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13. ABSTRACT ***(Maximum 200 Words)***

Our proposed research is focused on developing nitrone-based antioxidants as antidotes against chemical agents that induced excitatory neurotoxicity. We proposed to use kainic acid, an analog of the excitatory amino acid glutamate, to induce chronic neurological damage in adult rats. This model has been widely used as a model for studying human temporal lobe epilepsy. The delayed neuronal degeneration induced by kainic acid resembles CNS neuronal injury, repair, and plasticity. Our preliminary observation have shown that nitrone antioxidants, free radical trapping compounds, protected rats from kainic acid induced death. More recently, we have found that cotreatment with the experimental antioxidant, phenyl-N-tert-butylnitrone (PBN) resulted in a diminution of NFkB, AP-1, and p38 activation, suppressed cytokine and apoptotic gene expression, inhibited neuronal apoptosis, and diminished seizure activity. These data suggest that pharmacological antagonism of multiple signal transduction pathways is achievable in the brain, and that inhibition of these processes may prevent a cascade of gene-inductive events leading to neuronal apoptosis. These results clarify the molecular basis for KA-induced seizure activity and may indicate a novel therapeutic strategy for certain chronic neurodegenerative disorders.

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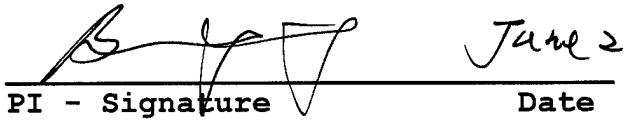

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Introduction:

With the goal of identifying new strategies for inhibiting neuronal apoptosis, which occurs in neuronal trauma and degeneration diseases, we have begun to explore the molecular basis for apoptosis in Kainic acid-induced neurodegeneration, a commonly used animal model for human temporal lobe epilepsy. Rats treated with kainic acid (KA) suffer recurrent convulsive seizures and apoptotic neuron loss in the CA1 and CA3 regions of the hippocampus. We hypothesized that KA chronically stimulates signal transduction pathways linked to apoptotic gene induction within sensitive populations of hippocampal neurons. In support of this hypothesis, we studied several distinct signal transduction pathways in the hippocampus following systemic exposure of KA. In particular, immunochemical studies and electromobility gel shift assays (EMSAs) demonstrate activation by KA of the NFκB (nuclear factor kappa B) system, the AP-1 (activator protein 1) system, and the p38 mitogen activated protein kinase (p38 MAPK) pathway. Most intriguingly, treatment of the KA-exposed animals with the compound phenyl-N-*tert*-butylnitrone (PBN) inhibits KA-induced neuronal apoptosis, down-regulates apoptosis-associated gene expression, and prevents seizure activity and death.

Body:

As an indication of KA-induced hippocampal damage, *in situ* TUNEL staining was performed to assess frank apoptosis. KA treatment caused DNA damage indicative of an apoptotic process within four days of subcutaneous administration (Fig. 1). Apoptosis was largely restricted to the CA1 and CA3 regions of the hippocampus wherein c-Fos was most strongly expressed. Administration of PBN 30 minutes after KA exposure strongly inhibited this apoptosis as indicated by diminished TUNEL staining in hippocampi from PBN treated animals (Fig. 1). Beginning approximately 30 minutes after KA injection, animals displayed archetypical epileptiform behavior including "wet dog" shakes, facial clonus, nodding, and forelimb clonus. Three hours after injection, KA-treated rats showed full limbic motor seizures including rearing and loss of postural control, as well as hypersalivation, circling and jumping. Rats treated with PBN 90 minutes after KA injection did not develop full limbic seizures by the 3 hour time point

(Table I). Moreover, PBN rescued the KA-treated animals from mortality when evaluated at the end of the four day experiment (Table I). No behavioral, physiologic or histologic alterations were observed in animals receiving PBN only.

The first immunochemical analysis of KA-treated rats was aimed at determining whether PBN could antagonize the AP-1 system *in vivo*. Immunocytochemical analysis was performed using well-characterized antibodies against the two AP-1 subunits, c-Fos and c-Jun. Within hours of KA treatment, c-Fos and c-Jun expression increased in hippocampal neurons, particularly within the CA1 and CA3 regions (Fig. 2). The c-Fos and c-Jun expression was maintained throughout the 4 day experiment (not illustrated), consistent with previously reported data (Bing et al., 1997). A single injection of PBN completely suppressed c-Jun expression in both CA regions and in the dentate gyrus (Fig. 2) while c-Fos expression was suppressed by PBN only in the CA1 and CA3 regions, where most of the pathological changes were manifest (Fig. 2 and discussed further below). It may be significant to note that while c-Jun expression can be induced rapidly in neurons during growth factor deprivation, c-Fos expression seems to be restricted to those populations of neurons that actually commit to an apoptotic program (Estus et al. 1994).

The AP-1 pathway is but one of numerous signal transduction pathways which have been associated with cellular stress and linked to ligand-induced neurotoxicity. In particular, the p38 MAPK pathway has been repeatedly linked to neuronal apoptosis and, in some circumstances, may indirectly activate both the AP-1 and NFkB pathways (Schulze-Osthoff et al. 1997; Vanden Berghe 1998; Hazzalin et al. 1997). The p38 mitogen-activated protein kinase pathway has been causally linked to neuronal apoptosis induced by growth factor withdrawal (Xia et al. 1995; Kummer et al. 1997). We therefore undertook an immunohistochemical analysis of p38 activation using an antibody specifically directed against the dual-phosphorylation motif which is present only on the active p38 kinase (Raingeaud et al., 1995). Within 4 hours of KA treatment, p38 activation was seen within the hippocampus in a pattern consistent with that of AP-1 activation (Fig. 3). As in the case of AP-1, PBN suppressed p38 phospho-activation (Fig. 3). The p38 system remained activated somewhat above the level of controls at the four

day timepoint, but this chronic activation was not as dramatic as in the AP-1 case (not shown).

The NFκB transcription factor is also ubiquitously activated by physiologic stress and may potentiate excitotoxic damage in striatal neurons (Qin et al. 1998). Alternatively, NFκB seems to serve a protective role in hippocampal neurons undergoing an oxidative insult (Mattson et al. 1997) and may actually play an anti-apoptotic role in TNFα-stimulated cells (Van Antwerp et al. 1996; Wang et al. 1998). NFκB is part of a signal transduction cascade which has traditionally been thought of as distinct from the Jnk and p38 cascade modules, though correlated activation of the three pathways is often noted in cell culture experiments. We therefore sought to determine whether NFκB was activated by KA in a PBN-sensitive manner. As shown in Fig. 4, NFκB-p65 immunoreactivity in the hippocampus increased dramatically within hours of KA treatment, and this effect was suppressed by PBN. The immunochemical data was corroborated by EMSA analysis which showed a dramatically-increased NFκB binding activity in hippocampal nuclei of KA treated rats, which was partially mitigated by PBN cotreatment (Fig. 4).

Hyperactivation of the Jnk, NFκB and p38 signal transduction pathways could be anticipated to have numerous detrimental consequences. All three signaling pathways have been linked to transcription of inflammatory cytokines and to modulation of apoptosis (Kawasaki et al., 1997; Kummer et al., 1997; Yang et al., 1997; Qin et al., 1998). We therefore sought to determine whether cytokine and proapoptotic genes were being transcribed at a greater rate in the KA treated rats than in normal rats, and whether PBN could abrogate such an effect. Using a multiprobe ribonuclease protection assay, several inflammatory cytokines were clearly found to be transcribed following KA treatment (Fig. 5). IL1α, IL1-β, IL-6 and TNF-α transcription were strongly induced by KA. Within the timeframe that cytokine transcription was enhanced, several proapoptotic genes were also induced. Most notably, the Fas antigen mRNA was strongly induced following KA and this elevation was maintained for at least four days (Fig. 6). PBN treatment suppressed transcription of both inflammatory cytokine gene products and proapoptotic gene products while having minimal effect on transcription of constitutively-expressed "housekeeping genes" including the L-32 ribosomal mRNA and

glyceraldehyde phosphate dehydrogenase mRNA (Figs. 5-6). PBN suppression of cytokine mRNA transcription was relatively unspecific. Interestingly, PBN displayed particular potency in suppressing Fas antigen and caspase 3 transcription, while other apoptosis-associated mRNA species analyzed by RPA were somewhat less affected by the nitrone (Fig. 6).

Disscussion: Kainic acid is a well-studied neurotoxin, which elicits an animal model of temporal lobe epilepsy and delayed neuronal death in the CA1 and CA3 hippocampal regions. Kainic acid kills neurons in culture by an apoptotic pathway involving binding to non-NMDA type glutamate receptors and consequent expression of immediate early genes including *c-Jun* (Cheung et al. 1998). In the present study, we extend upon these cell culture experiments by documenting increased c-Fos and c-Jun expression (i.e., activation of the AP-1 signaling pathway) within the rat hippocampus following systemic administration of KA. Furthermore, we document the activation of two distinct signal transduction pathways, the NF*k*B pathway and the p38 MAPK pathway, following the KA challenge. Activation of these three distinct signal transduction pathways correlated temporally with the transcription of both proinflammatory cytokines and proapoptotic mRNA species. Inhibition of these three pathways by the experimental compound phenyl-*tert*-butylnitrone was associated with diminished cytokine elaboration, prevention of neuronal apoptosis, reduced seizure activity, and reduced mortality. While the AP-1, NF*k*B, and p38 pathways are known to respond positively to oxidants and negatively to antioxidants in cell culture (Suzuki et al. 1994; Guyton et al. 1996; Robinson et al. 1999a), the data in this present study are the first to demonstrate the sensitivity of these three pathways to an antioxidant compound within the context of an established *in vivo* model of hippocampal neurodegeneration.

The findings of the present study extend upon previous observations concerning the broad-spectrum neuroprotective action of nitrone compounds, and provide a novel context for discussing the pathology of excitotoxicity. PBN and related nitrones have been shown to suppress striatal excitotoxic lesions induced by NMDA, KA, and AMPA, though not by virtue of any obvious direct interaction with glutamate receptors (Shultz et al., 1995). Similarly, PBN and a sulfated analog inhibit striatal lesions caused by mitochondrial inhibitors such as malonate and the 1-methyl-4-phenylpyridinium (MPP+;

Shultz et al. 1995). Nitrones suppress apoptosis and oxidative stress in cultured Down's syndrome neurons (Busciglio and Yankner 1995), and similarly inhibit chemically-induced thymocyte apoptosis *in vitro* (Slater et al. 1995), though the influence of nitrones on apoptosis *in vivo* has not been well-studied. Unfortunately, the pharmacologic effects of nitrones in most previous investigations were not correlated with biomarkers of oxidative stress, inflammation or apoptosis. The present data suggest that suppression of apoptosis by PBN in the KA model and possibly other models of neurodegeneration is likely due to mitigation of proinflammatory or proapoptotic gene expression under the control of the AP-1, NFkB, and p38 MAPK pathways. While the ultimate cellular target(s) for PBN action remain unclear, the present data suggest that the broad-spectrum neuroprotective action of the nitrone class of compounds (Hensley et al. 1997) might be due, in part, to antagonism of crucial oxidation-sensitive signal transduction elements linked to the initiation of apoptotic programs.

Pharmacological strategies designed to specifically inhibit individual signaling modules, such as the NFκB, AP-1 or p38 modules, might therefore be unnecessary and might be more difficult to execute *in vivo* than alternative strategies designed to suppress multiple signaling processes simultaneously. Evaluation of these issues must await future investigations aimed at identifying the central control systems which regulate proinflammatory and proapoptotic signaling in the hippocampus, particularly with respect to localizing specific oxidation-sensitive elements that may be targeted by nitrone-type neuroprotective agents.

Key Research Accomplishments:

- **Established kainic acid induced neuronal damage in adult rats as a model to study excitatory amino acid-induced neurodegenerative diseases by Terminal deoxyuridine nick-end labeling (TUNEL) for apoptotic cell death, Nissl staining and immunohistochemical assays.**
- **Demonstrated that nitrone antioxidant, PBN, inhibits KA_induced neuronal apoptosis, down regulating apoptosis-associate gene expression, and moreover, prevents seizure activity and death.**

- **Elucidated the molecular mechanisms underlying the nitrone antioxidants' protective functions against KA-induced neurodegeneration with signal transduction pathways by studying the activation of NFkB, p38, and AP-1.**

Reportable Outcomes:

Preprint:

Hensley, K., Stewart, C.A., Zheng, N.Y., Mou, S., Floyd, R.A., **Bing, G. (2000)** Phenyl-N-*tert*-butylnitrone inhibits neuronal apoptosis in the kainic acid model of Epilepsy by suppressing proapoptotic signal transduction pathways. Submitted to *J Neurosci.*

Abstracts and Presentations:

Nguyen, X.V., Hensley, K., Stewart, C.A., Zheng, N.Y., Jin, L., Zhu, M., Williamson, K.S., Floyd, R.A., **Bing, G. (1999)** Involvement of oxidant-sensitive signal transduction pathways in hippocampal excitotoxicity. Eighth Annual Symposium, *Oklahoma Center for Neuroscience (OCNS)*. The Neurobiology of Addiction: Neuroal, Behavioral, and Clinical Features, October 1, Oklahoma City, Oklahoma. p. 34 Abstr. #20.

Gabbita, S.P., Hensley, K., Floyd, R.A., Nael, R., Markesbery, W.R., Mou, S., Williamson, K.S., Zheng, N.Y., **Bing, G. (1999)** Potential Cytotoxic Role of Epoxide Hydrolase (EH) in Alzheimer's Disease (AD). Free Radical Biology & Medicine, 6^{Th} Annual Meeting of the Oxygen Society. The Marriot, New Orleans, November 18-22, Vol. 27:S139 Suppl. 1, Abstr. # 423.

Animal Model: We have successfully used KA-induced neurodegeneration as a animal model for delayed neuronal cell death that occurred in many neurodegeneration diseases such and Alzheimer's and Parkinson's diseases.

Conclusions:

The findings of the present study extend upon previous observations concerning the broad-spectrum neuroprotective action of nitrone compounds, and provide a novel context for discussing the pathology of excitotoxicity. PBN and related nitrones have been shown to suppress striatal excitotoxic lesions induced by KA. The present data suggest that suppression of apoptosis by PBN in the KA model and possibly other models of neurodegeneration is likely due to mitigation of proinflammatory or proapoptotic gene expression under the control of the AP-1, NFkB, and p38 MAPK pathways. While the ultimate cellular target(s) for PBN action remain unclear, the present data suggest that the broad-spectrum neuroprotective action of the nitrone class of compounds might be due, in part, to antagonism of crucial oxidation-sensitive signal transduction elements linked to the initiation of apoptotic programs.

References:

Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O (1997) Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc. Natl. Acad. Sci. USA 94: 10432-10437.

Berger ML, Lefauconnier JM, Tremblay E, Ben-Ari Y (1986) Limbic seizures induced by systemically applied kainic acid: How much kainic acid reaches the brain? Adv. Exp. Med. Biol. 203: 199-209.

Bernard C, Wheal HV (1995) Plasticity of AMPA and NMDA receptor-mediated epileptiform activity in a chronic model of temporal lobe epilepsy. Epilepsy Res. 21: 95-107.

Bing G, Wilson B, Hudson P, Jin L, Feng Z, Zhang W, Bing R, Jau-Shyong H (1997) A single dose of kainic acid elevates the levels of enkephalins and activator protein-1 transcription factors in the hippocampus for up to one year. Proc. Natl. Acad. Sci. USA 94: 9422-9427.

Busciglio J, Yankner, BA (1995) Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. Nature 378: 776-779.

Carney JM., Starke-Reed PE, Oliver CN, Landum RW, Cheng MS, Wu JF, Floyd RA (1991) Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound, N-*tert*-butyl-α-phenylnitrone. Proc. Natl. Acad. Sci. USA 88: 3633-3636.

Cheung NS, Carroll FY, Larm JA, Beart PM, Giardina SF (1998) Kainate-induced apoptosis correlates with c-Jun activation in cultured cerebellar granule cells. J. Neurosci. Res. 52: 69-82.

Culcasi M., Lafon-Cazal M., Pietri S., Bockaert J. (1994) Glutamate receptors induce a burst of superoxide via activation of nitric oxide synthase in arginine-depleted neurons. J. Biol. Chem. 269: 12589-12593.

Cronin J, Obenaus A, Houser CR, Dudek FE (1992) Electrophysiology of dentate granule cells after kainate-induced synaptic reorganization of the mossy fibers. Brain Res. 573: 305-310.

Estus S, Zaks WJ, Freeman RS, Gruda M, Bravo R, Johnson EM (1994) Altered gene expression in neurons during programmed cell death: Identification of c-Jun as necessary for neuronal apoptosis. J Cell Bio 127: 1717-1727.

Ferraro TN, Golden GT, Smith GG, Berrettini WH (1995) Differential susceptibility to seizures induced by systemic kainic acid treatment in mature DBA/2J and C57BL/6J mice. Epilepsia 36: 301-307.

Goodenough S, Davidson M, Chen W, Beckmann A, Pujic Z, Otsuki M, Matsumoto I, Wilce P (1997) Immediate early gene expression and delayed cell death in limbic areas of the rat brain after kainic acid treatment and recovery in the cold. Exp. Neurol. 145, 451-461.

Griffiths MR, Black EJ, Culbert AA, Dickens M, Shaw PE, Gillespie DA, Tavare JM (1998) Insulin-stimulated expression of c-fos, fra1 and c-jun accompanies the activation of the activator protein-1 (AP-1) transcriptional complex. Biochem. J. 335: 19-26.

Guyton KZ, Liu Y, Gorospe M., Xu Q, Holbrook NJ. (1996) Activation of mitogen-activated protein kinase by H_2O_2: Role in cell survival following oxidant injury. J. Biol. Chem. 271: 4138-4142.

Hazzalin CA, Cuenda A, Cano E, Cohen P, Mahadevan LC (1997) Effects of the inhibition of p38/RK MAP kinase on induction of five fos and jun genes by diverse stimuli. Oncogene 15: 2321-2331.

Hensley K, Carney JM, Stewart CA, Tabatabaie T, Pye QN, Floyd RA (1997) Nitrone-based free radical traps as neuroprotective agents in cerebral ischemia and other pathologies. Int. Rev. Neurobiol. 40: 299-317.

Hsu SM, Raine L, Fanger HJ (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabelled antibody (PAP) procedures. Histochem. Cytochem. 29: 577-480.

Kawasaki H, Morooka T, Shimohama S, Kimura J, Hirano T, Gotoh Y, Nishida E (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells. J Biol Chem 272 : 18518-18521.

Kotake Y, Sang H, Miyajima T, Wallis GL (1998) Inhibition of NF-κB, iNOS mRNA, COX2 mRNA, and COX catalytic activity by phenyl-N-*tert*-butylnitrone (PBN). Biochem. Biophys. Acta 1448: 77-84.
Kawasaki H, Morooka T, Shimohama S, Kimura J, Hirano T, Gotoh Y, Nishida E (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cereballar granule cells. J Biol Chem 272: 18518-18521.

Kummer JL, Rao PK, Heidenreich KA (1997) Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. J Biol Chem 272: 20490-20494.

Lafon-Cazal, M., Pietri, S., Culcasi, M., Bockaert J. (1993) NMDA-dependent superoxide production and neurotoxicity. Nature 364: 535-537.

Mathis C, Ungerer A (1992) Comparative analysis of seizures induced by intracerebroventricular administration of NMDA, kainate and quisqualate in mice. Exp. Brain Res. 88: 277-282.

Mattson MP, Goodman Y, Luo H, Fu W, Furukawa K (1997) Activation of NFkB protects hippocampal neurons against oxidative stress-induced apoptosis: Evidence for induction of manganese superoxide dismutase and suppression of peroxinitrite production and protein nitration. J Neurosci Res 49: 681-697.

Meier B., Radeke H.H., Selle S., Younes M., Sies H., Resch K., Habermehl G.G. (1989) Human fibroblasts release reactive oxygen species in response to interleukin or tumor necrosis factor alpha. Biochem. J. 263: 539-545.

Pisa M, Sanberg PR, Corcoran ME, Fibiger HC. Spontaneously recurrent seizures after iintracerebral injections of kainic acid in rat: A possible model of human temporal lobe epilepsy (1980) Brain. Res. 200: 481-487.

Qin ZH, Wang Y, Nakai M, Chase TN (1998) Nuclear factor-kappa B contributes to excitotoxin-induced apoptosis in rat striatum. Mol Pharmacol 53: 33-42.

Racine R, Okujava V, Chipashvili S. Modification of seizure activity by electrical stimulation 3: Mechanisms (1972) Electroencephalogr. Clin. Neurophysiol. 32: 295-299.

Raingeaud J, Gupta S, Rogers JS, Martin D, Han J, Ulevitch RJ, Davis RJ (1995) Proinflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J. Biol. Chem. 270: 7420-7426.

Rice N and Ernst, MK (1993) In vivo control of NFκB activation by IκBα. EMBO J. 12: 4685-4695.

Robinson KA, Stewart CA, Pye QN, Nguyen X, Kenney L, Salzman S, Floyd RA, Hensley K (1999a) Redox sensitive protein phosphatase activity regulates the phosphorylation state of p38 protein kinase in primary astrocyte culture. J Neurosci Res. 55: 724-732.

Robinson KA, Stewart CA, Pye QN, Floyd RA, Hensley K (1999b) Basal protein phosphorylation is decreased and phosphatase activity increased by an antioxidant and a free radical trap in primary rat glia. Arch. Biochem. Biophys. 365: 211-215.

Schwob JE, Fuller T, Price JL, Olney JW (1980) Widespread patterns of neuronal damage following systemic or intracerebral injections of kaiinic acid: A histological study. Neuroscience 5: 991-1014.

Shulz JB, Henshaw DR., Siwek D, Jenkins BG, Ferrante RJ, Cipolloni PB, Kowall NW, Rosen BR, Beal MF (1995) Involvement of free radicals in excitotoxicity in vivo. J. Neurochem. 64: 2239-2247.

Schulze-Osthoff K, Ferrari D, Riehemann K, Wesselborg S (1997) Regulation of NF-kappa B activation by MAP kinase cascades. Immunobiology 198: 35-49.

Simonian NA, Getz RI, Leveque JC, Konradi C, Coyle JT. (1996) Kainic acid induces apoptosis in neurons. Neuroscience 75: 1047-1055.

Slater AFG, Nobel CS, Maellaro E, Bustamante J, Kimland M, Orrhenius S. (1995) Nitrone spin traps and a nitroxide antioxidant inhibit a common pathway of thymocyte apoptosis. Biochem. J. 306: 771-779.

Sommer W. Erkrankung des ammonshorns als aetiologisches moment der epilepsie (1880) Arch. Psychiatr. Nervenkrankh. 10: 631-675.

Sonnenberg J, MacGregor-Leon P, Curran T, Morgan J (1989) Dynamic alterations occur in the levels and composition of transcription factor AP-1 complexes after seizure. Neuron 3: 359-365.

Sperk G, Lassmann H, Baran H, Seitelberger F, Hornykiewicz O (1996) Kainic acid-induced seizures: Dose-relationship of behavioral, neurochemical and histopathological changes. Brain Res. 338: 289-295.

Suzuki YJ, Mizuno M, Packer (1994) Signal transduction for nuclear factor-kB activation: Proposed location of antioxidant-inhibitable step. J. Immunol. 153: 5008-5015.

Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM (1996) Suppression of TNF-α-induced apoptosis by NFκB. Science 274: 787-789.

Vanden Berghe W, Plaisance S, Boone E, De Bosscher K, Schmitz ML, Fiers W, Haegeman G (1998) p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-kappa B p65 transactivation mediated by tumor necrosis factor. J. Biol. Chem. 273: 3285-3290.

Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldrin AS (1998) NF-κB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281: 1680-1683.

Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270: 1326-1331.

Yang DD, Kuan CY, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, Rakie P, Flavell RA (1997) Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk 3 gene. Nature 389: 865-870.

Appendices:

TABLE Suppression by PBN of limbic seizures and mortality in kainic acid-treated rats.

Figures 1-6

Hensley, K., Stewart, C.A., Zheng, N.Y., Mou, S., Floyd, R.A., **Bing, G. (2000)** Phenyl-N-*tert*-butylnitrone inhibits neuronal apoptosis in the kainic acid model of Epilepsy by suppressing proapoptotic signal transduction pathways. Submitted to *J Neurosci.*

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Guoying Bing, M.D., Ph.D., Curriculum Vitae

TABLE Suppression by PBN of limbic seizures and mortality in kainic acid-treated rats. Seizure activity was ranked on a five-point scale as described in the methods.

Treatment	Seizure Intensity	Mortality (4 days)
Kainic acid (N = 30)	4.9 ± 0.4	12 / 30 (38 %)
Kainic acid + PBN (N = 20) **	2.3 ± 0.3 *	0 / 20 (0 %)

* $P < 0.05$ (Student's t-test)
** $P < 0.02$ (χ^2 test)

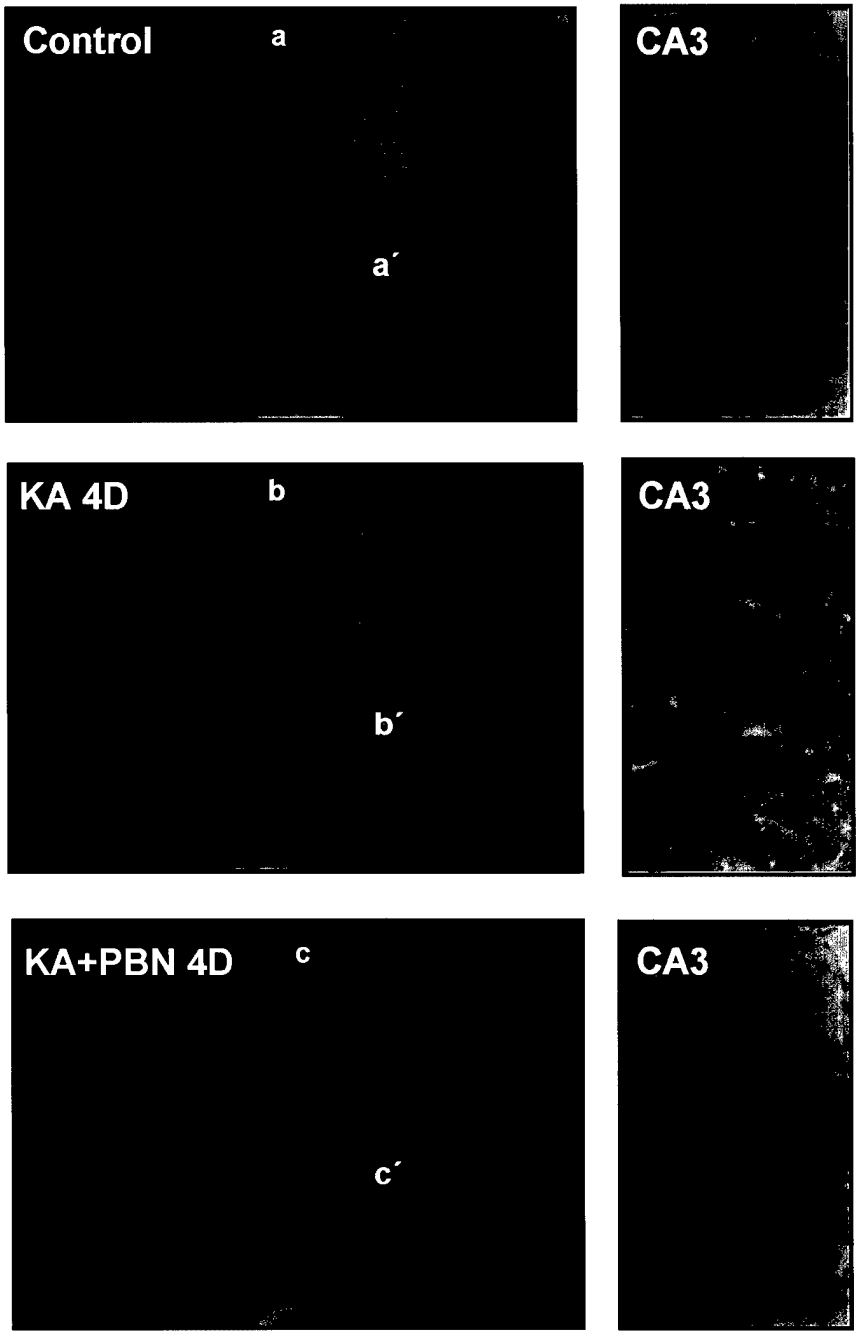

Figure 1. Kainic acid induces apoptosis in the hippocampus as indicated by TUNEL staining. Brown staining (arrows) indicates DNA fragmentation. Tissue was counterstained with methyl green. Boxed areas indicate magnification of CA1 (insets) and CA3 subregions (rightmost panels).

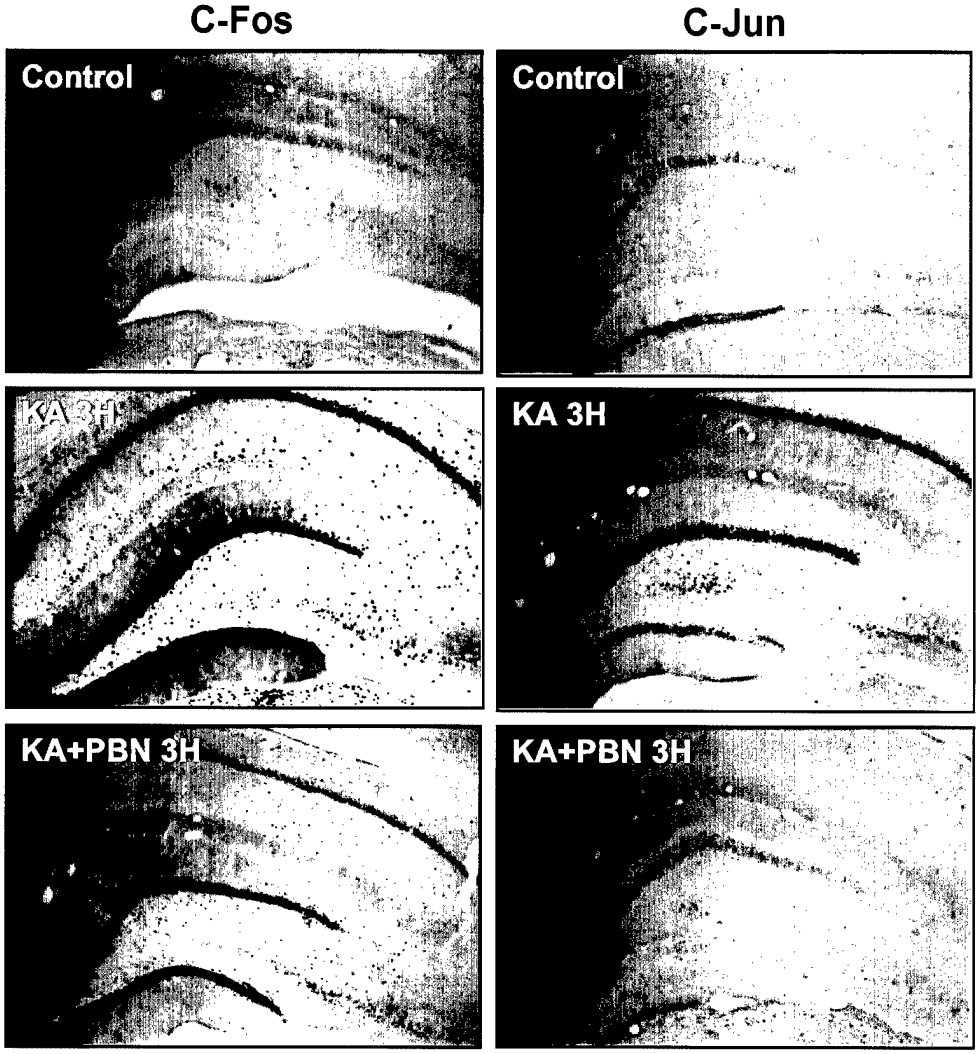

Figure 2. Kainic acid increases the expression of AP-1 transcription factor components in the hippocampus as evidenced by increases in c-Fos and c-Jun immunoreactivity. Cotreatment with PBN suppresses c-Jun expression globally, however, c-Fos expression is suppressed only in the CA1 and CA3 regions while c-Fos expression in the dentate gyrus was largely unaffected by PBN.

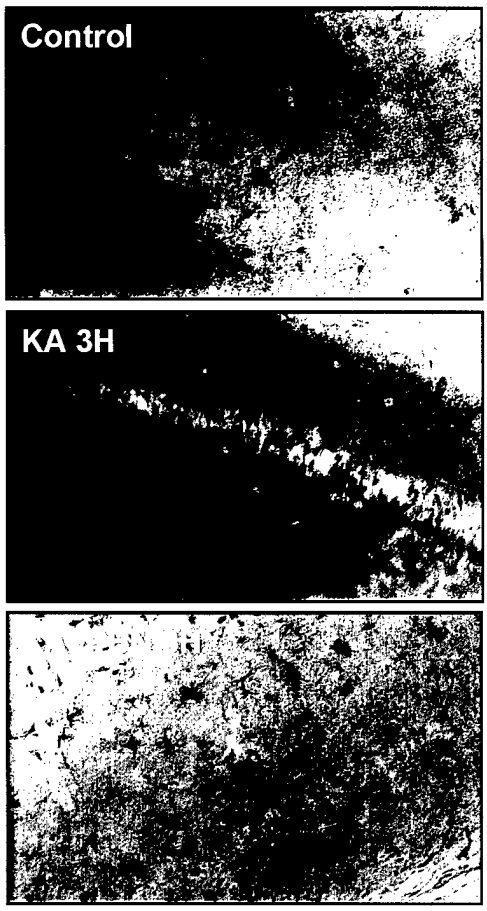

Figure 3. Kainic acid increases p38-MAPK activation in the hippocampus as indicated by increased phosphorylation of the p38-MAPK activation domain. The CA1 subregion is depicted. Immunohistochemistry was performed using an antibody directed against the phosphorylation domain of the active p38 MAPK enzyme ($pThr^{180}$-Gly^{181}-$pTyr^{182}$).

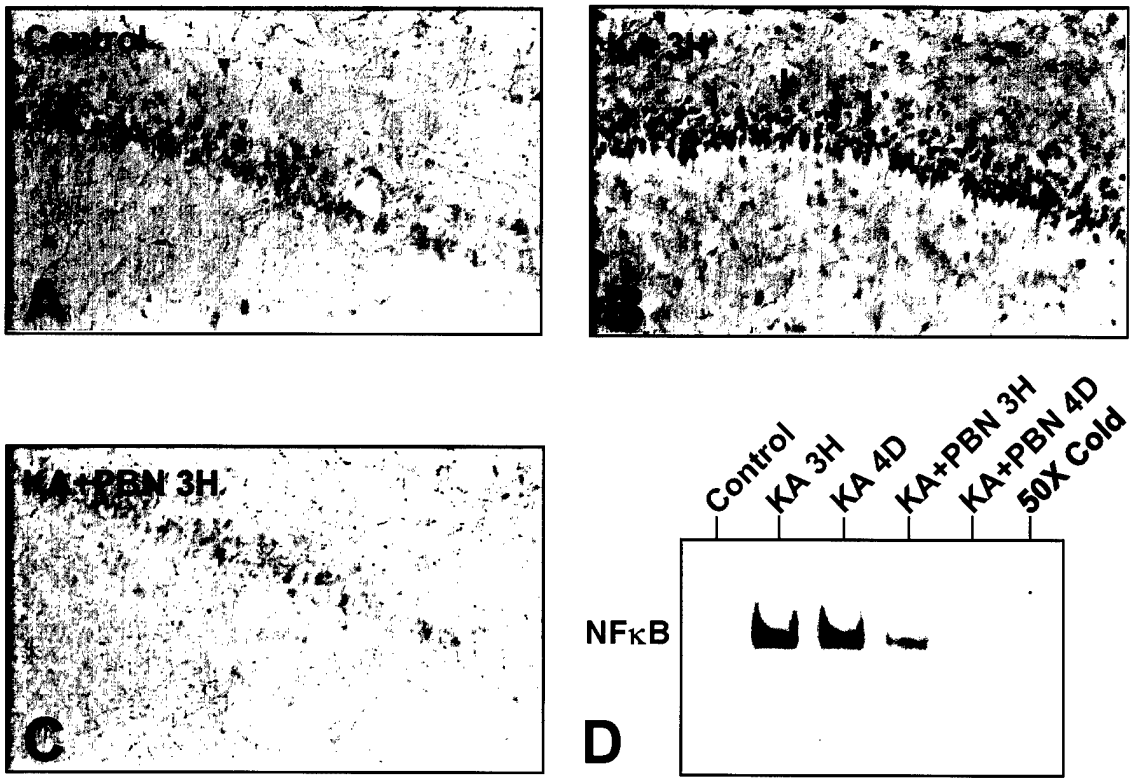

Figure 4. Kainic acid increases NFκB activation in the hippocampus. A,B, and C illustrate exposure of the p65 subunit of the NFκB complex following KA treatment (arrows). D: Electromobility gel shift assay demonstrating increased NFκB binding activity in nuclear extracts induced by KA treatment and suppressed by cotreatment with PBN. Specificity of binding was evidenced by competition for the NFκB complex by an unlabeled (cold) oligonucleotide probe (rightmost lane).

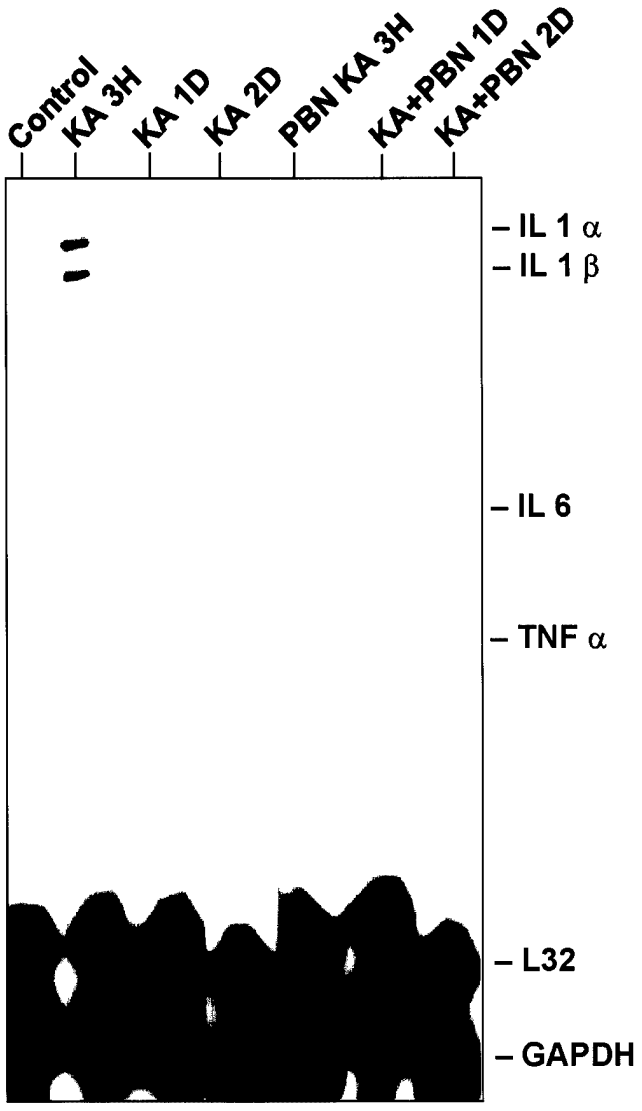

Figure 5. Kainic acid stimulates the transcription of proinflammatory cytokines in the hippocampus as determined by multiprobe ribonuclease protection assay (RPA).

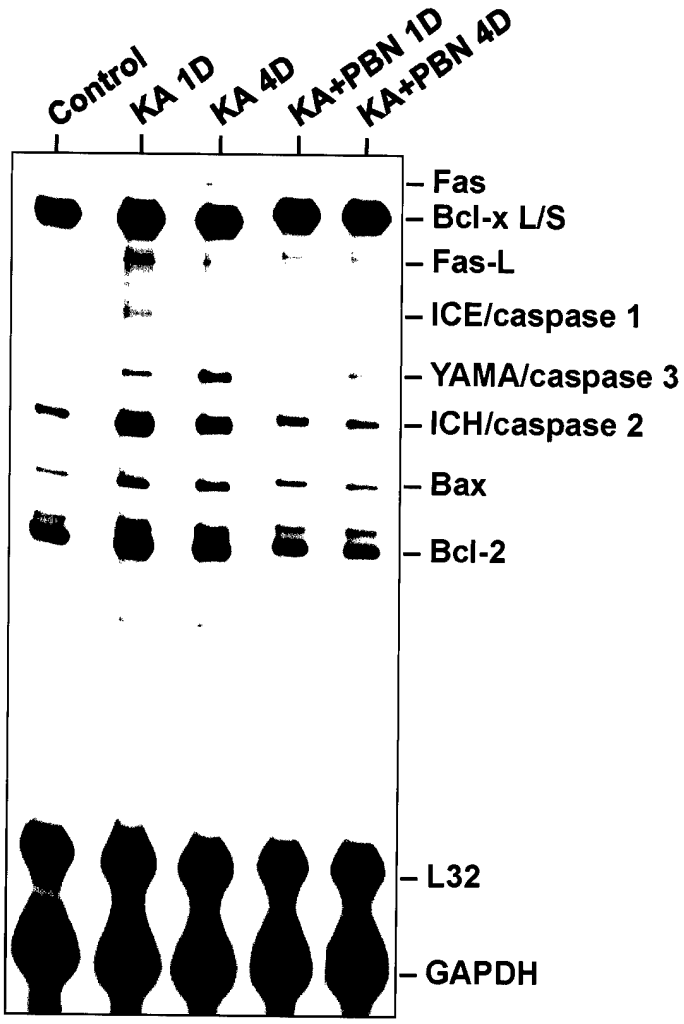

Figure 6. Kainic acid stimulates transcription of proapoptotic genes in the hippocampus as determined by multiprobe ribonuclease protection assay (RPA).

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Phenyl-N-*tert*-butylnitrone Inhibits Neuronal Apoptosis in the Kainic Acid Model of Epilepsy by Suppressing Proapoptotic Signal Transduction Pathways

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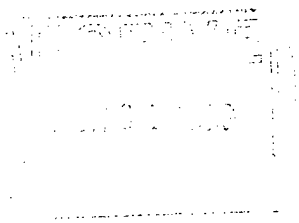

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ABSTRACT

Exposure of rats to kainic acid (KA), a non-NMDA type glutamate receptor agonist, induces recurrent (delayed) convulsive seizures and hippocampal neurodegeneration reminiscent of human epilepsy. In this study, the effects of KA were studied with respect to three separate signal transduction pathways likely to regulate inflammatory and apoptotic gene expression in the hippocampus. Immunohistochemical methods and electromobility gel shift assays (EMSAs) demonstrate the concerted activation of the NFκB pathway along with the activator-1 pathway (AP-1) and the p38 mitogen-activated protein kinase pathway (p38 MAPK). Activation of these three pathways occurred simultaneously with the expression of several proapoptotic biomolecules (most notably TNFα and the Fas antigen) and simultaneously with the onset of convulsive seizures but prior to the initiation of neuronal apoptosis. Cotreatment with the experimental antioxidant and antiinflammatory compound phenyl-N-*tert*-butylnitrone (PBN) resulted in a diminution of NFκB, AP-1 and p38 activation, suppressed cytokine and apoptotic gene expression, inhibited neuronal apoptosis, and diminished seizure activity. These data suggest that pharmacological antagonism of multiple signal transduction pathways is achievable in the brain, and that inhibition of these processes may prevent a cascade of gene-inductive events leading to neuronal apoptosis.

Keywords: Kainic acid, inflammation, apoptosis, nitrone, kinase.

The ability to commit apoptosis, or programmed self-destruction, is inherent to most somatic cells and doubtlessly serves a vital function during periods of tissue development or remodeling, or as a defense against neoplastic transformation. Apoptosis must be tightly regulated in order to avoid capricious destruction of healthy tissue. Nonetheless, apoptosis occurs in numerous pathological states, under conditions where deliberate cell death confers no obvious adaptive benefit. In these latter circumstances, an arrest of apoptosis by agents which antagonize the appropriate signal transduction pathways may result in a net benefit to the damaged tissue, and the organism.

With the goal of identifying new strategies for inhibiting neuronal apoptosis, we have begun to explore the molecular basis for apoptosis in a commonly used animal model of epilepsy. In the kainic acid (KA) model of epilepsy, a single systemic dose of the excitotoxin kainic acid initiates a process of hippocampal neurotoxicity (Bernard and Wheal 1995). Rats treated with kainic acid suffer recurrent convulsive seizures and apoptotic neuron loss in the CA1 and CA3 regions of the hippocampus (Pisa et al., 1980; Schwob et al., 1980; Ben Ari et al., 1985; Tauk et al., 1985). Seizure activity is correlated with neuroanatomical changes including mossy fiber sprouting in the dentate gyrus, hippocampal sclerosis, and eventually, neuronal death (Schwob et al., 1980; Sauk et al., 1985; Sperk et al., 1985; Cronin et al., 1992). The lesions produced by systemic kainic acid treatment resemble those seen in hippocampi of human epileptics (Sommer et al., 1880; Schwob et al., 1980; Pisa et al., 1980; Ben Ari et al., 1985; Sperk et al., 1985). Kainic acid appears to act directly on non-NMDA type ionotropic glutamate receptors (Bernard and Wheal 1995), leading to cell death which is predominantly apoptotic in nature (Simonian et al., 1996; Bengzon et al., 1997; Yang et al., 1997; Cheung et al., 1998).

We hypothesized that KA chronically stimulates signal transduction pathways linked to apoptotic gene induction within sensitive populations of hippocampal neurons. In support of this hypothesis, we present evidence that several distinct signal transduction pathways are activated in the hippocampus between 3 hours

and 4 days following systemic exposure to KA. In particular, immunochemical studies and electromobility gel shift assays (EMSAs) demonstrate activation by KA of the NFκB (nuclear factor kappa B) system, the AP-1 (activator protein 1) system, and the p38 mitogen activated protein kinase (p38 MAPK) pathway. Within the timeframe that these signal transduction systems become hyperactivated, numerous proinflammatory and proapoptotic genes are transcriptionally upregulated in a concerted fashion. Most intriguingly, treatment of the KA-exposed animals with the compound phenyl-N-*tert*-butylnitrone (PBN, a brain-accessible antioxidant with potent anti-inflammatory and anti-excitotoxic action) (Hensley 1997) inhibits KA-induced neuronal apoptosis, down-regulates apoptosis-associated gene expression, and moreover, prevents seizure activity and death. These results clarify the molecular basis for KA-induced seizure activity and may indicate a novel therapeutic strategy for certain chronic neurodegenerative disorders.

MATERIALS AND METHODS

Animals. Adult male Sprague Dawley rats (225-250 g each) were injected subcutaneously with KA (Sigma Chemical, St. Louis MO) at a dose of 10 mg/kg, or with vehicle alone (saline). Animals were observed for four hours following KA treatment and seizure activity was rated according to the scale developed by Racine (1972). Phenyl-N-*tert*-butylnitrone was synthesized at the Oklahoma Medical Research Foundation (Oklahoma City, OK) and was injected at a dose of 150 mg/kg intraperitoneally, in saline vehicle, 90 minutes after KA treatment.

Immunohistochemistry. For immunocytochemical studies, animals were anesthetized with pentobarbital and perfused with saline followed by 4 % paraformaldehyde in saline. Brains were sectioned into 30 μm slices, which were incubated in 4 % normal goat serum in saline for 30 min. at ambient temperature. After three washes with saline, the sections were incubated overnight at 4°C in saline plus 0.025 % triton X-100, 1 % goat

serum, and primary antibody. Immunoreactivity was visualized by the avidin-biotin-bridged immunoperoxidase method using 3,3'-diaminobenzidine (DAB) as the chromagen (Hsu et al., 1981). The anti-phospho-p38 antibody was an affinity purified IgG purchased from New England Biolabs (Beverly, MA), used at 1/300 dilution. Affinity purified antibodies against c-Fos, c-Jun and the p65 subunit of NFκB were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and were used at 1/1000, 1/1000, and 1/300 dilution, respectively. Photomicroscopy was performed on a Zeiss Axioplan 2 instrument (Carl Zeiss Inc., Thornwood, NY).

Electromobility gel-shift assays (EMSAs). EMSAs were conducted to determine binding of activated NFκB complexes to synthetic oligonucleotide consensus sequences. The NFκB-binding oligomer was a 22-mer: 5'-GATCGAGGGGACTTTCCCTAGC-3', purchased from Stratagene (La Jolla CA). Double-stranded oligomers were labeled with [γ-^{32}P]ATP using 10 u/reaction of T4 polynucleotide kinase (U.S. Biochemical Corp., Cleveland, OH). Hippocampi were dissected free and homogenized, and nuclear protein extracts were prepared as described (Sonnenberg et al., 1989). Binding reactions (30 µL) were performed at room temperature in reaction mixtures containing 40 µg protein, 20 mM Tris-HCL pH 7.8, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol, 50 µg/mL bovine serum albumin, 100 µg/mL sonicated salmon sperm DNA, 10 % glycerol, and approximately 0.2 ng (50,000 cpm) of the specific probe. Protein-DNA complexes were separated on 5 % nondenaturing polyacrylamide gels run at 150 V in 50 mM Tris/ 50 mM boric acid / 1 mM EDTA. Gels were then dried and autoradiographed overnight.

Terminal deoxyuridine nick-end labeling (TUNEL). DNA fragmentation characteristic of apoptosis was visualized by 3'end labeling with biotin-derivatized deoxynucleotides via terminal deoxynucleotidyl transferase catalysis. A commercially available TUNEL kit was used (TdT FragEL, Calbiochem, San Diego CA).

Biotinylated nucleotides were detected using streptavidin-conjugated horseradish peroxidase and diaminobenzidine (Hsu et al., 1989). Tissue sections thus labeled were counterstained with methyl green as an aid to morphological evaluation.

Ribonuclease protection assays. Approximately 100 mg of hippocampal tissue was homogenized in trizol isolation reagent (Life Technologies, Gaithersburg, MD) using a Dounce-type homogenizer. Total RNA in the extract was quantified by UV absorbance at 260 nm. Inflammation and apoptosis-associated mRNA species were selectively visualized using a multiprobe ribonuclease protection assay (RPA). Radiolabeled probes were synthesized from DNA templates containing a T7 RNA polymerase promoter (Pharmingen, San Diego, CA). Templates were transcribed in the presence of [γ-^{32}P]ATP to yield radioactive probes of defined size for each mRNA. Probes were hybridized with total hippocampal RNA, then samples were treated with RNAse A and T1 to digest single-stranded RNA. Intact double-stranded RNA hybrids were resolved on 5 % polyacrylamide / 8 M urea gels to produce bands detected by autoradiography.

RESULTS

Beginning approximately 30 minutes after KA injection, animals displayed archetypical epileptiform behavior including "wet dog" shakes, facial clonus, nodding, and forelimb clonus. Three hours after injection, KA-treated rats showed full limbic motor seizures including rearing and loss of postural control, as well as hypersalivation, circling and jumping. Rats treated with PBN 30 minutes after KA injection did not develop full limbic seizures by the 3 hour time point (Table I). Moreover, PBN rescued the KA-treated animals from mortality when evaluated at the end of the four day experiment (Table I).

KA treatment causes alteration in glutaminergic neurotransmission, which is intimately linked to recruitment of certain transactivating factors such as the AP-1 complex (Cheung et al., 1998). Transcriptional

activity of AP-1 is largely regulated by phosphorylation via the c-Jun amino terminal kinase (JNK). Interestingly, transgenic mice lacking the JNK-3 gene product (a brain-specific JNK isoform) are resistant to kainic acid-induced seizures and death (Yang et al., 1997). The first immunochemical analysis of KA-treated rats was therefore aimed at determining whether PBN could antagonize the AP-1 system *in vivo*. Immunocytochemical analysis was performed using antibodies against immediate early gene products c-Fos and c-Jun, which dimerize to form the AP-1 complex. Within hours of KA treatment, c-Fos and c-Jun expression increased in hippocampal neurons, particularly within the CA1 and CA3 regions (Fig. 1). The c-Fos and c-Jun expression was maintained throughout the four day experiment (not illustrated), consistent with previously reported data (Bing et al., 1997). A single injection of PBN completely suppressed c-Jun expression in both CA regions and in the dentate gyrus (Fig. 1) while c-Fos expression was suppressed by PBN only in the CA1 and CA3 regions, where most of the pathological changes were manifest (Fig.1 and discussed further below). It may be significant to note that while c-Jun expression can be induced rapidly in neurons during growth factor deprivation, c-Fos expression seems to be restricted to those populations of neurons which actually commit to an apoptotic program (Estus et al. 1994).

The AP-1 pathway is but one of numerous signal transduction pathways which have been associated with cellular stress and linked to ligand-induced neurotoxicity. The p38 mitogen-activated protein kinase pathway has been causally linked to neuronal apoptosis induced by growth factor withdrawal (Xia et al. 1995; Kummer et al. 1997). Moreover, pharmacological antagonism of p38 protects cultured neurons against glutamate excitotoxicity (Kawasaki et al. 1997) and we have shown PBN to antagonize cytokine- and hydrogen peroxide-induced p38 activation in cell culture (Robinson et al., 1999). We therefore undertook an immunohistochemical analysis of p38 activation using an antibody specifically directed against the dual-phosphorylation motif which is present only on the active p38 kinase (Raingeaud et al., 1995). Within 4 hours of KA treatment, p38 activation was seen within the hippocampus in a pattern consistent with that of AP-1

activation (Fig. 2). As in the case of AP-1, PBN suppressed p38 phospho-activation (Fig. 2). The p38 system remained activated somewhat above the level of controls at the four day timepoint, but this chronic activation was not as dramatic as in the AP-1 case (not shown).

The NFκB transcription factor is also ubiquitously activated by physiologic stress and may potentiate excitotoxic damage in striatal neurons (Qin et al. 1998). Alternatively, NFκB seems to serve a protective role in hippocampal neurons undergoing an oxidative insult (Mattson et al. 1997) and may actually play an antiapoptotic role in TNFα-stimulated cells (Van Antwerp et al. 1996; Wang et al. 1998). NFκB is part of a signal transduction cascade which is largely distinct from the Jnk and p38 cascade modules. We therefore sought to determine whether NFκB was activated by KA in a PBN-sensitive manner. NFκB activation can be indexed several ways. Immunologically, NFκB activation can be inferred from increased immunoreactivity of an epitope on the p65 subunit which is exposed upon NFκB recruitment (Rice and Ernst, 1993). As shown in Fig. 3, NFκB-p65 immunoreactivity in the hippocampus increased dramatically within hours of KA treatment, and this effect was suppressed by PBN. The immunochemical data was corroborated by EMSA analysis which showed a dramatically-increased NFκB binding activity in hippocampal nuclei of KA treated rats, which was partially mitigated by PBN cotreatment (Fig. 3).

Hyperactivation of the Jnk, NFκB and p38 signal transduction pathways could be anticipated to have numerous detrimental consequences. All three signaling pathways have been linked to transcription of inflammatory cytokines and to modulation of apoptosis (Kawasaki et al., 1997; Kummer et al., 1997; Yang et al., 1997; Qin et al., 1998). We therefore sought to determine whether cytokine and proapoptotic genes were being transcribed at a greater rate in the KA treated rats than in normal rats, and whether PBN could abrogate such an effect. Using a multiprobe ribonuclease protection assay, several inflammatory cytokines were clearly found to be transcribed following KA treatment (Fig. 4). IL1α, IL1-β, IL-6 and TNF-α transcription were strongly induced by KA. Within the timeframe that cytokine transcription was enhanced, several proapoptotic

genes were also induced. Most notably, the Fas antigen mRNA was strongly induced following KA and this elevation was maintained for at least four days (Fig. 5). PBN treatment suppressed transcription of both inflammatory cytokine gene products and proapoptotic gene products while having minimal effect on transcription of constitutively-expressed "housekeeping genes" including the L-32 ribosomal mRNA and glyceraldehyde phosphate dehydrogenase mRNA (Figs. 4-5). PBN suppression of cytokine mRNA transcription was relatively unspecific. Interestingly, PBN displayed particular potency in suppressing Fas antigen and caspase 3 transcription, while other apoptosis-associated mRNA species analyzed by RPA were somewhat less affected by the nitrone (Fig. 5).

As a final indication of KA-induced hippocampal damage, *in situ* TUNEL staining was performed to assess frank apoptosis. KA treatment caused DNA damage indicative of an apoptotic process within four days of subcutaneous administration (Fig. 6). Apoptosis was largely restricted to the CA1 and CA3 regions of the hippocampus wherein c-Fos was most strongly expressed. Administration of PBN 30 minutes after KA exposure strongly inhibited this apoptosis as indicated by diminished TUNEL staining in hippocampi from PBN treated animals (Fig. 6). TUNEL staining for apoptotic nuclei therefore corroborates the pattern of of KA-induced and PBN-sensitive immediate early gene expression depicted in Fig. 1, and the pattern of proapoptotic gene induction illustrated in Fig. 5.

DISCUSSION

Kainic acid is a well-studied neurotoxin which elicits an animal model of temporal lobe epilepsy and delayed neuronal death in the CA1 and CA3 hippocampal regions. Kainic acid kills neurons in culture by an apoptotic pathway involving binding to non-NMDA type glutamate receptors and consequent expression of immediate early genes including *c-Jun* (Cheung et al. 1998). In the present study, we extend upon these cell culture experiments by documenting increased c-Fos and c-Jun expression (i.e., activation of the AP-1 signaling

pathway) within the rat hippocampus following systemic administration of KA. Furthermore, we document the activation of two distinct signal transduction pathways, the NF*k*B pathway and the p38 MAPK pathway, following the KA challenge. Activation of these three distinct signal transduction pathways correlated temporally with the transcription of both proinflammatory cytokines and proapoptotic mRNA species. Inhibition of these three pathways by the experimental compound phenyl-*tert*-butylnitrone was associated with diminished cytokine elaboration, prevention of neuronal apoptosis, reduced seizure activity, and reduced mortality. While the AP-1, NF*k*B, and p38 pathways are known to respond positively to oxidants and negatively to antioxidants in cell culture (Suzuki et al. 1994; Guyton et al. 1996; Robinson et al. 1999), the data in this present study are the first to demonstrate the sensitivity of these three pathways to an antioxidant compound within the context of an established *in vivo* model of hippocampal neurodegeneration.

The findings of the present study extend upon previous observations concerning the broad-spectrum neuroprotective action of nitrone compounds, and provide a novel context for discussing the pathology of excitotoxicity. PBN and related nitrones have been shown to suppress striatal excitotoxic lesions induced by NMDA, KA, and AMPA, though not by virtue of any obvious direct interaction with glutamate receptors (Shultz et al., 1995). Similarly, PBN and a sulfated analog inhibit striatal lesions caused by mitochondrial inhibitors such as malonate and the 1-methyl-4-phenylpyridinium (MPP+; Shultz et al. 1995). Nitrones suppress apoptosis and oxidative stress in cultured Down's syndrome neurons (Busciglio and Yankner 1995), and similarly inhibit chemically-induced thymocyte apoptosis *in vitro* (Slater et al. 1995), though the influence of nitrones on apoptosis *in vivo* has not been well-studied. Unfortunately, the pharmacologic effects of nitrones in most previous investigations were not correlated with biomarkers of oxidative stress, inflammation or apoptosis. The present data suggest that suppression of apoptosis by PBN in the KA model and possibly other models of neurodegeneration is likely due to mitigation of proinflammatory or proapoptotic gene expression under the control of the AP-1, NFkB, and p38 MAPK pathways. While the ultimate cellular target(s) for PBN

action remain unclear, the present data suggest that the broad-spectrum neuroprotective action of the nitrone class of compounds (Hensley et al. 1997) might be due, in part, to antagonism of crucial oxidation-sensitive signal transduction elements linked to the initiation of apoptotic programs.

It may be impossible to separate the anti-inflammatory, anti-apoptotic and antioxidant effects of PBN or other, similar pharmacophores. For instance, stimulation of primary astrocytes or fibroblasts with the inflammatory cytokine IL1β elicits cellular H_2O_2 production (Meier et al., 1989, Robinson et al. 1999) which is partially inhibited by PBN (Robinson et al. 1999). Moreover, addition of exogenous H_2O_2 causes *de novo* cytokine expression in these cells (personal observations). These and similar findings have led to the postulate that H_2O_2 is an intracellular messenger involved with inflammatory signal transduction (Suzuki et al., 1994; Robinson et al. 1999). Agents such as PBN which uncouple ligand-receptor binding from intracellular oxidant production might therefore inhibit inflammation and apoptosis as well as diminish cellular oxidative stress. Consistent with such a notion and shown in the present study, PBN inhibits cytokine and apoptosis-associated gene expression *in vivo* following KA challenge. Thus, part of the reported antioxidant effects of PBN extracted from previous *in vivo* studies might reflect a secondary consequence of the gene suppressive and anti-inflammatory action of this compound, possibly mapping to an unknown but centrally-located immunologic target of action.

In the KA model of hippocampal neurodegeneration, pharmacologic inhibition of pathways leading to apoptosis is correlated with a positive physiologic outcome (survival and diminished seizure activity). Thus, aversion of apoptosis beneficial in this particular model. While the present study does not address the relative importance of the several signal transduction pathways which are inhibited by PBN, the results suggest that certain drugs may antagonize multiple stress-related signal transduction pathways. Pharmacologic strategies designed to specifically inhibit individual signaling modules, such as the NFκB, AP-1 or p38 modules, might therefore be unnecessary and might be more difficult to execute *in vivo* than alternative strategies designed to

suppress multiple signaling processes simultaneously. Evaluation of these issues must await future investigations aimed at identifying the central control systems which regulate proinflammatory and proapoptotic signaling in the hippocampus, particularly with respect to localizing specific oxidation-sensitive elements that may be targeted by nitrone-type neuroprotective agents.

REFERENCES

Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O (1997) Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc. Natl. Acad. Sci. USA 94: 10432-10437.

Bernard C, Wheal HV (1995) Plasticity of AMPA and NMDA receptor-mediated epileptiform activity in a chronic model of temporal lobe epilepsy. Epilepsy Res. 21: 95-107.

Bing G, Wilson B, Hudson P, Jin L, Feng Z, Zhang W, Bing R, Jau-Shyong H (1997) A single dose of kainic acid elevates the levels of enkephalins and activator protein-1 transcription factors in the hippocampus for up to one year. Proc. Natl. Acad. Sci. USA 94: 9422-9427.

Busciglio J, Yankner, BA (1995) Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. Nature 378: 776-779.

Carney JM., Starke-Reed PE, Oliver CN, Landum RW, Cheng MS, Wu JF, Floyd RA (1991) Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound, N-*tert*-butyl-α-phenylnitrone. Proc. Natl. Acad. Sci. USA 88: 3633-3636.

Cheung NS, Carroll FY, Larm JA, Beart PM, Giardina SF (1998) Kainate-induced apoptosis correlates with c-Jun activation in cultured cerebellar granule cells. J. Neurosci. Res. 52: 69-82.

Culcasi M., Lafon-Cazal M., Pietri S., Bockaert J. (1994) Glutamate receptors induce a burst of superoxide via activation of nitric oxide synthase in arginine-depleted neurons. J. Biol. Chem. 269: 12589-12593.

Cronin J, Obenaus A, Houser CR, Dudek FE (1992) Electrophysiology of dentate granule cells after kainate-induced synaptic reorganization of the mossy fibers. Brain Res. 573: 305-310.

Estus S, Zaks WJ, Freeman RS, Gruda M, Bravo R, Johnson EM (1994) Altered gene expression in neurons during programmed cell death: Identification of c-Jun as necessary for neuronal apoptosis. J Cell Bio 127: 1717-1727.

Guyton KZ, Liu Y, Gorospe M., Xu Q, Holbrook NJ. (1996) Activation of mitogen-activated protein kinase by H_2O_2: Role in cell survival following oxidant injury. J. Biol. Chem. 271: 4138-4142.

Hensley K, Carney JM, Stewart CA, Tabatabaie T, Pye QN, Floyd RA (1997) Nitrone-based free radical traps as neuroprotective agents in cerebral ischemia and other pathologies. Int. Rev. Neurobiol. 40: 299-317.

Hsu SM, Raine L, Fanger HJ (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabelled antibody (PAP) procedures. Histochem. Cytochem. 29: 577-480.

Kawasaki H, Morooka T, Shimohama S, Kimura J, Hirano T, Gotoh Y, Nishida E (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cereballar granule cells. J Biol Chem 272: 18518-18521.

Kummer JL, Rao PK, Heidenreich KA (1997) Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. J Biol Chem 272: 20490-20494.

Lafon-Cazal, M., Pietri, S., Culcasi, M., Bockaert J. (1993) NMDA-dependent superoxide production and neurotoxicity. Nature 364: 535-537.

Mattson MP, Goodman Y, Luo H, Fu W, Furukawa K (1997) Activation of NFkB protects hippocampal neurons against oxidative stress-induced apoptosis: Evidence for induction of manganese superoxide dismutase and suppression of peroxinitrite production and protein nitration. J Neurosci Res 49: 681-697.

Meier B., Radeke H.H., Selle S., Younes M., Sies H., Resch K., Habermehl G.G. (1989) Human fibroblasts release reactive oxygen species in response to interleukin or tumor necrosis factor alpha. Biochem. J. 263: 539-545.

Pisa M, Sanberg PR, Corcoran ME, Fibiger HC. Spontaneously recurrent seizures after iintracerebral injections of kainic acid in rat: A possible model of human temporal lobe epilepsy (1980) Brain. Res. 200: 481-487.

Qin ZH, Wang Y, Nakai M, Chase TN (1998) Nuclear factor-kappa B contributes to excitotoxin-induced apoptosis in rat striatum. Mol Pharmacol 53: 33-42.

Racine R, Okujava V, Chipashvili S. Modification of seizure activity by electrical stimulation 3: Mechanisms (1972) Electroencephalogr. Cliln. Neurophysiol. 32: 295-299.

Raingeaud J, Gupta S, Rogers JS, Martin D, Han J, Ulevitch RJ, Davis RJ (1995) Proinflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J. Biol. Chem. 270: 7420-7426.

Rice N and Ernst, MK (1993) In vivo control of NFκB activation by IκBα. EMBO J. 12: 4685-4695.

Robinson KA, Stewart CA, Pye QN, Nguyen X, Kenney L, Salzman S, Floyd RA and Hensley K (1999) Redox sensitive protein phosphatase activity regulates the phosphorylation state of p38 protein kinase in primary astrocyte culture. J Neurosci Res. 55: 724-732.

Schwob JE, Fuller T, Price JL, Olney JW (1980) Widespread patterns of neuronal damage following systemic or intracerebral injections of kaiinic acid: A histological study. Neuroscience 5: 991-1014.

Shulz JB, Henshaw DR., Siwek D, Jenkins BG, Ferrante RJ, Cipolloni PB, Kowall NW, Rosen BR, and Beal MF (1995) Involvement of free radicals in excitotoxicity in vivo. J. Neurochem. 64: 2239-2247.

Simonian NA, Getz RI, Leveque JC, Konradi C, Coyle JT. (1996) Kainic acid induces apoptosis in neurons. Neuroscience 75: 1047-1055.

Slater AFG, Nobel CS, Maellaro E, Bustamante J, Kimland M, Orrhenius S. (1995) Nitrone spin traps and a nitroxide antioxidant inhibit a common pathway of thymocyte apoptosis. Biochem. J. 306: 771-779.

Sommer W. Erkrankung des ammonshorns als aetiologisches moment der epilepsie (1880) Arch. Psychiatr. Nervenkrankh. 10: 631-675.

Sonnenberg J, MacGregor-Leon P, Curran T, Morgan J (1989) Dynamic alterations occur in the levels and composition of transcription factor AP-1 complexes after seizure. Neuron 3: 359-365.

Sperk G, Lassmann H, Baran H, Seitelberger F, Hornykiewicz O (1996) Kainic acid-induced seizures: Dose-relationship of behavioral, neurochemical and histopathological changes. Brain Res. 338: 289-295.

Suzuki YJ, Mizuno M, Packer (1994) Signal transduction for nuclear factor-kB activation: Proposed location of antioxidant-inhibitable step. J. Immunol. 153: 5008-5015.

Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM (1996) Suppression of TNF-α-induced apoptosis by NFκB. Science 274: 787-789.

Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldrin AS (1998) NF-κB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281: 1680-1683.

Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270: 1326-1331.

Yang DD, Kuan CY, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, Rakie P, Flavell RA (1997) Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking th Jnk 3 gene. Nature 389: 865-870 .

Figure Legends

Figure 1. Kainic acid increases the expression of AP-1 transcription factor components in the hippocampus as evidenced by increases in c-Fos and c-Jun immunoreactivity. Cotreatment with PBN suppresses c-Jun expression globally, however, c-Fos expression is suppressed only in the CA1 and CA3 regions while c-Fos expression in the dentate gyrus was largely unaffected by PBN.

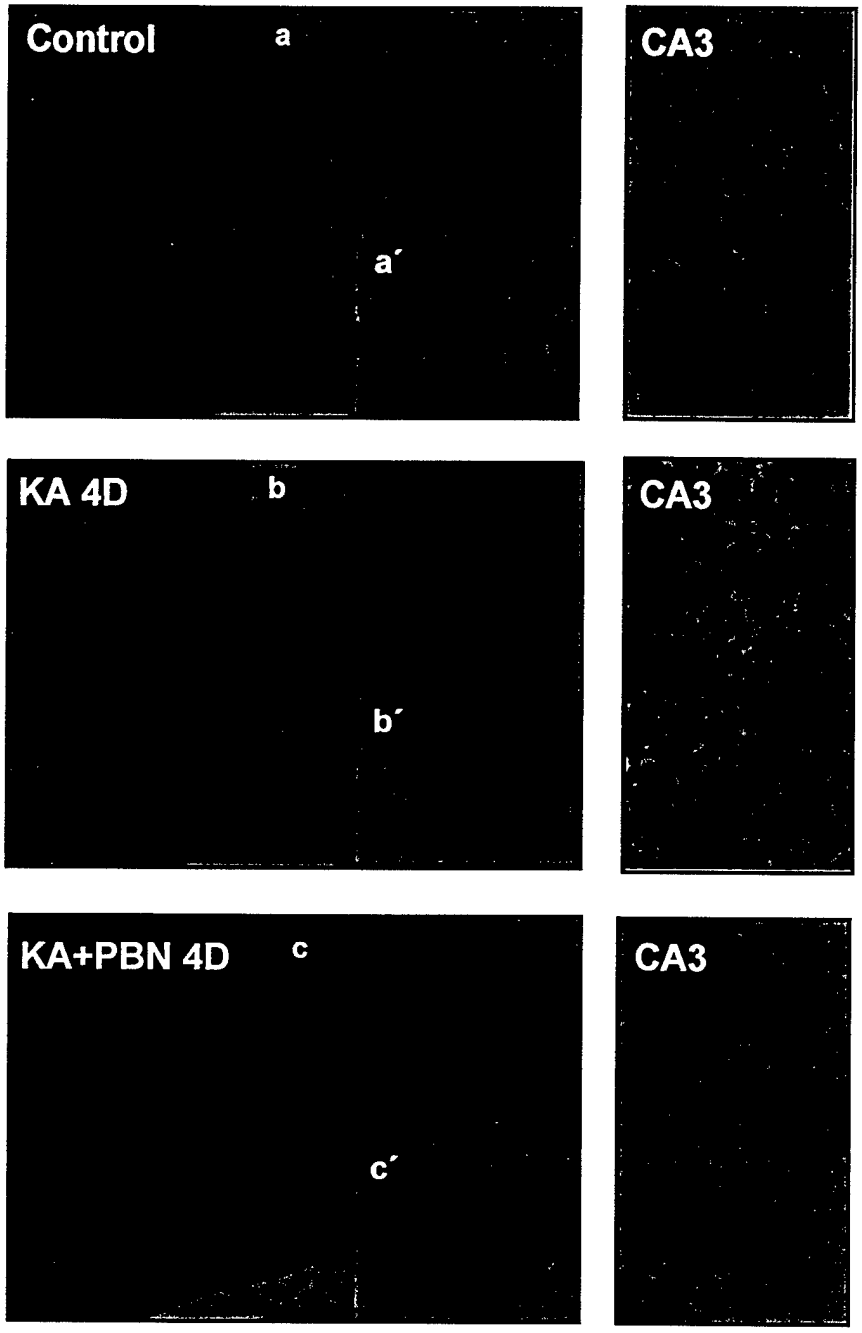
Figure 2. Kainic acid increases p38-MAPK activation in the hippocampus as indicated by increased phosphorylation of the p38-MAPK activation domain. The CA1 subregion is depicted. Immunohistochemistry was performed using an antibody directed against the phosphorylation domain of the active p38 MAPK enzyme ($pThr^{180}$-Gly^{181}-$pTyr^{182}$).

Figure 3. Kainic acid increases NFκB activation in the hippocampus. A,B, and C illustrate exposure of the p65 subunit of the NFκB complex following KA treatment (arrows). D: Electromobility gel shift assay demonstrating increased NFκB binding activity in nuclear extracts induced by KA treatment and suppressed by cotreatment with PBN. Specificity of binding was evidenced by competition for the NFκB complex by an unlabeled (cold) oligonucleotide probe (rightmost lane).

Figure 4. Kainic acid stimulates the transcription of proinflammatory cytokines in the hippocampus as determined by multiprobe ribonuclease protection assay (RPA).

Figure 5. Kainic acid stimulates transcription of proapoptotic genes in the hippocampus as determined by multiprobe ribonuclease protection assay (RPA).

Figure 6. Kainic acid induces apoptosis in the hippocampus as indicated by TUNEL staining. Brown staining (arrows) indicates DNA fragmentation. Tissue was counterstained with methyl green. Boxed areas indicate magnification of CA1 (insets) and CA3 subregions (rightmost panels).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a´
CA3
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b
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CA3
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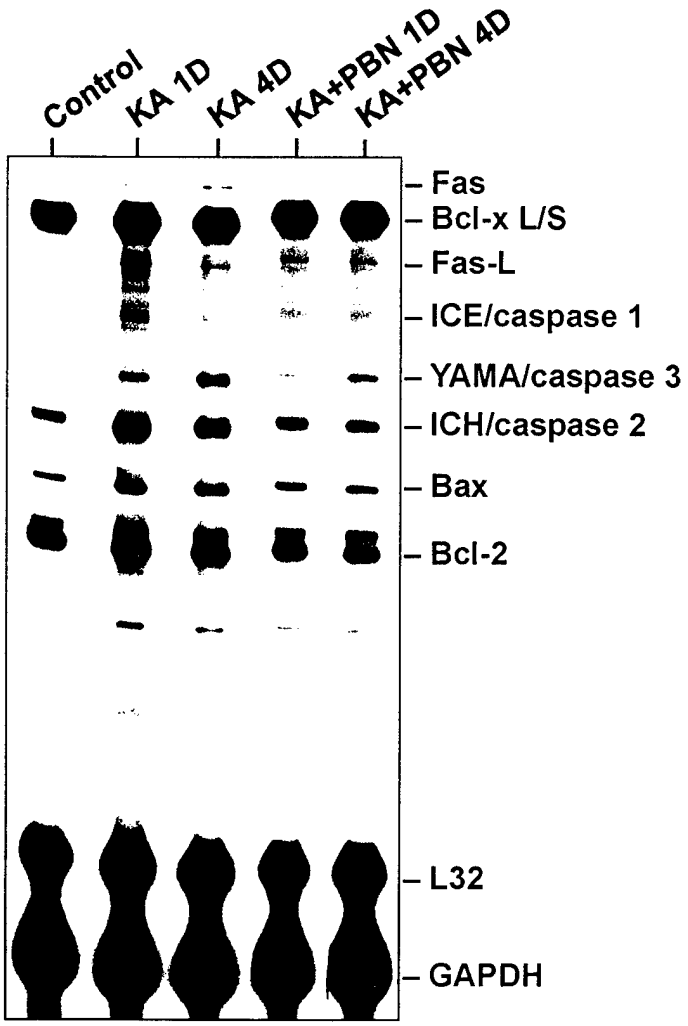
c
c´
CA3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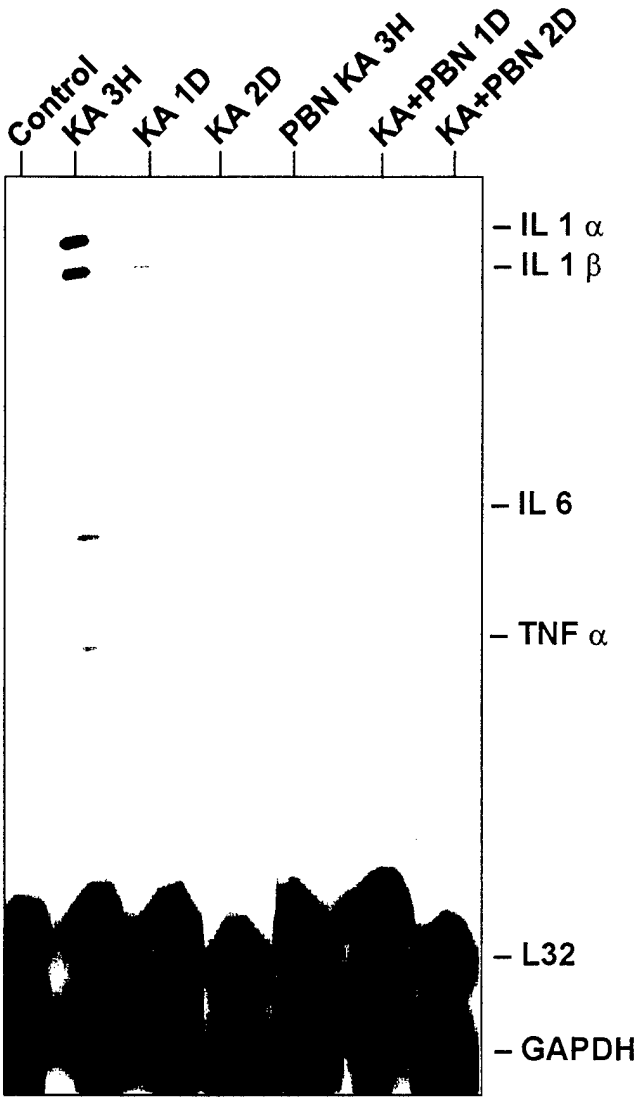
– ICH/caspase 2
– Bax
– Bcl-2
– L32
– GAPDH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PBN KA 3H
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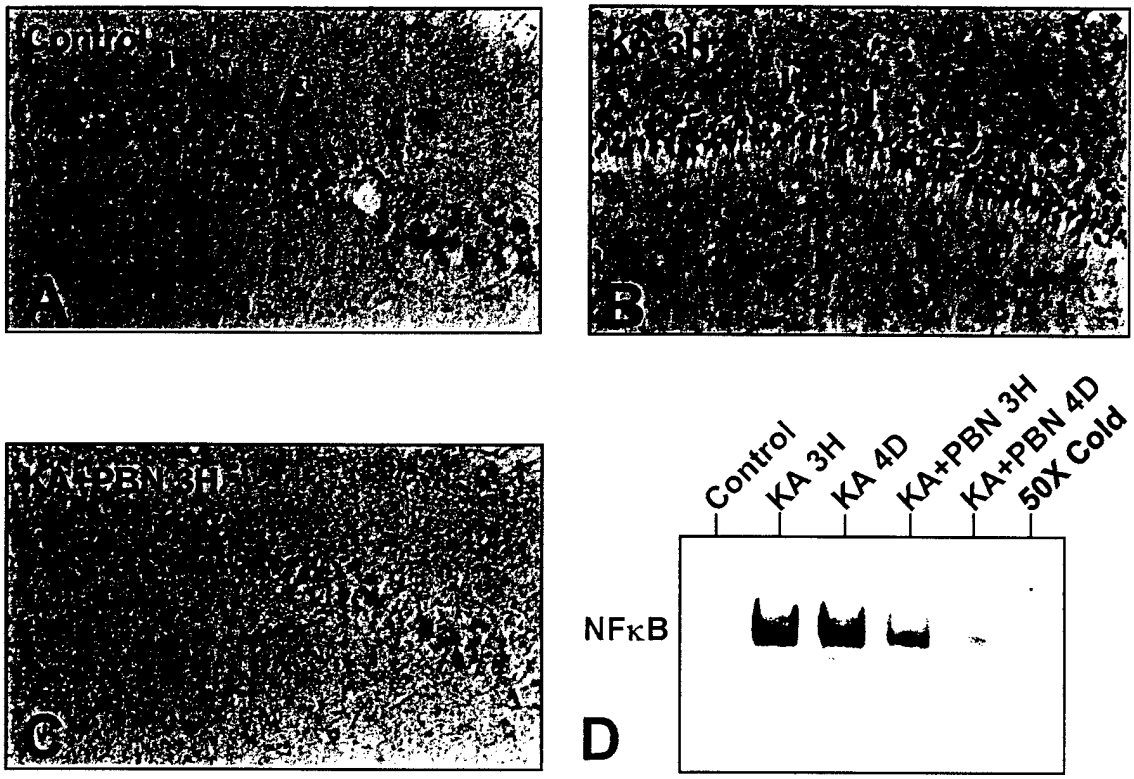
– IL 1 β
– IL 6
– TNF α
– L32
– GAPDH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B
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C
Control
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KA 4D
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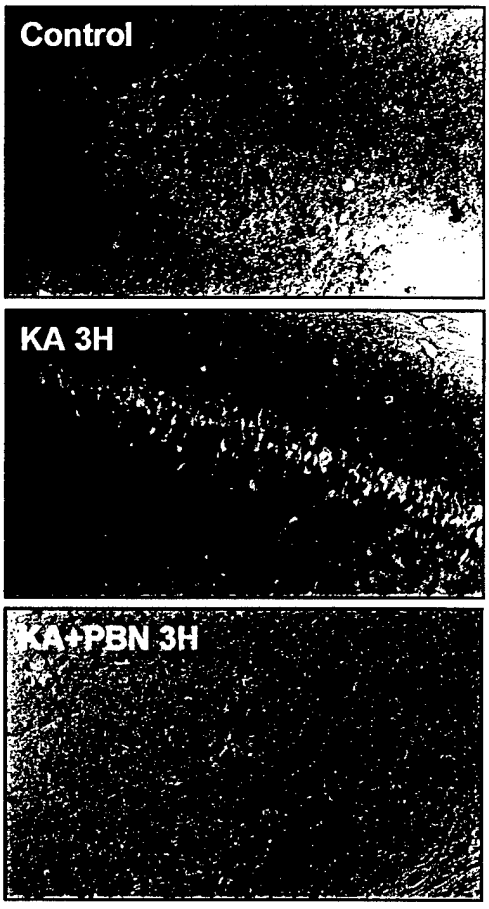
KA+PBN 4D
50X Cold
NFκB
D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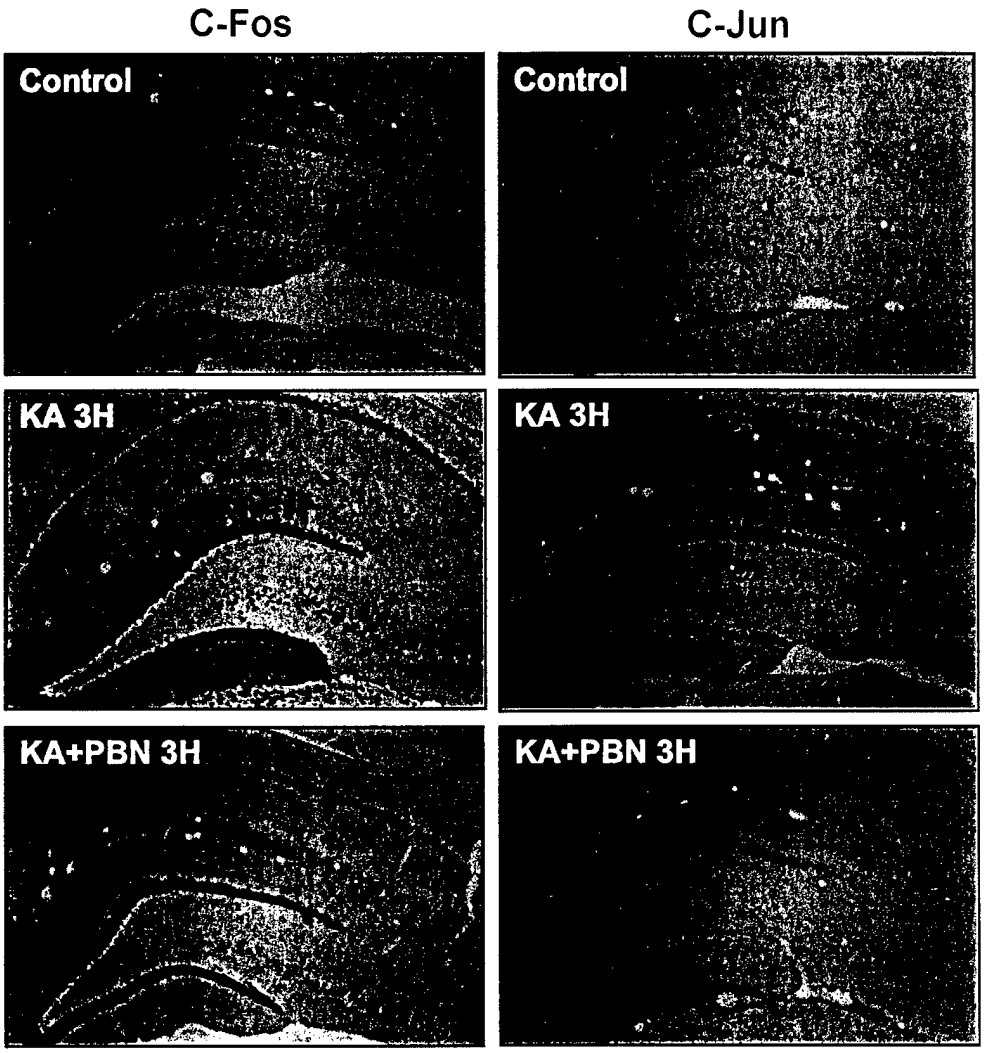
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C-Jun
Control
Control
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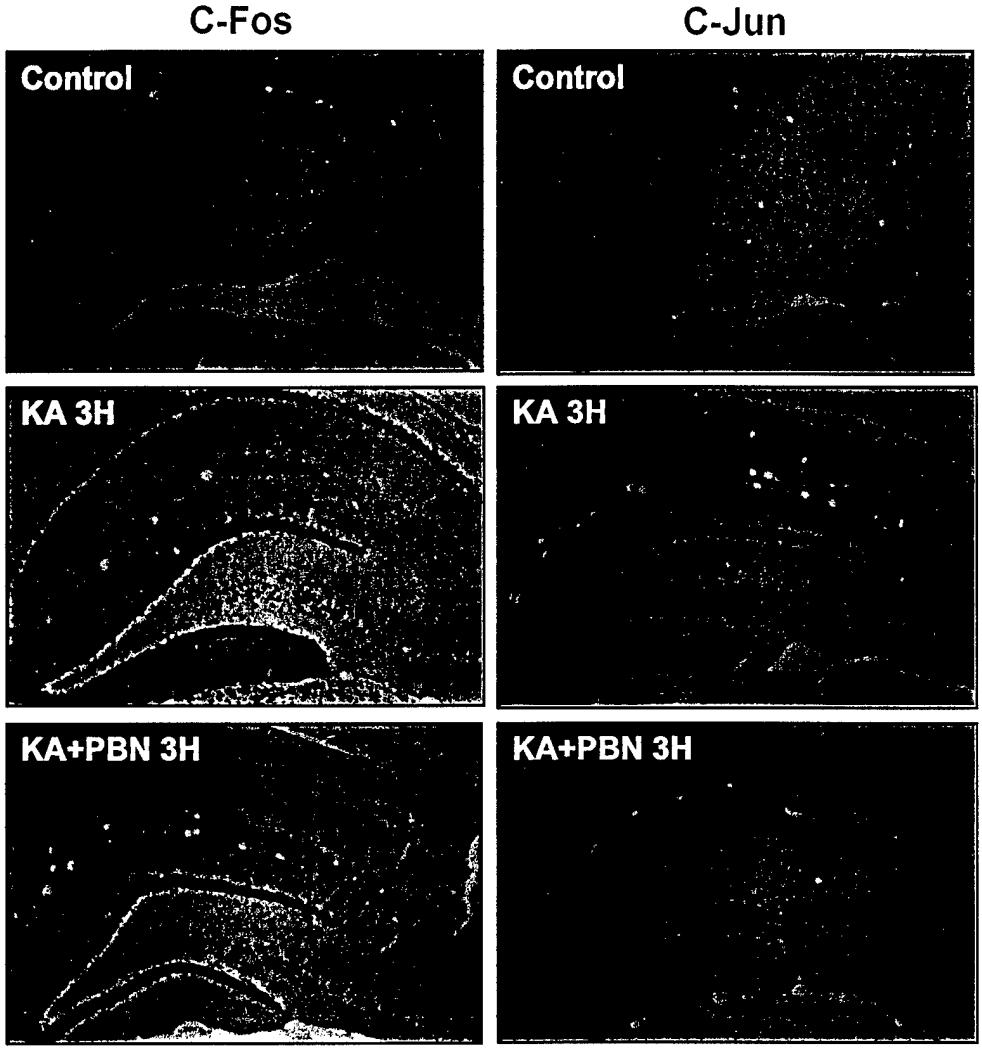
KA 3H
KA+PBN 3H
KA+PBN 3H


C-Fos
C-Jun
Control
Control
KA 3H
KA 3H
KA+PBN 3H
KA+PBN 3H

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admitted and had a CT scan of the brain and carotid doppler which were reportedly normal. There she was found to have a bradycardia and was transferred to the Cardiology service at Presbyterian Hospital. After a negative cardiac work up and Neurosurgical consultation she was discharged. On her way home in the parking lot, she had another episode of numbness of left side of her body involving face, arm and leg lasting approximately 40 minutes and she returned to the ER and was admitted to Neurology. Her past medical history was significant for Deep Vein Thrombosis at age 25, which was treated with warfarin for 6 months. Additional investigations showed Antithrombin 3 to be 57 (Normal: 70-140%), AT 3 AG (Immuno) to be 62 (Normal: 80-120%), Protein C to be 99 (Normal: 74-151%) and Protein S to be 49 (Normal: 60-185%). Her Factor V mutation was normal and Anticardiolipin antibody was negative.

19. F. Alan Stevens

CHANGES IN CEREBRAL PERFUSION AND PIAL CAPILLARY DIAMETER IN RESPONSE TO FLUID PERCUSSION INJURY. F.A. Stevens, P. Tompkins, S.C. Kim, C.M. Loftus, and P.C. Francel, Department of Neurosurgery, University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73104.

Traumatic brain injury results in a variety of brain abnormalities, both physiological and biochemical. There is evidence that the outcome of closed head injury may be dependent on early events such as reduced brain blood flow and vascular injury. Due to the time required to transport patients to the emergency room, little information is currently available from the clinical setting on these early vascular events. Because brain blood flow is so important in long term outcome, current treatment centers are returning brain blood flow to normal levels once it has been altered. Treatment includes mannitol to increase blood flow by reducing viscosity and resistance and the use of pressers to increase systemic blood pressure and improve cerebral perfusion pressure. While these attempts are sometimes successful, a lack of information on early chemical/vascular events following head injury impedes successful treatment of a majority of patients in this category. We have been using a closed cranial window model in the rat to assess both pial artery vessel diameter and laser Doppler perfusion in response to a moderate fluid-percussion cerebral injury (1.5 atm). The control group shows no change in either vessel diameter (PC based video imaging system) or laser Doppler perfusion over a two hour period. Fluid-percussion results in a 50% decrease in cerebral perfusion within 5 minutes of injury and at two hours post injury, perfusion is still reduced by 30% from pre-injury levels. No significant changes in pial artery diameter were detected over this time frame. Moderate cerebral trauma gives rise to prolonged alterations in cerebral perfusion that may contribute to long term vascular pathology. Continuing studies involve the use of pharmacologic agents to attenuate the vascular consequences of cerebral trauma.

20. *Xuan Nguyen

INVOLVEMENT OF OXIDANT-SENSITIVE SIGNAL TRANSDUCTION PATHWAYS IN HIPPOCAMPAL EXCITOTOXICITY. Xuan V. Nguyen[1], Kenneth Hensley, Charles A. Stewart, Nai-Ying Zheng[2], Lei Jin[2], Meili Zhu[2], Kelly Williamson[2], Robert A. Floyd[2], and Guoying Bing[2], [1]Graduate Student, Oklahoma Center for Neuroscience, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

Numerous neuropathological conditions are associated with stimulation of excitatory amino acid receptors in the central nervous system. In models of temporal lobe epilepsy, ionotropic glutamate receptor activation by kainic acid induces seizure activity and activates inflammatory and apoptotic pathways, resulting in hippocampal neurodegeneration. The nitrone antioxidant phenyl-N-*tert*-butylnitrone was found to reduce the severity of kainic acid-induced seizures, attenuate mRNA expression of inflammatory cytokine and apoptotic genes as evidenced by multiprobe ribonuclease protection assays, and reduce immunoreactivity toward NFB, AP-1, and phosphorylated p38-MAPK. Recently, we have observed that hippocampal lysate from kainic acid-treated mice exhibit changes in protein thiol and glutathione levels in a manner similar to that observed in cultured astrocytes exposed to hydrogen peroxide, further suggesting the involvement of oxidative mechanisms. These observations support a role of reactive oxygen species in mediating neurodegeneration in hippocampal excitotoxicity. Supported by grants from the Department of Defense, National Institutes of Health [NS35747], and Oklahoma Center for the Advancement of Science and Technology [HR97-067 and HR98-004]. Xuan is a Howard Hughes Medical Institute Predoctoral Fellow.

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ELSEVIER

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POTENTIAL CYTOTOXIC ROLE OF EPOXIDE HYDROLASE (EH) IN ALZHEIMER'S DISEASE (AD).

S. Prasad Gabbita, K. Hensley, R. A. Floyd, R.Nael, W. R. Markesbery, S. Mou, K. Williamson, N. Zheng, G.Bing. Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

EH is generally considered a cytoprotective, phase II enzyme converting toxic epoxide metabolites of xenobiotics to non-toxic trans-dihydro diols. Although, recent evidence has shown that during certain inflammatory processes, EH converts 9,10 and 12,13 linoleic acid epoxides (Leukotoxin) to the more cytotoxic trans dihydrodiols. The present study was designed to investigate the role of EH in the context of neuroinflammatory processes occurring within the AD brain mediated by lipid metabolites. A immunohistochemical evaluation of EH, quantitation of the EH protein by Western blotting and HPLC-based EH activity assay were performed on different brain regions of the AD and age-matched control subjects. A significant increase in the expression of EH was observed in the parietal lobe and the hippocampus whereas the cerebellum showed a very low level of expression of this enzyme. Our results suggest that activation of EH within astrocytes during a neuroinflammatory process may play a critical role in causing neuronal cell death in AD by the formation of highly cytotoxic lipid mediators. Grant Support: MCMR-AAA-BA-95-1; USAMRMC 98228627; NIH-NS 35747 & PO1-AG05119; Oklahoma Center for the Advancement of Science and Technology and the Abercrombie Foundation.

CURRICULUM VITAE

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Education:

Ph. D.	1988	Neurobiology and Anatomy, University of Rochester, Rochester New York, USA
M. D.	1977	Medicine, Jilin Medical College, Jilin, China

Honors/Awards:

1981-1982	Best in Teaching Award, Jilin Medical College
1982-1983	Fellowship for Chinese Graduate Student Study in USA, Chinese Government
1983-1984	Fellowship from Educational Commission for Foreign Medical Graduate, USA (ECFMG)
1984-1985	Teaching Assistantship, University of Rochester
1985-1986	Research Fellowship, Society of Physiology

Patent Awarded:

Methods of using alpha 2 agonist for the treatment of neurodegenerative diseases.

Inventor: **Guoying Bing,** and Eric Stone. 1993.

USA Patent Number: 5,252,816

Patent Pending:

A method for preventing and treating the degeneration of neurons.

Inventor, **Guoying Bing**, Nai-ying Zheng, Lei Jin, and Xin Lu, 1999

U.S. Serial No. **60,114,214**

Gene Bank Submission:

Molecular cloning of a new gene for Fos-related antigen (FRA) in the kainic acid treated hippocampus.

Submitted by: **Guoying Bing,** Qiping Qi, Zhihuei Feng and Jau-Shyong Hong.

Accession Number: **U34932**

Professional Position:

1997-Present	**Assistant Member** Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation
1997-Present	**Adjunct Professor** Department of Cell biology, University of Oklahoma, Health Science Center.
1988-Present	**Visiting Professor** Beijing Institute for Neuroscience, Capital University of Medical Science, China
1993-1997	**Senior Fellow** National Institute of Environmental Health Science
1991-1993	**Assistant Professor** NYU Medical Center, Department of Psychiatry
1989-1991	**Staff Fellow** NYU Medical Center, Department of Psychiatry
1983-1988	**Teaching Assistant and Tutor** University of Rochester, School of Medicine, Department of Neurobiology & Anatomy
1978-1983	**Instructor** Department of Anatomy, Jilin Medical College

Research Interests:

Major research interests focus on the molecular mechanisms underlying the neurodegenerative diseases. Currently, there are three research projects are actively carried on in the laboratory: 1) The role of

neuro-inflammatory processes in the etiology and pathophysiology of Parkinson's disease—A new animal model for Parkinson's disease. 2). Long-term neuronal adaptation to excitatory neurotoxicity---Molecular cloning long-term, differential expressed genes in the hippocampus after KA-induced epileptic seizures. and 3). The role of xenobiotic metabolite enzymes in the central nervous system---The effects of environmental and endogenous toxins on neurodegeneration.

Grant Support:

Active Support:

Principal Investigator for **OCAST Grant**-HR98-004

Project Title: Long-term Expressed Genes in Epileptic Seizures

Effective: June 1, 1998 through May 31, 2001

Total Amount: $105,000

Principal Investigator for **US Army Medical Research Grant**-MCMR-AAA-BA 95-1

Project Title: Protective Mechanisms of Nitrone Antioxidants in Kainic Acid Induced Neurodegeneration

Effective: June 1, 1999 through May 31, 2002

Total Amount: $540,000

Principal Investigator for **NIH Grant**, R01 NS39345-01

Project Title: Microglia Activation Induces Parkinsonism in rats

Effective: December 1, 1999 through November 31, 2003

Total Amount: $829,114

Pending Grants:

Principal Investigator for **NIH Grant**, R01

Project Title: Epoxide Hydrolase Involves Neurodegeneration

Effective: December 1, 2000 through November 30, 2005

Total Amount: $1,025,000

Principal Investigator for **NIH Grant** R03

Project Title: Expression of Epoxide Hydrolase in Reactive Astrocytes induced by Neurodegeneration

Effective: July 1, 2000 through June 31, 2001

Total Amount: $50,000

Reviewer Activity:

Brain Research

Neuroscience Protocols

Neuroscience

Neuroscience Letter

Neurodegeneration

J. Neuroscience

Invited Lectures:

1. "Cografts of Adrenal Medullary cells with Neurotrophic producing Cells" Veterans Administration Hospital , Bedford, MA 01730, 1987.

2. "Transplantation of Adrenal Medullary, Carotid Body Glomus Cells with C6 Glioma Cells into the rat brain" Department of Anatomy, Boston University School of Medicine Boston, MA 02118, 1987.

3. "Neurotransplantation: Present and Future" Capital Institute of Medicine, Beijing, China, 1988.

4, "Animal models used in neurotransplantation" New York University, Medical Center, New York, NY 10016, 1991

5. "Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra" NIEHS/NIH, Research Triangle Park, NC 27709, 1993.

6. "Long-term genomic effects of administration of kainic acid in the rat brain" Centaur Pharmaceutical Inc., Sunnyvale, CA 94086, 1995.

7. "The regulation of the opioid peptide by seizure activities ----Role of long-term AP-1 transcription factors". Oklahoma Medical Science Foundation, City, OK 73104, December, 1996.

8. "The regulation of the opioid peptide by seizure activities ----Role of long-term AP-1 transcription

factors". University of Oklahoma, Oklahoma Center for Neuroscience, Oklahoma City, OK 73104, January, 1997.

9. Capital University of Medical Science, Beijing, China. March,1997.

10. "Microglia mediated neuronal death----A new animal model for Parkinson's disease" Kangwon National University, Korea. April, 1997.

11. "Long-term gene induction in the hippocampus by excitatory amino acid----A PCR-selected subtractive cloning methods" Shanghai Medical University, Shanghai, China. September,1998.

12. "Current trends in research for neurodegenerative diseases" Shandong Medical University, Shandong, China. September,1998

13. "Microglia mediated neuronal death----A new animal model for Parkinson's disease" National Institute of Radiation Research, Ciba, Japan. June, 1999.

14. "Microglia mediated neuronal death----A new animal model for Parkinson's disease" Yamagata University, School of Medicine, Yamagata, Japan, June, 1999

15. "Recent development of Molecular biological techniques in Neuroscience Research". Capital University of Medical Science, Beijing, China. March, July, 1999.

16. "Microglia mediated neuronal death----A new animal model for Parkinson's disease" University of Kansas, School of Pharmacy, Kansas City, MS, August, 1999.

Professional Societies:

Society for Neuroscience
The New York Academy of Science

Committee:

Fleming Scholar Select Committee, Oklahoma Medical Research Foundation
Graduate Faculty Committee, University of Oklahoma Health Sciences Center

Major Expertise & Professional Activities:

Supervised one graduate student, four postdoctoral fellows, two research associates, and a technician since 1989 to conduct research involving following techniques:

Molecular Biology:
Molecular cloning,
cDNA library construction and library screening
PCR Techniques

Differential Display
Subtractive Cloning
TUNNEL Methods for in situ staining of apoptosis.

Neurobiology:

Light and Electronic Microscope Techniques
Major Histological and Pathological Staining
Immunohistochemistry
In situ Hybridization
Animal Models (rat, mouse, and monkey) for Parkinson's Disease
Neurotransplantation using fetal brain tissue, neuroblastoma, glioma, and primary cultured cells
Neurochemical Analysis by HPLC for Monoamine System.

Cell Biology:

Tissue Culture;
Primary Neuronal and Glial Cultures;
In situ hybridization on the Brian Slides and Culture Dishes.

Publications:

1. Gash, D.M., Notter, M.F.D., **Bing, G.**, Kodower, J.F. **(1986)** Neural implants into primates: Studies employing differentiated neuroblastoma cells. *Cell and Tissue Transplantation into the Adult Brain* pp. 37.

2. Hansen, J.T., **Bing, G.**, Notter, M.F.D., Gash, D.M. **(1987)** Ultrastructure of striatal implants of adult adrenal chromaffin cells in unilateral 6-OHDA lesioned rats. *Anat. Rec.* 218:56A.

3. **Bing, G**., Notter, M.F.D., Hansen, J.T., Gash, D.M. **(1988)** Comparison of adrenal medullary, carotid body and PC12 cell grafts in 6-OHDA lesioned rats. *Brain Res. Bull.* 20:399-406.

4. Hansen, J.T., **Bing**, G., Notter, M.F.D., Gash, D.M. **(1988)** Paraneuronal grafts in unilateral 6-OHDA lesioned rats: Morphological aspects of adrenal chromaffin and carotid body glomus cell implants. In: *Transplantation into Mammalian CNS* (D.M. Gash and J. R. Sladek, Jr., Editors) Elsevier, Amsterdam, *Prog Brain Res*, 78:535-542.

5. Gash, D.M., Notter, M.F.D., Hansen, J.T., **Bing, G.**, Okawara, S.H. **(1988)** Human organ donor adrenals: Fine structure, plasticity and viability. In: *Transplantation into Mammalian CNS* (D.M. Gash and J. R. Sladek, Jr., Editors) Elsevier, Amsterdam, *Prog Brain Res*. 78:559-565.

6. Kordower, J.H., **Bing, G.**, Fiandaca, M.S., Sladek Jr., J.R., Gash, D.M. **(1988)** Tyrosine hydroxylase-immunoreactivity somata within the primate subfornical organ: Species specificity. *Brain Res.* 461:221-229.

7. Hansen, J.T., **Bing, G.**, Notter, M.F.D., Gash, D.M. **(1989)** Adrenal chromaffin cells as transplants in animal models of Parkinson's disease. *J. Electron Microscopy Tech.* 12:308-315.

8. **Bing, G.**, Notter, M.F.D., Hansen, J.T., Kellogg, C., Gash, D.M. **(1990)** Cografts of adrenal medulla with C6 glioma cells in rats with 6-OHDA induced lesions. *Neurosci.* 34:687-697.

9. **Bing, G.**,. Neurotransplantation: The Present and Future. In: *Neurotransplantation* (S. Jiao and **G. Bing**, Editors) Science Press, Beijing, China, in press.

10. **Bing, G.**, Filer, D., Miller, J.C., Stone, E.A. **(1991)** Noradrenergic activation of immediate early genes in rat cortex. *Molec. Brain Res.* 11:43-46.

11. Stone, E.A., Zhang, Y., John, S., **Bing, G. (1991)** C-fos response to administration of catecholamine into brain by microdialysis. *Neurosci. Lett.* 133:33-35.

12. **Bing, G.**, Chen, S., Zhang, Y., Hillman, D., Stone, E.A. **(1992)** Noradrenergic-induced expression of c-fos in rat cortex: neuronal localization. *Brain Res.* 140:260-264.

13. Stone, E.A., **Bing G.**, John S.M., Zhang, Y., Filer, D. **(1992)** Cellular localization of responses to catecholamine in brain tissue. *Prog. Brain Res.* 94:303-307.

14. Stone, E.A., John, S.M., **Bing, G.**, Zhang, Y. **(1992)** Studies on the cellular localization of biochemical responses to catecholamines in the brain. *Brain Res. Bull.* 29:285-288.

15. **Bing, G.**, Stone, E.A., Zhang, Y., Filer, D. **(1992)** Immunohistochemical studies of noradrenergic-induced expression of c-fos in the rat CNS. *Brain Res.* 592:57-62.

16. Stone, E.A., Zhang, Y., John, S., Filer, D., **Bing, G. (1993)** Effect of locus coeruleus lesion on c-fos expression in the cerebral cortex caused by yohimbine injection or stress. *Brain Res.* 19:181-185.

17. Stone, E.A., Manavalan, J.S., Basham, D.A., **Bing, G. (1994)** Effect of yohimbine on nerve growth factor mRNA and protein levels in rat hippocampus. *Neurosci. Lett.* 14:11-13.

18. **Bing, G.**, Zhang, Y., Watanabe, Y., McEwen, B.S., Stone, E.A. **(1994)** Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra. *Brain Res.* 668:261-265.

19. Hiller, J., Zhang, Y., **Bing, G.**, Gioannini, T., Stone E., Simon, E. **(1994)** Immunohistochemical Localization of mu-opioid receptors in rat brain using antibodies generated against a peptide sequence present in a purified mu-opioid binding protein. *Neurosci.* 62:829-841.

20. McMillian, M., Kong, L.-Y., Sawin, S.M., Wilson, B., Das, K., Hudson, P., Hong, J.-S., **Bing, G. (1995)** Selective killing of cholinergic neurons by microglial activation in basal forebrain mixed neuronal/glial cultures. *Biochem. Biophys. Res. Commun.* 215:572-577.

21. Das, K.P., McMillian, M., **Bing, G.**, Hong, J.-S. **(1995)** Modulatory effects of [Met^5]- enkephalin on interleukin-1b secretion from microglia in mixed brain cell cultures. *J. Neuroimmuno.* 62:9-17.

22. Perez-Otano, I., McMillian, M., **Bing, G.**, Hong, J.-S., Pennypacker, K. **(1996)** Induction of NF-kB-like transcription factors in brain areas susceptible to kainate toxicity. *Glia.* 16:306-315.

23. **Bing, G.**, Wilson, B., McMillian, M., Feng, Z., Qi, Q., Kim, H., Wang, W., Jensen, K., Hong, J.-S. **(1996)** Long-term expression of Proenkephalin and prodynorphin in the rat brain after systemic administration of kainic acid ——an *in situ* hybridization study. in *Neurodegenerative Disease,* ed. by G. Flskum, Plenum Press, pp 8-18.

24. **Bing, G.**, McMillian, M.,Kim, H., Pennypacker, K., Feng, Z., Qi, Q., Kong, L.-Y, Iadarola, M., Hong, J.-S. **(1996)** Long-term expression of the 35-kDa fos-related antigen (FRA) in rat brain after kainic acid treatment. *Neurosci.* 73:1159-1174.

25. Kim, H., Pennypacker, K., **Bing, G.**, Bronstein, D., McMillian, M., Hong, J.-S. **(1996)** The effects of dextromethorphan on kainic acid-induced seizures in the rat. *J. Neurotoxic.* 17:375-386.

26. Kong, L.-Y., McMillian, M., **Bing, G.**, Hudson, P.M., Hong, J.-S. **(1996)** The effects of the HIV-1 envelope protein gp 120 on the production of nitric oxide and proinflammatory cytokins in mixed glial cell cultures. *Cell Immunol.* 172:77-83.

27. **Bing, G.**, Wang, W., Qi, Q., Feng, Z., Jin, L., Bing, R., Hong, J.-S. **(1997)** Long-term expression of Fos-related antigen and transient expression of Δ FosB associated with seizures in the hippocampus and striatum. *J. Neurochem.* 68:272-279.

28. Kim, H., **Bing, G.**, Hong, J.-S. **(1997)** Dextromethorphan blocks opioid peptide gene expression in the rat hippocampus induced by kainic acid. *Neuropeptides.* 31:05-112.

29. **Bing, G.**, Wilson, B., Hudson, P., Jin, L., Feng, Z., Zhang, W., Bing, R. **(1997)** A single dose of kainic acid elevates the levels of enkephalins and activator protein-1 transcription factors in the hippocampus for up to 1 year. *Proc. Natl. Acad. Sci., USA.* 94:9422-9427.

30. Simpson, J.N., Zhang, W.Q., **Bing, G.**, Hong, J.-S. **(1997)** Kainic acid-induced sprouting of dynorphin- and enkephalin-containing mossy fibers in the dentate gyrus of the rat hippocampus. *Brain Res.* 747:318-323

31. Feng, Z., Zhang, W., **Bing, G.**, Hundson, P., Feng, W., Hong, J.-S. **(1997)** Characterization of the long-lasting activator protein-1 complex induced by kainic acid treatment. *Brain Res.*770:53-59.

32. Chen, S., Ren, Y.Q., **Bing, G.**, Hillman, D.E. **(1998)** Transient c-*fos* gene expression in cerebellar development and functional stimulation. *Brain Res* 795:87-97.

33. Gupta, R.P., **Bing, G.**, Hong, J.S., Abou-Donia, M.B. **(1998)** cDNA cloning and sequencing of Ca^{2+}/calmodulin-dependent protein kinase IIα subunit and its mRNA expression in diisopropyl phosphorofluoridate (DFP)-treated hen central nervous system. *Mol Cell Biochem* 181:29-39.

34. Kim H.C., **Bing, G,** Jhoo, W.K., Ko, K.H., Kim, W.K., Lee, D.C., Shin, E.J., Hong, J.S. **(1999)** Dextromethorphan modulates the AP-1 DNA-binding activity induced by kainic acid. *Brain Res* 824:125-132.

35. Hensley, K., Floyd, R.A., Zheng, N.Y., Nael, R., Robinson, K.A., Nguyen, X., Pye, Q.N., Stewart, C.A., Geddes, J., Markesbery, W.R., Patel, E., Johnson, G.V.M., **Bing, G. (1999)** p38 Kinase is activated in the Alzheimer's disease brain. *J. Neurochem.* 72:2053-2058.

36. Feng, Z., Chang, R.C., **Bing, G.**, Hudson, P., Tiao, N., Jin, L., Hong, J.S. **(1999)** Long-term increase of Sp-1 transcription factors in the rat hippocampus after kainic acid treatment. *Brain Res.* 69:144-148.

37. Floyd, R.A., Robinson, K.A., Stewart, C.A., **Bing, G.,** Hensley, K. **(1999)** Neuroinflammatory events and signal transduction processes are involved in neurodegeneration. In: Free Radicals in Brain Pathophysiology. (Cadenas, E., Packer, L, Poli, G., Ed.) pp. 109-126, Marcel Decker, NY.

38. Kim, H.C., Jhoo, W.K., Choi, D.Y., Im, D.H., Shin, E.J., Suh, J.H., Floyd, R.A., **Bing, G. (1999)** Protection of methamphetamine nigrostriatal toxicity by dietary selenium. *Brain Res.* 851:76-86.

39. Kim, H.C., Jhoo, W.K., Ko, K.H., Kim, W.K., **Bing, G.,** Kwon, M.S., Shin, E.J., Suh, J.H., Lee, Y.G., Lee, D.W. **(2000)** Prolonged exposure to cigarette smoke blocks the neurotoxicity induced by kainic acid in rats. *Life Sci.* 66:317-326.

40. **Bing, G.**, Feng, Z., Qi, Q., Wilson, B., McMillian, M., Kim K.H., Hong, J. **(2000)** Cloning and expression of MP 13, an antigen immunoreactive with antibody against FOS-related antigen, from rat hippocampus after systemic kainic acid treatment. *Neurosci Lett.* In press.

41. Kim, H.C., **Bing, G.**, Jhoo, W.K., Ko, K.H., Kim, W.K., Suh, J.H., Kim, S.J., Kato, K., Hong, J.S. **(2000)** Changes of hippocampal Cu/Zn-superoxide dismutase after kainate treatment in the rat. *Brain Res.* 853:215-226.

42. Kim, H.C., Jhoo, W.K., Shin, E.J., **Bing, G. (2000)** Selenium deficiency potentiates methamphetamine-induced nigral neuronal loss; comparison with MPTP model. In press.

43. **Bing, G.**, Lu, X., Zheng, N., Jin, L., Kim, H. **(2000)** Microglia activation induced dopaminergic cell death in the substantia nigra. Submitted to *Nature Neurosci.*

43. **Bing, G.**, Neal, R., Zheng, N., Jin, L., Zhu, M., Kim, H. **(2000)** Induction of epoxide hydrolase in neurodegenerative diseases. Submitted to *J. Neurosci.*

44. Floyd, R.A., Hensley, K., **Bing, G. (2000)** Evidence for enhanced neuroinflammatory processes in neurodegenerative diseases and the action of nitrones as potential therapeutics. Submitted to *J. Neural Transmission.*

45. Hensley, K., Stewart, C.A., Zheng, N.Y., Mou, S., Floyd, R.A., **Bing, G. (2000)** Phenyl-N-*tert*-butylnitrone inhibits neuronal apoptosis in the kainic acid model of Epilepsy by suppressing proapoptotic signal transduction pathways. Submitted to *J Neurosci.*

Book Chapters:

1. Nael, R., **Bing, G. (1998)** Potential role of p38 MAP kinase in Alzheimer's disease. Summer Scholars Scientific Reports, The Oklahoma Medical Research Foundation, pp. 31-42.

2. Somers, R.C., **Bing, G. (1999)** Investigation of epoxide hydrolase mRNA in the rat hippocampus after kainic acid treatment. Summer Scholars Scientific Reports, The Oklahoma Medical Research Foundation, in press.

3. Kim, H.C., **Bing, G. (2000)** Alterations of antioxidant status in the neurodegenerative models; Study for Parkinson's disease. In Frontiers of the Mechanisms of Memory and Dementia (ed. T. Tato) Elsevier Science B.V. pp. 65-68.

Relevant Abstracts:

1. **Bing, G.**, Notter, M.F.D., Kellogg, C., Gash, D.M. **(1986)** Implants of PC 12 cells into rats with unilateral nigrostriatal lesions. *Neurosci Abstr.* 12:1288.

2. **Bing, G.**, Notter, M.F.D., Hansen, J.T., Kellogg, C., Kordower, J.H., Gash, D.M. **(1987)** Adrenal Medullary transplants IV. Cografts with growth factor producing cells. *Neurosci Abstr.* 13:16.

3. Lory, J., Kordower, J.H., **Bing, G.**, Sladek, J.R., Jr., Gash, D.M. **(1987)** Species specific tyrosine hydroxylase immunoreactive cell group in the subfornical organ: Presence in the monkey but not rat brain. *Neurosci Abstr.* 13:1336.

4. **Bing, G.**, Jiao, S., Notter, M.F.D., Hansen, J.T., Gash, D.M. **(1988)** Cografts of adrenal medulla with peripheral nerve in the dopamine denervated rat striatum. *Neurosci. Abstr.* 14:735.

5. **Bing, G.**, Vielkind, U., Bohn, M.C. **(1989)** Glucocorticoid receptor expression in primary hippocampal neuronal cultures. *Neurosci. Abstr.* 15:717.

6. **Bing, G.**, Stone, E.A., Miller, J.C., Friedhoff, A.J., Filer, D. **(1990)** Beta adrenoceptor-induced expression of early response genes in the rat cerebral cortex. *Neurosci. Abstr.* 16:2.

7. Watanabe, Y., Angulo, J., **Bing, G.**, Stone, E.A., McEwen, B.S. **(1991)** Effects of repeated restraint stress in rats on neuroendocrine and molecular markers of the brains response. *Neurosci. Abstr.* 17:83.

8. **Bing, G.**, Zhang, Y., Filer, D., Stone, E.A. **(1991)** Immunohistochemical identification of c-fos protein in rat brain after noradrenergic stimulation. *Neurosci. Abstr.* 17:1357.

9. Zhang, Y., **Bing, G.**, Filer, D., Stone, E.A. **(1992)** Effect of locus coeruleus (LC) lesion on c-Fos response to metrazol (PTZ) in rat brain. *Neurosci. Abstr.* 18:1375.

10. **Bing, G.**, Zhang, Y., Stone, E.A. **(1992)** Protective action of locus coeruleus noradrenergic system on substantia nigra (SN) of mice treated with MPTP. *Neurosci. Abstr.* 18:1375.

11. **Bing, G.**, Manavalan, J.S., Stone, E.A. **(1993)** Hippocampal NGF Mrna following yohimbine injection. *Neurosci. Abstr.* 19:50.

12. Stone, E.A., Zhang, Y., **Bing, G. (1993)** The role of the noradrenergic system in central c-fos responses. *Neurosci. Abstr.* 19:17.

13. Hiller, J.M., **Bing, G.**, Stone, E., Gioannini, T.I., Simon, E.J. **(1993)** Immunohistochemical localization of μ opioid receptors in rat brain with antibodies against a peptide sequence derived from a purified opioid receptor binding protein. *Neurosci. Abstr.* 19:116.

14. Perez-Otano, I., Pennypacker, M.K., McMillian, M.K., **Bing, G.**, Hong, J.S. **(1994)** NF-kB transcription factors are increased in brain areas susceptible to kainate toxicity. Neurosci. *Abstr.* 20:50.

15. Shao, Y., **Bing, G.**, Chen, K.H., Kufuor, N.K., Qi, Q., Mccarthy, K.D., Hu, P.C., Hong, J. **(1994)** Adenovirus and adeno-associate virus mediated gene transfer in rat brain cells. *Neurosci. Abstr.* 20:94.

16. Kim, H., Pennypacker, M.K., **Bing, G.**, J.S. Hong. **(1994)** Effects of dextromethorphan on kainate acid-induced seizure in rat. *Neurosci. Abstr.* 20:244.

17. **Bing, G.**, Wu, G., Kim, H., McMillian, M., Qi, Q., He, X., J.S. Hong. **(1994)** D1 dopaminergic agonists increase the expression of dynorphin and c-fos in primary striatal culture of rat. *Neurosci. Abstr.* 20:257.

18. **Bing, G.**, McMillian, M., Kim, H., Pennypacker, K., Feng, Z., Qi, Q., Kong, L., Chan, J., Wilson, B., Hong, J.S. **(1995)** Long-term expression of the 35-kDa fos-related antigen (FRA) in rat brain after kainic acid treatment. *Neurosci. Abstr.* 21:127.1.

19. Kong, L.-Y., McMillian, M., **Bing, G.**, Hudson, P.M., Hong, J.S. **(1995)** The effects of the HIV-1 envelope protein gp 120 on the production of nitric oxide and proinflammatory cytokins in unprimed or interferon g- primed glial cell cultures. *Neurosci. Abstr.* 21:352.18.

20. Feng, Z., **Bing, G.**, Qi, Q., Wilson, B., McMillian, M., Pennypacker, K., Iadarola, M., Hong, J.S. **(1995)** Cloning and analysis of fos-related antigen from rat hippocampus after systemic of kainic acid. *Neurosci. Abstr.* 21:521.14.

21. McMillian, M., Kong, L.-Y., Sawin, S.M., Wilson, B., Das, K., Hudson, P., Hong, J.S., **Bing, G.** **(1995)** Selective killing of cholinergic neurons by microglial activation cell cultures. *Neurosci. Abstr.* 21:679.18.

22. Kim, H.C., Kim, S.J., **Bing, G.**, Hong, J.S. **(1995)** The role of hippocampal Cu, Zn-superoxide dismutase (SOD-1) in kainic acid-induced neuronal degeneration. *Neurosci. Abstr.* 21:836.21.

23. Simpson, J., **Bing, G.**, Feng, Z., Wilson, B., Hong, J.S. **(1996)** Long-term effects of kainic acid-induced seizures on the opioid peptides and AP-1 transcription factors I. Morphological studies. *Neurosci. Abstr.* 22:519.7.

24. Feng, Z., Zhang, W., **Bing, G.**, Simpson, J., Hudson, P., Hong, J.S. **(1996)** Long-term effects of kainic acid-induced seizures on the opioid peptides and AP-1 transcription factors II. Biochemical studies. *Neurosci. Abstr.* 22:519.8.

25. **Bing, G.**, Wang, W., Qi, Q., Feng, Z., Hudson, P., Jin, L., Hong, J.S. **(1996)** Expression of ΔFosB in the rat hippocampus and striatum after systemic administration of kainic acid. *Neurosci. Abstr.* 22:519.9.

26. Kong, L., McMillian, M., Wilson, B., Hudson, P., Jin, L, **Bing, G.**, Hong, J.S. **(1996)** Inhibition of lipopolysaccharide-induced nitric oxide and cytokine mRNA expression in mixed glia cultures: suppression by protein tyrosine kinase inhibitors. *Neurosci. Abstr.* 22:537.12.

27. **Bing, G.**, Wilson, B., Hudson, P., Jin, L., Feng, Z., Zhang, W., Bing, R., Hong, J.S. **(1997)** A single dose of kainic acid elevates the levels of enkephalins and AP-1 transcription factors in the hippocampus for up to one year. *Neurosci. Abstr.* 23:537.12.

28. Hillman, D., Kim, E.J., Bing, R., **Bing, G. (1997)** Expression cloning of a rat organ of corti cDNA library. *Neurosci. Abstr.* 23:287.2.

29. Lu, X., **Bing, G.**, Hagg, T., Long, L.Y., Hong, J.S. **(1997)** Naloxone prevents lipopolysaccharide-induced neuronal degeneration in rat substantia nigra. *Neurosci. Abstr.* 23:738.11.

30. Feng, Z., Bing, G., Hudson, P., Jin, L., Tiao, N., Hong, J.S. **(1997)** Long-term effects of kainic acid-induced seizures on the expression of ENKCRE 2 and SP-1 transcription factors. *Neurosci. Abstr.* 23:904.9.

31. Jin, L., Zheng, N.Y., **Bing, G. (1998)** Long-term, differential effects of systemic kainic acid treatment on neuropeptide expression in the hippocampus. *Neurosci. Abstr.* 24:473.12.

32. **Bing, G.**, Lu, X., Zheng, N.Y., Jin, L., Stewart, C.A., Floyd, R.A., Kim, H.C. **(1998)** Microglia mediated dopaminergic cell death in the substantia nigra: A new animal model for Parkinson's disease. Society for Neuroscience 28th Annual Meeting, November 7-12, Los Angeles, CA. 24:574.20 page 1466.

33. Stewart, C.A., Zheng, N.Y., Jin, L., Floyd, R.A., Anderson, R.E., **Bing, G. (1998)** Microglial-mediatedapoptotic cell death in kainate treated rat hippocampus. Society for Neuroscience 28th Annual Meeting, November 7-12, Los Angeles, CA. 24:767.7 page 1933.

34. **Bing, G.**, Lu, X., Zheng, N.Y., Jin, L., Floyd, R.A., Kim, H.C. **(1998)** Microglia mediated dopaminergic cell death in the substantia nigra: A new animal model for Parkinson's disease. The 5th Annual Meeting of The Oxygen Society. *Oxygen '98*. November 19-23, Washington, D.C. Abstr. # 103, page S44.

35. Hensley, H., **Bing, G.**, Nael, R., Zheng, N.Y., Robinson, K.A., Nguyen, X., Patel, E., Markesbery, W.R., Floyd, R.A. **(1998)** Redox sensitive p38 kinase is activated in the Alzheimer brain. The 5th Annual Meeting of The Oxygen Society. *Oxygen '98*. November 19-23, Washington, D.C. Abstr. # 316, page S111.

36. Floyd, R.A., Hensley, K., **Bing, G.**, Markesbery, W. **(1999)** The role of neuro-inflammatory processes in brain aging and neurodegeneration. Oxygen Club of California. *Oxidants and Antioxidants in Biology*. March 3-6, Santa Barbara, CA. Page 96.

37. Hensley, K., **Bing, G.**, Markesbery, W., Floyd, R.A. **(1999)** Hyperphosphorylation of p38 kinase in Alzheimer's disease: Possible indications of a neuroinflammatory disease process. Oxygen Club of California. *Oxidants and Antioxidants in Biology*. March 3-6, Santa Barbara, CA. Page 146.

38. Floyd, R.A., Hensley, K., **Bing, G.**, Williamson, K., Markesbery, W. **(1999)** Enhanced Signal Transduction Processes Near Plaques in Alzheimer's Brain and Other Evidence of Neuro-Inflammatory Processes. *The FASEB Journal,* Biochemistry and Molecular Biology '99, May 16-20, San Francisco, California. p. A1389, Abstr. #336.

39. Feng, Z., Leong, Ho, S.L., Qi, Q., **Bing, G. (1999)** Different *C-FOS* isoforms were induced in brain by lipopolysaccharide, HIV envelope protein, GP120, and kainic acid. Thirteenth International Congress on Parkinson's Disease, July 24-28, Vancouver, Canada.

40. Nguyen, X.V., Hensley, K., Stewart, C.A., Zheng, N.Y., Jin, L., Zhu, M., Williamson, K.S., Floyd, R.A., **Bing, G. (1999)** Involvement of oxidant-sensitive signal transduction pathways in hippocampal excitotoxicity. Eighth Annual Symposium, *Oklahoma Center for Neuroscience (OCNS)*. The Neurobiology of Addiction: Neuroal, Behavioral, and Clinical Features, October 1,

Oklahoma City, Oklahoma. p. 34 Abstr. #20.

41. **Bing, G.**, Zheng, N.Y., Jin, L., Qi, Y., Neal, R., Kim, H. **(1999)** Neurodegeneration-induced microsomal epoxide hydrolase expression in reactive astrocytes. Society for Neuroscience 29th Annual Meeting, October 23-28, Miami Beach, Florida. Vol. 25:1830 Abstr. #732.8.

42. Jin, L., Zheng, N.Y., Zhu, M., **Bing, G. (1999)** Long term, differential gene expression in the rat hippocampus after systemic kainic acid injection. Society for Neuroscience 29th Annual Meeting, October 23-28, Miami Beach, Florida. Vol. 25:847 Abstr. #340.16.

43. Kim, H.C., **Bing, G.**, Kim, S.J., Wie, M.B., Cha, S.H., Jhoo, W.K., Hong, J.-S. **(1999)** Kainate treatment alters TGF-β3 gene expression in the hippocampus of rats. Society for Neuroscience 29th Annual Meeting, October 23-28, Miami Beach, Florida. Vol. 25:1112 Abstr. #452.17.

44. Kotake, Y., Sang, H., Wallis, G.,L., Stewart, C.A., Zheng, N.Y., **Bing, G. (1999)** PBN amplifies the production of the anti-inflammatory cytokine interleukin-10 in a rat endotoxin shock model. Free Radical Biology & Medicine, 6Th Annual Meeting of the Oxygen Society. The Marriot, New Orleans, November 18-22, Vol. 27:S62 Suppl. 1, Abstr. # 171.

45. Gabbita, S.P., Hensley, K., Floyd, R.A., Nael, R., Markesbery, W.R., Mou, S., Williamson, K.S., Zheng, N.Y., **Bing, G. (1999)** Potential Cytotoxic Role of Epoxide Hydrolase (EH) in Alzheimer's Disease (AD). Free Radical Biology & Medicine, 6Th Annual Meeting of the Oxygen Society. The Marriot, New Orleans, November 18-22, Vol. 27:S139 Suppl. 1, Abstr. # 423.