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Changes in BDNF gene expression correlate with rat strain differences in neuropathic pain

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Abstract

The Fischer 344 (F344) rat inbred strain differs from the inbred Lewis and the outbred Sprague–Dawley (SD) in the response to different pain stimuli, which has been partially attributed to differences in the endogenous opioid and noradrenergic systems. Since brain-derived neutrophic factor (BDNF) modulates both the endogenous opioid and noradrenergic systems, we have now studied specific changes in BDNF gene expression related to the maintenance of neuropathic pain in the three rat strains. F344 rats were found to be the only strain that completely recovered from neuropathic pain (mechanical allodynia) 28 days after chronic constriction injury (CCI) of the sciatic nerve. Real time RT-PCR studies revealed minimal changes in the expression of BDNF in the spinal cord after CCI despite the strain considered, but marked changes in dorsal root ganglia (DRG) were observed. A significant upregulation of BDNF gene expression was found only in injured DRG of F344 rats, thus correlating with higher resistance to neuropathic pain. The data suggest that BDNF could be involved in strain differences concerning CCI resistance.

Keywords: Lewis; F344; CCI; Allodynia; BDNF

Genetic differences underlying behavioural differences in the development and maintenance of neuropathic pain after peripheral nerve injury have been suggested through the use of different strains of rats that display different degrees of pain behaviours, such as the inbred rat strains Fischer 344 (F344) and Lewis [14,25,30]. Gene expression studies showed that Lewis rats have lower levels of proenkephalin mRNA in different brain areas such as the striatum and nucleus accumbens [15,23], confirming previous results at the protein level [20]. Importantly, a greater spinal induction of preprodynorphin mRNA levels was linked to a stronger hyperalgesia in F344 rats compared to Lewis and Sprague–Dawley after peripheral inflammation [31]. Furthermore, Lewis rats were found to have decreased mRNA levels of tyrosine hydroxylase, the rate-limiting enzyme of the catecholamine biosynthesis pathway, in hippocampus and striatum [11] what led us to suggest that strain differences in hippocampal noradrenergic activity could be involved in the different responses to pain of F344 and Lewis rats.

The combined data seemed very intriguing to us since the endogenous noradrenergic and opioid systems closely interact in the descending control of pain (see reviews [2,18]). In fact, it was shown that the analgesic efficacy of morphine is higher at lower doses (1 and 5 mg/kg) in F344 rats, but lower at higher doses (10 mg/kg) [9], data that could be related to a more effective stimulation of μ-opioid receptor in F344 than in Lewis rats [10,24]. On the other hand, the α_2-adrenergic agonist clonidine exhibited a more potent antinociceptive effect in F344 rats at all doses tested leading us to suggest a balanced contribution of opioid and α_2-adrenoceptor mechanisms to control pain transmission in both strains [9]. It is interesting to note that the mechanical allodynia induced by spinal nerve ligation (SNL) is almost completely reverted by the α-adrenergic receptor blocker phentolamine in Lewis but not in F344 rats [12], suggesting that the noradrenergic pathway may be less relevant in the latter strain and, thus, F344 rats may be more responsive to exogenous administration of α_2-adrenergic agonists [9].

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We saw brain-derived neutrophic factor (BDNF) as a perfect candidate to further look for genetic differences between F344 and Lewis rats that could underlie their behavioural differences in response to pain. BDNF is known to be involved in opiate-induced plasticity of the locus coeruleus [19] and has been shown to be essential for the expression of tyrosine hydroxylase (TH) after chronic opiate exposure [1]. Furthermore, BDNF has been shown to initiate opioid and noradrenergic activation [16,27], being some of these effects of critical importance for BDNF-induced neurite outgrowth [22]. Therefore, it seemed a reasonable hypothesis to us that BDNF could regulate the combined contribution of opioid and noradrenergic pathways to control pain behaviour, contribution that was found to be critical in the different response of F344 and Lewis rats in a model of acute pain [9].

Interestingly, the role of BDNF in neuropathic pain remains poorly understood and highly controversial. It is very well known that BDNF synthesis is significantly increased in dorsal root ganglion (DRG) following nerve injury (see review [21]), however controversy rises when considering the antinociceptive actions of BDNF [4,7,13,29] against its pronociceptive profile claimed by others [26,32]. Clearly, different experimental conditions partially underlie those contradictory reports. For example, Miki et al. [17] demonstrated that BDNF exhibits antinociceptive or pronociceptive actions depending on the dose used. For these reasons, with the present work, we pretended to better understand the role of BDNF in neuropathic pain from a different point of view: evaluating if possible differences in BDNF mRNA levels could correlate with strain differences in the development of neuropathic pain. To extend previous results in the literature, we chose the chronic constriction injury (CCI) of the sciatic nerve as a neuropathic pain model, since, to the best of our knowledge, it has been never used in F344 and Lewis rats. In addition, we expanded our experiments using Sprague–Dawley (SD) rats, not only as an outbred reference, but also because previous strain-related differences in pain behaviour between SD rats, and F344 and Lewis rats have been reported [25,30,31].

All studies followed the guidelines of the Institutional Animal Care and Use Committee (IACUC), in accordance with the guidelines of the National Institutes of Health. This chronic model of neuropathic pain was originally described by Bennett and Xie [3]. 10 male rats of each strain (SD, F344 and Lewis; Harlan, Indianapolis, IN) 8 weeks old were anesthetized with ketamine/xylazine i.p. before CCI surgery. The common sciatic nerve was then exposed and four loose ligatures of 4–0 chromic gut suture were tied around the nerve in 1 mm intervals to just constrict the surface of the nerve as seen under microscope. We used as controls for Taqman RT-PCR studies five more animals of each strain that did not undergo surgery to obtain the basal BDNF mRNA levels of each strain.

Mechanical allodynia was determined by applying five times in succession a series of calibrated Von Frey monofilaments ranging from 0.69 to 15.86 g to both hind paws in intact naive animals just before surgery (basal) and at 7 and 28 days after surgery until paw withdrawal to characterize the chronic pain responses [28]. The lowest monofilament in the series that evoked at least one withdrawal response was recorded as threshold.

Just after the 28-day behavioural measures, all animals with CCI were anesthetized with ketamine/xylazine i.p., decapitated, and immediately, the portion of ipsi and contralateral spinal cord comprised between the L4 and L5 spinal nerves and their DRGs from each one of these animals ($n = 10$/strain) were removed to perform gene expression studies. In addition, we used the same tissues from five naive animals from each strain as control to determine basal expression of BDNF in the same tissues. RNA was extracted following the TRIZOL (invitrogen) method. One microgram of RNA from each sample was used for cDNA synthesis that was performed as we previously described [11].

Probe (5′-TTCCCGGGTGATGCTCAGCAGTC-3′) and primer sequences (forward, 5′-CATAAGGACGCGGACTTGTACA-3′; reverse, 5′-AGCAGAGGAGGCTCCAAAGG-3′) for BDNF were designed using Primer Express software (Applied Biosystems). The probe synthesized with the fluorescent reporter dye FAM (6-carboxy-fluorescein) attached to the 5′-end and a quencher dye TAMRA (6-carboxy-tetramethylrhodamine) to the 3′-end, was purchased from PE Applied Biosystems.

We performed Taqman RT-PCR assays to measure the relative expression of BDNF following the protocol we previously used [11]. To correct for both RNA quality and quantity, data were normalized by dividing BDNF copies/ng by the copies/ng of an assay-dependent 18S (housekeeping) gene, and expressed as a percentage (relative expression).

Statistical comparisons were made with the use of two-way analyses of variance (ANOVAs: Bonferroni tests for post hoc comparison). $p < 0.05$ was considered significant.

Mechanical allodynia was determined by the Von Frey test in animals before CCI surgery (10/strain) and at 7 and 28 days after CCI (Fig. 1). F344 rats showed a higher sensitivity to mechanical stimuli at baseline than Lewis ($t = 3.959$, $p < 0.001$) and SD rats ($t = 2.540$, $p < 0.05$). We found that responses of naive animals before surgery and responses observed in the contralateral side of animals after CCI surgery was similar (data not shown). CCI of the sciatic nerve caused a significant allodynia 7 days after surgery in the ipsilateral side of F344 ($t = 5.578$, $p < 0.001$), Lewis ($t = 10.080$, $p < 0.001$) and SD animals ($t = 7.818$, $p < 0.001$) compared to basal values. Mechanical allodynia 28 days after CCI was maintained compared to basal values in Lewis ($t = 7.586$, $p < 0.001$) and SD rats ($t = 8.696$, $p < 0.001$), but not in F344 animals ($t = 1.877$, $p > 0.05$). We did not find statistical differences in the mechanical allodynia induced by CCI 28 days after surgery compared to 7 days after surgery in SD rats ($t = 0.878$, $p > 0.05$). However, we did find differences in the mechanical allodynia induced by CCI in Lewis rats 28 days after surgery compared to 7 days after surgery ($t = 2.491$, $p < 0.05$), suggesting that Lewis animals were initiating their recovery from CCI procedure (Fig. 1).

The levels of expression of BDNF in naive rats were found very similar in spinal cord and DRG of all the three strains (Table 1). The analysis of gene expression in DRG and spinal cord (ipsi and contralateral) from CCI-injured animals showed that the levels of expression in contralateral samples were very similar to those of the control samples from naive rats (data not shown). Minimal changes in BDNF expression were