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Role of poly(ADP-ribose)synthetase in inflammation

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Abstract

Peroxynitrite and hydroxyl radicals are potent initiators of DNA single strand breakage, which is an obligatory stimulus for the activation of the nuclear enzyme poly(ADP-ribose)synthetase (PARS). Rapid activation of PARS depletes the intracellular concentration of its substrate, NAD^+, slowing the rate of glycolysis, electron transport and ATP formation. This process can result in acute cell dysfunction and cell necrosis. Accordingly, inhibitors of PARS protect against cell death under these conditions. In addition to the direct cytotoxic pathway regulated by DNA injury and PARS activation, PARS also appears to modulate the course of inflammation by regulating the expression of a number of genes, including the gene for intercellular adhesion molecule 1, collagenase and the inducible nitric oxide synthase. The research into the role of PARS in inflammatory conditions is now supported by novel tools, such as novel, potent inhibitors of PARS, and genetically engineered animals lacking the gene for PARS. In vivo data demonstrate that inhibition of PARS protects against various forms of inflammation, including zymosan or endotoxin induced multiple organ failure, arthritis, allergic encephalomyelitis, and diabetic islet cell destruction. Pharmacological inhibition of PARS may be a promising novel approach for the experimental therapy of various forms of inflammation.

Keywords: Free radical; Peroxynitrite; Nitric oxide, (NO); Superoxide; Septic shock; Endotoxin; Inflammation; Stroke; Diabetes; Mitochondrial respiration; Nicotinamide; 3-Aminobenzamide; Poly(ADP-ribose)synthetase

1. The role of PARS activation in cell death

1.1. The poly(ADP-ribose)synthetase suicide pathway

Poly(ADP-ribose)synthetase (PARS) is a protein-modifying and nucleotide-polymerizing enzyme which is abundantly present in the nucleus. PARS consists of the DNA-binding N-terminal domain, the central automodification domain and the C-terminal catalytic domain. The DNA-binding domain utilizes two zinc fingers, which recognize breaks in double-stranded DNA. The central, highly conserved domain can be auto-poly-ADP-ribosylated by PARS. The C-terminal catalytic domain is involved in the synthesis of poly(ADP-ribose)polymer (for review, see Ueta and Hayashi, 1985; Lautier et al., 1993).

The obligatory trigger of PARS activation is DNA single strand breaks, which can be induced by a variety of environmental stimuli and free radical/oxidants, most notably hydroxyl radical and peroxynitrite (see below). In response to DNA damage, PARS becomes activated and, using NAD^+ as a substrate, catalyzes the building of homopolymers of adenosine diphosphate ribose units. Poly(ADP-ribose)acceptors include histones, topoisomerases I and II, DNA polymerases and DNA ligase 2, as well as PARS itself. Poly(ADP-ribose)catabolism and metabolism is a dynamic process, with poly(ADP-ribose)glycohydrolase catalyzing the degradation of the polymer (see Ueta and Hayashi, 1985; Lautier et al., 1993).

Cellular NAD^+ levels are known to regulate an array of vital cellular processes. NAD^+ serves as a cofactor for glycolysis and the tricarboxylic acid cycle, thus providing ATP for most cellular processes. NAD^+ also serves as the precursor for NADP, which acts as a cofactor for the pentose shunt, for bioreductive synthetic pathways, and is involved in the maintenance of reduced glutathione pools. The observation that activation of PARS can lead to massive NAD^+ utilization, and changes in the cellular NAD^+ levels led Berger to propose that consumption of NAD^+ due to DNA damage and activation of PARS can affect cellular energetics and function (Berger, 1991; Cochrane, 1991; Szabó, 1996a). In the 1980s, a variety of

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in vitro studies demonstrated that rapid depletion of NAD^+ due to PARS activation leading to cellular ATP depletion and functional alterations of the cell, with eventual cell death (Berger et al., 1986; Schraufstatter et al., 1986, 1988; for review, see Berger, 1991; Cochrane, 1991). The relative contribution of PARS to the cellular metabolic changes and cell injury is dependent on the cell type studied. In endothelial cells, epithelial cells, and fibroblasts PARS appears to play a greater role in the oxidant damage, whereas in hepatocytes inhibition of PARS has little influence on oxidant-induced cell damage (Junod et al., 1989; Yamamoto et al., 1993; Kirkland, 1991; Spragg, 1991; Szabó et al., 1996a,b, 1997a,c). The initial studies on the role of PARS were performed using pharmacological inhibitors of PARS (most frequently, 3-aminobenzamide and nicotinamide). These agents can have additional actions, for example as free radical scavengers. More recent studies, using cells from PARS knockout animals confirmed the role of the PARS pathway in oxidant-mediated cell injury. In the first such study, Heller et al. (1995) observed that islets of the $PARS^{-/-}$ mice are resistant to NO and oxidant-related injury, when compared to the response in islets of the wild-type mice. Similarly, we observed that pulmonary fibroblasts from the $PARS^{-/-}$ mice are protected from peroxynitrite-induced cell injury when compared to the fibroblasts of the corresponding wild-type animals (Szabó et al., 1998b). Furthermore, Eliasson et al. (1997) demonstrated protection by PARS negative phenotype in brain slices exposed to various oxidants. Thus, the more definitive studies utilizing PARS knockout cells have now confirmed the conclusions of the earlier pharmacological studies.

1.2. Hydroxyl radical and peroxynitrite, as triggers of DNA single strand breakage

DNA single strand breakage is an obligatory trigger of activation of the nuclear enzyme poly(ADP-ribose)synthetase (PARS). Peroxynitrite is a labile, toxic oxidant species produced from the reaction of superoxide and nitric oxide (NO) (Beckman et al., 1990; see for review: Beckman and Koppenol, 1996). This species, as well as hydroxyl radical, are the key pathophysiologically relevant triggers of DNA single strand breakage (see Szabó, 1996a,b). The fact that hydroxyl radical, produced during oxidant stress or radiation injury, induces DNA single strand breakage has been well known for longer than a decade (for review, see Halliwell and Aruoma, 1991). In recent years, peroxynitrite emerged as another major trigger of DNA single strand breakage. In 1992, King and colleagues demonstrated that potassium peroxynitrite induces DNA cleavage in solutions of end-labelled DNA restriction fragments (King et al., 1992). Over the last years, several groups have independently demonstrated the occurrence of DNA single strand breakage in various types of intact cells upon exposure to peroxynitrite. For instance, in calf thymus DNA, and in the bacteriophage PM2, DNA strand breakage has been reported after exposure to authentic peroxynitrite or to a sydnonimine compound that simultaneously generates NO and superoxide (Salgo et al., 1995a,c; Inoue and Kawanishi, 1995; Epe et al., 1996). Moreover, DNA single strand breakage has been demonstrated in our laboratory in cultured macrophages and smooth muscle cells exposed to peroxynitrite (Szabó et al., 1996a,b; Zingarelli et al., 1996a). Recently, the phenomenon of peroxynitrite-induced DNA single strand breakage has also been described in human pancreatic islet cells (Delaney et al., 1996). The mechanism of the DNA strand breakage is probably related to abstraction of hydrogen atoms from the ribose of the DNA moiety, thereby opening the sugar ring (Salgo et al., 1995a).

1.3. Endogenously produced oxidants can also trigger DNA single strand breakage

Before the early 90s it was generally assumed that triggers of DNA single strand breakage are restricted to severe, environmental toxic agents (e.g. genotoxic or cytotoxic drugs), or various forms of radiation (Larsen et al., 1982; Aubel-Sadron and Londos-Gagliardi, 1984; Collins, 1987; Halliwell and Aruoma, 1991). The research into the suicidal role of PARS gained a new momentum in the mid-90's by studies linking the formation of NO, an endogenously produced, reactive free radical species produced from L-arginine by a family of enzymes termed NO synthases, to DNA single strand breakage and PARS activation, with subsequent energetic changes in the cell (Radons et al., 1994; Zhang et al., 1994). Subsequent studies clarified that the actual trigger of DNA single strand breakage is peroxynitrite, rather than NO per se (Szabó et al., 1996b; for review, see Szabó, 1996a): 'pure' NO donors, even at high concentrations, are relatively weak inducers of DNA single strand breakage, when compared to the effect of peroxynitrite (see: Szabó et al., 1996a,b; Snyder, 1996; Endres et al., 1998).

The identification of NO and peroxynitrite as important mediators of the cellular damage in various forms of inflammation and reperfusion injury, stimulated interest into the role of the PARS-related suicide pathway plays a role in various pathophysiological conditions. Endogenous production of peroxynitrite and other oxidants has been shown to lead to DNA single strand breakage and PARS activation. For example, in immunostimulated macrophages and smooth muscle cells (which simultaneously produce NO and superoxide, and thus peroxynitrite from endogenous sources) (Ischiropoulos et al., 1992; Szabó and Salzman, 1995; Zingarelli et al., 1996a; Szabó et al., 1996a), DNA single strand breakage has been demonstrated, and the time course of the strand breakage paralleled the time course of NO and peroxynitrite production (Zingarelli et al., 1996a; Szabó et al., 1996a). Similarly in brain slices, activation of NMDA receptors (a trigger for enhanced NO,

superoxide and peroxynitrite production) led to peroxynitrite-mediated DNA single strand breakage and PARS related cell injury (Zhang et al., 1994; Snyder, 1996).

It is noteworthy that, although NO per se does not directly cause DNA single strand breaks, several hypotheses have been put forward for indirect mechanisms. Unrepaired abasic sites (due to NO-mediated injury) may lead to the development of DNA single strand breaks (Tamir et al., 1996). The development of these DNA strand breaks may involve AP endonucleases, excision repair, topoisomerase-mediated repair, Ca^{2+}/Mg^{2+} dependent endonucleases (Tamir et al., 1996). Furthermore, it has been proposed that inhibition of ribonucleotide reductase by NO may reduce the supply of deoxyribonucleotides for DNA synthesis and repair, which, in turn, may cause a delay in the repair process and lead to the prevalence of DNA single strand breaks (Zhang et al., 1995). Similarly, it is possible that inhibition by NO of a variety of DNA repair processes, as demonstrated, for example, for the Fpg protein and for *O*6-methylguanine-DNA-methyltransferase (Wink and Laval, 1994; Laval and Wink, 1994), may increase the degree of DNA single strand breakage in cells challenged with oxidants. Additional mechanisms of NO-related injury may involve the production of oxyradicals by the mitochondrial chain. In such scenario, NO may first inhibit the activity of mitochondrial enzymes, which subsequently triggers increased oxyradical generation from the mitochondria (Poderoso et al., 1996). One can hypothesize that this process may lead to the generation of peroxynitrite, and subsequent DNA single strand breakage. An example of such mechanism has recently been described in relation to tumor necrosis factor induced inhibition of mitochondrial respiration, oxyradical production, DNA injury and PARS activation in L929 cells (Shoji et al., 1995).

1.4. The nature of PARS-related cell death

In recent years, PARS has been implicated in apoptotic cell death (programmed cell death). In this scheme, the proposed that the role of PARS is to act as a 'death substrate' for caspases (Kaufmann et al., 1993; Nicholson et al., 1995; Nicholson and Thornberry, 1997). Since PARS is assumed to be an enzyme important in DNA repair, cleavage (i.e. inactivation) of PARS, in turn, would lead to enhanced apoptosis, because it would end the 'basal' poly(ADP-ribosylation) of Ca^{2+}/Mg^{2+} dependent endonuclease, and the ADP-ribosylation of histone H1, which are required for a 'basal' suppression of apoptosis (Wachsman, 1996; Yoon et al., 1996). In this context, the PARS-related mechanisms of apoptotic cell death are perceived as terminal, delayed effectors (Ohta et al., 1997; Bellosillo et al., 1997).

In contrast, in the scheme of PARS activation the process of 'DNA single strand breakage ≫ PARS activation ≫ energy depletion' induces necrotic, rather than apoptotic cell death, characterized by rapid cell injury, changes in membrane integrity, release of lactate dehydrogenase from the cells, and mitochondrial injury (Zhang et al., 1994; Heller et al., 1995; Watson et al., 1995; Virág et al., 1998a,b). In fact, in cells challenged with peroxynitrite or hydrogen peroxide, inhibition of PARS diverts the mode of cell death from the necrotic towards the apoptotic mode, or protects against cell injury altogether (Palomba et al., 1996; Virág et al., 1998a,b). In these latter studies, strong, oxidant-induced triggers of cell death were used, which induce substantial degree of energetic changes (and a substantial degree of necrosis-like cell death), while in many previous studies investigating the role of PARS in apoptosis, the stimuli used mainly induced milder cell injury and delayed apoptosis.

Recent studies implicated mitochondrial alterations in the process of necrotic cell death. In fact, damage to the mitochondria appears to be the primary early event in necrosis (Rosser and Gores, 1995; Zamzami et al., 1997). At least two pathways may be involved. In the first, inhibition of oxidative phosphorylation in the absence of the mitochondrial permeability transition leads to ATP depletion, ion dysregulation, and enhanced degradative hydrolase activity. If oxygen is present, toxic oxygen species may be generated and lipid peroxidation can occur. Subsequent cytoskeleton and plasma membrane damage result in plasma membrane bleb formation. If injury continues, bleb rupture and cell lysis occur. In the second pathway, mitochondrial damage results in an mitochondrial permeability transition. This step is believed irreversible and leads to cell death (Rosser and Gores, 1995; Richter, 1997). It is believed that mitochondrial permeability transition occurs secondary to changes in the first pathway (e.g. oxidative stress, increased intracellular Ca^{2+}, and ATP depletion) and that all the 'downstream events' occurring in the first pathway may result from permeability transition. Mitochondrial permeability transition involves the formation of proteaceous, regulated pores, probably by apposition of inner and outer mitochondrial membrane proteins which cooperate to form the mitochondrial megachannel (also known as the mitochondrial permeability transition pore). The permeability transition has important metabolic consequences, namely the collapse of the mitochondrial transmembrane potential, uncoupling of the respiratory chain, hyperproduction of superoxide anions, disruption of mitochondrial biogenesis, outflow of matrix Ca^{2+} and glutathione, and release of soluble intermembrane proteins (Rosser and Gores, 1995; Hirsch et al., 1997). Recent studies have demonstrated the occurrence of the above listed mitochondrial alterations in various cell types challenged with peroxynitrite (Gow et al., 1998; Virág et al., 1998c). Importantly, the changes in mitochondrial membrane potential, the mitochondrial permeability transition, the increase in reactive oxygen intermediate production, the increased Ca^{2+} mobilization, and the destruction of mitochondrial structure are attenuated by inhibition of the activation of PARS (Virág et al., 1998c).

Since cellular NAD^+ and ATP, are important regulators of mitochondrial functions (Takeyama et al., 1993; Zizi et al., 1994; Costantini et al., 1996; Lee et al., 1996; Devin et al., 1997; Rustin et al., 1996), maintenance of cellular energetic pools in cells where PARS is inhibited may explain the improvement of the mitochondrial functions (Fig. 1).

A widely held view is that necrosis is a process which cannot be influenced by pharmacological means, and apoptosis is the process which is under the control of a sophisticated cellular machinery. Recent reports, demonstrating protection against cell injury by inhibition of PARS, however, prove that the necrotic process, indeed, is amenable to pharmacological interventions. Additional mechanisms which can affect necrotic cell death include Bcl-2, which can protect against necrosis (Guenal et al., 1997; Tsujimoto et al., 1997; Bonfoco et al., 1996; Albina et al., 1996). The most likely mode of Bcl-2's action is inhibition of secondary free radical generation in the mitochondria of cells exposed to peroxynitrite. Similarly, heat shock protein 27 (Guenal et al., 1997) and certain free radical scavengers (which inhibit secondary, mitochondria-related free radical generation) are protect against necrotic cell death (Richter, 1997; Rosser and Gores, 1995). In some systems inhibition of interleukin-1 converting enzyme can protect against necrotic cell death (Tsujimoto et al., 1997). Thus, according to the more current models, necrosis does not equal overwhelming injury, but is controlled and can be modulated (i.e. suppressed by antioxidants, Bcl-2, heat shock protein 27, or PARS) to achieve survival benefit.

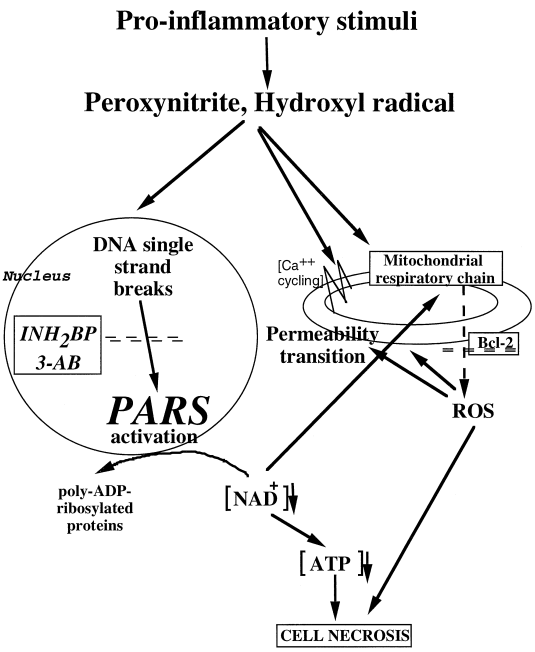

Fig. 1. Mechanisms of oxidant-induced cell necrosis. Rapid DNA single strand breakage activates PARS. Massive poly-ADP-ribosylation leads to NAD^+ depletion, which potentiates the oxidant-induced mitochondrial dysfunction. Massive energy depletion and mitochondrial free radical generation result in cell necrosis.

Necrotic cell death is an important pathway of cell death, which has direct relevance for various forms of reperfusion injury, and for various forms of inflammation. Under such conditions, overwhelming oxidant production can occur, and cells rapidly die via necrosis. The importance of PARS in mediating this process is underlined by recent in vivo experiments, where pharmacological inhibition or inactivation of PARS protects against cell necrosis in various forms of inflammation and reperfusion injury (see below).

2. Role of the PARS pathway in various forms of inflammation

2.1. Zymosan and carrageenan induced inflammatory models

Recent studies have clearly demonstrated the role of PARS activation in various forms of local or systemic inflammation induced by the prototypical inflammatory stimuli zymosan and carrageenan. For example, in carrageenan-induced paw edema, inhibition of PARS with 3-aminobenzamide reduced paw swelling and inhibited the infiltration of neutrophils into the inflamed paw (Szabó et al., 1997b). Furthermore, in a model of acute local inflammation (carrageenan-induced pleurisy) the poly(ADP-ribose)synthetase inhibitor 3-aminobenzamide (given at 1–30 mg/kg) inhibits the inflammatory response (pleural exudate formation, mononuclear cell infiltration, histological injury) (Cuzzocrea et al., 1998a,b). Similar to the effect of the pharmacological inhibitors, $PARS^{-/-}$ animals were resistant against zymosan-induced inflammation and multiple organ failure when compared to the response of wild-type mice (Szabó et al., 1997b).

Inhibition of PARS also reduced the formation of nitrotyrosine, an indicator of the formation of peroxynitrite, in the inflamed tissues (Szabó et al., 1997b; Cuzzocrea et al., 1998b). This finding was at first unexpected because PARS activation is distal to the generation of oxidants. The explanation for this finding is likely related to the fact that $PARS^{-/-}$ phenotype or pharmacological inhibition of PARS reduces the infiltration of neutrophils into inflammatory sites (Szabó et al., 1997b; Cuzzocrea et al., 1998b). Thus, the reduction in tissue injury by PARS inhibitors may result from a decreased inflammatory infiltrate, which would be associated with a reduction in both oxygen and nitrogen centered free radical production (hence, reduced nitrotyrosine staining) (Fig. 2). The basis for PARS-inhibitable neutrophil infiltration is not yet defined, but may relate to the effect of PARS activation on the expression of intercellular adhesion molecules, and/or due to modulation by PARS of a post-adhesion event (Roebuck et al., 1995; Szabó et al., 1997a,b,c,d; Zingarelli et al., 1998). Other mechanisms by which PARS modulates neutrophil

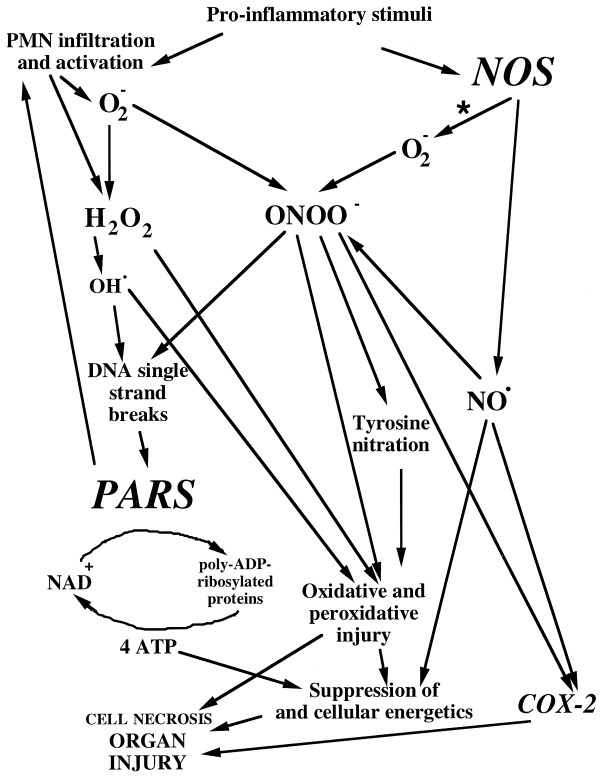

Fig. 2. Proposed scheme of PARS-dependent and PARS-independent cytotoxic pathways involving nitric oxide ($NO^\cdot$), hydroxyl radical ($OH^\cdot$) and peroxynitrite ($ONOO^-$) in inflammation. Proinflammatory mediators induce the expression of the inducible NO synthase (iNOS), whereas NMDA receptor ligands activate the constitutive neuronal NOS (bNOS). NO, in turn, combines with superoxide to yield peroxynitrite. Hydroxyl radical (produced from superoxide via the iron-catalyzed Haber–Weiss reaction) and peroxynitrite or peroxynitrous acid induce the development of DNA single strand breakage, with consequent activation of PARS. Depletion of the cellular NAD^+ leads to inhibition of cellular ATP-generating pathways leading to cellular dysfunction. NO alone does not induce DNA single strand breakage, but may combine with superoxide (produced from the mitochondrial chain or from other cellular sources) to yield peroxynitrite. Under conditions of low cellular L-arginine NOS may produce both superoxide (*) and NO, which then can combine to form peroxynitrite. There are PARS-independent, parallel pathways of cellular metabolic inhibition, and these pathways can be activated by NO, hydroxyl radical, superoxide and by peroxynitrite (alone or in combination or synergy). For instance, peroxynitrite can induce cell injury via protein tyrosine nitration. In inflammation, activation of the inducible cyclooxygenase (COX-2) by NO or peroxynitrite may also amplify the inflammatory response. PARS activation promotes neutrophil (PMN) recruitment, thereby triggering a positive feedback cycle.

tissue infiltration cannot be excluded, including an effect on endothelial integrity (Szabó et al., 1997a).

2.2. Pancreatic islet cell destruction—diabetes mellitus

The importance of the PARS pathway in the pathogenesis of pancreatic islet cell injury has been initially put forward by Yamamoto as early as 1981, based on studies in streptozotocin-treated islet cells. Inhibition of PARS inhibited NAD^+ depletion and the suppression of proinsulin synthesis without modifying the extent of DNA damage in streptozotocin-treated islet cells (Yamamoto et al., 1981; Uchigata et al., 1982). Moreover, in vivo experiments demonstrated that PARS inhibition by 3-aminobenzamide or nicotinamide prevented the onset of streptozotocin- and alloxan-induced diabetes (Uchigata et al., 1983; Masiello et al., 1985, 1990). These above observations can now be re-evaluated based on the current evidence demonstrating that (i) streptozotocin generates NO in aqueous solutions (Turk et al., 1993; Kwon et al., 1994) and (ii) scavenging NO protects against streptozotocin-induced DNA strand breakage (Kröncke et al., 1995). Coupling these previous data with the recent evidence showing that peroxynitrite is the potent trigger of DNA strand breakage (see above) it is logical to propose that PARS activation due to peroxynitrite formation underlies the pathogenesis of islet cell damage in response to streptozotocin or NO donor compounds. In this respect it is also noteworthy that heat shock and heat shock proteins also protects against peroxynitrite-induced cytotoxicity as well as against streptozotocin-induced islet cell injury (Bellmann et al., 1995, Szabó et al., 1996c; Bellmann et al., 1996).

The studies into the role of PARS in diabetes gained new momentum by a recent line of investigations demonstrating showing increased poly(ADP-ribosylation) in islet cells exposed to oxidants and NO donors, and protection against the associated cellular injury by inhibition of PARS (Radons et al., 1994; Inada et al., 1995; Heller et al., 1995; Eizirik et al., 1996). Most notably, Heller et al. (1995) demonstrated that islet cells of the $PARS^{-/-}$ knockout mouse are resistant against injury in response to NO generator and oxyradical generator compounds. Interestingly, the protection waned when islets are challenged with extremely high concentrations of the oxidants (Heller et al., 1995), demonstrating that at very high levels of oxidant stress, the PARS related pathway of cellular injury may be replaced by PARS-independent cytotoxic mechanism.

Although the evidence is solid regarding the effectiveness of PARS inhibitors against oxidant-induced islet cell injury, the relevance of these findings in relation to autoimmune diabetes is unclear. The relevance of the chemically induced diabetes models has frequently been questioned, and it appears that experimental models of spontaneous autoimmune diabetes (such as the nonobese diabetic mice are more relevant to the pathophysiology of the human disease. In this latter experimental model, the development of the disease is governed by the intraislet production of pro-inflammatory cytokines. These cytokines, in turn, induce the expression of the inducible isoform of NO synthase in the pancreatic islet cells of autoimmune diabetes-prone nonobese diabetic mice, with consequent overproduction of NO (Corbett et al., 1993; Suarez-Pinzon et al., 1994; Rabinovitch et al., 1996). In a recent set of studies, we have recently shown the massive presence of nitrotyrosine in pancreatic islet cells of the

diabetes-prone nonobese diabetic mice (Suarez-Pinzon et al., 1997). The potential role of PARS activation in the autoimmune model of diabetes in vivo requires further investigations.

2.3. Arthritis

The production and role of oxygen-derived free radicals and oxidants is well established in the pathophysiology of arthritis (Greenwald, 1991; Oyanagui, 1994; Santos and Tipping, 1994; Kaur et al., 1996). Furthermore, several lines of evidence suggest a role for NO overproduction in the pathogenesis of arthritis. The expression of the inducible isoform of NO synthase and the production of large amounts of NO have been demonstrated in chondrocytes from experimental animals and humans (Hauselmann et al., 1994; Sakurai et al., 1995; Murrell et al., 1995; Grabowski et al., 1996; Hayashi et al., 1997). An increase in the circulating levels of nitrite/nitrate (the breakdown products of NO) has been demonstrated in patients with arthritis (Farrell et al., 1992; Stichtenoth et al., 1995). Increased plasma and synovial fluid levels of nitrotyrosine, a marker of peroxynitrite formation have been demonstrated in patients with arthritis (Kaur and Halliwell, 1994). Similarly, increased nitrotyrosine formation was observed in the joints of mice suffering from collagen-induced arthritis (Szabó et al., 1998b).

The development of the disease has been shown to be ameliorated by various, non-isoform-selective inhibitors of NO synthase in various animal models of adjuvant-induced arthritis (Ialenti et al., 1993; McCartney-Francis et al., 1993; Stefanovic-Racic et al., 1993, 1994, 1995; Evans et al., 1995; Weinberg et al., 1994; Connor et al., 1995). In a recent study, mercaptoethylguanidine, an antiinflammatory agent with a combined mechanism of action (inhibition of the inducible isoform of NO synthase, scavenging pathophysiological and inhibition of cyclooxygenase provided marked beneficial effects in a collagen-induced arthritis (Brahn et al., 1998).

There are now direct experimental data implicating the role of PARS activation in the pathophysiology of arthritis. In murine models of arthritis, inhibition of PARS with nicotinamide, or with nicotinic acid amide, reduced the onset of the disease (Miesel et al., 1995; Ehrlich et al., 1995; Kroger et al., 1996). The onset, progression, and remission of arthritis positively correlated with the phorbol ester-activated respiratory burst of neutrophils and monocytes (Miesel et al., 1995). Inhibition of PARS not only prevented the development of arthritis, but also inhibited the progress of established collagen induced arthritis (Kroger et al., 1996; Szabó et al., 1998b). The combined application of thalidomide (as a drug that inhibits tumor necrosis factor-α expression in arthritis) and nicotinic acid amide provided a powerful synergistic inhibition of arthritis. (Kroger et al., 1996). Furthermore, recent studies with 5-iodo-6-amino-1,2-benzopyrone, a novel PARS inhibitor which lacks oxyradical scavenging properties also protected in a mouse model of collagen-induced arthritis: the PARS inhibitor reduces both the incidence of arthritis and the severity of the disease throughout the experimental period (Szabó et al., 1998b). Histological evaluation of the paws in the vehicle-treated arthritic animals revealed signs of severe suppurative arthritis, with massive mixed (neutrophil, macrophage and lymphocyte) infiltration. In the animals treated with the PARS inhibitors, the degree of arthritis was significantly reduced: a moderate, primarily neutrophil infiltration into several of the larger joints, coupled with mild to moderate necrosis and hyperplasia of the synovium (Szabó et al., 1998b). As in the other forms of inflammation, hydroxyl radical and peroxynitrite are the most likely triggers of PARS activation.

2.4. Inflammatory bowel disease

It is well established that inflammatory bowel disease is associated with the production of oxygen-derived free radicals and oxidants (Allgayer, 1991; Babbs, 1992; Keshavarzian et al., 1992; Chamulitrat and Spitzer, 1997). Increased NO production from the inducible NO synthase has also been proposed to be responsible for various experimental models of inflammatory bowel disease (Yamada et al., 1993; Miller et al., 1993; Aiko and Grisham, 1995; Ribbons et al., 1995; Rachmilewitz et al., 1995; Hogaboam et al., 1995; Mourelle et al., 1996; Kiss et al., 1997), and ulcerative colitis in humans, where inducible NO synthase activity and elevated levels of luminal nitrite have been detected in rectal dialysates and in biopsy specimens (Middleton et al., 1993; Boughton-Smith et al., 1993; Lundberg et al., 1994; Ikeda et al., 1997). During inflammatory bowel disease, the simultaneous production of superoxide and NO is likely to produce peroxynitrite and to promote oxidative reactions. Biochemical evidence for the formation of peroxynitrite has been provided in an experimental model of ileitis in guinea pigs by immunohistochemical staining of nitrotyrosine in epithelial cells (Miller et al., 1995). Similarly, in human samples of active Crohn's lesions, massive nitrotyrosine staining has been reported (Singer et al., 1996). The role of peroxynitrite in the pathogenesis of is further supported by the fact that intracolonic administration of exogenous peroxynitrite produces a severe mucosal damage in rats (Rachmilewitz et al., 1993). Experimental studies have showed that the inflammatory response can be reduced by administration of NO synthase inhibitors, such as N^{G}-nitro-L-arginine methyl ester and aminoguanidine (Yamada et al., 1993; Miller et al., 1993; Aiko and Grisham, 1995; Ribbons et al., 1995; Rachmilewitz et al., 1995; Hogaboam et al., 1995; Mourelle et al., 1996; Kiss et al., 1997). Recent preliminary studies in rodent models of experimental colitis support the role of PARS activation in the pathogenesis of the disease (Salzman et al., 1997). Intraluminal administration of the hapten trinitrobenzene sulfonic

acid in 50% ethanol induced mucosal erosion and ulceration associated with increased neutrophil infiltration, lipid peroxidation, an intense staining for nitrotyrosine, and progressive weight loss. Genetic ablation of the PARS gene or pharmacological inhibition of PARS with 3-aminobenzamide resulted significant resistance to the damage induced by trinitrobenzene sulfonic acid administration, reduced nitrotyrosine formation and tissue levels of malondialdehyde, and reduced the neutrophil recruitment into the injured tissue (Salzman et al., 1997). These in vivo data are in good agreement with recent in vitro studies demonstrating protection by pharmacological inhibition of PARS against intestinal epithelial cell injury induced by hydrogen peroxide (Watson et al., 1995) or peroxynitrite (Kennedy et al., 1998).

2.5. Inflammatory diseases of the central nervous system

2.5.1. Allergic encephalomyelitis—multiple sclerosis

Increased oxygen-derived free radical production and oxidative injury has been reported in central nervous system tissues from animals subjected to experimental allergic encephalomyelitis (Guy et al., 1989, 1994; Brett and Rumsby, 1994; Ruuls et al., 1995; Malfroy et al., 1997), and oxidative injury has been implicated in the pathogenesis of chronic central nervous system (CNS) inflammatory disorders such as multiple sclerosis. The production of NO by invading macrophages and/or CNS resident cells of the macrophage/monocyte lineage has also been implicated in the pathogenesis of chronic diseases of the CNS, such as multiple sclerosis (Lin et al., 1993; Koprowski et al., 1993; Cross et al., 1994, 1997; Akaike et al., 1995; Bagasra et al., 1995; Hooper et al., 1995). The overproduction of NO in inflammatory diseases of the CNS is due to the expression of the inducible NO synthase, which is strongly upregulated in experimental allergic encephalomyelitis and immune-mediated viral diseases of the CNS. Similarly, in brain tissue from multiple sclerosis patients where, unlike brain tissue from controls without neurological disease, cells expressing inducible NO synthase-specific mRNA have been reported (Koprowski et al., 1993; Bagasra et al., 1995). The overproduction of NO and oxyradicals in experimental allergic encephalomyelitis leads to the generation of peroxynitrite. Accordingly, increased nitrotyrosine staining has been reported in humans with multiple sclerosis, as well as in the active experimental allergic encephalomyelitis lesions (Koprowski et al., 1993; Cross et al., 1997; Van der Veen et al., 1997). Furthermore, the putative peroxynitrite scavenger has been shown to improve the outcome of experimental allergic encephalomyelitis in mice (Hooper et al., 1997, 1998).

Recent data directly implicate the role of the peroxynitrite–PARS axis in the pathogenesis of experimental allergic encephalomyelitis. In a rat model of experimental allergic encephalomyelitis in male Lewis rats, 3-aminobenzamide and the novel, potent PARS inhibitor 5-iodo-6-amino-1,2-benzopyrone delayed the course of the disease (Scott et al., 1988). PARS inhibition resulted in both a delay in the onset as well as a reduction in the incidence and severity of disease signs. Increased poly-ADP-ribose immunoreactivity was associated with the development of the brain lesions in vehicle-treated rats, while 5-iodo-6-amino-1,2-benzopyrone eliminated the development of the lesions and abolished poly(ADP-ribose)immunoreactivity (Scott et al., 1988). The mechanism by which inhibition of PARS suppresses the course of experimental allergic encephalomyelitis has not been clarified. In fact, even the exact pathogenesis of experimental allergic encephalomyelitis is unclear at present. Undoubtedly, one of the major features of multiple sclerosis and experimental allergic encephalomyelitis is demyelination. Experimental allergic encephalomyelitis (and presumably multiple sclerosis is triggered and amplified by a variety of interrelated immunological events. Immunological, clinical and pathological studies suggest that T lymphocytes directed against myelin antigens are involved in the pathogenesis of multiple sclerosis. It is now clear that myelin basic protein or proteolipidprotein-specific T cells mediate the destruction of CNS myelin in experimental allergic encephalomyelitis. While the autoimmune disease is initiated by antigen-specific autoreactive T cells, there is accumulating evidence that CNS injury is essentially mediated by CNS-infiltrating inflammatory cells, and inhibition of cell infiltration can suppress the course of experimental allergic encephalomyelitis (Kent et al., 1995; Eng et al., 1996; Miyagishi et al., 1997). In addition, it is established that activated inflammatory mononuclear cells contribute to tissue damage in several inflammatory diseases by releasing highly reactive oxygen metabolites (for example, Malfroy et al., 1997), and nitrogen metabolites (see above), and subsequent activation of matrix metalloproteinases (Gijbels et al., 1994). It is therefore possible that demyelination associated with experimental allergic encephalomyelitis/multiple sclerosis results from oxidative injury caused by a cascade of reactive oxygen and nitrogen metabolites produced by CNS-infiltrating activated macrophages and other inflammatory cells. The infiltration of mononuclear cells into the CNS is a process which is closely linked to the breakdown of the blood–brain barrier, a process related to the production of oxidants and free radicals in experimental allergic encephalomyelitis (Paul and Bolton, 1995; Karlik et al., 1996). Once mononuclear cells infiltrate the CNS, and myelin degradation begins, a variety of positive feedforward cycles initiate. For instance, phagocytosis of opsonized myelin can trigger the induction of the inducible NO synthase in macrophages, which can, in turn, further enhance the process of demyelination during multiple sclerosis or experimental allergic encephalomyelitis (Van der Laan et al., 1996). Induction of the inducible NO synthase and induction of pro-inflammatory cytokines may enhance each other during experimental allergic encephalomyelitis. This is supported by the

finding that aminoguanidine, an inhibitor of the inducible NO synthase, reduced the expression of tumor necrosis factor-α, in experimental allergic encephalomyelitis (Brenner et al., 1997).

Although the exact cell types involved have not yet been identified, recent data indicate that activation of NMDA receptors also plays an important role in the pathogenesis of experimental allergic encephalomyelitis. In fact, antagonists of these receptors has been shown to suppress the course of disease (Wallstrom et al., 1996; Bolton and Paul, 1997). Possibly this process is related to the decreased metabolism of glutamate in astrocytes during experimental allergic encephalomyelitis (Hardin-Pouzet et al., 1997). Considering the abundant evidence for a role of PARS activation in the pathogenesis of NMDA-mediated neuroinjury (Zhang et al., 1994; Eliasson et al., 1997; Endres et al., 1997), the above studies lend further support to our working hypothesis that PARS activation plays a role in experimental allergic encephalomyelitis, and inhibition of PARS is of beneficial effects.

Currently, the cellular and molecular targets where inhibition of PARS would interrupt the inflammatory cascade leading to demyelination in experimental allergic encephalomyelitis, are unclear. Nevertheless, several possibilities can be considered by which PARS inhibition prevents myelin degradation in experimental allergic encephalomyelitis, such as (i) protection against oligodendrocyte death, and improved myelin synthesis (ii) protection against astrocyte death; (iii) protection against the breakdown of the blood–brain barrier and the related (iv) inhibition of mononuclear cell infiltration into the CNS; (v) inhibition of the expression of the inducible NO synthase (see below) during experimental allergic encephalomyelitis and (vi) inhibition of NMDA-activation related cell injury. With respect to oligodendrocyte death, there are direct in vitro data to show that oligodendrocytes and astrocytes are susceptible to NO-induced mitochondrial damage, and also oligodendrocytes are extremely sensitive to NO-induced single stranded DNA breaks (Mitrovic et al., 1994). Thus, it is conceivable the oligodendrocytes and astrocytes would be injured in a PARS-dependent fashion during the course of experimental allergic encephalomyelitis. Furthermore, and similar to the inflammatory responses of peripheral organs, where inhibition or genetic inactivation of PARS suppresses inflammatory cell recruitment (Szabó et al., 1997b), it can be expected that inhibition of PARS would have similar actions in allergic encephalomyelitis as well.

2.5.2. Parkinson's disease

Parkinson's disease, a chronic progressive neurologic disorder, is related to the degeneration of the neuromelanin-containing neurons predominantly located in the pars compacta of the substantia nigra (Jenner et al., 1992). The synthetic heroin analogue, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, can selectively damage neurons in the nigrostriatal dopaminergic pathway and produce Parkinsonism in experimental animals (Kopin and Markey, 1988). There is good evidence for both the production of oxygen-derived free radicals and oxidants (Fahn and Cohen, 1992; Ebadi et al., 1996; Smith and Bennett, 1997; Lan and Jiang, 1997), and for the overproduction of NO and peroxynitrite (Youdim et al., 1994; Schulz et al., 1997; Bolanos et al., 1997) in the pathogenesis of: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. In contrast to experimental allergic encephalomyelitis, where the inducible NO synthase is the source of NO production, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neuroinjury, the neuronal NO synthase is the source of cytotoxic NO and peroxynitrite. Accordingly, the neuronal NO synthase inhibitor 7-nitro-indazole, is neuroprotective against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. The inhibitor dramatically protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced reductions in striatal dopamine content, decreases in numbers of nigral tyrosine hydroxylase-positive neurons and the numbers of silver-stained degenerating nigral neurons (Schulz et al., 1995; Przedborski et al., 1996). Furthermore, genetically engineered mice which lack the neuronal NO synthase gene are significantly more resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced neurotoxicity when compared with wild-type littermate controls (Przedborski et al., 1996).

There are now direct data demonstrating the role of the PARS pathway in the pathogenesis of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced neurotoxicity. In a mouse model of Parkinson's disease, where 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment reduced striatal dopamine and cortical noradrenaline levels by more than 50%, co-treatment with five different inhibitors of PARS ameliorated the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced catecholamine depletion. The protective activities of benzamide and its derivatives paralleled their in vitro efficacies and potencies both as neuroprotective agents and as inhibitors of PARP, while the activity of 1,5-dihydroxyisoquinoline, a structurally unrelated PARS inhibitor was without significant protective effect (Cosi et al., 1996). Further studies, with PARS inhibitors lacking oxyradical scavenging actions, or experiments using PARS$^{-/-}$ animals challenged with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine are needed to clarify the role of PARS in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity.

2.5.3. Other inflammatory diseases of the CNS

In addition to multiple sclerosis and Parkinson's disease, there are a number of other CNS inflammatory conditions associated with overproduction of NO and oxyradicals. For example, a markedly increased expression of human inducible NO synthase has been demonstrated in the brain in Alzheimer's disease (Dorheim et al., 1994) and acquired immunodeficiency syndrome (Bukrinsky et al., 1995). Production of NO and peroxynitrite have also

been implicated in the process of neurodegeneration associated with Huntington's disease (Galpern et al., 1996; Li et al., 1996). In acquired immunodeficiency syndrome-related neurodegeneration, the expression of the inducible NO synthase as well as the release of pro-inflammatory cytokines from macrophages have been implicated (Bukrinsky et al., 1995). These cytokines, released by macrophages or by the neuronal cells themselves, may then induce the expression of the inducible NO synthase in various cells including glial cells (Lipton et al., 1994; Koka et al., 1995). In addition, it has been directly demonstrated that gp120 is able to kill cortical neurons cultured in vitro, and the cytotoxicity is reduced by NO synthase inhibitors (Dawson et al., 1993). The mechanism of this process is very similar to the neuroinjury induced by NMDA receptor agonists, and so it is likely related to peroxynitrite, toxicity, and, possibly, PARS. However, there are no direct reports available to directly implicate PARS in these processes.

2.6. Systemic inflammatory response syndrome—circulatory shock

2.6.1. Role of PARS in the vascular alterations in shock

Various forms of circulatory shock is associated with the enhanced formation of oxyradicals (Parks et al., 1983; Deitch et al., 1990; French et al., 1994) and with the expression of a distinct inducible isoform of NOS, resulting in overproduction of NO (Szabó and Thiemermann, 1994; Szabó, 1995). NO and superoxide reacts to form peroxynitrite, which can be demonstrated in various organs and tissues (Szabó et al., 1995a,b; Wizemann et al., 1994; Zingarelli et al., 1997b). In isolated cells and tissues, authentic peroxynitrite is capable of mimicking many the pathophysiological alterations associated with shock (endothelial and epithelial dysfunction, vascular hyporeactivity, and cellular dysfunction), and these alterations are, in part, related to PARS activation (Szabó et al., 1996a, 1997a,c; Kennedy et al., 1998).

The vascular contractile failure associated with circulatory shock is closely related to overproduction of NO within the blood vessels. Expression of the inducible NO synthase within the vascular smooth muscle cells has been implicated in the pathogenesis of vascular hyporeactivity during various forms of shock (see: Rees, 1995; Szabó, 1995). Recent studies have demonstrated that a superoxide dismutase mimetic also offers a significant protection against the suppression of the vascular contractility of the thoracic aorta in a rat model of endotoxic shock, suggesting that the vascular hyporeactivity may be related to peroxynitrite generation, rather than NO per se (Zingarelli et al., 1997a). What, then, is the mechanism of the peroxynitrite-induced vascular hyporeactivity in endotoxic shock? In studies in anesthetized rats, inhibition of PARS with 3-aminobenzamide and nicotinamide were able to reduce the suppression of the vascular contractility of the thoracic aorta in ex vivo experiments (Szabó et al., 1996a; Zingarelli et al., 1996b). Similarly, in a murine model of cecal ligation and puncture, inhibition of PARS with 3-aminobenzamide improves microvascular contractility (Osman et al., 1998).

Peroxynitrite production has been suggested to contribute to endothelial injury in ischemia-reperfusion, circulatory shock and atherosclerosis (White et al., 1994; Zingarelli et al., 1997a; White et al., 1996). Peroxynitrite can impair the endothelium-dependent relaxations (Villa et al., 1994; Szabó et al., 1997a). Current data, demonstrating protective effects of 3-aminobenzamide against the development of endothelial dysfunction in vascular rings obtained from rats with endotoxic shock (Szabó et al., 1997a) suggest that DNA strand breakage and PARS activation occur in endothelial cells during shock and that the subsequent energetic failure reduces the ability of the cells to generate NO in response to acetylcholine-induced activation of the muscarinic receptors on the endothelial membrane. Indeed, several lines of in vitro data demonstrate DNA injury, PARS activation and consequent cytotoxicity in endothelial cells exposed to hydroxyl radical generators (Spragg, 1991; Thies and Autor, 1991; Junod et al., 1989), or in response to peroxynitrite (Szabó et al., 1997c). The relative contribution of peroxynitrite vs. hydroxyl radical in the PARS activation and endothelial injury in shock remains to be further investigated, since both species are known to be produced in shock, and the available scavengers (such as superoxide dismutase analogs) would be expected to reduce the production of both peroxynitrite and hydroxyl radical.

2.6.2. Role of PARS in the energetic alterations and lethality in shock

There is now good evidence suggesting that NO (or a related species, such as peroxynitrite) plays a role in the cellular energetic changes and the related organ dysfunction associated with endotoxic shock. This conclusion is chiefly based on the results of pharmacological studies, in which inhibition of NO synthesis, especially by agents that are selective towards the inducible isoform of NO synthase, reduce cellular injury and improve organ function in shock (Southan and Szabó, 1996). It is noteworthy that in the same experimental models of rodent endotoxic shock, the cell-permeable superoxide dismutase analog MnIII tetrakis (4-benzoic acid) porphyrin (Szabó et al., 1996d) also reduced the endotoxin-induced depression of mitochondrial respiration in peritoneal macrophages ex vivo (Zingarelli et al., 1997a), thereby suggesting that peroxynitrite, rather than NO per se plays a role in these alterations. Peroxynitrite-induced activation of the PARS pathway has also been implicated in the pathophysiology of the cellular energetic failure associated with endotoxin shock by demonstration of increased DNA strand breakage, decreased intracellular NAD^+ and ATP levels and mitochondrial respiration in peritoneal macrophages obtained from

rats subjected to endotoxin shock (Zingarelli et al., 1996a,b). This cellular energetic failure was reduced by pretreatment of the animals with the PARS inhibitors 3-aminobenzamide or nicotinamide (Zingarelli et al., 1996a,b).

In contrast to these encouraging results in peritoneal macrophages, it appears that the PARS pathway only plays a limited role in the liver dysfunction associated with endotoxin shock. In an endotoxic shock model in the rat, inhibition of PARS with 3-aminobenzamide and nicotinamide did not affect the alterations in most parameters of liver injury, whereas inhibition of PARS with 1,5-dihydroxyisoquinoline resulted in a marginal protective effect (Thiemermann, personal communication, 1997). These observations are perhaps not surprising when considering the fact that in in vitro studies, the oxidant-induced injury in cultured hepatocytes is not prevented by pharmacological inhibition of PARS (Yamamoto et al., 1993). The exact reason why inhibition of PARS does not affect the course of the oxidant injury in hepatocytes remains to be further investigated.

Pharmacological inhibition of PARS, either with 3-aminobenzamide (Szabó et al., 1996a) or with the potent, novel potent PARS inhibitor (Bauer et al., 1996) 5-iodo-6-amino-1,2,-benzopyrone (Szabó et al., 1997d) improves survival rate in mice challenged with high dose endotoxin. Based on these observations, one may suggest that, in response to pharmacological inhibition of PARS, the improved hemodynamic status due to improved vascular function, and possibly, the improved cellular energetic status in some organs, results in an overall survival benefit in this condition.

3. Additional roles of PARS in the regulation of the inflammatory response

In addition to the PARS-related energetic depletion and suicidal cycle, PARS may have another important functions in modulating the inflammatory response. Although highly controversial, PARS (or its cleavage) may have a role in the process of apoptosis. In addition, PARS appears to be directly regulating the expression of a variety of genes, some of them directly related to various inflammatory conditions.

3.1. Role of PARS in the process of apoptosis

Several reports demonstrated that peroxynitrite cause apoptosis in a variety of cell types (Salgo et al., 1995b; Bonfoco et al., 1995; Lin et al., 1995; Estevez et al., 1995). It appears that sustained exposure or low levels of peroxynitrite cause apoptosis whereas sudden exposure to high concentrations of peroxynitrite induce cell necrosis. However, the peroxynitrite-induced apoptosis, in all cell types studied so far, cannot be attenuated by pharmacological inhibitors of PARS, or $PARS^{-/-}$ phenotype (Leist et al., 1997; Wang et al., 1997; O'Connor et al., 1997).

It is important to emphasize that, although NO is one of the precursors of peroxynitrite, the chemical properties and the reactivity of these two species are quite different, and so are the modes of peroxynitrite and NO-induced apoptosis. With respect to cellular injury, NO can inhibit apoptosis, a process, which can occur, at least in part, via a thiol modification of caspases (Mannick et al., 1997; Mohr et al., 1997; Tzeng et al., 1997; Melino et al., 1997). Yet, large amounts of pure NO, can also cause apoptosis, via p53 generation, inhibition of mitochondrial respiration, and, perhaps by PARS cleavage (Messmer and Brune, 1996; Messmer et al., 1996). Under these conditions, no DNA strand breakage occurs, there are no changes in cellular NAD^+, and the NO-induced ATP depletion is not affected by inhibitors of PARS (Messmer and Brune, 1996; Szabó et al., 1996a; Kennedy et al., 1998).

Although a role for PARS in the development of apoptosis has previously been proposed in a variety of cell types (Monti et al., 1994, 1995; Nicholson et al., 1995), based on recent studies using PARS knockout cells, several groups concluded that PARS is dispensable for apoptosis (Leist et al., 1997; Wang et al., 1997). The role of PARS in the process of NO-induced apoptosis is also highly controversial. In human leukemia cells, 3-aminobenzamide and nicotinamide reduce NO-induced apoptosis (Kuo et al., 1996), whereas these inhibitors are ineffective in blocking apoptosis in RAW murine macrophages (Messmer and Brune, 1996). It is, nevertheless, clear, that the NO-induced apoptosis is mediated through mechanisms which are different from the apoptosis triggered by potent DNA single strand breaking agents, such as peroxynitrite or hydroxyl radical.

The NO-mediated apoptosis may be related to the cleavage of PARS, at least in some systems (Messmer et al., 1996). In immunostimulated cells the delayed proteolytic cleavage of PARS can be blocked by pharmacological inhibitors of NO synthase. Overexpression of the anti-apoptotic protein Bcl-2 in these cells has been shown to block the NO-induced apoptosis, and the NO-induced proteolytic cleavage of PARS (Messmer et al., 1996). Based on these results, the hypothesis has been put forward that PARS cleavage (with a consequent inhibition of the catalytic activity of PARS) is a process involved in endonuclease activation and apoptosis in NO-treated cells (Messmer et al., 1996). However, this proposal is somewhat in contrast with the finding that pharmacological inhibitors of PARS do not affect the course of immunostimulation-induced (and NO-dependent) apoptotic process in macrophages (Messmer and Brune, 1996; O'Connor et al., 1997). Clearly, the area of NO-related apoptosis and PARS cleavage is highly controversial, and no clear conclusions can be made as to whether pharmacological inhibition of PARS would affect the course of inflammation via modulation of a PARS-dependent apoptotic pathway.

3.2. Role of PARS in the regulation of gene expression

It appears that PARS plays an important role in the regulation of gene expression and cell differentiation (Nagao et al., 1991; Smulson et al., 1995). Under basal conditions, PARS is closely associated to DNA, with preference to regions of cruciform DNA, bent DNA, and in A–T rich regions (Sastry et al., 1989). PARS also appears to be more frequently associated with transcriptionally active regions of chromatin (Hough and Smulson, 1994; DeMurcia et al., 1988). Basal PARS activity has been proposed to regulate histone shuttling and nucleosomal unfolding (Althaus et al., 1994). In fact, a recent report proposes that PARS acts as a functional component of the positive cofactor 1 activity, its function being the enhancement activator-dependent transcription processes (Meisterernst et al., 1997).

Using pharmacological inhibitors of PARS, it has been demonstrated that the activity of PARS is required for the expression of the major histocompatibility complex class II gene (Hiromatsu et al., 1992; Taniguchi et al., 1993; Qu et al., 1994), *ras*, c-*myc* (Bauer et al., 1996; Nagao et al., 1991), DNA methyltransferase gene (Bauer et al., 1996), protein kinase C (Bauer et al., 1996) and collagenase (Ehrlich et al., 1995). Moreover, in several independent lines of investigations, it has been demonstrated that pharmacological inhibition of PARS (with nicotinamide, 3-aminobenzamide and 5-iodo-6-amino-1,2-benzopyrone) suppresses the expression of mRNA of the inducible NO synthase (Hauschildt et al., 1992; Pellat-Seceunyk et al., 1994; Zingarelli et al., 1996a; Szabó et al., 1997d). In studies using 5-iodo-6-amino-1,2-benzopyrone, inhibition of the expression of the inducible NO synthase in RAW macrophages was indicated by the inhibition of nitrite production, the expression of the inducible NO synthase mRNA protein (Szabó et al., 1997d). Similarly, the expression of the inducible NO synthase mRNA and protein, and the production of nitrite/nitrate was reduced in PARS$^{-/-}$ fibroblasts when compared to wild-type cells (Szabó et al., 1998b). However, the role of PARS in the regulation of the expression of the inducible NO synthase may be cell-type specific, as inhibition of PARS reduces the amounts of NO produced in cultured macrophages, but not in thioglycollate-elicited, bacterial lipopolysaccharide stimulated murine peritoneal macrophages (Szabó, Scott, O'Connor and Virág, unpublished observations). The regulation of the expression of the inducible NO synthase by PARS appears to be related to a specific part of the promoter of the inducible NO synthase: in transfection studies using the PARS inhibitor 5-iodo-6-amino-1,2-benzopyrone in murine RAW macrophages, it was found that this PARS inhibitor suppressed the transcription of the inducible NO synthase, when cells transfected with the full length (-1592 bp) promoter construct with the inhibitor. However, similar co-treatment of cells transfected with the -367 bp deletional construct did not significantly reduce the bacterial lipopolysaccharide-mediated increase in luciferase activity (Szabó et al., 1997d). The regulation by 5-iodo-6-amino-1,2-benzopyrone of the induction of the inducible NO synthase also occurred in whole animals challenged with bacterial lipopolysaccharide: pre-treatment, but not post-treatment of the animals with the inhibitor suppressed the bacterial lipopolysaccharide-induced increase in plasma nitrite/nitrate concentrations and reduced the bacterial lipopolysaccharide-induced increase in the expression of the inducible NO synthase in the lung (Szabó et al., 1997d). Although the mode of inhibition of the expression of the inducible NO synthase by inhibition of PARS (or the mode of promotion of the expression of the inducible NO synthase by poly-ADP-ribosylation) has not yet been fully explored, recent studies proposed that at a step involving polyADP-ribosylation is required to activate the process of NF κB-mediated gene transcription, and the consequent expression of the inducible NO synthase (Le Page et al., 1998).

From the above experimental data it appears that PARS, via a not yet characterized mechanism, regulates the expression of a variety of genes, including the inducible NO synthase, and intercellular adhesion molecule 1 and collagenase. Inhibition of the expression of these genes may represent an additional mode of beneficial action of PARS inhibition in various forms of inflammation. However, it appears that inhibition of the expression of the inducible NO synthase is not obligatory for the protective effect of PARS inhibitors in inflammation or reperfusion injury. In in vivo experiments, PARS inhibitors can have beneficial effects in concentrations which do not affect the expression of the inducible NO synthase (Zingarelli et al., 1996a,b; Szabó et al., 1997a), or even in conditions where the inducible NO synthase is not even expressed, such as the early phases of myocardial and splanchnic reperfusion (Cuzzocrea et al., 1997; Zingarelli et al., 1997c; Thiemermann et al., 1997). Nevertheless, it is possible that, during chronic administration, pharmacological inhibitors of PARS may suppress the expression of the inducible NO synthase, and thereby reduce the generation of NO and peroxynitrite.

4. Role of PARS in reperfusion injury

The subject of the current review is the role of PARS in inflammation. Nevertheless, we must mention that the production of hydroxyl radical, and peroxynitrite, triggers of PARS activation is also well established in the pathogenesis of ischemia/reperfusion injury. For example, the generation of peroxynitrite has been demonstrated or suggested in the reperfused heart (Matheis et al., 1992; Schulz and Wambolt, 1995; Naseem et al., 1995, Wang and Zweier, 1996; Liu et al., 1997; Zingarelli et al., 1997c), liver (Ma et al., 1995), kidney (Yu et al., 1994) intestine (Szabó et al., 1995b), brain (Dawson and Dawson, 1996; Keller et al., 1998; Forman et al., 1998) and lung

(Ischiropoulos et al., 1995). In these conditions, prevention of peroxynitrite generation by inhibition of NO biosynthesis markedly reduces reperfusion injury, as shown by reduced pulmonary lipid peroxidation in the lung (Ischiropoulos et al., 1995) or improved mechanical performance of the heart (Matheis et al., 1992; Schulz and Wambolt, 1995; Naseem et al., 1995, Wang and Zweier, 1996). Although it is likely that peroxynitrite and other oxidant species produced during the reperfusion phase initiate a multitude of interrelated cytotoxic processes, PARS activation represents a key pathway of cell injury: inhibition or pharmacological inactivation of PARS exerts protective effects in animal models of stroke (Endres et al., 1997; Eliasson et al., 1997; Takahashi et al., 1997), myocardial ischemia-reperfusion (Zingarelli et al., 1997c; Thiemermann et al., 1997); and splanchnic ischemia-reperfusion (Cuzzocrea et al., 1997; Szabó et al., 1998a). PARS inhibitors also improve survival in hemorrhagic shock, which can be considered a model of whole-body ischemia-reperfusion (Szabó, 1998).

5. Inhibition of PARS: a novel anti-inflammatory approach

Current strategies aimed at limiting NO-mediated cell/organ injury include agents which inhibit the induction of the inducible NO synthase, NO synthase enzyme inhibitors, preferably with selectivity for iNOS, agents that scavenge or inactivate NO, as well as agents that limit substrate or cofactor availability for the inducible NO synthase. Less attention has been directed to strategies that interfere with intracellular cytotoxic pathways initiated by NO or its toxic derivatives. Direct and indirect experimental evidence presented in this review supports the view that peroxynitrite-induced DNA strand breakage and PARS activation importantly contribute to the pathophysiology of various forms of inflammation. Although the enhanced formation of peroxynitrite has been demonstrated in a variety of pathophysiological conditions with inflammatory components not discussed in this review (e.g. transplant rejection, adult respiratory distress syndrome, atherosclerosis, carbon monoxide poisoning, etc.), the role of PARS in the associated cytotoxicity remains to be investigated.

We conclude that pharmacologic inactivation of PARS represents a novel, therapeutically viable strategy to limit cellular injury and improve the outcome of a variety of pathophysiological conditions associated with peroxynitrite production. The viability of this potential therapeutic strategy is strengthened by recent observations demonstrating that the absence of PARS does not compromise DNA repair (Wang et al., 1995). Furthermore, unlike many current anti-inflammatory approaches, PARS inhibition is unlikely to interfere with the antimicrobial defense systems, since invading bacteria do not contain PARS.

6. The physiological role of PARS: is there a price to pay?

It is surprising that PARS, an enzyme which can exert marked suicidal effects, did not become deleted during the evolution process, and suggests that PARS has important physiological roles, possibly in the maintenance of genomic stability (Wang et al., 1997). It is however, uncertain what side-effects long-term pharmacological inhibition of PARS would induce. In fact, the physiological function of PARS has been the subject of debate for at least two decades, the 'classical' proposition being that PARS is a DNA repair enzyme. According to the more recent evidence, PARS is not effective as a DNA repair enzyme, since cells from a PARS knock-out mice have normal DNA repair characteristics (Wang et al., 1995). PARS does not appear to be obligatory for normal development: PARS knockout animals are normal and viable (Wang et al., 1995; De Murcia et al., 1997). Although the lack of PARS does not compromise DNA repair induced by UV or the alkylating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (Wang et al., 1995), PARS$^{-/-}$ mice were found more sensitive to the methylating agent *N*-methyl-*N*-nitrosourea and to γ-irradiation than the wild-type littermates (De Murcia et al., 1997). The reason for the different outcome of the above two studies is unclear. It is uncertain whether or not radiation sensitization will be a potential side effect of pharmacological PARS inhibition. Cells lacking PARS have been shown to have an elevated level of spontaneous sister chromatid exchanges and chromosome aberrations (Wang et al., 1995; De Murcia et al., 1997). Clearly, further studies are required to understand the physiological roles of PARS and the potential side effects of short-term or long-term pharmacological inhibition of PARS. Currently, a range of PARS inhibitors are in various stages of development, for stroke (Takahashi et al., 1997) or for anti-viral and anti-cancer indications (Cole et al., 1991; Bauer et al., 1995, 1996). There are no published data available on the side-effects or toxicity of these agents. High-dose nicotinamide, as an inhibitor of PARS, is currently being tested in clinical trials for the treatment of diabetes mellitus, with encouraging preliminary results, and no apparent long-term toxicity (Gale, 1996; Elliott et al., 1996; Pozzilli et al., 1997).

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