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The actions of BDNF on dendritic spine density and morphology in organotypic slice cultures depend on the presence of serum in culture media

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Abstract

We have previously shown that brain-derived neurotrophin factor (BDNF) increases dendritic spine density and the proportion of stubby spines in apical dendrites of CA1 pyramidal neurons of hippocampal slice cultures maintained in serum-free media. We show here that serum withdrawal causes an increase in the proportion of thin spines and a decrease in the fraction of stubby spines, without changing the overall density of dendritic spines. When slices are maintained in serum-containing media, BDNF also increased spine density but had the opposite effect on spine morphology: it increased the proportion of mushroom and thin spines and decreased the proportion of stubby spines. Intriguingly, slices maintained in serum media showed a lower p75NTR-to-TrkB expression level than serum-free slices, even after BDNF exposure. The differential actions of BDNF on spine morphology depending on the presence of serum in culture media, together with the difference in neurotrophin receptor expression are reminiscent of opposing functional signaling by p75NTR and Trk receptors, and reveal a complex modulation of dendritic morphology by BDNF signaling.

Keywords: CA1 pyramidal neuron; Biolistic transfection; Hippocampus; Confocal microscopy; Enhanced yellow fluorescent protein; Organotypic slice culture

The establishment of proper dendrite architecture is a fundamental process in nervous system development, where the formation of dendritic spines is critical for the formation of excitatory synaptic networks. The characteristic morphology of dendritic spines has been long postulated to correlate with their function as biochemical or electrical compartments (Kasai et al., 2003; Shepherd, 1996). Based on their morphology, dendritic spines have been classically categorized into three major types: stubby (type-I), mushroom (type-II), and thin (type-III) spines (Peters and Kaiserman-Abramof, 1970, 1969). Differences in spine morphology have been postulated to play a fundamental role in synaptic plasticity models of memory formation and storage (Chapleau and Pozzo-Miller, 2007; Ethell and Pasquale, 2005; Nimchinsky et al., 2002; Yuste and Bonhoeffer, 2001). Several lines of evidence implicate neurotrophic factors in the formation and structural plasticity of existing dendritic spines in CNS pyramidal neurons (McAllister et al., 1999; Tyler et al., 2002a).

The mammalian neurotrophic factors, a family of growth factors that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5), have essential roles in neuronal survival and differentiation (Huang and Reichardt, 2001; Lewin and Barde, 1996). In addition to these classical functions, BDNF in particular has been shown to be one of the most potent modulators of synaptic transmission and plasticity, as well as neuronal and synaptic morphology (Amaral et al., 2007; Black, 1999; Poo, 2001; Tyler et al., 2002a; Vicario-Abejon et al., 2002). Each neurotrophin exerts its actions through binding and activation of specific, membrane-bound tropomyosin-related kinase (Trk) receptors, or a single pan-neurotrophin receptor, the so-called p75NTR (Barbacid, 1993). Individual Trk receptors have high affinity for specific neurotrophins: TrkA for NGF, TrkB for BDNF and NT-4, and TrkC for NT-3; on the other hand, all neurotrophins bind to p75NTR with equal affinity and no apparent selectivity (Chao and Hempstead, 1995). BDNF has been

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shown to modulate dendritic spine density and morphology in cortical pyramidal neurons (Horch and Katz, 2002; Horch et al., 1999; McAllister et al., 1996, 1997, 1995), cerebellar Purkinje, basket and stellate neurons (Mertz et al., 2000; Shimada et al., 1998), dentate granule neurons (Danzer et al., 2002; Tolwani et al., 2002), olfactory bulb granule cells (Matsutani and Yamamoto, 2004), and hippocampal pyramidal neurons (Alonso et al., 2004; Amaral and Pozzo-Miller, 2007b; Ji et al., 2005; Tyler and Pozzo-Miller, 2003, 2001). These observations clearly demonstrate the role of BDNF on dendritic spine formation, but unfortunately they were obtained from different types of culture systems without considering the potential confounding actions of serum in culture media. Since the presence of serum in culture preparations has been shown to affect neurotrophin actions (Morrison and Mason, 1998; Tyler and Pozzo-Miller, 2003), we compared the effects of BDNF on spine density and morphology in apical dendrites of CA1 pyramidal neurons of hippocampal slice cultures maintained in serum-containing media vs. its actions in serum-free media. As we shown before, BDNF increased spine density and the proportion of stubby spines in slices kept in serum-free media (Alonso et al., 2004; Amaral and Pozzo-Miller, 2007b; Tyler and Pozzo-Miller, 2003, 2001). Even though it also increased spine density in slices maintained in serum-containing media, BDNF had the opposite effect on spine morphology, i.e. it increased the proportion of thin (type-III) spines while reducing the fraction of stubby (type-I) spines.

1. Material and methods

1.1. Organotypic slice cultures

All animal procedures followed national and international ethics guidelines and were reviewed and approved by the IACUC at UAB on an annual basis. Hippocampal slice cultures were prepared from postnatal-day 7–10 (P7–P10) Sprague–Dawley rats and maintained *in vitro* as previously described (Pozzo Miller et al., 1993; Stoppini et al., 1991; Yamamoto et al., 1989). Briefly, rats were quickly decapitated and their brains aseptically dissected and immersed in ice-cold dissecting solution, consisting of Hanks' Balanced Salt Solution (HBSS), supplemented with glucose (36 mM) and antibiotics/antimycotics (1:100; penicillin/streptomycin/amphotericin B). Hippocampi were then dissected and transversely sectioned into ~500 μm slices using a custom-made tissue slicer (Katz, 1987) strung with 20 μm-thick tungsten wire (California Fine Wire Company; Groover Beach, CA). Slices were incubated at 4 °C for ~30 min, and then plated on tissue culture inserts (0.4 μm pore size, Millicell-CM, Millipore Corporation; Billerica, MA). Culture media contained minimum essential media (MEM; 50%), HBSS (25%), heat-inactivated equine serum (20%), L-glutamine (1 mM), and D-glucose (36 mM). Slices were maintained in incubators set at 36 °C, 5% CO_2, 98% relative humidity. Culture medium was first changed at 4 days *in vitro* (div) and every 2 days afterwards. It should be noted that, even though organotypic slice cultures are isolated *in vitro* for extended times, CA1 pyramidal neurons continue to develop acquiring dendritic and spine morphologies that are not significantly different than those of CA1 neurons from age-matched acutely prepared hippocampal slices (De Simoni et al., 2003).

To avoid potential confounding effects due to unknown factors in the equine serum used in standard organotypic slice culture protocols, our prior studies used slices maintained in defined serum-free media (Alonso et al., 2004; Amaral and Pozzo-Miller, 2007b; McCutchen et al., 2002; Pozzo-Miller, 2006; Tyler and Pozzo-Miller, 2003, 2001). When using serum-free media, equine serum was titrated down from 20% over 3 days (10% at 4 div; 5% at 5 div; serum-free at 6 div). Serum-free media consisted of Neurobasal-A plus B-27 supplement and L-glutamine (1 mM). All tissue culture reagents were obtained from InVitrogen (Carlsbad, CA), except for glucose, which was obtained from Sigma (St. Louis, MO).

1.2. Particle-mediated gene transfer

After 7 days *in vitro*, slices were transfected as previously described (Alonso et al., 2004; Amaral and Pozzo-Miller, 2007b). Briefly, plasmid cDNA for enhanced yellow fluorescent protein (eYFP; Clontech; Mountain View, CA) was introduced by biolistic transfection using a Helios gene-gun (Bio-Rad; Hercules, CA). Plasmid cDNA was precipitated onto 1.6 μm-diameter colloidal gold at a ratio of 2 μg DNA/1 mg gold, and then coated onto Tefzel tubing. Slices on tissue culture inserts were bombarded with gold particles accelerated by ~100 psi He from a distance of 15 mm using a modified gene-gun nozzle (Alonso et al., 2004). Prior to transfection, an antibiotic/antimycotic mixture (1:100; penicillin/streptomycin/amphotericin B) was added to culture media to prevent contamination during biolistic transfection. The antibiotic/antimycotic mixture was only used during biolistic transfection and was removed after 24 h to avoid the consequences of network desinhibition from their known actions on $GABA_A$ receptor channels (Twyman et al., 1992).

1.3. Treatment conditions

Slices were randomly assigned to the following groups: (1) serum-containing media controls; (2) slices kept in serum-free media; (3) BDNF (250 ng/mL; provided by Amgen; Thousand Oaks, CA) in the presence of serum; (4) BDNF (250 ng/mL) in serum-free media. A droplet (50 μL) of medium was gently applied onto each slice to facilitate penetration, followed by full medium exchange (1 mL per tissue culture well). All treatments lasted 48 h, beginning 48 h after biolistic transfection, and slices were coded for subsequent "blind" quantitative analyses of dendritic spine density and morphology by an investigator unaware of treatment groups.

1.4. Laser-scanning confocal microscopy

After 48 h in each of the treatment conditions, slices were fixed by immersion in 4% paraformaldehyde in 100 mM phosphate buffer (overnight at 4 °C), and washed in phosphate buffer saline (PBS). Filter membranes around each slice were trimmed, and each slice was individually mounted on glass