Chapter 4

Novel Approaches to Studying Activity-Dependent Regulation of Neurotrophins and Neuropeptides in Sensory Pathways from Orofacial Tissues

Agnieszka Balkowiec
Oregon Health & Science University, USA

Ewa Balkowiec-Iskra
Medical University of Warsaw, Poland

ABSTRACT

Growth factors are critical for normal development of craniofacial innervation. Recent evidence indicates that neuronal growth factors, and particularly members of the neurotrophin family, are also involved in activity-dependent synaptic plasticity, with wide-ranging implications for chronic orofacial pain syndromes. The authors’ laboratory has developed novel approaches to studying regulation of neurotrophin expression and release from neurons by activity. This chapter presents their advances in preparation of neuron-enriched cultures of trigeminal ganglia followed by stimulation with physiological patterns of electrical activity alone, and in combination with neuropeptides and inflammatory mediators. It also describes the authors’ modified, ultra-sensitive ELISA methods (ELISA in situ and ‘rapid capture’ ELISA) for detection of endogenous neurotrophic factors, and other neuropeptides that are expressed and released in quantities below the detectability thresholds of standard assays. These methodologies are presented using Brain-Derived Neurotrophic Factor (BDNF) and Endomorphin-2 as examples.

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Primary sensory neurons with cell bodies in the trigeminal ganglion (TG) carry nociceptive information from the craniofacial region, including several structures linked to chronic pain conditions, such as the meninges (migraine headaches), temporomandibular joint (TMJ; TMJ disorder), muscles of mastication (bruxism-associated muscle soreness), and teeth (odontalgias). TG neurons have commonly been used as a model for studies of mechanisms that govern development of mammalian primary sensory neurons during embryonic stages. The unambiguous definition and easy accessibility of the components of the trigeminal system throughout the embryonic development have given rise to investigations of many fundamental processes involved in neural development using this model (for review, see Davies, 1988, 1997). On the other hand, the mechanisms of postnatal maturation of TG neurons, including the role of neuronal growth factors, are largely unknown. Similarly, very little has been done to characterize activity-dependent plastic changes in trigeminal pathways in response to disease processes, such as those involving neuroinflammation.

One reason for this gap in the current understanding of the trigeminal system is the limited number of research tools to: 1) reliably mimic different levels of activity in TG neurons associated with various pathophysiological conditions, and 2) detect resulting changes in expression and release of peptide neuromodulators, such as neurotrophins. The objective of this chapter is to facilitate filling this gap by presenting new strategies developed in our laboratory to: 1) prepare neuron-enriched dissociated cultures of TG, 2) detect relatively small quantities of neurotrophins and other neuropeptides expressed and/or released from TG neurons, and 3) stimulate neurotrophin and other neuropeptide expression and release from TG neurons with patterns of electrical stimuli that closely mimic neuronal activity in vivo.

There is mounting evidence that neurotrophins play a critical role in mechanisms of activity-dependent plastic changes in spinal sensory pathways, including chronic pain phenomena, such as allodynia. Recent data from our and other laboratories indicate that TG neurons express the neurotrophin brain-derived neurotrophic factor (BDNF) after the time the neurons depend on growth factors for survival (Buldyrev et al., 2006, Ichikawa et al., 2006). This raises the possibility that BDNF is a mediator of postnatal maturation and plasticity in trigeminal pathways, including those carrying nociceptive signals. Given the enormous significance of the trigeminal system in etiology of several common, debilitating pain conditions, such as migraine headaches or trigeminal neuralgia (tic douloureux), a complete understanding of the role played by neurotrophins in this system seems critical.

The first step in understanding the roles of neurotrophins at trigeminal synapses is to characterize conditions under which neurotrophins are synthesized, transported to TG neuron terminals, and released from these neurons in order to exert synaptic actions. The conditions include the effects of different patterns of neuronal activity that can be associated with certain disease states, role of other neuropeptides and inflammatory mediators, etc. The approach taken by our laboratory to address these questions is based on primary cultures of dissociated rat trigeminal ganglion neurons at various postnatal ages. The neurons are activated by electrical field stimulation to closely mimic the activity of TG neurons in vivo. Moreover, various modulators of neuronal function are added in conjunction with the electrical activation. Changes in neurotrophin expression and release are detected and measured by customized immunoassays. The following paragraphs provide a detailed description of this approach.