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Neurodegeneration and Glia Response in Rat Hippocampus Following Nitro-L-Arginine Methyl Ester (L-NAME)

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Hippocampal neurodegeneration and glia response was examined following administration of the nitric oxide synthase inhibitor, N_W-nitro-L-arginine methyl ester (L-NAME). Male Long-Evans rats received L-NAME (50 mg/kg, ip) either once or twice a day for 4 days. Both dosing schedules decreased NOS activity by approximately 90%. At 10 and 30 days following cessation of L-NAME (2x/day), moderate neuronal death was evident in CA 1–2 pyramidal cells and dentate granule cells. Neurodegeneration was accompanied by increased astrocyte glial fibrillary acidic protein (GFAP) immunoreactivity yet, minimal astrocyte hypertrophy. Microglia response was limited to an increase in ramified microglia at 10 days, returning to normal by 30 days. As early as 4 days post-dosing (2x/day), GFAP mRNA levels were significantly elevated as were mRNA levels for tumor necrosis factor-α(TNFα), interleukin-1α (IL-1α), and interleukin 6 (IL-6). No alterations were seen with L-NAME dosing limited to once a day. The co-administration of a hippocampal neurotoxicant, trimethyltin (TMT), with the last dose of L-NAME (2x/day), produced an additive response pattern of neuronal degeneration including both CA1–2 and CA3–4 pyramidal neurons accompanied by TMT-induced astrocyte hypertrophy and prominent microglia reactivity. This was preceded by elevations in mRNA levels for GFAP, TNFα, IL-1α, and IL-6 similar to those seen with each substance alone. These data suggest that high levels of L-NAME can produce a pro-inflammatory environment in the brain and that neurodegeneration and neuroglia responses in the hippocampus can be induced by an alteration in the balance and regulation of local nitric oxide levels.

Keywords: Interleukin-1α; Interleukin-6; microglia; tumor necrosis factor α; trimethyltin

INTRODUCTION

Nitric oxide (NO) is involved in numerous physiological functions including regulation of immune function, vascular relaxation, neurotransmission, and cytotoxicity (see reviews: Moncada et al., 1991; Lowenstein and Snyder, 1992). NO is synthesized through enzymatic conversion of the amino acid arginine to nitric oxide and citrulline by nitric oxide synthase (NOS; Synder 1992) and occurs in two principal forms, a constitutive form (cNOS) and an inducible form (iNOS). In the brain, two isoforms of cNOS have been identified: neuronal NOS (nNOS) and endothelial NOS (eNOS). Both are calcium/calmodulin dependent and produce NO for short periods in response to receptor acti-

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vation (Bredt and Snyder, 1990; Hibbs et al., 1990; Moncada et al., 1991; Stuehr et al., 1991; Dawson et al., 1992; Sparrow, 1995). In the brain, iNOS is primarily localized to astrocytes and microglia where it is associated with response to infection, ischemia, or trauma (Boje and Arora, 1992; Chao et al., 1992; 1993; Galea et al., 1992; Lowenstein et al., 1992; Simmons and Murphy, 1992; Zielasek et al., 1992; Endoh et al., 1994b; Nakashima et al., 1995). Both nNOS and iNOS have been reported to be involved in various neural processes.

Cytokines and endotoxins increase the biological activity of NO through the stimulation of the inducible isoform of NOS (iNOS; Moncada et al., 1991; Galea et al., 1992; Simmons and Murphy, 1992). In macrophages, microglia, and astrocytes, NO is produced several hours after exposure to cytokines or microbial products (Zielasek et al., 1992; Betz-Corradin et al., 1993; Murphy et al., 1993; Nomura and Kitamura, 1993; Park and Murphy, 1994; Sakai et al., 1995). iNOS is transcriptionally regulated through activation of NF-kB/Rel and once activated, can generate large quantities of NO for extended periods of time (Xie et al., 1994). NO then functions as an effector molecule mediating cytostatic and cytotoxic effects (Zielasek et al., 1992; Betz-Corradin et al., 1993; Murphy et al., 1993; Nomura and Kitamura, 1993). It has been proposed that immunostimulated microglia mediate neurotoxicity by various released factors associated with oxidative stress such as NO (Boje and Arora, 1992; Chao et al., 1992; see Dawson and Dawson, 1998 for review).

Non-hydrolyzable analogues of L-arginine have been shown to inhibit NOS. L-NMMA (N^G-monomethyl-L-arginine) and L-NAME (N_W-nitro-L-arginine methyl ester) are two compounds that effectively block NO formation by inhibiting both the inducible and the constitutive isoforms of NOS. L-NAME inhibits NO synthase in vascular endothelium (Laszlo et al., 1995) and in nervous system tissue (Moncada et al., 1992; Ayers et al., 1997; Chen et al., 1998; Wada et al., 1999; de Oliveira et al., 2000; Wu et al., 2000). While an elevation in NOS is damaging to neurons, the inhibition of NOS is not uniformly protective (Kaufman, 1999 for review). For example, Zou and co-workers (1998) reported that daily administration of L-NAME to rats impaired both reference and working memory as measured in a radial-arm maze. In injury models, the effects of NOS inhibitors are also variable. Inhibitors of iNOS can decrease experimental allergic encephalomyelitis in the mouse (Cross et al., 1994) but enhance disease pathology in the rat (Zielasek et al., 1995). Neuronal loss and deficits in cognitive performance produced by brain tumors were exacerbated with treatment by iNOS inhibitors (Sinz et al., 1999). NO has been proposed to serve as a mediator of ischemic neurotoxicity (for review see Dawson and Dawson, 1994; 1998; Dawson and Snyder, 1994; Carreau et al., 1994; Dawson, 1995). Inhibition of NOS has been reported to reduce the size of focal brain ischemia infarct yet, failed to protect CA1 hippocampal neurons from focal ischemia (Moncada et al., 1992; Sancesario et al., 1994). Some of this variability may be associated with the dose and time of administration. For example, iNOS has been reported to have neuroprotectant properties (Billiar, 1999) and L-NAME to offer protection from traumatic brain injury when administered at lower doses (Carreau et al., 1994) and within the first hour after injury (Mesenge et al., 1996).

Given the proposed modulator role for NO in brain function, the present study was undertaken to examine alterations in microglia, astrocytes, and neurons following inhibition of NO synthase. Rats were treated under two different schedules of L-NAME and the hippocampus examined. Within 30 days, L-NAME produced neurodegeneration and astrogliosis in the CA1 region of the hippocampus that was preceded by elevations in both GFAP and pro-inflammatory cytokine mRNA levels. These data suggest that NO levels play a critical role in maintaining the overall cellular stability of the hippocampus.

METHODS

Materials

Trimethyltin hydroxide (TMT) was purchased from Alfa Products (Danvers, MA), glial fibrillary acidic protein (GFAP) polyclonal antibody from Dako Corp (Carpinteria, CA), Vectastain™ Elite immunohistochemistry kit from Vector Laboratories (Burlingame, CA), 3,3′-diaminobenzidine tetrahydrochloride (DAB), lectin (Bandeiraea Simplicifolia BS I-B_4), and N_W-nitro-L-arginine methyl ester (L-NAME) from Sigma Chemical Co. (St. Louis, MO). Alpha ^{32}[P]-dCTP (specific activity 3000 Ci/mM) and Gene Screen Plus nylon membranes were obtained from New England Nuclear (Wilmington, DE). PCR kits were purchased from Perkin Elmer Cetus (Norwalk, CT), reverse transcriptase enzyme (SuperScript™), PCR primers (Clontech Labs, Palo Alto, CA) and Ultrapure agarose from Gibco BRL (Gaithersburg, MD). All other reagents were of reagent grade.

Animals

Adult male Long-Evans hooded rats (175–250 g; Charles River Laboratories, Raleigh, NC) were housed in a dual corridor, semi-barrier animal facility at constant temperature (72° ± 0.5 °F), humidity (50% ± 5%), and lighting (7:00–19:00hr). Food (autoclaved NIH 31) and deionized, reverse osmotic-treated water were available ad libitum. Sentinel animals were negative for pathogenic bacteria, mycoplasma, viruses, ectoparasites, and endoparasites. Animals received either saline or L-NAME (50 mg/kg bwt. ip.) either once or twice a day for 4 consecutive days.

Recent reports suggest that chronic inhibition of nitric oxide synthesis can lead to inflammatory changes in the heart and vessel (Koyanagi et al., 2000) and may modify responses to other inflammatory injuries. Thus, for comparison and examination of interactions, a second group of animals were injected with a compound known to produce an early response of microglia and pro-inflammatory cytokines in the hippocampus of rats. Under each dosing regimen of L-NAME, a group of rats were administered a single injection of either saline or trimethyltin (6 mg TMT/kg bwt. sc.) immediately following the last injection of L-NAME. Animals were examined on either 4, 10 or 30 days post dosing.

Histological Procedures

At either 4, 10, or 30 days post-dosing, 6 animals from each dosing group were deeply anesthetized with pentabarbital (68 mg/kg bwt), perfused via cardiac puncture with saline followed by 4% paraformaldehyde in phosphate buffered saline (0.1 M, pH 7.2), and post-fixed overnight at 4°C. Brains were bisected in the midsagittal plane, dehydrated in ethanol, embedded in paraffin, and 6-micron sections cut. Neuronal necrosis characterized by pyknosis, karyorrhesis, or karyolysis was identified in brain sections stained with Hematoxylin and Eosin (H & E). Astrocytes were identified by immunohistochemistry for GFAP. Rehydrated sections were treated with 3% H_2O_2 for 10 min to remove endogenous peroxidase activity. Sections were then rinsed in PBS, incubated with non-immune goat serum (in 1 % BSA/PBS) for 20 min prior to a 60 min incubation with polyclonal rabbit anti-rat GFAP (1:2000, Dako) followed by a secondary IgG antibody for 30 min. Sections were rinsed in PBS, incubated in ABC reagent (Vectastain™ Elite immunohistochemistry kit) for 30 min, rinsed, and stained with DAB substrate containing $CoCl_2$ and $NiCl_2$.

Microglial cells, both resting and reactive, were identified by lectin (Bandeiraea Simplicifolia BS I-B_4) binding using the method of Streit and Kreutzberg (1987). Deparaffinized and hydrated brain sections were washed in PBS buffer containing 0.1 mM $CaCl_2$, $MgCl_2$, $MnCl_2$, and Triton X-100 for 20 min and bathed over-

night at 4°C in PBS buffer containing peroxidase-labeled lectin (BS-I-B_4). Sites containing microglia bound peroxidase-lectin conjugates were visualized by DAB substrate containing 1 % $CoCl_2$ and $NiCl_2$.

Northern hybridization for GFAP mRNA

Fifteen μg RNA isolated from the hippocampus of 4–7 rats per group (RNAzol B™; CINNA/BIOTECX Laboratories, Friendswood, TX) was fractionated by agarose gel electrophoresis, transferred to nylon filters (GeneScreen; New England Nuclear; Boston, MA) and hybridized overnight with a ^{32}[P] labeled cDNA probe for GFAP (ATCC/NIH # 37922; Rockville, MD). The total amount of radioactivity in each band was determined (Ambis Image Acquisition and Analysis system; San Diego, CA) prior to autoradiography (Hyperfilm MP, Amersham; Sweden). Filters were stripped and reprobed with ^{32}[P] labeled cDNA probe for actin (Oncor Inc.; Gaithersburg, MD).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for TNFα, IL-1α, and IL-6

Reverse transcription (RT) was performed using 3.0 μg total RNA from the hippocampus of 4 to 7 animals per group. Hot start PCR was conducted on 5 μl cDNA (0.1 μg RNA), and a 45 μl aliquot of a master mix PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.8 mM $MgCl_2$, 200 μM dNTPs), 0.2 μM 5′ sense primer and 3′ antisense primer (TNFα: 295 bp; IL-6; 614 bp; IL-1α: 623 bp; and G3PDH; 983 bp), and 1.25 units of Taq DNA polymerase. PCR products were separated by electrophoresis and the relative optical density of each ethidium bromide stained band was determined directly from the gel (Eagle Eye II Still Video System; Stratagene, La Jolla, CA). Peak area under the curve of each sample was averaged for each treatment group and expressed as mean (±SEM) densitometric units. Changes seen following dosing were confirmed by semi-quantitative PCR using Mimics™ as previously described (Maier et al., 1995).

Citrulline Assay for Constitutive Nitric Oxide Synthase Activity

NOS activity was measured by the method of Bredt and Snyder (1990) by monitoring the conversion of ^{3}[H]-arginine to ^{3}[H]-citrulline. Brain NOS, homogenates of specific brain regions from 3 animals per group, was incubated at 37°C for 15 min in a reaction volume of 100 μl with 50 mM Tris (pH 7.4), 1 mg/ml bovine serum albumin, 1 mM DTT, 2 mM $CaCl_2$, 10 μM FAD, 10 μM tetrahydrobiopterin, 3 μM L-arginine containing L-2,3-^{3}[H]-arginine at 300 cpm/pmole, 1 μM NADPH, and 50 nM calmodulin. The reaction was terminated by addition of a solution containing 10 mM EGTA, 100 mM HEPES, pH 5.5 and 1 mM citrulline. ^{3}[H]-Citrulline was separated by chromatography on Dowex 50 W X-8 cation exchange resin and radioactivity determined by liquid scintillation spectroscopy.

Statistical Analysis

Data for each mRNA transcript were analyzed by a 2x2 ANOVA with independent group mean analysis conducted using Fisher's LSD (least significance difference test). An accepted level for statistical significance was set at $p < 0.05$.

RESULTS

Morphological changes in hippocampus by L-NAME

At either 4 hours or one day following cessation of L-NAME (2x/day) dosing, no alterations were seen in either neurons or glia of the hippocampus. By 4 days, the morphological integrity of the neurons remained intact (Fig 1), while astro-

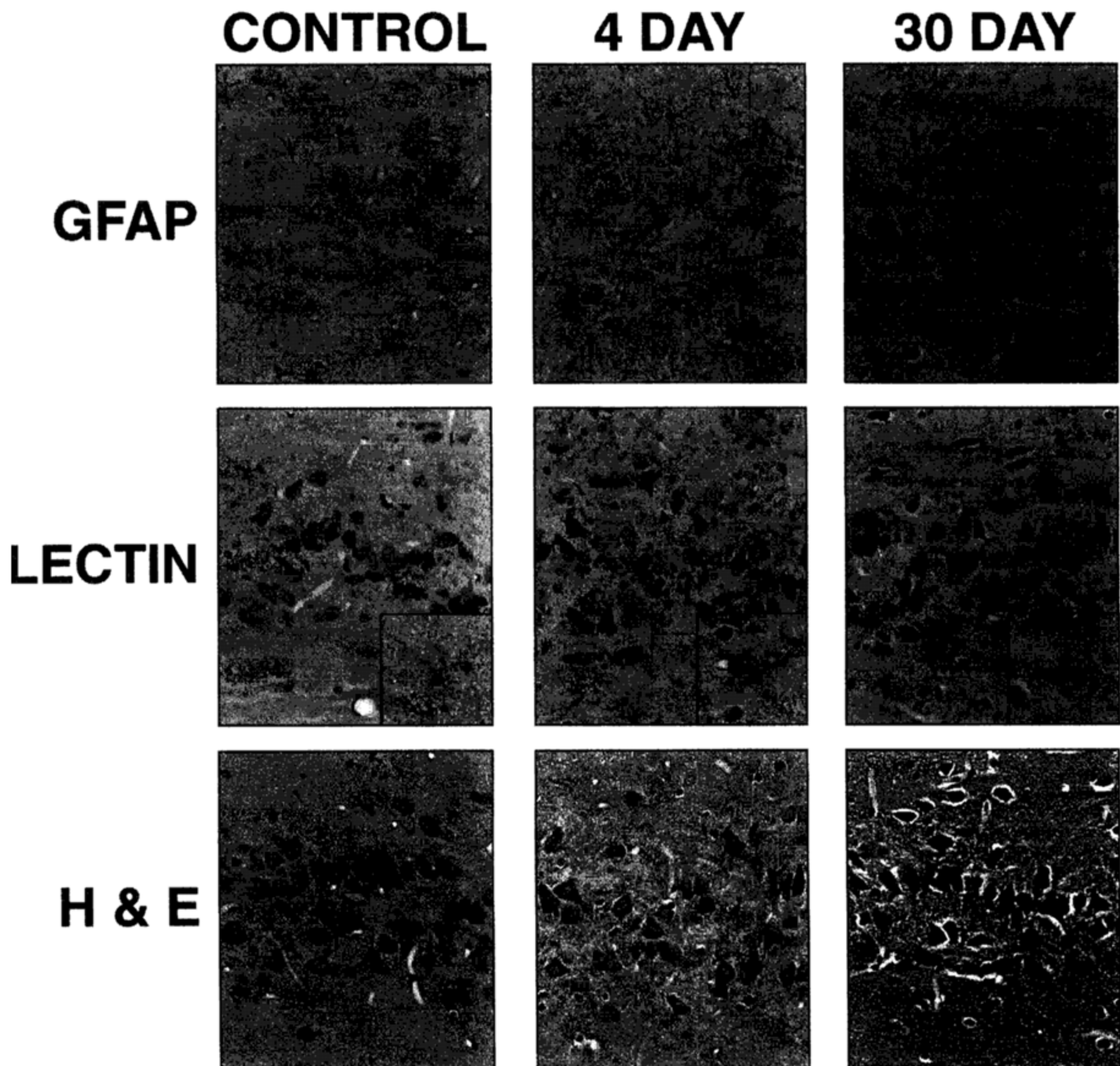


FIGURE 1 Representative hippocampal sections stained with GFAP, lectin, or H&E, 4 and 30 days following cessation of L-NAME administration (50 mg/kg bwt/2x/day/4 days). At 4 days, there was no evidence of neuronal degeneration. By 30 days, moderate neuronal degeneration was seen in the granular cell layer and in the CA1 pyramidal cell region. Compared to saline controls (4 days post injection with saline), there was an increased number of $GFAP^+$ astrocytes with no evidence of astrocyte hypertrophy. Lectin staining for microglia showed increased ramification of microglia at 4 days post-L-NAME and by 30 days post-L-NAME microglia displayed a resting phenotype similar to saline controls Inset (x400). (magnification x80). Saline injected animals displayed identical morphology at both 4 and 30 day post-injection periods (data not shown)

cyte GFAP immunoreactivity and lectin binding to fibrous microglia increased (Fig 1). By 10 days, neuronal necrosis was evident in the CA1 pyramidal cell region and progressed until examination at day 30 (Fig 2A). Further evaluation showed limited necrosis in the granular cell layer (Fig 2B,D) with lesser involvement of CA3–4 cells (Fig 2B,C). Degenerated neurons

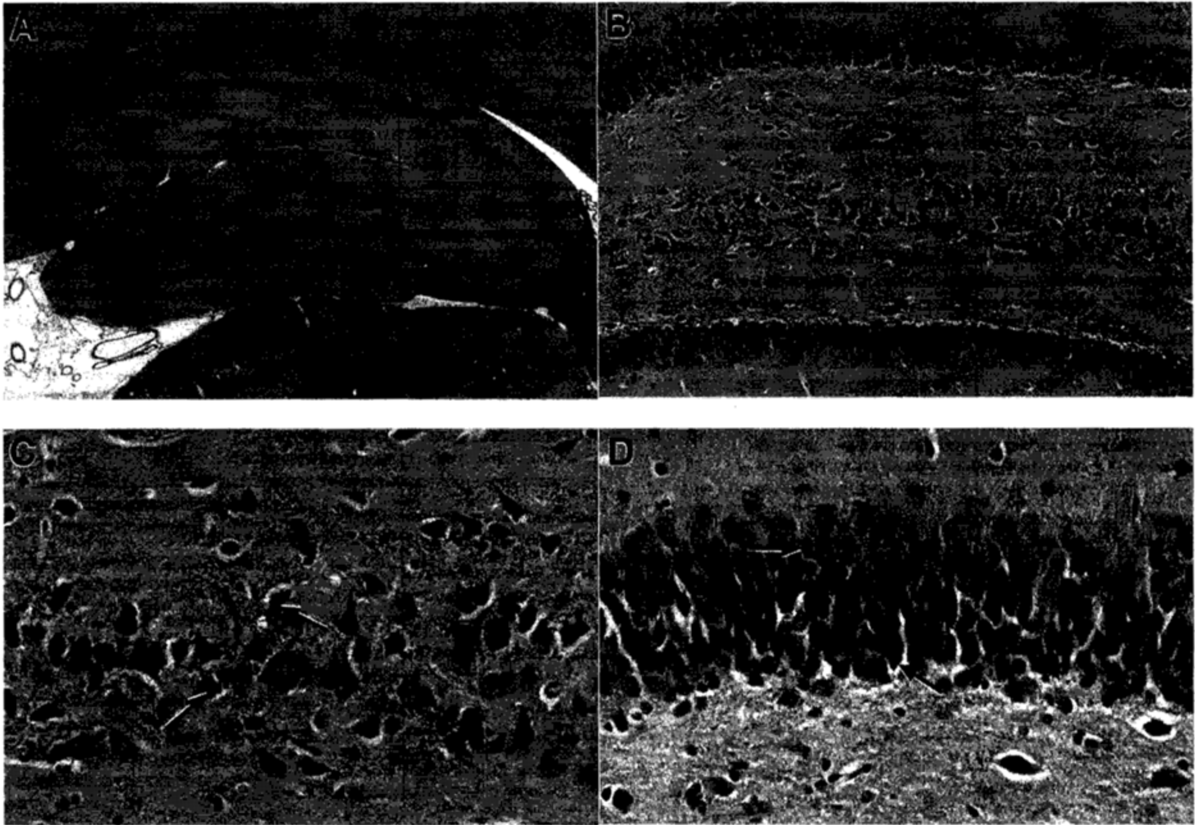


FIGURE 2 Representative H&E sections of the hippocampus 30 days following administration of L-NAME (50 mg/kg bwt/2x/day/4 days) (A: x8). Dentate and pyramidal cell layers (B, x33), CA 3–4 pyramidal cells (C, x80), Dentate granular cells (D, x100). In the granular and pyramidal regions, there was evidence of moderate neuronal degeneration characterized by shrunken, angular cells (arrow) with increased cytoplasmic and nuclear basophilia (arrows). Lesions were most prominent in the granular cell layer (D) and the CA1, with lesser involvement of CA3–4 (See Color Plate IV at the back of this issue)

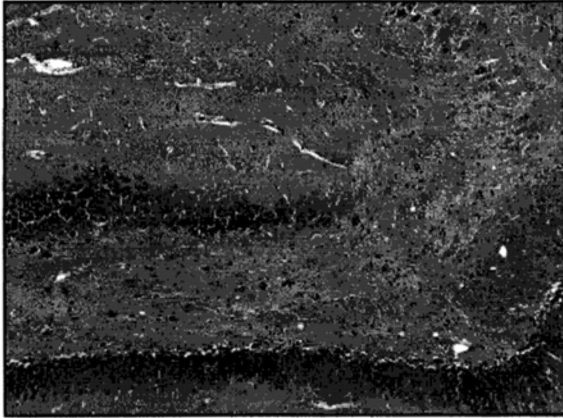
appeared shrunken and angular with increased cytoplasmic and nuclear basophilia. At 4 days post-dosing with L-NAME, lectin staining identified reactive microglia with ramified morphology with no evidence of activated phagocytic microglia (Fig 1). By 30 days, microglia had returned to a quiescent state (Fig 1). L-NAME administered once per day produced no neuronal injury or glia response (data not shown).

Given the lack of a phagocytic response of microglia, we were interested if alterations induced by L-NAME could be unmasked by additional exposure to a known hippocampal neurotoxicant, trimethyltin (TMT), to produce neuronal degeneration and microglia activation. TMT produced degeneration of CA3–4 pyramidal neurons (Fig 3). The administration of both L-NAME (2x/day) and TMT produced a combined pattern of damage involving pyramidal neurons in all layers providing no protection from TMT toxicity in the CA3–4 neurons (Fig 3). At 30 days post-dosing similar levels of astrocyte hypertrophy and microglia activation were seen in both TMT (Fig 4A,B) and TMT+L-NAME (2x/day) exposure groups (Fig 4C).

Relative mRNA Levels of GFAP

By 4 days, post-L-NAME administration (2x/day) GFAP mRNA levels were elevated

TMT



TMT+L-NAME

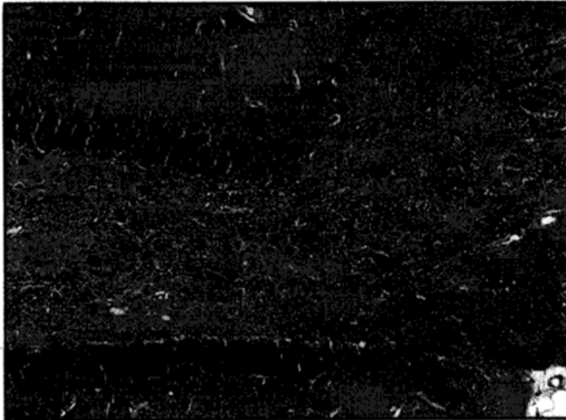


FIGURE 3 H&E sections of hippocampus 30 days post-injection of TMT hydroxide (6 mg/kg body wt) following injections (2x/day) of (A) saline or (B) L-NAME. Both panels display a distinct loss of neuronal cells in the CA3–4 region of the hippocampus (x40)

approximately 100%. No elevations were seen with L-NAME injections delivered only once per day. TMT injections alone also produced approximately 100% elevation in GFAP mRNA levels. The combined exposure to L-NAME (2x/day) and TMT resulted in an increase of approximately 300% above control levels(Fig 5). No significant interactions were seen with TMT when L-NAME was injected only once per day (data not shown).

TABLE I NOS Activity in Brain Regions following L-NAME

	Cerebellum	*Hippocampus*	*Remaining Brain*
Control	71.9 ± 10.9	16.8 ± 3.6	15.5 ± 4.0
L-NAME Once daily	6.43 ± 3.84	1.92 ± 0.45	1.36 ± 0.44
	(91%)[a]	(89%)	(91%)
L-NAME Twice daily	3.37 ± 1.40	2.26 ± 1.20	1.49 ± 0.68
	(95%)	(87%)	(90%)

NOS Activity = pmol citrulline formed/min/mg protein (±SEM).
At 4 days post-dosing, inhibition of NOS activity was approximately 80% for both dose groups.

a. Represents percentage inhibition as compared to control levels.

Relative mRNA Levels of TNFα, IL-1α, and IL-6

Within 4 days of cessation of exposure, TNFα, IL-1α, and IL-6 mRNA levels in the hippocampus increased following L-NAME administration twice a day for 4 days (Fig. 6). As previously reported (Maier et al., 1995), 4 days following an acute injection of TMT, mRNA levels for TNFα, IL-1α, and IL-6 were significantly elevated in the hippocampus (Fig 6). No significant interaction between L-NAME (2x/day) and TMT was evident in levels for each cytokine mRNA transcript (Fig 6). mRNA levels for TNFα, IL-1α, or IL-6 were not elevated following administration of L-NAME once a day for 4 days and no interactions were seen with the addition of TMT (data not shown). G3PDH mRNA levels were similar between all exposure groups. In comparison, tissue samples of the cerebellum from all dosing conditions showed no elevation in cytokine transcript levels (data not shown).

Levels of NOS Activity

Given the distinct difference in the neural response to L-NAME depending upon the dosing regimen of one or two doses per day, we

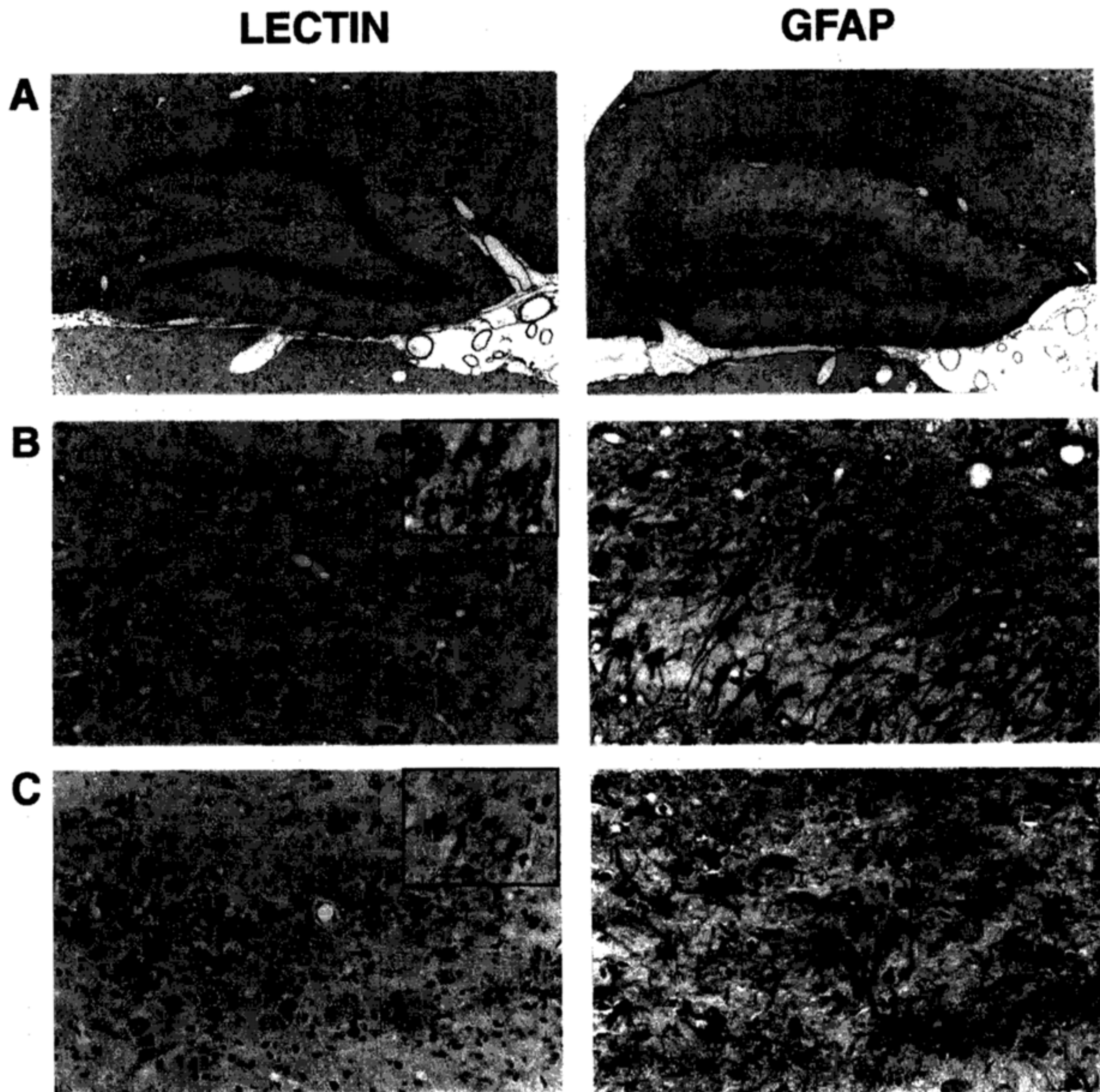


FIGURE 4 Representative staining for astrocytes and microglia in the hippocampus 30 days following administration of TMT (6 mg/kg) with pretreatment of (A,B) saline or (C) L-NAME (50 mg/kg bwt, 2x/day). (A) Following saline pretreatment, TMT-induced astrocyte hypertrophy was evident throughout the hippocampus with focal microglia response in the CA3 region. (Magnification × 20). (B) At higher magnification (x100) there was evidence of prominent staining with lectin for microglia and GFAP immunoreactivity in astrocytes undergoing hypertrophy (x70; Inset x200) (C) Animals that received pretreatment with L-NAME (50 mg/kg bwt/2x/day/4 days) showed a prominent staining with lectin for microglia and GFAP immunoreactivity in astrocytes undergoing hypertrophy (magnification x70; Inset x200)

examined the levels of constitutive NOS activity (primarily neuronal NOS) in the hippocampus, cerebellum, and cortex of 3 individual animals in each group. Twenty-four hours following cessa-

tion of L-NAME dosing, NOS activity was inhibited by approximately 90% under both dosing schedules (Table I). Consistent with previous reports (Dwyer et al., 1991; Iadecola et al., 1994), levels continued to be significantly decreased by approximately 75% at 4 days post-dosing under both dosing schedules. However, differential responses of glia cells was evident at this time suggesting a role for factors in addition to the inhibition of NOS activity.

DISCUSSION

Data presented in this study demonstrated that dosing regimens of L-NAME, one or two times per day for 4 days, resulted in essentially identical suppression of constitutive NOS activity levels. However, when L-NAME was administered twice a day, a pattern of neurodegeneration was seen in the hippocampus characterized by neuronal degeneration of the CA1–2 pyramidal cells, astrogliosis, and a transient increase in reactive ramified microglia. These morphological changes were preceded or accompanied by elevations in mRNA levels for GFAP and pro-inflammatory cytokines. These results suggest a synergistic interaction between nitric oxide regulation and the pro-inflammatory process in determining the pattern of nervous system injury.

Under such dosing regimens, the dysregulation of NOS by either direct enzyme inhibition or upon the return to normal basal level of NOS may result in the release of NO into the cellular environment. Nitric oxide reacts with super oxide anions and forms peroxynitrite anions that decompose to yield highly damaging hydroxyl free radicals, peroxynitrous acid, or N_2O_3 (Beckman et al., 1990). The generation of superoxide anion generated in the vicinity of newly formed NO may block effects of NO by destroying NO as it is formed. NO may also function as a negative feedback modulator of its own constitutive synthesis (Rogers and Ignarro 1992). For example, it has been shown that an induction of NOS in cells correlates with a decrease in the expression of constitutive NOS (Lamas et al., 1992; MacNaul and Hutchison, 1993). Little is known about the kinetics of NOS and its temporal and individual cellular pattern during recovery from inhibition. It is possible, that following a generalized inhibition with L-NAME, the temporal aspect of NOS inhibition and subsequent recovery is distinct for individual cell populations and possibly each NOS isoform. The distinct pattern of cellular responses seen in this study may be associated with such individual kinetics of inhibition and recovery.

It has been previously proposed that the protective or destructive role for NO in the nervous system is contingent on factors such as the cellular source of NO and its targets, the production level of NO, the stage of the injury, the degree of glutamate receptor activation, and the state of the tissue (Lipton et al., 1993). In the hippocampus, immunoreactivity of eNOS is highly concentrated within both the pyramidal cells of the CA1-CA3 region and the granule cells of the dentate gyrus (Dinerman et al, 1994). Neuronal NOS is uniformly distributed in the granular cell layer of the dentate gyrus and in the pyramidal cell layer of the Ammon's horn (Iwase et al., 1998). A distinct paucity has been reported for immunostaining of nNOS in pyramidal cells of the CA1 region (Dinerman et al., 1994) while message for constitutive NOS is expressed (Endoh et al., 1994a; Black et al., 1995). Thus, we speculate that the vulnerability of the CA 1 neurons demonstrated in this study may reflect a cell specific distribution of NOS isoform and response to factors in the extracellular environment.

The current data also suggest a regulatory role for NOS activity on both the astrocyte and microglia response. Previous studies have demonstrated a neurotoxic role of cytokine-activated microglia from rodents via a NO-mediated mechanism (Boje and Arora, 1992; Chao et al, 1992). Astrocytes release NO under basal as well

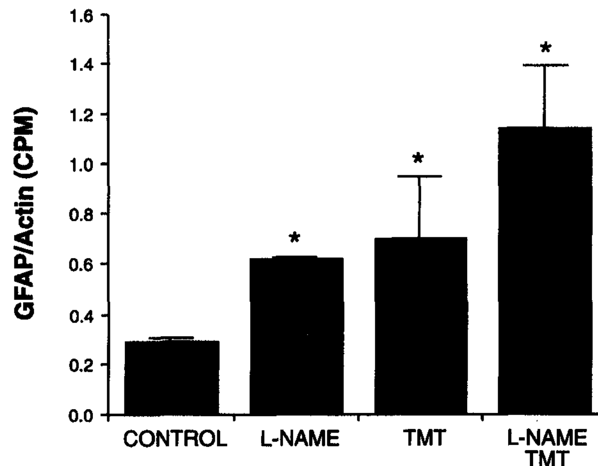


FIGURE 5 Northern hybridization for GFAP mRNA levels relative to β-actin mRNA levels following saline (CONTROL), L-NAME, TMT, or L-NAME + TMT administration. L-NAME (50 mg/kg bwt) or saline was administered twice a day for 4 days prior to injection of TMT (6 mg/kg bwt). Samples were acquired 4 days post-TMT thus comparable to 4 days post L-NAME time point in previous figures. Data represent mean radioactivity (CPM) ± SEM as determined by Ambis Image Acquisition and Analysis System from 4–7 animals per group *significance $p \leq 0.05$

as stimulated conditions such as activation following exposure to cytokines (Murphy et al., 1990; Galea et al., 1992; Mollace and Nistico, 1995; Nathan 1992; Salvemini et al., 1992; Marletta, 1993; Wallace and Bisland, 1994; Murphy et al., 1995). iNOS can be induced in response to cytokine secretion such as TNFα, by macrophages or microglia accumulating in damaged regions of the brain (Minc-Golomb et al., 1994). TNFα is know to increase NOS activity in endothelial cells as well as significantly shorten the halflife of NOS sugesting a dynamic interaction between the two factors in various host-responses to injury (Yoshizumi et al., 1993). In the present study, a microglia reactive response was demonstrated however, evidence of microglia activation to a phagocytic state was lacking. Thus, while neuronal degeneration was evident, loss of neuronal material was not a major pathological response even as late as 30 days. A similar finding was reported by Takeuchi et al., (1998) with L-NAME significantly decreasing microglia phagocytosis of injured neurons in the striatum. In addition, a role for TNFα is suggested in these studies in that cellular response to L-NAME exposure occurred only when the dosing regimen elevated TNFα mRNA levels. A similar pro-inflammatory phenotype has been previously reported for heart tissue following multiple administration of a relatively high dose of L-NAME (Luvara et al., 1998; Koyanagi et al., 2000).

In conclusion, data from the present study support a synergistic interaction between nitric oxide regulation and the pro-inflammatory processes in determining the pattern of nervous system injury. Additional studies are needed to examine the dynamics of individual cellular responses following inhibition of specific nitric oxide synthases as well as the potential for an inflammatory phenotype to occur in the brain following chronic low-level inhibition. A further understanding of both kinetics and interactive processes will offer information toward understanding the dynamics of nitric oxide in the nervous system and the individual cell responses to nitric oxide associated injury.

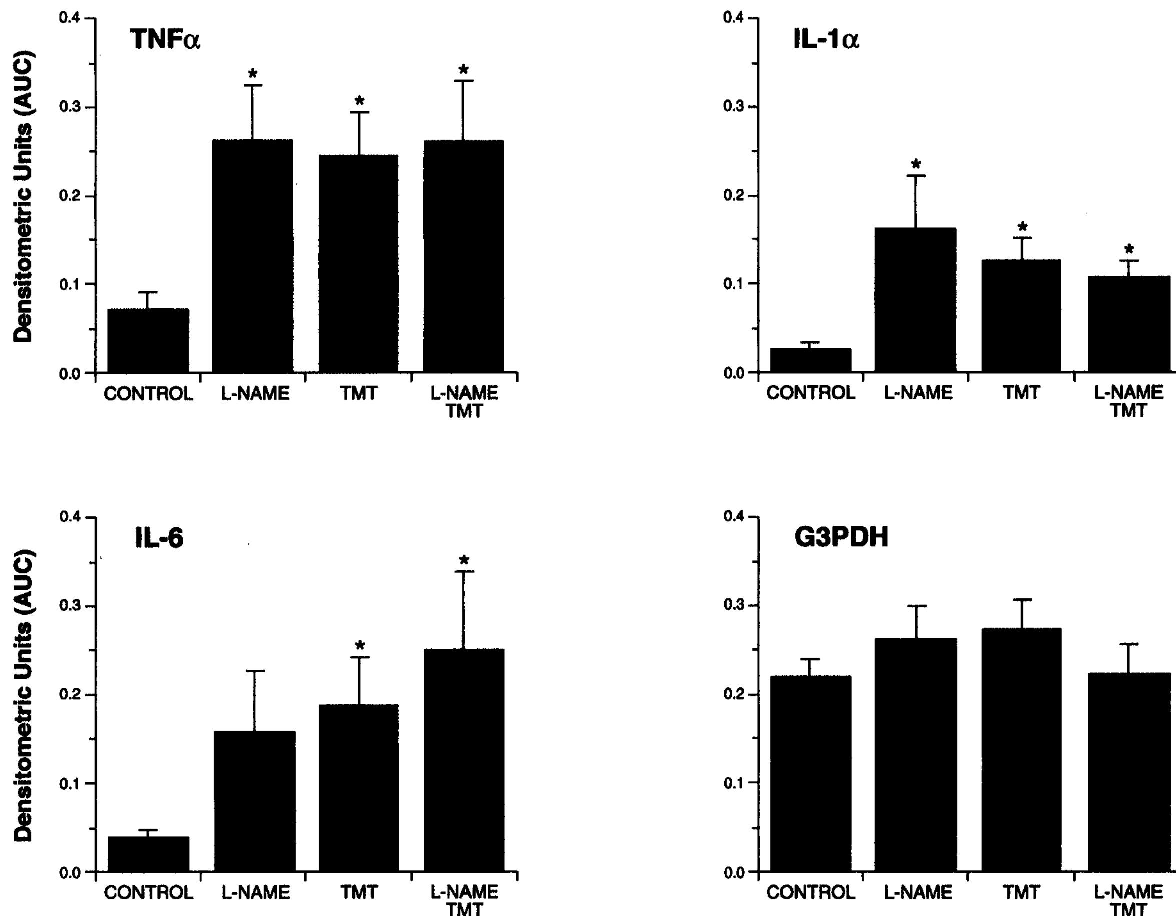


FIGURE 6 RT-PCR for mRNA levels of TNFα, IL-1α, IL-6, and G3PDH in hippocampal tissue of rats exposed to L-NAME, TMT, or L-NAME + TMT. L-NAME (50 mg/kg bwt) or saline was administered twice a day for 4 days prior to injection of saline or TMT (6 mg/kg bwt) and samples acquired 4 days post-TMT. Data represents mean optical density (±SEM) for 4–7 animals per group. *significance $p \leq 0.05$

Acknowledgements

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