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The control of alternative splicing by SRSF1 in myelinated afferents contributes to the development of neuropathic pain

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ABSTRACT

Neuropathic pain results from neuroplasticity in nociceptive neuronal networks. Here we demonstrate that control of alternative pre-mRNA splicing, through the splice factor serine-arginine splice factor 1 (SRSF1), is integral to the processing of nociceptive information in the spinal cord.

Neuropathic pain develops following a partial saphenous nerve ligation injury, at which time SRSF1 is activated in damaged myelinated primary afferent neurons, with minimal found in small diameter (IB_4 positive) dorsal root ganglia neurons. Serine arginine protein kinase 1 (SRPK1) is the principal route of SRSF1 activation. Spinal SRPK1 inhibition attenuated SRSF1 activity, abolished neuropathic pain behaviors and suppressed central sensitization. SRSF1 was principally expressed in large diameter myelinated (NF200-rich) dorsal root ganglia sensory neurons and their excitatory central terminals (vGLUT1 + ve) within the dorsal horn of the lumbar spinal cord.

Expression of pro-nociceptive VEGF-A_{xxx}a within the spinal cord was increased after nerve injury, and this was prevented by SRPK1 inhibition. Additionally, expression of anti-nociceptive VEGF-A_{xxx}b isoforms was elevated, and this was associated with reduced neuropathic pain behaviors. Inhibition of VEGF receptor-2 signaling in the spinal cord attenuated behavioral nociceptive responses to mechanical, heat and formalin stimuli, indicating that spinal VEGF receptor-2 activation has potent pro-nociceptive actions. Furthermore, intrathecal VEGF-A_{165}a resulted in mechanical and heat hyperalgesia, whereas the sister inhibitory isoform VEGF-A_{165}b resulted in anti-nociception. These results support a role for myelinated fiber pathways, and alternative pre-mRNA splicing of factors such as VEGF-A in the spinal processing of neuropathic pain. They also indicate that targeting pre-mRNA splicing at the spinal level could lead to a novel target for analgesic development.

1. Introduction

Insults to the peripheral nervous system usually result in pain and hypersensitivity to noxious (hyperalgesia) and innocuous (allodynia) stimuli. These abnormal sensations arise due to neuronal plasticity leading to alterations in sensory neuronal excitability. These alterations include peripheral sensitization (Djouhri et al., 2006), with enhanced evoked and on-going activity in primary afferents, and central sensitization, responsible for the generation and maintenance of chronic pain.

The most widely accepted model for establishment of central sensitization is that ectopic firing/increased activity in C-nociceptive afferents drives altered spinal sensory processing, particularly the processing of A-fiber inputs, resulting in secondary hyperalgesia and allodynia (pain remote from an area of damage) (Li et al., 1999; Woolf and King, 1990; Woolf, 2011) (Kramer and Doring, 2013; Torebjörk et al., 1992; Ziegler et al., 1999a). C-nociceptor changes are reported in the majority of studies of animal or human neuropathies (Ali et al., 1999; Chen and Levine, 2007; Djouhri et al., 2006; Khan et al., 2002; Kirillova et al., 2011; Serra et al., 2012; Serra et al., 2014; Shim et al., 2007; Zhu and Henry, 2012) (although not all e.g. (Chen and Levine, 2007; Khan et al., 2002)). Central sensitization can also occur through neuro-immune interactions, following injury-induced local immune cell infiltration and cytokine production/release (Uceyler and Sommer, 2008). After nerve injury there is activation of spinal glia, disruption of the blood-spinal cord barrier, and consequent infiltration of immune cells (Clark et al., 2013). These events can alter the central processing of peripheral inputs, implicated in the development of chronic pain (Geranton et al., 2009; Kim et al., 2001; Torsney, 2011b). There is, however still debate

Abbreviations: VEGF-A, vascular endothelial growth factor – A; SRSF1, Serine Arginine-rich Splicing Factor 1; SRPK1, Serine Arginine-rich Protein Kinase 1; VEGFR2, vascular endothelial growth factor receptor 2; PSNI, partial saphenous nerve ligation injury.

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on how the processing of A or C fiber inputs is differentially regulated to form the neuronal basis of chronic pain.

During chronic pain, changes in the complement of proteins result in alterations in sensory neuron excitability, as recently demonstrated whereby expression of voltage gated potassium channels in the DRG is altered in ATF3 positive sensory neurons following nerve injury (Tsantoulas et al., 2012). Furthermore, alternative mRNA splicing allows for functionally distinct proteins to arise from a single gene. This provides a vast repertoire of actions from a limited source of transcripts, allowing for cell-specific and stimulus-induced alteration in cellular function. Targeting regulation and expression of alternative RNA transcripts, and hence proteins, has been proposed as a potential route for novel drug discovery (Tavares et al., 2015), but this has not been widely investigated with respect to nociception/analgesia.

We recently demonstrated the analgesic effect of targeting alternative mRNA splicing, by inhibition of peripheral serine-arginine rich protein kinase 1, SRPK1 (Hulse et al., 2014). SRPK1 controls phosphorylation of serine-arginine rich splice factor 1 (SRSF1), which is fundamental to the control of the vascular endothelial growth factor A (VEGF-A) family alternative splicing (Amin et al., 2011; Bates et al., 2013; Nowak et al., 2010; Nowak et al., 2008). Inactive SRSF1 is located in the cytoplasm, but when phosphorylated by SRPK1 it translocates to the nucleus. There are two VEGF-A isoform families, VEGF-A_{xxx}a and VEGF-A_{xxx}b (Harper and Bates, 2008) where xxx refers to the number of amino acids encoded, and a and b denote the terminal amino acid sequence. SRSF1 phosphorylation results in preferential production of the proximal splice site isoforms, VEGF-A_{xxx}a (Nowak et al., 2010). Little is understood about the contribution of VEGF-A proteins to nociceptive processing. VEGF receptor-2 (VEGFR2), the principal receptor activated by both isoform families, has been implicated in nociceptive processing in animal (Grosios et al., 2004; Hulse et al., 2014; Liu et al., 2012), and clinical studies (Langenberg et al., 2011). VEGF-A isoforms and VEGFR2 are present in the spinal cord (Bates et al., 2002a), and contribute to neuroregeneration and neuroprotection (Verheyen et al., 2012).

We therefore tested the hypothesis that the SRPK1/SRSF1 system contributes to spinal nociceptive processing in rodent models of neuropathic pain, concentrating on the effects of SRPK1 inhibition, and VEGF-A_{xxx}a/VEGFR2 signaling in central terminals of myelinated afferents.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (total 72; 250–350 g, Harlan UK) and adult male 129Ola mice (total 20; 25–30 g inbred strain) were used. Animals were provided food and water ad libitum. All animal procedures were carried out in laboratories at the University of Bristol in accordance with the U.K. Animals (Scientific Procedures) Act 1986 plus associated U.K. Home Office guidance, EU Directive 2010/63/EU, with the approval of the University of Bristol Ethical Review Group.

2.2. Nociceptive behavior

Nociceptive behavioral testing was carried out as previously described (Hulse et al., 2014). All animals were habituated to both handling by the tester and the testing environment on the day prior to testing. Two days of baseline testing were carried out prior to any intervention (either drug or surgical) followed by testing post-intervention at discrete time-points as detailed in each experiment. Stimuli were applied to the partially innervated medial aspect of the plantar surface of the hind paw, an area innervated by the saphenous nerve. Mechanical withdrawal thresholds were calculated from von Frey hair force response curves. Animals were housed in Perspex holding chambers with metal mesh floors (Ugo Basile) and allowed to habituate for 10 min. A range of calibrated von Frey hairs were applied to the plantar surface of the hind paw (for a maximum of five seconds or until paw withdrawal), with a total of five applications per weighted hair. From these data, force response curves were generated and withdrawal values were calculated as the weight at which withdrawal frequency = 50%. Tactile allodynia was assessed in the metal mesh floored enclosures using a brush moved across the plantar surface of the hind paw where a withdrawal scored one, with no response zero. This was repeated a total of five times giving a maximum score of five per session. Cold allodynia: a single drop of acetone was applied to the plantar surface of the hind paw using a 1 ml syringe a maximum of five times giving a maximum score of five if the animal exhibited licking/shaking behavior in response to each application. Thermal hyperalgesia (Hargreaves test (Hargreaves et al., 1988): animals were held in Perspex enclosures with a glass floor. A radiant heat source was positioned under the hind paw, and the latency was recorded for the time taken for the animal to move the hind paw away from the stimulus. This was repeated three times and a mean value calculated for each test.

Formalin Testing: animals were habituated to glass floored testing enclosures as above. A single 50 μl injection of 5% formalin was administered to the plantar surface of the right hind paw by intradermal injection. Immediately following formalin injection, animals were placed into the testing enclosures. Time (seconds) spent exhibiting pain-like behaviors and the total number of pain-like behaviors was recorded in five minute bins for sixty minutes. Data are shown as the classical biphasic response with behavioral responses pooled for the first phase 0–15 min and second phase 20–60 min. Blinding of nociceptive behavioral studies are routine in the laboratory however where animal welfare/experimental design prohibits this, it cannot be implemented. For instance, in nerve-injured animals blinding is not possible as controls are naïve. The lack of blinding may have introduced some subjective bias into these experiments, which is in part mitigated by behavioral data is supported by the inclusion of experiments in which measurements are not subjective (e.g. in vivo noxious e.m.g. recording, expression analysis, and neuronal activation using c-fos).

2.3. Electromyographic experiments

A well-defined method for minimally invasive preferential selection of either C- or A- fiber mediated nociceptive pathways was used (Yeomans et al., 1996; Yeomans and Proudfit, 1996). Noxious withdrawal responses to A- and C-nociceptor selective stimulation were carried out as previously described (Leith et al., 2014; Leith et al., 2007; McMullan et al., 2004), by measurement of electromyographic activity in biceps femoris. Animals were anesthetized using isoflurane induction (4% in oxygen), and the external jugular vein and trachea were cannulated to allow maintenance of airway and anesthesia. Following surgery, anesthesia was switched to alfaxalone (~30 mg/kg/h i.v.), and animals were maintained at a steady level of anesthesia by continuous pump perfusion via the jugular vein for the remainder of the experiment. Bipolar electrodes were made with Teflon coated stainless steel wire (Advent Research Materials, Oxford UK) implanted into the bicep femoris. EMG recordings were amplified and filtered by a combination of in-house built and Neurolog preamplifier and band pass filters (Digitimer Neurolog System). Animals were maintained at a depth of anesthesia where a weak withdrawal to noxious pinch could be elicited for the duration of the experiment. A- and C-cutaneous nociceptors were preferentially activated to elicit withdrawal reflex EMGs using a well-characterized contact heating protocol (Leith et al., 2014; Leith et al., 2007; McMullan et al., 2004). Two different rates of heating (2.5 °C/s and 7.5 °C/s) were applied to the dorsal surface of the left hind paw as these are known to preferentially activate slow/C-nociceptors (2.5 $°C \cdot s^{-1}$) and fast/A nociceptors (7.5 $°C \cdot s^{-1}$) respectively. Contact skin temperature at the time of onset of the EMG response was taken as the threshold. A cutoff of 58 °C for A-nociceptors, 55 °C for C-nociceptors was put in place to prevent sensitization if no response was elicited. If a withdrawal response was not elicited, threshold