Brain Research 1643 (2016) 10–17

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research Report

Membrane lipid peroxidation in neurodegeneration: Role of thrombin and proteinase-activated receptor-1

Bruce A. Citron [a,b,1], Syed Ameenuddin [a,2], K. Uchida [f], William Z. Suo [a,b], Karen SantaCruz [a,d,3], Barry W. Festoff [a,b,c,e,*]

[a] Neurobiology Research Laboratory, Department of Veterans Affairs Medical Center, Kansas City, Missouri 64128
[b] Departments of Neurology, University of Kansas Medical Center, Kansas City, Kansas 66170
[c] Departments of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66170
[d] Departments of Pathology and Laboratory Medicine University of Kansas Medical Center, Kansas City, Kansas 66170
[e] Departments of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66170
[f] Laboratory of Food and Biodynamics, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form
10 April 2016
Accepted 28 April 2016
Available online 30 April 2016

Keywords:
Membrane lipid peroxidation
Reactive oxygen
Apoptosis
Neurodegenerative diseases
Spinal cord injury

ABSTRACT

Thrombin and membrane lipid peroxidation (MLP) have been implicated in various central nervous system (CNS) disorders from CNS trauma to stroke, Alzheimer's (AD) and Parkinson's (PD) diseases. Because thrombin also induces MLP in platelets and its involvement in neurodegenerative diseases we hypothesized that its deleterious effects might, in part, involve formation of MLP in neuronal cells. We previously showed that thrombin induced caspase-3 mediated apoptosis in motor neurons, via a proteinase-activated receptor (PAR1). We have now investigated thrombin's influence on the oxidative state of neurons leading to induction of MLP-protein adducts. Translational relevance of thrombin-induced MLP is supported by increased levels of 4-hydroxynonenal-protein adducts (HNEPA) in AD and PD brains. We now report for the first time that thrombin dose-dependently induces formation of HNEPA in NSC34 mouse motor neuron cells using anti-HNE and anti-acrolein monoclonal antibodies. The most prominent immunoreactive band, in SDS-PAGE, was at ~54 kDa. Membrane fractions displayed higher amounts of the protein-adduct than cytosolic fractions. Thrombin induced MLP was mediated, at least in part, through PAR1 since a PAR1 active peptide, PAR1AP, also elevated HNEPA levels. Of interest, glutamate and Fe_2SO_4 also increased the ~54 kDa HNEPA band in these cells but to a lesser extent. Taken together our results implicate the involvement of thrombin and MLP in neuronal cell loss observed in various CNS degenerative and traumatic pathologies.

1. Introduction

Thrombin is a serine protease widely known for its role in hemostasis. However, over the last several years thrombin has increasingly been implicated in a wide variety of central nervous system (CNS) functions and disorders. These include neuronal cell shape, development and death, neurodegenerative diseases and CNS trauma (Festoff et al., 1996; Festoff et al., 2001; Suo et al., 2004; Turgeon and Houenou, 1997; Wang and Reiser, 2003; Xi et al., 2003). In addition to both brain (TBI) and spinal cord (SCI) injury thrombin signaling has been implicated in Alzheimer's (AD) (Grammas et al., 2006; Ho et al., 1994) and Parkinson's (PD) diseases. Thrombin and PAR peptides cause neurodegeneration in the basal ganglia (Suo et al., 2003a), thrombin causes neurofibrillary tangle (NFT) formation in mice (Suo et al., 2003b) and both thrombin and its precursor prothrombin are produced and increased in neuritic plaques in AD brains (Arai et al., 2006). Hallmarks of these conditions include increased levels of reactive oxygen species (ROS) and oxidative stress (Choi, 1995; Markesbery and Carney, 1999) as well as cell death by apoptosis and or necrosis (Krantic et al., 2005; LeBlanc, 2005; Przedborski, 2005). Neurons are particularly vulnerable to the increased oxidative stress.

The presence of membrane lipid peroxidation (MLP) products associated with the oxidative stress in neurodegenerative diseases has prompted numerous mechanistic studies on lipid peroxidative

* Correspondence to: PHLOGISTIX Neurodiagnostics, 4220 Shawnee Mission Parkway Suite 350B, Fairway, KS 66205.
E-mail address: bwfestoff@mac.com (B.W. Festoff).
[1] Bay Pines VA Healthcare System and Dept. of Molecular Medicine, Univ. of South Florida Morsani College of Medicine; Research & Development 151, Bldg. 22, rm. 123, PO Box 4125, Bay Pines, FL 33744
[2] Cellular Neurobiology Laboratory, Mayo Clinic and Mayo Foundation, 1501 Guggenheim Bldg., 200 First St. SW, Rochester, MN 55905
[3] Department of Pathology, University of New Mexico, MSC8 4640, Albuquerque, New Mexico 87131-0001

http://dx.doi.org/10.1016/j.brainres.2016.04.071
0006-8993/

products like malonaldehyde (MDA), acrolein and 4-hydroxynonenal (HNE) leading to protein adducts in neuronal and glial cell cultures. Recent studies have reported the presence of MLP protein adducts in AD and PD brains (Ando et al., 1998; Rofina et al., 2004; Sayre et al., 1997). Formation of MLP may be involved in the earliest stages of AD pathogenesis that may provide a window for therapeutic intervention (Butterfield et al., 2006; Volkel et al., 2006). Several groups including our own have implicated thrombin, acting through one or more of its proteinase-activated receptors (PARs), as an extracellular signal that activates intracellular pathways which, if prolonged, culminate in apoptosis (Donovan et al., 1997; LeBlanc, 2005; Smirnova et al., 1998a; Smirnova et al., 1998b; Thirumangalakudi et al., 2009; Turgeon and Houenou, 1997).

We have also shown that certain modulators of these pathways may protect against thrombin neurotoxicity even when relatively high concentrations of the protease persist (Smirnova et al., 2001) These same PARs, upon activation by thrombin, are responsible for platelet aggregation, which is accompanied by an increase in HNE production (Hurst et al., 1987; Malle et al., 1995). In the present study, we utilized monoclonal antibodies (MAbs) directed against two different aldehyde protein adducts, HNE and acrolein, and studied AD and PD brain cytosolic and membrane fractions, comparing them with age matched control brain samples. Further translational studies were carried out in a murine motor neuron cell line to define potential mechanisms related to thrombin and MLP. Parts of this work were presented earlier in poster form[1].

Evidence that MLP occurs with frequency in acute and chronic disorders of the central nervous system (CNS) has prompted numerous studies in model systems. A number of reports indicate that both MDA and HNE induce MLP in neurons and glial cells in culture (Abarikwu et al., 2012; Blanc et al., 1998a; Blanc et al., 1998b; Blanc et al., 1997; Bruce-Keller et al., 1998; Kabuta et al., 2015a; Siddiqui et al., 2012). An additional relationship between HNE, MLP and β-amyloid (Aβ peptide) in AD (Mark et al., 1997a; Mark et al., 1997b; Montine et al., 1998) has also been identified. Of interest, the coagulation protease, thrombin (Smith-Swintosky et al., 1995), like HNE (Mattson and Pedersen, 1998), lowers the threshold of neuronal vulnerability to added Aβ. In turn, thrombin has been shown to stimulate the formation of both MDA and HNE in isolated rat platelets in a time and dose-dependent fashion that occurred in parallel with thromboxane B2 production and platelet aggregation (Gorog and Kovacs, 1995; Ilic et al., 2011; Nosal et al., 1993). By contrast, direct addition of HNE prevented aggregation of platelets induced by ADP or arachidonic acid but not by thrombin (Hurst et al., 1987).

To determine if a relationship existed between thrombin-mediated neurotoxicity and MLP injury we also compared, in parallel experiments, the effects of α-thrombin and a PAR1 active peptide, PAR1AP (TRAF-6), on mouse hybrid motor neurons previously found to have HNE protein adducts induced by Fe_2SO_4 (Pedersen et al., 1999). Our results indicate clear evidence of MLP in membrane fractions of murine neurons treated with nanomolar α-thrombin or micromolar PAR1AP. MLP was also detected in membranes of neurons exposed to conventional oxidative stress (Fe_2SO_4) but not with excitotoxic agents (glutamate). Of particular significance, we found that the principal antigenic band(s) induced by thrombin were in the ~50 kDa range, greater in membrane than in cytosolic proteins. One of these bands co-migrated with the murine neuronal PAR1, the principal G-protein-coupled thrombin receptor in these cells.

[1] Ameenuddin et al., Membrane lipid peroxidation (MLP) in Alzheimer's (AD) and Parkinson's (PD) brains and role of protease activated receptor-1 (PAR-1). American Society for Neurochemistry, Chicago, IL, March 25, 2000

2. Materials and methods

2.1. Materials

We purchased fetal bovine sera (FBS), Dulbecco's essential medium (DMEM), N1 neuronal defined medium (consisting of 0.5 g/L insulin, 0.5 g/L human transferrin, 0.5 mg/L sodium selenite, 1.6 g/L putrescine, and 0.73 mg/L progesterone) and Lubrol-PX from Sigma (St. Louis, MO). Tissue culture plates (6 well) were bought from Costar (Corning, NY). Pre-cast sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) gels were purchsed from Novex (San Diego, CA). ECF Western blotting kit was from Amersham (Pittsburgh, PA, U.S.A), where as the Anti-mouse IgG fluorescein-linked antibody and anti-fluorescein alkaline phosphatase conjugate were from Molecular Probes (Eugene, OR, U.S.A) Life Science (Arlington Heights, IL., U.S.A). Bicinchoninic acid (BCA) protein assay reagent were obtained from Pierce (Rockford, IL, U.S.A.). Protease inhibitor cocktail tablets were purchased from Boehringer Mannheim (Germany). Purified human α-thrombin, and thrombin receptor active peptide (TRAP-6; SFLLRN) were generous gifts of Drs. John Fenton, II, pH.D. (Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY) and Thomas Anderson (Albany Medical College, Albany, NY), respectively. Antibody to murine PAR1 was a generous gift of Drs. Patricia Andrade-Gordon and Michael D'Andrea (R. W. Johnson Pharmaceutical Research Institute, Spring House, PA).

2.2. Tissue culture

As in our previously-published studies (Citron et al., 1997; Smirnova et al., 1998a; Smirnova et al., 1998b), we used motor neuron-like NSC19 (or NSC34) cells (initially a gift from Dr. N. Cashman, Montreal Neurologic Hospital, Montreal, Canada), a mouse-mouse neural hybrid cell line produced through fusion of the aminopterin-sensitive neuroblastoma N18TG2 with motor neuron-enriched embryonic day 12-14 spinal cord cells, (Cashman et al., 1992). Relevant to our experiments, a prior report utilized these same cells to estimate the effects of HNE on glutamate transport (Pedersen et al., 1999). Following our previous experience with these cells, we grew them in 10% FBS/DMEM at 5% CO_2 and 37 °C. For 24 hours prior to addition of agents, we maintained the cells in the absence of serum using the N1 medium supplement for neural cell cultures (Bottenstein and Sato, 1979).

2.3. Human brain specimens

AD, PD and control human brain specimens were obtained from the University of Kansas Medical Center Alzheimer's Disease Center (ADC). Affected and aged matched controls ranged from 69-93 years of ages. Brain samples displayed prevalent Lewy bodies. AD samples were from patients diagnosed with AD prior to death and confirmed by plentiful neuritic plaques and neurofibrillary tangles in the cortex and hippocampus. The postmortem interval was less than 8 hours and samples were rapidly frozen in liquid nitrogen. All neuropathologic diagnoses were confirmed according to published criteria (Braak and Braak, 1994; Hyman and Trojanowski, 1997). All work was conducted in accordance with the VA Medical Center and University research oversight committees and programs.

2.4. Membrane and cytosol preparation

Following treatment of cultured motor neurons, washed monolayers were scraped in polycarbonate centrifuge tubes and then sonicated (Fisher Scientific 60 Sonic dismembrator) on ice