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Inflammation in dorsal root ganglia after peripheral nerve injury: Effects of the sympathetic innervation

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ABSTRACT

Following a peripheral nerve injury, a sterile inflammation develops in sympathetic and dorsal root ganglia (DRGs) with axons that project in the damaged nerve trunk. Macrophages and T-lymphocytes invade these ganglia where they are believed to release cytokines that lead to hyperexcitability and ectopic discharge, possibly contributing to neuropathic pain. Here, we examined the role of the sympathetic innervation in the inflammation of L5 DRGs of Wistar rats following transection of the sciatic nerve, comparing the effects of specific surgical interventions 10–14 days prior to the nerve lesion with those of chronic administration of adrenoceptor antagonists. Immunohistochemistry was used to define the invading immune cell populations 7 days after sciatic transection. Removal of sympathetic activity in the hind limb by transecting the preganglionic input to the relevant lumbar sympathetic ganglia (ipsi- or bilateral decentralization) or by ipsilateral removal of these ganglia with degeneration of postganglionic axons (denervation), caused less DRG inflammation than occurred after a sham sympathectomy. By contrast, denervation of the lymph node draining the lesion site potentiated T-cell influx. Systemic treatment with antagonists of α_1-adrenoceptors (prazosin) or β-adrenoceptors (propranolol) led to opposite but unexpected effects on infiltration of DRGs after sciatic transection. Prazosin potentiated the influx of macrophages and $CD4^+$ T-lymphocytes whereas propranolol tended to reduce immune cell invasion. These data are hard to reconcile with many in vitro studies in which catecholamines acting mainly via β_2-adrenoceptors have inhibited the activation and proliferation of immune cells following an inflammatory challenge.

1. Introduction

The sympathetic nervous system and the immune system are thought to interact during an inflammatory response, with the sympathetic system generally regarded as immunosuppressant (Bellinger et al., 2008; Benarroch, 2009). Catecholamines activate adrenoceptors on macrophages and $CD4^+$ T-lymphocytes in vitro. Activation of T-cells has commonly been found to reduce the secretion of pro-inflammatory cytokines via β_2-adrenoceptors (Sanders, 2012) whereas many responses of macrophages are mediated via α_1-adrenoceptors (Grisanti et al., 2011);Bellinger & Lorton (in this issue). In vivo, in response to an inflammatory challenge such as lipopolysaccharide (LPS), increased TNF production and T-cell proliferation were inhibited by β-adrenoceptor agonists and this effect was abrogated by the β-adrenoceptor antagonist, propranolol (Sekut et al., 1995). During the response to a hemorrhagic shock, propranolol treatment decreased the numbers of circulating $CD8^+$ lymphocytes (Oberbeck et al., 2002). However, following injection of bee venom in the paw, the inflammatory response and ensuing hyperalgesia were attenuated by blockade of α_1-adrenoceptors (Chen et al., 2010). In addition, de novo release of catecholamines from activated macrophages has been shown to auto-inhibit them via α_2-adrenoceptors, restraining LPS-induced lung inflammation (Flierl et al., 2007). It is evident that the roles of endogenous catecholamines and adrenoceptor sub-types in systemic inflammation are unclear.

What is even more unclear is how and where sympathetic nerve activity affects immune cells in vivo. One known site of interaction is the spleen where noradrenergic varicosities contact T-cells (Felten and Olschowka, 1987) and contacts probably also occur in lymph nodes where varicose noradrenergic terminals terminate among T-cells and macrophages (Felten et al., 1984). Little is known about the interaction between local sympathetic nerves and the immune system during an inflammatory response to injury. This question has been addressed here during the sterile inflammation that develops in dorsal root ganglia (DRGs) following damage to a peripheral nerve trunk.

Naïve DRGs lack a blood–nerve barrier (Jacobs et al., 1976) and contain populations of macrophages and T-lymphocytes that are presumed to be involved in immune surveillance (Esiri and Reading, 1989; Hu and McLachlan, 2002a). The density of T-cells is much lower than that of

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macrophages. Several types of macrophage are present in naïve DRGs, namely, resident macrophages that express CD163, some containing $CD68^+$ lysosomes, with or without major histocompatibility complex type II (MHC II) and others expressing only MHC II (Hu and McLachlan, 2003). The majority of T-cells that survey the healthy nervous system are $CD8^+$ (Hickey, 1991; Hu et al., 2007). In the first few days after axonal injury from transection or compression of a major nerve trunk, like the sciatic nerve, Schwann cells and later invading macrophages clear away degenerating axons and myelin debris at and beyond the injury site (Hirata and Kawabuchi, 2002). The draining lymph node contains the first immune cells to be exposed to the antigens from the degenerating nerve (Xin et al., 2008). In the days to weeks following this contact, activated macrophages and T-lymphocytes from lymph nodes and spleen are attracted to the injury site and also, selectively, to associated DRGs (Hu and McLachlan, 2002a, 2003; Hu et al., 2007; Kim and Moalem-Taylor, 2011; Moalem et al., 2004; Schmid et al., 2013) and sympathetic ganglia (Hu and McLachlan, 2004; Schreiber et al., 1995). The immune cells fill the ganglia where release of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), have been reported to directly raise neuronal firing rates (Ibeakanma and Vanner, 2010; Junger and Sorkin, 2000; Ozaktay et al., 2006) or indirectly modify gene expression (Grisanti et al., 2011; Ohtori et al., 2004; Schreiber et al., 1995). In this way, neurones projecting in the injured nerve trunk, and also uninjured neurones projecting in intact more proximal nerve branches (such as those supplying the lymph nodes that drain the injury site), are exposed to these cytokines and can be affected.

There is another way in which sympathetic axons may modify events in the affected DRG. Over several weeks after the injury, upregulation of neurotrophin production by satellite glia in the DRG (Zhou et al., 1999) attracts collateral sprouts from nearby perivascular noradrenergic terminals (McLachlan et al., 1993; Ramer et al., 1999). These noradrenergic sprouts form basket-like structures around some large diameter afferent somata. The role of these ectopic sympathetic terminals is not clear although functional studies have suggested that they potentiate only cold allodynia (Pertin et al., 2007; Zhao et al., 2007), consistent with the small number of afferent neurones that receive sympathetic baskets. A functional link is suggested by the similar arrangements of noradrenergic terminals and MHC II^+ macrophages around some large diameter neurones (Hu and McLachlan, 2002a), although their co-localization has not been demonstrated directly. The presence of these similar perineuronal aggregations suggest that interaction between sympathetic terminals and immune cells may occur within the injured DRG.

Most previous attempts to investigate the actions of sympathetic nerves on immune function in vivo have involved the use of pharmacological tools that produce systemic "sympathectomy", either by blocking all adrenoceptors (Benarroch, 2009) or by using agents like 6-hydroxydopamine (6-OHDA) that destroy noradrenergic neurones or their terminals (Ebbinghaus et al., 2012; Madden et al., 1989). These interventions lead to multiple autonomic and neuroendocrine disruptions throughout the body (Bellinger et al., 2008). However, it is notable that selective (local) denervation decreased the number of cells in the submaxillary lymph node and increased its response to LPS (Esquifino and Cardinali, 1994), consistent with an inhibitory role for the innervation of lymphoid tissue in systemic inflammation.

Here we have used several interventions to investigate the involvement of sympathetic activity in inflammation of L5 DRGs after sciatic transection. The sympathetic axons in the rat sciatic nerve arise from cell bodies in the lumbar sympathetic chain mainly lying in L3 and L4 ganglia (Baron et al., 1988; Sittiracha and McLachlan, 1986) which are commonly fused (L3/4) in our inbred strain of Wistar rats. The axons project down the chain and then via gray rami to L4 and L5 spinal nerves (see Fig. 1); only a few axons originate from cell bodies lying caudal to L3/4 ganglia. The preganglionic axons that innervate these sciatic-projecting neurones arise in T13–L2 segments of the spinal cord

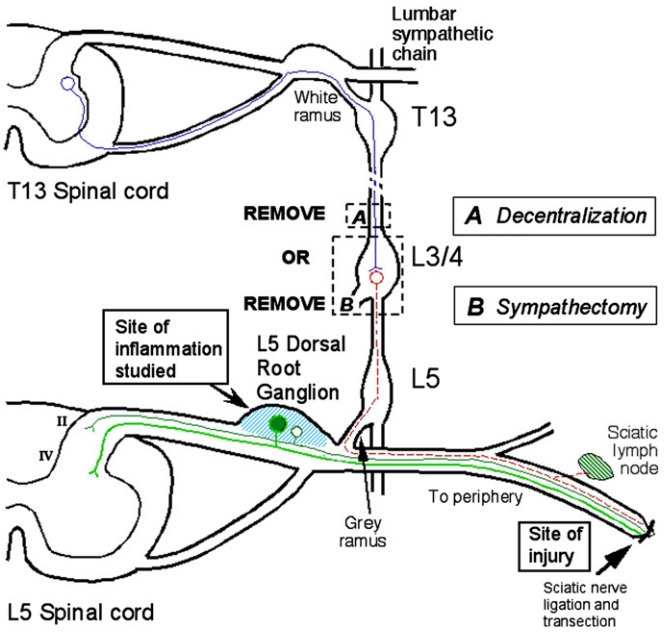

Fig. 1. Diagram of the anatomical arrangement of lumbar sympathetic pathways and sites of nerve lesions. The immunohistochemical analysis was performed on L5 dorsal root ganglion (DRG, *light blue shaded*). The dashed box *A* indicates the site where the preganglionic axons (*dark blue*) innervating the postganglionic neurones in L3/4 ganglia (*red*) were transected to "decentralize" them without direct damage. The dashed box *B* indicates the removal of L3/4 ganglia to destroy the postganglionic neurones projecting in the sciatic nerve. The sciatic lymph node (*green shaded*) lies at the sciatic notch proximal to the site of nerve transection above the sciatic trifurcation. Small and large diameter afferent axons shown in *green*.

(Anderson et al., 1989; Rathner and McAllen, 1998). After uni- or bilateral decentralization of L3/4 ganglia (removal of activity in postganglionic neurons by transection of their preganglionic inputs), unilateral surgical sympathectomy of L3/4 ganglia (with subsequent degeneration of postganglionic axons, including those that sprout into L5 DRGs) or a sham sympathectomy, we evaluated the populations of macrophages and lymphocytes recruited into L5 DRGs after sciatic transection. For comparison, we examined the effects of endogenous noradrenaline (NA) by chronic administration of antagonists of α_1-adrenoceptors (prazosin) or β-adrenoceptors (propranolol). The surgical interventions to the lumbar chain had effects opposite to those after denervation of the draining lymph node and the pharmacological interventions had opposing effects that did not fit with conventional views. The data leave many questions open about the role of sympathetic activity in this inflammatory response.

2. Materials and methods

Inbred female Wistar rats (8 weeks of age, 180–230 g at operation, conventionally housed) were anesthetized with intraperitoneal ketamine (60 mg/kg, Ketamil; Troy Laboratories Pty Ltd, Australia) plus xylazine (10 mg/kg; Ilium-Xylaxil-20; Troy Laboratories Pty Ltd) and the left sciatic nerve was ligated and transected distally above its trifurcation (*SciX*). Seven days later, the animals were reanesthetized with pentobarbitone (100 mg/kg i.p.) and perfused through the descending thoracic aorta, first with heparinized saline containing 0.1% $NaNO_2$, and then with Zamboni's fixative. Bilateral L5 dorsal root ganglia and attached nerve roots were dissected and postfixed overnight, washed and infiltrated with 30% sucrose prior to being blocked together in pairs and frozen at −80 °C. All experimental procedures were carried out following the Australian Code for the Care and Use of Animals in Research and approved by the Animal Care and Ethics Committee of the University of New South Wales.