Journal of Plastic, Reconstructive & Aesthetic Surgery (2006) **59**, 393–397

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

The expression of a motoneuron-specific serine protease, motopsin (PRSS12), after facial nerve axotomy in mice

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Received 28 August 2004; accepted 21 April 2005

KEYWORDS
Neurotrypsin;
Brain-specific serine protease;
Facial nucleus;
In situ hybridisation;
Growth associated protein-43;
Mouse

Summary Motopsin (PRSS12) is a mosaic serine protease that is preferentially expressed in motor neurons. To study the relationship between motopsin and motoneuron function, we investigated the expression of motopsin mRNA in facial nerve nuclei after facial nerve axotomy at the anterior margin of the parotid gland in mice. Neuronal function was monitored by assessing vibrissal motion in 3 months. Vibrissal behaviour on the injured side disappeared until the day 14 post-operation, and then recovered between the day 21 and 35. Motopsin expression decreased at the day 14, but markedly recovered by the day 21. In contrast, expression of growth-associated protein-43 (GAP-43) was induced at the day 3. These results suggest that the recovery of motopsin expression is correlated with the recovery of the facial motor neuronal function.

Evidence that serine proteases play important roles in neuronal plasticity is accumulating. Two serine proteases, tissue type plasminogen activator (tPA) and thrombin, exert a strong influence on central nervous system (CNS) development, neuronal activity, and cell death. tPA is induced as an immediately early gene during seizure, kindling and long-term potentiation (LTP). Hippocampal slice culture from tPA knock-out mice shows selective reduction in Schaffer collateral and mossy fibre pathways. In contrast, over-expression of tPA in post-natal neurons enhances hippocampal LTP. tPA secreted into the extracellular environment activates plasminogen to plasmin, which

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doi:10.1016/j.bjps.2005.04.018

digests laminin during LTP. Thrombin is well characterised as a negative factor for neuronal function, such as growth cone collapse, neurite retraction, and induction of apoptosis. However, it was recently reported that thrombin may potentiate *N*-methyl-D-aspartate (NMDA) receptor functions through activation of protease-activated receptor (PAR)-1.

We have previously identified serine proteases in the CNS,[1,2] and have isolated cDNA encoding a mosaic serine protease; the latter consists of a kringle domain, three scavenger receptor cysteine-rich domains, and a serine protease domain.[3] Expressed in all motor nuclei, the cerebral cortex, hippocampus, and amygdala, but not in sensory neurons,[4] this protease has been designated motopsin, and has been assigned to PRSS12 by the Human Gene Nomenclature Committee. Motopsin is identical to a protease previously reported by Gschwend et al.[5]

During the early developmental stage in mice, motopsin mRNA is expressed in various areas of the nervous system, including Schwann cell precursors, olfactory epithelium, trigeminal ganglion, midbrain, and floor plate.[6] Motopsin may play an important role in neuronal development and plasticity,[7] although the relationship between expression of motopsin in the CNS and neuronal functions is still obscure.

To investigate the physiological function of motopsin in adult motor neurons, the expression pattern of motopsin was analysed after facial nerve axotomy. Twenty female ICR mice (10 weeks old, Charles River Laboratory, Wilmington, MA, USA) were used in this study. All the animals were housed at a constant temperature (at 22 °C) with a 12-h light–dark cycle and given food and water as desired. All experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee in compliance with the Animal Research Centre of Kyoto Prefectural University of Medicine, Kyoto, Japan. The animals were anaesthetised with peritoneal administration of pentobarbital. Right facial nerves were transected at the anterior margin of the parotid gland and immediately repaired with 10/0 nylon sutures under a microsurgical microscope. The extent of injury and neuronal function was assessed using the score of vibrissal function as follows: 0, no vibrissal function; 1, slight motion; 2, improved motion; 3, the same motion as the contralateral side. For semi-quantitative analysis of motopsin mRNA expression, expression ratio was measured by counting total cell number. The expression ratio of the ipsilateral side was calculated by dividing the number of cells, which show motopsin mRNA expression by that of the contralateral side. The statistical analysis was performed with the nonparametric Mann–Whitney *U*-test.

No vibrissal function was observed until the day 14 post-operation (Fig. 1b). Vibrissal function markedly improved between the day 21 and 35, and by the day 70, it had recovered to nearly the same level as on the contralateral side, indicating re-innervation of the facial nerve. Expression of motopsin and growth associated protein (GAP)-43 was analysed by in situ hybridisation every week post-operation until the day 84 by using the procedures detailed in a previous report.[4] Both antisense and sense RNA probes were synthesised using cDNAs encoding full-length motopsin and GAP-43. GAP-43 mRNA was induced in the axotomised facial nucleus at the day 3 and remained strong at the day 14 (Fig. 1a (A–D)). Expression of GAP-43 mRNA gradually decreased after the day 14, although it was still apparent at the day 21 (Fig. 1a (E and F)); there was no observable difference in expression level between sides at the day 35 (Fig. 1a (G and H)) and later (data not shown). This pattern of expression of GAP-43 is consistent with previous reports,[8] the axotomy and nerve regenerating circumstances were well controlled.

In contrast, expression of motopsin mRNA in the axotomised facial nucleus was observed at the same level as in the contralateral side at the day 7 (Fig. 2a (A and B)). Compared with the contralateral side, however, motopsin mRNA expression in the injured side markedly decreased at the day 14; Fig. 2a (C and D) shows considerably reduced expression of motopsin mRNA in the axotomised facial neurons at this time. At the day 14, the level of motopsin mRNA expression in the injured side was statistically reduced, compared with that of contralateral side ($p<0.05$, Fig. 2b). The level of expression of motopsin mRNA in the axotomised facial nucleus gradually recovered to that of the contralateral side between the day 21 and 35 (Fig. 2a (E–H)). Vibrissal motor function markedly improved during this period of recovery of motopsin expression, suggesting that recovery of the neuronal function is involved in the re-induction of motopsin. After the initial recovery of motopsin mRNA expression in the injured side, there was no observable difference in expression level between sides at the day 35 (Fig. 2a (G and H)) and later (data not shown). Motopsin mRNA expression on injured side showed temporal reduction during the early re-innervation of the peripheral target and initial recovery, the induction of motopsin expression is consistent with the recovery of visual vibrissal behaviour, suggesting that recovery of the neuronal function is involved in the re-expression of motopsin mRNA.