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Sex-specific pain modulation: The growth factor, neuregulin-1, as a pro-nociceptive cytokine

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ABSTRACT

An increasing amount of evidence indicates that there are significant sex differences in clinical and experimental pain sensitivity in men and women. While it is now clear that the endogenous sex steroids are involved in mediating these sex differences, the cellular and molecular mechanisms that underlie their effects on nociceptive sensitivity remain elusive. Recent studies have shown that sex steroids are potent regulators of gene expression in glial cells, particularly astrocytes. This review specifically highlights some of the evidence of sex steroid regulation of growth factor expression. Growth factors have been shown to be potent pro-nociceptive mediators in rodents. Thus, regulation of their expression by sex steroids may be a general mechanism by which sex steroids exert their effect on pain sensitivity. One such mechanism, the progesterone specific regulation of the growth factor, neuregulin-1, following nerve root injury in the rat, is described in detail. Neuregulin-1 expression is increased in spinal cord astrocytes only in female rats with circulating progesterone. Neuregulin-1 has also been shown to produce transient tactile allodynia when delivered intrathecally in rats. Our understanding of growth factor regulation by sex steroids promises to open up new avenues of investigation into the mechanisms that drive sex differences in pain sensitivity.

It has been observed that differences exist between men and women in terms of sensitivity to painful stimuli and incidence of several pain syndromes. Women typically have lower pain thresholds and have less tolerance to painful stimuli than males [3]. Importantly, several pain syndromes have significant sex differences in clinical incidence [34]. Many studies to date have focused on the pivotal role of the sex steroid hormones in the modulation of sensory systems and nociception in order to understand their role in mediating sex differences in pain [1,6,12,19]. The major steroid molecules produced by the ovaries and testes are broadly classified as the sex steroids. These include the estrogens and progestins, and the androgen steroids testosterone and dihydrotestosterone.

Recent decades have seen a revolution in our understanding of the role that glial cells (particularly astrocytes and microglia) play in most, if not all, aspects of the pathological and inflammatory processes of the central nervous system (CNS) following injury or infection. Previously considered only supporting cells in the CNS, our current understanding now recognizes that glial cells are active participants in synaptic transmission the functional unit of neurotransmission must now be considered to include both neuronal *and* glial components [9]. In response to CNS pathology, glial cells become "reactive" and in doing so, they can alter neuronal excitability. This is particularly true in the case of peripheral nerve/nerve root-induced chronic pain syndromes, in which glial cell activation has been hypothesized be intimately involved in the initiation and maintenance of the chronic pain state.

Reactive glial cells produce and release a plethora of potential algesic mediators including cytokines. Cytokines are small, polypeptide regulatory molecules expressed and released by immune cells which act to modulate the inflammatory responses of other immune cells. Outside of their classical immune modulation functions, cytokines are recognized to be neuromodulatory molecules as well [32]. Exogenous delivery of several pro-inflammatory cytokines to the CNS and peripheral nerves results in a hyperalgesic state suggesting a link between pro-inflammatory cytokine expression and pain hypersensitivity [8,26,33]. Therefore, the regulation of cytokine expression and release may have profound effects on initiation and maintenance of chronic pain states.

The literature describing the effects of sex steroids on nociceptive behaviors in rodents is quite contradictory. While they certainly seem to have genuine biological effect, their actions (whether pro- or anti-nociceptive) appear to be dependent on a large number of variables, including: which sex steroid or combination of sex steroids, the dose (physiological or pharmacological), the duration of dosing and timing of exposure, and the genetic background of the animal. Despite all of these confounding variables, several general-

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izable truths have been gleaned from the literature: (1) sex steroids are generally neuroprotective and reduce inflammation in the CNS [10,25]. (2) Sex steroids are direct and indirect neuromodulators in many different brain regions [4]. (3) Sex steroids, as neuromodulators, affect nociceptive behavior in both rodents and humans [2]. The mechanisms in which these sex steroids act as neuromodulators, especially through astrocytes following CNS insult, are rapidly being uncovered.

Astrocytes have important homeostatic functions by removing ions and neurotransmitters from the extrasynaptic space through specialized transporters [36]. They also form an integral part of neurovascular coupling and the blood–brain barrier [37]. Like microglia, astrocytes transform into a reactive phenotype following CNS injury or infection, which is associated with morphological changes, intermediate filament upregulation, and increased expression and release of a large variety of pro-algesic mediators. Several lines of evidence suggest that these growth factors and neurotrophic factors in the CNS can increase pain-related behaviors in various animal models of pain [9,15,22]. Furthermore, astrocytes may be a key source for the production and secretion of these growth factors in the CNS, particularly after becoming activated in response to injury or infection [30]. An increase in the local production of growth factors is thought to act as a "double edged sword" following injury. That is, they support neurons from cell death by increasing the trophic environment (neuroprotection) while at the same time heightening nociceptive sensitivity.

The mechanisms by which growth factors act as neuromodulatory molecules are an area of active investigation. Growth factor receptors belong to the family of receptor tyrosine kinases. When a particular growth factor binds to its cognate receptor, the resulting signaling cascade(s) activate downstream second messengers such as the mitogen-activated protein kinases (MAPKs). In sensory neurons, this in turn regulates the transcription of a variety of genes known to be involved in the generation of central sensitization, including ion channels, neurotransmitter receptors, and neuropeptides [14]. In this manner, growth factors can profoundly affect the pathophysiologic changes that accompany chronic pain syndromes.

Astrocytes are known to express sex steroid receptors [16,17] and they respond to steroid molecules through alterations in cell morphology, cellular metabolic functions, and regulation of gene expression (see review; [18]). In particular, sex steroids are known to be potent inducers of growth factor expression in several brain regions [5,29] and in dorsal root ganglion sensory neurons [31]. This is especially true for growth factors that are known to sensitize nociceptive systems. Thus, it appears that one of the important actions of sex steroids in the CNS is to modulate growth factor expression in astrocytes, which may be one of the mechanisms by which glia cells can facilitate nociceptive sensitivity (Fig. 1). For example, it has been shown that gonadal hormone status modulates the expression of astrocyte-derived basic fibroblast growth factor (bFGF) following 5-hydroxydopamine lesion in the brain [35]. Likewise, several studies have demonstrated that estrogen and progesterone regulated nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) mRNA and protein expression in several of different brain regions [5,29]. Progesterone also was show to increase the expression of BDNF mRNA and protein following spinal cord injury [13]. Finally, transforming growth factor-β (TGF-β), a growth factor expressed and released by astrocytes, is known to have neuroprotective effects both *in vitro* and *in vivo*. Several studies have demonstrated the estrogen is a strong promoter of TGF-β expression and release in astrocytes [11]. This mechanism appears to be mediated by a non-classical, non-genomic estrogen receptor mechanism that activates cell signaling pathways [11]. These select examples highlight the importance of circulating sex steroids on the basal and injury-induced expression of growth factors in the central nervous system.

In a series of experiments, our laboratory has observed a pronociceptive effect of chronic, physiological levels of progesterone on both basal nociception and the development of allodynia following nerve root ligation, a rodent model of radiculopathy-associated low back pain in the rat. Like other groups, we observed that female rats displayed higher levels of pain behaviors and that this pain hypersensitivity was dependant on gonad derived, circulating sex steroids [19,20]. Hormone replacement of ovariectomized female rats with subcutaneous pellets that released physiological levels of progesterone was shown to completely rescue the ovariectomy-mediated decrease in pain behaviors [21]. In this chronic exposure paradigm it appears that progesterone drives the phenomenon of female behavioral hypersensitivity following nerve root injury.

In order to elucidate the molecular mechanisms that drive the effect of sex steroids on pain behaviors, we performed a high-density mRNA microarray screen on spinal cord tissue derived from male and female rats following nerve root injury. We observed a significant sex difference in the expression of both the neuregulin-1

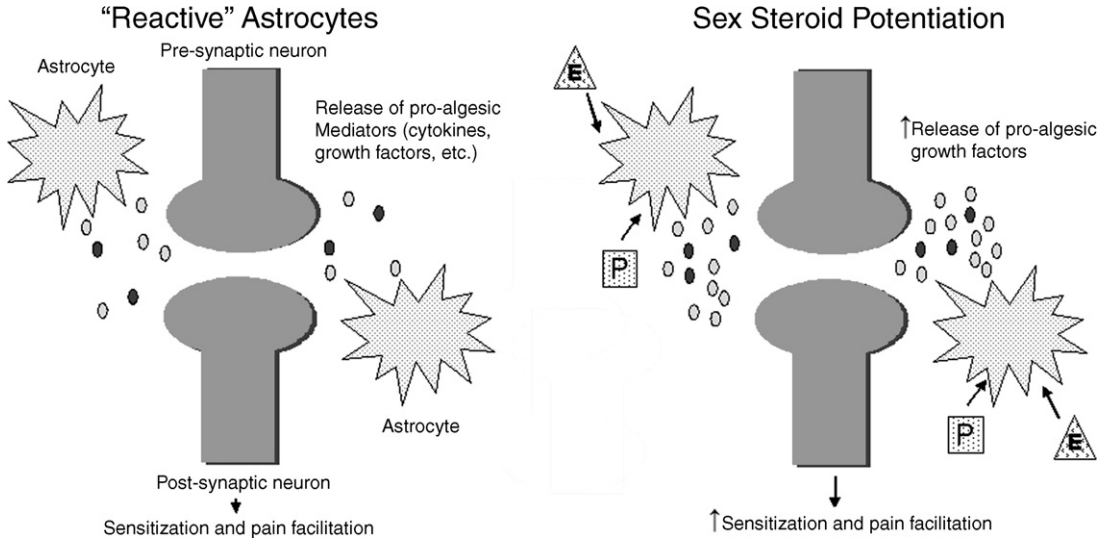

Fig. 1. Schematic depiction of neuronal sensitization and pain facilitation by reactive astrocytes through pro-algesic mediator release. The presence of estrogen (E) and/or progesterone (P) modulates cell signaling cascades that increase the expression and subsequent release of growth factors, which then further sensitize sensory neurons.