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Hypoxia/Reoxygenation Differentially Modulates NF-κB Activation and iNOS Expression in Astrocytes and Microglia

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ABSTRACT

Hypoxia/ischemic brain injury accompanies an inflammatory response involving an activation of glial cells. This study, using an *in vitro* model, investigated the signaling mechanisms mediating hypoxic responses of the two glial cell types (astrocytes and microglia) in relation to the expression of inducible nitric oxide synthase (iNOS). In cultures of rat brain microglia and astrocytes, hypoxia (8 h) followed by reoxygenation (24 h) (H/O) had little (microglia) or no (astrocytes) effect on the expression of iNOS. However, H/O elicited opposite effects on lipopolysaccharide (LPS) induction of iNOS in the two cell types: it potentiated LPS induction of iNOS in microglia but inhibited this response in astrocytes. Similar differential effects of hypoxia were observed on the production of tumor necrosis factor-α (TNFα). In contrast, there was an upregulation of hemoxygenase-1 (HO-1), a counter-regulatory pathway, with astrocytes showing a bigger induction than microglia. While hypoxic activation of mitogen-activated protein kinases (MAPKs) was similar in the two glial types, the activation pattern of NFκB was clearly different: hypoxia stimulated the activation of NFκB pathway and NFκB-dependent transcription in microglia but not in astrocytes. Lastly, the two cell types displayed differential vulnerabilities to hypoxia-induced cell death, the astrocytes being relatively more resistant than microglia. *Antioxid. Redox Signal.* 8, 911–918.

INTRODUCTION

Glial cells (astrocytes and microglia) play major roles in a variety of neurodegenerative and neuroinflammatory diseases as well as in brain injury resulting from trauma, ischemia, and stroke (5, 21, 36, 45, 50). They become activated by inflammatory and/or injury signals in a wide range of CNS pathologies and perform both protective and destructive roles via their production of trophic and toxic mediators. As one of the important physiological stimuli, hypoxia/reoxygenation or ischemia can induce glial activation in association with neuronal injury (20, 30, 38, 45). Experimental studies further indicate that neuronal injury resulting from hypoxia/reoxygenation is preceded by glial activation (1, 17) and that the production by activated glia of toxic mediators such as nitric oxide (NO) may underlie neurodegenerative changes (4, 18). In fact, the induction of inducible NO synthase (iNOS) and ensuing NO production by activated microglia under hypoxic conditions has been proposed as an important cause of neuronal death following stroke and hypoxia/ischemia (7, 9, 10, 46). However, conflicting results have been reported regarding the effects of NOS inhibitors on neuronal injury caused by hypoxia/ischemia (13, 25, 28, 43, 51). Moreover, activation of eNOS is known to play a beneficial role in contrast to neuronal NOS and iNOS (12, 23, 24, 37). A recent study also suggests that iNOS-derived nitric oxide, while exerting deleterious effects on the late stages of ischemic brain damage, can also be beneficial by promoting "ischemic tolerance" (8).

Since both astrocytes and microglia express iNOS that may be subject to complex regulation under hypoxic conditions, it was of interest to compare and contrast their responses under controlled *in vitro* conditions including an analysis of the activation of intracellular signaling pathways. It is well known

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that glial activation in response to inflammogens such as the bacterial lipopolysaccharide (LPS), cytokines, and other triggers, is mediated through the activation of cell signaling pathways including mitogen-activated protein kinase (MAPK) cascades and the nuclear factor kappa-B (NF-κB) pathway. The same two pathways have also emerged as major routes through which hypoxic stimulation is transduced into gene transcription events (5, 52). Brain ischemia rapidly triggers time-dependent phosphorylation of ERK1/2, p38 MAPK, and SAPK/JNK in both neurons and glia with potential functional consequences towards glial activation and neuronal injury (26, 32, 39, 49). *In vitro* studies show that an hypoxic environment can activate MAP kinases that may mediate glial production of mediators including NO (27, 41, 48). Besides MAPKs, an activation of NFκB, a transcription factor widely known for its ubiquitous roles in inflammation and immune responses (33), also occurs in activated glial cells after ischemia *in vivo* (11, 16), and in response to hypoxia–reoxygenation *in vitro* (44). NF-κB influences the expression of a complex array of injury responsive genes in the nervous system including iNOS and the cytokine, TNFα, which may play a pathogenic role in stroke (14).

Although both astrocytes and microglia are commonly activated following ischemia, they may differ in their response repertoire with distinct functional consequences. Astrocytes, in particular, are known to play dualistic roles, being either protective or detrimental to bystander neurons. Unlike chronically activated microglia, activated or reactive astrocytes may protect stressed neurons by multiple mechanisms: production of neurotrophic factors, counteracting oxidative stress, and via ischemic preconditioning (38, 47). As noted above, NO is an important byproduct of activated glia under hypoxia/ischemia and represents an important mediator impacting upon neuronal survival and death. In this study, we have determined the expression of iNOS in the two glial cell types subjected to H/O in the presence and absence of an exogenous inflammogen, LPS. The results show clear differences in the responses of astrocytes and microglia, including the expression of iNOS and other mediators as well as the activation of NFκB pathway.

MATERIALS AND METHODS

Dulbecco's modified Eagle's medium (DMEM), calf serum (CS) and antibiotics were purchased from Invitrogen Corporation (Grand Island, NY). Antibiotic-antimycotic mixture and bacterial LPS, polyclonal anti-iNOS and anti-β-actin antibodies were from Sigma (St Louis, MO). Anti-IκB-α and phospho-IκB-α antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against and phosphorylated ERK, p38 MAPK and JNK were from Cell Signaling Technologies (Beverly, MA). The reporter construct, pNFκB-Luc was purchased from Stratagene (La Jolla, CA). *Renilla luciferase* vector (PRL-TK) and dual luciferase assay kits were from Promega Corporation (Madison, WI).

Astrocytes and microglial cultures

Primary mixed glial cell cultures were prepared from the cerebral cortex of newborn rat as described previously (6). Briefly, cells isolated from cerebral hemispheres were dissociated in DMEM with 10% calf serum and plated in 75 cm^2 culture flasks (Falcon, Fort Worth, TX) and incubated at 37°C in an atmosphere of 5% CO_2 in air. The medium was changed after 3–4 days and twice a week thereafter. At confluency (12–14 DIV), mixed glial cultures were shaken to dislodge microglia that were loosely attached to the astrocytes bedlayer and the harvested cells plated into culture dishes and the contaminating cells removed by a brief incubation with Tris–EDTA. The mixed glial cultures were then shaken overnight to remove oligodendrocyte progenitors and the remaining astrocyte-enriched cultures were subcultured and used for the experiments. The purity of the microglial and astrocyte culture (after isolation of microglia) was determined immunocytochemically with OX-42 and anti-GFAP antibody. Of the cells in microglial cultures, 99.5 ± 0.3% were positive for OX-42, the marker for macrophage/microglial cell types (GFAP-positive cells were not present). In astrocyte culture, 95%–98% cells were anti-GFAP positive.

Induction of hypoxia

Hypoxia was induced by replacing culture medium with 95% N_2/5% CO_2 pre-equilibrated, serum-free DMEM and placing the cells into a 37°C incubator in a humidified atmosphere with 95% N_2/5% CO_2. Oxygen level was monitored with a Proox Model 110 (BioSpherix, Redfield, NY). Oxygen concentration was less than 0.2%. After the indicated time periods of hypoxia, cells were reoxygenated in a nomoxic incubator (95% air, 5% CO_2).

Cytotoxicity assays

Cytotoxicity assay based on the measurement of intracellular lactate dehydrogenase (LDH) activity released into the medium was performed using a Cytotoxicity Detection Kit (Roche, Indianapolis, IN). The percentage of LDH released was calculated by dividing the activity recovered in the medium by the sum of the cellular (after lysis) and released activity in the control cultures.

Assay of TNF-α release

Glial cells in 24-well plates were covered with 0.5 ml of culture medium and subjected to hypoxia. TNF-α release was measured by an enzyme-linked immunosorbent assay (ELISA), which was performed using a commercially available kit according to the instructions provided by the manufacturer (PeproTech, Princeton, NJ). All samples and standards were assayed in duplicate. Results were normalized per 1×10^6 cells.

Western blot analysis

Cells were harvested quickly after either hypoxic or normoxic incubation for the desired time, washed twice with ice-cold phosphate-buffered saline, and resuspended in radioimmunoprecipitation assay buffer (20 m*M* HEPES [pH 7.8], 350 m*M* NaCl, 0.5 m*M* EDTA, 0.1 m*M* EGTA, 1 m*M* DTT, 1 m*M* $MgCl_2$, 20% glycerol, 1% NP-40) with protease inhibitor cocktails I and phosphatase inhibitor cocktail II (Sigma, St Louis, MO), 1 m*M* phenylmethylsulfonyl fluoride (PMSF). Cells were lysed on ice for 15 min and centrifuged for 10 min

to separate cell debris. Protein concentrations were measured using the BCA protein assay kit (Pierce, Rockford IL). The cell extracts (30 μg protein equivalents) were mixed with Nu-Page LDS sample buffer (4×) (Invitrogen, Carlsbad, CA) with 5% β-mercaptoethanol, and heated at 95–100°C for 5 min and loaded onto 10% polyacrylamide gels containing SDS. Following electrophoresis, the resolved proteins were transferred onto polyvinylidene difluoride membranes (Millipore Corporation, Bedford, MA). The membranes were blocked with TBS containing 5% dry milk for 1 h and incubated with appropriately diluted primary antibody for 2 h at room temperature, washed three times for 15 min with TBS-T (PBS with 0.1%, v/v, Tween 20), and incubated with a 1:5,000–10,000 dilution of horseradish peroxidase-conjugated secondary antibody for 30 min at room temperature. Blots were again washed three times for 10 min each with TBS-T and developed by enhanced chemiluminescence (Amersham, Piscataway, NJ). Membranes were exposed to Kodak Biomax film (Rochester, NY), then stripped and reprobed for β-actin.

Reverse transcriptase–polymerase chain reaction (RT–PCR)

Total RNA was prepared from glial cells using TRIzol reagent (Invitrogen), and mRNA levels were estimated by RT–PCR. First strand cDNA synthesis by reverse transcription (RT) was carried out using oligo (dT) primer and M-MLV reverse transcriptase (Invitrogen) in a 20 μl reaction volume, according to the supplier's protocol. PCR was performed using 1 μl cDNA with primer sets for detecting iNOS mRNA (i.e., 2680F: 5′-CGT GTG CCT GCT GCC TTC CTG CTG T-3′ and 3327R: 5′-GTA ATC CTC AAC CTG CTC CTC ACT C-3′), yielding a 647-bp product. PCR was initiated by a hot start method, and conditions were 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 45 s, and extension at 72°C for 45 s, followed by 8 min at 72°C. PCR products were separated by electrophoresis through 1% agarose gels containing 0.1 μg/ml ethidium bromide.

Transfection and dual luciferase assays

Transient transfections of astrocytes and microglia were carried out using Polyfect and Effectene (Qiagen, Valencia, CA) transfection reagents, respectively. Cells were transfected with 0.3 μg of firefly luciferase reporter plasmids (NFκB–pGL3) and cotransfected with 0.1 μg of the control *Renilla* luciferase vector (PRL–TK). After transfection, cultures were maintained in normal growth medium for 24 h and then exposed to hypoxia (<0.2% O_2, 95% N_2, 5% CO_2) for 5 h in the presence or absence of LPS. Determinations of firefly and *Renilla* luciferase activities were carried out using the Dual Luciferase Reporter Assay System (Promega). Briefly, the cells were washed with phosphate-buffered saline and lysed with Passive Lysis Buffer. Cell lysates were mixed with Luciferase Assay Reagent II, and the firefly luminescence was measured using a luminometer. Next, samples were mixed with the Stop and Glo reagent and the *Renilla* luciferase activity was measured as an internal control. Relative luciferase activity was calculated as the ratio of firefly luciferase activity to *Renilla* luciferase activity.

RESULTS

Differential effects of hypoxia on iNOS and TNFα expression in primary cultures of astrocytes and microglia

The cultures of astrocytes and microglia were subjected to 8 h hypoxia, followed by 24 h reoxygenation in the presence and absence of LPS and analyzed for the expression of iNOS by immunoblot and RT–PCR. As shown in Fig. 1, although H/O itself had little (microglia) or no (astrocytes) effect on the expression of iNOS (protein and mRNA), it elicited opposite effects on LPS induction of iNOS in the two cell types. Thus, H/O potentiated LPS induction of iNOS in microglia but inhibited this response in astrocytes.

To determine the effect of hypoxia on TNFα production in primary astrocytes and microglia, aliquots of the medium from the cultures exposed to hypoxia in the presence and absence of LPS, were analyzed by ELISA for TNFα. As shown in Fig. 2, while LPS itself induced the release of TNFα by astrocytes, there was no difference in the amount of TNFα released between normoxic and hypoxic cultures of astrocytes after 8 h hypoxia either in the presence or absence of LPS. In contrast, hypoxia significantly increased TNFα secretion by microglia both in LPS-treated and -untreated cultures. These differential responses of astrocytes and microglia were main-

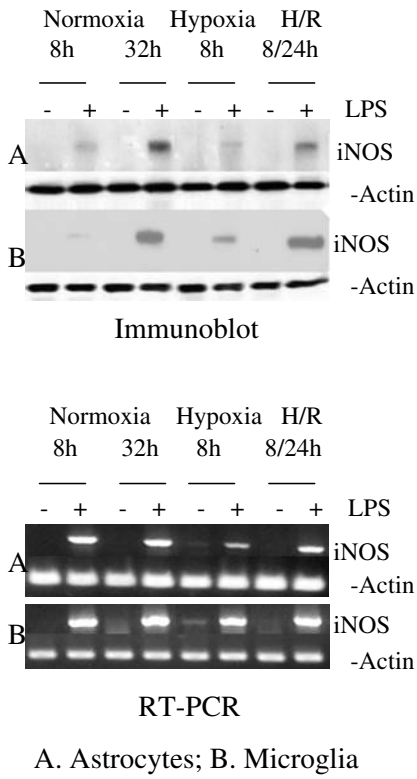

FIG. 1. Effects of hypoxia/reoxygenation on iNOS expression in primary glial cells. Primary astrocytes (**A**) and microglia (**B**) were exposed to hypoxia for 8 h in the presence and absence of LPS (200 ng/ml for astrocyte, 100 ng/ml for microglia) and then reoxygenated for 24 h. iNOS protein (*top*) and iNOS mRNA (*bottom*) were determined by Western blot and RT–PCR. β-actin served as the control.

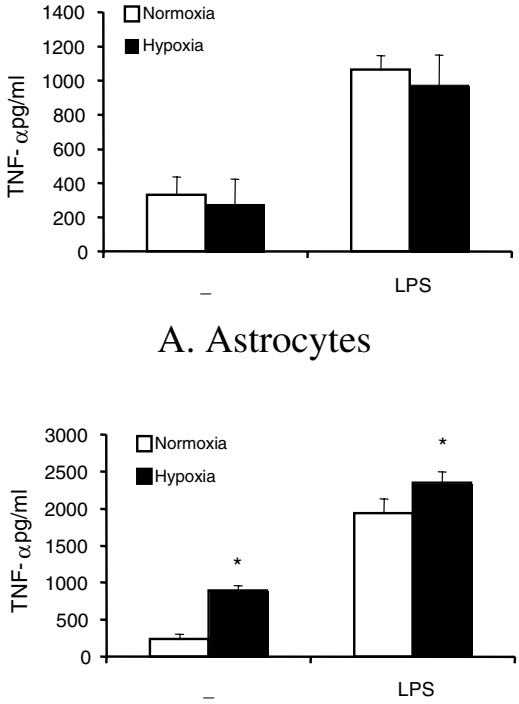

FIG. 2. Effect of hypoxia on TNF-α expression. Primary astrocytes (**A**) and microglia (**B**) were exposed to hypoxia for 8 h in the absence or presence of LPS (200 ng/ml for astrocytes, 100 ng/ml for microglia). Supernatants were collected from cell cultures to measure TNF-α production by ELISA. Values represent mean ± SD. *$p < 0.05$, statistically significant as compared to nomoxic controls.

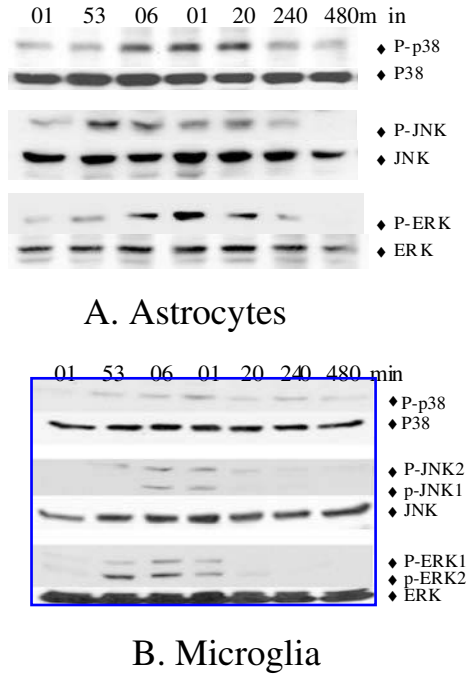

FIG. 3. Activation of p38 MAPK, ERK, and JNK kinases in response to hypoxia in primary glial cultures. Primary astrocytes (**A**) and microglia (**B**) were exposed to hypoxia for the indicated time periods, and cell lysates prepared and analyzed by immunoblot for phosphorylated and total p38 MAPK, JNK, and ERK using specific antibodies.

tained following reoxygenation of the hypoxic cultures (data not shown).

Activation of cell signaling pathways in glial cultures exposed to hypoxia: MAPKs

We reasoned that the differential regulation by hypoxia of the expression of iNOS and TNFα in the two glial cell types may be related to changes in hypoxia-induced activation of signaling pathways. To test this possibility, activation of the three MAP kinases (p38 MAPK, ERK, and JNK) were examined by determining hypoxia-induced phosphorylation of these kinases by immunoblot using phospho-specific antibodies. As shown in Fig. 3, activation of all three kinases occurred both in astrocytes and microglia exposed to hypoxia, albeit with some kinetic differences. Thus, maximal activation of the kinases was found to occur by 2 h in astrocytes with a rapid decline by 4 h. In contrast, maximal kinase activation in microglia was seen by 1 h posthypoxia with a rapid decline by 2 h.

Activation of NFκB pathway by hypoxia in primary glial cultures

Activation of NFκB involves its release from the complex, NFκB–IκB following signal-mediated phosphorylation and degradation of the inhibitory protein, IκB. To test the activation of this pathway in glial cells subjected to hypoxia, the cell extracts were analyzed for IκB phosphorylation and degradation by immunoblot using antiphospho-IκB and anti-total IκB antibodies, respectively. IκB-α phosphorylation and degradation in astrocytes occurred in two waves: first, within 30 min hypoxia, followed by a secondary phosphorylation and degradation after 4 h hypoxia. In contrast, IκBα phosphorylation and degradation in microglia occurred after 4 h hypoxia (Fig. 4A). We further determined changes in NFκB-dependent transcriptional activity using a NF-κB reporter gene assay in primary cultures transfected with a plasmid containing NFκB–luciferase. Luciferase activities were significantly increased after treatment with LPS in the two glial cells that are consistent with previous reports. However, after 6 h hypoxic exposure, luciferase activities were markedly reduced in astrocytes and enhanced in microglia in the presence and absence of LPS (Fig. 4B).

Induction of HO-1

Hypoxia induces the expression of HO-1, which can play a counter-regulatory role against hypoxia-induced inflammatory and cytotoxic responses (2, 42). It was therefore of interest to determine and compare the responses of the two glia in terms of an induction of the expression of this regulatory molecule. As revealed by immunoblot analysis (Fig. 5), there was a strong induction of the expression of HO-1 in response to hypoxia and even stronger expression following reoxygenation in astrocytes compared to a modest level of induction in microglia.

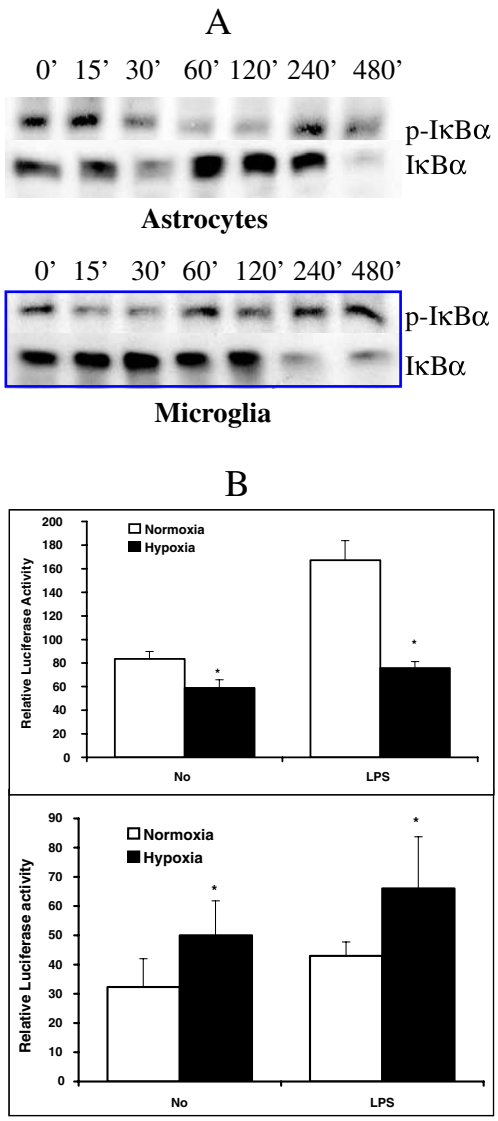

FIG. 4. The effect of hypoxia on NF-κβ activation in primary glial cells. (**A**) NFκB-dependent transcriptional activation. Primary cultures were transfected with p(NF-κB)-Luc along with Renilla luciferase (internal control). After 24 h, the cells were exposed to hypoxia in the absence or presence of LPS (200 ng/ml for astrocytes, 100 ng/ml for microglia) for 6 h, and the cells lysed and assayed for NFκB-luciferase activity. Values represent mean ± SD. *$p < 0.05$, statistically significant as compared to nomoxic controls. (**B**) IκB phosphorylation/degradation. Primary cultures were exposed to hypoxia for the indicated times followed by immunoblot analysis of IκB-α phosphorylation and degradation using anti-phospho-specific IκB-α and anti-total IκB-α antibodies.

Hypoxia-induced changes of cell viability

Finally, it was of interest to determine the effect of H/R on the viability of the two glial cell types. Cell viability was determined by the measurement of LDH release into the culture medium. As shown in Figure 6, while no significant difference was found during 8 h hypoxia exposure between hypoxic and normoxic astrocytes, a significant difference was only observed following 24 h reoxygenation. In contrast, in primary microglial cultures, LDH release increased significantly after both 8 h hypoxia and 24 h reoxygenation either with or without LPS treatment. There was also an additive effect of the two stimuli (hypoxia and LPS) on LDH release in these cultures (Fig. 6). In general, microglia exhibited a greater vulnerability to hypoxia than astrocytes.

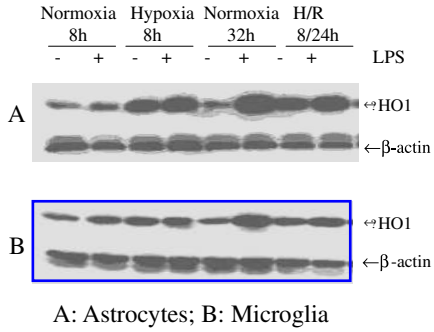

FIG. 5. Effect of hypoxia/reoxygenation on HO-1 induction. The experiments were carried out as in Fig. 1 and the cell extracts analyzed by immunoblot for the expression of HO-1 in astrocytes (**A**) and microglia (**B**). β–Actin was used as the internal control. The ratio of HO-1 to β-actin immunoreactivity (**C**) was determined by densitometric analysis of the blots.

DISCUSSION

The goal of this work was to study molecular events triggered by hypoxic stimulation of primary glial cells, with an emphasis on the induction of iNOS gene expression in astrocytes and microglia in relation to an activation of intracellular signaling pathways. The results presented show that astrocytes and microglia respond differently to H/O *in vitro* in terms of their expression of pro- and antiinflammatory mediators. Thus, LPS induction of iNOS expression was downregulated in astrocytes by H/O, whereas in microglia, this response was potentiated by H/O. H/O by itself was ineffective in inducing iNOS expression in astrocytes, whereas in microglia it seems to induce iNOS mRNA to some degree but far less than was reported in a previous study using a microglial cell line (41). Our observation of an increased expression of iNOS in response to a combination of LPS and hypoxia in microglia is in agreement with a recent *in vivo* study showing that LPS microinjection into rat corpus callosum results in accelerated cerebral ischemic injury involving activated macrophages/microglia (31). The injury was well correlated with increased expression of iNOS and nitrotyrosine immunoreactivity. Immunohistochemical studies further showed that iNOS was mostly expressed in activated microglia/macrophages, but not astrocytes. Our studies also revealed a differential hypoxic modulation of the expression of a proinflammatory cytokine (i.e., TNFα in the two glial cell types). Thus, there was an induction of TNFα release in microglia subjected to hypoxia even in the absence of LPS, but

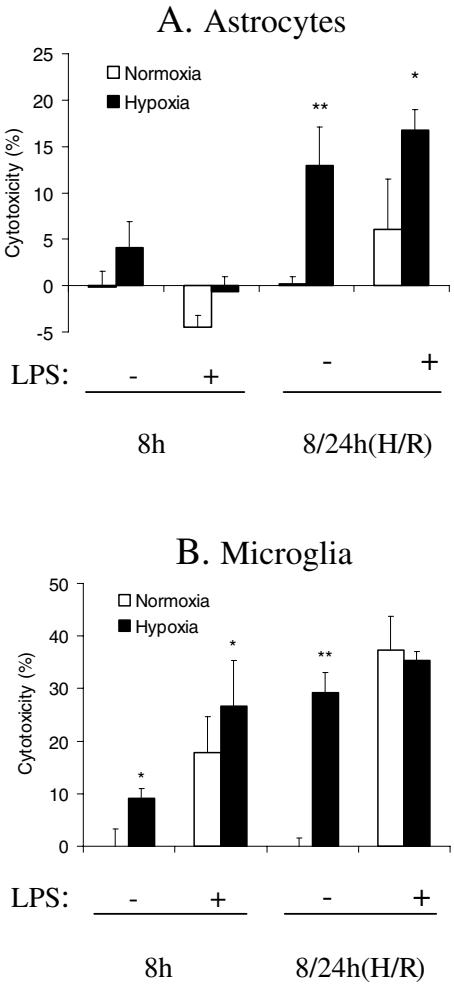

FIG. 6. Hypoxia–reoxygenation-induced glial cell death as measured by LDH release. The LDH activities were measured in the medium of primary astrocytes (**A**) and microglia (**B**) cultures subjected to 8 h hypoxia, followed by 24 h normoxic reoxygenation. The LDH activities are presented as percentages of the total (cellular plus released) activity in control cultures kept normoxic for comparable periods (mean ± SD.; $n = 4$). *$p < 0.05$, **$p < 0.01$ for normoxic versus hypoxia/reoxygenation.

in the presence of LPS, the cytokine release was further enhanced. In contrast, hypoxia alone failed to induce the expression of TNFα in astrocytes, neither did it enhance LPS effect. The expression of HO-1 followed an opposite course in that the enzyme was superinduced by H/O in astrocytes compared to its modest increase in microglia. HO-1 (or heat shock protein-32) is a stress-inducible form of hemoxygenase that catalyzes the oxidative degradation of heme, producing carbon monoxide (CO), ferrous iron, and biliverdin, which is converted into bilirubin (34, 40). Its induction has been demonstrated in models of ischemia where it may play a neuroprotective role (15, 29). Through its production of bilirubin and CO, both with antioxidant and antiinflammatory properties, HO-1 is known to counter oxidative stress and inflammation (2, 42). It is possible that a higher level of expression of HO-1 in astrocytes exposed to H/O may account for a suppression of inflammatory response. Further studies are needed to confirm such a reciprocal regulation of the expression of HO-1 vs iNOS and TNFα in the two cell types.

An analysis of hypoxic activation of the signaling pathways revealed that all three MAPK members (ERK, JNK, and p38MAPK) as well as NFκB, are activated in both astrocytes and microglia. Hypoxia activation of multiple MAP kinases and NFκB that we observed is consistent with previous findings with models of hypoxia/ischemia (26, 32, 39). However, there have been no comparable studies with the two glial cell types. The results of our study indicate that astrocytes respond to hypoxia with longer-lasting activation profiles of the MAPKs compared to microglia. The mechanisms underlying this subtle difference are likely to be complex and the implications are yet unclear. Although we have not carried out additional experiments to delineate the functional significance of individual kinases, each of the MAPKs potentially mediate transcriptional and post-transcriptional regulation of hypoxia-inducible genes.

NFκB is a key transcription factor required for the expression of many immune-, inflammation-, and stress-related genes. It is present within the cytoplasm in an inactive state, bound to the inhibitory κB (IκB) protein. Stimulation of cells with cytokines, LPS, viruses, or oxidants triggers a series of signaling events leading to the phosphorylation and proteolytic degradation of IκB, and activation of NFκB. Once translocated into the nucleus NFκB stimulates transcription by binding to cognate κB sites in the promoter regions of target genes including cytokines, chemokines, and cell adhesion molecules (33). In the brain, members of the NF-κB family of transcription factors have been identified in neurons, astrocytes, and microglial cells, all of which show activation of the transcription factor in response to hypoxia/ischemia (3, 11,16, 44). In the present study, although astrocytes and microglia did not differ much in their MAPK activation responses (above), they did differ in the activation of NFκB pathway in response to hypoxic stimulation. First, in the case of astrocytes, there was a biphasic response in IκB degradation, a key step in NFκB activation pathway. IκB phosphorylation, which renders the protein susceptible to signal-regulated degradation, seemed to parallel this biphasic response. Thus, in astrocytes, primary IκB-α phosphorylation and degradation occurred within 30 min hypoxia, followed by a secondary phosphorylation and degradation after 4 h hypoxia. In contrast, there was a steady decline in the level of IκB-α in microglia exposed to hypoxia. The difference was even more dramatic when NFκB-dependent transcriptional activity was measured in astrocytes and microglia. Thus, hypoxic exposure stimulated the transfected NFκB–Luc activity in microglia, whereas it inhibited this activity in astrocytes, either in the presence or absence of LPS. Although we do not yet know the mechanisms of this dual regulation of NFκB activation, it seems likely that a delayed accumulation of IκB following the initial downregulation would interfere with sustained activation of NFκB in astrocytes and subsequent inhibition of NFκB-dependent transcription as evidenced by an inhibition of NFκB-Luc activity in hypoxic astrocytes. The differential regulation of NFκB signaling in the two glial

cells potentially translates into differential regulation of the expression of the target genes including iNOS and TNFα.

Finally, microglia and astrocytes displayed differential vulnerability to hypoxia-induced cell death. Thus, in microglial cultures, hypoxia alone caused significant cell death that was further exacerbated in the presence of LPS. Reoxygenation for 24 h following 8 h hypoxia in the absence of LPS also resulted in an enhanced cell death. In contrast, astrocytes were resistant to hypoxia alone (8 h) either in the presence or absence of LPS. However, reoxygenation for 24 h following 8 h hypoxia induced a significant—yet lower than that observed in microglia—level of cell death (i.e., approx. 15% cell death in astrocytes compared to approx. 30% in microglia). Nevertheless, there is evidence that astrocytes do undergo programmed cell death upon ischemic insult *in vivo* (19). The differential vulnerability of the two glial cell types to hypoxia that we observed perhaps stems from their differential expression of cell protective (e.g., HO-1) versus cytotoxic (e.g., NO, TNFα) molecules. These mediators would also determine the fate of bystander neurons as demonstrated using *in vivo* (35) and *in vitro* (22) models of ischemia superimposed with LPS exposure.

ACKNOWLEDGMENTS

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ABBREVIATIONS

ERK, extracellular signal-regulated kinase; HO-1, hemoxygenase-1; H/O, hypoxia–reoxygenation; IFNγ, interferon-γ; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEKK1, MAPK/ERK kinase kinase-1; NO, nitric oxide.

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