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Plasminogen activator induction facilitates recovery of respiratory function following spinal cord injury

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The possibility that plasminogen activator (PA) plays a role in synaptic plasticity was explored in the spinal cord during the crossed phrenic phenomenon (CPP), where respiratory functional plasticity develops following spinal cord injury. Synaptic remodeling on phrenic motorneurons occurs during the characteristic delay period following spinal cord injury before CPP recovery of respiratory function. The molecular mechanisms underlying this plasticity are not well-defined. During the critical 1–2 h delay period required for this synaptic plasticity following a C2 hemisection in mice, uPA and tPA mRNAs are rapidly induced in C4–5 ventral spinal cord neurons in the ipsilateral phrenic motor nucleus (PMN), as are uPA and tPA protein levels. A role for uPA in CPP spinal cord plasticity is confirmed by the impaired ability of uPA knockout mice to acquire a good CPP response by 6 h post-hemisection and their lack of structural remodeling of PMN synapses that underlies development of the CPP response.

Keywords: Plasminogen activator; Crossed phrenic phenomenon; Knockout mice; Synaptic plasticity; Diaphragmatic function

Introduction

Each year thousands of individuals suffer spinal cord injuries (SCI), leading to loss of motor function and varying degrees of paralysis. Many of these are traumatic injuries to the cervical spinal cord that interrupt descending bulbospinal respiratory pathways from the medulla often rostral to the level of the phrenic motor nucleus. Such injuries cause respiratory muscle paralysis and require mechanical ventilator support. In animal models and some human SCI cases there is often some degree of respiratory recovery. Therefore, a greater understanding of the cellular mechanisms leading to respiratory pathway recovery and plasticity in the injured spinal cord may lead to new therapies for treating these spinal cord injuries.

The crossed phrenic phenomenon (CPP) recovery of respiratory function following a spinal cord injury is one of the most dramatic examples of spinal cord plasticity (for a review, see Goshgarian, 2003). Several mammalian species (Porter, 1895; Deason and Robb, 1911; Rosenblueth and Ortiz, 1936; Goshgarian, 1979; Minor et al., 2006) exhibit the CPP following cervical spinal cord hemisection rostral to the phrenic motor nucleus (PMN) which paralyzes the ipsilateral hemidiaphragm by interrupting the descending flow of respiratory impulses from the medulla to phrenic motorneurons in the spinal cord (Fig. 1 schematic). This leads to a stronger respiratory drive to the contralateral PMN via the rostral ventral respiratory group (rVRG). A small population of these contralateral rVRG fibers cross-over the spinal cord midline during development and contact phrenic motorneurons. The loss of ipsilateral rVRG input to the phrenic motorneurons coupled with this increased activity from crossed contralateral rVRG fibers converts some of their presumptive synaptic contacts on phrenic motorneurons on the paralyzed side of the spinal cord from a "functionally ineffective" state pre-hemisection to a "functionally latent" state post-hemisection. As defined by Goshgarian (2003) a "functionally latent" synapse is presumably an anatomically/physiologically modified cell contact that requires a specific time interval for conversion in rats and mice, yet still does not restore hemidiaphragm functional activity under normal conditions. If the animal is then subjected to additional respiratory stress by transecting the contralateral phrenic nerve, these latent respiratory synapses become "activated" and function is restored to the paralyzed hemidiaphragm (Goshgarian, 2003). Activation of these synapses and the crossed pathway requires an interoperative delay of several hours between hemisection and phrenicotomy to elicit the CPP (O'Hara and Goshgarian, 1991; Minor et al., 2006). Several ultrastructural changes including an elongation of synaptic active zones and increased numbers of multiple axo-dendritic synapses on phrenic motorneurons are seen during this delay period in rats (Castro-Moure and Goshgarian, 1997). However, the molecular mechanisms underlying this cellular

Abbreviations: CPP, crossed phrenic phenomenon; PA, plasminogen activator; PMN, phrenic motor nucleus; rVRG, rostral ventral respiratory group; WGA, wheat germ agglutinin.

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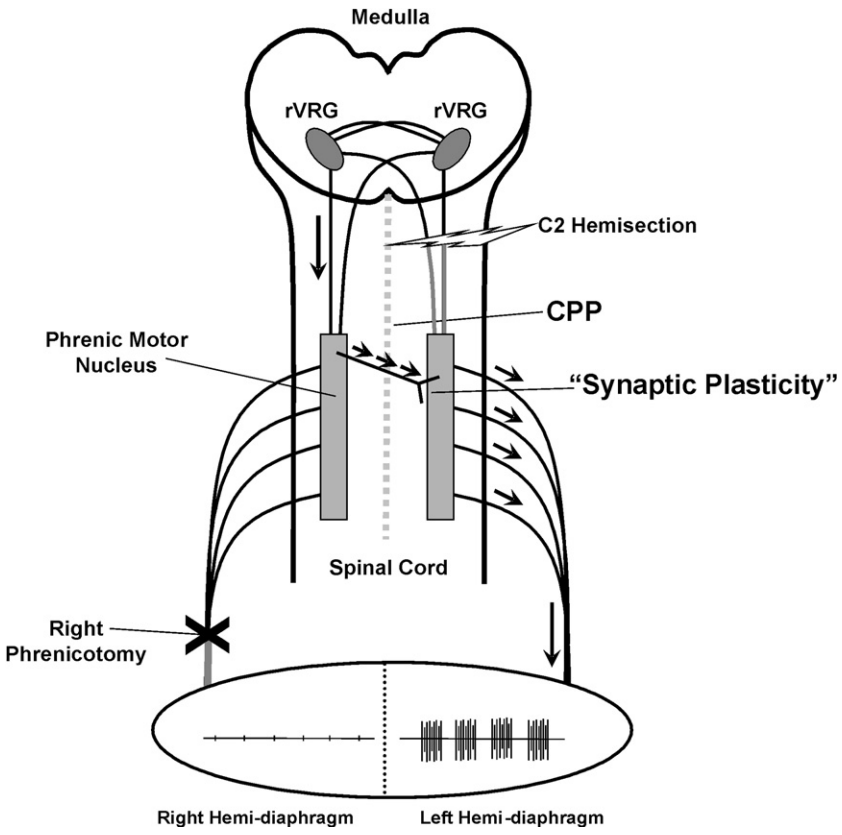

Fig. 1. Schematic of the crossed phrenic pathway. A spinal hemisection was performed at C2 disrupting the rostral ventral respiratory group (rVRG) descending axons that innervate the phrenic motor nucleus, a column of motorneurons extending from C3 to C6, thus blocking phrenic motorneuron axonal output and silencing the ipsilateral left hemidiaphragm. Subsequent transection of the contralateral phrenic nerve leads to asphyxia; however, if performed after a critical delay period of synaptic remodeling, the rVRG axons on the contralateral side of the spinal cord now convey respiratory impulses across the midline (arrows) via activated synaptic contacts on the silent PMN and recovery of hemidiaphragm function (lower recording) on the hemisected left side.

remodeling and the synaptic plasticity associated with the CPP and its interoperative delay are not well defined.

Plasminogen activator (PA) has been implicated in aspects of cerebral synaptic remodeling associated with cerebellar motor learning, visual cortex ocular dominance columns and hippocampal and corticostriatal LTP (Seeds et al., 1995, 2003; Muller and Griesinger, 1998; Mataga et al., 2004; Huang et al., 1996; Baranes et al., 1998), in addition to its well known roles in the vascular system, neural development and excitotoxic cell death (Collen, 1980; Krystosek and Seeds, 1981; Pittman, 1985; Seeds et al., 1999; Tsirka et al., 1995). Therefore, we have explored the possibility that PA may also play a role in spinal cord synaptic plasticity associated with the CPP and the recovery of respiratory function.

Results

We recently showed that mice, an animal model more amenable to a molecular genetic approach, also express the CPP (Minor et al., 2006). A C2 spinal cord hemisection in mice removes all respiratory activity in the ipsilateral hemidiaphragm (i.e., a functionally complete hemisection) (Fig. 2). If the contralateral phrenic nerve is then cut, both hemidiaphragms are silent and the animal must be ventilated. However as shown previously (Minor et al., 2006), if the contralateral phrenicotomy is delayed for several hours then the ipsilateral hemidiaphragm shows some recovery of function upon phrenicotomy; i.e., the CPP response. More complete recovery of function is seen as the delay time is increased, and by 6 h post-hemisection about 90% of the mice show a CPP response upon phrenicotomy. This immediate CPP recovery of diaphragm function following a phrenicotomy at 24 h post-spinal cord lesion is illustrated in Fig. 2. Anatomical completeness of hemisection is shown in Fig. 3. Although structural remodeling has been seen at the ultrastructural level (Goshgarian et al., 1989; Sperry and Goshgarian, 1993), the molecular mechanisms occurring during this critical delay period prior to CPP recovery are not well characterized.

Prior studies with tissue remodeling events and synaptic plasticity associated with different learning paradigms suggested that the extracellular protease plasminogen activator may play a role in spinal cord synaptic remodeling associated with the CPP response. This possibility was explored initially by assessing the expression of the plasminogen activators uPA (urokinase) and tPA (tissue plasminogen activator) in the PMN during the critical latent period following a C2 hemisection. Using a ^{35}S-cRNA antisense probe complementary to uPA mRNA, a rapid and specific induction of uPA mRNA within the first 2 h post-hemisection was seen in a limited population of motorneurons in the PMN region of the ipsilateral C4–5 ventral spinal cord (Fig 4A). This uPA mRNA induction was restricted to large cells in the ipsilateral PMN region (Fig. 4A inset) and was not seen anywhere at the C2–3 interface or the C6–7 interface ventral spinal cord above or below the PMN, nor was any reactivity seen with the "uPA-sense" ^{35}S-cRNA control probe in these spinal cord sections (data not shown). Interest-