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Upregulation of brain-derived neurotrophic factor and neuropeptide Y in the dorsal ascending sensory pathway following sciatic nerve injury in rat

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Abstract

An immunohistochemical study was undertaken to examine the changes of brain-derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) in the nucleus gracilis of rats following sciatic nerve transection. The results showed that BDNF-immunoreactivity (-ir) in the gracile nucleus was significantly increased after the nerve injury. The upregulation was apparent 24 h after nerve lesion, remaining robust up to 56 days postlesion. The increase in BDNF-ir was blocked by hemisection of the spinal cord, or by dorsal rhizotomy ipsilateral to the lesion. NPY-ir changes were similar to those of BDNF-ir, but the onset was delayed by 7 days. No NPY-ir was detected in dorsal root ganglion (DRG) from normal animals. Following sciatic nerve lesion, most of the NPY-immunoreactive neurones were found to be colocalized with BDNF-immunoreactive neurones. Neutralization of endogenous BDNF with its antiserum had no effects on NPY-ir in either the gracile nucleus or DRG. These results indicate that neurones contributing to the dorsal ascending sensory pathway upregulate the expression of both BDNF and NPY in response to sciatic nerve injury.

Keywords: Brain-derived neurotrophic factor; Neuropeptide Y; Sciatic nerve injury; Gracile nucleus; Dorsal root ganglia

Peripheral nerve injury causes dramatic changes in sensory neurone function, resulting frequently in chronic pain, but the underlying mechanism remains poorly understood. Changes in the number of neuropeptides in neurones in the dorsal root ganglia (DRG) have been implicated in the aetiology of neuropathic pain. Neuropeptide-Y (NPY) in many large and some medium-sized neurones in the DRG and their central projections is upregulated following sciatic nerve injury [12,14]. The neurotrophin, brain-derived neurotrophic factor (BDNF), is synthesized in some sensory neurones [9], and may maintain their survival [1]. Our recent studies indicate that BDNF in sensory neurones is anterogradely transported to the periphery and the spinal cord [15]. This transportation is significantly enhanced by peripheral nerve lesion [11]. BDNF-immunoreactivity (-ir) accumulates on both sides of the transected dorsal column of the spinal cord [4]. In the present study, we focus on changes of BDNF and NPY in the gracile nucleus of the brain following unilateral transection of the sciatic nerve.

All experiments were approved by the Animal Welfare Committee of Flinders University. Male Sprague–Dawley rats weighting 250–300 g were anaesthetised with Halothane, the left sciatic nerve exposed, ligated and sectioned at the mid-thigh level. Animals were allowed to survive for 1, 3, 7, 14, 28 or 56 days postlesion (three rats for each time point, plus three control rats). For the 7-day time point, ipsilateral to the left sciatic nerve cut, a hemisection of the spinal cord at T_{12} was performed in one additional rat, and a dorsal rhizotomy in two other rats. Rats were sacrificed with an overdose of sodium pentobarbitone and perfu-

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sion fixed in phosphate buffer containing 2% formaldehyde and 15% saturated picric acid. Brain stems, spinal cords and DRG were dissected, postfixed for 2 h, placed in 30% sucrose buffer solution overnight, cut frozen at 50 μm, and stained for BDNF and NPY.

Rabbit antibodies to BDNF have been fully characterized previously [15]. BDNF immunohistochemistry was performed as described [15], but the staining was enhanced with a Tyramide Signal Amplification kit (NEN Life Science Products). Sheep antiserum against NPY (Chemicon, Temecula, CA) was used at a dilution of 1:5000. Double staining of BDNF and NPY in DRG was performed using fluorescein-conjugated donkey anti-rabbit and rhodamine-conjugated donkey anti-sheep IgG (Jackson ImmunoResearch Laboratory, West Grove, PA).

In normal L4 and L5 DRG, BDNF-ir was mainly found in small to medium-sized sensory neurones (Fig. 1A), consistent with our previous finding [15]. No NPY-ir was detected. The number of large sensory neurones immunoreactive for BDNF in the ipsilateral L4 and L5 DRG increased by 7 days post sciatic nerve lesion (Fig. 1B). In the contralateral DRG, the labelling pattern was similar to normal control DRG. In the normal gracile nucleus, only a few nerve terminals were immunoreactive for BDNF whereas sciatic nerve transection led to pronounced BDNF-ir (Fig. 1C,D). The increase was apparent 24 h after the lesion, and remained robust at all time points studied (1–56 days). To quantify the staining intensity, all the micrographs were taken using the same conditions without changing the intensity of input light. Grey scales were read from an area 150 × 50 μm over the gracile nuclei on both sides using an NIH Image programme (V.1.59). Values were calculated as differences between contralateral and ipsilateral sides. Significant elevation of BDNF-ir persisted for 56 days after the injury (one-way ANOVA, $P < 0.01$; Fig. 2A,B and Fig. 3). BDNF-ir was never found in nerve tracts in the dorsal column of the spinal cord. Lesion to the dorsal column or rhizotomy of L4 and L5 dorsal roots prevented the increase in BDNF-ir in the ipsilateral gracile nucleus 7 days after sciatic nerve lesion.

Consistent with previous findings [12,14], a marked increase of NPY-ir in large sensory neurones of the ipsilateral DRG was observed (Fig. 4B). Since large sensory neurones became immunoreactive for both BDNF and NPY after nerve lesion, we sought to determine whether they were colocalized. Over 96% NPY-immunoreactive sensory neurones in the ipsilateral DRG were colocalized with BDNF-ir. Usually, small BDNF-immunoreactive neurones lacked NPY-ir (Fig. 4A,B). NPY-immunoreactive fibres were also found in the ipsilateral gracile nucleus after the sciatic nerve transection, but the time course of their appearance was different from that of BDNF-immunoreactive fibres. NPY-ir was unchanged during the first 3 days after the injury, but began to increase after 7 days. The immunoreactivity was restricted to nerve terminals and varicosities, became more obvious after 2 weeks and remained positive

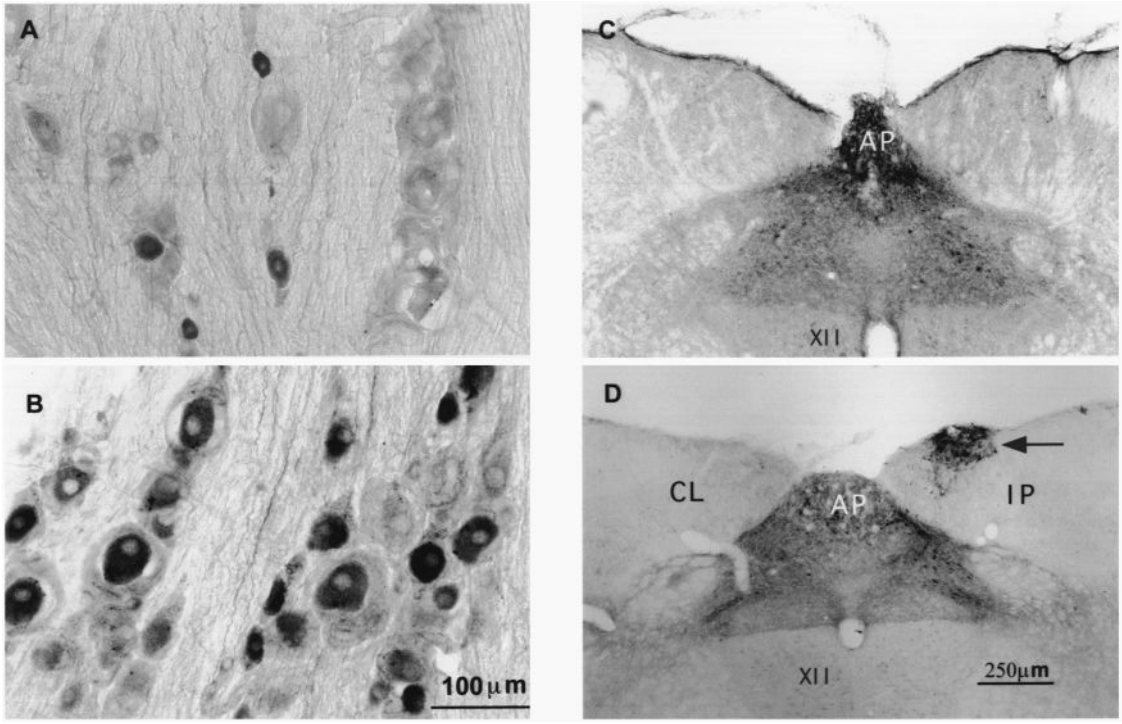

Fig. 1. (A) BDNF-ir in the normal L5 DRG and (B) in the L5 DRG 7 days after sciatic nerve transection. (C) Effect of sciatic nerve lesion on BDNF-ir in the brain stem; a normal control section. (D) A brain stem section from a rat 7 days after unilateral transection of the sciatic nerve. The arrow indicates the increase in BDNF-ir. IP: ipsilateral to the sciatic nerve lesion. CL, contralateral to the sciatic nerve lesion; AP, area postrema; XII, hypoglossal nucleus.

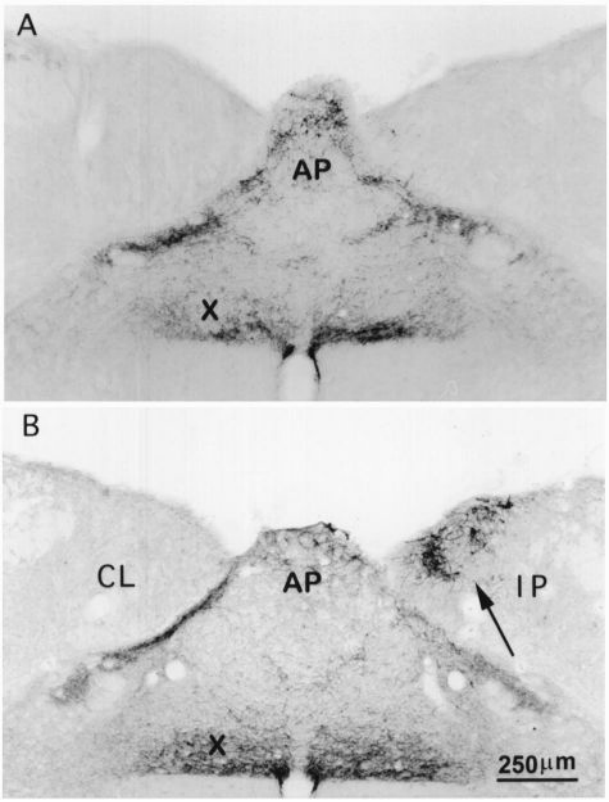

Fig. 2. Effect of sciatic nerve lesion on NPY-ir in the brain stem. (A) A normal control section. (B) A brain stem section from rat 7 days after unilateral transection of sciatic nerve. The arrow indicates the increase in NPY-ir. IP, ipsilateral to the sciatic nerve lesion; CL, contralateral to the sciatic nerve lesion; X, dorsal motor nucleus of vagal nerve.

for at least 56 days (one-way ANOVA, $P < 0.01$, Fig. 3). No NPY-ir was detected in the contralateral or normal control gracile nuclei.

Since BDNF-ir and NPY-ir were colocalized following sciatic nerve injury and the increase in NPY-ir appeared much later than BDNF-ir, it is possible that endogenous BDNF was involved in the regulation of the expression of NPY. To address this issue, BDNF antiserum was injected intraperitoneally to neutralize any extracellular BDNF in the periphery. BDNF antiserum (5 ml/kg body weight, twice a week) was injected into two rats, and non-immune normal rabbit serum into one rat as control, for 7 days after the sciatic nerve transection [15]. No significant changes of NPY-ir were found in the gracile nucleus or DRG after the antiserum treatment (not shown). However, injection of this antiserum substantially reduced BDNF accumulation in the distal, but not proximal side of a ligature on the vagal nerve, indicating that this antiserum has a biological activity in neutralization of extracellular, but not intracellular, BDNF. While this result supports the conclusion that endogenous BDNF does not regulate NPY expression, it does not exclude the possibility of central regulation.

NPY may exert anti-nociceptive actions by inhibiting the release of neurotransmitters from trigeminal and DRG neurones [6]. NPY is not expressed in normal DRG neurones but is upregulated in large-diameter neurones following peripheral nerve injury. Local treatment with FGF-1 or FGF-2, but not NGF, counteracts the axotomy-induced increases in NPY expression [13]. Local application of NT-3 also significantly diminished the transganglionic NPY response in the gracile nucleus and spinal cord [8]. Upregulation of NPY in these neurones was attenuated in leukaemia inhibitory factor-deficient, as compared with wild-type mice [2]. Taken together, this body of evidence suggests neurotrophic factors are likely to be involved in NPY regulation, however, our results suggest that peripheral BDNF is probably not involved.

In this study, we found large sensory neurones projecting to the gracile nucleus become BDNF-immunoreactive in response to sciatic nerve lesion. The increase of BDNF-ir in the gracile nucleus was abolished following combined transection of the sciatic nerve and the ipsilateral dorsal column or dorsal root rhizotomy, suggesting BDNF in large neurones is also anterogradely transported. It is likely that the upregulation of BDNF in large sensory neurones is involved in triggering morphological changes in the DRG and spinal cord after the nerve injury [7]. BDNF is anterogradely transported [15], stored in vesicles [5] and thus, may be released in a manner similar to other neurotransmitters. The BDNF high affinity receptor, TrkB, is also upregulated in injured sensory neurones [3]. In a recent study, antibodies to BDNF, injected systemically, attenuated the pain response to a heat stimulus after nerve lesion [10]. It is possible that BDNF synthesised in large sensory neurones is released within the DRG and spinal cord, playing a role in neuropathic pain after nerve lesion. In addition, the upregulation of BDNF in large diameter neurones may play a role

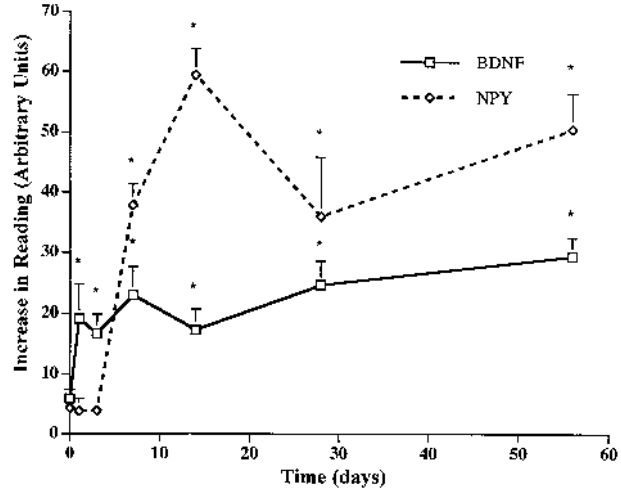

Fig. 3. The increase of BDNF-ir and NPY-ir quantified in gracile nucleus at several time points following sciatic nerve transection. The values were converted from grey scale reading from ipsilateral gracile nuclei, which were subtracted by reading from the corresponding contralateral side. All readings were taken from a fixed optical condition. Each point represents mean ± SE ($n = 3$); *$P <$ 0.01.

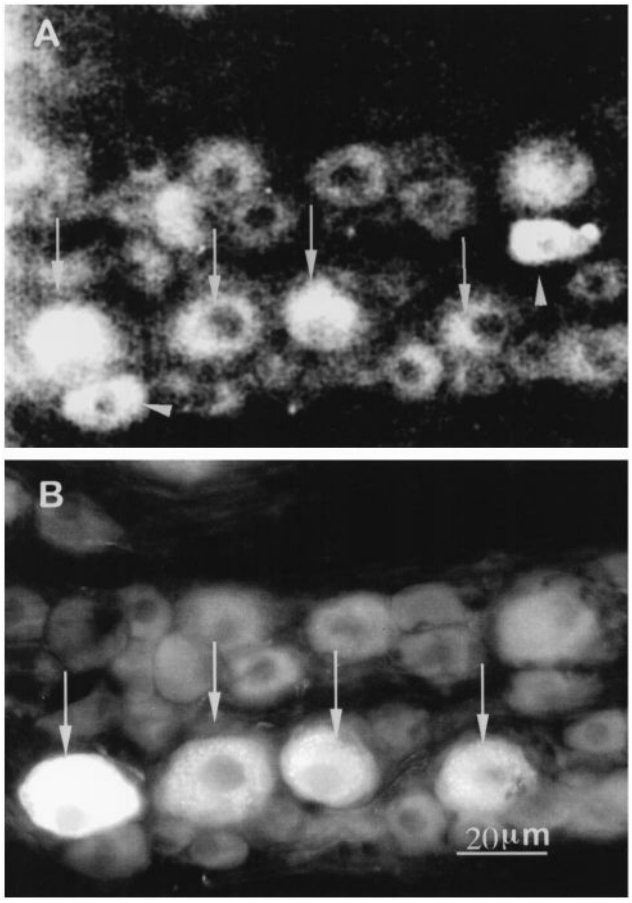

Fig. 4. Colocalization of BDNF-ir (A) and NPY-ir (B) in the L5 DRG by 7 days after sciatic nerve transection. Over 96% of all NPY-ir sensory neurons in the ipsilateral DRG was colocalized with BDNF (arrows). BDNF-ir neurons containing no NPY-ir were generally of a small diameter (arrow heads).

in the survival of axotomized neurones through an autocrine mechanism [1].

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