Activation of Protease-Activated Receptor-1 Triggers Astrogliosis after Brain Injury

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We have studied the involvement of the thrombin receptor [protease-activated receptor-1 (PAR-1)] in astrogliosis, because extravasation of PAR-1 activators, such as thrombin, into brain parenchyma can occur after blood–brain barrier breakdown in a number of CNS disorders. PAR1+/− animals show a reduced astrogliotic response to cortical stab wound, suggesting that PAR-1 activation plays a key role in astrogliosis associated with glial scar formation after brain injury. This interpretation is supported by the finding that the selective activation of PAR-1 in vivo induces astrogliosis. The mechanisms by which PAR-1 stimulates glial proliferation appear to be related to the ability of PAR-1 receptor signaling to induce sustained extracellular receptor kinase (ERK) activation. In contrast to the transient activation of ERK by cytokines and growth factors, PAR-1 stimulation induces a sustained ERK activation through its coupling to multiple G-protein-linked signaling pathways, including Rho kinase. This sustained ERK activation appears to regulate astrocytic cyclin D1 levels and astrocyte proliferation in vitro and in vivo. We propose that this PAR-1-mediated mechanism underlying astrocyte proliferation will operate whenever there is sufficient injury-induced blood–brain barrier breakdown to allow extravasation of PAR-1 activators.

Key words: protease receptor; thrombin; astrogliosis; trauma; MAP kinase; G-protein

Introduction
Glial cells participate in several important CNS processes, including ion homeostasis, neurotransmitter uptake, neuremodulation, and neuroinflammation. After traumatic or ischemic brain insults, astrocytes undergo proliferation (Liu et al., 2000), phenotypic changes (Ridet et al., 1997), and cellular hypertrophy. The latter is characterized by increased expression of the astrocyte-specific glial fibrillary acidic protein (GFAP) and emission of GFAP-positive processes. This astrogliosis is a shared feature of acute and chronic neurodegenerative diseases that are characterized by an inflammatory component. Although astrogliosis is typically considered a barrier to repair (Menet et al., 2003; Silver and Miller, 2004), reactive astrocytes also have some regenerative effects (Hatten et al., 1991; Ridet et al., 1997; Fawcett and Asher, 1999; Liberto et al., 2004; Hamill et al., 2005).

The molecular trigger for astrogliosis in vivo remains unknown. In addition to cytokines and growth factors implicated in glial reaction to injury, serine proteases such as thrombin also control astrocyte function (Rogove et al., 1999; Gingrich and Traynelis, 2000; reactive astrocytes also have some regenerative effects (Hatten et al., 1991; Ridet et al., 1997; Fawcett and Asher, 1999; Liberto et al., 2004; Hamill et al., 2005).

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effects by using a genetically altered mouse lacking PAR-1 and a small peptide-selective PAR-1 activator with the amino acid sequence TFLLR-NH$_2$ (TFLLR) to test specifically whether thrombin extravasation can trigger astrogliosis through PAR-1 activation.

**Materials and Methods**

**Animals.** PAR1$^{-/-}$ and wild-type controls were generated by breeding male PAR1$^{-/-}$ mice, a gift from Dr. Shaun Coughlin (University of California, San Francisco, CA) (Connolly et al., 1996), with female C57BL/6 wild-type mice from The Jackson Laboratory (Bar Harbor, ME). Breeding heterozygous littermates generated homozygous null mutants and genetically matched wild-type controls that were 99.9% C57BL/6. Animals used for these studies were within five generations of the initial homozygous null mutant or wild-type breeding pairs.

**Cortical stab wound.** All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee at Emory University. PAR1$^{-/-}$ or littermate controls were anesthetized with a 1:1 mixture of rompun and ketamine (0.12 ml/100 mg), a midline incision was made through the scalp, and the skin was retracted laterally. The periosteum was cleaned from the skull, and a rectangular window was drilled over the left cerebral hemisphere, exposing the dura. The implant to be inserted was a specially shaped nitrocellulose filter (3 x 3 mm; Millipore, Bedford, MA), which was boiled in several changes of distilled water to remove residual surfactants, dried, and stored at −20°C. A number 11 scalpel blade was inserted horizontally into the cortex. The filter implant was then inserted 2–5 mm into the stab wound with a pair of self-closing forceps. The skin was sutured closed, and, 7 d after injury, animals were killed and transcardially perfused with 4% paraformaldehyde (PFA). Each brain was removed and cryoprotected in 20% sucrose.
Specific sets of oligonucleotides: β-actin sense, 5′-GTGGGCCCCTCCTAGGCACAA-3′; β-actin antisense, 5′-CTCTTGTGTGACAGGCGATTC-3′ (Ali et al., 2000); cyclin D1 sense, 5′-ATGGAAACACGAGCTCTG-3′; cyclin D1 antisense, 5′-AGCACGTGCGCTCTTTTG-3′; and PAR-1 antisense, 5′-CTCGAGCGCTAGCCCTCTT-3′. Amplification conditions using a Minicycler thermocycler (MJ Research, Watertown, MA) were as follows: 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s. Amplified products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The levels of cyclin D1 expression have been analyzed, and the procedures have been standardized relative to actin expression levels.

Statistics. Statistical evaluation was performed using Student’s t tests or ANOVA with Bonferroni–Dunn’s post hoc test, where appropriate; p < 0.05 was considered to be significant. Data are given as mean ± SEM.

Results
Role of PAR-1 in astrogliosis
To directly test whether PAR-1 activation contributes to glial scar formation, we evaluated astrogliosis in an in vivo model of cortical injury in PAR1−/− mice and littermate wild-type controls (Connolly et al., 1996). In this model, a cortical stab wound is accompanied by hemorrhage, thrombosis, and extravasation of large-molecular-weight plasma proteins. Figure 1 shows the extensive intraparenchymal penetration of extravasated Evan’s Blue-bound albumin 2 h after injury (representative of six animals); sham-operated animals (n = 3) showed little or no cortical extravasation of Evan’s Blue-bound albumin.

Because increased GFAP immunoreactivity has been used extensively as a diagnostic feature for CNS reactive gliosis, we used GFAP immunostaining to compare the astrocytic reaction to a cortical stab wound between wild-type and PAR1−/− mice (Rudge et al., 1989) (Fig. 2A, B) (n = 6). A nitrocellulose filter was used to assist in identifying the cortical stab wound during sectioning many days after injury. Seven days after filter insertion in wild-type mice, a high level of GFAP reactivity could be seen in astrocytes at the surface of the filter insert. Strong GFAP immunoreactivity was absent or greatly reduced 7 d after filter implantation in the cerebral cortex of PAR1−/− mice (Fig. 2A, right) (n = 6). To eliminate any potential effects of nitrocellulose, the same stab wound was made in wild-type mice without filter implantation and caused an increase in GFAP-positive astrocytes extending radi- ally away from the lesion (Fig. 2B) (n = 3). Evaluation of PAR1−/− animals showed a dramatic reduction in GFAP immunoreactivity in response to injury (Fig. 2B) (n = 2), indicating that PAR-1 activation may be important for gliosis.

To evaluate the participation of PAR-1 activation in glial scar formation, astrocytic reactions to intracortical injection of
10 nmol of the selective PAR-1 agonist TFLLR were studied in PAR1−/− mice as well as wild-type littermate control mice. The selectivity of TFLLR was confirmed by comparison of the calcium response between astrocytes from wild-type and PAR1−/− mice (n = 3; data not shown). Five days after TFLLR injection, a marked increase in GFAP immunoreactivity was evident in the ipsilateral (Fig. 2C, left panels) (n = 3), but not contralateral (n = 3; data not shown), cortex of wild-type mice. However, no enhancement of GFAP immunoreactivity was found in PAR1−/− mice injected with TFLLR (Fig. 2C, middle panels) (n = 3) or in wild-type animals injected with buffer (PBS–BSA 0.1%; n = 3) (Fig. 2C, right panels). Together, these data suggest an important role for PAR-1 activation in reactive gliosis and scar formation. Cellular mechanism underlying PAR-1-triggered astrogliosis

During the early phase of astrogliosis (3–5 days after injury), reactive astrocytes increase expression of the structural protein GFAP and begin to populate the periphery of the damaged region by proliferation and migration from adjacent regions (Ridet et al., 1997; Matyash et al., 2002). We considered three ways by which PAR-1 activation might contribute to astrogliosis: PAR-1 activation could stimulate astrocyte proliferation, GFAP expression, and/or migration. We tested each of these possibilities in primary cultures of murine astrocytes. First, cultured astrocytes were challenged with either 3 or 30 μM TFLLR, and proliferation was monitored by the incorporation of BrdU into newly synthesized DNA of dividing cells (Fig. 3A, B). TFLLR induced a dose-dependent
increase in BrdU incorporation into cultured astrocytes from wild-type, but not from PAR-1−/− (Fig. 3C). Second, we determined the effect of TFFLR on GFAP expression in astrocyte cultures using Western immunoblots. Treatment of cells with forskolin (50 µM), which is known to increase GFAP levels in astrocytes (Kaneko et al., 1994), increased GFAP immunoreactivity at 24 and 48 h (p < 0.05; unpaired t test; n = 3) (Fig. 3D), confirming our ability to measure changes in GFAP. However, in side-by-side experiments with forskolin, TFFLR did not significantly alter the level of GFAP immunoreactivity (unpaired t test; n = 3 at each time point). Third, we examined the effect of TFFLR on astrocyte motility, which could also play an important role in astrocytic response to injury (Matyash et al., 2002). To examine the role of PAR-1 activation on astrocyte migration, we used an in vitro wound-healing model (Hou et al., 1995) in which a cell-free zone was created within a confluent astrocyte monolayer. We subsequently analyzed by time-lapse microscopy the degree to which astrocytes repopulated the cell-free zone at 0 and 24 h under different experimental conditions. To eliminate the contribution of cell proliferation to wound closure, all experiments were performed in the presence of 10 µM AraC. As described previously (Matyash et al., 2002), we observed lamellipodial extension of cells (Fig. 3E, arrowhead) located in the immediate vicinity to the wound margin. As summarized in Figure 3F, ~22% of the wound area was repopulated within 24 h in the absence of serum, and TFFLR (30 µM) did not significantly alter the degree of repopulation at 24 h compared with serum-free control (n = 10–12). Application of serum served as a positive control and stimulated an increase in astrocyte motility that resulted in 95% wound closure (p < 0.0001; unpaired t test). Thus, PAR-1 signaling does not appear to alter the migratory processes of astrocytes in vitro.

Together, these results suggest that PAR-1 activation may contribute to glial scar formation through stimulation of astrocyte proliferation rather than through enhanced migration to the injured area or upregulation of GFAP. To test whether PAR-1 can influence proliferation in vivo, animals received one intrastriatal injection of TFFLR (0.5 µl/10 nmol/5 min) or vehicle (0.5 µl PBS–0.1% BSA/5 min) and were subsequently pulsed with BrdU to mark newly proliferating cells. To determine whether these newly generated cells within the glial scar had differentiated into astrocytes, double immunostaining for GFAP/BrdU was performed 5 d after injection (Fig. 3G). Increased numbers of GFAP- and BrdU-positive cells were present around the TFFLR injection site when compared with vehicle injection site. Furthermore, the areas that showed the strongest GFAP immunoreactivity coincided with the highest BrdU staining (p < 0.01; unpaired t test) (Fig. 3H), confirming in vivo that the activation of thrombin receptor PAR-1 induces the proliferation of astrocytes. Cresyl violet staining in parallel slices was homogenous, suggesting that changes did not result from nonspecific neuronal injury at the site of injection (data not shown).

Biochemical mechanism underlying PAR-1-triggered astrocyte proliferation

Several studies, mostly in cell lines, suggest that the activation of mitogen-activated protein kinase (MAPK) (also known as ERK) is required for cell proliferation (Roovers and Assoian, 2000). To examine whether stimulation of PAR-1 can elicit ERK phosphorylation and activation in astrocytes, we challenged serum-starved astrocytes with TFFLR (30 µM) for varying lengths of time, ranging from 15 min to 24 h, and quantified by immunoblot the amount of phosphorylated ERK (p42 and p44). As shown in Figure 4A1, TFFLR induced a time-dependent increase in ERK phosphorylation in primary cultured astrocytes. TFFLR stimulated a strong early peak of ERK activation, which declined over 1 h (n = 3). Because recent studies have demonstrated that the duration of ERK activation seems to control cell proliferation (Weber et al., 1997; Bottazzi et al., 1999; Roovers et al., 1999), we determined the time course of PAR-1 agonist-induced ERK phosphorylation over 12 h. After the initial response, we found a second phase of persistent ERK activation above basal at 12 h (Fig. 4A1) (n = 3).
Delayed treatment of astrocytes with U0126 at 1, 3, and 6 h after TFLLR at 30 μM for 24 h, and U0126 (10 μM) was added at indicated times after TFLLR application (top). Delayed blockade of ERK activation with U0126 blocks TFLLR-induced proliferation, as measured with BrdU incorporation (middle; n = 8; *p < 0.001; ANOVA). A representative immunoblot is shown below demonstrating the blockade of TFLLR-induced ERK phosphorylation by delayed U0126 treatment (bottom). Con, Control. Error bars represent SEM.

Because ERK is a key component for mitogenic signal transduction, we tested the effect of specific MAP kinase kinase (MEK) inhibitors [10 μM 1,4-diamino-2,3-dicyano-1,4-bis(o-aminophenylmercapto) butadiene (U0126) or 10 μM 2-(2-amino-3-methoxyphenyl)-4-H-1-benzopyran-4-one (PD98059)] on TFLLR-stimulated ERK phosphorylation in cultured astrocytes. As shown in Figure 4B, 30 min of preincubation with U0126 completely suppressed ERK activation induced by 30 μM TFLLR for up to 1 h (n = 3) and totally blocked the TFLLR-induced proliferation in astrocytes (n = 6; p < 0.001; unpaired t test) (Fig. 4B). Similar data were obtained with the MEK1 inhibitor PD98059 (10 μM; n = 6; p < 0.001), which decreased TFLLR-induced astrocyte proliferation from 246 ± 3.4% above baseline to control levels (89.9 ± 2.1%). In contrast, an inhibitor of the MEK1/2, SB203580, 108.2 ± 2.0%.

Figure 5. Block of ERK by delayed U0126 inhibits PAR-1-induced proliferation. Serum-starved murine primary cortical astrocytes were treated with TFLLR (30 μM) for 24 h, and U0126 (10 μM) was added at indicated times after TFLLR application (top). Delayed blockade of ERK activation with U0126 blocks TFLLR-induced proliferation, as measured with BrdU incorporation (middle; n = 8; *p < 0.001; ANOVA). A representative immunoblot is shown below demonstrating the blockade of TFLLR-induced ERK phosphorylation by delayed U0126 treatment (bottom). Con, Control. Error bars represent SEM.

TFLLR was added reduced ERK activation and proliferation to control levels (n = 8 per condition; p < 0.001). At 12 h, U0126 significantly reduced proliferation and ERK activation, but the levels of proliferation were still significantly higher than control. These data suggest that continued MEK activity is required to fully stimulate proliferation. Because U0126 modestly decreased proliferation at 12 h, stabilization of ERK activity may also contribute to the mitogenic response of astrocytes to PAR-1 activation. However, ERK activation was mostly suppressed by U0126 at 12 h, suggesting that other signaling mediators may also be involved in PAR-1-induced astrocyte proliferation. Neither TFLLR (Fig. 4A) nor U0126 (data not shown) altered PAR-1 expression, as determined by reverse transcription (RT)-PCR analysis of astrocytes.

Cellular actions induced by PAR-1 activation typically involve one or several heterotrimeric G-protein subunits, such as GαGβγ, Gαi1/GαGβγ, Gαi2/GαGβγ, and Gαi3/GαGβγ (Grand et al., 1996). To dissect the pathway(s) responsible for the coupling of TFLLR activation of PAR-1 to astrocyte proliferation, cultured astrocytes were treated with the Gaq/11 inhibitor pertussis toxin (PTX) (1 μg/ml) for 12 h before challenge with the agonist TFLLR. This treatment induced a partial reduction of ERK activation (Fig. 4C) (n = 3) and a partial reduction of the proliferative effect of PAR-1 activation (Fig. 4D) (n = 10; p < 0.05; unpaired t test).

Similar experiments using the Rho kinase inhibitor (R)-trans-N-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide (Y27632) (10 μM) (Welsh et al., 2001) suggest that activation of the Gaq/Rho effector is not required for the coupling of TFLLR activation of PAR-1 to the phosphorylation of ERK in cultured astrocytes within the first hour (Fig. 4D). However, the Gaq/Rh effector, Rho kinase, appears necessary to sustain activation of ERK beyond 1 h (Fig. 4D), because Y27632 pretreatment blocks ERK activation at 12 h after TFLLR treatment. Y27632 also reduces astrocyte proliferation (Fig. 4D).

In most cell types, including glia, proliferation is controlled by the sequential expression of cyclins and activation of cyclin-dependent kinases (Durand et al., 1997; Roberts, 1999). Among G1-phase cyclins, the D types (including D1, D2, and D3) are growth factor responsive. Cyclins D1 and D3 are expressed in a large number of tissues, including the brain, whereas cyclin D2 seems to have more restricted patterns of expression (Bartkova et al., 1998). To further understand the molecular basis of the proliferative response of astrocytes to selective PAR-1 activation, we determined the kinetics of G1-phase cyclin expression in response to the PAR-1-activating peptide TFLLR. We evaluated the levels of expression of cyclin D1 and p27kip1 which also controls the cell cycle by counteracting cyclin D1 (Durand et al., 1997; Roberts, 1999). Preconfluent primary astrocytes were treated with 30 μM TFLLR, and total cell lysates were prepared. RT-PCR analysis showed that the expression level of cyclin D1 mRNA was clearly increased after PAR-1 activation, with the maximum expression after 12–24 h (Fig. 6A, E) (n = 3). In contrast, the level of p27kip1 was not modified by TFLLR treatment (Fig. 6A) (n = 3). As summarized in Figure 6E, we observed a clear temporal correlation between TFLLR-stimulated cyclin D1 mRNA expression and increasing BrdU incorporation into proliferating astrocytes. To determine whether the increase in cyclin D1 mRNA is followed by an increase in protein synthesis, we performed an immunoblot experiment with a cyclin D1 antibody. As observed for the mRNA expression, treatment of astrocytes with TFLLR increased cyclin D1 protein expression at 3, 6, and 12 h (Fig. 6C) (n = 2). To determine whether ERK activation and Rho kinase activation control cyclin D1 protein expression in astrocytes, we tested by...
by incorporation of $[^{3}H]$thymidine, by 5.16 ± 0.8-fold. These data suggest that astrocytes do not release a stable mitogenic agent after TFLLR treatment and thus further support our working hypothesis.

Microglia, similarly to astrocytes, show ERK phosphorylation in response to PAR-1 activation and are known to release cytokines and growth factors when activated (Suo et al., 2003). We therefore tested the potential cross talk between microglia and astrocytes in response to PAR-1 activation by coculturing purified PAR1$^{-/-}$ or littermate wild-type astrocytes with microglia from either PAR1$^{-/-}$ or littermate wild-type animals. Figure 7 summarizes the proliferative response of astrocytes to 10 nM thrombin when cultured alone or with PAR1$^{-/-}$ or wild-type microglia. Thrombin significantly stimulated BrdU incorporation into GFAP-positive wild-type cells in all conditions, suggesting that activation of microglial PAR-1 is not necessary for astrocyte proliferation. Interestingly, there was a slight enhancement of the proliferative response to thrombin in the presence of PAR1$^{-/-}$ microglia, suggesting that thrombin may act on wild-type microglia to slow astrocyte proliferation. However, activation of PAR-1 by the selective peptide activator TFLLR produced identical levels of astrocyte proliferation under the same conditions ($n = 3$; data not shown), suggesting that the enhanced effect seen with thrombin may reflect other substrates or PARs. As a complement to this experiment, PAR1$^{-/-}$ astrocytes did not show enhanced proliferation in response to PAR-1 agonists when cocultured with wild-type microglia, whereas the wild-type microglia in these cocultures showed significant proliferation (165 ± 14% of control; $p \leq 0.02; n = 8$). Together, these experiments demonstrate that activation of microglial PAR-1 is not required for astrocytic proliferation in response to PAR-1 agonists.

Discussion

The protective blood–brain barrier normally allows the diffusion of small molecules and transport of essential nutrients into brain parenchyma but excludes large proteins and other blood constituents from the interstitial space of the CNS. However, head trauma, stroke, status epilepticus, and other pathological conditions can all compromise the integrity of this barrier and allow blood proteins such as albumin to gain access to the extracellular spaces that surround neurons and glia. Given their possible entry into brain tissue during cerebrovascular insult, the effects of blood-derived proteases such as thrombin in the CNS have come under increasing scrutiny (Akiyama et al., 1992; Cunningham et al., 1993; Nishino et al., 1993; Lee et al., 1996; Gingrich and Traynelis, 2000). Preliminary data show that subdural hematoma can elevate thrombin levels 250-fold in CSF from 100 pM to 25 nM for a period of <1 week (Suzuki et al., 1992), suggesting that appreciable amounts of thrombin can be generated and persist at sites of cerebrovascular injury. When bleeding occurs directly.
within the brain tissue, active thrombin and other proteases will freely penetrate the interneuronal spaces by diffusion, until clotting closes the injured vessels and thrombin becomes depleted from the clots. Because prothrombin circulates in blood at high concentrations (1 μM) (Lee et al., 1996), and because vascular injury triggers its rapid conversion to thrombin, direct entry of thrombin into interstitial space could result in the activation of neuronal and glial PAR-1, given that the thrombin EC_{50}, for activation of PAR-1 is in the subnanomolar range (Vu et al., 1991). Thus, it is imperative to understand all actions of thrombin and PAR-1 on each component of the injury process.

Considerable evidence suggests that PAR-1 activation can influence neuronal survival. A number of studies exist showing both harmful as well as beneficial effects of PAR-1 activation (Akiyama et al., 1992; Festoff et al., 1992; Cunningham et al., 1993; Gingrich and Traynelis, 2000; Vivien and Buisson, 2000; Lee et al., 1996), and because vascular injury triggers its rapid conversion to thrombin, direct entry of thrombin into interstitial space could result in the activation of neuronal and glial PAR-1, given that the thrombin EC_{50} for activation of PAR-1 is in the subnanomolar range (Vu et al., 1991). Thus, it is imperative to understand all actions of thrombin and PAR-1 on each component of the injury process.

PAR-1 activation in vivo

Previous work has suggested that thrombin may play multiple roles in the glial response to injury. Indeed, infusion of exogenous thrombin into the rat caudate nucleus causes reactive gliosis and scar formation, infiltration of inflammatory cells, proliferation of mesenchymal cells, induction of angiogenesis, and other effects that resemble inflammation (Nishino et al., 1993). Although these intriguing data suggest that thrombin may be a candidate trigger or regulator of the processes of gliosis in vivo, Wang et al. (2002b) showed that thrombin can use PAR-1, PAR-3, and PAR-4 for the signal transduction in astrocytes, making it difficult to fully interpret the results from Nishino et al. (1993). Our data demonstrate that PAR-1 activation plays a key role in the initial phases of the astrocytic response to injury in vivo. Moreover, a sustained activation of the ERK/MAPK signaling pathway has been shown in perilesional reactive glia after a forebrain stab lesion and suggests a critical role for this cascade in astrogliosis (Carbonell and Mandell, 2003). This sustained activation of ERK is consistent with the effects of PAR-1 activation described here.

Interestingly, PAR-1 involvement in glial scar formation has been suggested previously in the wobbler (wr) mutation model, which shows muscular atrophy associated with developmental motoneuron degeneration. Wobbler is detectable in the third week of life, when homoyogotes (wr/wr) exhibit prominent gliosis and significant motoneuron loss (Laage et al., 1988). In wr/wr mice, reactive astrogliosis was correlated with increased PAR-1 protein expression (Festoff et al., 2000). Our data suggest that the increased PAR-1 expression in the wr/wr mice might explain, in part, the prominent astrogliosis observed in these animals. Moreover, a developmental distribution of GFAP-positive astrocytes in the spinal cord of wobbler mice during the course of the disease has shown that astrocyte overgrowth may be a fundamental feature of the wobbler disease, and is consistent with the function of PAR-1 in astrocytic proliferation (Hantaz-Ambroise et al., 1994).
Cellular mechanism underlying PAR-1-triggered gliosis

The mechanisms by which PAR-1 stimulates astrocyte proliferation appear related to the ability of PAR-1 to control ERK activation. These results are in line with previous reports that thrombin acts as a mitogen for astrocytes through PAR-1 activation (Grabham and Cunningham, 1995). This and other studies (Wang et al., 2002b; Sorensen et al., 2003) have demonstrated that stimulation of PAR-1 rapidly activates ERK1/ERK2 and that inhibition of these pathways with the MEK inhibitor U0126 completely inhibits the proliferative response to the PAR-1 agonist. Although several studies (Teixeira et al., 2000; Wang et al., 2002b; Carbonell and Mandell, 2003) have examined the effect of ERK signaling in astrocyte proliferation and the relationships between astrocyte proliferation and cell-cycle proteins, our data are the first to suggest a link between these two processes. To establish this link, the coupling of PAR-1 activation and proliferation was pharmacologically dissected using inhibitors of PAR-1 signaling pathways. PTX and Y27632 were used to define the role for G\textsubscript{i/o} and Rho kinase pathways in these processes, respectively. Both PTX and Y27632 disrupted astrocyte proliferation but displayed differential effects on MAPK activation. Our data show that G\textsubscript{j}

References


