Neuroscience 233 (2013) 174–183

THE ERYTHROPOIETIN-DERIVED PEPTIDE ARA290 REVERSES MECHANICAL ALLODYNIA IN THE NEURITIS MODEL

K. G. T. PULMAN, M. SMITH, M. MENGOZZI, P. GHEZZI AND A. DILLEY*

Division of Clinical and Laboratory Investigation, Brighton and Sussex Medical School, Medical Research Building, University of Sussex, Falmer, Brighton BN1 9PS, United Kingdom

Abstract—Studies on the neuritis model suggest that in many patients with neuropathic pain, symptoms may be due to nerve inflammation rather than frank nerve injury. Treatments for these patients are often ineffective. The neuroprotective and hematopoietic agent erythropoietin (EPO) has been shown to reverse pain behaviors in nerve injury models and therefore may be of therapeutic benefit. However, EPO can cause thrombosis. ARA290 is an analog of EPO that has the neuroprotective activities of EPO without stimulating hematopoiesis. The present study has examined the effects of ARA290 on pain behavior in the neuritis model. Following neuritis induction, 30 or 120 μg/kg ARA290 or saline vehicle was injected intraperitoneally into rats daily from day 1 post surgery. Animals were assessed for mechanical allodynia and heat hyperalgesia. Levels of the cytokine tumor necrosis factor-α (TNF-α) and chemokine (C—C motif) ligand 2 (CCL2) mRNA were also assessed using polymerase chain reaction. Vehicle-treated neuritis animals ($n = 20$) developed signs of mechanical allodynia and heat hyperalgesia that reached a maximum on day 4 and 3 of testing, respectively. Treatment with either 30 ($n = 11$) or 120 μg/kg ARA290 ($n = 9$) prevented the development of mechanical allodynia. However, ARA290 did not significantly affect heat hyperalgesia. There was no significant difference between the effects of each drug dose ($p < 0.05$, unpaired *t* test comparing area under the curve for mechanical allodynia). The levels of CCL2 and TNF-α mRNA in the nerve and Gelfoam were not significantly different following 120 μg/kg ARA290 treatment ($n = 3$–7) compared to vehicle-treated animals ($n = 3$–7; $p = 0.24$; unpaired *t* tests). In summary, ARA290 may be beneficial in the treatment of neuropathic pain symptoms where signs of nerve injury are absent on clinical assessment. The mechanisms of action do not appear to involve the inhibition of TNF-α or CCL2 production.

Key words: neuritis, erythropoietin, allodynia, hyperalgesia, tumor necrosis factor, chemokine (C—C motif) ligand 2.

INTRODUCTION

Neuropathic pain is a complex and debilitating disorder. Treatments are often ineffective, which may reflect our limited understanding of the underlying mechanisms. Much of our current knowledge has been gained through the use of animal models, such as the chronic constriction injury, spinal nerve ligation and spared nerve injury models. These models, which are all associated with significant nerve injury, produce pain behaviors that are consistent with clinical signs of allodynia and hyperalgesia (Bennett and Xie, 1988; Kim and Chung, 1992; Decosterd and Woolf, 2000). In the clinic however, many patients with the symptoms of neuropathic pain do not appear to have signs of nerve injury on routine clinical testing. These patients may be diagnosed with conditions such as complex regional pain syndrome type 1, non-specific arm pain (also known as repetitive strain injury) or back pain. Studies on the neuritis model, a model of localized peripheral nerve inflammation, suggest that symptoms in these patients may be due to inflammation and not frank nerve injury (Eliav et al., 1999; Bove et al., 2003; Dilley et al., 2005). In the neuritis model, animals develop signs of mechanical allodynia and heat hyperalgesia in the absence of axonal degeneration or demyelination (Eliav et al., 1999; Chacur et al., 2001; Bove et al., 2003). These behavioral changes are rapid and short-lived, and begin to resolve at 1 week. On electrophysiological examination, intact nociceptive (C-fiber) axons develop ongoing (spontaneous) activity and axonal mechanical sensitivity at the inflamed site (Bove et al., 2003; Dilley et al., 2005).

A novel modulator of inflammation that may be beneficial in the treatment of the symptoms of neuropathic pain is the agent erythropoietin (EPO). EPO is typically associated with hematopoiesis but has also been shown to be tissue protective in other tissues, which include the nervous system (Brines et al., 2000; Villa et al., 2003). Both EPO and its receptors are expressed within the peripheral nervous system and, following nerve injury, EPO is upregulated in Schwann cells (Campana and Myers, 2003). Binding of EPO to receptors on injured neurons can prevent axonal degeneration (Keswani et al., 2004). The neuroprotective activity of EPO in the peripheral nervous system has led to several studies that have examined the systemic administration of recombinant human EPO as a potential treatment for neuropathic pain. These studies have shown that the administration of human recombinant EPO is effective in

*Corresponding author. Tel: +44-(0)1273-877094; fax: +44-(0)1273-877884.
E-mail address: a.dilley@bsms.ac.uk (A. Dilley).

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CCL2, chemokine (C—C motif) ligand 2; EPO, erythropoietin; qPCR, quantitative polymerase chain reaction; TNF-α, tumor necrosis factor.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.12.022

reversing nerve injury-induced pain behaviors, such as mechanical allodynia and heat hyperalgesia (Campana and Myers, 2003; Keswani et al., 2004; Campana et al., 2006; Jia et al., 2009a,b). EPO is reported to perform this function by preventing neuronal apoptosis and axonal degeneration (Sirén et al., 2001; Campana and Myers, 2003; Keswani et al., 2004; Campana et al., 2006) or by reducing the production of cytokines (Villa et al., 2003; Campana et al., 2006; Jia et al., 2009a). Proinflammatory cytokines are reputed to play a significant role in the development of neuropathic pain. For example, tumor necrosis factor-α (TNF-α) can cause primary sensory neurons to develop ongoing (spontaneous) activity (Sorkin et al., 1997; Leem and Bove, 2002; Schafers et al., 2003; Richards et al., 2011), which may drive central mechanisms that lead to the symptoms of allodynia (LaMotte et al., 1991; Gracely et al., 1992; Campbell and Meyer, 2006; Woolf, 2011).

There are a number of issues with administering EPO for the treatment of neuropathic pain. Activation of erythropoiesis by EPO increases the hematocrit. This effect, along with platelet activation and raised blood pressure, will increase the risk of thrombosis (Corwin et al., 2007). More recently an EPO analog, ARA290, has been developed that mimics the neuroprotective activities of EPO without stimulating hematopoiesis (Brines et al., 2008). This 11-amino acid peptide has been shown to provide long-term relief of nerve injury-induced mechanical and cold allodynia in the spared nerve injury model via activation of the tissue-protective receptor (the EPO receptor–β-common-receptor complex) (Swartjes et al., 2011). The present study has expanded on this previous investigation by focusing on the potential beneficial effects of ARA290 on pain behavior in the neuritis model, which, in contrast to the spared nerve injury model, lacks gross nerve pathology. Specifically, it has examined the effects of ARA290 administration on the development of mechanical allodynia and heat hyperalgesia. A previous study from our laboratory has inferred a role for the cytokine TNF-α and chemokine (C—C motif) ligand 2 (CCL2) in the maintenance of neuritis-induced ongoing activity (Richards et al., 2011). Therefore, the effects of ARA290 on the levels of mRNA for TNF-α and CCL2 were also examined at the peak of the neuritis (day 4) and at a later time point (day 11) when most behavioral effects have resolved.

EXPERIMENTAL PROCEDURES

Animals and surgery

Experiments were carried out in strict accordance with the UK Animals (Scientific Procedures) Act (1986). A total of 58 adult male Sprague Dawley rats (240–355 g; Harlam, UK) were used in this study.

Forty-six animals underwent neuritis surgery as previously described (Eliav et al., 1999; Bove et al., 2003; Dilley et al., 2005). Animals were anesthetized and maintained on isoflurane (1.75%) in oxygen. The left sciatic nerve was exposed at the mid-thigh by blunt dissection through the biceps femoris muscle, and a 7–8 mm length carefully separated from adjacent connective tissue. The nerve was loosely wrapped in a strip (approximately 3 mm × 3 mm × 10 mm) of sterile Gelfoam (Spongostan; Ferrosan, Denmark) saturated with approximately 150 μl Complete Freund's adjuvant (Sigma, Dorset, UK; diluted 1:1 using sterile saline). The muscle and skin were closed using 4/0 monofilament sutures (Vicryl; Ethicon, West Lothian, UK) and the animals were allowed to recover for 24 h.

Behavioral testing

Mechanical allodynia. Mechanical allodynia was tested by applying von Frey hairs of increasing stiffness (Ugo Basile, Varese, Italy) to the glabrous skin on the plantar surface of the foot. The current protocol was modified from previously published methods (Tal and Bennett, 1994).

The test apparatus consisted of Perspex animal enclosures that were raised on a metal-perforated floor. Animals were habituated to the apparatus for 1 h on three consecutive days before the start of the testing period. Behavioral testing was always performed at the same time on each day. On the day of testing, animals were acclimated for 15 min to allow for exploration and major grooming activities to cease. Each von Frey hair was presented perpendicular to the plantar surface of the foot, with sufficient force to cause slight buckling against the paw, and held for 5 s. A positive response was noted if the paw was sharply withdrawn. Each hair was applied a maximum of five times at 10-s intervals or until two consecutive positive responses were recorded. The minimum size von Frey hair that produced two consecutive responses was designated the withdrawal threshold. Testing commenced using a 4-g von Frey hair, which avoided unnecessary repeated application of finer von Frey hairs. If the animal failed to respond, von Frey hairs of increasing stiffness (6, 9, 12 and 15 g) were applied with an interval of 1 min between hairs until two consecutive responses with the same hair were recorded. If there were no responses by the final testing of the 15-g filament, a threshold of 15 g was assigned. If the animal responded to the 4-g von Frey hair, the finest von Frey hair (0.4 g) was applied followed by hairs of ascending stiffness (0.7, 1.2, 1.5 and 2 g). Each repeat test was applied to a different area of the glabrous skin. Both ipsilateral and contralateral sides were tested. The side to be tested first was randomized and the investigator waited 5 min before starting the second side. Three pre-surgery withdrawal thresholds were established on three separate days prior to neuritis surgery and averaged as the baseline value. Withdrawal thresholds were further determined on days 1, 2, 3, 4 and 7 post-surgery.

Heat hyperalgesia. Heat-hyperalgesia was tested using the Hargreaves method, as described elsewhere (Hargreaves et al., 1988). The apparatus consisted of Perspex animal enclosures that were raised on an elevated glass platform (Ugo Basile, Italy). Similar to the mechanical allodynia protocol, animals were habituated to the apparatus for 1 h on three consecutive days before the start of the testing period. Behavioral testing was always performed at the same time on each day. On each test day, animals were acclimated for 15 min prior to testing. A calibrated movable radiant heat source beneath the floor was aimed at the mid-plantar hind paw. Onset of the stimulus activated a timer that automatically stopped when the animal withdrew its foot. Prior to commencing the study, the intensity of the heat source was set such that the mean baseline withdrawal latency was 10–15 s for the size and strain of the rat being tested. A cut off of 20 s was set to avoid damage to the skin.

To avoid a sensitisation effect of repeated testing, only one test was performed on each side. The investigator waited 10 min before starting on the second side. The ipsilateral foot was always tested first since, during preliminary experiments, there was a tendency for the second foot to be tested to