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Research report

Normal hypothalamo–pituitary–adrenal axis function in a rat model of peripheral neuropathic pain

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Abstract

Chronic pain conditions such as rheumatoid arthritis and fibromyalgia are associated with profound hypothalamo–pituitary–adrenal (HPA) axis dysfunction which may exacerbate symptoms of chronic pain. HPA axis dysfunction has also been well documented in animal models of chronic inflammatory pain. However, the role of the HPA axis in animal models of neuropathic pain is currently unknown. Rats with a chronic constriction injury (CCI) of the sciatic nerve that developed marked mechanical allodynia and hyperalgesia of the injured hindpaw were used to determine basal and stimulatory levels of HPA axis activity. Plasma ACTH and corticosterone levels were increased significantly ($P < 0.05$) in CCI rats after 20 min restraint stress compared with baseline; however, the magnitude of the increase was no different from sham rats. Furthermore, the temporal profile of ACTH release over the 60 min period after termination of restraint was similar between CCI and sham rats suggesting normal glucocorticoid-mediated feedback. Restraint stress also significantly increased ($P < 0.05$) expression of the immediate early genes c-Fos and FosB within the hypothalamic PVN to a similar extent in CCI and sham rats. Within the parvocellular PVN basal expression of both CRF and AVP mRNA was no different between CCI and sham rats; restraint stress induced a significant 2.5 fold increase ($P < 0.05$) in CRF mRNA expression in sham rats only. These results suggest that, in contrast to inflammatory immune-mediated pain models where HPA axis function is profoundly altered, in the CCI model of neuropathic pain, basal HPA axis function is unchanged. Furthermore, the HPA axis responds normally to a novel stressor in the face of ongoing nociceptive input, a stimulus known to activate the HPA axis.

Theme: Sensory systems
Topic: Pain modulation: anatomy and physiology

Keywords: ACTH; Allodynia; Corticosterone; CRF; Hyperalgesia; Nerve injury; Stress

1. Introduction

Prolonged changes in the activity and function of nociceptive pain pathways can occur as a result of inflammation or damage to nerves within both the peripheral and central nervous systems [60]. Sensory deficits, which often manifest as allodynia (pain evoked by normally non-painful stimuli) and hyperalgesia (an increased response to painful stimuli), are key diagnostic criteria of chronic pain conditions in both animal models and humans [6,17]. Neuropathic pain conditions in particular remain difficult to treat with available analgesics [17]. Thus, there is a clearly defined need to better understand the array of pathophysiological changes that may contribute to or arise as a consequence of neuropathic pain.

Corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) are synthesised by parvocellular neurones within the paraventricular nucleus (PVN) of the hypothalamus. Both neuropeptides are secreted into the portal circulation to act as major regulators of pituitary adrenocorticotrophic hormone (ACTH) secretion during stress.

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ACTH in turn acts upon the adrenal cortex in order to enhance release of the glucocorticoid corticosterone in rats [26]. Normally, CRF is the primary ACTH secretagogue, whilst AVP is a weaker ACTH secretagogue that acts synergistically with CRF [19]. However, several studies support the concept that AVP is the dominant secretagogue during repeated and chronic stress [22,25]. The stress response is controlled by glucocorticoid-mediated feedback at multiple levels of the hypothalamo–pituitary–adrenal (HPA) axis [26].

Animal models of chronic inflammatory disease such as adjuvant-induced arthritis (AA) are associated with overt behavioural hyperalgesia and allodynia in response to hindpaw sensory stimulation [43]. Increased self-administration of analgesics together with altered sleep and increased ultrasonic vocalisation (USV) patterns further indicate that AA animals experience chronic unrelenting pain [1,11,14,37]. AA rats also display profound HPA axis dysfunction as typified by increased basal plasma levels of ACTH and corticosterone [23,59]. At the cellular level, CRF mRNA expression is decreased within the PVN of AA rats whilst the CRF mRNA response to physical and psychological stress is impaired [21,23,24]. In contrast, AVP mRNA is up-regulated within the PVN of AA rats [13]. Importantly, whether chronic pain per se acts as a chronic stressor in this model or whether inflammation-induced release of cytokines contributes to these changes in HPA axis function is currently unknown [48,51].

In animal models of neuropathic pain, the occurrence of spontaneous pain behaviours, which can consist of guarding or scratching of the injured hindpaw, have been well documented [3,6,33,34,36,50]. However, marked alterations in other pain-related behaviours (e.g. sleep patterns and USVs) have been less and/or inconsistently reported [2,30,35,42]. Long term increases in regional cerebral blood flow within the PVN of neuropathic rats suggest that alterations in neuronal activity may occur after injury [46], although to our knowledge HPA axis function in neuropathic animals has not been directly investigated. Thus, we have sought to determine whether disturbances in HPA axis function are present in the chronic constriction injury (CCI) model of neuropathic pain in rats, using a combination of plasma hormone profiling together with measurement of immediate early gene and peptide gene expression within the hypothalamic PVN.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Harlan Scandinavia, Allerød, Denmark) were used. They were housed in groups (3–4 per cage) on soft bedding with food and water available ad libitum in a temperature-controlled environment with a light–dark cycle of 12:12 h. Rats were allowed to habituate to the housing facilities for at least 1 week prior to surgery or behavioural testing. All experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain [61], and all procedures were conducted in accordance with the Danish National Guide for Care and Use of Laboratory animals.

2.2. Neuropathic pain; chronic constriction injury procedure

A chronic constriction injury (CCI) was performed in rats (body weight 204–245 g at the time of surgery) as described previously [5]. Anaesthesia was induced and maintained by 2% isoflurane (Baxter, Allerød, Denmark), combined with oxygen (30%) and nitrous oxide (68%). The sciatic nerve was exposed at the mid-thigh level proximal to the sciatic trifurcation. Four chromic gut ligatures (4/0) (Ethicon, New Jersey, USA) were tied loosely around the nerve, 1–2 mm apart, such that the vascular supply was not overly compromised. In all animals, the overlying muscle was closed in layers with 4/0 synthetic absorbable surgical suture and the skin closed with 9 mm autoclips. Animals that subsequently showed overt behavioural disturbances unrelated to the nerve injury procedure were excluded from the experiments. Sham-operated animals underwent the same surgical procedure, but no ligatures were placed around the nerve. Control animals were kept under the same conditions as CCI and sham animals but were not exposed to any surgical procedures.

2.3. Behavioural testing of nerve-injured animals

CCI, sham and control rats were tested twice weekly for the presence of pain-like behaviours for up to 21 days after surgery (CCI and sham), according to previously described methods [8,10,53]. Body weight (g) was also measured at 4, 7, 14 and 21 days after injury prior to behavioural testing.

During testing, the rats were placed in open mesh steel cages positioned on an elevated metal grid allowing access to the plantar surface of the injured hindpaw. The animals were allowed to habituate for at least 15 min prior to initiation of behavioural testing. To test for the presence of mechanical allodynia, a series of calibrated von Frey hairs (0.059–19.4 g, Stoelting, IL, USA) were applied to the plantar surface of the hindpaw with increasing force until the individual filament used just started to bend. The filament was applied for a period of 1–2 s and was repeated 8–10 times at 1–2 s intervals. The filament that induced a reflex paw withdrawal in 50% of the total number of applications was considered to represent the threshold level for a mechanical allodynic response to occur. The presence of mechanical hyperalgesia was determined by pressing the mid plantar glabrous surface of the hindpaw with the point of a safety pin, 3 cm in length. The intensity applied was sufficient to produce a reflex withdrawal response in normal unoperated animals, but insufficient to penetrate the skin. A