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Review

The role of activin in neuropeptide induction and pain sensation

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Abstract

Signals from target tissues play critical roles in the functional differentiation of neuronal cells, and in their subsequent adaptations to peripheral changes in the adult. Sensory neurons in the dorsal root ganglia (DRG) provide an excellent model system for the study of signals that regulate the development of neuronal diversity. DRG have been well characterized and contain both neurons that convey information from muscles about limb position, as well as other neurons that provide sensations from skin about pain information. Sensory neurons involved in pain sensation can be distinguished physiologically and antigenically, and one hallmark characteristic is that these neurons contain neuropeptides important for their functions. The transforming growth factor (TGF) beta family member activin A has recently been implicated in neural development and response to injury. During sensory neuron development, peripheral target tissues containing activin or activin itself can regulate pain neuropeptide expression. Long after development has ceased, skin target tissues retain the capacity to signal neurons about changes or injury, to functionally refine synapses. This review focuses on the role of activin as a target-derived differentiative factor in neural development that has additional roles in response to cutaneous injuries in the adult.

Keywords: Activin; Neuropeptides; Pain; Tissue repair; Sensory; Wound; Inflammation

Introduction

During development of the nervous system, neurons extend axons to other neurons within the central and peripheral nervous system, or to target tissues in the body where they form structural and chemical synapses. These peripheral targets may include skeletal muscle for motor neurons that eventually develop acetylcholine as a chemical neurotransmitter, blood vessels for autonomic neurons that use norepinephrine as a transmitter, or skin and spinal cord for cutaneous sensory neurons that use glutamate as a transmitter. In addition to these classical neurotransmitters, a neuron may also utilize one or more neuropeptides as a co-transmitter or modulator of function. An important challenge in developmental neurobiology has been to learn how neurons acquire their chemical identities, and how target tissues concomitantly "match" their receptor expression to these neurons for functional efficacy.

The specification and regulation of neuronal functional identity is likely to involve some combination of neural activity (Spitzer et al., 2005), expression of intrinsic transcription factors (Chen et al., 2006; Hippenmeyer et al., 2002; Kramer et al., 2006), and the focus of this review, target-derived differentiative factors (Nishi, 1994; Schotzinger and Landis, 1994). Evidence that neural activity in developing neurons contributes to neurotransmitter specification comes from studies in which calcium spike activity modulated the transmitter spinal neurons utilized, without affecting other cell identity markers (Borodinsky et al., 2004). Interestingly, this regulation appears to be homeostatic, that is, suppression of activity leads to increased excitatory transmitters, while enhanced activity leads to increased inhibitory transmitters.

Instructive cues from embryonic tissues can promote specific neural phenotypes and limit cellular potential of precursors for the peripheral nervous system originating in the neural crest. Elegant transplantation studies by Le Douarin and colleagues in which small pieces of quail were placed in heterologous

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locations in chick (and vice versa) demonstrated that peripheral nervous system components originating from the neural crest are quite responsive to environmental cues (Le Douarin et al., 1977, 1981; Le Lievre et al., 1980). Indeed, both *in vivo* transplantation studies and *in vitro* mechanistic studies led to the identification of a number of candidate differentiation factors that act upon neural crest cells. For example, neural crest cells that encounter bone morphogenetic proteins (BMPs) in the dorsal aorta (or in exogenous locations experimentally) initiate sympathetic neuron differentiation including neurotransmitter specification (Reissmann et al., 1996; Schneider et al., 1999).

Later aspects of neuronal cell fate are also regulated by target-derived factors. One example of the role of target is found in studies of sympathetic innervation of the sweat gland. While most sympathetic neurons contact blood vessels and utilize noradrenaline as a neurotransmitter, a few neurons contact sweat glands, switch their neurotransmitter phenotype and utilize acetylcholine as a transmitter (Francis and Landis, 1999). Key experiments in understanding the role of target tissues in this system involved transplanting foot pad tissue rich in sweat glands to areas that normally receive adrenergic sympathetic innervation (Schotzinger and Landis, 1988). Although factors such as leukemia inhibitory factor, ciliary neurotrophic factor, or cardiotrophin-1 are capable of causing this adrenergic to cholinergic switch *in vitro* (Habecker et al., 1995, 1997), it is still not clear what actual factor from sweat gland produces the transmitter switch *in vivo*.

Activin functions in neuropeptide induction

Current work suggests that activin may act as a target-derived factor to promote pain-sensing neuron differentiation during development. Activin was initially identified as a regulator of pituitary follicle-stimulating hormone in the reproductive axis (Schwall et al., 1988) and has been implicated in many functions in nervous system development. For example, studies suggest activin may be a survival factor for rodent CNS hippocampal and cortical neurons (Iwahori et al., 1997; Schubert et al., 1990). More recently, activin's biological activity has emerged in a surprising arena: the specification and differentiation of neurons.

Like the other members of the TGF-β superfamily, activins are dimeric proteins consisting of disulphide-linked βA and βB subunits: the homodimeric activin A (βA-βA), activin B (βB-βB) and heterodimeric activin AB (βA-βB) (Massague, 2000). Activins signal through a type I receptor called activin receptor like kinases or ALK4/ActRIB, type II receptors including activin receptor IIA and ActRIIB and an intracellular smad pathway. When activin binds to ActRII, ALK4 is recruited and its Ser/Thr kinase activity is activated. Smad2 or smad3 is then phosphorylated by activated ALK4 and binds to smad4 to form a heteromeric complex. This activated smad complex translocates into the nucleus and, in conjunction with other nuclear binding proteins, regulates the transcription of target genes (Fig. 1A). The translocation of specific smads, or appearance of phosphorylated smads is a useful tool for assaying activin signaling. Although activin clearly activates smads during its actions on embryonic sensory neurons (Ai et al., 1999), the identification of the complement of target genes regulated by activin forms an active area of investigation.

Target-regulated control of neurotransmitter phenotype involves activin. In the parasympathetic chick ciliary ganglion, large ciliary neurons that use acetylcholine as a transmitter innervate striated muscle in the iris and ciliary muscle, while small choroid neurons with the neuropeptide somatostatin innervate blood vessels in the choroid of the eye. Somatostatin appearance coincides with target tissue contact, and indeed, in cell culture, target choroid cells release material that stimulates somatostatin differentiation And this target-derived material turns out to be activin (Coulombe et al., 1993). *In vivo*, it appears that somatostatin differentiation is the result not of activin expression alone, which occurs in both choroid and iris target tissues, but by the absence of follistatin, a natural inhibitor, in the choroid (Darland et al., 1995).

Similarly, in the *Drosophila* CNS, peptidergic neurons are regulated in part by target-derived factors in the family that contains activin (Allan et al., 2003). In this case, six ventral nerve cord neurons within the group specified by the homeodomain gene apterous express the neuropeptide gene FMRFamide. Appropriate FMRFamide expression appears due to a target-derived transforming growth factor beta (TGFβ) family member called glass bottom boat activating a presynaptic receptor and acting via a retrograde signal within neurons also expressing an intrinsic transcription factor.

Studies on developing rodent sensory neurons also point to activin as a skin-derived factor that induces *de novo* calcitonin gene related peptide (CGRP) expression (Ai et al., 1999; Hall et al., 2001, 2002). Early embryonic (E14) rat DRG contain no detectable CGRP mRNA or protein (Hall et al., 1997), although after peripheral connections are functional (E18–19) neuropeptides become apparent and increase (Hall et al., 1997; Kessler and Black, 1981; Marti et al., 1987; Senba et al., 1982). These data point to target-derived factors in the development of subsets of neuropeptide-containing sensory nociceptors. Not only is the appearance of neuropeptides coincident with target contact, but the co-culture of embryonic DRG neurons with an embryonic skin cell line or treatment with skin conditioned medium from a skin cell line induces *de novo* CGRP expression, and this function is blocked with antibody to activin (Hall et al., 1997, 2001).

Application of recombinant activin or the related BMPs specifically induces neuropeptide expression in naïve embryonic rat sensory neurons in a concentration-dependent manner, such that about 60% of neurons become CGRP-immunoreactive (IR), and neuronal survival is not affected (Ai et al., 1999). Although both activin and BMPs can induce CGRP expression, only anti-activin antibody blocked the ability of skin-conditioned medium to induce CGRP expression in embryonic neurons suggesting activin is the active ligand (Hall et al., 2001). Further, embryonic skin contains activin protein and embryonic gut contains detectable BMP4, suggesting that these specific ligands may affect subsets of sensory neurons that project to different targets. By contrast, although NGF supports embryonic neuron survival, and can increase CGRP expression