

SUMMARY

Local extracellular cues are regarded as crucial for the establishment of neuronal connectivity, as they may facilitate neurite development, mutual recognition of the pre- and postsynaptic targets, and/or stabilization of the synaptic contacts. The aim of this study was to further clarify the role of brain-derived neurotrophic factor (BDNF) and plasticity-related gene-1 (PRG-1) in dendrite growth and synapse formation of primary hippocampal or entorhinal cortical neurons. Effects of BDNF on neurite outgrowth and synaptic transmission have been reported earlier, but based on experiments with exogenous BDNF, BDNF-neutralizing antibodies, BDNF gene inactivation or BDNF overexpression in wild-type neurons. The role of PRG-1 in dendrite development and synapse formation/stabilization in single neurons has not yet been explored.

To characterize the local effects of BDNF, an experimental model was established to provide a neuronal source of local BDNF. Single neurons in low density primary neuronal cultures from the hippocampus of E18 *bdnf*^{-/-} mice were transfected with a BDNF::EGFP plasmid construct. After an expression time of 16 h (or in few experiments, 72 h) the cultures were fixed and subjected to immunocytochemical analysis for a change in dendrite geometry and synapse numbers. Specific antibodies and combined immunostaining were used to visualize and quantify glutamatergic and GABAergic synaptic terminals separately. The data were complemented by experiments with quantitative PCR, siRNA-transfection and treatment with a variety of receptor-specific blockers.

In the present study, evidence to support the following conclusions is reported: 1) Neurons expressing BDNF display changes in dendrite morphology: they exhibited weaker dendrite elongation (fewer dendritic trees with a length >50 μm), but stronger dendrite initiation (a larger number of primary dendrites and higher-order branches). 2) Neurons expressing BDNF attract a larger total number of synaptic terminals. 3) A cellular source of BDNF produces stronger up-regulation of synaptic terminal numbers than ambient BDNF at high concentration (100 ng/ml). 4) Glutamatergic and GABAergic synaptic terminals react in a differential manner to postsynaptic BDNF. The strength of the glutamatergic input increases, while the strength of GABAergic input decreases. 5) The upregulation of glutamatergic

synaptic input by the postsynaptic BDNF requires TrkB activity. 6) The transfected BDNF (t-BDNF)-induced downregulation of GABAergic synaptic terminal numbers and dendrite elongation is prevented by block of TrkB. In addition, these two suppressive actions of t-BDNF are precluded by GluR block suggesting that the suppression is associated with t-BDNF-stimulated GluR activity. 7) Block of p75 cannot prevent the BDNF-induced changes in dendrite length and synapse numbers, but it reduces the branching response to BDNF transfection. 8) Suppression of PRG-1 expression results in a decrease of synaptic terminal numbers, whereas PRG-1-overexpression attenuates the LPA-induced loss of glutamatergic synapses.

Based on these results we propose that (i) postsynaptic expression and release of BDNF and its binding to presynaptic TrkB receptors boosts the glutamatergic synaptic input. The contribution of p75 to this glutamatergic upregulation is small, if at all existing. (ii) The arrest of dendritic length growth and the down-regulation of GABAergic synaptic input are consequences of the enhanced glutamate receptor activity rather than a result of direct action of BDNF on TrkB receptors on the postsynaptic dendrites or presynaptic GABAergic terminals, respectively. (iii) Dendrite initiation is controlled by p75 and reflects the level of ambient neurotrophin concentration (and the action of other ligands of p75). In this process, the impact of glutamatergic synaptic input is less obvious. (iv) PRG-1 can act as membrane-bound postsynaptic stabilizer of glutamatergic synaptic terminals.