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Poly(ADP-Ribose) Polymerase

The Nuclear Target in Signal Transduction and Its Role in Brain Ischemia–Reperfusion Injury

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Abstract

Poly(ADP-ribose) polymerase (PARP)-1 is a DNA nick sensor that transforms ADP-ribose from βNAD$^+$ in the form of polymer to over 40 nuclear proteins, particularly to histones, several transcription factors, and PARP itself, modulating their activities and functions. PARP-1 activated by DNA breaks facilitates transcription, replication, and DNA base excision repair. The last studies indicate that PARP-1 is the new nuclear target for fast signals evoked in cell membranes by depolarization and cholinergic and glutaminergic receptors stimulation. Excessive activation of PARP-1 by peroxynitrate-evoked DNA damage during oxidative stress can cause cell death by NAD$^+$/ATP depletion after ischemia–reperfusion injury, inflammation, and diabetes mellitus. The PARP-1 through interaction with nuclear factor-κB, p53, and other transcription factors might significantly modulate cell survival and death and a type of death pathway. The pharmacological modulation of PARP-1 might offer a new effective approach for neuroprotection.

Index Entries: PARP; PARP-1; brain; ischemia; reperfusion; NF-κB; p53; neuroprotection.

Introduction

Poly(ADP-ribose) polymerase (PARP-1, E.C. 2.4.2.30) is a highly conserved protein (113 kDa) localized in the nucleus. PARP-1 is involved in a variety of physiological and pathological events such as DNA replication, DNA repair, gene expression, cellular differentiation, chromatin decondensation, malignant transformation, and apoptosis *(1–5)*. Activation of PARP-1 was demonstrated as the earliest and the most sensitive response of cell to DNA damage and it was recognized as "a molecular nick sensor" *(6)*. This signaling model proposes that PARP-1 recognizes and rapidly binds to DNA single- and

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double-strand breaks and then cleaves βNAD$^+$ into nicotinamide and ADP-ribose. Subsequently, it synthesizes polymers of ADP-ribose [poly(ADP-ribose) or (ADP-ribose)$_n$] to nuclear acceptor proteins. The size of the branched polymer varies from a few to 200 ADP-ribose units *(7–10).* Because of its high negative charge, the covalently attached ADP-ribose polymer alters the function of the target proteins. Histones are a major acceptor for poly(ADP-ribose) *(11).* Poly(ADP-rybosyl)ation leads to automodification of PARP itself and to a significant covalent alteration of histones and other chromatin proteins. Modification of PARP-1 and other chromatin proteins might act as a strong signal that rapidly recruits other DNA damage signaling molecules *(11–13).* DNA damage signaling is a major determinant for the maintenance of genomic stability *(14,15).* PARP-1 is involved in the poly(ADP-ribosyl)ation of more than 40 nuclear chromatin-associated proteins, among them are p53, AP-1, AP-2, nuclear factor (NF)-κB, Oct-1, histones, topoisomerase I and II, DNA ligase, Ca^{2+}-Mg^{2+}-dependent endonuclease, and DNA polymerase *(7,12,13,16–18).* Poly(ADP-ribosylation) is a dynamic process, that has a half-life of less than 1 min in vivo. Two enzymes are involved in the catabolism of poly (ADP-ribose). Poly(ADP-ribose) glycohydrolase cleaves ribose–ribose bonds of both linear and branched portions of poly(ADP-ribose), and ADP-ribosyl protein lyase removes the protein proximal ADP-ribose monomer *(16).*

Excessive PARP-1 activation leads to βNAD$^+$ and, subsequently, to ATP depletion and to cell death. PARP-1 is considered as the molecular switch between cell life and death (Fig. 1). Ziegler and Oei (3) proposed that a major function of PARP-1 is silencing of transcription that prevents expression of damage genes. This hypothesis is based on the observation that modification of transcription factors by poly(ADP-ribose) prevents their binding to a promotor. Such a situation precluded the initiation of transcription when PARP-1 is stimulated by a DNA strand break. However, ongoing transcription is not disturbed by PARP-1. Grube and Burkle *(20)* observed that long-lived mammalian species expressed much higher specific PARP-1 activity (but not protein) compared to short-lived species. This enzyme might contribute to the maintenance of genome stability and integrity during aging (20). The link between poly(ADP-ribosyl)ation and the aging process was presented and discussed in several studies and is still not completely explained and understood *(21–24).*

PARP-1 interacts with different proteins through its automodification domain. The N-terminal domain contains two unique zinc fingers that bind atypical structures of DNA such as single-strand breaks. The catalytic domain is located at the C-terminus. The structure of PARP-1 and the other six isoenzymes from the whole family of poly (ADP-ribose) polymerases is presented in Fig. 2. The properties of PARP-1 compared with the other family members are presented in Table 1.

In the last years, 16 new PARPs were discovered. Until now, only seven isoenzymes are characterized; among them, PARP-1 and PARP-2 are a nuclear proteins activated by DNA breaks *(25,27–29).* Other PARPs do not appear to take part in DNA repair.

It is important to note that PARP-1 is responsible for almost 97% of protein ribosylation in the brain. Poly(ADP-ribose) formation is drastically reduced in PARP-1 knockout animals in the brain, pancreas, liver, small intestine, and colon testis (3–14%), but not in skeletal muscle and the eye *(37).* Lower levels of PAR formation (20–60%) were observed in the thymus, heart, lung, and kidney *(37).* In the brain, PARP-1 has significantly higher activity and higher expression in neurons comparing to glia cells (Table 2). The biochemical properties of purified PARP-1 and the selected biological activators and inhibitors of this enzyme are presented in Table 3. There are three ways in which PARP-1 can influence other proteins. First, direct modification of the target protein usually inhibits its DNA binding and/or enzymatic activity. Second, a noncovalent interaction occurs between PARP-1 and many of its targets; often it is independent of its enzymatic activity. Third, a

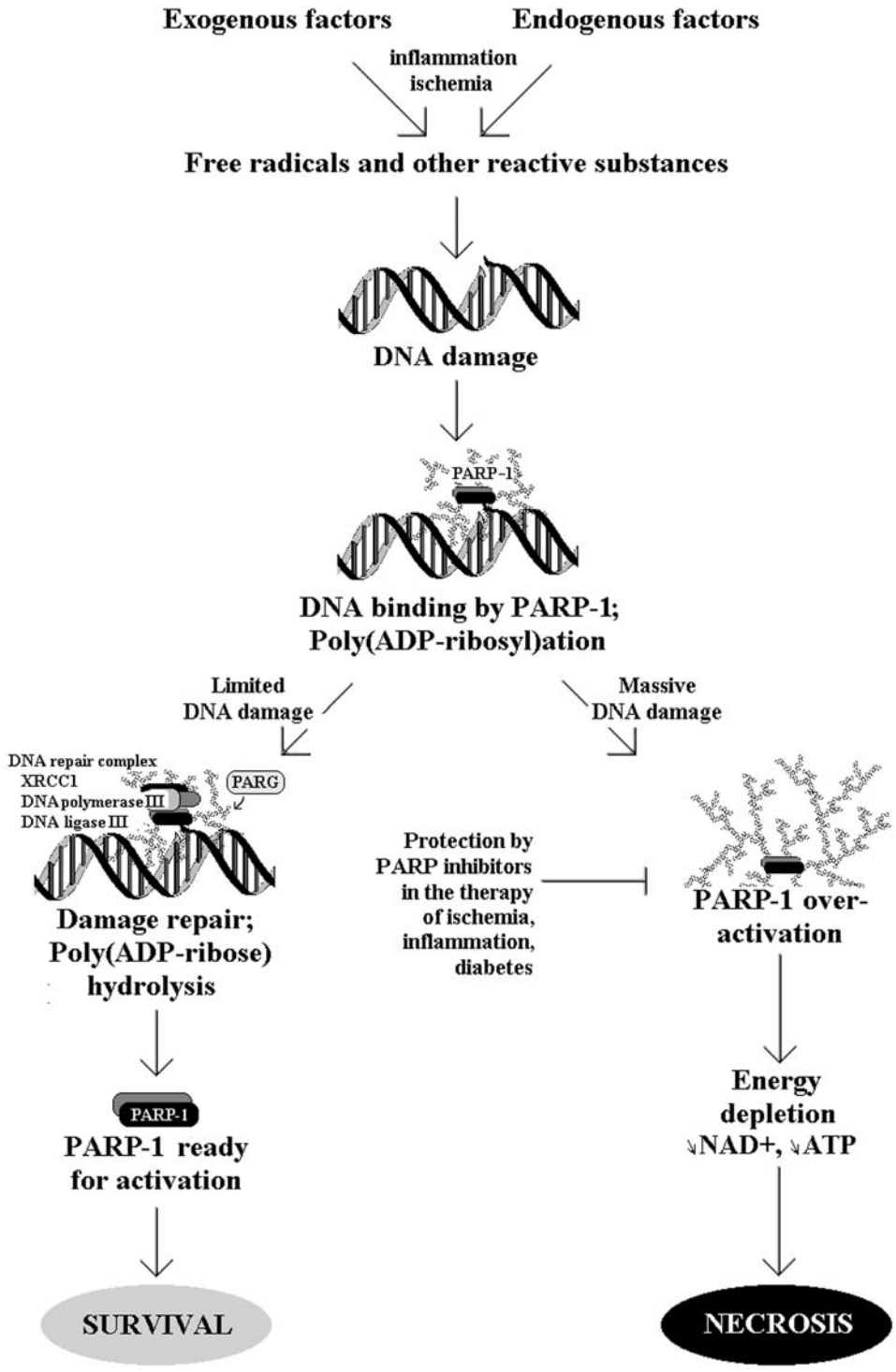

Fig. 1. Poly(ADP-ribose) polymerase-1 is the molecular switch for cell life and death under oxidative stress. PARG: poly(ADP-ribose) glycohydrolase; XCRR1: X-ray cross-complementing 1. (Modified from ref. *19*.)

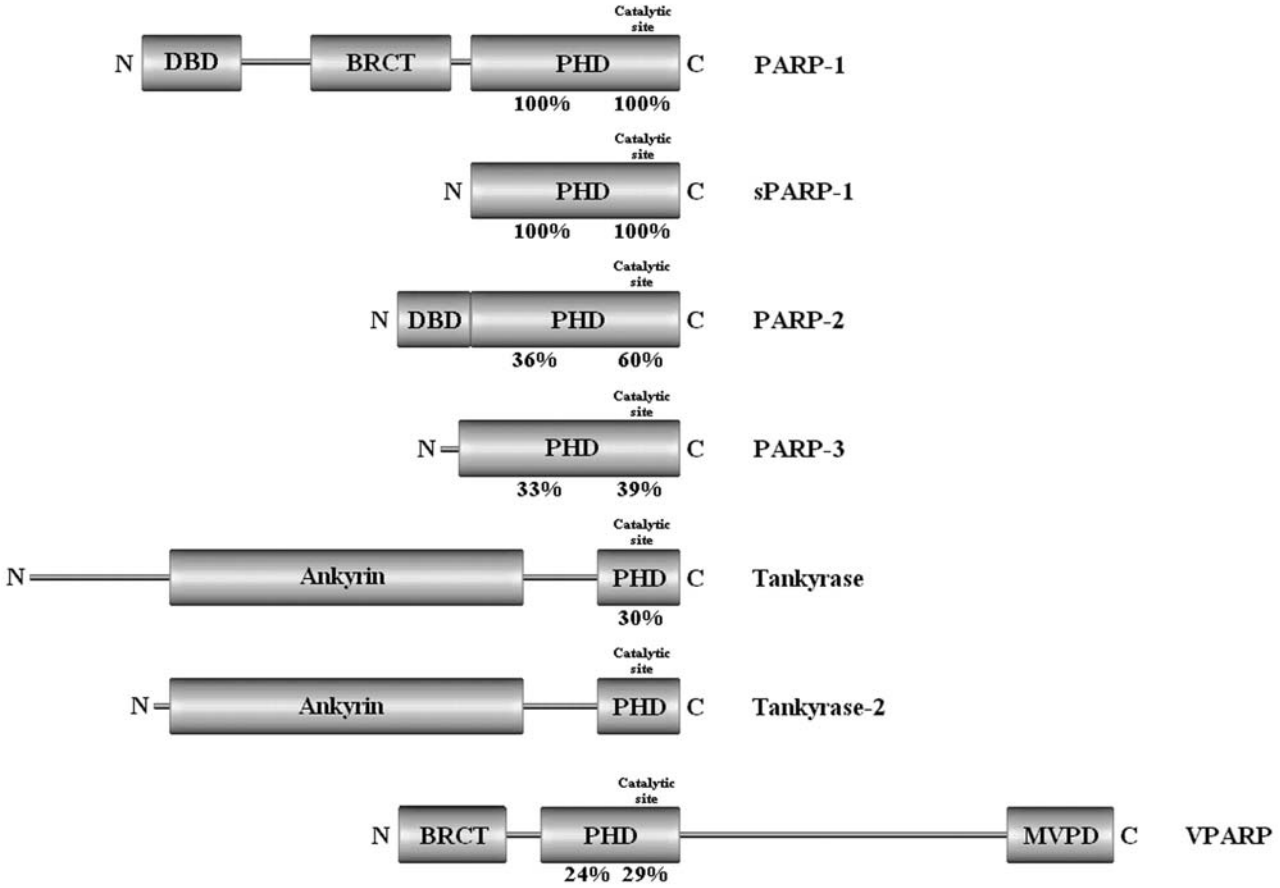

Fig. 2. The structure of PARP-1 and the other six isoenzymes from the whole family of poly(ADP-ribose) polymerases, according to Chiarugi *(25)* and Smith *(4)*. The percentages reflect their amounts in the PARP-1 sequence. BRCT: BRCA1 C-terminal domain within the automodification domain; DBD: DNA-binding domain; PHD: PARP homology domain with NAD binding and catalytic sites; MVPD: major vault protein-binding domain; VPARP: vault poly(ADP-ribose) polymerase.

recently discovered conserved motif present in target proteins binds poly(ADP-ribose) that is attached, for example, to PARP-1, p53, and the specific domain in the other DNA checkpoint proteins *(14,15)*.

The classical hypothesis describes the role of PARP-1 in DNA single-strand break repair as an agent causing local disassembly of chromatin. The results of Masson et al. *(38)* and others suggest that there is a second mechanism in which the rapid activation of PARP-1 at sites of DNA strand breakage facilitates DNA repair by recruiting the base excision repair (BER) complex because automodified PARP-1 has a strong affinity to the BER scaffold protein XRCC1. Some findings show the possibility that PARP-1 can also affect the DNA double-strand break (DSB) repair through poly(ADP-ribosyl)ation of DNA-dependent protein kinase, the DNA-PK catalytic subunit *(42)*. The last data of Susse et al. *(43)* demonstrated that PARP-1 overexpression counteracts DSB repair independently of its enzymatic activity and of poly (ADP-ribosyl)ation of p53.

The fact that PARP-1 is DNA break dependent and uses βNAD^+ as a substrate has led to the hypothesis that it might be a crucial mediator of cytotoxicity of nitric oxide and other genotoxins.

Table 1
Properties of Recently Identified PARP Family Members

	Protein size				Inhibition by PARP	Localization			
	Amino acid	kDa	DNA dependence	Species	inhibitors	Gene	Protein	Function	Ref.
PARP-1	1014	113	Yes	Human, mouse	Yes	1q41-1q42	Nuclear	Regulation of DNA base excision repair complex, TFs, chromatin structure, etc.	*4–9, 16*
sPARP-1	493	55	No	Mouse	Yes	—	Nuclear	Probably an alternatively spliced form of PARP-1	*26*
PARP-2	559	62	Yes	Human, mouse	Yes	14q11.2-q12	Nuclear	Regulation of DNA base e xcision repair complex (?), maintenance of telomere integrity (?)	*27, 28, 29, 30*
PARP-3	533	60	?	Human	?	3p21.1-p22.2	Nuclear?		*29*
Tankyrase	1314	142	No	Human	Yes	Chromosome 8	Nuclear + cytoplasmic	Regulation of telomere length	*31, 32, 33*
Tankyrase-2	1166	130	?	Human	?	10q23.2	Cytoplasmic	*34, 35*	
vPARP	1724	193	No	Human	Yes	Chromosome 13	Nuclear + cytoplasmic	Cellular transport?	*36*

Data according to Bürkle *(22)* and Chiarugi *(25)* and from publications cited in the table.

Table 2
Poly(ADP-Ribosyl)ation, Expression of PARP-1, Levels of NAD^+, and DNA Damage in Rat Neuronal and Glial Cells

Cells	PARP activity (nmol/mg DNA/min.)	PARP expression (nmol/mg DNA)	Specific PARP activity (nmol/min./nmol of PARP)	Source	Ref.
Brain neurons	9.8 ± 1.2^a	0.21 ± 0.01^b	47	Permeabilized neurons and glial cells isolated from 3-mo-old rats	*23*
Brain glia	1.5 ± 0.1^a	0.07 ± 0.006^b	21		
Cells	PARP activity (pmol [^{32}P]dCTP incorporated/mg protein)	DNA damage (pmol [^{32}P]dCTP incorporated/mg protein)	NAD^+ (µmol/mg protein)		
Cortex neurons	92.2 ± 5.2	3.25 ± 0.04	8.68 ± 0.22	Primary cultured cortical neurons and astrocytes	*37*
Cortex astrocytes	1.33 ± 0.69	0.05 ± 0.0031	19.00 ± 0.74		

Table 3
Biochemical Properties and Selected Biological Activators and Inhibitors of PARP-1

Molecular mass (depending on species)	110–130 kDa
K_M for NAD	20–80 μ*M* (depending on type of DNA and proteins present in the reaction medium)
Optimal temperature	25°C
Optimal pH	8.0–8.5
Selected biological activators	
Damaged DNA (single- and double-strand breaks)	
Histones	
Ca^{2+} (depending on DNA, histones and spermine concentration)	
Mg^{2+} (depending on DNA, histones, spermine, and Ca^{2+} concentration)	
Polyamines (spermine, spermidine, putrescine)	
Selected biological inhibitors	
Arachidonic acid	
ATP	
Histamine	
Linoleic acid	
Linolenic acid	
Nicotinamide	
Oleic acid	
Poly(ADP-ribose)	
Purines (hypoxanthine, inosine, adenosine)	
Thymidine	
Thymine	

Data according to Banasik and Ueda *(39),* Hayaishi and Ueda *(16),* Kun et al. *(40),* and Virag and Szabo *(41).*

Extensive poly(ADP-ribosyl)ation after massive DNA damage might trigger a decrease of cellular NAD^+ content, leading to depletion of ATP, which is used for NAD^+ resynthesis. This phenomenon is indeed found in many processes involving NO, such as cerebral ischemia and inflammation. The overactivation of PARP seems to activate the cell loss or switch the mode of cell death from energy-consuming apoptosis to a more passive necrosis.

PARP: A New Nuclear Target for Signal Transduction

The data from Homburg et al. *(44),* Strosznajder et al. *(21,24,45),* and Zambrzycka and Strosznajder *(46)* demonstrated a fast activation of PARP-1 by signals evoked in the cell by membrane depolarization and glutaminergic and cholinergic receptors stimulation. They demonstrated that activation of PARP was mediated by inositol-1,4,5-trisphosphate-Ca^{2+} mobilization. These findings identified PARP as a novel downstream target of phospholipase C and were the first evidence for a fast activation of PARP that did not involve DNA breaks. Homburg et al. *(44)* showed that membrane depolarization induced by high potassium concentration significantly activated poly(ADP-ribosyl)ation of nuclear proteins in the absence of extracellular Ca^{2+}. The results indicated that PARP was activated in the depolarized neurons by intracellular Ca^{2+} mobilization evoked by a physiological concentration of inositol(1,4,5)trisphosphate (IP_3) in the range of 0.05–5 μ*M*. IP_3 in a dose-dependent manner enhanced poly(ADP-ribosyl)ation of PARP in crude nuclei of brain cortical neurons. It was also found that IP_3

was specifically bound to its receptor in the crude nuclei and that [Ca^{2+}] ions concentration was markedly increased in the nucleoplasm of nuclei isolated from depolarized neurons *(44).* The data indicated that Ca^{2+} mobilized from intracellular stores was released into the nucleoplasm. Concomitantly, the influence of cholinergic and glutaminergic receptor stimulation on PARP activity was studied in the brain *(24,45,46).* Activation of cholinergic receptor by nonhydrozylable analog of acetylocholine, carbachol, and guanosine-5′-0-(3-thiotriphosphate) (GTPγS) enhanced PARP activity in the hippocampus and brain cortex by about 100% and 50%, respectively, compared to control (nonstimulated) conditions *(46).* Inhibition of IP_3 receptor by 8-(*N*,*N*-diethylamino)-octyl3,4,5-trimethoxybenzoate (TMB-8) eliminated the cholinergic receptor evoked PARP activation *(21).* This result indicated that IP_3-induced Ca^{2+} mobilization played a significant role in the cholinergic receptor signal transduction pathway to a new nuclear target PARP-1. Amyloid-β peptides known to accumulate in the brain during aging eliminate cholinergic receptor evoked stimulation of PARP activity in the brain cortex and hippocampus *(46).* However, amyloid-β peptides enhanced significantly PARP activity by 100% exclusively in hippocampus probably by free-radical-evoked DNA damage *(46).* Moreover, it was demonstrated that activation of glutaminergic NMDA receptor also enhanced significantly PARP activity in hippocampus but not in the brain cortex. The data indicated that nitric oxide (NO) was involved in signal transduction processes from the NMDA receptor into PARP *(45).* The studies carried out by Pieper et al. *(37)* presented high basal levels of PARP activity and DNA strand breaks in the neurons of hippocampal dentate gyrus, cerebellar granule cells, olfactory bulb in ventricular ependymal, and subependymal cells as well as in peripheral tissues such as the heart, spleen, and kidney. These new data altered our understanding of PARP under basal conditions. Previously, PARP has been thought to be inactive under basal physiological conditions. Pieper et al. *(37)* indicated that poly(ADP-ribosyl)ation basally activated by DNA strands breaks reflects glutamate neurotransmission that involves NMDA receptor and neuronal isoform of nitric oxide synthase (nNOS) activity. NMDA receptor antagonist and nNOS inhibitors protected against PARP-1-evoked NAD^+ decrease, indicating that basal glutamate–PARP activity regulates neuronal energy dynamics *(37).* This new role of PARP in signal transduction together with its involvement in regulation of transcription function and gene expression makes PARP-1 a very important and unique molecular regulator of cell life.

PARP in Ischemia–Reperfusion Injury

Recent studies using PARP-1 knockout mice demonstrated that the genetic inactivation of PARP-1 protected neurons against death evoked by ischemia–reperfusion injury *(47,48)* Moreover, it was presented that PARP-1 played a crucial role in inflammation and in neurodegenerative disorders. This enzyme is involved in the pathogenesis of septic and hemorrhagic shock, arthritis and diabetes, and in Parkinson's and Alzheimer's diseases. Mandir et al. *(49)* investigated the role of PARP-1 in NMDA and non-NMDA receptor-mediated neurotoxicity. They found that mice lacking PARP-1 were highly resistant to exitotoxicity induced by NMDA but remained susceptible to AMPA exitotoxicity. Restoring PARP-1 protein in mice by viral transfection restores susceptibility to NMDA. These data supported the requirement of PARP-1 in NMDA neurotoxicity. The experiments carried out in mice lacking the gene for nNOS confirmed the role of NO in NMDA receptor-evoked PARP stimulation. During ischemic stroke, there is a significant increase of extracellular glutamate that causes neuronal damage. Nitric oxide produced by neuronal NO synthase (nNOS) plays a preferencial role in NMDA receptor-mediated neurotoxicity observed in primary brain cultures *(50)* and in brain ischemia *(51–54).* NMDA receptor activa-

tion increases intracellular calcium concentration preferentially, leading to activation of nNOS. The toxic effect of NO appears to be a result of its reaction with superoxide radical O_2^- and with the formation of the highly toxic compound peroxynitrite ($ONOO^-$) *(55,56).* $NO^{\cdot}$ and peroxynitrite and its degradation products hydroxyl radical $^{\cdot}OH$ and $NO_2^{\cdot}$ can cause DNA damage and PARP activation. There are several other sources of reactive oxygen species that can contribute to the DNA strand breakage and PARP activation. Free radicals are liberated during mitochondrial electron transport and metabolism of arachidonic acid via the cyclo-oxygenase and lipoxygenase pathways. Free radicals are also formed during oxidation of hypoxanthine and xanthine by xanthine oxidase and by cooper and ferrum ions in the Fenton reaction. The last data indicate that PARP-1-evoked depletion of NAD^+ leads to the alteration of mitochondria potential and to the release of apoptosis-inducing factor (AIF) and to its translocation into the nucleous *(57–59).* These events are responsible for a novel, caspase-independent pathway of programmed cell death. Figure 3 presented the involvement of PARP-1 and AIF in NMDA receptor-evoked cell death. There are evidences that PARP-1 activation is responsible for neuronal death after focal brain ischemia *(48,54,56)* and that PARP inhibitors have a protective effect on neuronal survival *(60,61).* The ischemic infarct volume in the brain and in heart significantly decreases in animals treated with PARP inhibitors and in gene-deficient PARP-1 mice *(47,48,62,63).* Until now, there are controversial results on the effect of PARP inhibitors in global brain ischemia. Plaschke et al. *(64)* have observed the neuroprotective effect of PARP inhibition. However, Nagayama et al. *(65)* suggested that activation of poly(ADP-ribose) polymerase in the rat hippocampus might contribute to cellular recovery following sublethal transient global ischemia. Moroni et al. *(66)* did not find an ameliorating effect of PARP inhibitors in the gerbil model of brain ischemia. In that study, benzamide was administrated intraperitonealy in a very high dose of 160 mg/kg body weight 2 h before ischemia *(66).* The studies of Strosznajder et al. *(67)* indicated that 3-aminobenzamide (3-AB) or benzamide had no effect if these compounds were administrated intraperitonealy. Also, Thiemermann et al. *(68)* and Plaschke et al. *(64)* used intra-arterial or intraventricular injection. Tokime et al. *(69)* reported the lack of the PARP-inhibitor ameliorating effect after intraperitoneal injection in a model of focal brain ischemia. Benzamide and its derivatives have been reported to have an effect on cell viability, glucose metabolism, and *de novo* synthesis of DNA. These compounds inhibited activity of nicotinamide-*N*-methyltransferase at a high concentration *(70,71).* These side effects might counteract the neuroprotective effect of PARP inhibition. The activation of PARP in the reperfused brain has been assumed in previous studies but has not been directly presented. The time-course of PARP activation after different times of brain ischemia–reperfusion was demonstrated by Strosznajder et al. *(67).* Depending on the duration of the ischemic insult, there was more or less massive cell death in the CA1 layer of the hippocampus *(67).* The brief 3-min ischemia in the gerbil induced the necrotic death of more as 70% of neurons in the CA1 layer of the hippocampus. The data indicated that PARP inhibitor(s) exerted a potent neuroprotective effect at the cellular and ultrastructural levels after a short global ischemia *(72).*

The effect of the PARP inhibitor 3-aminobenzamide (3-AB) on cell survival after a 3-min transient forebrain ischemia is demonstrated in Fig. 4. It was found that the NMDA receptor was responsible for PARP activation during reperfusion *(67).* The inhibition of PARP protects the brain against edema and neuronal death induced by ischemic insult *(67).* The ameliorating effect of 3-AB significantly depended not only on the type and severity of ischemia but also on the dose and the method of PARP inhibitor administration. Both necrotic and apoptotic neuronal death have

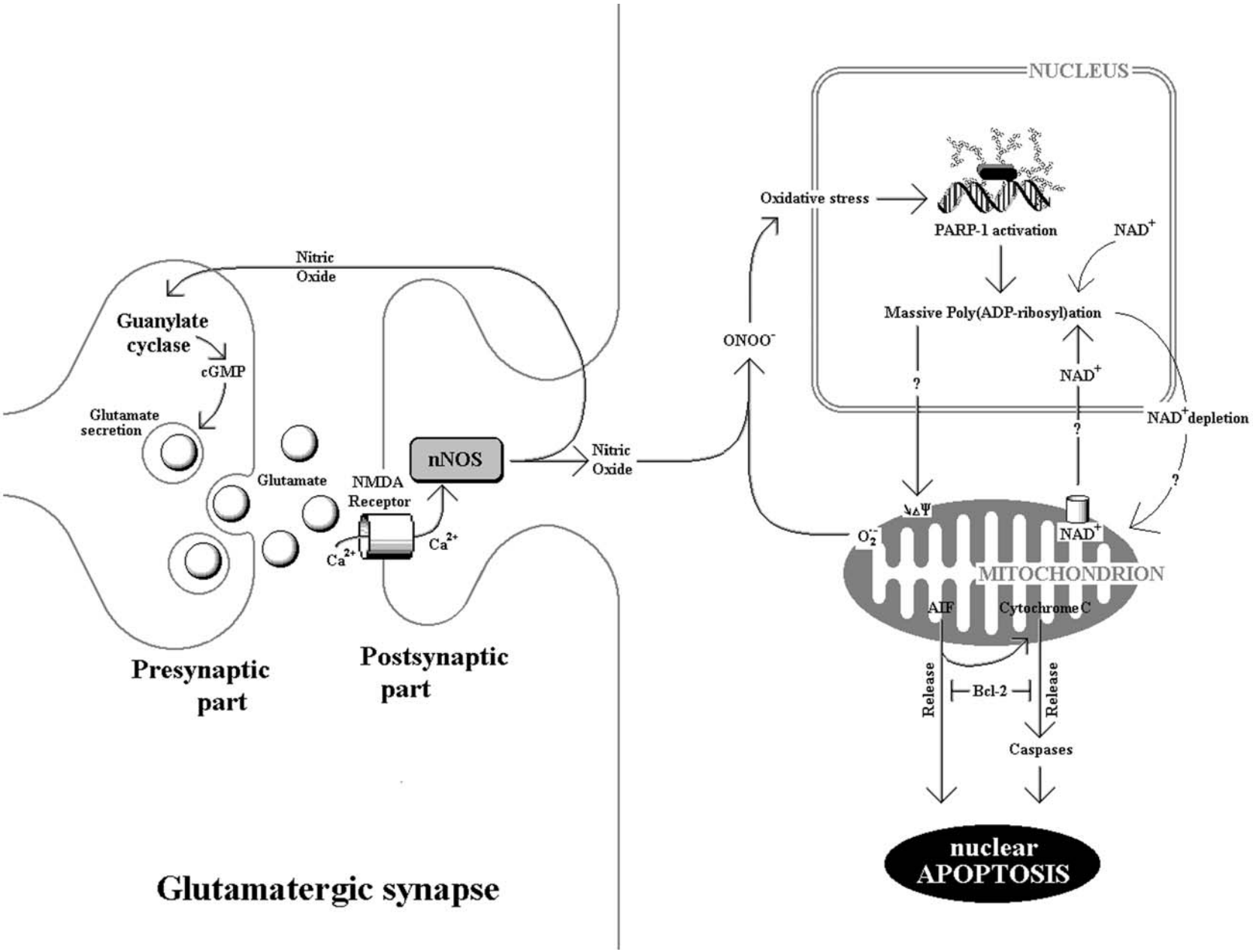

Fig. 3. PARP-1 and AIF in neurotoxicity evoked by activation of glutamatergic NMDA receptors. (Modified from Chiarugi and Moskovitz *[59]* and Yu et al. *[57]*.)

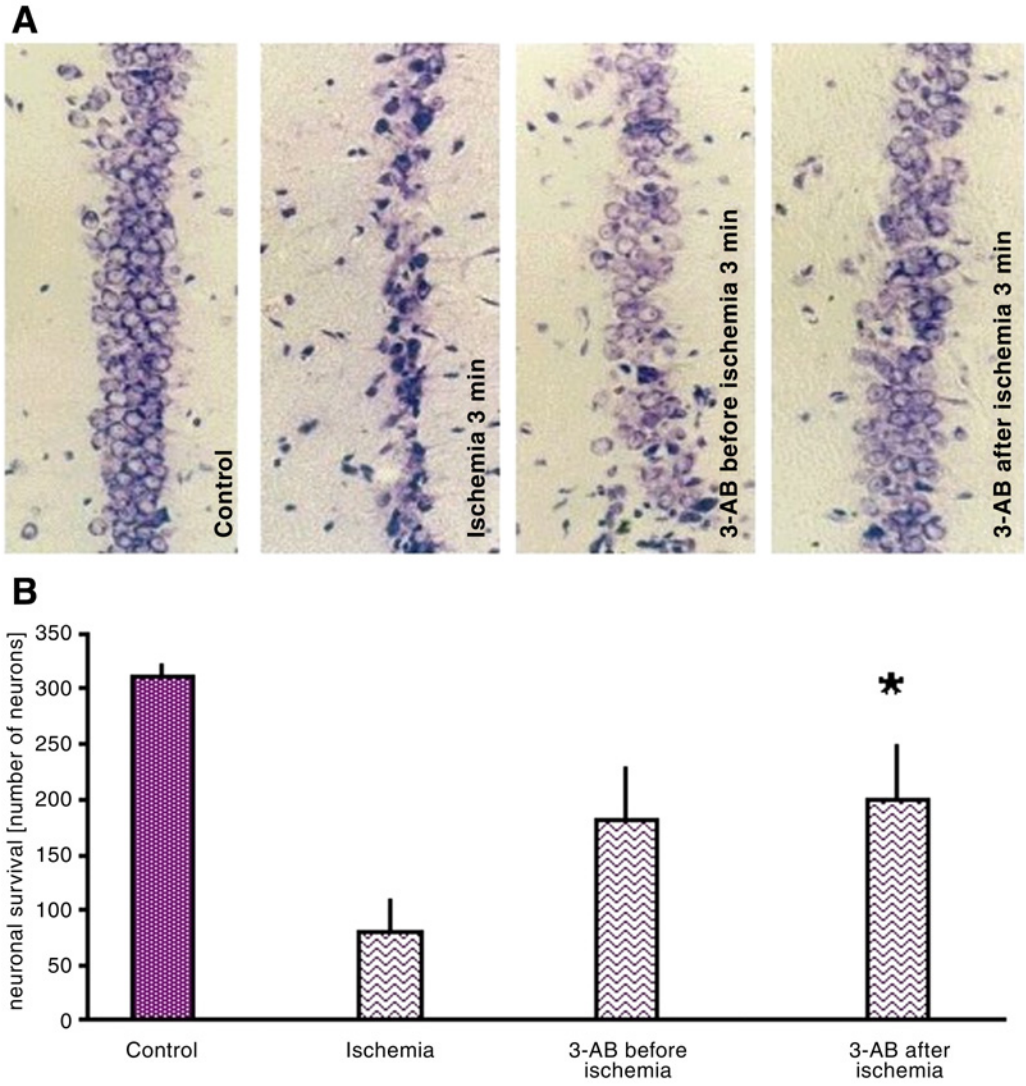

Fig. 4. Effect of the PARP-1 inhibitor 3-AB on cell survival in the CA1 layer of the hippocampus 7 d after 3 min of forebrain ischemia in gerbils. (From ref. *67*.)

been described following cerebral ischemia *(73–75)*. In our study, we have found that the integrity of PARP in the hippocampus was preserved during the reperfusion after 3 and 10 min of brain ischemia *(67)*. These results demonstrated that caspase-3-induced PARP cleavage was not significantly activated. Liu et al. *(76)* using both monoclonal and polyclonal antibodies to PARP cleavage products have found little evidence of PARP cleavage in gerbil brains within the first 3 d after 10 min of global ischemia. However, they have found a substantial increase in PARP mRNA levels in the granule cell layer of the dentate gyrus in the brain. It might play a role in DNA integrity in dentate granule cells that generally survive these types of injury. Liu et al. *(76)* for the first time presented the altered PARP gene expression in the brain. The increased PARP mRNA expression in this gerbil ischemia model might

also lead to enhancement of PARP protein levels in the dentate gyrus. Whole hippocampal PARP protein levels did not change after global ischemia in gerbils and rats *(65,67,76).* The study by Fukuda et al. *(77)* suggested that novel nonapoptotic death occurred in the hippocampus following global ischemia. They demonstrated that increased PARP enzymatic activity following global ischemia correlated with DNA damage. The neuroprotective effect of PARP-1 inactivation in focal and global brain ischemia is presented in Table 4.

PARP-1 is now recognized as a key regulator of cell death and survival through interaction with the transcription factors (Fig. 5). This type of interaction might play an important role in brain ischemia–reperfusion injury.

PARP-1 might interact with (NF)-κB *(18,82),* p53 *(15,83,84),* and AP-1, AP-2, YY1, and Sp1 *(85–88).* PARP-1 binds NF-κB in the nucleus. This binding is stable and independent of DNA *(12,18,87,88).* Moreover, NF-κB contains the poly(ADP-ribose)-binding motif *(14)* and can be poly(ADP-ribosyl)ated in vitro and in vivo *(89).* PARP inhibition by 3-AB has a negligible effect on NF-κB, indicating that PARP protein rather than its activity is required for NF-κB transcriptional activity. In the absence of PARP-1, NF-κB is either totally or partially inhibited depending on the promoter used. This suggests that PARP-1 plays different roles in the expression of particular NF-κB-dependent genes.

PARP-1/NF-κB cooperation might play an important role in brain ischemia pathology and other diseases that have an inflammatory component. PARP in brain ischemia exerts its effects also by influencing tumor suppressor protein p53. This protein, which in normal conditions is destabilized by MDM2 protein (murine double minute 2 protein) after a stress insult such as DNA damage or heat shock, undergoes stabilization and accumulates. The accumulation and activation of p53 leads to cell cycle arrest and, in some cases, to apoptosis. It was found that p53 binds PARP-1 in the nucleus. Central and carboxy-terminal fragments of p53 bind N-terminal and central domains of PARP-1. The complex formation between p53 and PARP-1 is dependent on p53 phosphorylation status *(90).* Moreover, p53 is poly(ADP-ribosyl)ated in vitro and in vivo. This modification affects binding of p53 to its DNA-consensus sequence *(91,92).* It was demonstrated that p53 binds also poly(ADP-ribose) noncovalently *(15).* The presence of PARP-1 stabilizes wild conformation and increases the half-life of p53 *(93–95).* In PARP-$1^{-/-}$ cells a decrease of p53 stability was observed *(83,94,96–98).* A reduced level and reduced activity of p53 are also noted in cells treated with PARP inhibitors *(99,100).* Inhibition or lack of PARP-1 protein usually lowers the transactivation of p53 target genes comparing to wild-type (wt) cells *(101,102).*

The data of Tomasevic et al. *(103)* presented the activation of p53 and its response genes in the brain following lethal as well as nonlethal ischemic insults. The effects of PARP-1/p53 interactions are dependent on the type of DNA damage and are important in the regulation of the cell cycle and apoptosis *(98).* PARP-1 takes part in early events of apoptosis, ensuring the proper function of p53 by regulating its activity by poly(ADP-ribosyl)ation and binding of p53 *(91,104,105).* The role of PARP and poly(ADP-ribosyl)ation in the regulation of apoptosis is confirmed by the fact that the subsequent degradation of ADP-ribose polymers co-occurs with the transactivation of p53-dependent genes and the activation of caspases *(91,104).*

The inhibitors of PARP-1 could, therefore, prevent brain ischemia pathology by reducing NF-κB activity or p53. In evaluating the role of PARP-1 in brain ischemia–reperfusion, it is important to take into consideration its active interaction with several transcriptions factors such as: NF-κB, p53, AP-1, AP-2, Sp1, and others *(86,87,106–108).* Depending on the transcription factors and cell types, PARP-1 modulates gene expression differently. However, the alteration of PARP, p53, and NF-κB in the ischemic sensitive parts of brain as well as in the resistant parts indicate that their role is much more complex than previously assumed. The interaction of PARP-1 with other nuclear proteins remains a challenging area for further studies in the field

Table 4
Neuroprotection by Pharmacological Inactivation of PARP-1 in Brain Ischemia

Animal model	Mode of PARP-1 inhibition	Dose, (mg/kg body wt)	Mode of administration	Effect of PARP-1 inhibition	Ref.
Focal cerebral ischemia					
Rat	DPQ	5–40	i.p. 2 h before ischemia	Reduction of infarct volume	*60*
Rat	3-AB	10	Microdialysis, after ischemia	Reduction of infarct volume, attenuation of glutamate elevation	*79*
Rat	DPQ	40	i.p. 30 min after ischemia	Significant decrease of infarct volume	*81*
Rat	3-AB	250	i.p. 15 before reperfusion	Significant decrease in the volume of damaged tissue	*61*
Rat	3-AB	1–30	i.p. ventricular, 30 min before ischemia	Decrease of infarct volume in a dose-dependent manner	*69*
Mice	3-AB	40	i.p. 15 min before reperfusion	Neuroprotection, 30% decrease in the infarct volume, improvment the neurological score	*78*
Rat	3-AB	15, 55		Decrease of infarct volume, improvment the neurological outcome and motor function	*80*
Global cerebral ischemia					
Rat	3-AB	5, 10, 20	Intracerebroventricularly, 10 min before ischemia	Protection of energy state by preserving cortical phosphocreatine and NAD^+	*64*
Gerbil	3-AB	30	i.v. directly after ischemia	Protect >60% of neuronal cells against death, decreased forebrain edema	*67*
Rat	3-AB	3–30	i.v. before ischemia	Decreased the number of surviving neurons	*65*
Gerbil	Benzamide, DPQ	10–160	i.p. before and after reperfusion	No improvment of the survival of neuronal cells	*66*

3-AB: 3-aminobenzamide; DPQ: 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2*H*)-isoquinolinone; i.p.: intraperitoneally, i.v.: intravenously.

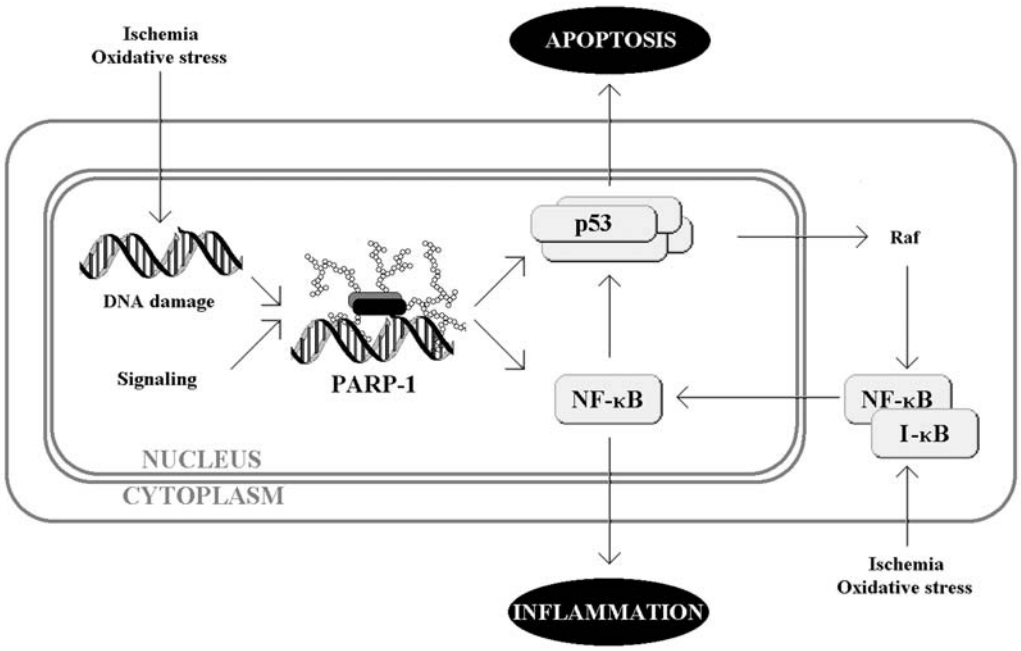

Fig. 5. PARP-1 and its interaction with transcriptions factors in signal transduction. (Modified from ref. *25*.)

of proteomics and genomics. These studies should be helpful in a better understanding of the role of PARP-1 in signal transduction and in the pathomechanism of brain ischemia–reperfusion injury.

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