ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 9 & 10, 2005

Forum Review

Oxidative and Antioxidative Potential of Brain Microglial Cells

RALF DRINGEN

ABSTRACT

Microglial cells are the resident immune cells of the central nervous system. These cells defend the central nervous system against invading microorganisms and clear the debris from damaged cells. Upon activation, microglial cells produce a large number of neuroactive substances that include cytokines, proteases, and prostanoids. In addition, activated microglial cells release radicals, such as superoxide and nitric oxide, that are products of the enzymes NADPH oxidase and inducible nitric oxide synthase, respectively. Microglia-derived radicals, as well as their reactive reaction products hydrogen peroxide and peroxynitrite, have the potential to harm cells and have been implicated in contributing to oxidative damage and neuronal cell death in neurological diseases. For self-protection against oxidative damage, microglial cells are equiped with efficient antioxidative defense mechanisms. These cells contain glutathione in high concentrations, substantial activities of the antioxidative enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, as well as NADPH-regenerating enzymes. Their good antioxidative potential protects microglial cells against oxidative damage that could impair important functions of these cells in defense and repair of the brain. *Antioxid. Redox Signal.* 7, 1223–1233.

INTRODUCTION

THE BRAIN is a complex organ that contains besides neurons a variety of glial cell types. Among these, astrocytes and oligodendrocytes, the so-called macroglial cells, are well known for their functions in neuronal support and myelin formation (8, 63, 85). In addition, the brain contains microglial cells in substantial numbers. Microglial cells are the major neuroprotective immunocompetent cell type in the CNS (106). This cell type is often considered as the "macrophage of the brain." The origin of microglial cells is still a matter of intensive debate. Fetal macrophages, as well as cycling monocytes, have been discussed as precursors of the microglial cells in the mature brain (7, 44, 107). In addition to resident microglial cells, perivascular macrophages are present in low number as immunocompentent cells in normal brain and hematogenous macrophages infiltrate the brain in most pathological conditions (44, 105). The phenotypic differentiation and clear identification of the different immunocompetent cells in brain are of high importance, and large numbers of membranous, biochemical, and morphological markers have been used to discriminate between resident microglial cells and macrophages in brain (44).

Microglial cells have important functions especially after brain injury and during inflammation. In the healthy brain, microglial cells are quiescent and have a ramified morphology (107). However, they respond rapidly to disturbances in their environment with a program of protective activities that are part of the innate defense mechanisms of the brain and assist in specific immune functions. Thus, in any pathological condition of the brain, microglial cells become activated and change their morphology from the resting ramified to the reactive amoeboid one (107). In this activated state, the cells are able to produce and release a large number of neuroactive substances that include cytokines, prostanoids, and proteases, as well as radicals such as nitric oxide (NO) and superoxide (49, 62, 78, 82, 83). Microglial activation aims at the protection of the CNS, because microglia-derived compounds

Faculty 2 (Biology/Chemistry), University of Bremen, Bremen, Germany; and Department of Psychology, Monash University, Clayton, Victoria, Australia.

contribute to the defense of the brain against pathogens and are important for the stimulation of repair processes after brain damage. However, inappropriate activation may lead to cell degeneration. During recent years, the importance of microglial cells in acute and chronic pathological conditions in the brain was intensively studied. Microglial cells have been considered the enemy within the brain that is harmful due to the generation of inflammatory cytokines and toxic reactive oxygen species (ROS). The various aspects of microglial functions and dysfunctions for physiological and pathological situations in brain have recently been extensively reviewed (73, 81, 101, 106). The present article will focus on the oxidative potential of microglial cells, as well as the antioxidative mechanisms that contribute to the self-defense against the ROS that are produced by these cells.

OXIDATIVE STRESS AND THE GENERATION AND DISPOSAL OF ROS

In mammalian cells, peroxides and oxygen radicals are continuously generated during aerobic metabolism (47). For example, superoxide is generated as a by-product of the respiratory chain or as a product of enzymes such as xanthine oxidase. Superoxide disproportionates quickly to oxygen and hydrogen peroxide (H_2O_2). In addition, various cellular oxidases, such as monoamine and amino acid oxidases, generate H_2O_2. In the presence of redox-active iron, highly reactive and toxic hydroxyl radicals are generated from H_2O_2 and superoxide in the Fenton reaction and the Haber–Weiss cycle. To avoid ROS-mediated cellular damage, such as lipid peroxidation, protein inactivation, and DNA strand breaks, antioxidative mechanisms are present in cells that remove ROS or even prevent their generation. Low-molecular-weight antioxidants like glutathione (GSH), ascorbate, and α-tocopherol contribute to the antioxidative potential of cells. In addition, antioxidative enzymes such as superoxide dismutases (SODs), catalase, glutathione peroxidases (GPx), and glutathione reductase (GR) are important for the cellular defense against ROS (Fig. 1). An imbalance in the intracellular generation and disposal of ROS that leads to oxidative damage by an elevated intracellular concentration of ROS is described as oxidative stress. Cellular damage by oxidative stress is considered to be an important component in the progression of many diseases (47).

The toxic potential of ROS is used in the defense of the body against invading microorganisms. Contact with pathogens activates macrophages in the periphery or resting microglial cells in brain. As a consequence, these cells generate radicals such as superoxide and NO that are the products of the reactions catalyzed by the enzymes NADPH oxidase (Nox) and nitric oxide synthase (NOS), respectively (12, 80, 116). Superoxide and NO support the defense of the organism against invading pathogens, but have also the potential to harm the ROS-producing cell, as well as neighboring cells. As the ROS-producing cells are likely to encounter high concentrations of the generated superoxide and NO, as well as their reactive reaction products H_2O_2 and peroxynitrite, efficient antioxidative mechanisms are required in these cells to prevent unwanted oxidative damage.

The first line of cellular defense against radicals is the low-molecular-weight antioxidants GSH, ascorbate, and α-tocopherol. These compounds react easily with radicals such as superoxide and NO. Most of the superoxide that is generated extracellularly by Nox (12) or as a side product of the respiratory chain (69) is converted to oxygen and H_2O_2. Mitochondrial superoxide is a substrate of the manganese-containing SOD (MnSOD), whereas cytosolic and extracellular superoxide are substrates of SODs that contain copper and zinc (Cu/ZnSODs) and are localized in the cytosol or are excreted from cells (36, 89). In addition to the SOD-catalyzed reaction, superoxide spontaneously disproportionates to oxygen and H_2O_2 (36), reacts with GSH (117), or reacts with NO to form peroxynitrite (4, 104).

H_2O_2 generated as a product of SODs is substrate of the heme-containing catalase that converts H_2O_2 to oxygen and water. In addition, GPx use GSH to reduce H_2O_2 to water (Fig. 1). In this reaction, GSH is oxidized to glutathione disulfide (GSSG). In cells, GSH is regenerated from GSSG in the reaction that is catalyzed by the flavoenzyme GR. This enzyme needs NADPH as cosubstrate (Fig. 1). Thus, the cycling of GSH in the reactions catalyzed by GPx and GR depends on the availability of reduction equivalents in the form of NADPH.

The radical NO has been implicated in numerous biological functions in the immune system, the cardiovascular sys-

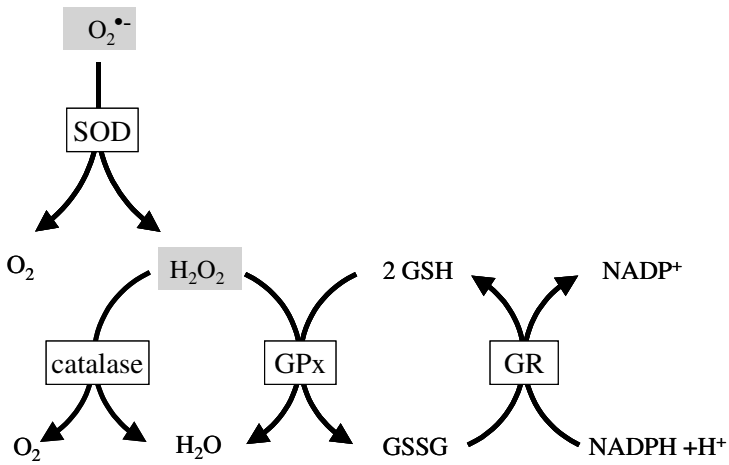

FIG. 1. Generation and disposal of ROS during cellular metabolism. Superoxide ($O_2\cdot^-$), which is a product of enzymatic reactions and by-product of the mitochondrial respiratory chain, is disproportionated by SODs to oxygen and H_2O_2. This peroxide is disposed of by catalase and/or GPx. GSH serves as electron donor for the reactions catalyzed by GPx and is oxidized to GSSG. The GSH consumed in the GPx reaction is regenerated by GR in a reaction that requires NADPH as cosubstrate.

tem, and also the CNS (5, 13, 80, 116). NO is synthesized by NOS (80, 116). All isoforms of NOS use arginine and molecular oxygen as substrates to generate NO and citrulline in a five-electron transfer reaction. NADPH serves as electron donor in NOS-catalyzed reactions. The constitutive NOS-1 and NOS-3 produce NO only for short periods after activation by a rise in intracellular Ca^{2+} concentration. In contrast, the induction of the expression of NOS-2 [inducible NOS (iNOS)] causes a long-lasting generation of high amounts of NO (116). NO reacts easily with other radicals. For example, NO and superoxide form peroxynitrite in a diffusion-limited reaction. GSH appears to be involved in the detoxification of NO and peroxynitrite, because this tripeptide reacts with NO to form nitrosoglutathione (20) and is discussed to serve as electron donor in the GPx-catalyzed reduction of peroxynitrite (4). In addition to its oxidative potential, NO has also been discussed as a powerful antioxidant that, even at the low physiological concentrations that occur in brain, could be able to prevent oxidation of cellular compounds by Fenton chemistry (102).

GENERATION OF ROS BY ACTIVATED MICROGLIAL CELLS

Different treatments and signaling pathways lead to superoxide or NO production in microglial cells. Phorbol esters or opsonized zymosan are frequently used to activate superoxide production by Nox in cultured microglial cells (21, 99), whereas microglial NO production is usually stimulated by induction of iNOS after application of bacterial lipopolysaccharide (LPS) plus interferon-γ (IFγ) (17, 87). Activation of Nox results in a rapid superoxide production within minutes (99), whereas induction of iNOS requires protein synthesis that causes a delay of several hours before a sustained microglial NO production can be observed (26).

Cultured microglial cells generate superoxide upon activation with phorbol esters (21, 99). However, microglial cells produce only up to 5% of the superoxide that is generated by an equal number of neutrophils (99). This superoxide production is catalyzed by Nox. Active Nox is a multisubunit protein complex that uses electrons derived from NADPH to reduce molecular oxygen to superoxide. The Nox of phagocytic leukocytes is understood best (12). Activation of these cells leads to the association of cytosolic protein subunits of Nox (Rac2, $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$) with the membrane-associated proteins $gp91^{phox}$ (Nox2) and $p22^{phox}$ to the active complex that produces superoxide. Expression of mRNAs of all the subunits of Nox have been reported for microglial cultures (43). In addition, the presence of the proteins $gp91^{phox}$, $p22^{phox}$, and $p47^{phox}$ has been demonstrated for microglial cells (43, 68, 99). The presence of $gp91^{phox}$ and $p47^{phox}$ is essential for superoxide production, because microglial cultures that are derived from mice deficient in one of these two subunits are unable to produce superoxide upon stimulation (68, 95). In the CNS, Nox is expressed predominantly in microglial cells and to a lesser extent in other cell types (122). Recently, six novel ROS-generating oxidases with homology to the Nox2 of granulocytes have been discovered, three of which are also expressed in brain cells (122).

NO is synthesized in the reaction catalyzed by NOS. Of the three isoforms of NOS (80, 116), iNOS is induced upon activation in microglial cells *in vitro* and *in vivo* (26, 51, 92). Besides microglial cells, astrocytes also have been reported to express iNOS (80). However, after intrahippocampal injection of LPS, iNOS was more quickly induced in microglial cells than in astrocytes (39). In addition, the low concentration of 1 ng/ml LPS caused already a substantial production of NO in microglial cultures, but not in cultured astrocytes (74). Thus, microglial cells appear to be more susceptible to stimulation of iNOS expression than astrocytes, at least after application of LPS.

As arginine is the only physiological substrate of NOS (116), the availability of cells to take up and/or to synthesize arginine is crucial for their ability to produce NO. This aspect is important, because iNOS produces superoxide in the presence of low concentrations of arginine (121). Thus, insufficient arginine availability in activated microglial cells could lead to iNOS-mediated production of NO and superoxide and to subsequent formation of toxic peroxynitrite. In order to secure sustained NO production by microglial iNOS, sufficient replenishment of the substrate arginine is required. Both uptake and intracellular regeneration of arginine from citrulline are likely to contribute to the provision of arginine for the proper iNOS reaction. Microglial cells express an arginine transporter that mediates uptake of extracellular arginine (22), as well as the two enzymes argininosuccinate synthetase (50) and argininosuccinate lyase (B. Fischmann and H. Wiesinger, personal communication) that are required for synthesis of arginine from citrulline. The regeneration of arginine from citrulline appears to be especially important in activated microglial cells *in vivo*, because argininosuccinate synthetase is strongly and almost exclusively induced in microglial cells after injection of immunostimulants into rat brain (50). In addition, argininosuccinate synthetase and iNOS are expressed and colocalized in a minority of microglial cells in Alzheimer's disease (AD) brain, but not in control brain (51).

EFFECTS OF ROS ON MICROGLIAL CELLS

The superoxide and NO generated by the reactions of Nox and iNOS, respectively, have the potential to harm the producing cells. However, induction of iNOS or activation of Nox alone appears not to cause substantial damage to microglial cells. In contrast, the cells are damaged if the concentration of superoxide is strongly elevated in mitochondria (56) or if the cellular superoxide concentration is increased in NO-producing microglial cells (87). As superoxide and NO react at a diffusion-limited rate to form peroxynitrite, this oxidant is likely to be generated under such conditions and may contribute to the oxidative damage observed. This conclusion is supported by the quick removal of NO and formation of peroxynitrite after application of NO to superoxide-producing microglial cells (5) and by the strong peroxynitrite formation in NO-producing microglial cells after activation of Nox (93). In this context, the cellular compartmentalization of superoxide and NO production is important to consider. As NO, but not the anion superoxide, easily crosses cell membranes, peroxynitrite

is likely to be formed extracellularly in NO-producing cells after activation of Nox. In contrast, formation of peroxynitrite in cells will occur if NO production by iNOS is accompanied by elevated intracellular levels of superoxide, *e.g.,* in mitochondria that have an altered respiration. As peroxynitrite is well known to inhibit the respiratory chain (15, 104, 109), mitochondrial ATP production could be impaired in activated microglial cells that produce superoxide and NO. Thus, efficient antioxidative defense mechanisms are especially required in mitochondria of microglial cells in order to prevent oxidative damage caused by superoxide, NO, and their products.

The superoxide generated by Nox in activated microglial cells quickly disproportionates to oxygen and H_2O_2. This peroxide has the potential to influence microglial functions. For example, application of H_2O_2 to microglial cells in culture has been reported to enhance vesicle formation, suggesting that ROS facilitate phagocytosis by amoeboid microglial cells (110). Effects of H_2O_2 on microglial functions appear to depend on the activation status of the cells. Exogenous H_2O_2 has been reported to induce only small responses in untreated microglial cells, but large calcium influxes and cation currents in LPS-treated cells (67).

In addition to the oxidative potential of superoxide and NO, these radicals have important functions as signaling molecules in cellular regulation (33, 48, 80). Little is known about such regulatory functions in microglial cells. However, recent studies on microglial cultures from gp91^{phox}-deficient mice revealed that Nox-derived superoxide is involved in the stimulation of expression and release of tumor necrosis factor-α (TNFα) by LPS-treated cells (95).

IMPLICATIONS OF MICROGLIAL ROS PRODUCTION IN NEUROLOGICAL DISEASES

Microglial cells play an important role in the inflammatory processes that occur in neurological disorders such as stroke (24, 32), Parkinson's disease (PD) (9, 23, 66, 90, 120), AD (10, 31, 76, 97), amyotrophic lateral sclerosis (77), multiple sclerosis (16), human immunodeficiency virus-associated neurodegeneration (40), and brain tumors (42). As microglial cells release a large number of neuroactive substances upon activation (49, 78, 81–83), proof for a direct contribution of the microglia-derived radicals in the pathological processes of human brain disorders is difficult to obtain.

Good evidence for a contribution of Nox- and iNOS-derived radicals in neuronal cell death during the progression of a human neurodegenerative disease comes from experiments using animal models of PD. This disease is characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta. The etiology of PD is unknown, but biochemical analyses of postmortem tissues provide evidence for oxidative stress in the substantia nigra during the disease (60, 66). Application of the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes cell death of dopaminergic neurons that is paralleled by activation and proliferation of microglial cells. In the MPTP model, mice with an improved defense against superoxide or with deficiencies in iNOS are at least partially protected from MPTP toxicity, suggesting that superoxide and NO contribute to the death of dopaminergic neurons (112). A contribution of superoxide to the cell death of dopaminergic neurons is demonstrated by the protection observed in MPTP-treated transgenic mice that overexpressed either Cu/ZnSOD or MnSOD (64, 94). Also iNOS-deficient mice are more resistant to MPTP than their wild-type littermates (25, 70), suggesting that iNOS-derived NO contributes to dopaminergic cell death. Inhibition by minocycline of microglial activation in the MPTP model prevented the activation of Nox and the induction of iNOS in mouse brain and subsequently protected against MPTP-induced neuronal cell death (118). As minocycline provided also significant protection against MPTP toxicity in iNOS-deficient mice (118), other sources of radicals than iNOS have to be involved in MPTP-induced neurotoxicity. A contribution of superoxide in this process is strongly supported by a recent report demonstrating that gp91^{phox}-deficient mice exibit less dopaminergic cell death and protein oxidation in the substantia nigra after application of MPTP than controls (119).

Nox-derived superoxide appears also to be involved in an inflammatory model of dopaminergic cell death. Chronic infusion of LPS into rat or mouse brain causes activation of microglial cells that is followed by a gradual loss of up to 70% of nigral dopaminergic neurons (37). This loss of dopaminergic neurons is substantially reduced in Nox-deficient mice (95). In addition, iNOS inhibitors significantly reduce dopaminergic cell death after LPS injection into rat brain (59), indicating that iNOS-derived NO also is involved in the inflammatory cell death in this model system.

NO and superoxide are not exclusively produced by activated microglial cells in brain. As other types of brain cell also express NOS and Nox (74, 80, 116, 122), these cells could contribute to radical-mediated brain damage in pathological conditions. For example, astroglial Nox has recently been suggested as a primary target of β-amyloid-induced neurotoxicity (1). Indirect evidence that an increased superoxide production in microglial cells may be a prominent factor in the pathogenesis of human neurodegenerative diseases is given by the observations that in AD brain the cytosolic Nox components p47^{phox} and p67^{phox} are markedly translocated to the membranes of microglial cells (103) and that in PD brain the gp91^{phox} subunit of Nox is up-regulated in microglial cells (119).

ANTIOXIDATIVE MECHANISMS OF MICROGLIAL CELLS

As activated microglial cells are able to produce superoxide and NO, these cells are in immediate contact with these radicals, as well as with their reactive reaction products H_2O_2 and peroxynitrite. Thus, microglial cells have to be equiped with sufficient antioxidative defense mechanisms to prevent oxidative and nitrosative damage that would compromise their functions in defense and repair of the brain.

Fluorescence labeling techniques demonstrated that, among the different glial cell types in mixed glial cultures, microglial cells contain the highest concentration of GSH (18). This observation was confirmed by comparison of the GSH contents

of rat brain cell cultures that were enriched for various brain cell types. Microglial cultures contain a higher specific GSH content than cultured astrocytes, neurons, and oligodendrocytes (18, 54–56, 88). The high intracellular GSH concentration in microglial cells is likely to contribute strongly to the defense against radical- and peroxide-mediated damage.

Little is known about the regulation of GSH synthesis in microglial cells and the consequences of microglial activation on the GSH content in these cells. Treatment of mixed glial cultures with LPS/IFγ caused induction of iNOS and lowered the cellular GSH content specifically in microglial cells (19). This reduction in microglial GSH was accompanied by the formation of nitrosoglutathione in the stimulated cultures. No evidence for cellular loss of GSH or nitrosoglutathione was reported (19). In addition, induction of NO production by application of LPS/IFγ to a microglial cell line lowered the cytosolic GSH content, whereas the mitochondrial GSH content remained nearly unaffected (98). In contrast, stimulation with TNFα for 48 h doubled the specific GSH content of microglial cultures (27). Thus, depending on the type of stimulation, the microglial GSH content may increase by improvement of GSH synthesis or decrease by accelerated consumption of GSH.

The cellular contents and the metabolism of ascorbate and α-tocopherol in microglial cells have not been explored in detail so far. However, these vitamins have interesting effects on microglial functions. For example, treatment of microglial cultures with α-tocopherol and ascorbate improves the survival of the cells, induces a ramified microglial morphology, and down-regulates adhesion molecules (52). Pretreatment with α-tocopherol reduces also LPS-induced lipid peroxidation and interleukin-6 production in cultured microglial cells and in brain (41). In addition, preincubation of porcine microglial cells with α-tocopherol attenuates the translocation of $p67^{phox}$ to the plasma membrane, thereby preventing superoxide production by Nox (30). Moreover, ascorbate inhibited cyclooxygenase 2 activity and prostaglandin E_2 synthesis in LPS-treated rat microglial cells (34). These reports suggest that α-tocopherol and ascorbate can modulate microglial pathways that are associated with acute or chronic inflammation in the CNS. Whether the well known antioxidative functions of ascorbate and α-tocopherol and/or other functions of these vitamins (14, 96) contribute to their effects on microglial cells remains to be elucidated.

The expression and presence of several antioxidative enzymes have been reported for microglial cells in brain sections, as well as in brain cell cultures. By immunocytochemical staining of rat brain slices, a low basal content of cytosolic Cu/ZnSOD has been demonstrated for microglial cells, whereas under unstressed conditions the mitochondrial MnSOD was not detectable in microglial cells (72). In contrast, after excitotoxically induced neurodegeneration following injection of quinolinic acid into rat brain, MnSOD-immunolabeled mitochondria were observed in activated microglial cells (86). In addition, after transient cerebral ischemia, microglial cells in rat hippocampus were stained both for Cu/ZnSOD and MnSOD (75). In brain cell cultures, both isoforms of SOD were detected in microglial cells by immunocytochemical staining (72, 87, 91) and by activity measurements (56). The specific activity of MnSOD in microglial cultures is 20 times and four times higher than that in cultured astrocytes and oligodendrocytes, respectively, whereas specific activities of Cu/ZnSOD are almost identical in these three types of neural cell cultures (56).

The expression of mitochondrial MnSOD in microglial cells is up-regulated by activation and by oxidative stress. Treatment of mixed glial cell cultures with the ROS-forming xanthine/xanthine oxidase system increases MnSOD immunoreactivity in microglial cells (91). In addition, stimulation of cultured microglial cells with LPS/IFγ causes induction of iNOS and up-regulation of mitochondrial MnSOD, whereas the contents of Cu/ZnSOD, catalase, or GPx are not affected (87). Moreover, treatment of microglial cultures with TNFα strongly increases the specific MnSOD activity in the cultures (27). Such an increase in the activity of MnSOD in microglial cells is likely to improve the potential of the cells to decompose mitochondrial superoxide. As SODs compete for superoxide with the iron-catalyzed Haber–Weiss cycle and with NO, an elevated activity of MnSOD in activated microglial cells is likely to reduce the risk of mitochondrial damage by superoxide-derived hydroxyl radicals and peroxynitrite.

Immunocytochemical studies revealed strong immunoreactivity for GPx in microglial cells in brain sections from rat (71) and man (53). In rat brain, GPx immunoreactivity is up-regulated after administration of quinolinic acid (71). This up-regulation of GPx in microglial cells was discussed as an important mechanism to withstand oxidative stress (71). GPx was also localized in microglial cells by immunocytochemical staining of glial primary cell cultures (87). In microglial cultures, the specific activity of GPx is similar to that in cultured astrocytes and significantly higher than in cultured neurons (54, 56). In contrast to SODs and GPx, little is known on GR in microglial cells. Immunocytochemical staining demonstrated that, among the different glial cell types in astroglia-rich cultures, microglial cells stained strongly for GR (45). The prominent expression of GR in microglial cells was confirmed by activity measurements. Microglial cultures contain higher specific GR activities than cultured neurons and astrocytes (54).

Besides GPx, catalase is involved in cellular H_2O_2 clearance. In human brain sections, catalase immunoreactivity was found in all types of brain cells, including microglial cells (36). In addition, microglial cells in mixed glial cultures from rat brain were immunopositive for catalase (87). This immunocytochemical evidence for expression of catalase was confirmed by activity measurements. The specific catalase activity of microglia cultures was reported to be similar to (56) or significantly lower than that of cultured neurons, astrocytes, and oligodendrocytes (54, 55).

Little is known about the expression and antioxidative functions of other antioxidative defense mechanisms in microglial cells. Some proteins that have been considered to contribute to the antioxidative defense have been reported to be induced in activated microglial cells. For example, peroxiredoxin 1 is induced in microglial cells after hemorrhage (84). In addition, the bilirubin-generating enzyme heme oxygenase 1 has been reported to be induced in brain microglial cells following transient focal brain ischemia (65), intracerebral hemorrhage (84), cortical lesions (11), or thiamine deficiency-induced neurodegeneration (61).

Among the different peroxides generated in cells, H_2O_2 is quantitatively the most important one. Under experimental

conditions, extracellular H_2O_2 concentrations of up to 100 μ*M* have been reported for brain in a microdialysis study (57). After activation, microglial cells encounter substantial extracellular concentrations of H_2O_2 (113) that are generated by spontaneous disproportionation of the superoxide produced in the Nox-catalyzed reaction. Cultures of microglial cells have been studied for their ability to dispose of exogenous H_2O_2. These cultures clear exogenous H_2O_2 in a reaction that follows first-order kinetics (55). The long half-life of H_2O_2 in the media of cultured microglial cells does not reflect a low detoxification capacity of the cells, but is a consequence of the low cell number in microglial cultures (55). The specific H_2O_2-detoxification rate constant of cultured microglial cells is almost identical to those calculated for astrocyte and neuron cultures (55), demonstrating the high potential of microglial cells to clear extracellular H_2O_2.

GSSG is hardly detectable in untreated microglial cultures, but exposure to H_2O_2 or to a H_2O_2-generating system increases the microglial GSSG content to 30% of the total cellular glutathione (54). This oxidation of GSH during peroxide exposure demonstrates that GPx is involved in the peroxide clearance by microglial cells. GSSG is only detectable in the cells as long as H_2O_2 is present in the extracellular medium (54), demonstrating that GR quickly restores the initial GSH to GSSG ratio after removal of the peroxide. As catalase is present in microglial cells (54, 56, 87), this enzyme also is likely to contribute to the detoxification of H_2O_2.

Regarding antioxidative mechanisms of microglial cells, it can be summarized that these cells are equiped with a prominent glutathione system and substantial activities of various antioxidative enzymes, suggesting a strong antioxidative potential of these cells. As microglial cells are able to up-regulate antioxidative enzymes, these cells can even adapt to stress conditions that require an improved antioxidative defense.

NADPH SUPPLY FOR THE GENERATION AND DISPOSAL OF ROS

The availability of cellular NADPH is an important factor for both the prooxidative and the antioxidative potential of microglial cells, because this reduced cofactor supplies the essential reduction equivalents for the reactions catalyzed by iNOS, Nox, and GR (Fig. 2). If microglial cells become activated, NADPH is quickly consumed in the superoxide-forming Nox reaction. In addition, induction of iNOS will lead to an increased NADPH consumption in microglial cells. On the other hand, NADPH is required to regenerate GSH from GSSG. Thus, efficient replenishment of the cellular pool of NADPH appears to be crucial in activated microglial cells. A high ratio of NADPH to $NADP^+$ in cells is maintained by the activities of NADPH-regenerating enzymes (NREs). If the rate of NADPH regeneration is insufficient in these cells, the prooxidative enzymes Nox and iNOS would compete with the antioxidative enzyme GR for the substrate NADPH (Fig. 2). Such a scenario is likely to occur in activated microglial cells, because the K_M values of GR, iNOS, and Nox for NADPH are in the low micromolar range (45, 100, 108).

Several enzymes can participate in cellular $NADP^+$ reduction. Besides the two pentose phosphate pathway enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH), also malic enzymes (MEs), $NADP^+$-dependent isocitrate dehydrogenases (ICDHs), and mitochondrial transhydrogenase contribute to the NADPH formation in brain cells (3, 6, 79, 115). Of these enzymes, G6PDH and 6PGDH are exclusively localized in the cytosol (6), whereas transhydrogenase is exclusively localized in mitochondria (3). For both ME and ICDH, isoforms are known that are localized either in the cytosol or in mitochondria (79, 115).

Prevention of oxidative damage in mitochondria appears to be essential for microglial cells (56), and the mitochondrial GSH system is likely to contribute strongly to the antioxidative potential of microglial mitochondria. As GR requires NADPH as electron donor, the availability of this reduced cosubstrate is crucial for efficient mitochondrial peroxide reduction by GSH redox cycling. The mitochondrial isoforms of ME and ICDH, as well as transhydrogenase, contribute to the NADPH regeneration for GSSG reduction in brain mitochondria (115). Cultured microglial cells contain substantial activities of mitochondrial ICDH (79), whereas no evidence for the presence of mitochondrial ME in microglial cells was reported (114). Expression of transhydrogenase has been suggested for all types of brain cells (3). Thus, among the different NREs, at least mitochondrial ICDH and transhydrogenase are likely to supply the NADPH that is required for GSSG reduction in mitochondria of microglial cells.

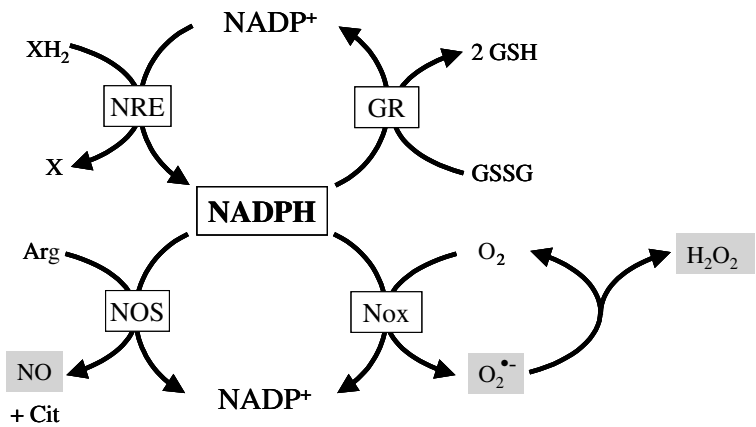

FIG. 2. Consumption and regeneration of NADPH in microglial cells. NADPH-regenerating enzymes (NREs) use reduced cellular metabolites (XH_2) as substrates to reduce $NADP^+$. In the cytosol of microglial cells, the enzymes glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and cytosolic $NADP^+$-dependent isocitrate dehydrogenases contribute to the regeneration of NADPH. The NADPH provided by NREs is used as electron donor in the reactions catalyzed by GR, NOS, and Nox. The superoxide ($O_2\cdot^-$) produced by Nox is disproportionated to oxygen and H_2O_2. Note that the antioxidative enzyme GR may compete with NOS and Nox for cytosolic NADPH in activated microglial cells. Arg, arginine; Cit, citrulline.

Microglial cells require NREs in the cytosol to supply Nox, iNOS, and cytosolic GR with their substrate NADPH. Substantial activities of G6PDH and 6PGDH were measured in cultured microglial cells, whereas ME activity was not detectable in these cultures (L. Kussmaul and R. Dringen, unpublished observations). In addition, expression and activity of cytosolic ICDH have recently been reported for cultured microglial cells (79). The presence of G6PDH, 6PGDH, and cytosolic ICDH in microglial cells demonstrates that two independent pathways can contribute to the NADPH regeneration in the cytosol. As no information is available on fluxes of metabolites through the pentose phosphate pathway and the cytosolic ICDH in activated microglial cells, the contribution of these pathways for NADPH regeneration in microglial cells remains to be elucidated.

CONCLUSIONS

Activated microglial cells release superoxide and NO, which are products of Nox and iNOS, respectively. To prevent oxidative damage by these ROS and their reactive reaction products H_2O_2 and peroxynitrite, microglial cells contain a high cellular concentration of GSH and express the antioxidative enzymes MnSOD, Cu/ZnSOD, GPx, GR, and catalase. In addition, the up-regulation of MnSOD and the induction of heme oxygenase 1 and peroxiredoxin 1 may further improve the protection against ROS-mediated cell damage in activated microglial cells. Microglia-derived ROS have the potential to damage neighboring cells in brain. Microglial proliferation and ROS production are therefore interesting targets to prevent oxidative damage and neuronal decay in neurodegenerative diseases (2, 9, 10, 38, 46, 111). Such strategies that target the oxidative and inflammatory potential of microglial cells will help to gain a better understanding of the functions of microglial cells in brain, as well as the beneficial and/or detrimental consequences of microglia-derived ROS for brain cells under physiological and pathological conditions.

ACKNOWLEDGMENTS

R.D. would like to thank Professor Heinrich Wiesinger (Tübingen) for critically reading the manuscript and for valuable suggestions, as well as Neurosciences Victoria (Australia) for a Senior Research Fellowship.

ABBREVIATIONS

AD, Alzheimer's disease; Cu/ZnSOD, copper- and zinc-containing superoxide dismutase; G6PDH, glucose-6-phosphate dehydrogenase; GPx, glutathione peroxidase(s); GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; ICDH, $NADP^+$-dependent isocitrate dehydrogenase; IFγ, interferon-γ; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; ME, malic enzyme; MnSOD, manganese-containing superoxide dismutase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NOS, nitric oxide synthase; Nox, NADPH oxidase; NRE, NADPH-regenerating enzyme; PD, Parkinson's disease; 6PGDH, 6-phosphogluconate dehydrogenase; ROS, reactive oxygen species; SOD, superoxide dismutase; TNFα, tumor necrosis factor-α.

REFERENCES

1. Abramov AY, Canevari L, and Duchen MR. β-Amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. *J Neurosci* 24: 565–575, 2004.
2. Aisen PS. The potential of anti-inflammatory drugs for the treatment of Alzheimer's disease. *Lancet Neurol* 1: 279–284, 2002.
3. Arkblad EL, Egorov M, Shakhparonov M, Romanova L, Polzikov M, and Rydstrom J. Expression of proton-pumping nicotinamide nucleotide transhydrogenase in mouse, human brain and *C. elegans. Comp Biochem Physiol B Biochem Mol Biol* 133: 13–21, 2002.
4. Arteel GE, Briviba K, and Sies H. Protection against peroxynitrite. *FEBS Lett* 445: 226–230, 1999.
5. Bal-Price A, Matthias A, and Brown GC. Stimulation of the NADPH oxidase in activated rat microglia removes nitric oxide but induces peroxynitrite production. *J Neurochem* 80: 73–80, 2002.
6. Baquer NZ, Hothersall JS, and McLean P. Function and regulation of the pentose phosphate pathway in brain. *Curr Top Cell Regul* 29: 265–289, 1988.
7. Barron KD. Microglia: history, cytology, and reactions. *J Neurol Sci* 207: 98, 2003.
8. Baumann N and Pham-Dinh D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev* 81: 871–927, 2001.
9. Beal MF. Mitochondria, oxidative damage, and inflammation in Parkinson's disease. *Ann N Y Acad Sci* 991: 120–131, 2003.
10. Benveniste EN, Nguyen VT, and O'Keefe GM. Immunological aspects of microglia: relevance to Alzheimer's disease. *Neurochem Int* 39: 381–391, 2001.
11. Bidmon HJ, Emde B, Oermann E, Kubitz R, Witte OW, and Zilles K. Heme oxygenase-1 (HSP-32) and heme oxygenase-2 induction in neurons and glial cells of cerebral regions and its relation to iron accumulation after focal cortical photothrombosis. *Exp Neurol* 168: 1–22, 2001.
12. Bokoch GM and Knaus UG. NADPH oxidases: not just for leukocytes anymore! *Trends Biochem Sci* 28: 502–508, 2003.
13. Bredt DS. Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Radic Res* 31: 577–596, 1999.
14. Brigelius-Flohe R, Kelly FJ, Salonen JT, Neuzil J, Zingg JM, and Azzi A. The European perspective on vitamin E: current knowledge and future research. *Am J Clin Nutr* 76: 703–716, 2002.
15. Brown GC and Bal-Price A. Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. *Mol Neurobiol* 27: 325–355, 2003.

16. Carson MJ. Microglia as liaisons between the immune and central nervous systems: functional implications for multiple sclerosis. *Glia* 40: 218–231, 2002.
17. Chao CC, Hu S, Molitor TW, Shaskan EG, and Peterson PK. Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. *J Immunol* 149: 2736–2741, 1992.
18. Chatterjee S, Noack H, Possel H, Keilhoff G, and Wolf G. Glutathione levels in primary glial cultures: monochlorobimane provides evidence of cell type-specific distribution. *Glia* 27: 152–161, 1999.
19. Chatterjee S, Noack H, Possel H, and Wolf G. Induction of nitric oxide synthesis lowers intracellular glutathione in microglia of primary glial cultures. *Glia* 29: 98–101, 2000.
20. Clancy RM, Levartovsky D, Leszczynska-Piziak J, Yegudin J, and Abramson SB. Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: evidence for *S*-nitrosoglutathione as a bioactive intermediary. *Proc Natl Acad Sci U S A* 91: 3680–3684, 1994.
21. Colton CA and Gilbert DL. Production of superoxide anions by a CNS macrophage, the microglia. *FEBS Lett* 223: 284–288, 1987.
22. Czapiga M and Colton CA. Microglial function in human APOE3 and APOE4 transgenic mice: altered arginine transport. *J Neuroimmunol* 134: 44–51, 2003.
23. Czlonkowska A, Kurkowska-Jastrzebska I, Czlonkowski A, Peter D, and Stefano GB. Immune processes in the pathogenesis of Parkinson's disease—a potential role for microglia and nitric oxide. *Med Sci Monit* 8: RA165–RA177, 2002.
24. Danton GH and Dietrich WD. Inflammatory mechanisms after ischemia and stroke. *J Neuropathol Exp Neurol* 62: 127–136, 2003.
25. Dehmer T, Lindenau J, Haid S, Dichgans J, and Schulz JB. Deficiency of inducible nitric oxide synthase protects against MPTP toxicity in vivo. *J Neurochem* 74: 2213–2216, 2000.
26. Ding M, St Pierre BA, Parkinson JF, Medberry P, Wong JL, Rogers NE, Ignarro LJ, and Merrill JE. Inducible nitric-oxide synthase and nitric oxide production in human fetal astrocytes and microglia. A kinetic analysis. *J Biol Chem* 272: 11327–11335, 1997.
27. Dopp JM, Sarafian TA, Spinella FM, Kahn MA, Shau H, and de Vellis J. Expression of the p75 TNF receptor is linked to TNF-induced NFκB translocation and oxyradical neutralization in glial cells. *Neurochem Res* 27: 1535–1542, 2002.
28. Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* 62: 649–671, 2000.
29. Dringen R and Hirrlinger J. Glutathione pathways in the brain. *Biol Chem* 384: 505–516, 2003.
30. Egger T, Hammer A, Wintersperger A, Goti D, Malle E, and Sattler W. Modulation of microglial superoxide production by α-tocopherol in vitro: attenuation of $p67^{phox}$ translocation by a protein phosphatase-dependent pathway. *J Neurochem* 79: 1169–1182, 2001.
31. Eikelenboom P, Bate C, Van Gool WA, Hoozemans JJ, Rozemuller JM, Veerhuis R, and Williams A. Neuroinflammation in Alzheimer's disease and prion disease. *Glia* 40: 232–239, 2002.
32. Emsley HC and Tyrrell PJ. Inflammation and infection in clinical stroke. *J Cereb Blood Flow Metab* 22: 1399–1419, 2002.
33. Esplugues JV. NO as a signalling molecule in the nervous system. *Br J Pharmacol* 135: 1079–1095, 2002.
34. Fiebich BL, Lieb K, Kammerer N, and Hull M. Synergistic inhibitory effect of ascorbic acid and acetylsalicylic acid on prostaglandin E_2 release in primary rat microglia. *J Neurochem* 86: 173–178, 2003.
35. Fouquet F, Zhou JM, Ralston E, Murray K, Troalen F, Magal E, Robain O, Dubois-Dalcq M, and Aubourg P. Expression of the adrenoleukodystrophy protein in the human and mouse central nervous system. *Neurobiol Dis* 3: 271– 285, 1997.
36. Fridovich I. Superoxide anion radical ($O_2^{-}\cdot$), superoxide dismutases, and related matters. *J Biol Chem* 272: 18515–18517, 1997.
37. Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, and Liu B. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *J Neurochem* 81: 1285–1297, 2002.
38. Gao HM, Liu B, Zhang W, and Hong JS. Novel anti-inflammatory therapy for Parkinson's disease. *Trends Pharmacol Sci* 24: 395–401, 2003.
39. Garcion E, Sindji L, Montero-Menei C, Andre C, Brachet P, and Darcy F. Expression of inducible nitric oxide synthase during rat brain inflammation: regulation by 1,25-dihydroxyvitamin D_3. *Glia* 22: 282–294, 1998.
40. Garden GA. Microglia in human immunodeficiency virus-associated neurodegeneration. *Glia* 40: 240–251, 2002.
41. Godbout JP, Berg BM, Kelley KW, and Johnson RW. α-Tocopherol reduces lipopolysaccharide-induced peroxide radical formation and interleukin-6 secretion in primary murine microglia and in brain. *J Neuroimmunol* 149: 101–109, 2004.
42. Graeber MB, Scheithauer BW, and Kreutzberg GW. Microglia in brain tumors. *Glia* 40: 252–259, 2002.
43. Green SP, Cairns B, Rae J, Errett-Baroncini C, Hongo JA, Erickson RW, and Curnutte JT. Induction of gp91-phox, a component of the phagocyte NADPH oxidase, in microglial cells during central nervous system inflammation. *J Cereb Blood Flow Metab* 21: 374–384, 2001.
44. Guillemin GJ and Brew BJ. Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. *J Leukoc Biol* 75: 388–397, 2004.
45. Gutterer JM, Dringen R, Hirrlinger J, and Hamprecht B. Purification of glutathione reductase from bovine brain, generation of an antiserum, and immunocytochemical localization of the enzyme in neural cells. *J Neurochem* 73: 1422–1430, 1999.
46. Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging* 18: 685–716, 2001.
47. Halliwell B and Gutteridge JMC. *Free Radicals in Biology and Medicine.* New York: Oxford University Press, 1999.
48. Hancock JT, Desikan R, and Neill SJ. Role of reactive oxygen species in cell signalling pathways. *Biochem Soc Trans* 29: 345–350, 2001.

49. Hanisch UK. Microglia as a source and target of cytokines. *Glia* 40: 140–155, 2002.
50. Heneka MT, Schmidlin A, and Wiesinger H. Induction of argininosuccinate synthetase in rat brain glial cells after striatal microinjection of immunostimulants. *J Cereb Blood Flow Metab* 19: 898–907, 1999.
51. Heneka MT, Wiesinger H, Dumitrescu-Ozimek L, Riederer P, Feinstein DL, and Klockgether T. Neuronal and glial coexpression of argininosuccinate synthetase and inducible nitric oxide synthase in Alzheimer disease. *J Neuropathol Exp Neurol* 60: 906–916, 2001.
52. Heppner FL, Roth K, Nitsch R, and Hailer NP. Vitamin E induces ramification and downregulation of adhesion molecules in cultured microglial cells. *Glia* 22: 180–188, 1998.
53. Hirato J, Nakazato Y, Koyama H, Yamada A, Suzuki N, Kuroiwa M, Takahashi A, Matsuyama S, and Asayama K. Encephalopathy in megacystis-microcolon-intestinal hypoperistalsis syndrome patients on long-term total parenteral nutrition possibly due to selenium deficiency. *Acta Neuropathol* (Berl) 106: 234–242, 2003.
54. Hirrlinger J, Gutterer JM, Kussmaul L, Hamprecht B, and Dringen R. Microglial cells in culture express a prominent glutathione system for the defense against reactive oxygen species. *Dev Neurosci* 22: 384–392, 2000.
55. Hirrlinger J, Resch A, Gutterer JM, and Dringen R. Oligodendroglial cells in culture effectively dispose of exogenous hydrogen peroxide: comparison with cultured neurones, astroglial and microglial cells. *J Neurochem* 82: 635–644, 2002.
56. Hollensworth SB, Shen C, Sim JE, Spitz DR, Wilson GL, and LeDoux SP. Glial cell type-specific responses to menadione-induced oxidative stress. *Free Radic Biol Med* 28: 1161–1174, 2000.
57. Hyslop PA, Zhang Z, Pearson DV, and Phebus LA. Measurement of striatal H_2O_2 by microdialysis following global forebrain ischemia and reperfusion in the rat: correlation with the cytotoxic potential of H_2O_2 in vitro. *Brain Res* 671: 181–186, 1995.
58. Ignarro LJ, Cirino G, Casini A, and Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol* 34: 879–886, 1999.
59. Iravani MM, Kashefi K, Mander P, Rose S, and Jenner P. Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. *Neuroscience* 110: 49–58, 2002.
60. Jenner P. Oxidative stress in Parkinson's disease. *Ann Neurol* 53 Suppl 3: S26–S36, 2003.
61. Ke ZJ, DeGiorgio LA, Volpe BT, and Gibson GE. Reversal of thiamine deficiency-induced neurodegeneration. *J Neuropathol Exp Neurol* 62: 195–207, 2003.
62. Kielian T. Microglia and chemokines in infectious diseases of the nervous system: views and reviews. *Front Biosci* 9: 732–750, 2004.
63. Kirchhoff F, Dringen R, and Giaume C. Pathways of neuron–astrocyte interactions and their possible role in neuroprotection. *Eur Arch Psychiatry Clin Neurosci* 251: 159–169, 2001.
64. Klivenyi P, St Clair D, Wermer M, Yen HC, Oberley T, Yang L, and Flint BM. Manganese superoxide dismutase overexpression attenuates MPTP toxicity. *Neurobiol Dis* 5: 253–258, 1998.
65. Koistinaho J, Miettinen S, Keinanen R, Vartiainen N, Roivainen R, and Laitinen JT. Long-term induction of haem oxygenase-1 (HSP-32) in astrocytes and microglia following transient focal brain ischaemia in the rat. *Eur J Neurosci* 8: 2265–2272, 1996.
66. Koutsilieri E, Scheller C, Grunblatt E, Nara K, Li J, and Riederer P. Free radicals in Parkinson's disease. *J Neurol* 249 Suppl 2: II1–II5, 2002.
67. Kraft R, Grimm C, Grosse K, Hoffmann A, Sauerbruch S, Kettenmann H, Schultz G, and Harteneck C. Hydrogen peroxide and ADP-ribose induce TRPM2-mediated calcium influx and cation currents in microglia. *Am J Physiol Cell Physiol* 286: C129–C137, 2004.
68. Lavigne MC, Malech HL, Holland SM, and Leto TL. Genetic requirement of p47phox for superoxide production by murine microglia. *FASEB J* 15: 285–287, 2001.
69. Lenaz G. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB Life* 52: 159–164, 2001.
70. Liberatore GT, Jackson-Lewis V, Vukosavic S, Mandir AS, Vila M, McAuliffe WG, Dawson VL, Dawson TM, and Przedborski S. Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. *Nat Med* 5: 1403–1409, 1999.
71. Lindenau J, Noack H, Asayama K, and Wolf G. Enhanced cellular glutathione peroxidase immunoreactivity in activated astrocytes and in microglia during excitotoxin induced neurodegeneration. *Glia* 24: 252–256, 1998.
72. Lindenau J, Noack H, Possel H, Asayama K, and Wolf G. Cellular distribution of superoxide dismutases in the rat CNS. *Glia* 29: 25–34, 2000.
73. Liu B and Hong JS. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J Pharmacol Exp Ther* 304: 1–7, 2003.
74. Liu B, Gao HM, Wang JY, Jeohn GH, Cooper CL, and Hong JS. Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann N Y Acad Sci* 962: 318–331, 2002.
75. Liu XH, Kato H, Nakata N, Kogure K, and Kato K. An immunohistochemical study of copper/zinc superoxide dismutase and manganese superoxide dismutase in rat hippocampus after transient cerebral ischemia. *Brain Res* 625: 29–37, 1993.
76. McGeer PL and McGeer EG. Local neuroinflammation and the progression of Alzheimer's disease. *J Neurovirol* 8: 529–538, 2002.
77. McGeer PL and McGeer EG. Inflammatory processes in amyotrophic lateral sclerosis. *Muscle Nerve* 26: 459–470, 2002.
78. Minghetti L and Levi G. Microglia as effector cells in brain damage and repair: focus on prostanoids and nitric oxide. *Prog Neurobiol* 54: 99–125, 1998.
79. Minich T, Yokota S, and Dringen R. Cytosolic and mitochondrial isoforms of $NADP^+$-dependent isocitrate dehydrogenases are expressed in cultured rat neurons, astrocytes, oligodendrocytes and microglial cells. *J Neurochem* 86: 605–614, 2003.

80. Murphy S. Production of nitric oxide by glial cells: regulation and potential roles in the CNS. *Glia* 29: 1–13, 2000.
81. Nakajima K and Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. *Curr Drug Targets Cardiovasc Haematol Disord* 4: 65–84, 2004.
82. Nakamura Y. Regulating factors for microglial activation. *Biol Pharm Bull* 25: 945–953, 2002.
83. Nakanishi H. Microglial functions and proteases. *Mol Neurobiol* 27: 163–176, 2003.
84. Nakaso K, Kitayama M, Mizuta E, Fukuda H, Ishii T, Nakashima K, and Yamada K. Co-induction of heme oxygenase-1 and peroxiredoxin I in astrocytes and microglia around hemorrhagic region in the rat brain. *Neurosci Lett* 293: 49–52, 2000.
85. Nedergaard M, Ransom B, and Goldman SA. New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci* 26: 523–530, 2003.
86. Noack H, Lindenau J, Rothe F, Asayama K, and Wolf G. Differential expression of superoxide dismutase isoforms in neuronal and glial compartments in the course of excitotoxically mediated neurodegeneration: relation to oxidative and nitrergic stress. *Glia* 23: 285–297, 1998.
87. Noack H, Possel H, Rethfeldt C, Keilhoff G, and Wolf G. Peroxynitrite mediated damage and lowered superoxide tolerance in primary cortical glial cultures after induction of the inducible isoform of NOS. *Glia* 28: 13–24, 1999.
88. Noack H, Possel H, Chatterjee S, Keilhoff G, and Wolf G. Nitrosative stress in primary glial cultures after induction of the inducible isoform of nitric oxide synthase (i-NOS). *Toxicology* 148: 133–142, 2000.
89. Noor R, Mittal S, and Iqbal J. Superoxide dismutase—applications and relevance to human diseases. *Med Sci Monit* 8: RA210–RA215, 2002.
90. Orr CF, Rowe DB, and Halliday GM. An inflammatory review of Parkinson's disease. *Prog Neurobiol* 68: 325–340, 2002.
91. Pinteaux E, Perraut M, and Tholey G. Distribution of mitochondrial manganese superoxide dismutase among rat glial cells in culture. *Glia* 22: 408–414, 1998.
92. Possel H, Noack H, Putzke J, Wolf G, and Sies H. Selective upregulation of inducible nitric oxide synthase (iNOS) by lipopolysaccharide (LPS) and cytokines in microglia: in vitro and in vivo studies. *Glia* 32: 51–59, 2000.
93. Possel H, Noack H, Keilhoff G, and Wolf G. Life imaging of peroxynitrite in rat microglial and astroglial cells: role of superoxide and antioxidants. *Glia* 38: 339–350, 2002.
94. Przedborski S, Kostic V, Jackson-Lewis V, Naini AB, Simonetti S, Fahn S, Carlson E, Epstein CJ, and Cadet JL. Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. *J Neurosci* 12: 1658–1667, 1992.
95. Qin L, Liu Y, Wang T, Wei SJ, Block ML, Wilson B, Liu B, and Hong JS. NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. *J Biol Chem* 279: 1415–1421, 2003.
96. Rice ME. Ascorbate regulation and its neuroprotective role in the brain. *Trends Neurosci* 23: 209–216, 2000.
97. Rogers J, Strohmeyer R, Kovelowski CJ, and Li R. Microglia and inflammatory mechanisms in the clearance of amyloid beta peptide. *Glia* 40: 260–269, 2002.
98. Roychowdhury S, Wolf G, Keilhoff G, and Horn TF. Cytosolic and mitochondrial glutathione in microglial cells are differentially affected by oxidative/nitrosative stress. *Nitric Oxide* 8: 39–47, 2003.
99. Sankarapandi S, Zweier JL, Mukherjee G, Quinn MT, and Huso DL. Measurement and characterization of superoxide generation in microglial cells: evidence for an NADPH oxidase-dependent pathway. *Arch Biochem Biophys* 353: 312–321, 1998.
100. Sasada M, Pabst MJ, and Johnston RB Jr. Activation of mouse peritoneal macrophages by lipopolysaccharide alters the kinetic parameters of the superoxide-producing NADPH oxidase. *J Biol Chem* 258: 9631–9635, 1983.
101. Schwartz M. Macrophages and microglia in central nervous system injury: are they helpful or harmful? *J Cereb Blood Flow Metab* 23: 385–394, 2003.
102. Sharpe MA, Robb SJ, and Clark JB. Nitric oxide and Fenton/Haber–Weiss chemistry: nitric oxide is a potent antioxidant at physiological concentrations. *J Neurochem* 87: 386–394, 2003.
103. Shimohama S, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T, Hayakawa T, Nunomura A, Chiba S, Perry G, Smith MA, and Fujimoto S. Activation of NADPH oxidase in Alzheimer's disease brains. *Biochem Biophys Res Commun* 273: 5–9, 2000.
104. Stewart VC and Heales SJ. Nitric oxide-induced mitochondrial dysfunction: implications for neurodegeneration. *Free Radic Biol Med* 34: 287–303, 2003.
105. Stoll G and Jander S. The role of microglia and macrophages in the pathophysiology of the CNS. *Prog Neurobiol* 58: 233–247, 1999.
106. Streit WJ. Microglia as neuroprotective, immunocompetent cells of the CNS. *Glia* 40: 133–139, 2002.
107. Streit WJ, Walter SA, and Pennell NA. Reactive microgliosis. *Prog Neurobiol* 57: 563–581, 1999.
108. Stuehr DJ, Cho HJ, Kwon NS, Weise MF, and Nathan CF. Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein. *Proc Natl Acad Sci U S A* 88: 7773–7777, 1991.
109. Szabo C. Multiple pathways of peroxynitrite cytotoxicity. *Toxicol Lett* 140–141: 105–112, 2003.
110. Takeda H, Tomita M, Tanahashi N, Kobari M, Yokoyama M, Takao M, Ito D, and Fukuuchi Y. Hydrogen peroxide enhances phagocytic activity of ameboid microglia. *Neurosci Lett* 240: 5–8, 1998.
111. Thomas M and Le WD. Minocycline: neuroprotective mechanisms in Parkinson's disease. *Curr Pharm Des* 10: 679–686, 2004.
112. Tieu K, Ischiropoulos H, and Przedborski S. Nitric oxide and reactive oxygen species in Parkinson's disease. *IUBMB Life* 55: 329–335, 2003.
113. Twig G, Jung SK, Messerli MA, Smith PJ, and Shirihai OS. Real-time detection of reactive oxygen intermediates from single microglial cells. *Biol Bull* 201: 261–262, 2001.
114. Vogel R, Jennemann G, Seitz J, Wiesinger H, and Hamprecht B. Mitochondrial malic enzyme: purification from

bovine brain, generation of an antiserum, and immunocytochemical localization in neurons of rat brain. *J Neurochem* 71: 844–852, 1998.
115. Vogel R, Wiesinger H, Hamprecht B, and Dringen R. The regeneration of reduced glutathione in rat forebrain mitochondria identifies metabolic pathways providing the NADPH required. *Neurosci Lett* 275: 97–100, 1999.
116. Wiesinger H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. *Prog Neurobiol* 64: 365–391, 2001.
117. Winterbourn CC and Metodiewa D. The reaction of superoxide with reduced glutathione. *Arch Biochem Biophys* 314: 284–290, 1994.
118. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H, and Przedborski S. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci* 22: 1763–1771, 2002.
119. Wu DC, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, and Przedborski S. NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *Proc Natl Acad Sci U S A* 100: 6145–6150, 2003.
120. Wüllner U and Klockgether T. Inflammation in Parkinson's disease. *J Neurol* 250 Suppl 1: I35–I38, 2003.
121. Xia Y, Roman LJ, Masters BS, and Zweier JL. Inducible nitric-oxide synthase generates superoxide from the reductase domain. *J Biol Chem* 273: 22635–22639, 1998.
122. Zekry D, Epperson TK, and Krause KH. A role for NOX NADPH oxidases in Alzheimer's disease and other types of dementia? *IUBMB Life* 55: 307–313, 2003.

Address reprint requests to:
Ralf Dringen, Ph.D.
Center for Biomolecular Interactions Bremen
Faculty 2 (Biology/Chemistry)
University of Bremen
P.O. Box 33 04 40
D-28334 Bremen
Germany

E-mail: ralf.dringen@uni-bremen.de

Received for publication July 21, 2004; accepted February 12, 2005.

This article has been cited by:

1. Ivana Dokic, Christian Hartmann, Christel Herold-Mende, Anne Régnier-Vigouroux. 2012. Glutathione peroxidase 1 activity dictates the sensitivity of glioblastoma cells to oxidative stress. *Glia* **60**:11, 1785-1800. [CrossRef]
2. Marshall Miller, Barbara Shukitt-Hale, James JosephBerry Fruit and Nuts 181-204. [CrossRef]
3. Feng Bao , Sandy R. Shultz , Jeff D. Hepburn , Vanessa Omana , Lynne C. Weaver , Donald P. Cain , Arthur Brown . 2012. A CD11d Monoclonal Antibody Treatment Reduces Tissue Injury and Improves Neurological Outcome after Fluid Percussion Brain Injury in Rats. *Journal of Neurotrauma* **29**:14, 2375-2392. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. S.W.A. Himaya, BoMi Ryu, Zhong-Ji Qian, Se-Kwon Kim. 2012. Paeonol from Hippocampus kuda Bleeler suppressed the neuro-inflammatory responses in vitro via NF-#B and MAPK signaling pathways. *Toxicology in Vitro* **26**:6, 878-887. [CrossRef]
5. K. Larsen, R. Najle, A. Lifschitz, G. Virkel. 2012. Effects of sub-lethal exposure of rats to the herbicide glyphosate in drinking water: Glutathione transferase enzyme activities, levels of reduced glutathione and lipid peroxidation in liver, kidneys and small intestine. *Environmental Toxicology and Pharmacology* . [CrossRef]
6. Derrick F. MacFabe. 2012. Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. *Microbial Ecology in Health & Disease* **23**:0. . [CrossRef]
7. Anna Sophie Berghoff, Hans Lassmann, Matthias Preusser, Romana Höftberger. 2012. Characterization of the inflammatory response to solid cancer metastases in the human brain. *Clinical & Experimental Metastasis* . [CrossRef]
8. Mustafa Naz#ro#lu. 2012. Molecular role of catalase on oxidative stress-induced Ca 2+ signaling and TRP cation channel activation in nervous system. *Journal of Receptors and Signal Transduction* 1-8. [CrossRef]
9. Long-Jun Wu, Gongxiong Wu, M Reza Akhavan Sharif, Amanda Baker, Yonghui Jia, Frederic H Fahey, Hongbo R Luo, Edward P Feener, David E Clapham. 2012. The voltage-gated proton channel Hv1 enhances brain damage from ischemic stroke. *Nature Neuroscience* . [CrossRef]
10. Inmaculada Tasset, Eduardo Agüera, Fernando Sánchez-López, Montserrat Feijóo, Ana I. Giraldo, Antonio H. Cruz, Félix Gascón, Isaac Túnez. 2012. Peripheral oxidative stress in relapsing-remitting multiple sclerosis. *Clinical Biochemistry* . [CrossRef]
11. Elena Galea, Nathalie Launay, Manuel Portero-Otin, Montserrat Ruiz, Reinald Pamplona, Patrick Aubourg, Isidre Ferrer, Aurora Pujol. 2012. Oxidative stress underlying axonal degeneration in adrenoleukodystrophy: A paradigm for multifactorial neurodegenerative diseases?. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [CrossRef]
12. Sandy R. Shultz , Feng Bao , Vanessa Omana , Charlotte Chiu , Arthur Brown , Donald Peter Cain . 2012. Repeated Mild Lateral Fluid Percussion Brain Injury in the Rat Causes Cumulative Long-Term Behavioral Impairments, Neuroinflammation, and Cortical Loss in an Animal Model of Repeated Concussion. *Journal of Neurotrauma* **29**:2, 281-294. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
13. Xi Shuhua, Liu Ziyou, Yan Ling, Wang Fei, Guifan Sun. 2012. A Role of Fluoride on Free Radical Generation and Oxidative Stress in BV-2 Microglia Cells. *Mediators of Inflammation* **2012**, 1-8. [CrossRef]
14. F. Fionnuala Cox, Donal Carney, Anne-Marie Miller, Marina A. Lynch. 2011. CD200 fusion protein decreases microglial activation in the hippocampus of aged rats. *Brain, Behavior, and Immunity* . [CrossRef]
15. S.W.A. Himaya, Ryu BoMi, Qian Zhong-Ji, Yong Li, Se-Kwon Kim. 2011. 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone [SE1] suppresses pro-inflammatory responses by blocking NF-#B and MAPK signaling pathways in activated microglia. *European Journal of Pharmacology* . [CrossRef]
16. Celia A. Dodd, Nikolay M. Filipov. 2011. Manganese potentiates LPS-induced heme-oxygenase 1 in microglia but not dopaminergic cells: Role in controlling microglial hydrogen peroxide and inflammatory cytokine output. *NeuroToxicology* . [CrossRef]
17. Qing-Shan Xue, Wolfgang J. Streit. 2011. Microglial pathology in Down syndrome. *Acta Neuropathologica* . [CrossRef]
18. Francesca L'Episcopo , Cataldo Tirolo , Nunzio Testa , Salvatore Caniglia , Maria Concetta Morale , Francesco Impagnatiello , Bianca Marchetti . 2011. Switching the Microglial Harmful Phenotype Promotes Lifelong Restoration of Subtantia Nigra Dopaminergic Neurons from Inflammatory Neurodegeneration in Aged Mice. *Rejuvenation Research* **14**:4, 411-424. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

19. Ertu#rul Uzar, Abdullah Acar, Osman Evliyao#lu, U#ur F#rat, Ka#an Kamasak, Cüneyt Göçmez, Harun Alp, Adnan Tüfek, Nebahat Ta#demir, Atilla #lhan. 2011. The anti-oxidant and anti-apoptotic effects of nebivolol and zofenopril in a model of cerebral ischemia/reperfusion in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* . [CrossRef]
20. Sandy R. Shultz, Derrick F. MacFabe, Kelly A. Foley, Roy Taylor, Donald P. Cain. 2011. A single mild fluid percussion injury induces short-term behavioral and neuropathological changes in the Long–Evans rat: Support for an animal model of concussion. *Behavioural Brain Research* . [CrossRef]
21. Jan Lewerenz , Pamela Maher . 2011. Control of Redox State and Redox Signaling by Neural Antioxidant Systems. *Antioxidants & Redox Signaling* **14**:8, 1449-1465. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
22. Anya M.Y. Lin, Y.H. Ping, G.F. Chang, J.Y. Wang, J.H. Chiu, C.D. Kuo, C.W. Chi. 2011. Neuroprotective effect of oral S/B remedy (Scutellaria baicalensis Georgi and Bupleurum scorzonerifolfium Willd) on iron-induced neurodegeneration in the nigrostriatal dopaminergic system of rat brain. *Journal of Ethnopharmacology* **134**:3, 884-891. [CrossRef]
23. Derrick F. MacFabe, Nathan E. Cain, Francis Boon, Klaus-Peter Ossenkopp, Donald P. Cain. 2011. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. *Behavioural Brain Research* **217**:1, 47-54. [CrossRef]
24. Peter Röhnert, Ulrich H. Schröder, Iryna Ziabreva, Michael Täger, Klaus G. Reymann, Frank Striggow. 2011. Insufficient endogenous redox buffer capacity may underlie neuronal vulnerability to cerebral ischemia and reperfusion. *Journal of Neuroscience Research* n/a-n/a. [CrossRef]
25. H. C. Yu, S. F. Feng, P. L. Chao, A. M. Y. Lin. 2010. Anti-inflammatory effects of pioglitazone on iron-induced oxidative injury in the nigrostriatal dopaminergic system. *Neuropathology and Applied Neurobiology* **36**:7, 612-622. [CrossRef]
26. Jianzheng Zheng, Oscar A. Bizzozero. 2010. Accumulation of protein carbonyls within cerebellar astrocytes in murine experimental autoimmune encephalomyelitis. *Journal of Neuroscience Research* **88**:15, 3376-3385. [CrossRef]
27. Dongmei Wang, Alan S. Hazell. 2010. Microglial activation is a major contributor to neurologic dysfunction in thiamine deficiency. *Biochemical and Biophysical Research Communications* **402**:1, 123-128. [CrossRef]
28. Jung-Hee Jang, Young-Joon SurhTherapeutic Potential of Ginseng for the Prevention and Treatment of Neurological Disorders **20105472**, 147-171. [CrossRef]
29. A. Kanamori, M.-M. Catrinescu, N. Kanamori, K. A. Mears, R. Beaubien, L. A. Levin. 2010. Superoxide is an associated signal for apoptosis in axonal injury. *Brain* **133**:9, 2612-2625. [CrossRef]
30. eMalick G. Njie, Ellen Boelen, Frank R. Stassen, Harry W.M. Steinbusch, David R. Borchelt, Wolfgang J. Streit. 2010. Ex vivo cultures of microglia from young and aged rodent brain reveal age-related changes in microglial function. *Neurobiology of Aging* . [CrossRef]
31. Joseph Lemire, Yves Milandu, Christopher Auger, Adam Bignucolo, Varun P. Appanna, Vasu D. Appanna. 2010. Histidine is a source of the antioxidant, #-ketoglutarate, in Pseudomonas fluorescens challenged by oxidative stress. *FEMS Microbiology Letters* no-no. [CrossRef]
32. Yu-Yi Chan, Chan-Hao Li, Yuh-Chiang Shen, Tian-Shung Wu. 2010. Anti-inflammatory Principles from the Stem and Root Barks of Citrus medica. *CHEMICAL & PHARMACEUTICAL BULLETIN* **58**:1, 61-65. [CrossRef]
33. Pamela Maher. 2009. Modulation of multiple pathways involved in the maintenance of neuronal function during aging by fisetin. *Genes & Nutrition* **4**:4, 297-307. [CrossRef]
34. Mariana Astiz, María J.T. de Alaniz, Carlos Alberto Marra. 2009. Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environmental Toxicology and Pharmacology* **28**:3, 465-473. [CrossRef]
35. Pamela MaherModulation of Multiple Pathways Involved in the Maintenance of Neuronal Function by Fisetin **20091218**, . [CrossRef]
36. Ralf Dringen, Johannes HirrlingerAntioxidative Defense of Brain Microglial Cells **20091218**, . [CrossRef]
37. Silvia Sorce , Karl-Heinz Krause . 2009. NOX Enzymes in the Central Nervous System: From Signaling to Disease. *Antioxidants & Redox Signaling* **11**:10, 2481-2504. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
38. Patricia D.S. Spada , Caroline Dani , Giovana V. Bortolini , Claudia Funchal , João A.P. Henriques , Mirian Salvador . 2009. Frozen Fruit Pulp of Euterpe oleraceae Mart. (Acai) Prevents Hydrogen Peroxide-Induced Damage in the Cerebral Cortex, Cerebellum, and Hippocampus of Rats. *Journal of Medicinal Food* **12**:5, 1084-1088. [Abstract] [Full Text PDF] [Full Text PDF with Links]
39. Élodie Pastural, Shawn Ritchie, Yingshen Lu, Wei Jin, Amir Kavianpour, Khine Khine Su-Myat, Doug Heath, Paul L. Wood, Maura Fisk, Dayan B. Goodenowe. 2009. Novel plasma phospholipid biomarkers of autism: Mitochondrial dysfunction as a putative causative mechanism. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **81**:4, 253-264. [CrossRef]

40. Maarten E Witte, Lars Bø, Richard J Rodenburg, Jeroen A Belien, Rene Musters, Thierry Hazes, Liesbeth T Wintjes, Jan A Smeitink, Jeroen JG Geurts, Helga E De Vries, Paul van der Valk, Jack van Horssen. 2009. Enhanced number and activity of mitochondria in multiple sclerosis lesions. *The Journal of Pathology* **219**:2, 193-204. [CrossRef]
41. Xiang Yang Zhang, Da Chun Chen, Mei Hong Xiu, Fan Wang, Ling Yan Qi, Hong Qiang Sun, Song Chen, Shu Chang He, Gui Ying Wu, Colin N. Haile. 2009. The novel oxidative stress marker thioredoxin is increased in first-episode schizophrenic patients. *Schizophrenia Research* **113**:2-3, 151-157. [CrossRef]
42. Bogdan O. Popescu, Emil C. Toescu, Lauren#iu M. Popescu, Ovidiu Bajenaru, Dafin F. Muresanu, Marianne Schultzberg, Nenad Bogdanovic. 2009. Blood-brain barrier alterations in ageing and dementia. *Journal of the Neurological Sciences* **283**:1-2, 99-106. [CrossRef]
43. Martin A. Bruno, Wanda C. Leon, Gabriela Fragoso, Walter E. Mushynski, Guillermina Almazan, A. Claudio Cuello. 2009. Amyloid #-Induced Nerve Growth Factor Dysmetabolism in Alzheimer Disease. *Journal of Neuropathology & Experimental Neurology* **68**:8, 857-869. [CrossRef]
44. Mariana Astiz, Graciela E. Hurtado de Catalfo, María J. T. Alaniz, Carlos Alberto Marra. 2009. Involvement of Lipids in Dimethoate-Induced Inhibition of Testosterone Biosynthesis in Rat Interstitial Cells. *Lipids* **44**:8, 703-718. [CrossRef]
45. Ji-Yeon Kim, Tae-Hyong Kim, Soung-Soo Kim. 2009. NSA9, a human prothrombin kringle-2-derived peptide, acts as an inhibitor of kringle-2-induced activation in EOC2 microglia. *BMB Reports* **42**:6, 380-386. [CrossRef]
46. Sandy R. Shultz, Derrick F. MacFabe, Samantha Martin, Jordana Jackson, Roy Taylor, Francis Boon, Klaus-Peter Ossenkopp, Donald P. Cain. 2009. Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long–Evans rat: Further development of a rodent model of autism. *Behavioural Brain Research* **200**:1, 33-41. [CrossRef]
47. Mariana Astiz, María J.T. de Alaniz, Carlos Alberto Marra. 2009. The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat. *Pesticide Biochemistry and Physiology* **94**:2-3, 93-99. [CrossRef]
48. Mie Østergaard Pedersen, Agnete Larsen, Dan Sonne Pedersen, Meredin Stoltenberg, Milena Penkowa. 2009. Metallic gold reduces TNF# expression, oxidative DNA damage and pro-apoptotic signals after experimental brain injury. *Brain Research* **1271**, 103-113. [CrossRef]
49. Kenneth Maiese, Zhao Zhong Chong, Yan Chen Shang, Jinling Hou. 2009. A "FOXO" in sight: Targeting Foxo proteins from conception to cancer. *Medicinal Research Reviews* **29**:3, 395-418. [CrossRef]
50. Yoichi Chiba, Atsuyoshi Shimada, Naoko Kumagai, Keisuke Yoshikawa, Sanae Ishii, Ayako Furukawa, Shiro Takei, Masaaki Sakura, Noriko Kawamura, Masanori Hosokawa. 2009. The Senescence-accelerated Mouse (SAM): A Higher Oxidative Stress and Age-dependent Degenerative Diseases Model. *Neurochemical Research* **34**:4, 679-687. [CrossRef]
51. Xia Chen, In Young Choi, Tong-Shin Chang, You Hyun Noh, Chan Young Shin, Chun-Fu Wu, Kwang Ho Ko, Won-Ki Kim. 2009. Pretreatment with interferon-# protects microglia from oxidative stress via up-regulation of Mn-SOD. *Free Radical Biology and Medicine* **46**:8, 1204-1210. [CrossRef]
52. Radovan Murin, Marija Cesar, Bhavani S. Kowtharapu, Stephan Verleysdonk, Bernd Hamprecht. 2009. Expression of Pyruvate Carboxylase in Cultured Oligodendroglial, Microglial and Ependymal Cells. *Neurochemical Research* **34**:3, 480-489. [CrossRef]
53. Jadwiga Turchan–Cholewo , Vanessa M. Dimayuga , Sunita Gupta , R.M. Charlotte Gorospe , Jeffrey N. Keller , Annadora J. Bruce–Keller . 2009. NADPH Oxidase Drives Cytokine and Neurotoxin Release from Microglia and Macrophages in Response to HIV-Tat. *Antioxidants & Redox Signaling* **11**:2, 193-204. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
54. John H. T. Power, Peter C. Blumbergs. 2009. Cellular glutathione peroxidase in human brain: cellular distribution, and its potential role in the degradation of Lewy bodies in Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathologica* **117**:1, 63-73. [CrossRef]
55. Rebecca L. Miller, Marilyn James-Kracke, Grace Y. Sun, Albert Y. Sun. 2009. Oxidative and Inflammatory Pathways in Parkinson's Disease. *Neurochemical Research* **34**:1, 55-65. [CrossRef]
56. Ming Lu, Li-Fang Hu, Gang Hu, Jin-Song Bian. 2008. Hydrogen sulfide protects astrocytes against H2O2-induced neural injury via enhancing glutamate uptake. *Free Radical Biology and Medicine* **45**:12, 1705-1713. [CrossRef]
57. Albert Y. Sun, Qun Wang, Agnes Simonyi, Grace Y. Sun. 2008. Botanical Phenolics and Brain Health. *NeuroMolecular Medicine* **10**:4, 259-274. [CrossRef]

58. Chuan-Chung Tsao, Yuh-Chiang Shen, Chung-Ren Su, Chia-Ying Li, Meei-Jen Liou, Nguyen-Xuan Dung, Tian-Shung Wu. 2008. New diterpenoids and the bioactivity of Erythrophleum fordii. *Bioorganic & Medicinal Chemistry* **16**:22, 9867-9870. [CrossRef]
59. R.S. Rao, B. Medhi, U.N. Saikia, S.K. Arora, Jaideep S. Toor, K.L. Khanduja, P. Pandhi. 2008. Experimentally induced various inflammatory models and seizure: Understanding the role of cytokine in rat. *European Neuropsychopharmacology* **18**:10, 760-767. [CrossRef]
60. Kryslaine O. Lopes, D. Larry Sparks, Wolfgang J. Streit. 2008. Microglial dystrophy in the aged and Alzheimer's disease brain is associated with ferritin immunoreactivity. *Glia* **56**:10, 1048-1060. [CrossRef]
61. Y-H Wang, Y-C Shen, J-F Liao, C-H Li, C-Y Chou, K-T Liou, Y-C Chou. 2008. Anti-inflammatory effects of dimemorfan on inflammatory cells and LPS-induced endotoxin shock in mice. *British Journal of Pharmacology* **154**:6, 1327-1338. [CrossRef]
62. Michela G. Schäppi, Vincent Jaquet, Dominique C. Belli, Karl-Heinz Krause. 2008. Hyperinflammation in chronic granulomatous disease and anti-inflammatory role of the phagocyte NADPH oxidase. *Seminars in Immunopathology* **30**:3, 255-271. [CrossRef]
63. Mathilde Gavillet, Igor Allaman, Pierre J. Magistretti. 2008. Modulation of astrocytic metabolic phenotype by proinflammatory cytokines. *Glia* **56**:9, 975-989. [CrossRef]
64. Phullara B. Shelat, Malgorzata Chalimoniuk, Jing-Hung Wang, Joanna B. Strosznajder, James C. Lee, Albert Y. Sun, Agnes Simonyi, Grace Y. Sun. 2008. Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A 2 in cortical neurons. *Journal of Neurochemistry* **106**:1, 45-55. [CrossRef]
65. Kenneth Maiese, Zhao Zhong Chong, Faqi Li, Yan Chen Shang. 2008. Erythropoietin: Elucidating new cellular targets that broaden therapeutic strategies. *Progress in Neurobiology* **85**:2, 194-213. [CrossRef]
66. Packiasamy A.R. Juliet, Xiaoyan Mao, Marc R. Del Bigio. 2008. Proinflammatory cytokine production by cultured neonatal rat microglia after exposure to blood products. *Brain Research* **1210**, 230-239. [CrossRef]
67. J KIM, T KIM, S KIM. 2008. Anti-inflammatory effect of a human prothrombin fragment-2-derived peptide, NSA9, in EOC2 microglia. *Biochemical and Biophysical Research Communications* **368**:3, 779-785. [CrossRef]
68. Kenneth Maiese, Faqi Li, Zhao Zhong Chong, Yan Chen Shang. 2008. The Wnt signaling pathway: Aging gracefully as a protectionist?. *Pharmacology & Therapeutics* **118**:1, 58-81. [CrossRef]
69. J BURDO, D SCHUBERT, P MAHER. 2008. Glutathione production is regulated via distinct pathways in stressed and non-stressed cortical neurons. *Brain Research* **1189**, 12-22. [CrossRef]
70. Donato A. Di MonteParaquat-induced Neurodegeneration: a Model of Parkinson's Disease Risk Factors 207-217. [CrossRef]
71. Jennifer Vila, Carmelina Gemma, Paula Bickford, Adam Bachstetter, Ingrid Strömberg, Yun WangSpirulina, Aging, and Neurobiology 271-291. [CrossRef]
72. Paul L. Wood, M. Amin Khan, Joseph R. Moskal. 2007. The concept of “aldehyde load” in neurodegenerative mechanisms: Cytotoxicity of the polyamine degradation products hydrogen peroxide, acrolein, 3-aminopropanal, 3-acetamidopropanal and 4-aminobutanal in a retinal ganglion cell line. *Brain Research* **1145**, 150-156. [CrossRef]
73. Chaohui Zhao, Zaodung Ling, Mary B. Newman, Ankush Bhatia, Paul M. Carvey. 2007. TNF-# knockout and minocycline treatment attenuates blood–brain barrier leakage in MPTP-treated mice. *Neurobiology of Disease* **26**:1, 36-46. [CrossRef]
74. Ann-Sofi Johansson, Jonas Bergquist, Christiane Volbracht, Anna P??ivi??, Marcel Leist, Lars Lannfelt, Anita Westlind-Danielsson. 2007. Attenuated amyloid-?? aggregation and neurotoxicity owing to methionine oxidation. *NeuroReport* **18**:6, 559-563. [CrossRef]
75. D. C. Duke, L. B. Moran, R. K. B. Pearce, M. B. Graeber. 2007. The medial and lateral substantia nigra in Parkinson’s disease: mRNA profiles associated with higher brain tissue vulnerability. *Neurogenetics* **8**:2, 83-94. [CrossRef]
76. Silvana Franceschetti, Giulio Sancini, Andrea Buzzi, Silvia Zucchini, Beatrice Paradiso, Giuseppina Magnaghi, Carolina Frassoni, Maia Chikhladze, Giuliano Avanzini, Michele Simonato. 2007. A pathogenetic hypothesis of Unverricht–Lundborg disease onset and progression. *Neurobiology of Disease* **25**:3, 675-685. [CrossRef]
77. Kenneth T. Kishida , Dr. Eric Klann . 2007. Sources and Targets of Reactive Oxygen Species in Synaptic Plasticity and Memory. *Antioxidants & Redox Signaling* **9**:2, 233-244. [Abstract] [Full Text PDF] [Full Text PDF with Links]
78. ANNE-KARIN WELIN, PERNILLA SVEDIN, RISTO LAPATTO, BO SULTAN, HENRIK HAGBERG, PIERRE GRESSENS, INGEMAR KJELLMER, CARINA MALLARD. 2007. Melatonin Reduces Inflammation and Cell Death in White Matter in the Mid-Gestation Fetal Sheep Following Umbilical Cord Occlusion. *Pediatric Research* **61**:2, 153-158. [CrossRef]

79. H KO, Y WANG, K LIOU, C CHEN, C CHEN, W WANG, S CHANG, Y HOU, K CHEN, C CHEN. 2007. Anti-inflammatory effects and mechanisms of the ethanol extract of Evodia rutaecarpa and its bioactive components on neutrophils and microglial cells. *European Journal of Pharmacology* **555**:2-3, 211-217. [CrossRef]

80. Filomena O. Dimayuga, Chunmei Wang, Jordan M. Clark, Edgardo R. Dimayuga, Vanessa M. Dimayuga, Annadora J. Bruce-Keller. 2007. SOD1 overexpression alters ROS production and reduces neurotoxic inflammatory signaling in microglial cells. *Journal of Neuroimmunology* **182**:1-2, 89-99. [CrossRef]

81. M.E.C. Robbins, W. ZhaoOxidative stress and radiation-induced late normal tissue injury 135-164. [CrossRef]

82. 2006. Rhei Rhizoma Extracts Have Antiproliferative Properties and Differential Effects on NO Production in Macrophages. *Journal of Food Science and Nutrition* **11**:4, 273-277. [CrossRef]

83. Kenneth T. Kishida, Eric Klann. 2006. Sources and Targets of Reactive Oxygen Species in Synaptic Plasticity and Memory. *Antioxidants & Redox Signaling*, ahead of print061121054212009. [CrossRef]

84. David W. Infanger , Ram V. Sharma , Robin L. Davisson . 2006. NADPH Oxidases of the Brain: Distribution, Regulation, and Function. *Antioxidants & Redox Signaling* **8**:9-10, 1583-1596. [Abstract] [Full Text PDF] [Full Text PDF with Links]

85. 2006. Immunomodulatory Activity of Pine Needle (Pinus densiflora) Extracts in Macrophages. *Journal of Food Science and Nutrition* **11**:2, 105-109. [CrossRef]

86. Prof. Jürgen Bernhagen . 2005. Macrophage Migration and Function: From Recruitment in Vascular Disease to Redox Regulation in the Immune and Neuroendocrine Networks. *Antioxidants & Redox Signaling* **7**:9-10, 1182-1188. [Citation] [Full Text PDF] [Full Text PDF with Links]