturase-1: PDS-95, postsynaptic density protein; BBB, blood–brain barrier

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the pre- and postsynaptic compartments. In addition, confocal microscopy revealed the occurrence of points of colocalization between synaptotagmin and PDS-95, which is consistent with the idea that oleic acid promotes synapse arrangement.

1. Introduction

Mounting evidence suggests the occurrence of an astrocyte–neuron crosstalk that synchronizes astrocytes and neurons so that they can carry out tasks in which the collaboration of both cells is needed. Thus, astrocytes synthesize and release the growth factors that mediate in the development of neurons and glia (Farina et al., 2007, Liberto et al., 2004, Blondel et al., 2000). Also, thrombospondins synthesized and released by astrocytes seem to control synaptogenesis during development (Christopherson et al., 2005, Barres, 2008). In addition, astrocytes synthesize cholesterol, which is released together with Apo E for use by neurons to build neurite membranes (Vance et al., 2005, Pfrieger, 2002).

In this context, our previous work has shown that the oleic acid synthesized and released by astrocytes behaves as a neurotrophic factor for neurons. The synthesis and release of oleic acid takes place in cultured astrocytes exposed to serum albumin, a protein that is internalized in astrocytes through endocytosis, mediated by megalin, caveolins and Dab-1 (Bento-Abreu et al., 2008, Bento-Abreu et al., 2009). Albumin uptake is followed by transcytosis, including passage through the endoplasmic reticulum (Tabernero et al., 2002, Bento-Abreu et al., 2009), where oleic acid is synthesized. Albumin sequestrates the fatty acid in the endoplasmic reticulum, promoting the induction of stearoyl-CoA desaturase-1 (SCD-1) by increasing the active form of the SREBP-1 transcription factor (Tabernero et al., 2002), which catalyzes a critical committed step in oleic acid synthesis: *i.e.*, the introduction of the *cis*-double bond at position delta 9 (Miyazaki et al., 2001). The oleic acid synthesized is released to the extracellular space, thereby becoming available as a neurotrophic factor for neurons (Tabernero et al., 2002). Thus, oleic acid promotes axonal and dendritic growth and the expression of growth-associated protein 43 (GAP-43) and of the microtubule-associated protein 2 (MAP-2), respectively markers for axonal and dendrite growth (Tabernero et al., 2001, Rodríguez-Rodríguez et al., 2004). This effect is synergistic with the neurotrophins NT-3 and NT-4/5 but not with NGF or BDGF (Granda et al., 2003). The neurotrophic effect of oleic acid in neurons is mediated by PPAR-alpha, protein kinase A (PKA) and Neuro D2 (Rodriguez-Rodriguez et al., 2004, Bento-Abreu et al., 2007), which comprise the signal chain for the neurotrophic effect of oleic acid. In addition, oleic acid concentrations increase sharply (6-fold) *in vivo* in the brain of neonatal rats during the first day after delivery (Polo-Hernandez et al., 2010). This is accompanied an increase in the levels of the active form of SREBP-1 and an enhancement of the expression of SCD-1 and GAP-43 (Velasco et al., 2003), suggesting that the neurotrophic effect observed in cultured neurons may participate in postnatal development of the brain.

Together with the effect of oleic acid on axonal and dendrite growth, we have also observed that the presence of oleic acid promoted neuron migration, resulting in the aggregation of neurons by somata, which aggregated in clusters that are connect with each other by axonal processes (Tabernero et al., 2001, Medina and Tabernero, 2002). In order to gain further insight into this phenomenon, in the present work we studied the effect of oleic acid on neuron migration in brain explants from the lateral periventricular zone. Our results show that oleic acid synthesized *in situ* by the presence of serum albumin enhances neuron migration, a phenomenon that is prevented if SCD, a key enzyme in the synthesis of oleic acid, is silenced with the RNA interference technique.

2. Results

2.1. *Oleic acid promotes neurite growth and cell migration in neurons from primary culture.*

We have previously shown that rat astrocytes specifically synthesize and release oleic acid in the presence of serum albumin (Tabernero et al., 2002). In addition, the exposure of neurons in primary culture to oleic acid promotes axonal and dendrite growth, accompanied by enhanced expression of GAP-43 and MAP-2, which are respectively markers of axonal and dendritic growth (Tabernero et al., 2001). Here, we monitored the effect of oleic acid by live-cell microscopy. Fig. 1 shows the first and the final frames of pictures taken over 15 h of neurons in primary culture in the presence of albumin or albumin plus oleic acid (for all pictures, see "Supporting information"). Under these circumstances, oleic acid enhanced neuron migration, axon and dendrite elongation, and the aggregation of neurons by their somata (for the still photograph at 72 h see: (Medina and Tabernero, 2002)).

2.2. *Oleic acid increases DCX expression in neurons from primary culture*

To test the ability of oleic acid *per se* to induce migration in neurons, we used neurons in primary culture and measured the expression of DCX, a microtubule-associated protein involved in the migration process (Francis et al., 1999). To accomplish this, neurons were cultured in the presence of albumin or albumin plus oleic acid and the expression of DCX was analyzed by Western blot. Western blot analyses revealed a transient increase in DCX expression after treatment with oleic acid. The expression of DCX increased at 24 and 48 h but no statistical differences were observed after 72 h of treatment (Fig. 2).