TGF-β1-Induced Long-Term Changes in Neuronal Excitability in Aplysia Sensory Neurons Depend on MAPK

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Submitted 20 July 2005; accepted in final form 18 January 2006

Chin, Jeannie, Rong-Yu Liu, Leonard J. Cleary, Arnold Eskin, and John H. Byrne. TGF-β1-induced long-term changes in neuronal excitability in Aplysia sensory neurons depend on MAPK. J Neurophysiol 95: 3286–3290, 2006; doi:10.1152/jn.00770.2005. Transforming growth factor beta-1 (TGF-β1) plays important roles in the early development of the nervous system and has been implicated in neuronal plasticity in adult organisms. It induces long-term increases in sensory neuron excitability in Aplysia as well as a long-term enhancement of synaptic efficacy at sensorimotor synapses. In addition, TGF-β1 acutely regulates synapsin phosphorylation and reduces synaptic depression induced by low-frequency stimuli. Because of the critical role of MAPK in other forms of long-term plasticity in Aplysia, we examined the role of MAPK in TGF-β1-induced long-term changes in neuronal excitability. Prolonged (6 h) exposure to TGF-β1 induced long-term increases in excitability. We confirmed this finding and now report that exposure to TGF-β1 was sufficient to activate MAPK and increase nuclear levels of active MAPK. Moreover, TGF-β1 enhanced phosphorylation of the Aplysia transcriptional activator cAMP response element binding protein (CREB), a homologue to vertebrate CREB. Both the TGF-β1-induced long-term changes in neuronal excitability and the phosphorylation of CREB1 were blocked in the presence of an inhibitor of the MAPK cascade, confirming a role for MAPK in long-term modulation of sensory neuron function.

INTRODUCTION

Neurotrophins and cytokines are essential for the survival and differentiation of nerve cells, and they modulate synaptic transmission and neuronal plasticity in mature organisms. Neurotrophins have been studied extensively for their roles in modulating synaptic transmission in developing systems as well as adult animals (reviewed in Kojima et al. 2002; Lu 2003). A role for the cytokine TGF-β1 in neuronal plasticity has also emerged. Recombinant TGF-β1 induces long-term facilitation of Aplysia sensory-motor synapses (Zhang et al. 1997). Moreover, 5-HT-induced long-term facilitation is blocked by a soluble fragment of the TGF-β1 type II receptor, which presumably acts by scavenging an endogenous TGF-β1-like molecule. TGF-β1 also induces long-term increases in neuronal excitability in Aplysia (Chin et al. 1999; Farr et al. 1999). Short-term modulation is also induced by TGF-β1. Synaptic depression is reduced, in association with regulation of synapsin function (Chin et al. 2002). Finally, mice deficient in Smad, a component of the TGF-β1 signaling pathway, have impaired spatial memory (Frankland et al. 2001).

The TGF-β1 superfamily controls an array of cellular processes regulating development, growth, homeostasis, extracellular matrix production and regulation and wound repair (reviewed in Massagué, 1998; ten Dijke and Hill 2004). Recently, the regulation and roles of TGF-β1 signaling in neurons have been investigated extensively (reviewed in Bottner et al. 2000; Sanyal et al. 2004). It has become clear that the pathways activated by TGF-β1 in neurons overlap with those engaged by other factors that modulate neuronal and synaptic plasticity. Of particular interest are the MAPK pathway and the transcriptional activator cAMP response element binding protein (CREB), which have both been widely implicated for their roles in activity-dependent plasticity. TGF-β1 transiently activates MAPK in chick ciliary ganglion neurons, which is necessary for acute and sustained effects of TGF-β1 on KCa channel expression (Lhuillier and Dryer 2000). In addition, a number of studies have implicated MAPK as being an important mediator of TGF-β1 signaling (reviewed in Mulder 2000). TGF-β1 also activates the MAPK pathway in Aplysia sensory neurons, which is critical for the TGF-β1-mediated reduction in synaptic depression at the sensorimotor synapse (Chin et al. 2002). The MAPK pathway plays a crucial role in the synaptic plasticity underlying learning and memory in both invertebrates and vertebrates (reviewed in Sweatt 2004). Among the targets of the MAPK pathway is the transcription factor CREB, which has been well-characterized for its role in long-term plasticity (reviewed in Lonze and Ginty 2002). CREB is also engaged by TGF-β1 signaling (Liu et al. 2005; Warner et al. 2003; Zhang et al. 2004), representing a point of convergence between activity-dependent and neurotrophin/cytokine-dependent modulation of neuronal function. Of particular interest to the present study, long-term facilitation of Aplysia sensorimotor synapses induced by 5-HT involves the activation of MAPK and CREB1 (Bartsch et al. 1998; Martin et al. 1997; Michael et al. 1998; Purcell et al. 2003; Sharma et al. 2003).

In the present study, we demonstrate that TGF-β1 induces the activation and increased nuclear localization of MAPK in sensory neurons and that this activation is necessary for TGF-β1 to induce long-term changes in neuronal excitability. Moreover, the transcription factor CREB1 is activated by TGF-β1 in a MAPK-dependent manner, suggesting that it may play a role in long-term changes induced by TGF-β1. These results underscore the role of TGF-β1 in modulating neuronal properties and the engagement of the MAPK/CREB signaling pathway in long-term neuronal plasticity.

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Culturing procedures followed those described in Shacher and Proshansky (1983) and Chin et al. (2002). Mechanosensitive sensory neurons from the ventral-caudal cluster of the pleural ganglia were isolated and allowed to grow for 3–5 days at 18°C before experiments were begun. For immunocytochemistry, neurons were fixed in 4% paraformaldehyde in PBS containing 30% sucrose and incubated with anti-dually-phosphorylated MAPK (1:2500, Promega) or anti-phosphorylated CREB1 (1:1000) followed by tetramethylrhodamine-conjugated goat-anti-rabbit IgG. The anti-dually-phosphorylated MAPK antibody was specific for Aplysia MAPK (Fioravante et al. 2006). The rabbit polyclonal anti-phosphorylated-CREB1 (pCREB1) antibody was raised against Ser85-phosphorylated Aplysia CREB1 as previously described (Mohamed et al. 2005). Immunofluorescence was viewed with a confocal microscope (Biorad, MRC 1024MP). A z-series of optical sections through the cell body (0.25 μm increments) were taken, and the section through the middle of the nucleus was used for analysis of mean fluorescence intensities by means of ImageTool 2.1 software (Univ. of Texas Health Science Center at San Antonio). Eight to 10 neurons on each coverslip were analyzed, and measurements from neurons on the same coverslip were averaged. For electrophysiology, all recordings were performed at 22°C in solution containing 50% L15 and 50% ASW. Neurons were impaled with one microelectrode (resistance approximately 10 MΩ). Resting potential and input resistance were measured, and the neuron was held at −45 mV by passing current. Excitability was measured by counting the number of action potentials triggered by a 1-nA, 2-s depolarizing current pulse. Measurements from cells in one dish were averaged (2–3 cells per dish). After baseline measurements, cells were treated with TGF-β1 (1 ng/ml), U0126 (20 μM in 0.2% DMSO, Promega), or 0.2% DMSO alone for 1 h prior to application of TGF-β1 or BSA for 5 min (short-term experiments) or 6 h (long-term experiments). After the treatment, neurons were rinsed and returned to culture media. Excitability was assessed either 5 min later (short term) or 24 h later (long term). The individual performing the electrophysiological tests did not know which treatment the neurons had received.

RESULTS

Cultured sensory neurons exposed to 1 ng/ml TGF-β1 for 6 h exhibited increased excitability measured 24 h later (Chin et al. 1999). Farr et al. (1999) also showed that TGF-β1 increased the excitability of Aplysia sensory neurons measured 24 h after treatment. Moreover, injection of active MAPK into sensory neurons induced long-term increases in neuronal excitability (Sung et al. 2001). On the basis of the ability of both TGF-β1 and MAPK to induce long-term changes in excitability, we examined whether MAPK activity was necessary for the long-term regulation of neuronal excitability by TGF-β1. U0126, a specific inhibitor of MEK, an upstream activator of MAPK (Favata et al. 1998), inhibits the activation of MAPK in Aplysia sensory neurons (Chin et al. 2002). Using isolated sensory neurons grown in culture, we replicated our previous finding that TGF-β1 increases neuronal excitability measured 24 h after 6-h treatment with TGF-β1 (Chin et al. 1999). TGF-β1 increased the excitability of neurons by 238 ± 30% (mean ± SE) of baseline (Fig. 1A1, n = 7, representing 18 neurons). However, the excitability of neurons treated with U0126 + TGF-β1 was only 151 ± 24% of baseline (Fig. 1A2, n = 8, representing 20 neurons). The excitability of neurons treated with U0126 + BSA was 147 ± 13% (Fig. 1A3, n = 7, representing 18 neurons) indicating that the effect of U0126 + TGF-β1 was the same as the effect of U0126 + BSA. A two-way analysis of variance (ANOVA), repeated measures design, revealed a significant difference among these three groups (F2,19 = 4.65, P < 0.05), and Tukey post hoc tests revealed that the group of neurons treated with DMSO + TGF-β1 was significantly different from the other two groups. These results indicate that MAPK plays a critical role in the induction of long-term changes in excitability by TGF-β1.

Because TGF-β1 acutely stimulates MAPK activation in sensory neurons (Chin et al. 2002), we examined whether acute (5 min) exposure to TGF-β1 was sufficient to induce short-term changes in excitability. These experiments were performed in a manner identical to that described above, but the excitability of sensory neurons was immediately restested after the end of the 5 min treatment with TGF-β1. Five minutes of treatment with TGF-β1 did not alter sensory neuron excitability (111 ± 17%, mean ± SE, for TGF-β1 vs. 95 ± 11% for BSA, n = 8, t14 = 0.81, P = 0.43). We also examined whether the brief, 5-min treatment would induce the long-term increase in neuronal excitability. Twenty-four hours after the 5-min treatment, the excitability of the sensory neurons was not different from that of BSA-treated controls (149 ± 27%, mean ± SE, for TGF-β1 vs. 146 ± 24% for BSA, n = 4, t6 = 0.89, P = 0.41).
there was a trend for an increase after 30 min (20 MAPK immunoreactivity is back to basal levels after 6 h TGF-

- A
- B

FIG. 2. TGF-β1 increases nuclear levels of active MAPK in sensory neurons. A: optical sections imaged from neurons treated for 5 min with either TGF-β1 or BSA. B: summary data indicates a significant increase in phospho-

- A
- B

FIG. 3. TGF-β1 increases levels of nuclear phospho-CREB1 in sensory neurons. A: optical sections imaged from neurons treated for 5 min with either TGF-β1 or BSA. B: summary data indicates that 5 min treatment with TGF-β1 was not sufficient to significantly increase nuclear phospho-CREB1 staining (1 ± 8%, mean ± SE; n = 4, representing 23 BSA-treated and 20 TGF-β1-treated neurons, t_{1} = 0.34, P = 0.38), 30 min treatment with TGF-β1 significantly increased nuclear phospho-CREB1 staining (15 ± 4%, n = 4, representing 20 BSA-treated and 18 TGF-β1-treated neurons, t_{3} = 3.24, P < 0.05). TGF-β1-induced increases in nuclear phospho-

- A
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- A
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- C
found that the increase in nuclear phospho-CREB1 was blocked with U0126 even when induced by a higher concentration (10 ng/ml) of TGF-β1 (33 ± 10%, mean ± SE, increase in TGF-β1–treated vs. 2 ± 6% increase in U0126+TGF-β1–treated neurons, n = 5, representing 41 DMSO+BSA-treated, 42 DMSO+TGF-β1–treated, 42 U0126+BSA-treated and 47 U0126+TGF-β1–treated neurons). ANOVA revealed a significant effect of treatment between the three groups, and Tukey post hoc tests revealed that the group of neurons treated with DMSO + TGF-β1 was significantly different from the other two groups (F_{2,12} = 5.00, P < 0.05, Fig. 3C).

**Discussion**

MAPK is both necessary (Fig. 1) and sufficient (Sung et al. 2001) for the induction of long-term changes in the excitability of sensory neurons by TGF-β1. Moreover, TGF-β1 leads to an activation of MAPK in sensory neurons (Fig. 2 and Chin et al. 2002) and results in increased nuclear levels of active MAPK (Fig. 2). The rapid activation of MAPK by TGF-β1 is in contrast to a much slower activation by 5-HT, with undetectable activation at the end of a 5-min pulse of 5-HT and a graded activation/translocation with additional pulses of 5-HT (Martin et al. 1997; Michael et al. 1998; Sharma et al. 2003). Although a 5-min treatment of TGF-β1 was sufficient to activate MAPK and cause an increase in nuclear levels of activated MAPK (Fig. 2), a 6-h treatment was necessary to induce a long-term change in excitability (Fig. 1). Presumably, at some point during the 6-h treatment, levels of activated MAPK became sufficient to activate gene expression necessary for long-term changes in excitability. This hypothesis is supported by our finding that whereas 5 min treatment with TGF-β1 did not increase nuclear levels of phospho-CREB1, 30 min treatment with TGF-β1 did increase nuclear levels of phospho-CREB1 in a MAPK-dependent manner. Such an increase in nuclear phospho-CREB1 suggests that ≥30 min of TGF-β1 treatment is necessary to induce the transcriptional events necessary for long-term increases in neuronal excitability. By 6 h of TGF-β1 treatment, events necessary for long-term changes in excitability have been achieved, and active MAPK levels are back to basal levels. The intermediate steps between the activation of the TGF-β1 receptor and the activation of MAPK are not known. The most likely possibility is that the activated receptor leads directly to the activation of MAPK through Ras (Derynk and Zhang 2003). However, PKC is also known to couple to the MAPK pathway (reviewed in Sweatt 2004). An alternative possibility that needs to be examined is that TGF-β1 engages the PKC cascade. PKC is activated by TGF-β1 (Farr et al. 1999) and induces long-term changes in sensory neuron excitability (Manseau et al. 1998), and at least one MAPK-dependent protein phosphorylation in *Aplysia* is blocked by a PKC inhibitor (Yamamoto et al. 1999).

The neurotrophins have been well characterized for their roles in development as well as in adult organisms (Kojima et al. 2002; Lu 2003; Tyler et al. 2002). Although each of these growth factors can have distinct effects in neuronal plasticity, one common factor in the signaling mechanisms of both neurotrophins and cytokines seems to be the engagement of the MAPK pathway. Our results implicating MAPK in TGF-β1-induced long-term changes in sensory neuron excitability in *Aplysia* add to this body of evidence. In addition, the present results provide a starting point from which to begin examining the substrate proteins such as CREB1 that are the targets of MAPK-dependent phosphorylation and the ways those proteins lead to the induction and expression of long-term changes induced by TGF-β1.

**Acknowledgments**

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**Grants**

This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-38100 to L. J. Cleary, NS-28462 to A. Eskin, and NS-19895 to J. H. Byrne.

**References**


