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TYROSINE KINASE RECEPTOR IMMUNOREACTIVITY IN TRIGEMINAL MESENCEPHALIC AND MOTOR NEURONS FOLLOWING TRANSECTION OF MASSETERIC NERVE OF THE RAT

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Abstract—Neurotrophins are known to promote survival after neural injury. To determine the relative importance of tyrosine kinase receptors on the survival of axotomized trigeminal nuclear neurons, we examined the temporal expression profile of tyrosine kinase A, tyrosine kinase B and tyrosine kinase C receptors in the mesencephalic trigeminal nucleus and the motor trigeminal nucleus following transection of the masseteric nerve in rats. Axotomized neurons in these nuclei were retrogradely identified with FluoroGold. We found increase in tyrosine kinase A-immunoreactive mesencephalic trigeminal nucleus neurons in the second week after axotomy but no change in the number of tyrosine kinase A-immunoreactive motor trigeminal nucleus neurons. There was no change in the number of tyrosine kinase B-immunoreactive mesencephalic trigeminal nucleus neurons but the significant increase of tyrosine kinase B-immunoreactive motor trigeminal nucleus neurons throughout the period of observation (3 weeks) peaked at ~1 week after axotomy. There was no alteration in the number of tyrosine kinase C-immunoreactive mesencephalic trigeminal nucleus neurons but significant increase in tyrosine kinase C-immunoreactive motor trigeminal nucleus neurons observable by 4 days post-axotomy was followed by decline to levels lower than the control in 2 weeks. Temporal changes in the expression of individual tyrosine kinase receptors in mesencephalic trigeminal nucleus and motor trigeminal nucleus neurons following transection of the masseteric nerve suggest differential contribution of tyrosine kinase-specific neurotrophins to the survival of these neurons after axotomy. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: retrograde tracing, trigeminal nucleus, immunohistochemistry, tyrosine kinase receptor, axotomy.

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Abbreviations: ABC, avidin–biotin complex; BDNF, brain-derived neurotrophic factor; FG, FluoroGold; Me5, trigeminal mesencephalic nucleus; MNT, masseteric nerve trunk; Mo5, motor trigeminal nucleus; NGF, nerve growth factor; NGS, normal goat serum; NT, neurotrophin; PBS, phosphate-buffered saline; TR, Texas Red; Trk, tyrosine kinase.

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Nerve injury often results in atrophy or even death of affected neurons (Berkelaar et al., 1994; Groves et al., 1997, 1999, 2003; Krueger-Naug et al., 2003). Neurodegeneration is attributed in part to deficiency in neurotrophins (Barde, 1989; Huang and Reichardt, 2001, 2003; Kobayashi et al., 1997; Giehl et al., 1998, 2001; Groves et al., 1999; Krueger-Naug et al., 2003). Neurotrophins (NTs), including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3 and NT-4/5, are crucial for neuron differentiation and survival during development as well as synaptic plasticity and neuronal survival in adult life (Barbacid, 1994; Huang and Reichardt, 2001, 2003). Following axotomy, administration of exogenous BDNF, NTs or recombinant adenoviral vector carrying genes for BDNF enhanced the survival of primary sensory neurons (retina ganglion neurons and dorsal root ganglion neurons: Eriksson et al., 1994; Groves et al., 1999; Krueger-Naug et al., 2003), motoneurons (in facial nucleus and spinal cord: Koliatsos et al., 1993; Eriksson et al., 1994; Baumgartner and Shine, 1997; Gimenez y Ribotta et al., 1997; Gravel et al., 1997), and spinal-projecting central neurons (Kobayashi et al., 1997; Giehl et al., 1998, 2001). Because of the pronounced effect of NTs in promoting survival following nerve injuries, both transient and long-term modulations of tyrosine kinase (Trk) receptor expression have been extensively studied in primary sensory neurons (Behnia et al., 2003; Bergman et al., 1999; Foster et al., 1994; Krekoski et al., 1996; Li et al., 2000; Wheeler et al., 1998) and central neurons after different injury paradigms (Barbany and Persson, 1993; Frisen et al., 1992; Numan and Serogy, 1997; Mudo et al., 1993).

The trigeminal mesencephalic and motor nuclei (Me5 and Mo5, respectively) are essential components of the jaw-closing reflex (Holstege et al., 1995; Lingenhöhl and Friauf, 1991). It has been well established that cranial proprioceptive signals, mainly from masticatory muscle spindles, reach Me5 neurons and are then transmitted, either directly or via brainstem premotor neurons, to Mo5 for the control of jaw movement (Lingenhöhl and Friauf, 1991; Holstege et al., 1995). Previous studies have reported that Me5 and Mo5 neurons expressed NT receptors which, in Me5 neurons, mediate trophic or modulatory effects (Yan et al., 1997; Jacobs and Miller, 1999; Yamuy et al., 2000a). In addition, TrkB and TrkC null mice exhibited deficits in muscle spindles and Me5 neurons during development (Fan et al., 2000; Matsuo et al., 2000). Application of exogenous NGF and NT-3 however increased the excitability of mature Me5 neurons (Yamuy et al., 2000b). Although NT/Trk signaling plays important roles in

maintaining the neuronal phenotype and function of Me5 and Mo5 neurons, little is known about their expression in Me5 and Mo5 neurons following axotomy. In the present study, we aim to document the expression of Trk receptors within 3 weeks after transection of the masseteric nerve trunk (MNT).

EXPERIMENTAL PROCEDURES

Thirty Sprague–Dawley rats (200–220 g) of either sex, divided into six groups of five each, were used in the present study. All animal protocols and procedures were performed in compliance with the *Principles of Laboratory Animal Care* (NIH publication no. 86-23, revised 1985) and were approved by the University Committee on the Use of Live Animals in Research. All efforts were made to minimize animal suffering and to reduce the number of animals used. One group of rats was used for immunohistochemical expression of Trk receptors in normal animals, and four groups received transection of the MNT. Under pentobarbital sodium anesthesia (60 mg/kg body weight, i.p; Sagatal, RMB Animal Health Ltd., Dagenham, UK), a lateral incision of the skin was made on the left side of the face from the ear to the caudal edge of the eye. Part of the zygomatic arch adjacent to the temporomandibular joint was removed to expose the MNT, which contains both the peripheral axons of Me5 neurons and axons of Mo5 neurons. Following transection of MNT at the site where it forks to enter the masseter muscle, a small piece of aluminum foil was placed under the cut end of the MNT. This was intended to prevent spilling of FluoroGold (FG, 2%; Fluorochrome, Denver, CO, USA) to neighboring tissues when applied via a FG-soaked cotton pellet to the proximal cut end of the MNT for 1 h. After the application of FG, the cut end was washed with physiological saline for several times. The muscles and skin were sutured and the operated rats were returned to single cages. Appropriate postoperative care was provided. Both lidocaine (Astra, Sweden) and antibiotic ointment (Furacin) were applied four times daily to the skin wound. The health status of the operated rats was closely monitored. These rats were allowed to survive for 4, 7, 14 and 21 days postoperation, respectively. We estimated that post-traumatic reorganization would occur in this period given that facial motoneurons regenerated within 3 weeks of crush injury to the facial nerve of adult animals (Moran and Graeber, 2004).

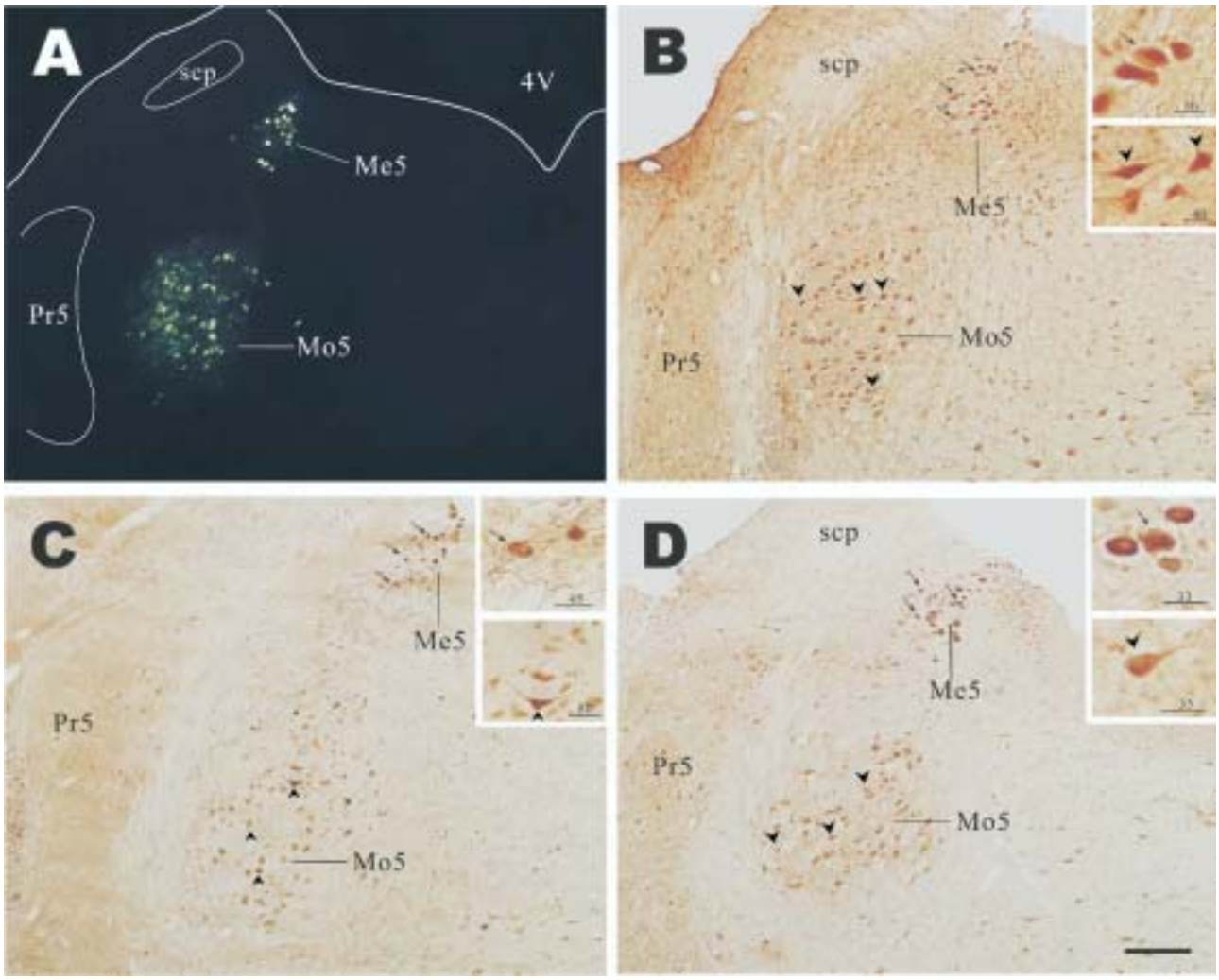

Fig. 1. Photomicrographs of sections through Me5 and Mo5. (A) Fluorescent image showing Me5 and Mo5 neurons retrogradely labeled by FG. B, C and D show brainstem neurons with immunoreactivities for TrkA, TrkB and TrkC, respectively. Neurons in Me5 (indicated by arrows) and Mo5 (arrowhead) are displayed at a higher magnification in the upper and lower insets, respectively. Abbreviations: Pr5, principal sensory trigeminal nucleus; scp, brachium conjunctivum; 4V, 4th ventricle. Scale bar=250 μm, unless otherwise indicated in the insets.