Inhibition of Poly(ADP-Ribose) Polymerase
Reduction of Ischemic Injury and Attenuation of N-Methyl-d-Aspartate–Induced Neurotransmitter Dysregulation

Eng H. Lo, PhD; Prince Bosque-Hamilton, BA; Wei Meng, MD

Background and Purpose—The nuclear enzyme poly(ADP-ribose) polymerase (PARP) may play a role in DNA repair. However, in cerebral ischemia, excessive PARP activation may lead to energy depletion and exacerbation of neuronal damage. We examined the effect of inhibiting PARP on (1) the degree of cerebral injury in a rat model of transient focal ischemia and (2) the degree of neurotransmitter dysregulation induced by local cortical perfusion of N-methyl-d-aspartate (NMDA).

Methods—In experiment 1, rats were subjected to transient ischemia for 90 minutes by occlusion of the middle cerebral artery. After 22.5 hours of reperfusion, lesions were quantified by tetrazolium staining. Untreated rats were compared with those treated with the PARP inhibitor 3-aminobenzamide (10 mg/kg). In experiment 2, rats were implanted with microdialysis probes in the cortex, and 1 mmol/L NMDA was perfused for 2 hours. Extracellular concentrations of neurotransmitter and neuropeptide amino acids were measured. Untreated rats were compared with those given 10 mg/kg 3-aminobenzamide.

Results—In experiment 1, PARP inhibition significantly reduced lesion volumes: 204±43 mm³ (untreated) versus 90±24 mm³ (treated). Neuroprotection was primarily manifested in the cortex. In experiment 2, NMDA perfusion resulted in large elevations of glutamate, taurine, and the lipid component phosphoethanolamine. Levels of the NMDA site modulator d-serine were reduced, and glycine levels appeared unchanged. 3-Aminobenzamide significantly attenuated the elevations in glutamate and phosphoethanolamine but had no effects on d-serine and glycine.

Conclusions—Inhibition of PARP reduced injury after transient focal ischemia in rats and attenuated NMDA-induced glutamate efflux and overall neurotransmitter dysregulation. The deleterious effects of excessive PARP activation may be related in part to amplification of excitotoxicity, possibly by cellular energy depletion and additional transmitter release and/or reduced reuptake.

Key Words: DNA repair ■ excitotoxicity ■ glutamates ■ nitric oxide ■ stroke, ischemic ■ rats

The role of NMDA-mediated injury in cerebral ischemia is well documented. After ischemia, energy deficits lead to efflux of various neurotransmitters and neuromodulators, including the excitatory amino acids such as aspartate and glutamate. Abnormal activation of the NMDA-type glutamate receptors can lead to intracellular calcium accumulation followed by a wide spectrum of calcium-related pathologies. Among the many proposed downstream pathways of action, the effects of NO have received much attention. NOS activity is elevated after NMDA receptor stimulation, and the associated free radical cascades involving NO and ONOO can lead to membrane phospholipid and DNA damage. It has been shown that DNA damage after ischemic/NMDA/NO insults may activate the nuclear enzyme poly(ADP-ribose) polymerase (PARP, EC 2.4.2.30). ADP-ribose polymer synthesis comes at a very high energy cost, and it is therefore conceivable that excessive activation of PARP may result in...

See Editorial Comment, page 836

NAD rundown and subsequently ATP depletion. Further depletion of energy stores under already ischemic conditions can lead to loss of membrane potentials and amplification of neurotransmitter and neuromodulator efflux. Additional release and/or reduced reuptake of excitatory amino acids such as glutamate would certainly exacerbate excitotoxic mechanisms of ischemic damage. Indeed, we have previously shown that altered release/reuptake kinetics can amplify the secondary accumulation of excitotoxic compounds during reperfusion injury after transient focal ischemia.

In this study we examined the hypothesis that excessive NMDA receptor stimulation in focal cerebral ischemia results in overactivation of PARP, thus leading to amplification of excitotoxic amino acid efflux and additional neuronal damage. We tested this hypothesis by conducting two experi-
Selected Abbreviations and Acronyms

3-AB = 3-aminobenzamide
dCSF = artificial cerebrospinal fluid
Cit = citrulline
D-Ser = D-serine
Gly = glycine
L-Ala = l-alanine
L-Arg = l-arginine
L-Glu = l-glutamate
L-Ser = l-serine
NIH = National Institutes of Health
NMDA = N-methyl-D-aspartate
NO = nitric oxide
NOS = nitric oxide synthase
PARP = poly(ADP-ribose) polymerase
PEA = phosphoethanolamine
Tau = taurine

Results

Materials and Methods

Rat Focal Ischemia Model

All procedures were conducted under an institutionally approved protocol in accordance with guidelines set by the Guide for Care and Use of Laboratory Animals (NIH publication 85-23, revised 1985). Male adult Sprague-Dawley rats (weight, 250 to 320 g) were anesthetized with halothane (1% to 1.5% in 2:1 air/oxygen) under spontaneous respiration. For the measurement of mean arterial blood pressure, pH, and gases, catheters were placed into femoral arteries. Rectal core temperatures were monitored, and normothermia was maintained with a heat lamp. The standard intraluminal technique was used to induce focal ischemia. Briefly, a surgical cutdown was performed in the ventral neck region, and the internal, external, and common carotid arteries were visualized. A 4.0 nylon monofilament suture coated with silicon was inserted into the internal carotid artery until its tip was placed approximately 19 to 20 mm from the internal-external carotid bifurcation. Immediately after arterial occlusion, rats were treated with either normal saline (controls, n = 7) or 10 mg/kg of 3-AB (n = 7). After 90 minutes of transient focal ischemia, the monofilament suture was withdrawn to allow for cerebral reperfusion. Rats were allowed to recover, and 22.5 hours later brains were extracted and stained with 2,3,5-triphenyltetrazolium chloride. Ischemic lesion volumes were quantified with the use of standard computer-assisted image analysis techniques. We also sought to determine the degree of brain swelling by calculating the ratio of ipsilateral versus contralateral hemispheric volumes.

In Vivo Microdialysis

All procedures were conducted under an institutionally approved protocol in accordance with guidelines set by the NIH Guide for Care and Use of Laboratory Animals. Male Sprague-Dawley rats (weight, 250 to 320 g) were anesthetized with halothane (1% to 1.5% in 2:1 air/oxygen) under spontaneous respiration. Rectal core temperatures were monitored, and normothermia was maintained with a heat lamp. Rats were then secured in a stereotactic frame (Kopf Instruments), and microdialysis probes (2 mm long, CMA-10, CMA/Microdialysis) were inserted into the frontoparietal cortex (from bregma: 0 mm anteroposterior, 2 mm lateral, 2 mm deep). An infusion pump (Harvard Apparatus) was used to perfuse the probes with aCSF at a rate of 2 μL/min. Samples were collected at 10-minute intervals, resulting in 20-μL volumes for each sample. A liquid switch (Valco Systems) was used to switch between normal aCSF and aCSF solutions containing 1 mmol/L of NMDA. aCSF was composed of 125 mmol/L NaCl, 2.5 mmol/L K+, 1.2 mmol/L CaCl2, 0.5 mmol/L NaH2PO4, 5 mmol/L Na2HPO4, 1 mmol/L MgCl2, 6H2O, and 0.2 mmol/L ascorbic acid. In this protocol, 90 minutes of stabilization after probe insertion was allowed before sample collection. Baseline samples were collected for 30 minutes with the use of normal aCSF. Then the perfusing solutions were switched to that containing 1 mmol/L NMDA for 2 hours. Rats treated with normal saline (controls, n = 5) were compared with those treated with 10 mg/kg of 3-AB (n = 5) given 20 minutes before NMDA perfusion. NMDA was purchased from RBI. All other chemicals were purchased from Sigma.

HPLC Measurements

Microdialysate samples were analyzed by reversed-phase high-performance liquid chromatography with fluorescence detection (CMA250, CMA/Microdialysis), as we have previously described. Derivatives of microdialysate amino acids were formed by reaction with N-acetyl-L-cysteine and o-phthalaldehyde. The chromatography system resolves the D- and L-enantiomers of chiral amino acids on a 4.5×250-mm Phenomenex Primesphere “HC” octadecylsila column (5-μm particle size) with a gradient of 0% to 40% methanol in 15 mmol/L sodium phosphate, pH 6.2 (0.13% tetrahydrofuran) over 65 minutes at 0.7 mL/min. Based on standards purchased from Sigma, concentrations of L-Glu, D-Ser, L-Ser, PEA, Gly, L-Arg, Cit, Tau, and L-Ala were determined. To determine effects of NMDA perfusion, absolute increases in concentrations were calculated. In addition, we also calculated a concentration ratio of Cit to L-Arg. Since L-Arg is converted by NOS into NO and Cit with a 1-to-1 stoichiometry, this ratio may serve as an indirect index of NO formation. Others have recently found that microdialysate concentrations of Cit can be used to estimate NOS activity. In our system, microdialysate γ-aminobutyric acid concentrations were barely detectable (~0.05 μmol/L), and no robust NMDA-induced efflux was discernable. Therefore, we did not analyze γ-aminobutyric acid data for this study. Finally, to estimate the total amount of efflux for the amino acids measured, we integrated the cumulative release above baseline levels after NMDA perfusion. These “area under the curve” data were expressed as micromolar minute products and served to provide an estimate of the total neuronal exposure to the elevated concentrations of the various neurotransmitters and neuromodulators.

Statistical Analysis

All data were expressed as mean±SEM. Unpaired two-tailed Student’s t tests was used for comparison of ischemic lesion sizes. Two-way repeated-measures ANOVA was used to examine the time course data on microdialysis amino acid concentrations. Cumulative effluxes integrated over time were compared by Student’s t tests. P values less than .05 were considered significant.

Results

Experiment 1: Transient Focal Cerebral Ischemia

All systemic parameters were within normal range, and no significant differences were observed between control and treated rats (Table 1). Slight elevations in mean arterial blood pressure were noted after ischemic onset, most likely reflecting the central nervous system ischemic response. Triphenyltetrazolium chloride staining performed 24 hours after transient focal ischemia revealed lesions primarily located in the middle cerebral artery territory, ie, frontoparietal cortex, basal ganglia, and in cases in which lesions were large, parts of the thalamus as well. In untreated controls, total lesion volumes were 204±43 mm3. The PARP inhibitor 3-AB reduced lesion volumes by approximately 55% to
90±24 mm³ (Fig 1). Most of the neuroprotection was present in the cortical areas only; no apparent lesion reduction was observed in the subcortical regions (Fig 1, right panel). The degree of hemispheric brain swelling was somewhat reduced by PARP inhibition, although the effect did not reach statistical significance: 6.3±2% in controls versus 2.1±2% in treated rats (P=.08).

**Experiment 2: NMDA Perfusion**

Basal concentrations of the various extracellular amino acids were in the range expected for microdialysis recoveries in rat cortex in vivo (Table 2). On switching to the aCSF solutions that contained 1 mmol/L NMDA, significant alterations in neurotransmitter and neuromodulator amino acid profiles were seen in the control rats (Table 2 and Fig 2). L-Glu concentrations appeared to show a biphasic temporal pattern. An initial increase occurred within the first 30 minutes, reaching a maximum of more than double baseline concentrations. This was followed by a slight decrease to a plateau that remained above baseline until the end of the 2-hour measurement period (Fig 2).

Significantly different profiles were seen for the NMDA site modulators Gly and D-Ser (Fig 2). No changes were apparent for the NMDA receptor site modulator Gly, but D-Ser concentrations were decreased after NMDA perfusion. In contrast, no significant alterations were seen in the non-NMDA active enantiomer L-Ser (Fig 2). The neuromodulator Tau and the lipid component PEA showed temporal patterns similar to those of L-Glu, with large elevations reaching a maximum at approximately 30 to 50 minutes, followed by

**TABLE 1. Systemic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>3-AB Treated</th>
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</thead>
<tbody>
<tr>
<td><strong>Preischemia</strong></td>
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<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>108±8</td>
<td>99±6</td>
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<tr>
<td>pH</td>
<td>7.33±0.01</td>
<td>7.36±0.04</td>
</tr>
<tr>
<td>Pco₂, mm Hg</td>
<td>40.8±4.7</td>
<td>43.3±3.1</td>
</tr>
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<td>Po₂, mm Hg</td>
<td>185±31</td>
<td>169±17</td>
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<tr>
<td>Rectal temperature, °C</td>
<td>37.3±0.4</td>
<td>36.7±0.2</td>
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<tr>
<td><strong>Postischemia</strong></td>
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<td></td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>125±7</td>
<td>118±4</td>
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<tr>
<td>pH</td>
<td>7.30±0.04</td>
<td>7.33±0.03</td>
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<tr>
<td>Pco₂, mm Hg</td>
<td>46.8±2.7</td>
<td>45.3±2.6</td>
</tr>
<tr>
<td>Po₂, mm Hg</td>
<td>179±2</td>
<td>154±28</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>37.8±0.4</td>
<td>37.3±0.4</td>
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</table>

**TABLE 2. Microdialysate Concentrations**

<table>
<thead>
<tr>
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<th>Controls</th>
<th>3-AB Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
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</tr>
<tr>
<td>L-Glu</td>
<td>0.37±0.10</td>
<td>0.27±0.05</td>
</tr>
<tr>
<td>D-Ser</td>
<td>0.53±0.07</td>
<td>0.46±0.06</td>
</tr>
<tr>
<td>L-Ser</td>
<td>2.53±0.51</td>
<td>2.02±0.50</td>
</tr>
<tr>
<td>PEA</td>
<td>0.79±0.09</td>
<td>0.72±0.06</td>
</tr>
<tr>
<td>Gly</td>
<td>1.69±0.50</td>
<td>1.24±0.30</td>
</tr>
<tr>
<td>Tau</td>
<td>4.53±0.61</td>
<td>3.90±0.61</td>
</tr>
<tr>
<td>L-Ala</td>
<td>2.01±0.44</td>
<td>1.99±0.53</td>
</tr>
<tr>
<td>Cit</td>
<td>0.51±0.18</td>
<td>0.29±0.08</td>
</tr>
<tr>
<td>L-Arg</td>
<td>1.54±0.17</td>
<td>1.03±0.17</td>
</tr>
<tr>
<td><strong>NMDA-Induced maximum or minimum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glu</td>
<td>0.83±0.17</td>
<td>0.56±0.10*</td>
</tr>
<tr>
<td>D-Ser</td>
<td>0.35±0.03</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>L-Ser</td>
<td>1.97±0.42</td>
<td>1.18±0.20</td>
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<tr>
<td>PEA</td>
<td>4.53±0.30</td>
<td>2.72±0.45*</td>
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<tr>
<td>Gly</td>
<td>2.75±0.83</td>
<td>1.91±0.26</td>
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<tr>
<td>Tau</td>
<td>12.1±1.5</td>
<td>11.0±1.9</td>
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<tr>
<td>L-Ala</td>
<td>2.61±0.63</td>
<td>2.37±0.51</td>
</tr>
<tr>
<td>Cit</td>
<td>0.65±0.22</td>
<td>0.43±0.09</td>
</tr>
<tr>
<td>L-Arg</td>
<td>1.20±0.15</td>
<td>0.81±0.09</td>
</tr>
<tr>
<td><strong>NMDA-Induced plateau</strong></td>
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<tr>
<td>L-Glu</td>
<td>0.55±0.08</td>
<td>0.44±0.08</td>
</tr>
<tr>
<td>D-Ser</td>
<td>0.42±0.04</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>L-Ser</td>
<td>2.60±0.49</td>
<td>1.76±0.54</td>
</tr>
<tr>
<td>PEA</td>
<td>3.04±0.28</td>
<td>1.78±0.31*</td>
</tr>
<tr>
<td>Gly</td>
<td>2.01±0.52</td>
<td>1.45±0.21</td>
</tr>
<tr>
<td>Tau</td>
<td>9.11±1.24</td>
<td>6.36±1.26</td>
</tr>
<tr>
<td>L-Ala</td>
<td>2.10±0.54</td>
<td>1.62±0.19</td>
</tr>
<tr>
<td>Cit</td>
<td>0.56±0.20</td>
<td>0.38±0.09</td>
</tr>
<tr>
<td>L-Arg</td>
<td>1.60±0.15</td>
<td>1.08±0.18</td>
</tr>
</tbody>
</table>

Values are micromolar. Maximum or minimum was calculated from the initial response phase, ie, samples 4–8. For the plateau concentrations, the last three samples (13–15) were averaged.

*P<.05 for controls vs 3-AB group.

NMDA active enantiomer L-Ser (Fig 2). The neuromodulator Tau and the lipid component PEA showed temporal patterns similar to those of L-Glu, with large elevations reaching a maximum at approximately 30 to 50 minutes, followed by

Figure 1. Reduction of ischemic cerebral injury by PARP inhibition with 3-AB. Significant neuroprotection is mainly manifested in the cortex. *P<.05.
elevated plateaus that were sustained over the entire NMDA perfusion period (Fig 2). However, the elevations for PEA were much greater (up to fivefold above baseline). Concentrations of the nonneuroactive amino acid L-Ala remained unchanged throughout.

In rats treated with 3-AB at 20 minutes before NMDA perfusion, these alterations in extracellular amino acid profiles were significantly attenuated (Table 2 and Fig 2). Elevations in L-Glu were decreased, with the most prominent effect occurring during the initial maximal response phase at 30 to 40 minutes. The large elevations in PEA were also greatly reduced. For Tau, however, the initial maximal response to NMDA perfusion was unchanged, but final “plateau” levels at the end of the measurement periods appeared to be slightly reduced. No effects on D-Ser, Gly, or L-Ser efflux were observed.

The cumulative efflux integrated over the 2-hour NMDA perfusion period was calculated to estimate the total neuronal exposure to elevated amino acids induced by NMDA perfusion. PARP inhibition with 3-AB significantly reduced the total efflux of L-Glu and PEA (Fig 3). Cumulative Tau efflux showed some slight decrease after 3-AB treatment, but the differences did not reach statistical significance \((P=0.07, \text{Fig } 3)\).

Finally, concentration ratios of Cit to L-Arg were calculated as an indirect indicator of NO formation since these amino acids are involved in a 1-to-1 stoichiometric relationship with NOS activity (see “Materials and Methods”). Ratios of Cit to L-Arg showed significant elevations after NMDA perfusion (Fig 4). However, no differences were observed between control and 3-AB–treated rats.

**Discussion**

PARP [also known as poly(ADP-ribos) synthetase or PARS] is a highly conserved nuclear enzyme that has been proposed to play a role in the maintenance of genomic integrity by promoting DNA repair pathways.\(^\text{18}\) This enzyme is a member of a class of ADP ribosyl transferases that cleave NAD and transfer ADP-ribose moieties to various target molecules.\(^\text{19}\) The enzyme molecule has a DNA binding domain with two zinc finger motifs that recognize and preferentially bind to DNA strand break locations. After binding, intense polymerase activity ensues with the construction of long branched chains of poly(ADP-ribose) attached to various Glu residues within the PARP molecule. This rapid polymerization activity has a high energy cost and can rapidly deplete cellular NAD and ATP levels. Although an obligatory role for PARP in DNA repair remains to be clarified,\(^\text{20}\) some of its proposed actions include sensing of DNA nicks, stabilization of V-shaped DNA conformations, facilitation of DNA access for various repair enzymes, and feedback control of G2 cell cycle checkpoints.\(^\text{18,21}\)

In the context of cerebral ischemia, PARP activity may play a slightly different role related to the high energy cost of the enzyme. After ischemia, sustained stimulation of the NMDA-type glutamate receptor may lead to potentiation of NO formation and other free radical species, DNA damage, and PARP activation.\(^\text{22,23}\) Excessive PARP activity may lead to rapid NAD rundown and subsequently drastic ATP depletion.\(^\text{8}\) In the face of ischemia, these additional energy deficits may exacerbate cerebral injury. In this report we showed that PARP inhibition with the nicotinamide analogue 3-AB sig-
significantly reduced lesion size after transient focal ischemia in the rat. These results are consistent with recent findings showing that PARP knockout mice are more resistant to cerebral ischemia than wild-type mice. In fact, the deleterious effects of PARP overactivation may not only apply to brain since others have found that inhibition with 3-AB can also reduce tissue injury from ischemia-reperfusion in heart and skeletal muscle as well as retina.

One possible mechanism for the observed neuroprotection may be related to the ability of PARP to amplify acute excitotoxicity. Additional energy deficiency in the face of ischemia may lead to further rundown of membrane potentials and decreased reuptake rates of various excitatory amino acid neurotransmitters. Increased release and decreased reuptake can amplify the accumulation of excitotoxic compounds, thus leading to additional neuronal damage. In the present study we used NMDA perfusion by means of microdialysis into the rat cortex to test this hypothesis. In control rats, NMDA perfusion resulted in large elevations of the excitotox neurotransmitter L-Glu. Treatment with 3-AB significantly attenuated these elevations. NMDA-induced elevations in the lipid component PEA were also attenuated by 3-AB. Extracellular PEA levels may reflect not only membrane damage but also membrane recycling and signaling. For the inhibitory neuromodulator Tau, the initial phase of maximal NMDA-induced efflux was unchanged by PARP inhibition. However, plateau levels and cumulative total efflux were slightly lower after 3-AB treatment, although the differences did not reach statistical significance. This may be of interest since there have been some suggestions that, under certain conditions, Tau may attenuate NMDA neurotoxicity. No significant effects of 3-AB were seen for the NMDA site neuromodulators Gly and D-Ser. Interestingly, however, D-Ser concentrations were reduced after NMDA perfusion in all cases. We have previously shown that D-Ser is not released on K1-evoked depolarization, and others have shown that it is also not released after veratrine stimulation. At present, it is unclear what the reductions in D-Ser mean; further studies are warranted to examine this intriguing phenomenon. Taken together, however, these data suggest that PARP overactivation can lead to amplification of L-Glu efflux and overall dysregulation of neurotransmitter and neuromodulator systems.

NO formation follows a 1-to-1 stoichiometry with respect to L-Arg and Cit. Others have demonstrated that Cit levels measured by microdialysis may be used as an indirect index of NOS activity. In this study we showed that concentration ratios of Cit to L-Arg were elevated after NMDA perfusion, but these changes were unaffected by 3-AB treatment. These data suggest that 3-AB did not inhibit the NMDA-induced stimulation of NO formation.

Recently, an increasing amount of evidence has supported a role for programmed modes of cell death or apoptosis in cerebral ischemia. During the apoptotic cascade, a variety of cysteine proteases or caspases are activated. Among them, caspase-3 or CPP32 cleaves and inactivates PARP as one of its substrate molecules. Indeed, it has been shown in several model systems that CPP32-mediated cleavage of PARP is one of the early events in the apoptotic cascade, occurring within 2 to 3 hours after induction. Since it appears that PARP may be inactivated relatively early, it is relevant to ask whether PARP can play a significant role in ischemic cell death. The answer is most likely affirmative and is related to the timing of pathophysiological events in cerebral ischemia. It has been shown that CPP32 is not upregulated until 8 to 16 hours after ischemia in a rat focal occlusion model. Therefore, a window of opportunity exists

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**Figure 3.** PARP inhibition by 3-AB significantly reduced cumulative L-Glu and PEA efflux integrated over time after NMDA perfusion (*P** < .05). Differences in Tau did not reach statistical significance (P = .07).

**Figure 4.** Ratios of Cit to L-Arg may serve as an indirect index for NO formation after NMDA perfusion. No differences were seen between controls and 3-AB–treated rats. Data are pooled for both groups. Baseline samples were collected for 30 minutes (samples 1 to 3) followed by NMDA perfusion over 2 hours (samples 4 to 15). *P** < .05 vs pre-NMDA baseline levels.
for PARP to amplify acute excitotoxicity during ischemia-reperfusion before it is cleaved by CPP32. The present study demonstrated that PARP inhibition was neuroprotective, but the data do not address the issue of whether PARP plays a role in necrotic or apoptotic pathways of neuronal damage.

Upregulation of DNA repair pathways such as PARP and GADD45 may represent a neuroprotective response to DNA damage after ischemia. On the other hand, data also exist that support a function for PARP as a possible mediator of a cell suicide pathway. Somewhat contradictory findings have been reported with PARP knockout mice, showing both decreased and unaltered DNA repair capabilities. The contributions of PARP in programmed cell death will likely be dependent on model system and context. In the context of ischemia, our present data suggest that PARP plays a deleterious role in cerebral injury.

One potential limitation of the present study is related to the question of 3-AB selectivity as a PARP inhibitor. Others have shown, however, that 3-AB prevents oxygen radical–induced cell death in PARP+/+ but not PARP −/− islet cells, thus suggesting that PARP is indeed the major target of 3-AB action. Furthermore, experiments using subcloned PARP cDNA in dominant negative mutants encoding only the DNA-binding domain and lacking polymerase activity yielded results similar to those obtained with 3-AB in terms of increased sensitivity to DNA damage. Finally, a recent study using PARP knockout mice demonstrated that the neuroprotective effects of 3-AB treatment were similar to those resulting from genetic deletion of PARP and furthermore, 3-AB decreased PARP activation after ischemia when measured immunohistochemically. Therefore, we believe that the major effects of 3-AB observed in this study were due to PARP inhibition.

In conclusion, we have presented data showing that inhibiting PARP activity in vivo may protect brain from injury after transient focal ischemia. These data in rats complement other published findings in which PARP knockout mice were used. In addition, our data suggest that the deleterious effects of PARP in cerebral ischemia may result in part from an amplification of excitotoxic mechanisms of neuronal damage. After ischemia, excessive PARP activation may exacerbate energy deficits, resulting in further excitotoxic transmitter release and/or reduced reuptake.

Acknowledgments

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References

Poly(ADP-ribose) polymerase (PARP) is a chromatin-bound enzyme that utilizes β-NAD as the ADP ribose substrate and is activated by DNA strand breaks. The amino acid sequence of PARP reveals a tripartite domain structure, one of the functions of which is to bind DNA. Protein poly(ADP-ribose)ylation appears to be required for the accurate rejoining of nicks and breaks on DNA that occur during DNA replication, gene expression, and excision-repair of DNA.

The article by Lo and colleagues reveals new data implying that nicotinamide analogue 3-AB significantly reduced lesion size in experimental transient cerebral ischemia. The authors suggest that 3-AB acts by an NO-independent mechanism. This finding signifies that other (not yet elucidated) mechanisms of cellular injury may be responsible for postischemic activation of PARP. Needless to say, some forms of programmed cell death may be PARP independent.

Obviously, the use of PARP inhibitors is a promising new direction in the treatment of stroke and cerebral ischemia. However, further animal experiments will need to be conducted to warrant future clinical trials.
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