0031-3998/09/6606-0642
PEDIATRIC RESEARCH

Vol. 66, No. 6, 2009
Printed in U.S.A.

Postnatal Age Influences Hypoglycemia-Induced Poly(ADP-ribose) Polymerase-1 Activation in the Brain Regions of Rats

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ABSTRACT: Poly(ADP-ribose) polymerase-1 (PARP-1) overactivation plays a significant role in hypoglycemia-induced brain injury in adult rats. To determine the influence of postnatal age on PARP-1 activation, developing and adult male rats were subjected to acute hypoglycemia of equivalent severity and duration. The expression of PARP-1 and its downstream effectors, apoptosis-inducing factor (*Aifm1*), caspase 3 (*Casp3*), NF-κB (*Nfkb1*) and bcl-2 (*Bcl*2), and cellular poly(ADP-ribose) (PAR) polymer expression were assessed in the cerebral cortex, hippocampus, striatum, and hypothalamus at 0 h and 24 h posthypoglycemia. Compared with the control group, PARP-1 expression increased in the cerebral cortex of adult rats 24 h posthypoglycemia, but not at 0 h, and it was accompanied by increased number of PAR-positive cells. The expression was not altered in other brain regions. *Aifm1*, *Nfkb1*, *Casp3,* and *Bcl*2 expressions also increased in the cerebral cortex of adult rats 24 h posthypoglycemia. Conversely, hypoglycemia did not alter PARP-1 expression and its downstream effectors in any brain region in developing rats. These data parallel the previously demonstrated pattern of hypoglycemia-induced brain injury and suggest that PARP-1 overactivation may determine age- and region-specific vulnerability during hypoglycemia. **(*Pediatr Res* 66: 642–647, 2009)**

Hypoglycemia is a common metabolic problem in newborn infants. Severe and recurrent hypoglycemia during the neonatal period is associated with brain injury (1). The effects of hypoglycemia of moderate severity on the developing brain are poorly understood.

We have recently demonstrated that the developing brain is less vulnerable than the mature brain to injury during moderate hypoglycemia in rats (2). Compared with the adult rats, neuronal injury was 4-fold less severe in postnatal day (P) 14 (*i.e.*, developing) rats (2). This study and previous studies have also demonstrated that neuronal injury is primarily confined to the cerebral cortex in moderate hypoglycemia (2–4). The factors responsible for the age- and region-specific vulnerability are not well understood.

Activation of poly(ADP-ribose) polymerase-1 (PARP-1) is an important component of hypoglycemia-induced neuronal injury in adult rats (5). PARP-1 is a nuclear enzyme responsible for maintaining the genomic integrity and chromatin structure under basal conditions (6–9). On activation by DNA strand breaks, PARP-1 catalyzes the formation of poly(ADP-ribose) (PAR) polymers that bind to acceptor proteins near the site of DNA damage and facilitate its repair (reviewed in Refs. 7,8). However, PARP-1 overactivation leads to cell death by depletion of cellular NAD^+/ATP and release of apoptosis-inducing factor (AIF) from the mitochondria (9–15). Although the trigger for AIF release during PARP-1 overactivation has yet to be conclusively established, loss of mitochondrial membrane potential and presence of PAR in the cytosol are considered major factors (13,14). AIF-mediated cell death is primarily caspase independent, even though caspase activation may occur during the process (9,16,17). In addition, as a coactivator of nuclear factor kappa B (NF-κB), PARP-1 may potentiate injury by promoting the synthesis of proinflammatory mediators at the site (7,8,18,19).

The objective of this study was to determine the influence of postnatal age on hypoglycemia-induced PARP-1 activation in the brain regions of rats. We evaluated PARP-1 expression in the cerebral cortex, hippocampus, striatum, and hypothalamus because of their dissimilar vulnerability during hypoglycemia (2–5). To differentiate physiologic up-regulation from pathologic overactivation, the expression of PARP-1 activation-dependent proapoptotic genes, AIF (*Aifm1*), caspase 3 (*Casp3*), and NF-κB (*Nfkb1*), and the antiapoptotic gene, bcl-2 (*Bcl*2), were assessed.

METHODS

Animal preparation. P14 and P60 (adult) Sprague-Dawley rats ($n = 76$) were used. Only male rats were studied, based on the established gender-specific effects of PARP-1 in hypoxia-ischemia (12,20). Pregnant rats were purchased (Harlan Sprague Dawley, Indianapolis, IN) and allowed to deliver spontaneously. The litter size was culled to 8–10 on P3 and pups were weaned on P21. Rats were maintained on a 12 h day-and-night cycle and allowed to feed and drink *ad libitum*. The Institutional Animal Care and Use Committee approved all experiments.

Induction of hypoglycemia. Acute hypoglycemia was induced as previously described (2). The target blood glucose concentration was <2.3 mM (<40 mg/dL), a value conventionally used to define hypoglycemia in newborn infants (1). Briefly, after overnight fasting, human regular insulin (Novo Nordisk Inc., Clayton, NC) was injected in a dose of 6 IU/kg s.c. to half the

Received June 5, 2009; accepted August 4, 2009.

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Supported by grants from NICHD (HD047276), Minnesota Medical Foundation, and Viking Children's Fund.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pedresearch.org).

Abbreviations: AIF, apoptosis inducing factor; **FJB,** Fluoro-Jade B; **PAR,** poly(ADP-ribose); **PARP-1,** poly(ADP-ribose) polymerase-1; **P,** postnatal day

number of rats in a litter (hypoglycemia group). The other half was injected with equivalent volume of 0.9% saline (control group). Ambient temperature was maintained at 34.0 ± 1.0°C and fasting was continued for 240 min, based on previous studies (2,21). Blood glucose concentration was measured every 30 min using a glucometer (Roche Diagnostics, Indianapolis, IN). Hypoglycemia was terminated by injecting 10% dextrose 200 mg/kg i.p., a dose that corrects brain glucose concentration in hypoglycemic newborn rats (22).

Tissue preparation. Rats were killed 24 h later ($n = 8$ per group) using sodium pentobarbital (100 mg/kg i.p.). The brain was removed and the cerebral cortex, hippocampus, striatum, and hypothalamus were dissected on ice, flash-frozen in liquid nitrogen, and stored at −80°C. Some rats ($n = 6$ per group) were killed immediately after the termination of hypoglycemia (*i.e.*, at 0 h) and their cerebral cortex was collected. Rats used for histochemistry ($n = 4–6$ per group) underwent *in situ* perfusion fixation before removal of the brain (2). Serial 20-μm coronal sections were obtained from the brain using a cryostat.

Quantitative RT-PCR. Experiments were performed as previously described (23). Total RNA was isolated using RNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA) and complementary DNA (cDNA) was generated using 500 ng of RNA (Affinity Script, Stratagene, LA Jolla, CA). The quantitative PCR (qPCR) experiments were performed using 4 μL of diluted cDNA and 0.5 μL 20× primer/probes (TaqMan Gene Expression Assays; Applied Biosystems Inc., Foster City, CA) (Table S1, supplemental data, http://links.lww.com/PDR/A51). Each sample was assayed in duplicate and normalized against ribosomal protein S18.

Western blot analysis. PARP protein was isolated using published methods (11,24). Twenty microgram of protein from the homogenized cerebral cortex was separated on 4–12% gradient SDS-PAGE gels (Invitrogen, Carlsbad, CA) and blotted onto nitrocellulose membranes. After blocking in 10% nonfat powdered milk and 1% BSA in Tris-buffered saline with 0.1% Tween-20 for 1 h at room temperature, the membranes were incubated with rabbit anti-PARP (1:1000; Abcam, Cambridge, MA) and mouse anti-β-actin (1:500; Sigma Chemical Co., St. Louis, MO) antibodies overnight at 4°C. After incubation with biotinylated goat anti-rabbit and anti-mouse antibodies (1:1000, each; Vector Laboratories, Burlingame, CA) for 30 min at room temperature, the bound antibodies were visualized (BCIP/NBT Substrate Kit; Vector Laboratories), and the intensity of PARP protein relative to β-actin was determined.

PAR immunohistochemistry. PAR immunohistochemistry was performed as previously described (11,25). Brain sections were incubated with mouse monoclonal anti-rat PAR (1:100; Enzo Life Sciences International, Plymouth Meeting, PA) overnight, followed by incubation with anti-mouse biotinylated secondary antibody and avidin-horseradish peroxidase conjugate solution (Vector Elite ABC Kit; Vector Laboratories) for 30 min. The protein/antibody complex was visualized using a chromogen kit (Vector NovaRed; Vector Laboratories).

Double immunofluorescence staining was performed using primary antibodies (1:100 dilution) against PAR and AIF (Abcam, Cambridge, MA) and secondary antibodies conjugated with Alexa Fluor 555 or 488 (1:500 dilution) (Invitrogen, Eugene, OR), followed by Fluoro-Jade B (FJB; Chemicon, Temecula, CA) staining (2). Sections were cover slipped using mounting medium containing 4′6-diamidino-2-phenylindole (DAPI).

PAR immunohistochemical analysis. Digital photomicrographs were collected and the brain regions were identified. Cells with intense cytosolic PAR were quantified using ImageJ program (Research Services Branch, National Institutes of Health, http://rsb.info.nih.gov). As nuclear PAR staining may represent PARP-1-mediated DNA repair, cells with staining confined to the nucleus were used to set threshold. All cells with staining intensity above this threshold inside 0.1 mm^2 grids placed on the cerebral cortex, striatum, and hippocampal subareas, CA1, CA3, and dentate gyrus, were counted.

Statistical analysis. The effects of age, brain region, and hypoglycemia on *Parp1* expression were determined using ANOVA. Inter- and intragroup differences were determined using unpaired *t* tests with Bonferroni correction when indicated. A software program was used for the analysis (SPSS version 15; SPSS, Chicago, IL). Data are presented as mean ± SEM. Significance was set at $p < 0.05$.

RESULTS

Blood glucose concentration. The target glucose concentration [<2.3 mM (<40 mg/dL)] was achieved within 30 min of insulin administration and maintained until 240 min in both hypoglycemia groups. The blood glucose concentrations were similar in the two hypoglycemia groups: P14, 1.5 ± 0.1 mM/L (27.4 ± 1.9 mg/dL) and P60, 1.7 ± 0.1 mM/L (30.2 ± 1.3 mg/dL), $p = 0.22$. Rats were conscious and seizure free during hypoglycemia. There was no mortality.

Parp1 expression under basal conditions. *Parp1* expression in the brain regions differed between the two control groups. Compared with the P14, the expression was lower in the cerebral cortex (−43%; $p = 0.002$) and hippocampus (−33%; $p = 0.05$) in the P60 control group (Fig. 1). *Parp1* expressions in the striatum and hypothalamus were comparable in the two control groups.

PARP-1 expression posthypoglycemia. Compared with the control group, *Parp1* expression was increased 1.9-folds in the cerebral cortex in P60 hypoglycemia group at 24 h ($p = 0.001$; Fig. 2*A*) with a corresponding 1.6-fold increase in PARP protein expression (Fig. 2*B*). The expression was not altered in other brain regions. Hypoglycemia did not alter *Parp1* expression in any brain region and PARP protein levels in the cerebral cortex in the P14 hypoglycemia group at 24 h (Fig. 2). There was no *Parp1* up-regulation at 0 h in either hypoglycemia group (Figure S1, supplemental data, http://links.lww.com/PDR/A51).

Cellular PAR expression posthypoglycemia. PAR-positive cells were absent in the control group and present in the hypoglycemia group of both ages. PAR-positive cells were primarily present in the cerebral cortex (Fig. 3). Whereas nuclear PAR expression was predominantly seen in P14 group (Fig. 3*C* and *E*), both nuclear and cytosolic staining were present in the P60 hypoglycemia group (Fig. 3*D* and *F*). Few PAR-positive cells were present in the hippocampus and striatum. There was no difference among the hippocampal subareas. The hypothalamus was devoid of PAR-positive cells in both hypoglycemia groups. Compared with the P60 control and P14 hypoglycemia groups, there were more PAR-positive cells in the P60 hypoglycemia group ($p < 0.02$, each; Fig. 3*G*). Cells expressing cytosolic PAR had condensed nucleus and labeled for AIF and FJB (Fig. 3*H* and *I*).

PARP-1 activation-dependent downstream effectors. Similar to *Parp1*, the expression of *Nfkb1, Bcl*2, and *Casp3* in the cerebral cortex differed in the two control groups. Compared with the P14 group, *Nfkb1* and *Casp3* levels were 45–75%

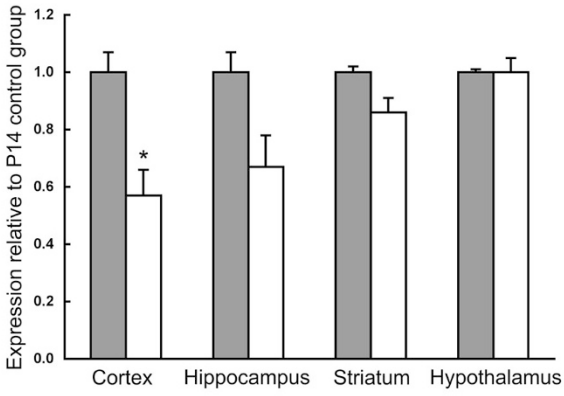

Figure 1. Regional poly(ADP-ribose) polymerase-1 mRNA (*Parp1*) expression in the control group of postnatal day (*P*) 14 (▩) and P60 (□) rats. Values are mean ± SEM normalized to P14 control group; $n = 8$ per group. There was an effect of age and brain region on *Parp1* expression ($p < 0.02$, each; ANOVA). *$p = 0.002$ *vs* P14 control group (Bonferroni-adjusted *t* tests).

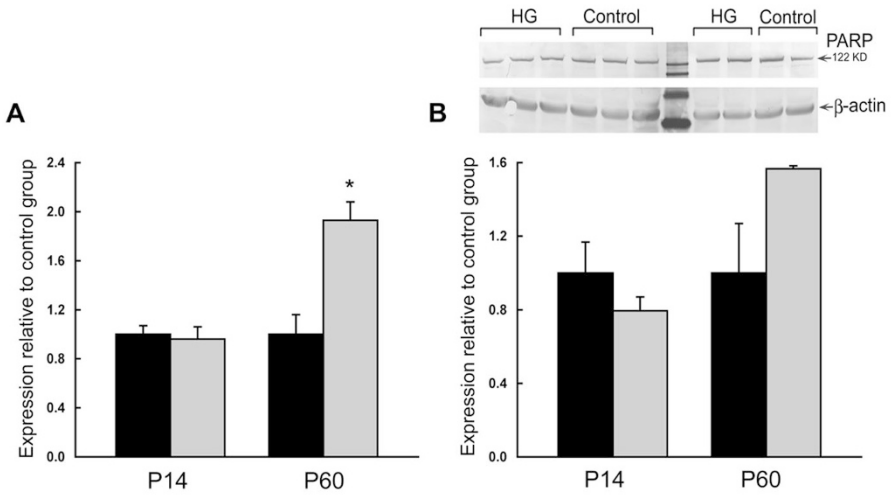

Figure 2. Poly(ADP-ribose) polymerase-1 (*PARP*) mRNA (*Parp1*) (*A*) and PARP protein (*B*) expression in the cerebral cortex in control (■) and hypoglycemia (▨) groups of postnatal day (*P*) 14 and P60 rats 24 h posthypoglycemia. Values are mean ± SEM normalized to the control group; $n = 8$ per group (mRNA), and $n = 2$–3 per group (protein). *$p = 0.001$ *vs* P60 control group. HG, hypoglycemia group.

lower, and *Bcl*2 level was 80% higher in the adult control group ($p < 0.04$, each; Figure S2, supplemental data, http://links.lww.com/PDR/A51). There was a trend toward lower *Aifm1* expression in the adult control group ($p = 0.06$). Although none of the transcripts was up-regulated in P14 rats (Fig. 4*A*), the expression of all four transcripts increased between 1.9- and 2.3-folds in the cerebral cortex of P60 rats 24 h posthypoglycemia ($p < 0.02$, each; Fig. 4*B*).

DISCUSSION

In this comparative study of developing and adult rats, postnatal age influenced hypoglycemia-induced PARP-1 activation in the brain regions. PARP-1 expression increased in the cerebral cortex of adult rats with concurrent up-regulation of cell death-promoting genes. Conversely, no such induction was observed in the developing rats. These results parallel the pattern of regional injury previously reported in this model (2,4) and suggest that PARP-1 overactivation may underlie the regional vulnerability during hypoglycemia.

Among the control groups, *Parp1* levels were higher in the cerebral cortex and hippocampus of P14 rats. This is consistent with previous studies in mice (26,27) and potentially reflects the neuroprotective role of PARP-1 during development (7,26,28). Unlike the striatum and hypothalamus, whose

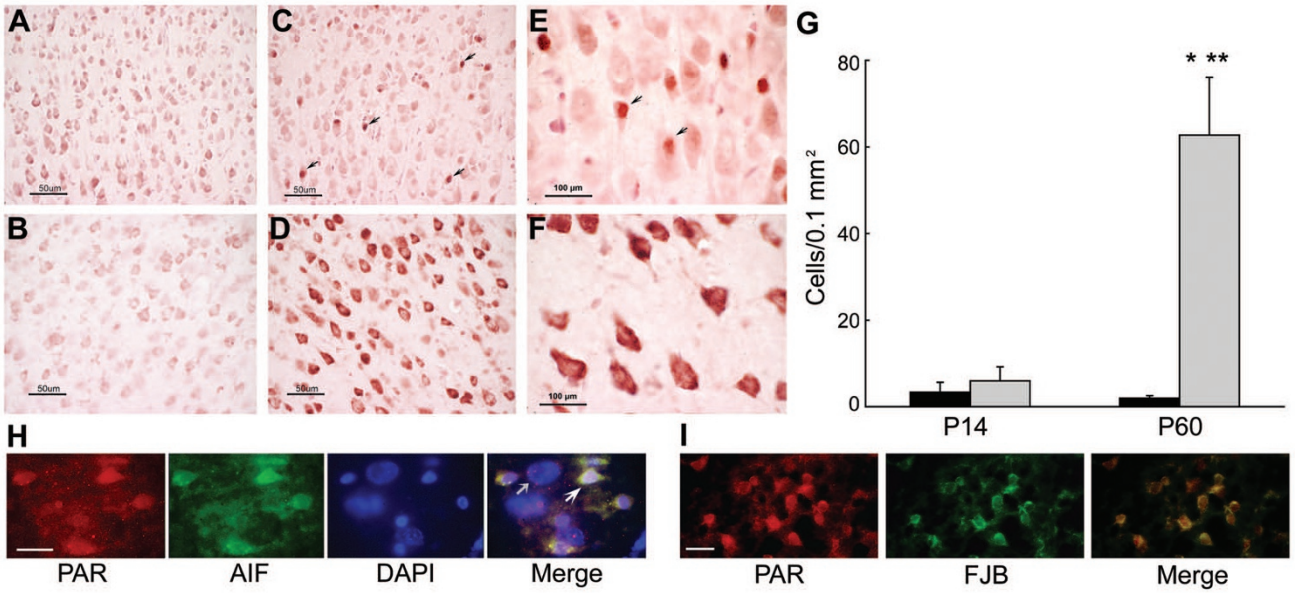

Figure 3. Poly(ADP-ribose) (*PAR*) expressing cells in the cerebral cortex in control (■) and hypoglycemia (▨) groups of postnatal day (*P*) 14 and P60 rats 24 h posthypoglycemia. *Panels A*, *C*, and *E* are from P14 groups, and *B*, *D*, and *F* from P60 groups. *E* and *F* are higher magnification photomicrographs of *C* and *D*, respectively. PAR-positive cells were absent in the control groups (*A* and *B*) and present in the hypoglycemia groups (*C*–*F*). Whereas nuclear staining (*arrows* in *C* and *E*) was primarily seen in the P14 hypoglycemia group, intense nuclear and cytosolic PAR staining was present in the P60 hypoglycemia group (*D* and *F*). Compared with the control group and P14 hypoglycemia group, there were more PAR-positive cells in the cerebral cortex of P60 hypoglycemia group (*G*). PAR-positive cells colocalized with cells expressing AIF (*H*) and had condensed nucleus (*white arrow*), compared with the nucleus of an unaffected cell (*gray arrow*). PAR-positive cells also stained for FJB; *I*. Values are mean ± SEM; $n = 4$–6 per group. *$p = 0.02$ *vs* P60 control group; **$p = 0.02$ *vs* P14 hypoglycemia group (*t* tests). Bar = 40 µm (*A* through *D*), Bar = 100 µm (*E* and *F*), and Bar = 20 µm (*H* and *I*).

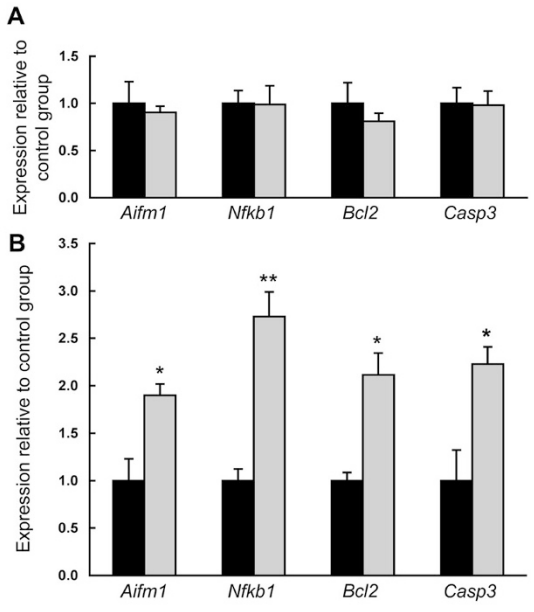

Figure 4. The mRNA expression of apoptosis-inducing factor (*Aifm1*), nuclear factor kappa B (*Nfkb1*), bcl-2 (*Bcl*2), and caspase 3 (*Casp3*) in the cerebral cortex in control (■) and hypoglycemia (▩) groups of postnatal day (*P*) 14 (*A*) and P60 (*B*) rats 24 h posthypoglycemia. Values are mean ± SEM, normalized to the control group; $n = 8$ per group. $*p < 0.02$ *vs* P60 control group; $**p = 0.008$ *vs* P60 control group (Bonferroni-adjusted *t* tests).

development is completed soon after birth, maturational changes continue in the cerebral cortex and hippocampus during the second postnatal week in rats (29). The potential risk of oxidant-mediated DNA damage, secondary to the increased metabolic demand in these regions (30), requires close genomic surveillance during this period. PARP-1 potentially serves this role because inhibition of its activity leads to apoptosis and mutagenesis in developing cells (7,8,28,31).

PARP-1 expression increased in the adult hypoglycemia group, but not in the P14 group, suggesting that postnatal age influences hypoglycemia-induced PARP-1 activation in the brain. The lack of PARP-1 up-regulation in P14 rats is consistent with the known resistance of the developing brain to injury during hypoglycemia of moderate severity (2,32). An inability to up-regulate PARP-1 expression is unlikely to be responsible for our results because PARP-1 overexpression has been reported in other injuries during development (11,12,24). It is possible that increasing the severity or duration of hypoglycemia will alter our results. NMDA receptor activation and oxidant stress that lead to PARP-1 activation in the hypoglycemic mature brain (33,34) are also observed in the developing brain during severe hypoglycemia (35,36). Alternately, the higher PARP-1 levels present during development may be adequate for repairing minor hypoglycemia-induced DNA damage at this age (7,27,28).

In the adult rats, hypoglycemia-induced PARP-1 up-regulation was limited to the cerebral cortex, unlike a previous study that demonstrated parallel up-regulation in the hippocampus (5). A lesser severity of hypoglycemia is potentially responsible for our results. Although extensive injury in the cerebral cortex, hippocampus, and striatum is characteristic of the profound hypoglycemia induced in the previous study (5,37), neuronal injury is primarily confined to the cerebral cortex in moderate hypoglycemia produced in our study (2–4). Collectively, these studies suggest that PARP-1 expression reflects the regional vulnerability of the mature brain during hypoglycemia. The lack of *Parp1* expression at 0 h suggests that PARP-1 activation is initiated after the resolution of hypoglycemia (5). This offers a therapeutic window for preventing hypoglycemia-induced injury in the mature brain using PARP-1 inhibitors (5,8,38).

Presence of cells with intense cytosolic PAR staining and up-regulation of cell death promoters in the cerebral cortex of hypoglycemic adult rats further support the role of PARP-1 in the pathogenesis of regional injury in hypoglycemia (5,6,11,14). Typically, PAR expression is restricted to the nucleus during PARP-1-mediated DNA repair, and PAR entering the cytosol is rapidly degraded by the PAR glycohydrolase enzyme (6,14). Thus, the nuclear PAR expression seen in some hypoglycemic P14 rats may represent PARP-1-mediated DNA repair. Conversely, the increased cytosolic PAR staining in the adult hypoglycemia group likely represents excessive PAR synthesis and/or impaired degradation and potential for AIF-mediated cell death, as suggested by labeling of these cells with AIF and FJB (6,7,13,14) (Fig. 5). Proinflammatory mediators may also be involved in this injury because *Nfkb1* was up-regulated in these rats (8,18,19,39,40). Conversely, despite *Casp3* up-regulation, cell death is likely to be caspase independent because the energy-depleted state during PARP-1 overactivation is not conducive for caspase-dependent apoptosis (8–10,41). Similarly, even though the antiapoptotic *Bcl*2 was up-regulated in the adult hypoglycemia group, potentially mediated by NF-κB (42), it is unlikely to prevent PARP-1-mediated cell death (15).

The potential reasons for the age- and region-related variations in hypoglycemia-induced PARP-1 expression were not determined in this study. Local energy demands, neuronal activity, and ability to transport and use glucose and nonglu-

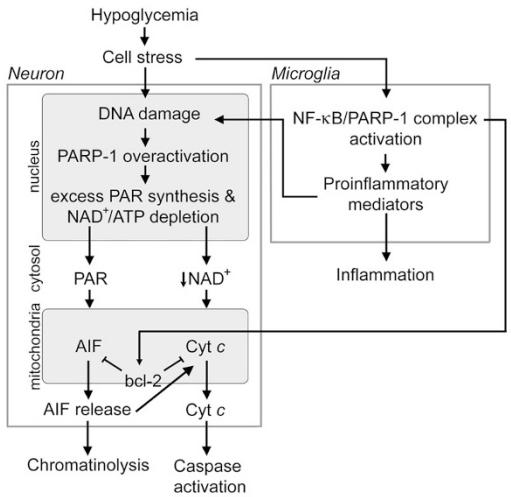

Figure 5. Proposed mechanism of poly(ADP-ribose) polymerase-1 (*PARP-1*)-mediated neuronal injury during hypoglycemia in the mature rat brain. Cyt c, cytochrome *c*; NF-κB, nuclear factor kappa B.

cose substrates determine the vulnerability of a brain region during hypoglycemia (32,43–45). Variations in any of these factors among the developing and mature brain regions may be responsible for our results. Factors intrinsic to PARP-1-targeted genes probably had a minor role because most were expressed higher in the developing brain under basal conditions. However, variations in factors that trigger the expression of these genes, such as cellular NAD^+/ATP depletion, PAR degradation, and mitochondrial depolarization (6,9), as well as posttranscriptional factors, such as the efficacy of protein synthesis during energy depletion (46) may be responsible for the dissimilar effects in the developing and mature brains. As our assessment was limited to 0 h and 24 h, it is possible that we may have missed transient PARP-1 expression at an intermediate time, especially in the developing brain (11). Similarly, determining the cytosolic and nuclear protein levels of the target genes would have enhanced our results. Further studies are necessary to address these limitations and to determine gender-specific effects of PARP-1 in hypoglycemia (12,20).

In summary, the study demonstrates that postnatal age influences hypoglycemia-induced regional PARP-1 expression in the rat brain. The results also imply that PARP-1 overactivation potentially underlies regional vulnerability during hypoglycemia. The interspecies differences in neurodevelopment and substrate utilization (45) preclude extrapolation of these results to human infants and children without additional research. Nevertheless, the study may have clinical implications. The results emphasize the need for devising age-specific interventions for hypoglycemia. The lack of PARP-1 up-regulation in the developing brain demonstrates the futility and potential harm of PARP inhibitors at this age (7,28,31). The relative resistance of the developing brain also argues against invasive diagnostic and therapeutic procedures for brief episodes of moderate hypoglycemia. Finally, understanding the neuroprotective factors in the developing brain may help in designing optimal preventive and therapeutic strategies for hypoglycemia-induced brain injury in the adult.

Acknowledgments. We thank Anirudh R. Rao for providing some tissue samples and Christopher Traudt, MD for statistical analysis and critical review of the manuscript.

REFERENCES

1. Burns CM, Rutherford MA, Boardman JP, Cowan FM 2008 Patterns of cerebral injury and neurodevelopmental outcomes after symptomatic neonatal hypoglycemia. Pediatrics 122:65–74
2. Ennis K, Tran PV, Seaquist ER, Rao R 2008 Postnatal age influences hypoglycemia-induced neuronal injury in the rat brain. Brain Res 1224:119–126
3. Yamada KA, Rensing N, Izumi Y, De Erausquin GA, Gazit V, Dorsey DA, Herrera DG 2004 Repetitive hypoglycemia in young rats impairs hippocampal long-term potentiation. Pediatr Res 55:372–379
4. Tkacs NC, Pan Y, Raghupathi R, Dunn-Meynell AA, Levin BE 2005 Cortical Fluoro-Jade staining and blunted adrenomedullary response to hypoglycemia after noncoma hypoglycemia in rats. J Cereb Blood Flow Metab 25:1645–1655
5. Suh SW, Aoyama K, Chen Y, Garnier P, Matsumori Y, Gum E, Liu J, Swanson RA 2003 Hypoglycemic neuronal death and cognitive impairment are prevented by poly(ADP-ribose) polymerase inhibitors administered after hypoglycemia. J Neurosci 23:10681–10690
6. David KK, Andrabi SA, Dawson TM, Dawson VL 2009 Parthanatos, a messenger of death. Front Biosci 14:1116–1128
7. Schreiber V, Dantzer F, Ame JC, de Murcia G 2006 Poly(ADP-ribose): novel functions for an old molecule. Nat Rev Mol Cell Biol 7:517–528
8. Jagtap P, Szabo C 2005 Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. Nat Rev Drug Discov 4:421–440
9. Chiarugi A, Moskowitz MA 2002 Cell biology. PARP-1—a perpetrator of apoptotic cell death? Science 297:200–201
10. Formentini L, Macchiarulo A, Cipriani G, Camaioni E, Rapizzi E, Pellicciari R, Moroni F, Chiarugi A 2009 Poly(ADP-ribose) catabolism triggers AMP-dependent mitochondrial energy failure. J Biol Chem 284:17668–17676
11. Martin SS, Perez-Polo JR, Noppens KM, Grafe MR 2005 Biphasic changes in the levels of poly(ADP-ribose) polymerase-1 and caspase 3 in the immature brain following hypoxia-ischemia. Int J Dev Neurosci 23:673–686
12. Hagberg H, Wilson MA, Matsushita H, Zhu C, Lange M, Gustavsson M, Poitras MF, Dawson TM, Dawson VL, Northington F, Johnston MV 2004 PARP-1 gene disruption in mice preferentially protects males from perinatal brain injury. J Neurochem 90:1068–1075
13. Yu SW, Andrabi SA, Wang H, Kim NS, Poirier GG, Dawson TM, Dawson VL 2006 Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell death. Proc Natl Acad Sci USA 103:18314–18319
14. Andrabi SA, Kim NS, Yu SW, Wang H, Koh DW, Sasaki M, Klaus JA, Otsuka T, Zhang Z, Koehler RC, Hurn PD, Poirier GG, Dawson VL, Dawson TM 2006 Poly(ADP-ribose) (PAR) polymer is a death signal. Proc Natl Acad Sci USA 103:18308–18313
15. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM, Dawson VL 2002 Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 297:259–263
16. Russo VC, Kobayashi K, Najdovska S, Baker NL, Werther GA 2004 Neuronal protection from glucose deprivation via modulation of glucose transport and inhibition of apoptosis: a role for the insulin-like growth factor system. Brain Res 1009:40–53
17. Beal MF 2005 Mitochondria take center stage in aging and neurodegeneration. Ann Neurol 58:495–505
18. Hassa PO, Buerki C, Lombardi C, Imhof R, Hottiger MO 2003 Transcriptional coactivation of nuclear factor-kappaB-dependent gene expression by p300 is regulated by poly(ADP)-ribose polymerase-1. J Biol Chem 278:45145–45153
19. Kauppinen TM, Swanson RA 2005 Poly(ADP-ribose) polymerase-1 promotes microglial activation, proliferation, and matrix metalloproteinase-9-mediated neuron death. J Immunol 174:2288–2296
20. McCullough LD, Zeng Z, Blizzard KK, Debchoudhury I, Hurn PD 2005 Ischemic nitric oxide and poly (ADP-ribose) polymerase-1 in cerebral ischemia: male toxicity, female protection. J Cereb Blood Flow Metab 25:502–512
21. Kim M, Yu ZX, Fredholm BB, Rivkees SA 2005 Susceptibility of the developing brain to acute hypoglycemia involving A1 adenosine receptor activation. Am J Physiol Endocrinol Metab 289:E562–E569
22. Vannucci RC, Vannucci SJ 1978 Cerebral carbohydrate metabolism during hypoglycemia and anoxia in newborn rats. Ann Neurol 4:73–79
23. Tran PV, Fretham SJ, Carlson ES, Georgieff MK 2009 Long-term reduction of hippocampal brain-derived neurotrophic factor activity following fetal-neonatal iron deficiency in adult rats. Pediatr Res 65:493–498
24. Williams BL, Hornig M, Yaddanapudi K, Lipkin WI 2008 Hippocampal poly(ADP-Ribose) polymerase 1 and caspase 3 activation in neonatal bornavirus infection. J Virol 82:1748–1758
25. Joly LM, Benjelloun N, Plotkine M, Charriaut-Marlangue C 2003 Distribution of Poly(ADP-ribosyl)ation and cell death after cerebral ischemia in the neonatal rat. Pediatr Res 53:776–782
26. Hakme A, Huber A, Dolle P, Schreiber V 2008 The macroPARP genes Parp-9 and Parp-14 are developmentally and differentially regulated in mouse tissues. Dev Dyn 237:209–215
27. Schreiber V, Ame JC, Dolle P, Schultz I, Rinaldi B, Fraulob V, Menissier-de Murcia J, de Murcia G 2002 Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. J Biol Chem 277:23028–23036
28. Visochek L, Steingart RA, Vulih-Shultzman I, Klein R, Priel E, Gozes I, Cohen-Armon M 2005 PolyADP-ribosylation is involved in neurotrophic activity. J Neurosci 25:7420–7428
29. Rice D, Barone S Jr 2000 Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108:511–533
30. Slotkin TA, Oliver CA, Seidler FJ 2005 Critical periods for the role of oxidative stress in the developmental neurotoxicity of chlorpyrifos and terbutaline, alone or in combination. Brain Res Dev Brain Res 157:172–180
31. Dantzer F, de La Rubia G, Menissier-De Murcia J, Hostomsky Z, de Murcia G, Schreiber V 2000 Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1. Biochemistry 39:7559–7569
32. Vannucci RC, Vannucci SJ 2001 Hypoglycemic brain injury. Semin Neonatol 6:147–155
33. Mandir AS, Poitras MF, Berliner AR, Herring WJ, Guastella DB, Feldman A, Poirier GG, Wang ZQ, Dawson TM, Dawson VL 2000 NMDA but not non-NMDA excitotoxicity is mediated by Poly(ADP-ribose) polymerase. J Neurosci 20:8005–8011
34. Suh SW, Hamby AM, Gum ET, Shin BS, Won SJ, Sheline CT, Chan PH, Swanson RA 2008 Sequential release of nitric oxide, zinc, and superoxide in hypoglycemic neuronal death. J Cereb Blood Flow Metab 28:1697–1706
35. McGowan JE, Chen L, Gao D, Trush M, Wei C 2006 Increased mitochondrial reactive oxygen species production in newborn brain during hypoglycemia. Neurosci Lett 399:111–114

36. McGowan JE, Haynes-Laing AG, Mishra OP, Delivoria-Papadopoulos M 1995 The effect of acute hypoglycemia on the cerebral NMDA receptor in newborn piglets. Brain Res 670:283–288
37. Auer RN, Wieloch T, Olsson Y, Siesjo BK 1984 The distribution of hypoglycemic brain damage. Acta Neuropathol 64:177–191
38. Suh SW, Gum ET, Hamby AM, Chan PH, Swanson RA 2007 Hypoglycemic neuronal death is triggered by glucose reperfusion and activation of neuronal NADPH oxidase. J Clin Invest 117:910–918
39. Chiarugi A, Moskowitz MA 2003 Poly(ADP-ribose) polymerase-1 activity promotes NF-kappaB-driven transcription and microglial activation: implication for neurodegenerative disorders. J Neurochem 85:306–317
40. Oliver FJ, Menissier-de Murcia J, Nacci C, Decker P, Andriantsitohaina R, Muller S, de la Rubia G, Stoclet JC, de Murcia G 1999 Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. EMBO J 18:4446–4454
41. Cipriani G, Rapizzi E, Vannacci A, Rizzuto R, Moroni F, Chiarugi A 2005 Nuclear poly(ADP-ribose) polymerase-1 rapidly triggers mitochondrial dysfunction. J Biol Chem 280:17227–17234
42. Tamatani M, Che YH, Matsuzaki H, Ogawa S, Okado H, Miyake S, Mizuno T, Tohyama M 1999 Tumor necrosis factor induces Bcl-2 and Bcl-x expression through NFkappaB activation in primary hippocampal neurons. J Biol Chem 274:8531–8538
43. Paschen W, Siesjo BK, Ingvar M, Hossmann KA 1986 Regional differences in brain glucose content in graded hypoglycemia. Neurochem Pathol 5:131–142
44. Sutherland GR, Tyson RL, Auer RN 2008 Truncation of the Krebs cycle during hypoglycemic coma. Med Chem 4:379–385
45. Nehlig A 1997 Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycaemia. Diabetes Metab 23:18–29
46. Hand SC, Menze MA 2008 Mitochondria in energy-limited states: mechanisms that blunt the signaling of cell death. J Exp Biol 211:1829–1840