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Uncovering molecular elements of brain–body communication during development and treatment of neuropathic pain

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Abstract

Integral to neuropathic pain is a reciprocal interaction between tumor necrosis factor-α (TNF) production and the α_2-adrenergic receptor response, offering an attractive therapeutic target. The effects of varying levels of brain TNF on α_2-adrenergic regulation of cyclic AMP (cAMP) production in the hippocampus and sciatic nerve were investigated during the development and amitriptyline treatment of chronic pain. Increased levels of TNF during the development of chronic pain transform α_2-adrenergic inhibition of cAMP production in the brain to potentiation. While α_2-adrenergic receptors regulate TNF production, they also affect descending noradrenergic pathways. Increases in levels of TNF in the brain deeply impact peripheral inflammation through regulating α_2-adrenergic receptors, offering insight into brain–body interactions during neuropathic pain. Amitriptyline as an analgesic inhibits pain-induced increases in brain-associated TNF and transforms peripheral α_2-adrenergic receptors. The dynamic equilibrium between TNF levels and α_2-adrenergic functioning is uniquely altered during development and treatment of neuropathic pain. Proper manipulations of this interaction offer efficacious treatment of neuropathic pain.

Keywords: Neuropathic pain; Tumor necrosis factor; Chronic constriction injury; Hyperalgesia; α_2-Adrenergic receptor; Cyclic adenosine monophosphate (cAMP); Hippocampus; Sciatic nerve; Rat

1. Introduction

Neuropathic pain, a chronic pain state arising from injury to the nervous system, remains a widely prevalent clinical problem due to our incomplete understanding of its pathogenesis. Over three million people in the U.S. alone suffer from chronic pain of neuropathic origin (Ariniello, 1999; Marx, 2004). Unfortunately, the treatment regimes currently followed target only the resulting symptoms, an unfruitful approach as shown by clinical experience. Clearly, it is imperative to identify proximal mechanisms that cause pain to become chronic. Supraspinal mechanisms are intricately involved in the processing of pain signals, as revealed by functional magnetic resonance imaging (Borsook et al., 2004; Buffington et al., 2005). These mechanisms drive the pathology of chronic pain, ultimately contributing to the disabilities culminating from the initial insult. Understanding these mechanisms is therefore key to the development of effective treatments of chronic pain.

A principal mechanism identified in neurons that may be targeted for therapy is the functional interactive relationship between production of the cytokine tumor necrosis factor-α (TNF) and the activation of α_2-adrenergic receptors by the neurotransmitter norepinephrine (Ignatowski et al., 1997; Ignatowski and Spengler, 1994; Nickola et al., 2000; Renauld and Spengler, 2002). Activation of the α_2-adrenergic receptor can either enhance or inhibit the production of TNF, dependent on the extra-cellular levels

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of TNF (Renauld et al., 2004). TNF, in turn, regulates coupling of the α_2-adrenergic receptor to specific G-proteins (Reynolds et al., 2005a), thereby directing the signal transduction of that receptor. These events occur within an equilibrium by which physiologic levels of TNF and normal functioning of the α_2-adrenergic receptor are preserved. A perturbation of this system, such as a pathologic increase in the levels of brain-derived TNF (Ignatowski et al., 1999), induces a dysfunctional adaptation of the α_2-adrenergic receptor (Covey et al., 2000), and disrupts this natural balance thereby directing the pathology of chronic pain.

Antidepressant drugs such as amitriptyline are commonly used in the treatment of chronic pain (Briley, 2004; Rizzati-Barbosa et al., 2003). It appears that a molecular basis for the analgesic action of antidepressants is perturbation of the aforementioned interactions between TNF production and α_2-adrenergic activation, in a direction opposite to that observed during the development of chronic pain (Ignatowski et al., 2005). This hypothesis is based on the decreased production of TNF that occurs in the brain following antidepressant drug administration (Ignatowski et al., 1997), which in turn, transforms the function of the α_2-adrenergic receptor from inhibiting to increasing norepinephrine release (Reynolds et al., 2004a,b). This transformation is significant because during the development of chronic pain, α_2-adrenergic inhibition of norepinephrine release in the brain is enhanced, which would contribute to the development of chronic pain (Covey et al., 2000). However, it remains to be elucidated as to how these effects of amitriptyline relate to its analgesic properties. Therefore, we administered amitriptyline during the onset and the development of neuropathic pain to investigate its effect on the functional interactions between TNF production and α_2-adrenergic receptor activation, which we propose, constitutes a mechanism of its analgesic action.

The production of TNF, by virtue of its effect on the α_2-adrenergic receptor, regulates the activity of noradrenergic neurons in the brain (Covey et al., 2000; Ignatowski et al., 1996, 1997; Ignatowski and Spengler, 1994; Reynolds et al., 2004b). Since noradrenergic neurons in the brain direct descending modulation of pain, it was our goal to determine whether this relationship between TNF production and the α_2-adrenergic receptor activation in the brain directs the development and resolution of a peripheral nerve injury.

Implications of brain–body communication are emerging concepts in pain research. Therefore, in the present investigation, we determined whether varying the levels of TNF in the hippocampus can modify a peripheral injury. The hippocampus was investigated since it is involved in processing painful stimuli (Khanna and Sinclair, 1989; McEwen, 2001), and is replete with α_2-adrenergic receptors (Scheinin et al., 1994). Additionally, an increase in levels of TNF in the hippocampus is pivotal in the development of chronic pain (Ignatowski et al., 1999).

The production of cyclic adenosine monophosphate (cAMP), a second messenger in α_2-adrenergic receptor signaling, was assessed both in the hippocampus and in a peripheral nerve (sciatic nerve). We hypothesize that increased levels of TNF in the brain supports a pro-inflammatory response from α_2-adrenergic receptor activation in the periphery, while a decrease in levels of TNF in the brain supports an anti-inflammatory response. In particular, since cAMP is a second messenger that decreases the production of the pro-inflammatory cytokine TNF, a receptor-stimulated increase in cAMP production would, therefore, be anti-inflammatory. Determining the effects that changes in brain levels of TNF have on a peripheral injury of the sciatic nerve will enable a better understanding of the molecular basis of brain–body communication, and provide a promising target for effective therapeutic management of neuropathic pain.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (300–350 g) (Harlan Sprague-Dawley Inc., Indianapolis, IN) were used for all experiments. The rats were housed in Laboratory Animal Facility-accredited pathogen-free quarters at 23 ± 1 °C, with access to food and water *ad libitum*. The animals were maintained on a 12 h light/dark cycle. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University at Buffalo as well as with the guidelines for the ethical treatment of animals established by the National Institute of Health. All efforts were made to ensure minimal animal suffering, as well as to use the minimum number of animals necessary to achieve statistically significant results.

2.2. Chronic constriction injury (CCI) model

Animals were anesthetized with an intra-peritoneal (i.p.) injection of ketamine (60 mg/kg) and xylazine (3 mg/kg). For inducing chronic constriction injury (CCI) to the right sciatic nerve, the nerve was exposed at mid-thigh level, freed of adherent tissue, and four loose chromic gut ligatures (Roboz Surgical Instrument Co., Inc., Rockville, MD) were applied, proximal to the trifurcation in the nerve (Bennett and Xie, 1988). In sham procedures, the nerve was similarly exposed, but no ligatures were applied.

2.3. Intra-peritoneal drug administration schedule

In experimental animals receiving i.p. treatment with amitriptyline (10 mg/kg) (Sigma–Aldrich, St. Louis, MO), or its vehicle, saline, were administered 1 h before sciatic nerve surgery, and continued every 12 h. Animals were decapitated 12 h after the last injection.

2.4. Thermal hyperalgesia

The rats were tested for increased sensitivity to a noxious thermal stimulus every other day throughout the eight-day testing period. Hyperalgesia (increased sensitivity to sensory stimuli) was measured by determining changes in paw withdrawal latency (PWL) using a plantar algesia apparatus (Analgesia Meter model #33, IITC Life Science Instruments, Woodland Hills, CA) (Hargreaves et al., 1988). A "difference score" generated from subtracting the contralateral PWL from the ipsilateral PWL was used as an index of hyperalgesia. PWL was measured using a radiant heat source (58 ± 0.1 °C) to stimulate thermal receptors in the foot. The use of this apparatus is based on the fact that peripheral nerve injury results in increased sensitivity to a sensory (thermal) stimulus. A maximal automatic cut-off latency of 15 s was used to prevent tissue damage. Rats were placed in Plexiglas chambers, on top of a temperature maintained (32 ± 0.1 °C) glass surface. Rats were acclimated to the testing apparatus