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Research Paper

Bilateral tactile hypersensitivity and neuroimmune responses after spared nerve injury in mice lacking vasoactive intestinal peptide

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ABSTRACT

Vasoactive intestinal peptide (VIP) is one of the neuropeptides showing the strongest up-regulation in the nociceptive pathway after peripheral nerve injury and has been proposed to support neuropathic pain. Nevertheless, the story may be more complicated considering the known suppressive effects of the peptide on the immune reactivity of microglial cells, which have been heavily implicated in the onset and maintenance of pain after nerve injury. We here used mice deficient in VIP and the model of spared nerve injury, characterized by persistent tactile hypersensitivity. While tactile hypersensitivity developed similarly to wild type mice for the ipsilateral hindpaw, only transgenic mice showed a mirror-image tactile hypersensitivity in the contralateral hindpaw. This exacerbated neuropathic pain phenotype appeared to be mediated through a local mechanism acting at the level of the lumbar spinal cord as a distant nerve lesion in the front limb did not lead to hindpaw hypersensitivity in VIP-deficient mice. Innocuous tactile hindpaw stimulation was found to increase a neuronal activation marker in the bilateral superficial laminae of the lumbar dorsal horn of VIP-deficient, but not wild type mice, after SNI. A deeper study into the immune responsiveness to the nerve lesion also proved that VIP-deficient mice had a stronger early pro-inflammatory cytokine response and a more pronounced microglial reactivity compared to wild type controls. The latter was also observed at four weeks after spared nerve injury, a time at which bilateral tactile hypersensitivity persisted in VIP-deficient mice. These data suggest an action of VIP in neuropathic states that is more complicated than previously assumed. Future research is now needed for a deeper understanding of the relative contribution of receptors and fiber populations involved in the VIP-neuropathic pain link.

1. Introduction

Vasoactive intestinal peptide (VIP) is a widely expressed 28-amino acid peptide with functions that reach well beyond blood vessel dilation and gastrointestinal tract motility (Delgado and Ganea, 2013; Delgado et al., 2004). During the last decades, VIP has been implicated in the development of neuropathic pain (Dickinson and Fleetwood-Walker, 1999). This condition is frequently caused by peripheral nerve damage and associated with a variety of symptoms of which tactile hypersensitivity has been considered as one of the most debilitating (Meldrum, 2000). Previous research has shown that peripheral nerve injury triggers an up-regulation of VIP in neurons of the dorsal root ganglia and leads to increased VIP expression in the superficial dorsal horn of the spinal cord (Hokfelt et al., 1994; Shehab, 2014; Villar et al., 1989). Within the dorsal horn, which is a critical site for nerve injury-induced neuroplasticity that underlies persistent pain states (Berger et al., 2011), the gene expression of the two VIP receptors, i.e. VPAC1 and VPAC2 has been reported to undergo a down-regulation and up-regulation, respectively (Dickinson et al., 1999). Spinal neurons express both types of receptors and their binding by specific agonists can induce neuronal activation. Pharmacological studies on VIP and pain have thus far focused on VPAC2, demonstrating that a specific antagonist effectively reduced tactile and heat hypersensitivity in a rat model of peripheral nerve injury (Garry et al., 2005). These data suggest that nerve injury causes modification in the VIP system, which promote neuropathic pain symptomatology.

Within the central nervous system (CNS), VIP does not exclusively target neuronal cells as VIP receptors are also found on glial cells such as astrocytes and microglia (Ashur-Fabian et al., 1997; Cholewinski and Wilkin, 1988; Delgado et al., 2002). VIP has been found to exert a strong suppressant effect on immune-challenged response through

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binding of the VPAC1 receptor, reducing the production of immune mediators such as pro-inflammatory cytokines and chemokines (Delgado et al., 2002; Delgado et al., 2003). Considering the importance of these immune mediators together with microglial reactivity in the development of neuropathic pain (Aldskogius and Kozlova, 2013; Austin and Moalem-Taylor, 2010; Gao and Ji, 2010), it might be speculated that VIP, through immunomodulation, negatively influences neuropathic pain symptomatology through immunomodulation.

While a role of VIP in neuropathic pain is well established, the nature of this role remains unclear. We here aimed to understand the effect of a full depletion of VIP in a mouse model of spared nerve injury, which is characterized by persistent tactile hypersensitivity (Bourquin et al., 2006). Previous work had already shown that mice lacking pituitary adenylate cyclase-activating peptide (PACAP), a neuropeptide structurally related to VIP, had normal nociception, but reduced inflammatory and neuropathic pain (Mabuchi et al., 2004). Even though PACAP can bind both VPAC1 and VPAC2, the latter effects are likely mediated through its high-affinity PAC1 receptor as PAC1 knock-out mice were found to show normal nociception (Jongsma et al., 2001) as well as reduced pathological pain (Jongsma et al., 2001; Martin et al., 2003).

In order to study putative effects of VIP depletion on tactile hypersensitivity after peripheral nerve injury we here used genetically modified mice lacking VIP. The mice have been generated through homologous recombination disrupting the VIP gene (Colwell et al., 2003) and leading to absence of VIP as well as peptide histidine-isoleucine, another neuropeptide encoded by the same gene (Girard et al., 2006).

2. Materials and methods

2.1. Animals

In this study we used male and female adult homozygous $VIP^{-/-}$ mice (at start of experiments: about 3 months old and 24 ± 2 g), bred on a C57BL/6 background and kindly donated by prof Vincent Lelièvre of the University of Strasbourg. $VIP^{+/+}$ mice served as their wild type controls. For algesimetric experiments, we further used female $VIP^{-/-}$ mice and $VIP^{+/+}$ mice, which were studied separately from male mice. Mice were genotyped after birth with genomic DNA extracted from a tail biopsy using PCR (data not shown) as described elsewhere (Colwell et al., 2003). All experiments were performed in strict adherence with the EU directive of 22/09/2010 (2010/63/EU) and approved by the ethical committee on animal experimentation (2010/UCL/MD/023). Our animal laboratory received the number LA2230419 from the Belgian Ministry of Agriculture. Mice were kept in groups of 5–10 animals in makrolon cages (43 × 26 × 15.5 cm) with ad libitum access to water and food, and at a regular 12 h/12 h light/dark cycle.

2.2. Surgery

Animals were subjected to three different groups with respect to nerve injuries; spared nerve injury (SNI), median nerve injury (MNI), or sham surgery as previously described (Bourquin et al., 2006; Tos et al., 2008). Additionally, hindpaw incision (PI) was used as a model of transient tactile hypersensitivity (Brennan et al., 1996; Pogatzki and Raja, 2003). In brief, anesthesia was induced and maintained using 2.5–3% sevoflurane in oxygen. For SNI, the left sciatic nerve was exposed at its trifurcation at the mid-thigh region through blunt dissection. The tibial and common peroneal branches were gently freed from surrounding connective tissues and were then transected at approximately 2 mm distal to the point of trifurcation. The nerve stumps were oriented in opposite directions, followed by closing of the skin with 6/0 prolene sutures. Sham-operated mice underwent the same procedure with the exception that no nerve injury was inflicted. For MNI, the left median nerve was exposed in the forelimb region between the axilla and elbow. The median nerve was gently isolated from surrounding tissues at the level of the pectoralis major muscle and then transected using spring scissors (Fine Science Tools GmbH, Heidelberg, Germany). The nerve stumps were oriented in opposite directions and the skin was closed using 6/0 prolene sutures (Ethicon, Livingston, Scotland). For PI, a 5-mm longitudinal incision was made through the skin, fascia and muscle of the plantar hindpaw using a surgical scalpel blade no. 11 (Swann Morton, Sheffield, England). The incision extended from 2 mm proximal to the heel toward the toes. The wound was closed with two single knot sutures of 6/0 nylon (Ethicon, Livingston, Scotland).

2.3. Von Frey algesimetry

The animals were first habituated to the experimenter and then placed in the experimental setting, which consisted of transparent plastic chambers on top of an elevated wire mesh. The mice were allowed to acclimatize to the environment and explorative behaviors and major grooming typically reduced after about 20 min. Then, the von Frey hair filament test was performed. The filament set used for the assessment consisted of seven calibrated von Frey hair filaments; 0.03, 0.07, 0.17, 0.4, 0.7, 1.2, and 2.0 g (Stoelting, Wood Dale, IL, US). In this test, filaments are applied perpendicular to the sural nerve territory at the lateral aspect of the plantar hindpaw surface and maintained in a slightly buckled position for a maximum duration of 6 s. Only in case of a paw withdrawal with aversive behavioral signs (brisk withdrawal with postural change, attacking of filament, licking of stimulated paw, etc.), the response is considered as positive. The test is started at the 0.4 g filament and positive responses are followed by stimulation with the next-lower force filament; negative responses are followed by stimulation with the next-higher force filament in the set. This 'up-down' procedure is continued until maximally six responses have been obtained that started with a combination of 'no response-response'. In case of a positive response to the very first filament application, a total of maximally five further applications are performed. In case negative responses are obtained at the highest force filament or in case positive responses are obtained at the lowest force filament, no further applications are performed. On the basis of this assessment, the 50% paw withdrawal threshold (PWT) is calculated as described previously (Chaplan et al., 1994). Mice which consistently show positive responses to all filaments or negative responses to all filaments receive higher (2.0 g) or lower (0.04 g) cut-off scores, respectively.

2.4. Innocuous tactile hindpaw stimulation of SNI animals

The responsiveness of spinal neurons in the lumbar spinal cord toward innocuous tactile hindpaw stimulation was investigated. $VIP^{-/-}$ mice and $VIP^{+/+}$ mice that had undergone an SNI surgery 7 days earlier were subjected to tactile innocuous hindpaw stimulation. Briefly, the animals received one stroke of the plantar hindpaw surface every 2 s for a period of 10 min. Strokes were made with the flat portion of the investigator's thumb and toward the distal footpad. During the entire time, the animals were maintained under anesthesia using 2.5–3% sevoflurane in oxygen. For both genotypes, control animals that had undergone SNI 7 days earlier were kept under anesthesia for the same duration, but received no tactile hindpaw stimulation. At 1.5 h after stimulation, the animals were sacrificed and spinal cord tissue was processed according to the same procedures as described in Section 2.6.

2.5. qRT-PCR

Quantitative PCR was performed on ipsilateral and contralateral quadrants of the lumbar spinal cord of mice at 3 d and 7 d after SNI or sham surgery. Briefly, animals were asphyxiated using CO_2 and the spinal cord was extracted through hydro-extrusion. The lumbar enlargement was selected for further processing and divided in four quadrants. Only the ipsilateral and contralateral dorsal quadrants