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Differential pharmacological modulation of the spontaneous stimulus-independent activity in the rat spinal cord following peripheral nerve injury

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Abstract

Peripheral nerve injury is a significant clinical problem that is often difficult to treat. The major clinical symptoms are numbness, tactile and cooling allodynia, hyperalgesias as well as ongoing pain. In animal models of neuropathy, abnormal responses to applied (or evoked) stimuli can be gauged, but spontaneous pain, a major clinical issue, has proved very difficult to assess. In neuropathic animals, spinal neuronal hyperexcitability indicative of peripheral and central changes with high levels of spontaneous neuronal firing has been reported. This latter stimulus-independent firing of sensory neurones may be a measure related to ongoing pain.

Two weeks after L5/6 spinal nerve ligation, deep dorsal horn neurones were recorded in halothane-anesthetized rats. The majority of neurones in neuropathic rats showed increased levels of spontaneous firing with irregular firing patterns. We examined and compared the effects of 5 centrally acting pharmacological agents: morphine (i.t. or i.v.), gabapentin, ketamine, memantine and mepyramine on stimulus-independent neuronal firing. This ongoing activity showed high sensitivity to gabapentin (s.c.) and morphine (i.t.) administration, being significantly reduced in a dose-dependent manner. Morphine administered via the systemic route produced modest but non-significant reductions of spontaneous activity. The two NMDA receptor antagonists, ketamine and memantine, and the histamine H_1 receptor antagonist, mepyramine, produced minor effects at doses known to be effective on stimulus evoked measures of deep dorsal horn neurones. This may form an electrophysiological basis for the efficacy of gabapentin and spinal morphine on ongoing pain in patients with peripheral neuropathy.

Keywords: Neuropathic pain; NMDA receptor antagonist; Morphine; Gabapentin; Mepyramine; Central sensitization

Introduction

Injury to peripheral nerves commonly results in neuropathic pain, characterized by a combination of symptoms such as ongoing pain, sensory loss, hyperalgesia (increased sensitivity to painful stimuli) and allodynia (perception of pain to a normally innocuous stimulus). The underlying mechanisms are multiple and complex, which in turn translates to difficulties in the assessment, treatment and management of this distressing condition. Currently, therapy has its limitations. Insight into the pathological mechanisms of nerve injury has been made possible through development of animal models that reproduce some of the symptoms of human neuropathic pain (Bennett and Xie, 1988; Decosterd and Woolf, 2000; Hama and Borsook, 2005; Kim and Chung, 1992; Seltzer et al., 1990; Walczak et al., 2005). Whereas abnormal responses to evoked stimuli can be readily measured (e.g., tactile and thermal hypersensitivity), ongoing pain and sensory loss are harder to assess and quantify. Similarly, pharmacological evaluation of analgesic compounds has largely focused on effects on allodynia or hyperalgesia (De Vry et al., 2004; Decosterd et al., 2004; Fox et al., 2003; Hama and Borsook, 2005; Hofmann et al., 2003; Labuda and Little, 2005; Urban et al., 2005; Walczak et al., 2005; Yamamoto and Yaksh, 1992) with little emphasis on spontaneous ongoing pain. The lack of animal data is largely attributed to the technical difficulty in measuring signs of ongoing/spontaneous pain behaviorally. Unlike tests for mechanical allodynia or thermal hyperalgesia where animals show clear demonstrable pain

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behaviors – such as foot withdrawal accompanied by licking, shaking and gnawing of the paw – behaviors indicative of ongoing pain are much more subtle. Despite the clinical finding that a large proportion of neuropathic pain patients report ongoing pain (Rasmussen et al., 2004), difficulties in assessing this in animals have resulted in only very few studies attempting to characterize the magnitude and time course of this measure and its pharmacological sensitivity (Attal et al., 1990; Choi et al., 1994; Mao et al., 1992; Yoon et al., 1996).

In vivo electrophysiological recordings of spinal neurones reveal physiological and pharmacological changes after nerve injury (Suzuki et al., 2005). These include increased receptive fields, spontaneous activity and de novo sensitivity to gabapentin. Since proportions of these spinal sensory neurones project to the brain, stimulus-independent activity may be a measure of ongoing pain.

There could be many causal mechanisms for this activity. Following peripheral nerve lesion, there is an initial burst of high-frequency injury discharge, followed by ectopic activity generated in the DRG and neuroma at the site of damage (Chen and Devor, 1998; Wall and Devor, 1983; Xie et al., 1995). Spontaneous firing is recorded in both injured and neighboring uninjured fibers (Wu et al., 2001). The development of such activity occurs early postlesion (12 h–3 days) and persists over 2 months (20–53 days) (Liu et al., 2000; Sun et al., 2005), paralleling that of abnormal pain behaviors (Kim et al., 1997; Sun et al., 2005). One possible neural basis for the abnormal hyperexcitability is the Na^+ channels that undergo significant plasticity following injury (Baker and Wood, 2001; Waxman et al., 1999). Blocking the abnormal expression of sodium channels through intrathecal GDNF infusion prevented ectopic activity generation in injured afferents and furthermore attenuated behavioral signs of allodynia (Boucher et al., 2000). Changes in the expression and function of other ion channels have additionally been reported (Kim et al., 2002; Rasband et al., 2001; Lee et al., 2005). Thus, manipulation of these channels blocks pain-related behaviors, confirming their role in the pathogenesis of abnormal pain (Lai et al., 2002; Lyu et al., 2000). Injury-induced ectopic activity therefore acts as a trigger in driving spinal hypersensitivity. This is supported by studies that demonstrate that blocking abnormal afferent input – using a local anesthetic or a channel blocker – transiently reverses some of the manifestations of nerve injury (Xiao and Bennett, 1995; Yoon et al., 1996; Sukhotinsky et al., 2004; but see Suter et al., 2003). Ectopic activity in afferents induced by nerve injury could promote hyperexcitability in spinal neurones, characterized by increased spontaneous activities, lowered activation thresholds to peripheral inputs and expansion of neuronal receptive fields (Chapman et al., 1998a,b; Chu et al., 2004; Palecek et al., 1992; Pertovaara et al., 1997; Suzuki et al., 2000). Another possibility is that the sensory loss causes central neurones to exhibit ongoing activity either as they become hypersensitive after loss of normal input or as a result of deafferentation. Peripherally generated aberrant activity and central spinal cord changes therefore form an essential substrate of neuropathic symptoms. The magnitude of this ectopic discharge can show good correlation with the degree of pain behavior (Han et al., 2000), at least in the early stages of nerve injury (Sun et al., 2005). These findings would further support the contribution of sustained injury discharges and spontaneous activities to the pathogenesis of neuropathic pain.

The aim of the present study is to compare the electrophysiological effects of 4 classes of pharmacological agents – opioid (morphine, i.v. or spinal routes), calcium channel blocker (gabapentin), NMDA receptor antagonists (ketamine, memantine), histamine H1 receptor antagonist (mepyramine) – on the spontaneous activities of deep dorsal horn neurones, 2 weeks after L5/6 spinal nerve ligation. Following injury to spinal nerves (Chapman et al., 1998a,b), dorsal horn neurones without peripheral receptive fields are frequently encountered (indicative of sensory loss), and a characteristic stimulus-independent activity is induced. To our knowledge, this is the first study to provide a direct comparison of mechanistically different drugs – some of which are employed clinically – on stimulus-independent neuronal measures. Using in vivo electrophysiology, we demonstrate that agents, in particular those with predominant presynaptic actions, produce strong inhibitions of the spontaneous activity in neuropathic animals. On the other hand, agents known to be effective against stimulus-evoked measures failed to suppress the stimulus-independent ongoing activity suggesting that different pain measures display variable sensitivities to pharmacological treatments. This will have important clinical implications in the treatment of this difficult condition.

Methods

A total of 48 spinal neurones were recorded from male Sprague–Dawley rats (Central Biological Services, University College London, UK). All experimental procedures were approved by the UK Home Office and follow the guidelines under the International Association for the Study of Pain (Zimmermann, 1983).

Surgery for peripheral nerve injury

Two weeks before in vivo electrophysiological recordings, animals (130–150 g) were subjected to surgery for spinal nerve ligation using methods as previously described (Kim and Chung, 1992). Briefly, the left L5 and L6 spinal nerves were isolated and tightly ligated with 6-0 silk thread under halothane anesthesia (50% O_2: 50% N_2O). Hemostatis was confirmed, and the wound was sutured.

Behavioral testing

Behavioral testing was carried out over a 2-week period. Mechanical sensitivity was assessed through measurement of foot withdrawal frequencies to a trial of 10 applications of a calibrated von Frey hair (8 g) onto the ipsilateral and contralateral hindpaw. Withdrawal frequency (%) was quantified as = (number of foot withdrawals/10) × 100. Ongoing pain was assessed using methods previously described (Choi et al., 1994). Briefly, rats were placed on a neutral temperature plate