Brain Ischemic Preconditioning Does Not Require PARP-1

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**Background and Purpose**—Poly(ADP-ribose) polymerase-1 (PARP-1) is involved in ischemic preconditioning of the heart and cultured neurons, but its role in brain ischemic preconditioning is unknown.

**Summary of Report**—We report that 5-minute bilateral common carotid artery occlusion (BCCAO) in the mouse prompted reduction of infract volumes triggered 24 hours later by 20-minute middle cerebral artery occlusion (MCAO). Pharmacological PARP-1 inhibition between BCCAO and MCAO did not impair preconditioning. The contents of the PARP-1 substrate NAD, those of its product poly(ADP-ribose), caspase-3 activation, and PARP-1 expression did not change after BCCAO within the preconditioned tissue. PARP-1 KO mice were similarly protected by the 5-minute BCCAO.

**Conclusion**—Data demonstrate that, at variance with the heart, PARP-1 is dispensable for brain ischemic preconditioning.

**Key Words:** neuroprotectants □ poly(ADP-ribose) □ PARP □ NAD □ ATP

Ischemic preconditioning (IPC) occurs in animals and humans affected by cardiovascular disorders. Understanding the biochemical events activated by IPC is of relevance to stroke therapy. Poly(ADP-ribose) polymerase-1 (PARP-1) uses nicotinamide adenine dinucleotide (NAD) to form poly(ADP-ribose) (PAR) and is a fundamental mediator of experimental stroke injury. Prior work indicates that myocardial IPC worsens infarct in PARP-1 KO mice, and the PARP-1 inhibitor 3-aminobenzamide impairs heart preconditioning in WT animals. Conversely, caspase-3-dependent PARP-1 cleavage underlies IPC in cultured neurons. PARP-1 cleavage also occurs in Zinc-preconditioned cultured neurons, but not in ischemically preconditioned ratinas. We sought to determine whether PARP-1 is involved in brain ischemic preconditioning.

**Materials and Methods**

C57Bl/6 male PARP-1 KO and WT mice (Harlan; Milan, Italy) obtained from homozygous breeding pairs were used. For IPC, we simplified an IPC protocol described by Cho and colleagues. Mice were anesthetized and common carotid arteries occluded (BCCAO) for 5 minutes with microclips. After 24 hours, mice underwent transient middle cerebral artery occlusion (MCAO) as reported. Mice were killed by decapitation and their brains obtained from homozygous breeding pairs were used. For IPC, we simplified an IPC protocol described by Cho and colleagues. Mice were subjected to 2-hour MCAO (74 ± 2 mm$^3$ in sham and 69 ± 8 mm$^3$ in preconditioned mice; n = 8/group; Figure 1B and 1C) and afforded significant neuroprotection with a 20-minute MCAO (27 ± 3 mm$^3$ in sham and 19 ± 8 mm$^3$ in preconditioned mice; n = 8/group; Figure 1D). Five-minute BCCAO showed a tendency toward neuroprotection with a 30 minutes MCAO (46 ± 8 in sham and 39 ± 9 mm$^3$ in preconditioned mice; n = 8/group; Figure 1B and 1C) and afforded significant protection with a 20-minute MCAO (27 ± 3 in sham and 10 ± 0.8 mm$^3$ in preconditioned mice; n = 8/group; Figure 1D and 1E).

Next, mice were injected with the PARP-1 inhibitor N-(6-Oxo-5,6-dihydropenanthridin-2-yl)-N,N-dimethylacetamide (PJ34) in the time window comprised between BCCAO and MCAO (Figure 1A). PJ34 was chosen because of its potency (IC$_{50}$ 110 nmol/L) and neuroprotective activity. Mice received a neuroprotective dose of 20 mg/kg i.p. at 0, 8, and 24 hours after BCCAO, followed by 20 minutes MCAO. ATP was measured in the striatum because, as evidenced by histological analysis, together with the deeper layers of the adjacent parietal cortex it was the preconditioned brain region. Yet, it was impossible to collect the ischemic parietal cortex because it was not feasible to identify the boundaries between the preconditioned and nonpreconditioned cortex in the frozen brain.

Indirect infarct determination, Western blotting, NAD, and ATP measurement were conducted as described. Infarcts were measured 3 days after MCAO to rule out transient neuroprotection. The anti-PAR antibody and the Caspase-3 activity determination kit were from Alexis, the anti–PARP-1, and anti–caspase-3 antibodies were from Cell Signaling.

**Results**

Five-minute BCCAO had no effect on infract volumes in mice subjected to 2-hour MCAO (74 ± 2 in sham and 69 ± 8 mm$^3$ in preconditioned mice; n = 5/group) or 1-hour MCAO (57 ± 7 in sham and 55 ± 8 mm$^3$ in preconditioned mice; n = 5/group) (not shown). Five-minute BCCAO showed a tendency toward neuroprotection with a 30 minutes MCAO (46 ± 8 in sham and 39 ± 9 mm$^3$ in preconditioned mice; n = 8/group; Figure 1D and 1E) and afforded significant protection with a 20-minute MCAO (27 ± 3 in sham and 10 ± 0.8 mm$^3$ in preconditioned mice; n = 8/group; Figure 1D and 1E).

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16 hours after BCCAO. In preconditioned animals, PJ34 had no effect on infarct volumes (10 ± 1.7 mm³; n = 10; Figure 1D and 1E) or areas (Figure 1D and 1F). PJ34 (20 mg/Kg i.p.) was also injected 24, 16, and 8 hours before a 20-minute MCAO in naive mice. PJ34 pretreatment did not affect infarct volumes (25 ± 5 in vehicle- and 23 ± 6 mm³ in PJ34-pretreated mice; n = 5/group; not shown), thereby excluding drug carryover during and after MCAO.

Figure 2. Effects of BCCAO on PAR, PARP-1, and NAD/ATP in the mouse striatum. Effect of 5-minute BCCAO premature reperfusion (A) or 5- to 15-minute BCCAO and 30-minute reperfusion (REP; C) on striatal PAR. B and D, Densitometric evaluation of PAR content shown in control and preconditioned striatum. Effect of 5-minute BCCAO ±15-minute reperfusion on NAD (E) and ATP (F) striatal content. In A, basal brain PAR contents from a PARP-1 WT and KO are shown. The reduced PAR content in the KO brain is in keeping with evidence that null mice synthesize ADP-ribose polymers. The effect of a 60-minute pretreatment with PJ34 20 mg/kg i.p. on NAD content in reperfused mice is shown in E. The effect of 5-minute BCCAO and different times of reperfusion on levels of 17 kDa-Caspase-3, Caspase-3 activity, and PARP-1 cleavage in the striatum are shown in G, I, and L, respectively. H, Densitometric evaluation of 17-kDa caspase-3 in control and preconditioned striatum. L, PARP-1 expression in extracts from control or etoposide-treated (Eto) Jurkat cells (J. Cells) is shown as positive control of PARP-1 cleavage. An experiment representative of 3 or 2 is shown in A, L and C, G, respectively. In E, F, and I, columns represent the mean ± SEM of 3 experiments.

Striatal PAR content was not changed after 5 minutes BCCAO or the following 30 minutes (Figure 2A and 2B), 4, 6, 12, and 24 hours of reperfusion (not shown). PAR contents did not increase even after longer BCCAO of 10 and 15 minutes (Figure 2C and 2D). Findings indicate that PARP-1 is not activated by 5- to 15-minute BCCO. Although PARP-1 activation prompts NAD and ATP consumption, striatal NAD contents were not affected by BCCAO±reperfusion or
by 60-minute pretreatment with 20 mg/kg PJ34 (Figure 2E). BCCAO prompted a drop of striatal ATP content that vanished on 15 minutes reperfusion (Figure 2F). Neither the active fragment of Caspase-3 (Figure 2G and 2H) nor caspase-3 activity (Figure 2I) increased in the preconditioned brain tissue in the time window comprised between BCCAO and MCAO. Accordingly, the 85-kDa PARP-1 fragment, a prototypical caspase-3 proteolytic product, was not present in the preconditioned brain tissue (Figure 2L).

In keeping with prior work,2 infarct volumes in KO mice were smaller than in WT (19±2 versus 27±3 mm³, respectively; *P*<0.05, Student *t* test). Five-minute BCCAO protected KO mice from 20 minutes MCAO (infarct volumes 9±1.6 mm³, *n* = 7, Figure 3A and 3B). The extent of protection by preconditioning in KO animals was similar to that in WT (59 and 63%, respectively).

**Discussion**

We report that PARP-1 expression and activity are not required for IPC in the mouse brain. Given that the PARP-1 inhibitor 3-aminobenzamide impairs myocardial IPC,3 data suggest that ischemic tolerance of the heart mechanistically differs from that of brain. Evidence that in PARP-1 KO mice IPC reduces heart ischemic tolerance4 but increases that of brain corroborates this hypothesis. Caution, however, should be exercised when comparing IPC in different tissues. Our findings indicate that PARP-1 is dispensable for brain ischemic preconditioning, but do not exclude the possibility that it contributes to IPC when activated.

Caspase-3–dependent PARP-1 cleavage underlies IPC of cultured neurons,4 as confirmed by Lee and colleagues.5 Caspase-3 activation is also involved in brain IPC of hypertensive rats.13 The findings that the active fragment of caspase-3, its enzymatic activity or PARP-1 cleavage do not increase in preconditioned mouse brains suggest that caspase-3 activation is not a prerequisite for brain ischemic preconditioning.

Overall, data suggest that PARP-1 plays different roles during IPC or maturation of brain infarct, and furthers our understanding of the molecular signaling responsible for setting the threshold of ischemic tolerance. Given that PARP-1 inhibitors are being investigated in clinical trials,2,10 our findings may have important clinical implications.

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**Disclosures**

None.

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