

USING PROMEGA MAPK ANTIBODIES AND REAGENTS TO STUDY SIGNALING IN NEURONS

Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology

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Introduction

Activity-dependent biochemical changes have been observed in mammalian neurons, but the pathways that control activity-dependent morphological changes in dendritic spines remain unknown. Reports indicate that the MAPK signaling pathway might mediate persistent structural changes in mammalian neurons, where MAPK is activated in a stimulus-dependent fashion and helps support neuronal excitability, synaptic potentiation, nuclear signaling and memory formation. In this paper, the authors focused on MAPK signaling in dendrites of cultured hippocampal pyramidal cells and explants of dentate gyrus.

Immunological Investigation of MAPK Signaling

MAPK signaling in dendrites was detected by immunocytochemistry in cultured hippocampal neurons. Potassium depolarization for three minutes gave rise to intense dually phosphorylated MAPK immunoreactivity, which peaked at 10 minutes and returned to close to baseline by 30 minutes. Administration of 4 spaced, depolarizing pulses greatly increased the persistence of phosphorylated MAPK (60 minutes). However, massing the four pulses together failed to generate prolongation of MAPK phosphorylation. Immunoblots of pMAPK/MAPK revealed a similar pattern to that obtained by immunocytochemical analysis.

Pharmacology

A pharmacological profile study showed that the initial MAPK phosphorylation was completely blocked by L-type Ca^{2+} channel inhibitors (nimodipine, D-APV), inhibitors of calmodulin (W7) and CaM kinases (KN-93), inhibitor of carboxyl methylation of Ras (AFC), or PD98059, a specific inhibitor of MEK. These data indicate that the initial phase of dendritic MAPK phosphorylation was supported by a Ca^{2+} -, CaM kinase-, Ras- and MEK-dependent pathway.

After persistent MAPK phosphorylation was induced either by 4 spaced, depolarizing stimuli or by application of BDNE, the MEK inhibitor PD98059 was added and caused the sustained pMAPK to return to baseline levels. This indicated that the stabilization of dendritic MAPK phosphorylation was supported by persistent kinase activity. In contrast to MEK inhibition, post-stimulus inhibition of CaM kinases, PKA, PKC or Ras did not affect the sustained MAPK phosphorylation. Thus, the early

peak and sustained phase of MAPK phosphorylation displayed very different pharmacological profiles.

To determine how the activation of dendritic MAPK was sustained, the authors applied U0126, a MEK antagonist, after the peak MAPK phosphorylation. It caused a prompt drop of pMAPK to baseline levels. Subsequent removal of U0126 in the absence of any further stimulation led to a rapid recovery of MAPK phosphorylation, indicating that the persistence evidently lies upstream of MEK in a form of biochemical memory that causes its sustained activation.

Morphology

The authors then examined dendritic morphology in explant cultures of hippocampal dentate gyrus transfected with green fluorescent protein (GFP) as a cellular marker. The spaced, repeated application of high- K^{+} stimuli caused two distinct forms of stable, robust morphological changes in dendritic spines: numerous filopodia protruding from the spines or the dendritic shaft, and a new spine-like structure with bulbous head. Both types of new structure persisted beyond 30–60 minutes after stimulation ended. In contrast, massed or single exposures to high K^{+} failed to produce persistent morphological changes. Also, the activity-dependent morphological changes in hippocampal dendrites were inhibited by antagonists of NMDA receptors and L-type calcium channels, an inhibitor of CaM kinases (KN-93), or the specific MEK antagonist U0126, indicating that the MAPK signaling pathway may be involved in morphological plasticity.

The findings presented here revealed that the persistent activation of the MAPK pathway was produced by multiple spaced membrane depolarizations but not by prolonging a single stimulus. Also, spaced, repeated stimuli and MAPK activation were critical for protrusion of new dendritic filopodia that remained stable. Thus, the pattern of activity not only determined the persistence of the biochemical changes but also controlled the subsequent changes in dendritic architecture. This research provides new perspective on mechanisms of long-term memory, showing that memory formation depends on repetitive stimulation through spaced training, MAP kinases, and dendritic structural changes.

Anti-ACTIVE® MAPK pAb, (Cat.# V8031), the inhibitors U0126 (Cat.# V1121) and PD98059 (Cat.# V1191), and the BDNF (Cat.# G1491) were obtained from Promega.

Ordering Information

Product	Size	Cat.#
Anti-ACTIVE® MAPK pAb, (pTEpY)	40µl	V8031
PD 98059	5mg	V1191
MEK Inhibitor U0126	5mg	V1121
Human Brain-Derived Neurotrophic Factor (rhBDNF)	5µg	G1491

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