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Growth-associated gene expression after stroke: Evidence for a growth-promoting region in peri-infarct cortex

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Abstract

Stroke induces axonal sprouting in peri-infarct cortex. A set of growth-associated genes important in axonal sprouting in peripheral nervous system regeneration and cortical development has recently been defined. The expression profiles of these growth-associated genes were defined during the post-stroke axonal sprouting response using a model of stroke in barrel field cortex. Stroke induces sequential waves of neuronal growth-promoting genes during the sprouting response: an early expression peak (SPRR1), a mid expression peak (p21, Ta1 tubulin, L1, MARCKS), a late peak (SCG10, SCLIP), and an early/sustained pattern (GAP43, CAP23, c-jun). These expression peaks correspond to specific time points in the sprouting response. The expression of the growth-inhibiting chondroitin sulfate proteoglycans aggrecan, brevican, versican, and phosphacan are induced late in the sprouting process; except neurocan, which is increased during the peak of the growth-promoting gene expression. The developmentally associated growth inhibitors ephrin-A5, ephB1, semaphorin IIIa, and neuropilin 1 are also induced in the early phases of the sprouting response. At the cellular level, chondroitin sulfate proteoglycans, in the form of peri-neuronal nets, are reduced in the region of axonal sprouting, during the peak of growth-promoting gene expression. These results identify a unique profile of growth-promoting gene expression in adult cortex after stroke, the inhibitory molecules that are present during the sprouting response, and a region in which growth-promoting genes are increased, growth-inhibitory proteins are diminished and axonal sprouting occurs. This region may be a growth-promoting zone after stroke.

Keywords: Plasticity; Repair; Sprouting; Barrel cortex; GAP43

Introduction

Recovery after stroke is limited, making it the leading cause of adult disability (American Heart Association, 2004). However, stroke induces a process of axonal sprouting in peri-infarct tissue that results in a substantial re-mapping of the connections of the somatosensory cortex adjacent to the infarct (Carmichael et al., 2001). This post-stroke axonal sprouting response is correlated in location and magnitude with functional recovery after stroke (Calautti and Baron, 2003; Carmichael, 2003a; Dijkhuizen et al., 2003). An understanding of the molecular mechanisms of post-stroke axonal sprouting may provide a framework for developing therapies to improve functional recovery.

Nervous system injury induces expression of both growth-promoting and growth-inhibitory genes that together determine the location and degree of axonal sprouting. Injury to peripheral nerve, and spinal cord or brain trauma, upregulate a set of growth-promoting genes, termed regeneration-associated genes (Bulsara et al., 2002; Carulli et al., 2004; Plunet et al., 2002). These gene products mediate growth cone membrane signaling events, transcriptional control in the regenerating neuron, cytoskeletal reorganization and axonal extension, and include GAP43, CAP23, MARCKS, c-jun, members of the stathmin family, Tα1 tubulin, L1, p21/waf1, and SPRR1. These proteins are specifically upregulated during sprouting, reduce axonal sprouting when knocked down or out, and mediate

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enhanced axonal sprouting when overexpressed (Benowitz et al., 2002; Bomze et al., 2000; Bonilla et al., 2002; Herdegen et al., 1997; Tetzlaff et al., 1994; Zagrebelsky et al., 1998; Laux et al., 2000; Carmichael, 2003b; Mason et al., 2002; Mori and Morii, 2002). Peripheral nerve and spinal cord injury also induce the expression of the three major classes of growth-inhibitory genes, myelin-associated proteins, chondroitin sulfate proteoglycans (CSPGs), and developmentally associated axonal growth inhibitors (Sandvig et al., 2004; Silver and Miller, 2004). CSPG expression limits axonal sprouting in glial scar adjacent to traumatic spinal cord or brain injury (Bradbury et al., 2002; Silver and Miller, 2004). Myelin-associated genes, such as Nogo A, block axonal sprouting after brain and spinal cord trauma (Lee et al., 2004a,b). Developmentally associated axonal growth inhibitors, such as members of the ephrin and semaphorin classes, participate in the glial/meningeal boundary formation during spinal cord trauma that limits local axonal sprouting (Bundesen et al., 2003; Sandvig et al., 2004; Silver and Miller, 2004).

Though stroke induces a robust process of axonal sprouting (Carmichael et al., 2001), and is the major form of CNS injury in the adult, there is limited data on the pattern of growth-associated gene expression after stroke. GAP43 immunohistochemical staining is increased in peri-infarct cortex during the first 2 weeks after stroke (Kawamata et al., 1997; Stroemer et al., 1995; Gregersen et al., 2001). The transcription factor c-jun is induced for up to 1 month in peri-infarct cortex (Ohba et al., 2003). The regeneration-associated gene small proline repeat rich protein (SPRR1) is increased in the first day in peri-infarct cortex after stroke (Lu et al., 2003). In terms of growth-inhibitory molecules, semaphorin IIIa (semaIIIa) and its receptor neuropilin 1 (NP1) are increased in the first 12 h after stroke (Fujita et al., 2001), and blockade of NogoA increases long distance axonal sprouting after stroke (Papadopoulos et al., 2002). These data indicate that some growth-promoting and growth-inhibiting genes are induced in tissue adjacent to the infarct, but there has been no systematic study of the gene systems that underlie post-stroke axonal sprouting.

The study of growth-associated gene expression after stroke has been hampered by the inability to relate changes in these genes to the location of axonal sprouting and to biological events in the sprouting process. Most studies have measured changes in growth-associated genes in a broad region of peri-infarct tissue at a small number of arbitrarily determined time points. This problem of localization and time point analysis was addressed in the present study by using a new, but well-characterized model of stroke in the rat somatosensory barrel cortex, with precisely mapped regions of axonal sprouting, glial scarring, and cell injury responses (Carmichael et al., 2001, 2004; Katsman et al., 2003). This anatomical localization was used to determine the expression pattern and cellular location of growth-promoting and growth-inhibitory genes within the area that undergoes axonal sprouting and relate these changes to time points in the initiation, maintenance, and termination phases of axonal sprouting (Kawamata et al., 1997; Stroemer et al., 1995; Carmichael and Chesselet, 2001; Carmichael et al., 2001). The data show that stroke induces a unique expression pattern of growth-promoting genes, time-locked to a reduction in the tissue distribution of a class of inhibitory molecules, the CSPGs. Together, this transient upregulation of growth-promoting genes and elimination of CSPGs define a time window and cortical region of axonal sprouting after stroke.

Materials and methods

Stroke model

All animal procedures were performed in accordance with the National Institutes of Health Animal Protection Guidelines and were approved by the UCLA Chancellor's Committee on Animal Research. Focal cortical strokes were produced on 2–4 months old adult male F344 rats as described (Carmichael et al., 2004; Katsman et al., 2003). Briefly, under isoflurane anesthesia, a small craniotomy was produced over the parietal cortex. One to two anterior branches of the distal middle cerebral artery were permanently occluded (Fig. 1). Both common carotid arteries were then clamped for 1 h. This produces three circumferential cortical regions: an area of permanent ischemia and pan-necrosis in the anterolateral and posteromedial barrel fields; an adjacent region of reperfusion injury, gliosis, apoptotic cell death; and a further region in which there is no cell death or injury (Carmichael et al., 2004; Katsman et al., 2003; Li et al., in press). Control animals received craniotomy alone.

Primer design

Primers for each gene were designed from NCBI sequences in the rat (Table 1) (Lasergene 5.0, DNAStar) and confirmed to be specific with BLAST search (NCBI). SPRR1 exists in two separate isoforms in the human, but only one has been described in the rat (NCBI). Primers were designed for the rat SPRR1. Primer sets were designed to cross intron/exon boundaries for 13 genes (Table 1), as a control against amplifying possible contaminating DNA in the RNA preparations. PCR conditions for each primer set were optimized with standard temperature and magnesium gradients. PCR products were confirmed to be the predicted size by gel electrophoresis. The efficiency of the PCR reaction at serial dilutions of cDNA in real-time PCR (Lightcycler, Roche Diagnostics, Indianapolis, Indiana) was used to generate an efficiency curve for each primer set. The log/log plot of PCR amplification vs. dilution should produce a slope near the theoretical PCR value of 2.0 (Pfaffl, 2001). If this was not the case, the primer set was redesigned.