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(54) Title: TREATMENT OF NEURODEGENERATIVE CONDITIONS WITH NIMESULIDE

(57) Abstract

The present invention relates to the use of nimesulide and structurally related compounds in the prevention and/or treatment of neurodegenerative conditions. It is based, at least in part, on the discovery that nimesulide exhibits a neuroprotective effect against β–amyloid induced cell death. Without being bound to any particular theory, it appears that nimesulide inhibits a non–inflammatory mechanism of neurodegeneration.

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Description

Treatment of Neurodegenerative Conditions With Nimesulide

1. Introduction

The present invention relates to the use of nimesulide and structurally related compounds in the prevention and/or treatment of neurodegenerative conditions such as Alzheimer's Disease. It is based, at least in part, on the discovery that nimesulide, in effective concentrations, inhibits cell death.

2. Background Of The Invention

2.1. Alzheimer's Disease

Sporadic Alzheimer's Disease is the major neurodegenerative disease associated with aging, the risk of developing the disease rising exponentially between the ages of 65 and 85, doubling every five years. Histologically, the hallmarks of Alzheimer's Disease are the deposition of amyloid in senile plaques and in the walls of cerebral blood vessels; the presence of neurofibrillary tangles, and neurodegeneration. The etiology of Alzheimer's Disease, however, is not well understood. Genetic factors have been proposed to play a role, including trisomy 21, mutations in the amyloid β-protein precursor ("APP") gene, the presenilin-1 (PS1) and presenilin-2 (PS2) genes, and the presence of the apolipoprotein E type 4 allele (Younkin, 1995, Ann. Neurol. 37:287-288; Lendon et al., 1997, JAM A 277:825). Several studies have indicated that β-amyloid induces apoptosis in cultured neurons (Loo et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:7951-7955; Li et al., 1996, Brain Res. 738:196-204). Such induction may involve the immediate early gene proteins, c-jun and fos (Anderson et al., 1995, J. Neurochemistry 65:1487-1498; Anderson et al., 1994, Experimental Neurology 125:286-295; Anderson et al., 1996, J. Neurosci. 16:1710-1719).

It has been proposed that apoptosis may be involved in the pathogenesis of Alzheimer's disease (Smale et al., 1995, Exp. Neurol. 133:225-230; Anderson et al., 1996, J. Neurosci. 16:1710-1719; Anderson et al., 1994, Exp. Neurol. 125:286-295). Inflammatory mechanisms have also been implicated; (Pasinetti, 1996, Neurobiol. Ageing 17:707-716) supportive of such a mechanism are the

observations that acute phase proteins are elevated in the serum of Alzheimer's Disease patients, and are deposited in amyloid plaques; activated microglial cells tend to localize in the vicinity of senile plaques, and complement components have been localized around dystrophic neurites and neurofibrillary tangles (Aisen and Davis, 1994, Am.J.Psychiatry 151:1105-1113). Antiinflammatory agents have been suggested as potential therapeutic agents (Aisen et al., 1996, Dementia 7:201-206; McGeer et al., 1996, Neurology 47: 425-432). Because they tend to have fewer adverse side effects, selective inhibitors of the enzyme cyclooxygenase-2 have been advanced as agents for treating such inflammation (International Publication No. WO 94/13635 by Merck Frosst Canada Inc.). Prior to the present invention, however, it had not been believed that such agents could be used to inhibit non-inflammatory aspects of neurodegeneration in the context of Alzheimer's Disease or otherwise.

2.2. Cyclooxygenase-2

Cyclooxygenases ("COXs") are enzymes that catalyze the formation of prostaglandin ("PG")-H_2 from arachidonic acid (AA). PG-H_2 is further metabolized to physiologically active PGs (*e.g.*, PG-D_2, PG-E_2 and PG-$F_{2\alpha}$), prostacyclin (PG-I_2) and thromboxanes. Specific PGs have diverse, often antagonistic effects on different tissues. For example, PG-I_2 and PG-E_2 are potent vasodilators that may contribute to the inflammatory response, whereas PG-$F_{2\alpha}$ is a vasoconstrictor.

There are two known COX isoforms, COX-1 and COX-2, which, though physiologically distinct, are similar in amino acid sequence and enzymatic functions. COX-1 is constitutively expressed at different levels in different cell types. COX-2, however, is not constitutively expressed, and is generally undetectable in normal peripheral tissues (Kujubu et al., 1991, J. Biol. Chem. 266:12866-12872; O'Banion et al. 1992, Proc. Natl. Acad. Sci. U.S.A. 89:4888-4892). Rather, COX-2 expression is inducible (for example, by mitogens) and COX-2 mRNA levels have been observed to rise rapidly in response to inflammatory stimuli such as interleukin-1β and lipopolysaccharide, and to decrease in response to glucocorticoids. When subjected to these same factors, COX-1 mRNA levels remain substantially unchanged, suggesting that COX-2 is the isoform which mediates inflammation (Cao

et al., 1995, Brain Res. 697:187-196; O'Banion et al. 1992, Proc. Natl. Acad. Sci. U.S.A. 89:4888-4892).

Recent evidence suggests that COX-2 may play a role in mechanisms of cell survival and cell adhesion in peripheral cells (Lu et al., 1996, Proc. Natl Acad. Sci. U.S.A. 92:7961-7965; Tsujii et al., 1995, Cell 83:493-501). Tsuji et al. reports that epithelial cells engineered to express elevated levels of COX-2 were resistant to butyrate-induced apoptosis, exhibited elevated BCL2 protein expression, and reduced transforming growth factor β2 receptor levels (Tsuji et al., 1995, Cell 83:493-501). Lu et al. indicates that non-steroidal antiinflammatory drugs may induce an apoptotic mechanism involving the COX system.

The roles of COX-1, COX-2 and PG synthesis in normal brain, and in the context of Alzheimer's Disease, have not been fully characterized to date. The importance of PGs in brain physiology may be independent of inflammatory mechanisms. In the brain, PG receptors have been identified in the hypothalamus, thalamus, and limbic system (Watanabe et al., 1989, Brain Res. 478:143-148). PGs are involved in hypothalamic-pituitary hormone secretion (Kinoshita et al., 1982, Endocrinol. 110:2207-2209), regulation of temperature and the sleep-wake cycle (Hayaishi, 1988, J. Biol. Chem. 263:14593-14596). There is recent evidence that COX-2 mRNA is expressed and regulated in rat brain by synaptic activity and glucocorticoids (Adams et al., 1996, J. Neurochem. 66:6-13; Kaufmann et al., Proc. Natl. Acad. Sci. U.S.A. 93:2317-2321; Yamagata et al., 1993, Neuron 11:371-386). These studies indicate that COX-2 is regulated as an immediate early gene in the brain, and suggest that PGs may be important in trans-synaptic signaling and long-term potentiation. Chang et al. (1996, Neurobiol. of Aging 17:801-808) report that COX-2 mRNA expression is decreased in Alzheimer's disease.

3. Summary Of The Invention

The present invention relates to the use of nimesulide and structurally related compounds in the prevention and/or treatment of neurodegenerative conditions. It is based, at least in part, on the discoveries that (i) COX-2 expression in models of neurodegeneration is increased in neurons rather than glial cells (consistent

with a non-inflammatory mechanism), and (ii) nimesulide exhibits a neuroprotective effect against β-amyloid induced neuronal cell death. This latter finding is particularly unexpected in view of the ability of COX-inhibitors to increase apoptosis of non-neuronal cells. Without being bound to any particular theory, it appears that nimesulide inhibits a non-inflammatory mechanism of neurodegeneration.

4. Description of the Figures

FIGURE 1. **Regional expression of COX-2 mRNA in control unlesioned male rat brain.** COX-2 mRNA expression was assessed by *in situ* hybridization and visualized by X-ray autoradiography. Abbreviations: DG, dentate gyrus; CA1, CA2 and CA3 subregions of the neuronal pyramidal layer of the hippocampal formation; PAC, parietal cortex; PYC, pyriform cortex; AC, amygdaloid complex; THAL, ventroposterior thalamic nucleic. Adapted from plate 24 of Paxinos and Watson, 1986 (*The Rat Brain in Steriotaxic Coordinates*, Academic Press, NY).

FIGURE 2. **Maturational regulation of COX-2 mRNA expression in rat brain.** Optical densities were quantified from autoradiographic images. Abbreviations, as above and as follows: PYC/AC, pyriform and amygdaloid complex that were quantified for COX-2 mRNA expression as a single brain region. N=5-8 per time point.

FIGURE 3. **Maturational influence on COX-2 mRNA expression during response to KA-induced seizures.** Micrographs were generated from autoradiographic images. For anatomical distribution of changes, refer to Figure 1. Control, unlesioned vehicle-injected rats; KA 8 H, KA-treated rats 8 hours prior to sacrifice; postnatal days P-7, P-14, and P-21.

FIGURE 4 A-D. **Time course of COX-2 mRNA changes in rat brain during responses to KA treatment: maturational influences and regional distribution of changes.** Optical densities were quantified from autoradiographic images. Data are expressed as means ± SEM, N=4=6 per group. *P<0.01 vs 0 H group (saline injected group); 4h, 8h, 16h, 30h, 120h, time in hours after onset of KA-induced seizures.

FIGURE 5 A-D. **Maturational influence on the distribution of COX-2 mRNA expression and induction in rat hippocampus.** COX-2 mRNA in P21 (A,B) and adult (C,D) hippocampal formations as assessed by *in situ* hybridization assay and visualized by emulsion autoradiography using dark-field illumination. In A and C, COX-2 mRNA expression in control rats (vehicle injected); in B and D, COX-2 mRNA 8 hours after onset of KA-induced seizures. Arrows point toward the superficial layer of the DG (stratum granulosum). Scale bar= 200 μm.

FIGURE 6. **Selective induction of hippocampal COX-2 but not COX-1 mRNAs during response to KA-induced seizures as assessed by gel blot hybridization assay.** CTL, control saline-injected rats; KA, KA-treated rats 12 hours post lesioning.

FIGURE 7 A-I. **KA-induced COX-2 and apoptosis in adult rat brain.** In (A,B), COX-2 mRNA expression in CA3 hippocampal pyramidal neurons of control and KA-treated rat, respectively; in (C), CA3 subregion of the hippocampal formation of KA-lesioned rats showing neurons with apoptotic features (arrows). In (D,E), COX-2 mRNA expression in pyriform cortex of control and KA-treated rat respectively. In (F), arrows point toward apoptotic cells of pyriform cortex of KA-lesioned rats. In (G,H), COX-2 mRNA expression in cells of the amygdaloid complex of control and KA-treated rats, respectively. In (I), arrows point toward apoptotic cells of the amygdaloid complex of KA-lesioned rats. COX-2 mRNA was assayed by *in situ* hybridization and visualized by emulsion autoradiography under dark field illumination. *In situ* 3' end-labeling was used to assess apoptotic features following KA treatment. Scale bar: in A,B,D,E,G,and H=200 μm; in C,F and I=40 μm.

FIGURE 8 A-D. **Immunocytochemical evidence of neuronal COX-2 expression/regulation in response to glutamate *in vitro*.** COX-2-like immunoreactivity in monotypic cultures of rat primary hippocampal neurons in (A) control and (B) after glutamate exposure (12 hours). In (C,D) control and glutamate treated cultures immunoreacted with immunoadsorbed COX-2 antibody, respectively. Scale bar = 50μm.

FIGURE 9 A-C. **Effect of nimesulide on endotoxin-mediated synthesis/secretion of cytokines and nitrites in glia.** The effect of 10^{-9} M nimesulide on the synthesis/secretion of (A) TNF, (B) NO intermediates (Griess reaction) and (C) PGE_2, as assessed in BV2 mouse immortalized microglial cells. Mean ±SEM, n=8-10 per group. Lipopolysaccharide ("LPS")=5 μg/ml. LPS and nimesulide were added in combination to cultures; incubation time was 24 hours. Statistics used ANOVA, $p<0.05$.

FIGURE 10 A-E. **Time course of changes of COX-2 protein in P19 cells during response to conditions leading to apoptotic death.** In (A), quantitative

analysis of COX-2 induction in P19 cells, n=4-6 per group, $p<0.05$ vs. t=0. In (B), changes were assessed by western analysis, using chemiluminescent detection. In (C,D) the morphological appearance of apoptotic nuclei in P19 cells was assessed by Hoechst H33258 24 hours after serum removal (and replacement with N2 medium). In (E), the electrophoretic profile of DNA showing DNA laddering degradation was assessed 14 hours after serum removal (lane 1, control cells at t=0; lane 2, DNA laddering 14 hours after serum removal; lane 3, DNA markers). The COX-2 polyclonal antibody used in these studies was as described in Section 6. The COX-2 specific antibody recognizes two major bands having estimated molecular weights of about 70 and 65 kDa in total homogenates of mouse, rat and human brains.

FIGURE 11A-E. **Regulation of COX-2 during response to Aβ1-40 mediated oxidative stress in SH-SY5Y neuronal cells.** (A) Bar graph showing the amount of COX-2 immunoreactivity present relative to control ("CTL"). The immunoreactivity measurement was derived from the data shown in (B). (B) Western blot of proteins prepared from control SH-SY5Y cells or cells exposed to Aβ peptides for 48 or 72 hours, reacted with COX-2 antisera. (C) Results of MTT assays of control or Aβ-treated SH-SY5Y cells. (D) The immunoblot shown in (B) stripped and immunoreacted with actin antisera. (E) SH-SY5Y cultures were examined for apoptotic mechanisms.

FIGURE 12. **Protective effect of nimesulide on Aβ1-40 mediated neurotoxicity as assessed by MTT assay using SH-SY5Y neuronal cells.** Abbreviations: CTL control; NIM nimesulide; A B-β-amyloid (1-40). *$P<0.01$ vs CTL, **$P<0.05$ vs. CTL.

FIGURE 13. **Micrographs showing induction of COX-2 immunoreactivity in temporal cortex of AD and age-matched neurological controls.** The micrographs show a selective induction of COX-2 immunostaining in neuron-rich grey matter ("gm") but not glia-rich white matter ("wm") regions.

FIGURE 14 A-D. **COX-2 immunostaining of neurons in frontal cortex of AD brain and co-localization of COX-2 immunostaining with AD plaques in frontal cortex of AD brains.** In (A,B), immunostaining of COX-2 neurons of AD are shown (A, low power; B, high power magnification). In (C),

immunostaining of diffuse plaques. In (D), COX-2 in neuritic plaques as assessed by Aβ immunostaining on adjacent tissue sections.

FIGURE 15A-D .**COX-2 expression in AD brain frontal cortex.** (A) Northern blots of COX-2 and COX-1 mRNA in AD frontal cortex relative to controls quantitative analysis of data is in (B). (C) Western blots of COX-2 protein in AD frontal cortex and control; quantitative analysis of data is in (D).

FIGURE 16. **COX-2 mRNA regulation in neurons of the anterior and posterior horn of the spinal cord of ALS and neurological controls.** Autoradiographic images were visualized by X-ray film. The arrow pointed toward the ventral horn shows intense induction of COX-2 mRNA signal in the ALS case.

FIGURE 17A-D. **COX-2 mRNA elevation in a biopsy of human cortical epileptic foci as assessed by in situ hybridization assay.** Autoradiographic images were visualized by X-ray film. Arrow pointed toward the cortical layers showing intense COX-2 mRNA signal in epileptic brain (A) versus control (B). Parts (C) and (D) show analysis of compiled data relating to COX-2 and COX-1 mRNA levels.

FIGURE 18. **Potentiation of cyclooxygenase and peroxidase activities by aggregated Aβ peptides.**

FIGURE 19A-B. **Transient expression of COX-2 in neuronal cells potentiates Aβ-mediated impairment of redox activity.** SH-SY5Y neuronal cells were transfected with either the human COX-2 (A) or COX-1 (B) gene and treated with $A\beta_{25-35}$, and then redox activity was assessed by MTT assay.

FIGURE 20A-B. **Generation of Transgenic Mice Expressing COX-2.** (A) *In situ* hybridization demonstrates COX-2 mRNA expression in TgNHC32 mice but not wild-type (mice one month old). (B) Regional expression of hCOX-2 mRNA in NHC32 and NHC5 relative to wild-type, as quantified using Bioquant image-analysis.

FIGURE 21. **hCOX-2 over-expression potentiates Aβ-mediated oxidative impairment in primary mouse neuronal cultures.**

FIGURE 22. **Nimesulide protects B12 neuronal cells against glutamate mediated oxidative stress.**

FIGURE 23. **Control studies for FIGURE 22.** Dose (A) and time course (B) association with glutamate mediated oxidative impairment as measured by LDH assay. (C) demonstrates lack of toxicity of nimesulide to B12 cultures.

5. Detailed Description of the Invention

The present invention relates to the use of the cyclooxygenase-2 ("COX-2") selective inhibitor, nimesulide, and structurally related compounds in the prevention and/or treatment of neurodegenerative conditions. It is based, at least in part, on the discoveries that COX-2 expression increased in parallel with neuronal lesions produced by kainic acid and with induction of neuronal apoptosis in an established *in vitro* system (see Sections 6 and 8, below). Contrary to reports in the prior art that COX-2 plays a primary role in inflammation and may be involved in an inflammatory component of neurodegeneration (for example, in the context of Alzheimer's Disease), it has further been discovered that in the human brain, COX-2 expression appears to be restricted to neurons rather than to glial cells (whereas expression in glial cells would be expected if an inflammatory mechanism were operative: see Section 9, below). A correlation between COX-2 expression and the characteristic pathological change associated with Alzheimer's Disease, amyloid plaque, has been observed (see Section 9). Further, it has been demonstrated that nimesulide exerts a neuroprotective effect against β-amyloid induced cell death in neuronal cultures. In view of these findings, according to the invention, nimesulide and related compounds may be used to intervene in the process of apoptotic neuronal cell death associated with Alzheimer's Disease and other neurodegenerative conditions. Without being bound to any particular theory, the action of nimesulide and related compounds may be mediated by the reduction of the apoptotic effects of oxidative stress.

The term "nimesulide", as used herein, refers to a compound, 4-nitro-2-phenoxymethanesulfonaninide, having a structure as set forth for Formula I below.

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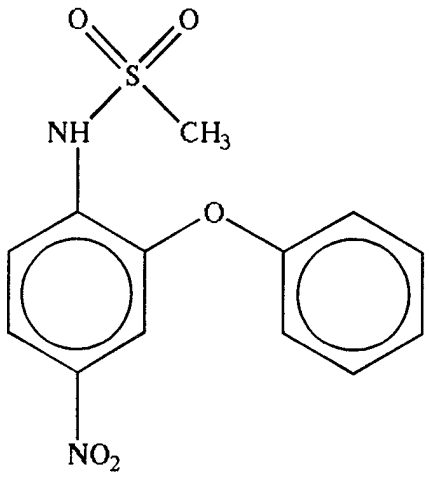

Compounds which are considered structurally related are compounds which have a similar bicyclic structure and which selectively inhibit COX-2. For example, substituents, analogs, and enantiomers of nimesulide are considered to be structurally related compounds. As a further example, a structurally related compound may compete with nimesulide for binding to COX-2, or may bind to substantially the same location of COX-2, as determined crystallographically. Moreover, nimesulide, conjugated to another compound, is also considered to be a structurally related compound as defined herein.

Nimesulide, or a structurally related compound, may be administered so as to provide an effective concentration in the nervous system of the subject being treated. An effective concentration is defined herein as that concentration which inhibits neuronal cell death by at least 20 percent. In specific, nonlimiting embodiments of the invention, the concentration of nimesulide is at least 1 nanomolar, and preferably at least 1 micromolar in the location of neuronal cells which are desired to be treated. Desirably, the concentration of nimesulide is less than 10^{-3} molar to avoid toxicity. In one such specific embodiment, where the neurodegenerative condition to be treated is Alzheimer's Disease, the concentration of nimesulide in the hippocampal formation of the subject is at least 1 picomolar, preferably at least 1 nanomolar, and more preferably at least 1 micromolar. The corresponding serum concentrations may be at least 10 picomolar, preferably at least 10 nanomolar, and more preferably at least 10 micromolar. Equivalent amounts of structurally related compounds (adjusted to compensate for differences in potency) may also be used.

Nimesulide or a structurally related compound may be administered in any manner which achieves the desired effective concentration. For example, suitable routes of administration include oral, intravenous, subcutaneous, intramuscular, transdermal and intrathecal routes.

Nimesulide or a structurally related compound may be comprised in a suitable pharmaceutic carrier. Formulations may provide for sustained release.

For example, but not by way of limitation, nimesulide may be administered orally at doses of 2-800 mg/day, preferably 50-400 mg/day, and most preferably 200 mg/day.

Neurodegenerative conditions which may be treated according to the invention include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, seizure-associated neurodegeneration, amyotrophic lateral sclerosis, spinal cord injury and other diseases wherein the condition is not conventionally regarded as an inflammation-mediated or autoimmune disorder.

6. Example: Maturational Regulation and Regional Induction of Cyclooxygenase-2 in rat Brain

6.1. Materials and Methods

Animals and excitotoxic lesions. Male adult Sprague/Dawley rats of different ages were maintained in a controlled light and temperature environment, with food and water *ad libidum*. In adult rats (250-300g), hippocampal excitotoxic lesions were induced by subcutaneous injection of kainic acid ("KA"; 10 mg/kg, Sigma). Because KA uptake is higher in young rats relative to adults (Berger et al., 1986, in Schwartz and Ben-Ari, *Advances in Experimental Medicine and Biology*, Plenum, NY, pp. 199-209), KA doses were adjusted to produce maximal excitotoxicity without reaching lethal doses (from 2 mg/kg at postnatal day P-7 to 6 mg/kg at postnatal day P25). Saline injected rats were used as controls (0 hour time point).

COX-1 and COX-2 cDNA probes. Bluescript plasmid (Stratagene) containing the full length rat COX-1 cDNA (2.7 kb) was linearized by digestion with *Cla*I; PCRII plasmid (Invitrogen) containing the coding sequence for rat COX-2 (1.8 kb) was linearized by digestion with *PflMI* (Feng et al.,1993, Arch. Biochem. Biophys. 307:361-368). Linearized plasmids were purified using Elu-Quick (Schleicher & Schuell) after agarose gel electrophoresis.

***In situ* hybridization.** At various intervals after the onset of KA-induced seizures, the rats were sacrificed, and the brains quickly removed, rinsed in cold phosphate buffer (PBS, 10 mM, pH 7.4) and immersed in methylbutane at -25°C for three minutes. The brains were sliced into 10 micrometer sections, frozen, and the resulting frozen sections were mounted on polylysine-coated slides and stored at -70°C. For immunocytochemistry ("ICC") or *in situ* hybridization ("ISH"), frozen sections were post-fixed in PBS containing 4 percent paraformaldehyde (30 minutes at room temperature) and then rinsed in PBS. For ISH, tissue sections were rinsed in 0.1M triethanolamine ("TEA"), pH 8.0, incubated in acetic anhydride ("AAH"; 0.25% v/v in TEA, 10 minutes) and rinsed in TEA and PBS. Following AAH treatment, tissue sections were hybridized with [^{35}S]-cRNA probes (0.3 μg/ml, 2 x 10^9 dpm μg^{-1}) made from COX-2 linearized cDNA transcription vectors (Feng et al.,1993, Arch.

Biochem. Biophys. 307:361-368). Sense strand hybridization was used as a control and gave negative results. Following hybridization for 3 hours at 50°C, stringent washes (0.1x SSC at 60°C) and dehydration, slides were exposed to X-ray film for seven days for quantification. Slides were then exposed to NTB-2 emulsion (Kodak, Rochester, NY) for microscopic analysis of COX-2 mRNA distribution. Following development, tissue sections were counterstained with cresyl violet. Film autoradiograms were analyzed quantitatively using an image analysis system with software from Drexel University (Tocco et al., 1992, Eur. J. Neurosci. 4:1093-1103). Statistics were calculated by ANOVA followed by additional posthoc analysis.

***In situ* end labeling.** In parallel studies, paraformaldehyde-fixed brain tissue sections were dehydrated, air dried and incubated with dATP, dCTP, dGTP (0.2 mM), dTTP (13 μM), digoxigenin-11-dUTP and DNA polymerase I (Boehringer Mannheim) at 10 units/100 μl at 37°C for 2 hours. The reaction was stopped by addition of 20mM EDTA, pH 8.0. Sections were incubated at room temperature overnight with an alkaline-phosphatase-conjugated digoxigenin antibody (Genius System, Boehringer Mannheim) diluted 1:200 in 5 percent sheep serum diluted in 150 mM NaCl, 100 mM TRIS-HCl, pH 7.5. Colorimetric detection with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate was performed with the Genius System by the manufacturer's protocol (Sakhi et al.,Proc. Natl. Acad. Sci. U.S.A. 91:7525-7529).

Northern blot hybridization assay. RNA extraction was performed as follows. After sacrifice, rat brains were dissected and stored at -75°C prior to processing. Total RNA was extracted from pools of hippocampal tissue (Pasinetti et al.,1994, J. Comp. Neurol. 339:387-400). Briefly, tissues were homogenized for 1 minute in 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.5), 0.5% sarcosyl and 0.1 M β-mercaptoethanol, in a final volume of .5 ml. After acidified phenol/chloroform extraction and ethanol precipitation, the RNA pellet was rinsed consecutively with 70% and 100% ethanol. The purified RNA was then dissolved in 0.5% sodium dodecyl sulfate ("SDS") and stored at -75°C. Total RNA was quantified in a UV spectrophotometer. Total RNA (5μg) from tissue was electrophoresed on denaturing (0.2M formaldehyde) agarose gels and transferred to a nylon membrane

(Nylon 66 plus; Hoeffer, San Francisco CA) in 2x SSC. Blot hybridization was carried out with 10^6 cpm/ml of antisense COX-1 or COX-2 [^{32}P]-cRNA probes in 50% formamide, 1.5x SSPE, 1% SDS, 0.5% dry milk, 100 μg/ml yeast total RNA and 500 mg/ml salmon sperm DNA at 53°C for about 15 hours. Blots were washed to a final stringency of 0.2x SSC, 0.2% SDS at 72°C. Blots were exposed to Kodak X-ray film (XAR-5) with intensifying screens at -70°C.

Cell cultures. Hippocampal neuron cultures were derived from embryonic rat brain. E16-E18 embryos were dissected in Hank's balanced salt solution and cultured (Pasinetti et al.,1994, J. Comp. Neurol. 339:387-400; Peterson et al., 1989, Dev. Brain Res. 48:187-195). Culture media were changed every 3 days. For glutamate neurotoxicity studies, eight day old cultures were treated with glutamate (250 μM, Sigma) in the presence of 2.4 mM calcium ion and 0.8 mM magnesium ion. After 6 hours of glutamate exposure, culture medium was replaced with fresh medium; COX-2-like immunoreactivity was assessed in neurons 12 hours later.

Immunocytochemical detection of COX-2 in monotypic cultures of primary rat neurons. Control and glutamate treated cultures were post-fixed in PBS containing 4% paraformaldehyde (30 minutes, room temperature), rinsed in PBS, pre-treated with normal serum and incubated overnight at 4°C with primary antibodies. COX-2 antisera (rabbit IgG) was raised against a synthetic peptide (CNASASHSRLDDINPT; SEQ ID NO:1) encompassing the C-terminal region of the murine COX-2. The antisera reacts with human and rat COX-2 but not with COX-1, as assessed by Western blot analysis. Vectastain ABC kit (Vector, Burlingame, CA) was used in subsequent steps to complete the diaminobenzene staining (Pasinetti et al.,1994, J. Comp. Neurol. 339:387-400). Immunoadsorption of COX-2 antisera with synthetic COX-2 peptides controlled for specificity; adsorption was carried out overnight at 4°C with synthetic COX-2 peptides at 30 μg/ml.

6.2. Results

COX-2 expression in adult rat brain. Figure 1 shows by ISH that the regional distribution of COX-2 mRNA was most notable in limbic structures, but was

also present in neocortex (consistent with the reports of Kaufmann et al., Proc. Natl. Acad. Sci. U.S.A. 93:2317-2321 and Yamagata et al., 1993, Neuron 11:371-386). In the hippocampal formation, COX-2 mRNA was selectively expressed in cells of the granule and pyramidal neuron layers. COX-2 mRNA expression was also found in the outer layer of the parietal cortex, the pyriform cortex and cells of the amygdaloid complex.

Maturational regulation of hippocampal COX-2 mRNA expression. ISH results indicated that during maturation, COX-2 mRNA expression is differentially regulated in subsets of cells of neuronal layers of the hippocampal formation (Figure 2, Figure 3 top). From postnatal days P7-P14, COX-2 mRNA showed greater than two-fold increase in the granule cell layer of the dentate gyrus and in the CA3 subdivision of the pyramidal cell layer ($p<0.001$, Figure 2). Though the expression of COX-2 mRNA was lower in the other brain regions examined, the pattern of maturational expression was similar (Figure 2). By postnatal day P21, COX-2 mRNA expression approximated adult levels in all subregions examined (Figure 2, Figure 3 top).

Response to KA-induced seizures. To further explore maturational regulation of COX-2 in brain, COX-2 mRNA expression during responses to KA-induced seizures was examined postnatally. Despite intense seizure activity after KA treatment, no detectable change of COX-2 mRNA expression was found in any brain region examined at P7 (Figure 3, Figure 4A). Changes in COX-2 mRNA expression at 120 hours post-KA treatment in the P7 group indicate developmental maturation rather than response to KA toxicity (Figure 4A). In contrast to the P7 group, at P14 and P21 COX-2 mRNA increased within 4-8 hours after onset of KA-induced seizures in all the hippocampal subregions examined (Figures 4B and 4C). The level of COX-2 mRNA expression returned toward control levels within 120 hours after treatment in P14, P21 and adult rat brain.

Within the dentate gyrus, control COX-2 expression in P14 and P21 rats was asymmetric, selectively localized to the more superficial neurons of the stratum granulosum rather than the deeper granule cells of the dentate gyrus blade (Figure 5A). In response to KA treatment, COX-2 mRNA induction showed similar

asymmetry of expression (Figure 5B). In contrast, the asymmetry within the dentate gyrus was less notable in control animals and after KA-induction of the adult group (Figures 5C and 5D).

In parallel studies, northern blot hybridization of total RNA from hippocampus of adult rats 12 hours after KA-induced seizures confirmed COX-2 mRNA induction (Figure 6). No detectable induction of COX-1 mRNA was found in the same rat brain (Figure 6).

KA-induced COX-2 and apoptosis in adult rat. By 8 hours after onset of KA-induced seizures, COX-2 mRNA induction in cells of the CA3 region of the hippocampal formation (Figure 7B), pyriform cortex (Figure 7E) and amygdaloid complex (Figure 7H) of the adult brain paralleled temporally and overlapped anatomically the onset of apoptosis as assessed by *in situ* end-labeling in the same brain regions (Figure 7C, CA3 regions of the hippocampus; Figure 7F, pyriform cortex; Figure 7I, amygdaloid complex). Cellular COX-2 mRNA expression in adult, control (Figures 7A, 7D and 7G) and KA-treated (Figures 7B, 7E and 7H) rats was identified by emulsion autoradiography using ISH assays.

Immunocytochemical evidence of neuronal COX-2 expression/regulation in response to glutamate *in vitro*. Primary cultures of rat hippocampal neurons were exposed to glutamate *in vitro*. At baseline, constitutive COX-2 expression was demonstrated by immunocytochemistry (Figure 8B). Twelve hours after exposure to glutamate, an increase in COX-2 immunoreactivity was observed which coincided with marked reduction in the number of neurons (Figure 8D).

COX-2 expression in human epilepsy. Increased expression of COX-2 mRNA was detected by ISH in a biopsy of human brain at epileptic foci (Figure 17A-D). It was found that COX-2 mRNA, but not COX-1 mRNA, was significantly elevated in the cortex of epileptic patients. the p vlaue was <0.05.

7. Example: Nimesulide Suppresses Cytokine and Nitrite Production in Microglia Cultures

In experiments conducted *in vitro*, nimesulide at low concentration (1

nanomolar) was found to be effective in the suppression of endotoxin-mediated induction of tumor necrosis factor ("TNF") production by immortalized brain-derived microglia (BV-2) and astrocytes (Figure 9A). In parallel, nimesulide was equally effective in blocking nitrite production (Griess reaction; Figure 9B). This latter observation is particularly relevant in view of evidence showing that blockade of neuronal nitric oxide ("NO")-synthase protects against glutamate neurotoxicity. Nimesulide was also found to be effective in blocking endotoxin-mediated induction of prostaglandin PGE_2) in brain-derived microglia (Figure 9C).

8. Example: COX-2 Expression in Apoptotic Cells

The regulation of COX-2 expression was studied using an established *in vitro* model of apoptosis. Specifically, the regulation of COX-2 was studied in P19 embryonic carcinoma cells during responses to serum deprivation. Under such conditions, the P19 cells underwent apoptotic cell death, showing characteristic DNA fragmentation and nuclear morphology. As shown in Figure 10, using this system, coincidental onset of apoptosis and elevation of COX-2 expression was observed.

These studies were extended to the human neuronal cell line SH-SY5Y. β-amyloid has been demonstrated to play a role in inducing neurodegeneration in SH-SY5Y cells (Oda et al., 1995, Alzheimers Res. 1:29-34). SH-SY5Y cells were treated with synthetic aggregated $A\beta_{1-40}$ peptides at a concentration of 20μM for various periods of time. Western blot analysis was performed of cell extracts, using COX-2 antisera or actin antisera as control (FIGURES 11B and D, respectively). In FIGURE 11A, COX-2 protein from control or Aβ-treated SH-Sy5Y neuronal cells was quantified from the immunoblots shown in FIGURE 11B (at the 72 hr. time point; only the 70kDa mw species being quantified). COX-2 signal was abolished by immunoadsorption of COX-2 antisera with purified human recombinant COX-2 ("hrCOX-2") peptides. FIGURE 11C shows that diminished cell redox activity was observed 72 hours after treatment with aggregated $A\beta_{1-40}$ (at 20μm), as assessed by MTT assay in parallel cultures. Bar graphs represent the mean ± SEM, n=4-5 per group, $p<0.05$ (t-test). COX-2 integrated optical densities were analyzed from digitized images using a Bioquant image analysis

(Biometrics, Nashville, TN). It was also found that COX-2 expression occurred in parallel to DNA laddering (FIGURE 11E), an index of apoptosis. Figure 12 shows that nimesulide at 10^{-6} and 10^{-9} molar was able to block Aβ1-40 toxicity, as assessed by the MTT assay.

Interestingly, when aggregated synthetic $A\beta_{1-40}$ peptides (150μM) were coincubated with purified hr-holoCOX-2 (COX-2 plus heme-cofactor, 50nM) for 16 hours, at 37°C in 1X PBS, and cyclooxygenase and peroxidase activities were measured (Murphy et al., 1989, Neuron 2:1547-1558) cyclooxygenase and peroxidase activities increased (relative to activity levels of enzyme in the absence of Aβ; FIGURE 18). Cyclooxygenase and peroxidase levels in the absence of heme gave negative results.

9. Example: COX-2 Expression in Alzheimer's Disease

The expression of COX-2 in normal human brains and in brains of Alzheimer's Disease ("AD") patients was studied. Immunocytochemical data indicates that COX-2 expression is primarily localized to cells with neuronal morphology in human brain; minimal localization of COX-2 immunostaining in cells with glial morphology was found in any region examined. These results tend to suggest a role for COX-2 in a non-inflammatory function. While the immunocytochemical signal for COX-2 was at the limit of detection in temporal cortex of neurological control cases, COX-2 showed elevation in AD brain (Figure 13A, 13B) The selective induction of COX-2 immunoreactivity in grey matter is consistent with the findings showing neuronal induction of COX-2 in rat brain during responses to lesions leading to neuronal death (see Section 6).

The cellular immunodistribution of COX-2 in AD brain was also explored. It was observed that neuronal COX-2 is not only localized to the perikarya (Figure 14A, but is found in neuronal projections as well (Figure 14B). Moreover, intense immunostaining was also found in diffuse plaques (Figure 14C) and in AD neuritic plaques identified by Aβ immunostaining on adjacent tissue sections of the hippocampal formation. These findings suggest a role for COX-2 in mechanisms of neuronal death or survival.

Studies were performed to quantify COX-2 expression in brains of AD and age-matched controls. We used quantitative dot-blot analysis and chemiluminescence detection. Western analysis was used for qualitative assessment. A greater than 2-fold elevation of COX-2 content was found in hippocampal homogenates of AD brains compared to neurological age-matched controls.

Because of the immunocytochemical evidence showing COX-2 immunoreactivity in AD plaques, levels of COX-2 and total Aβ in AD cases were compared. A direct correlation was found.

COX-2 mRNA and protein were found to be increased in AD brain frontal cortex. FIGURE 15A shows Northern blots of total RNA from neurological control (C) and AD frontal cortex hybridized to [^{32}P] COX-2 and COX-1 mRNAs. The mRNA species detected by COX-2 and COX-1 cRNA probes represent an RNA of the same size found in peripheral tissues. FIGURE 15B shows quantified results of the Northern studies. Specifically, COX-2 mRNA prevalence was increased in the frontal cortex of AD vs control, where the presence of equal amounts of RNA in both lanes was confirmed by normalization to 28S rRNA in the hybridization membrane, and complete transfer of RNA to the membrane was evidenced by the absence of 28S and 18S rRNAs in the gel post transfer. Integrated optical densities were analyzed from digitized images using a Biquant image analysis. Bar graphs represent n=9 per group, and the results were significant by a t-test value of $p<0.05$.

Similarly, when Western blot analysis was performed of AD frontal cortex vs. controls, the amount of COX-2 protein was found to increase. FIGURE 15C shows the results of such a western blot. Lanes 1 and 2 show the COX-2 protein content of the same tissues uses for Northern analysis. Lanes 3-4 show the absence of COX-2 bound when COX-2 antisera was immunoadsorbed using hrCOX-2 peptides, demonstrating the specificity of the signal.

The expected 70kDa new species of hrCOX-2 (lane 5) also was absent when antisera was preadsorbed with hr COX-2 peptides. These results are quantified in FIGURE 15D (with respect to the 70kDa species). Further, the immunoblots were stripped and immunoreacted with actin antisera to verify the specificity of changes (actin values: control group 100±8; AD 94±7% of control Bar graphs represent n=9

per group; t-test value is $p<0.05$). The inset in FIGURE 15D shows a correlation of COX-2 content and the number of plaques per mm^2, $n=8$, $r=0.72$, $p<0.03$. COX-2 and actin integrated optical densities were analyzed from digititzed images using Biquant image analysis.

10. Example: COX-2 Expression in Amyotrophic Lateral Sclerosis

In situ hybridization has demonstrated increased COX-2 mRNA in the anterior horn cells of spinal cords of patients suffering from amyotrophic lateral sclerosis ("ACS"); Figure 16.

11 Example: Transient Expression of COX-2 In Neuronal Cells Potentiates Aβ Mediated Impairment of Redox Activity

The role of COX-2 in neuronal death and/or survival was studied in cultured human SH-SY5Y neuronal cells. Cells were transiently transfected with a mammalian expression vector (pRc/CMV/hCOX, Invitrogen) containing either a full length human (h) COX-2 cDNA (pRc/CMV/hCOX-2), or a bacterial chloramphenicol acetyltransferase (CAT) gene (pRc/CMV2/CAT). Following Aβ25-35 treatment, impairment of redox activity as assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetraxolium bromide (MTT)-assay (FIGURE 19). Overexpression of hCOX2 in SH-SY5Y cells transfected with pRc/CMV/hCOX-2 was confirmed immunocytochemically in a parallel tissue culture chamber slide using an α-COX-2 antibody as previously described. In control cells transfected with pRc/CMV2/CAT, the same α-COX antibody generated minimal immunocytochemical signal. We found that transient overexpression of hCOX-2 in SH-SY5Y neuronal cells potentiated Aβ25-35 (25 μM, 48 hrs treatment) mediated impairment of redox activity when compared to SH-SY5Y cultures transfected with control vector (±± $p<0.01$ vs Aβ/control vector; *$p<0.001$ vs CTL. These data are consistent with evidence showing induction of COX-2 mRNA coincidentally with impairment of redox activity in SH-SY5Y neuronal cells (FIGURE 11).

12. Example: Experiments Using COX-2 Transgenic Mice

Based on the evidence that overexpression of COX-2 in neuronal cells may potentiate Aβ -mediated redox impairment and the observation that neuronal COX-2 is elevated in AD brain and in experimental neurodegeneration, we generated a transgenic mouse model with neuronal overexpression of human (h) COX-2. To obtain overexpression of hCOX-2 in neurons, a hybrid gene was prepared in which the expression of a cDNA sequence containing the entire hCOX-2 coding region is regulated by the rat neuron-specific enolase (NSE) promoter.

We obtained four lines with high expression of hCOX-2 mRNA expression. In one of them (NHC32), COX-2 mRNA was assessed by in situ hybridization analysis (FIGURE 20A). We found that in NHC32 mouse line, has high levels of hCOX-2 mRNA in the hippocampal formation, cerebral cortex and in other neuronal layers (Fig. 20B). No white matter level of HCOX-2 mRNA were found using combined immunocytochemistry for NSE and hCOX-2 in situ hybridization on the same tissue section, we confirmed the selectivity of hCOX-2 transgene expression to neuronal cells.

It has further been shown, using neuronal cultures derived from the transgenic mice, that hCOX-2 overexpression potentiated Aβ mediated response. As depicted in FIGURE 21, studies showed that primary neuronal cultures derived from transgenic mice with neuronal COX-2 overexpression (NHC32, n=3), are most susceptible to aggregated Aβ25-35 peptides mediated impairment of redox activity (MTT assay, Aβ25-35 25 μM for 48 hrs), when compared to equally treated neuronal cultures derived from wild type/control littermates (n=4). Moreover, when examined morphologically, Aβ25-35 treated neurons derived from transgenic hCOX-2 mice revealed an intensified regression of neuronal processes (B, inset) when compared to wild type control Aβ25-35 treated neuronal cultures (A, inset).

13. Example: Nimesulide Protects B12 Neuronal Cells Against Glutamate Toxicity

We examined the role of nimesulide, indomethacin and NS398 (another COX-2 preferential inhibitor) on glutamate mediated toxicity. In this study we used B12 neuronal cells; toxicity was assessed by amount increase of lactate

dehydrogenase LDH levels in the conditioned medium after treatment. LDH is an index of cell toxicity and its elevation is generally accepted as marker of neurotoxicity. As shown in FIGURES 22-23, we found that nimesulide protected against glutamate toxicity (10 mM) at 10-6, 10-9 and 10-12 M with pretreatment (24 hrs before) and cotreatment (with glutamate for 24 hrs). Indomethacin, protected at 10-6 M and lost its protective effect at 10-9 and 10-12 M. Marginal protection of glutamate mediated toxicity by indomethacin was observed at 10-9 M (pretreatment 24 hrs). No protection was observed with NS398 pretreatment. In the pretreatment condition drugs remained in the media during glutamate treatment.

Various publications are cited herein, the contents of which are hereby incorporated in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Pasinetti, Giulio M. and Aisen, Paul S.

(ii) TITLE OF THE INVENTION: TREATMENT OF NEURODEGENERATIVE CONDITIONS WITH NIMESULIDE

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 30 Rockefeller Plaza
(C) CITY: New York
(D) STATE: NY
(E) COUNTRY: USA
(F) ZIP: 10112-0228

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/831,402
(B) FILING DATE: April 1, 1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Clark, Richard S.
(B) REGISTRATION NUMBER: 26,154
(C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 212-705-5000
(B) TELEFAX: 212-765-2519
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Asn Ala Ser Ala Ser His Ser Arg Leu Asp Asp Ile Asn Phe Thr 16
5 10 15

WHAT IS CLAIMED IS:

1. A composition comprising an effective amount of nimesulide, to be used in a method of preventing non-inflammatory neuronal cell death in a patient suffering from a neurodegenerative condition.

2. The composition of claim 1, where the neurodegenerative condition is Alzheimer's Disease.

3. A composition comprising an effective amount of nimesulide, to be used in a method of preventing neuronal cell death in a patient suffering from amyotrophic lateral sclerosis.

4. A composition comprising an effective amount of nimesulide, to be used in a method of preventing neuronal cell death in a patient suffering from a neurodegenerative condition caused by chronic epilepsy.

5. The composition of claim 1, wherein neurons affected by the neurodegenerative condition are exposed to a concentration of nimesulide of at least one micromolar.

6. The composition of claim 2, wherein neurons affected by the neurodegenerative condition are exposed to a concentration of nimesulide of at least one micromolar.

7. The composition of claim 3, wherein neurons affected by the neurodegenerative condition are exposed to a concentration of nimesulide of at least one micromolar.

8. The composition of claim 4, wherein neurons affected by the neurodegenerative condition are exposed to a concentration of nimesulide of at least one micromolar.

9. A transgenic animal carrying a gene encoding the human cyclooxygenase-2 gene.

10. A neuronal cell culture prepared from the transgenic animal of claim 9.

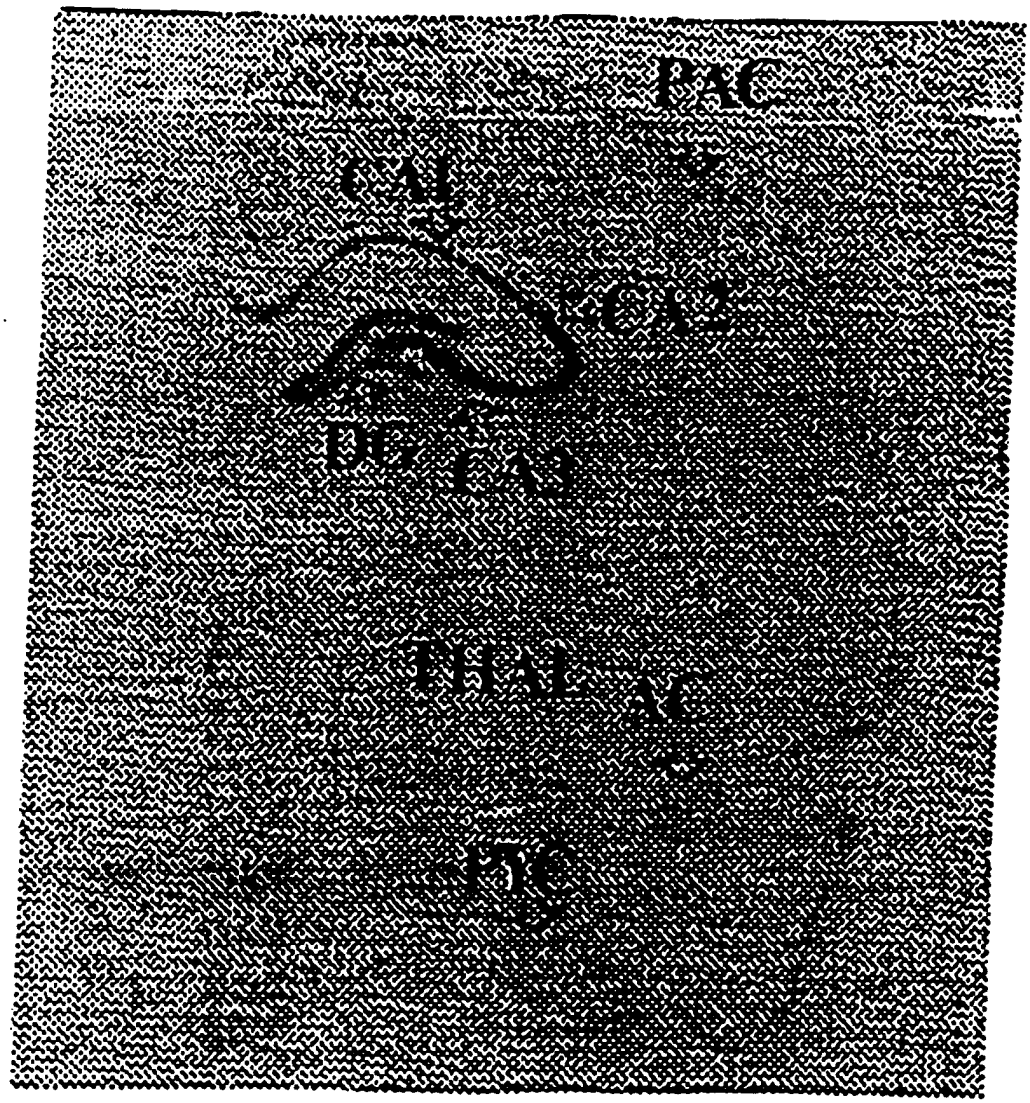


FIGURE 1

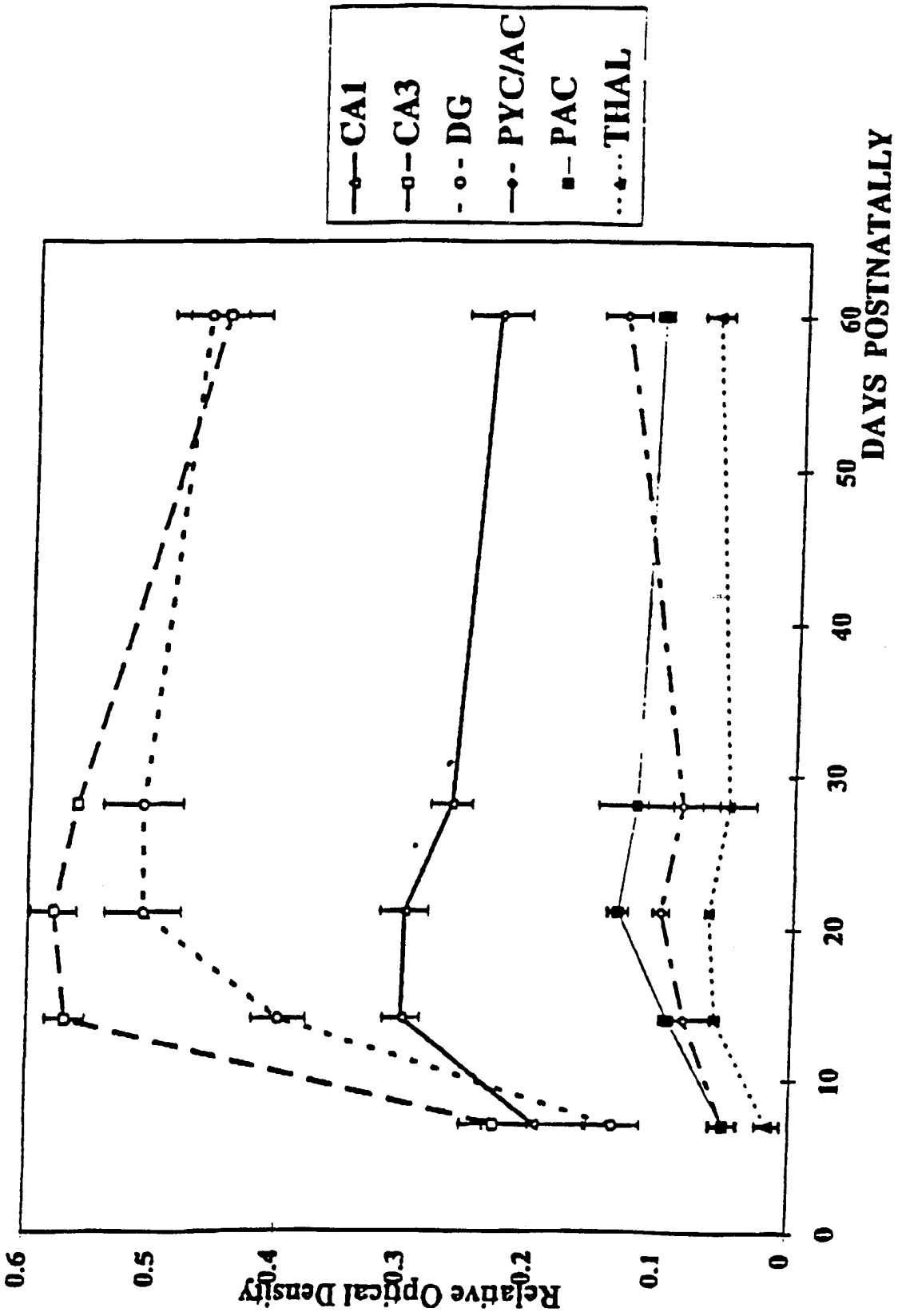

CA1
CA3
DG
PYC/AC
PAC
THAL
Relative Optical Density
0
0.1
0.2
0.3
0.4
0.5
0.6
0
10
20
30
40
50
60
DAYS POSTNATALLY

Fig. 2

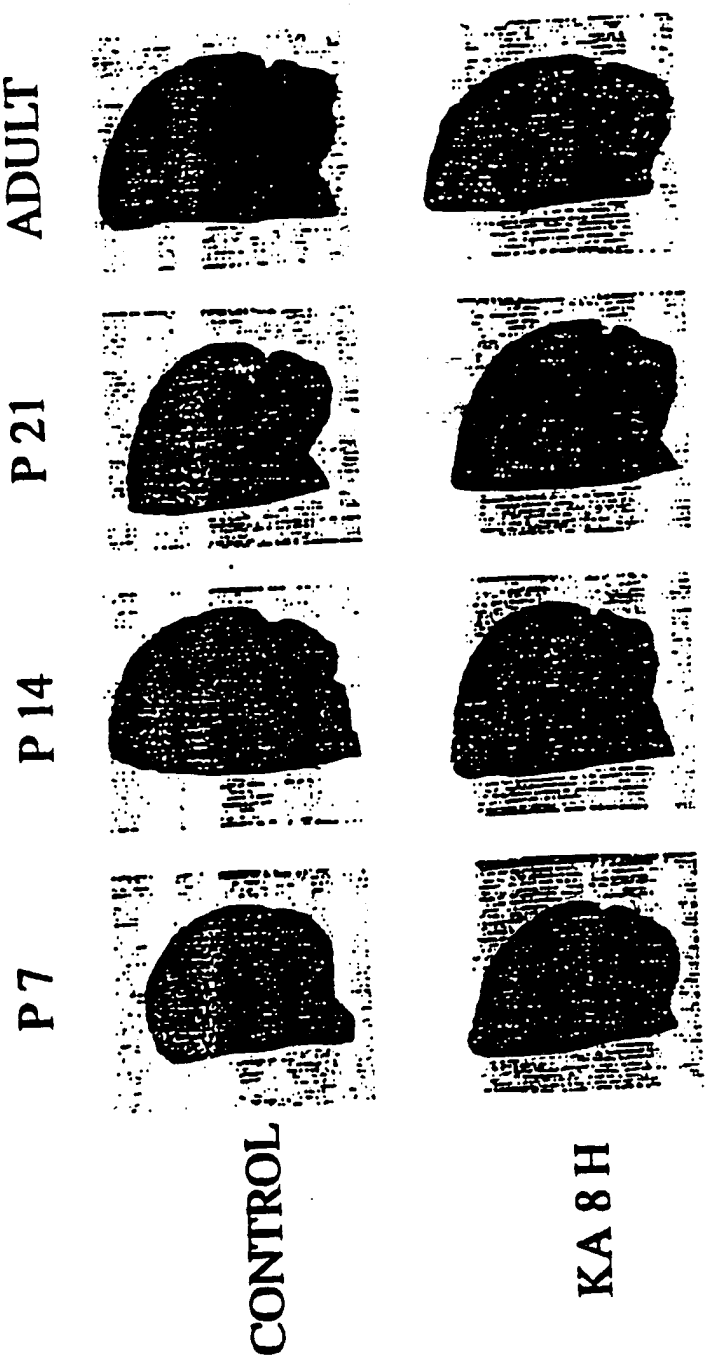
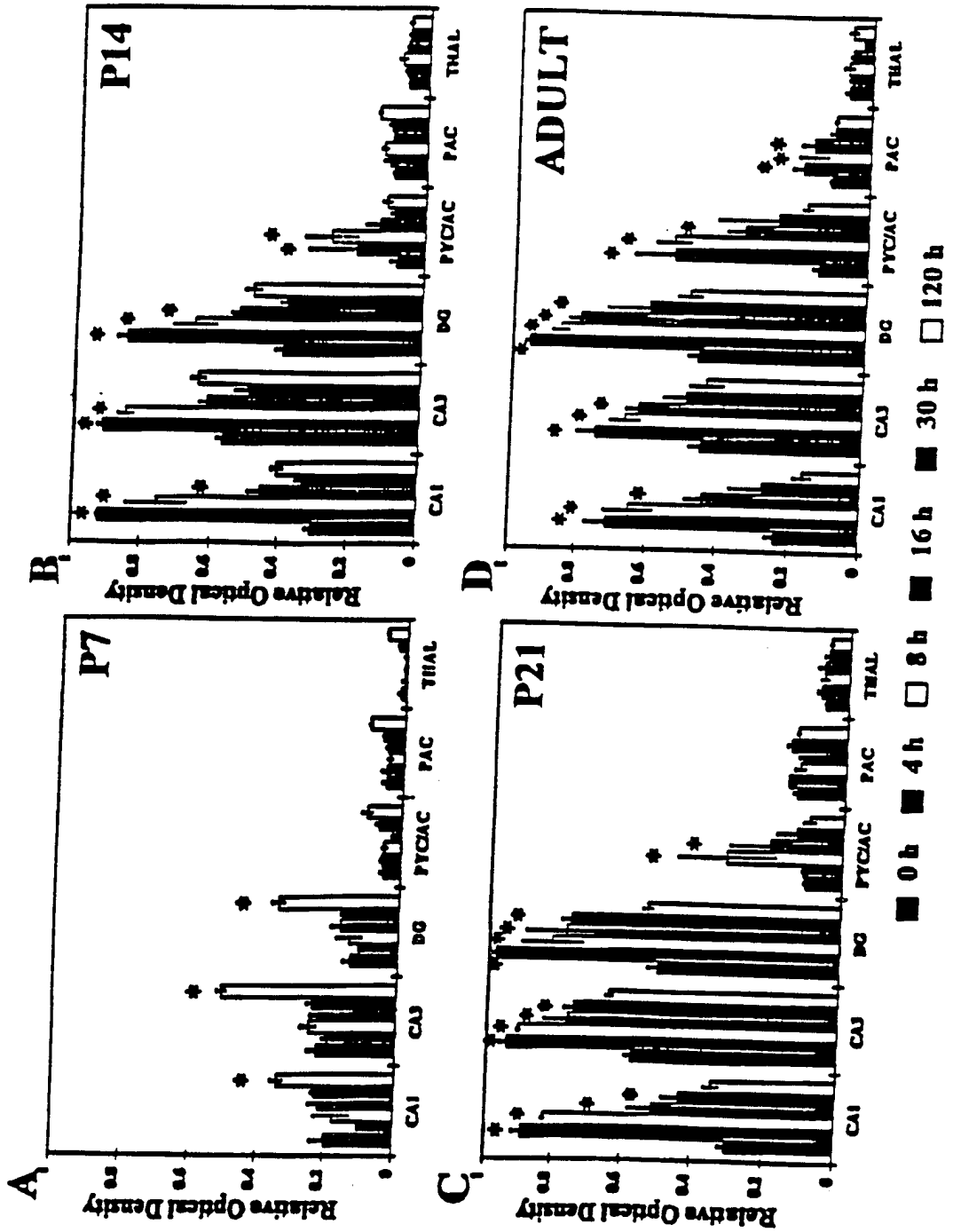


FIG. 3


A
P7
B
P14
C
P21
D
ADULT
Relative Optical Density
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CAJ
DG
PYC/AC
PAC
THAL
0 h
4 h
8 h
16 h
30 h
120 h

Fig. 4

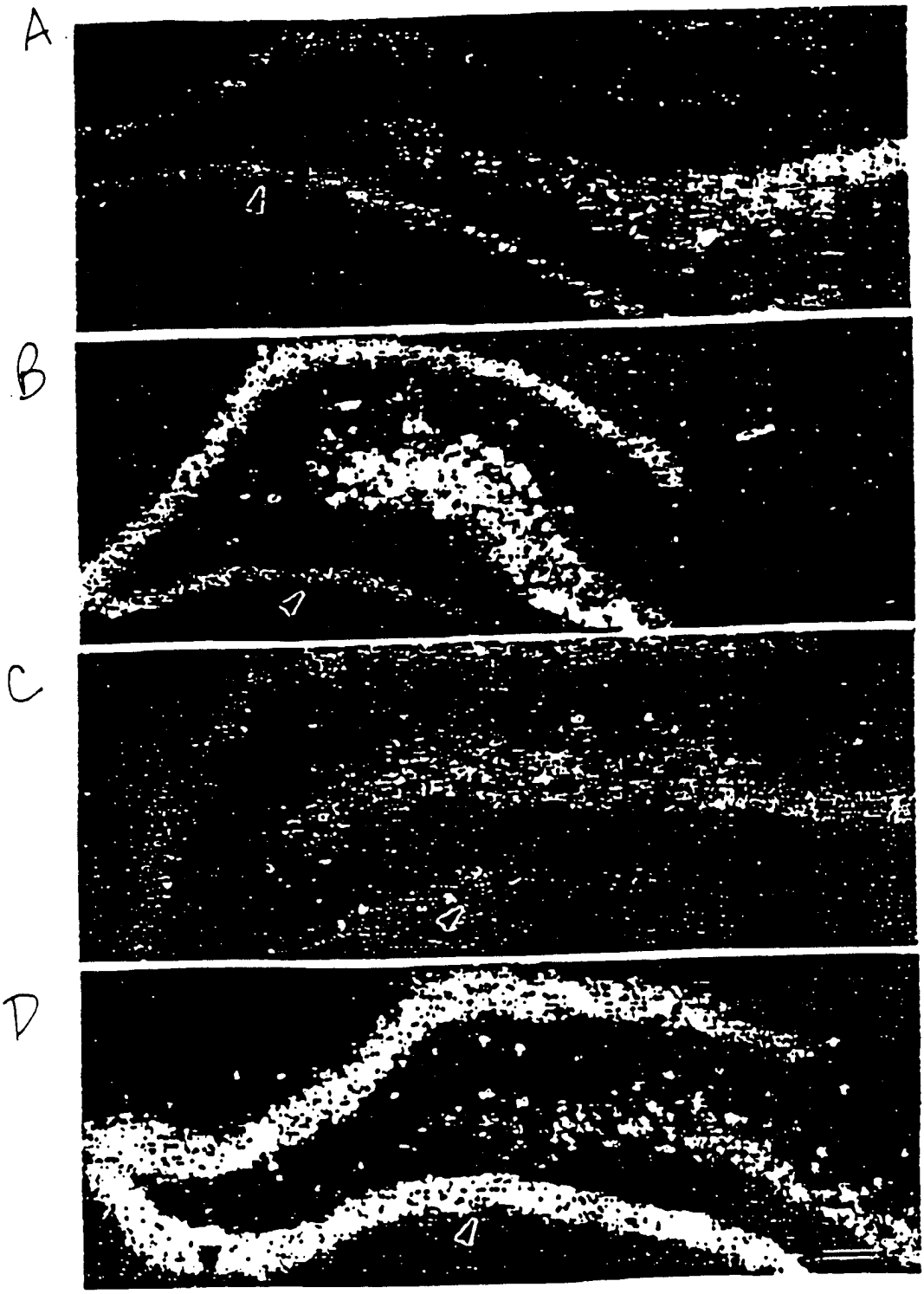


Fig. 5

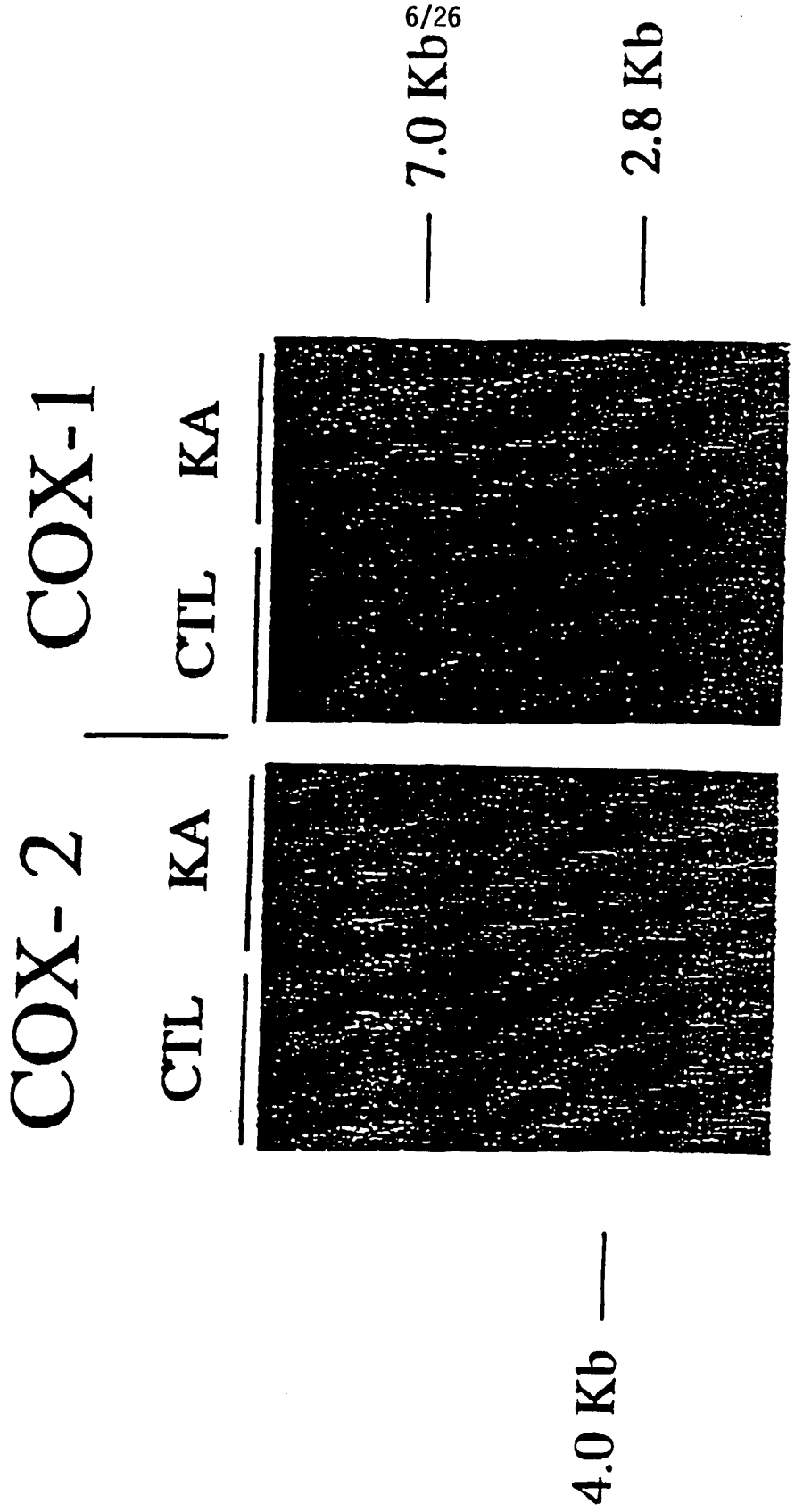


Fig. 6

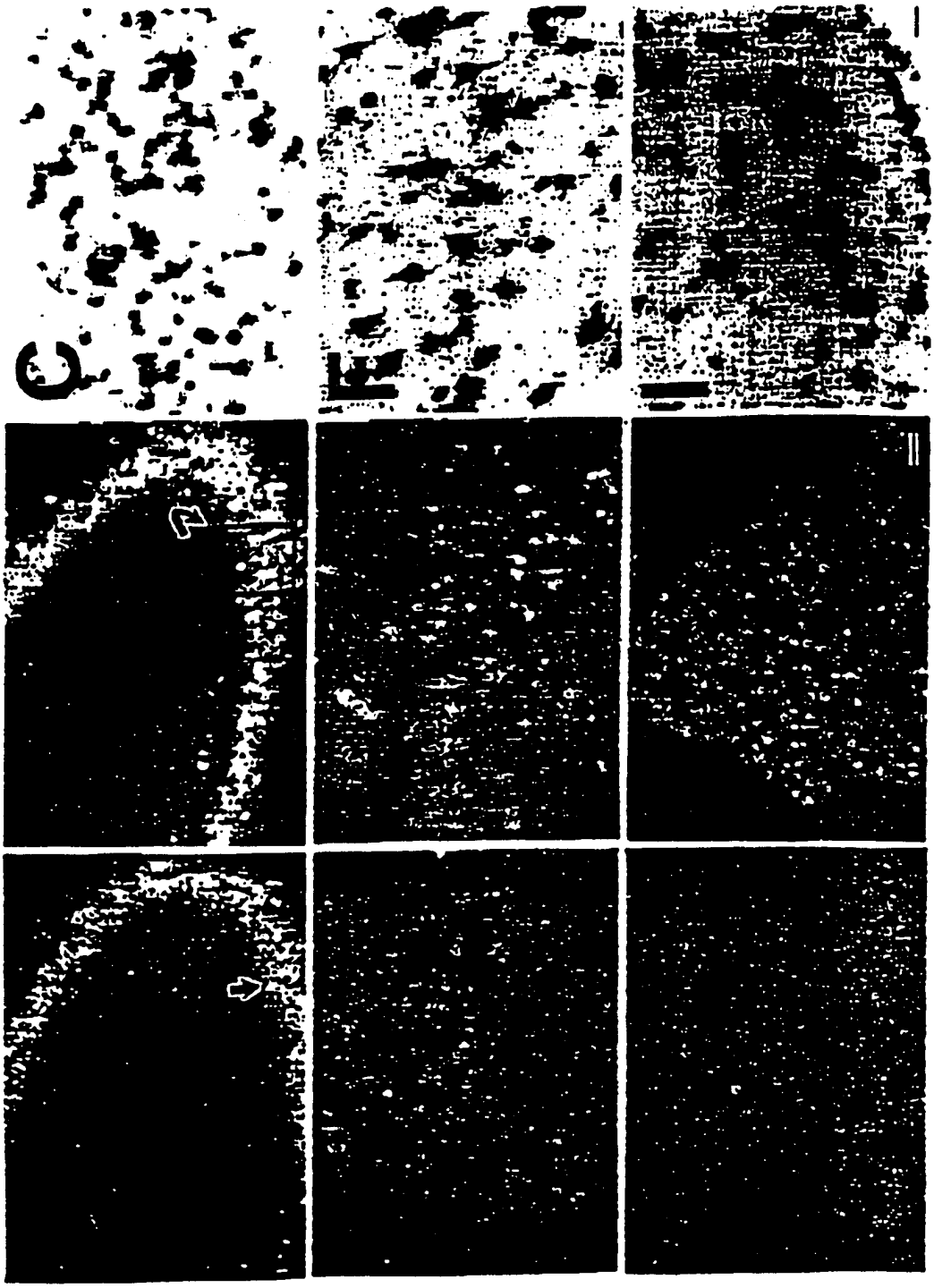


Fig. 7

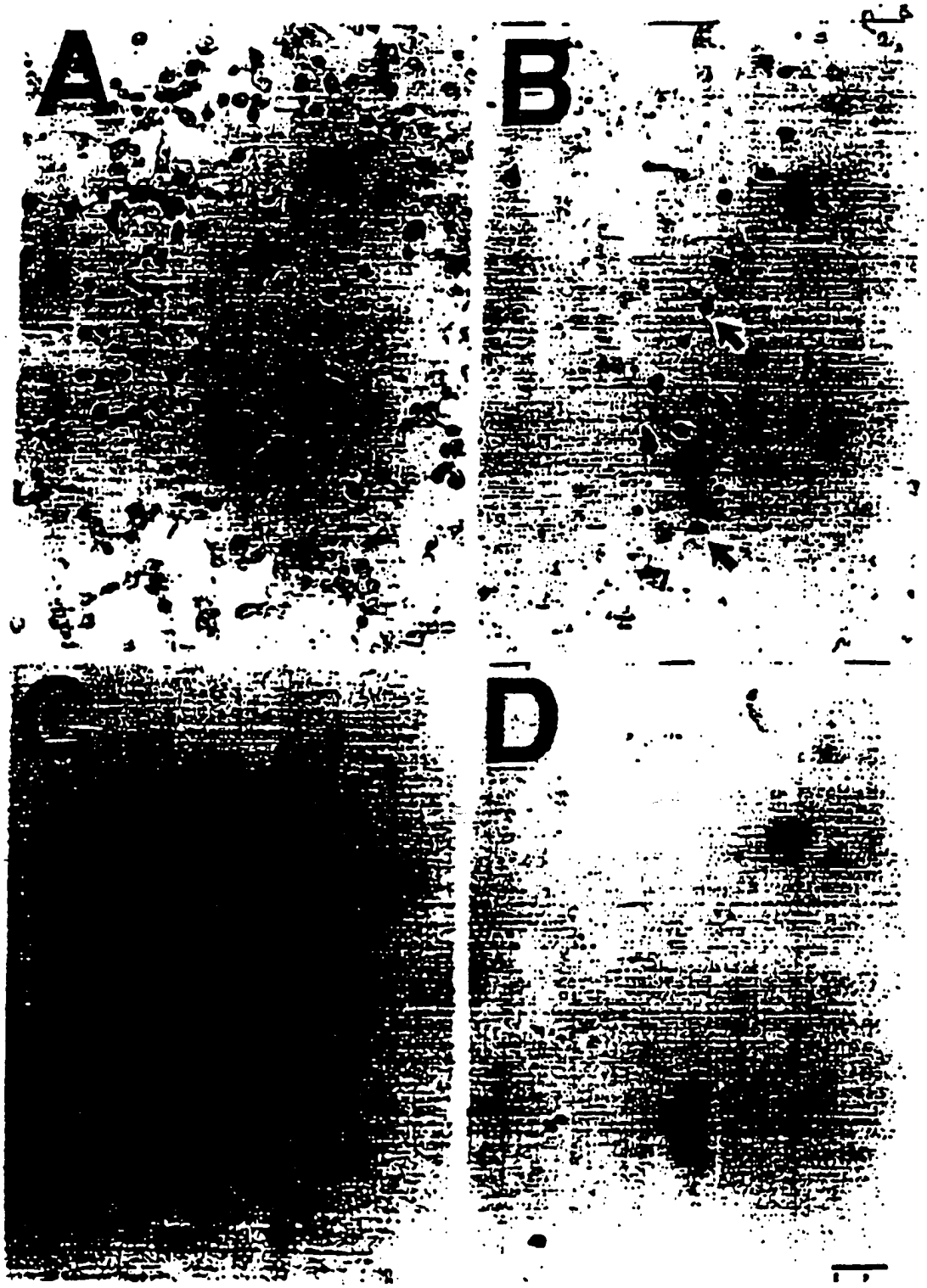


FIG. 8

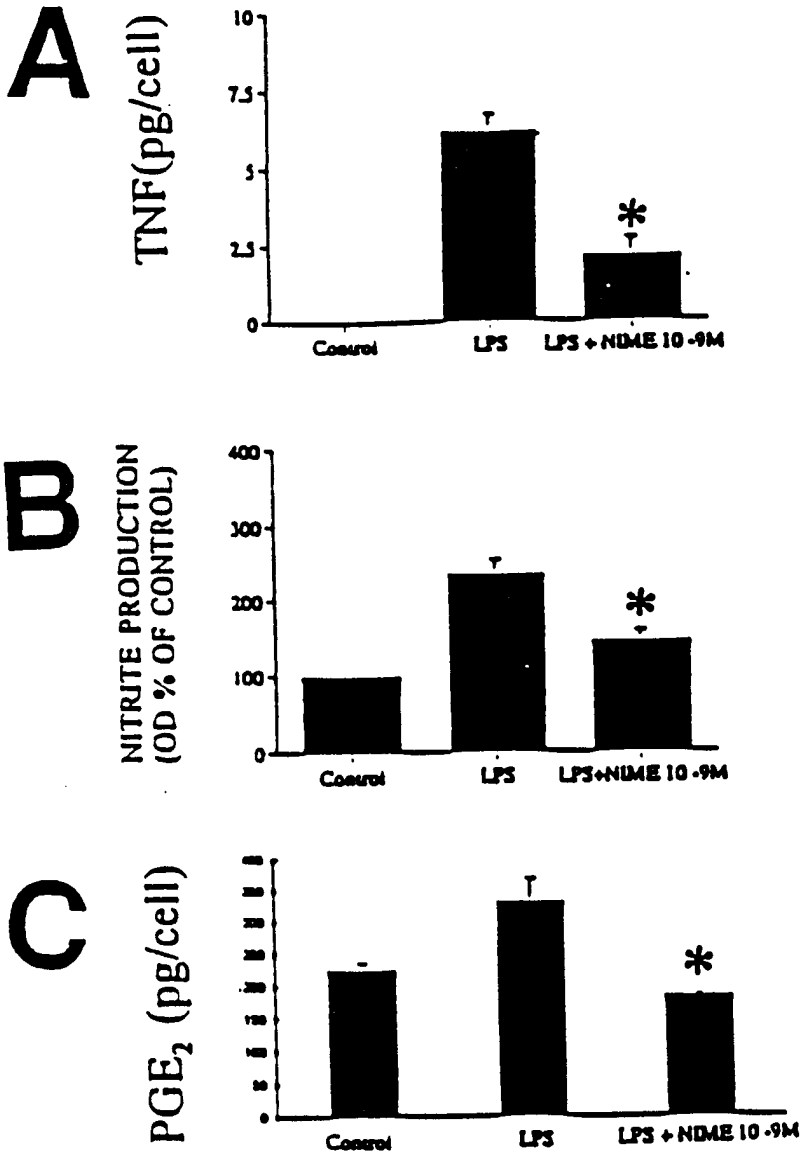

A
TNF(pg/cell)
10
7.5
5
2.5
0
Control
LPS
LPS + NIME 10 -9M
B
NITRITE PRODUCTION
(OD % OF CONTROL)
400
300
200
100
0
Control
LPS
LPS+NIME 10 -9M
C
PGE2 (pg/cell)
Control
LPS
LPS + NIME 10 -9M

Fig. 9

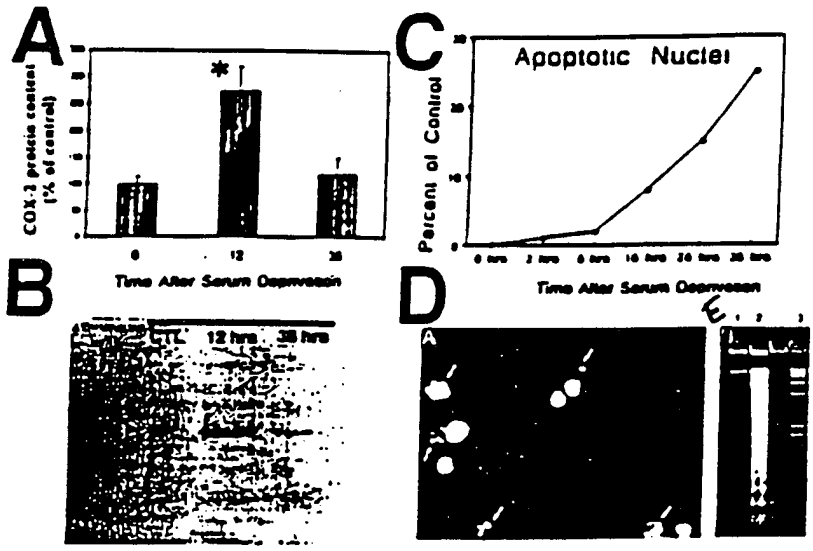

A
B
C
D
E
Apoptotic Nuclei
Percent of Control
Time After Serum Depression
12 hrs
36 hrs

Fig. 10

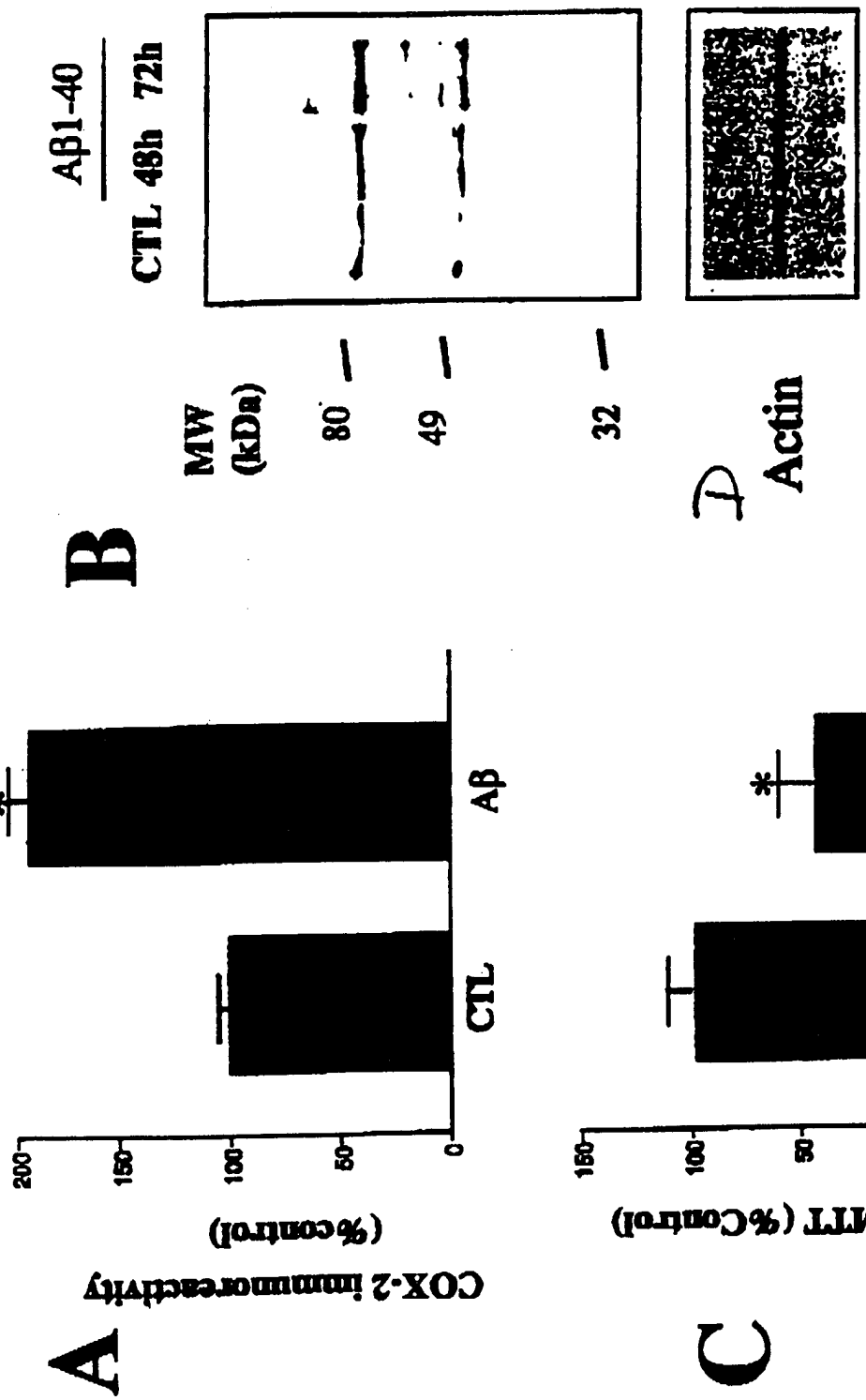

A
COX-2 immunoreactivity
(%control)
200
150
100
50
0
CTL
Aβ
*
B
Aβ1-40
CTL 48h 72h
MW
(kDa)
80
49
32
D
Actin
C
MTT (%Control)
150
100
50
0
CTL
Aβ
*

Fig. 11

E

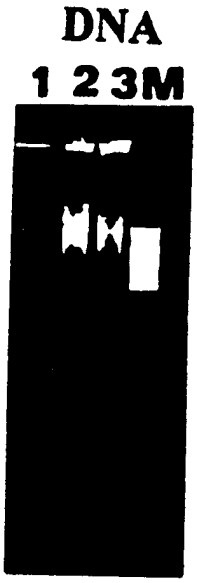


Fig. 11

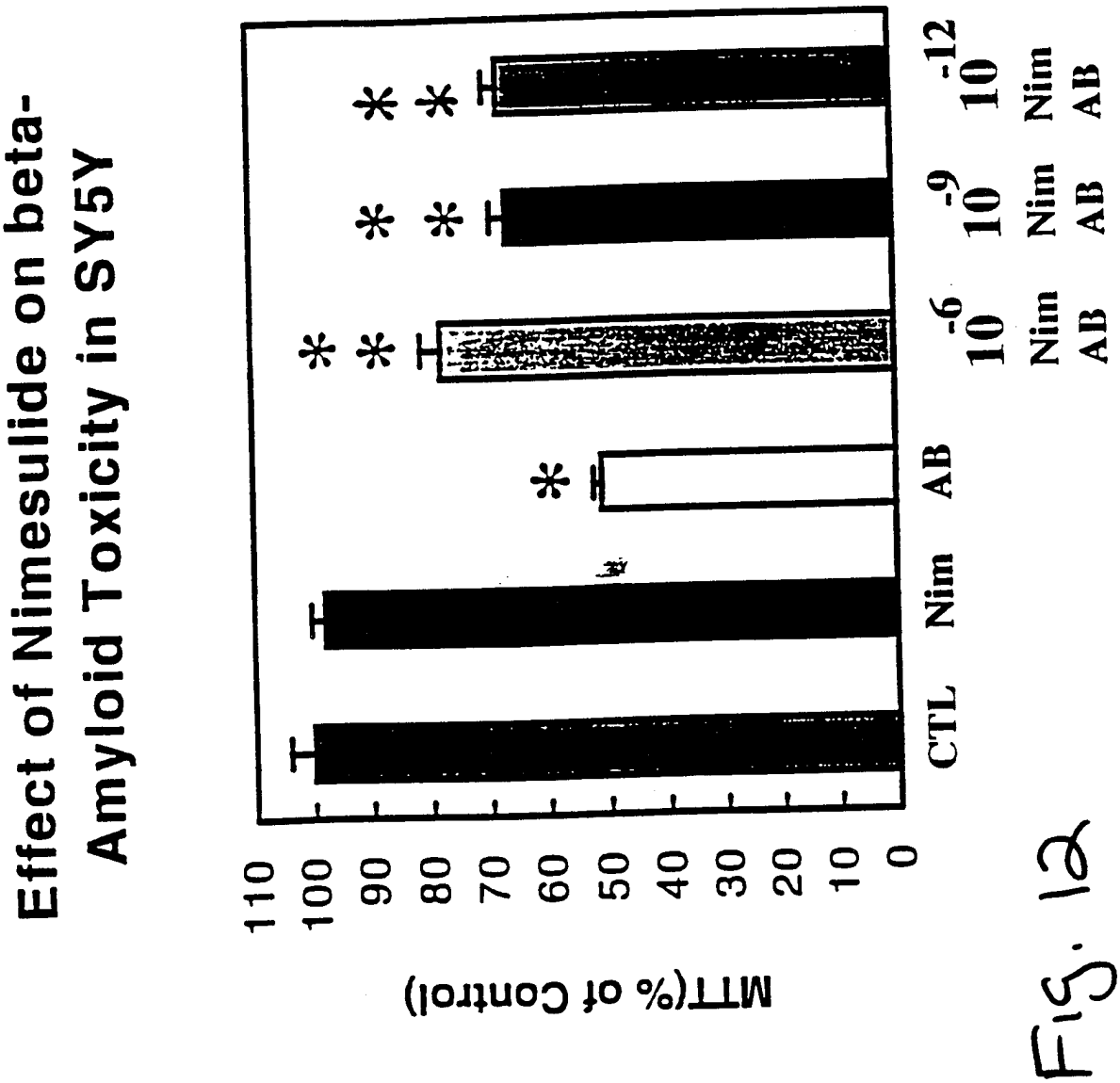

Effect of Nimesulide on beta-Amyloid Toxicity in SY5Y
MTT(% of Control)
110
100
90
80
70
60
50
40
30
20
10
0
CTL
Nim
AB
10 -6 Nim
AB
10 -9 Nim
AB
10 -12 Nim
AB
*
*
*
*
*
*
*

Fig. 12

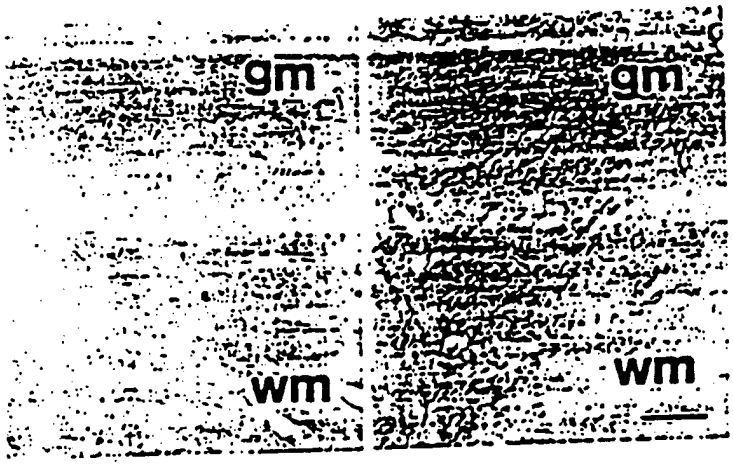
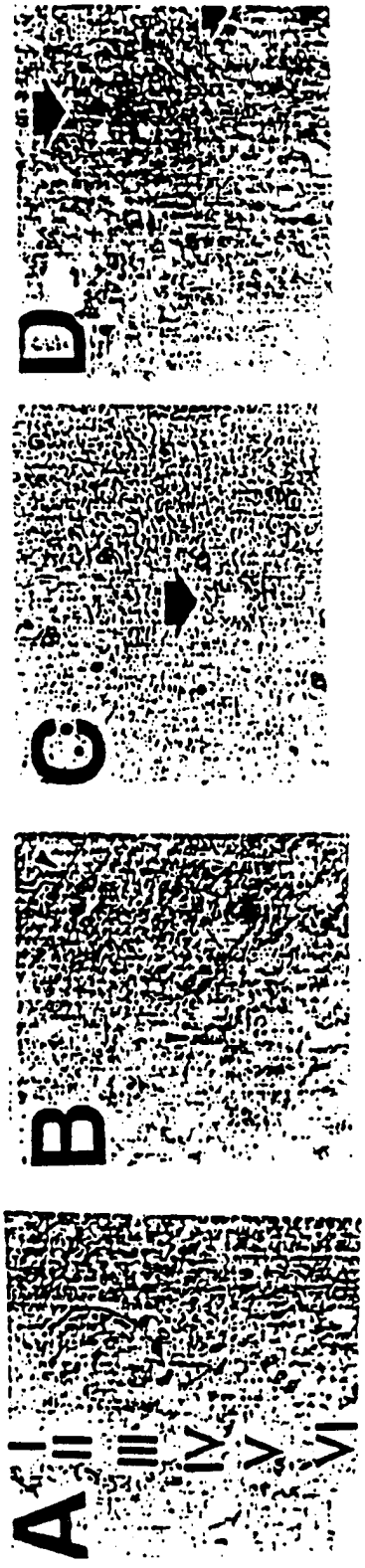


Fig. 13

Fig. 14



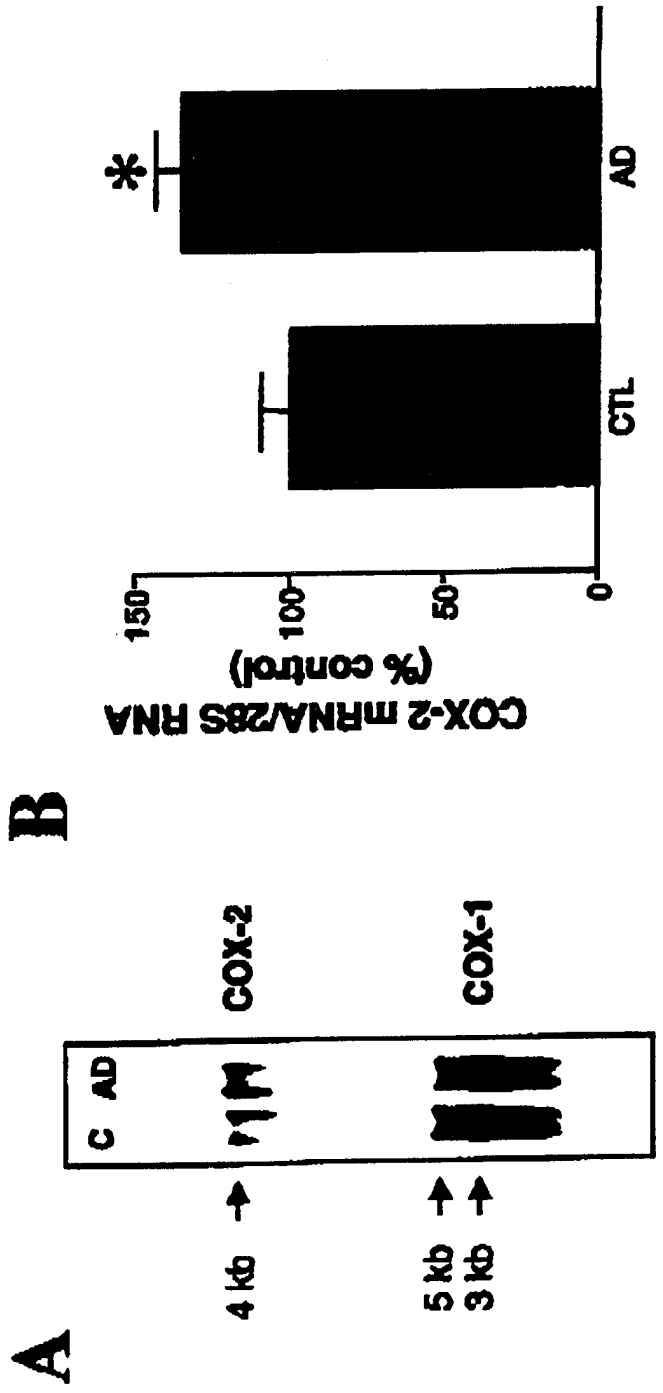

COX-2 mRNA/28S RNA
(% control)
150
100
50
0
*
AD
CTL
B
COX-2
COX-1
C
AD
4 kb
5 kb
3 kb
A

FIG. 15A, B

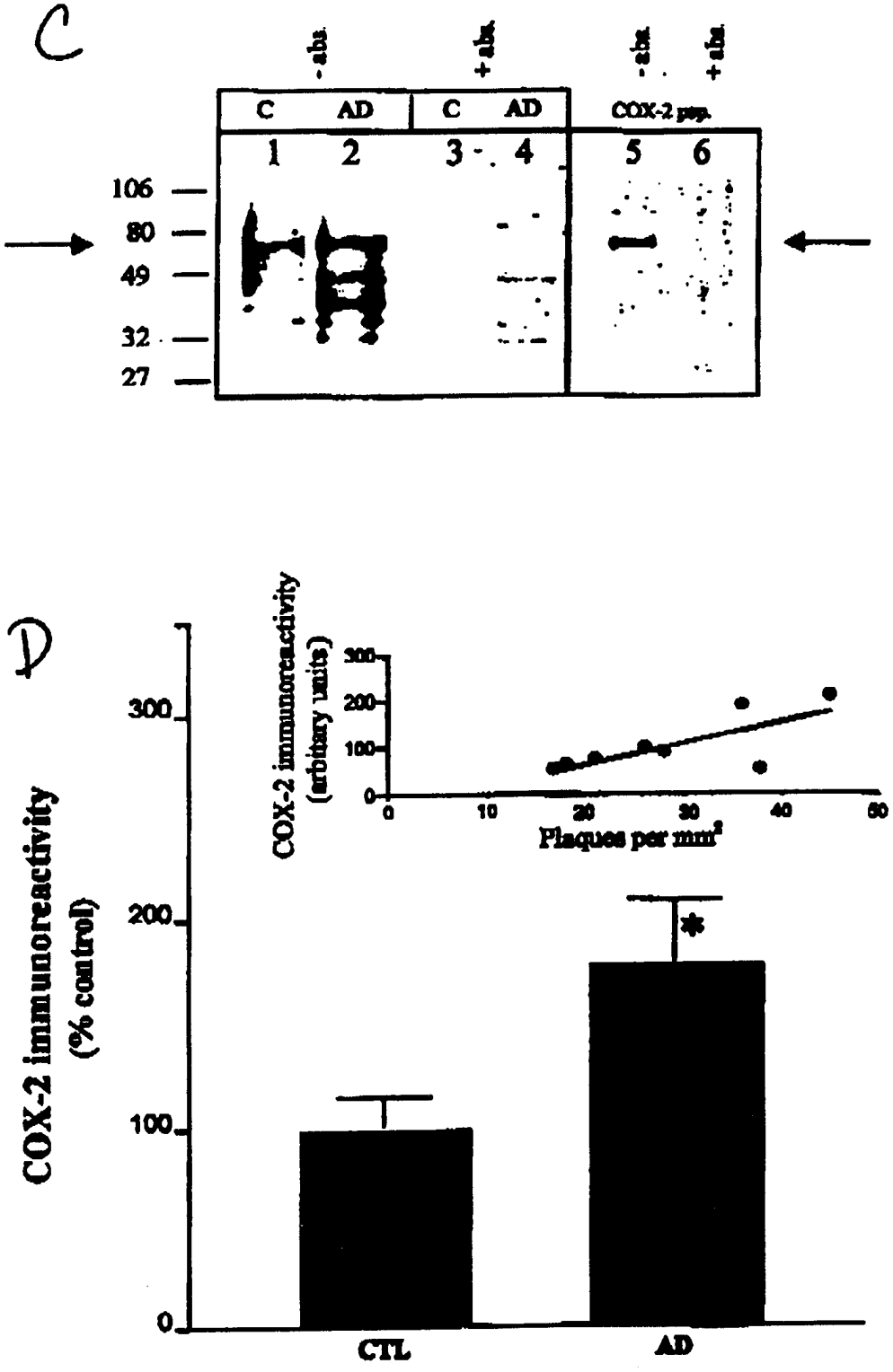

C
- abs.
+ abs.
- abs.
+ abs.
C
AD
C
AD
COX-2 pep.
1
2
3
4
5
6
106
80
49
32
27
D
COX-2 immunoreactivity (% control)
300
200
100
0
CTL
AD
*
COX-2 immunoreactivity (arbitrary units)
300
200
100
0
0
10
20
30
40
50
Plaques per mm²

Fig. 15C,D

Fig. 16

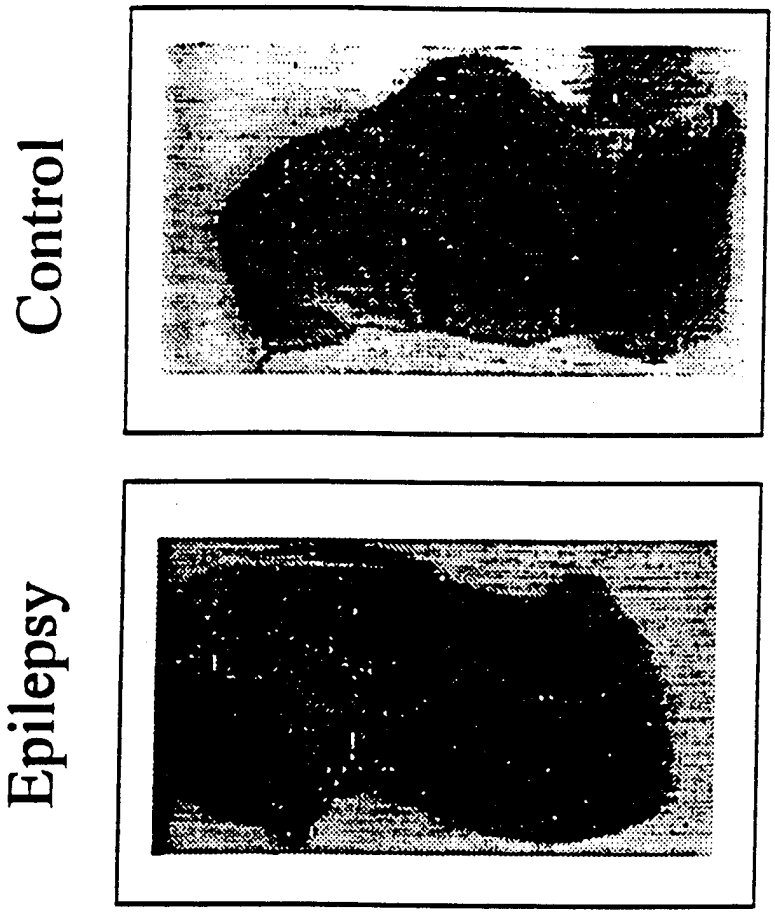
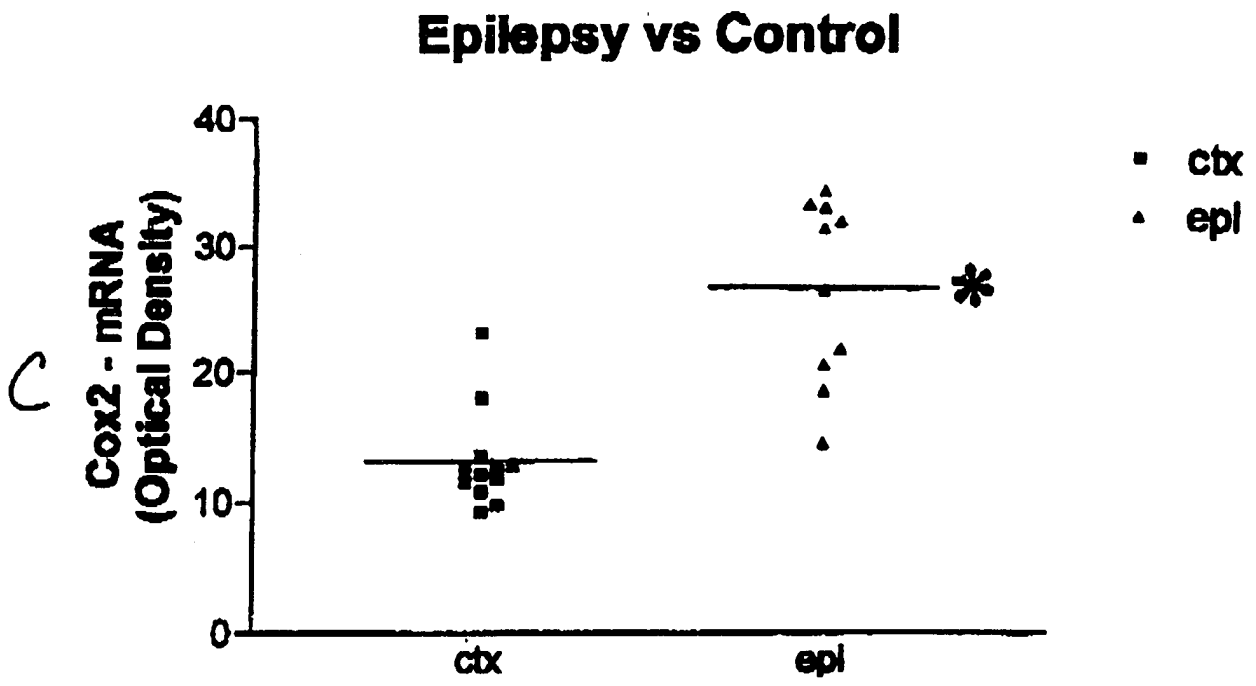


FIG 17 A, B


Epilepsy vs Control
C
Cox2 - mRNA (Optical Density)
40
30
20
10
0
ctx
epi
*
ctx
epi

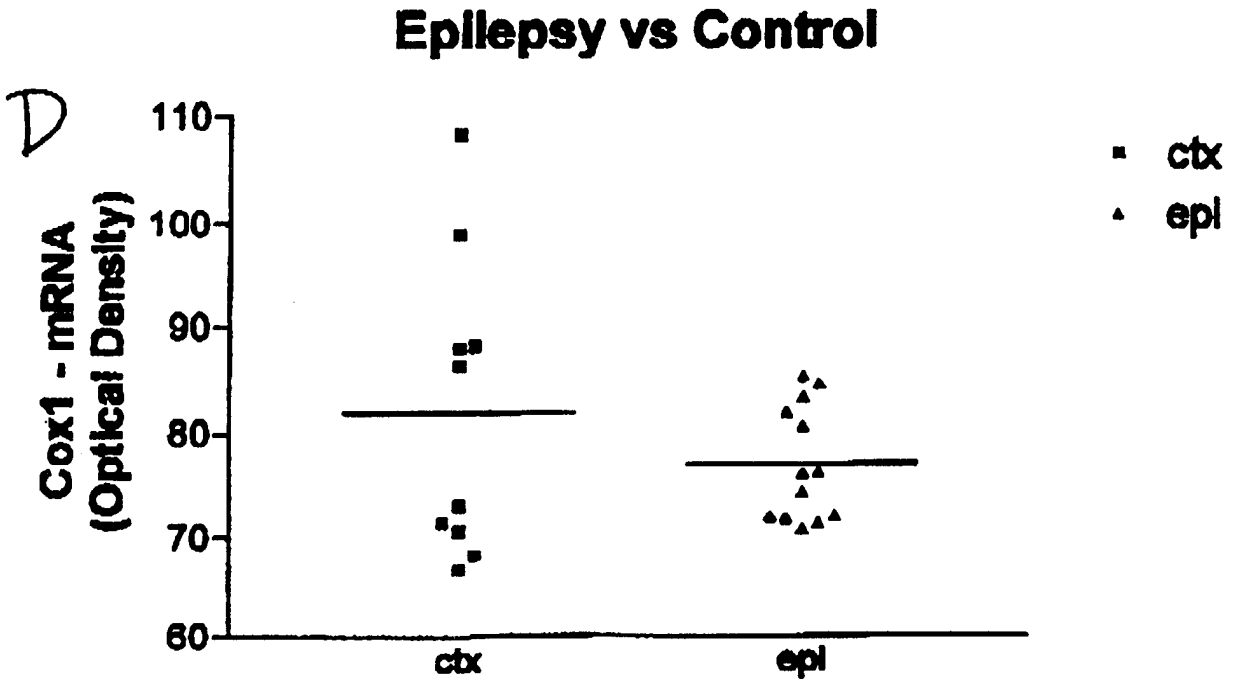

Epilepsy vs Control
D
Cox1 - mRNA (Optical Density)
110
100
90
80
70
60
ctx
epi
ctx
epi

FIG. 17C, D

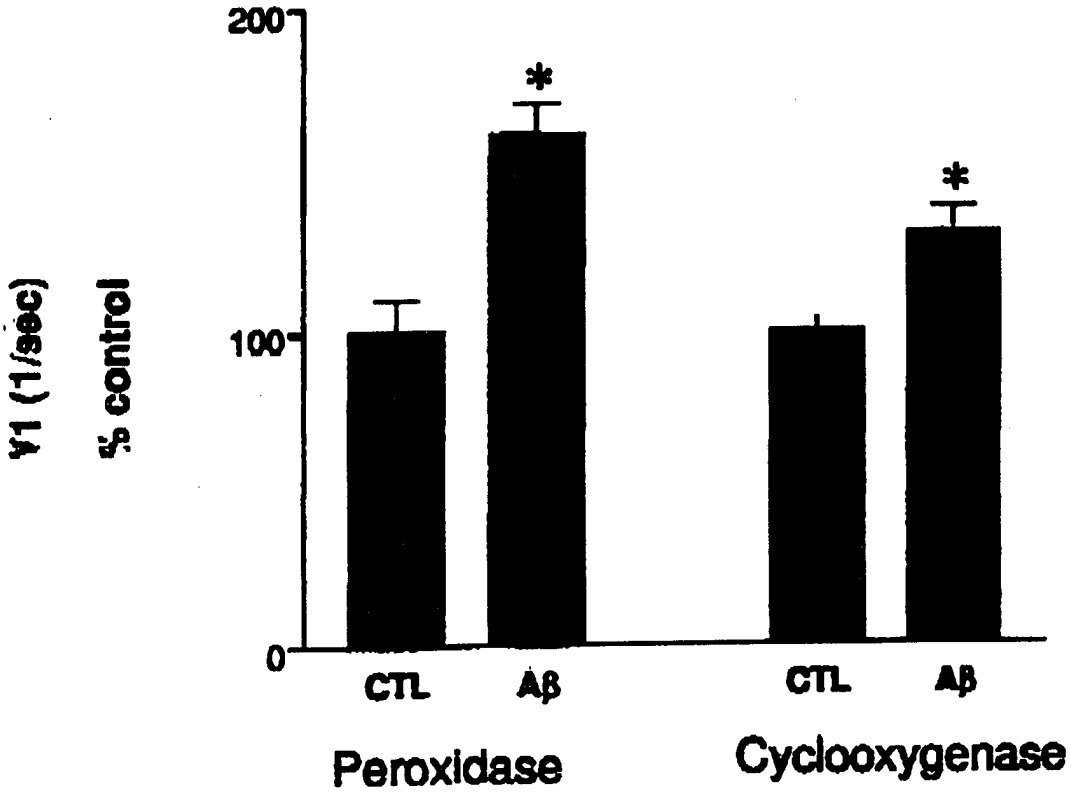

200
100
0
*
*
V1 (1/sec)
% control
CTL
Aβ
CTL
Aβ
Peroxidase
Cyclooxygenase

FIG. 18

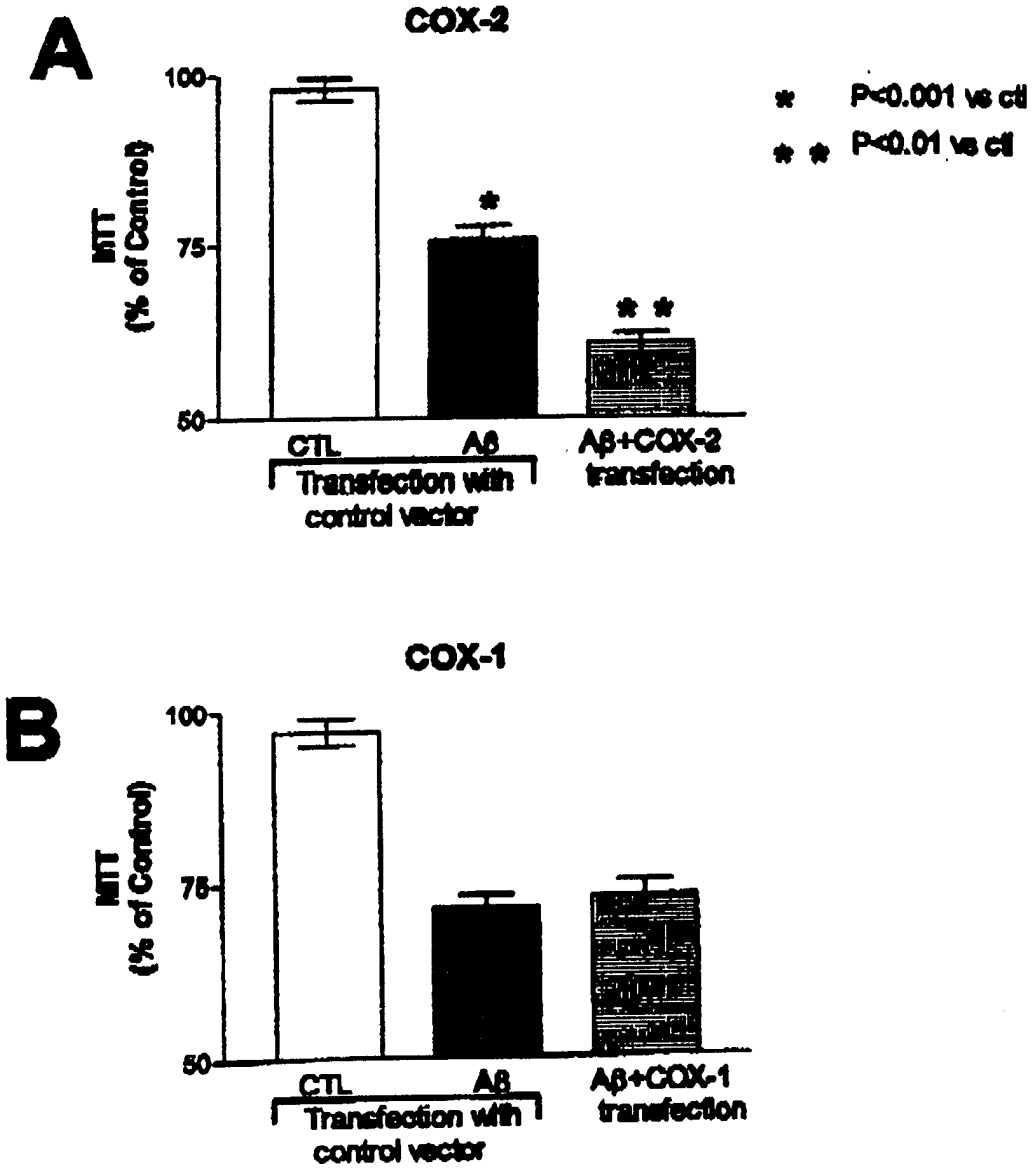
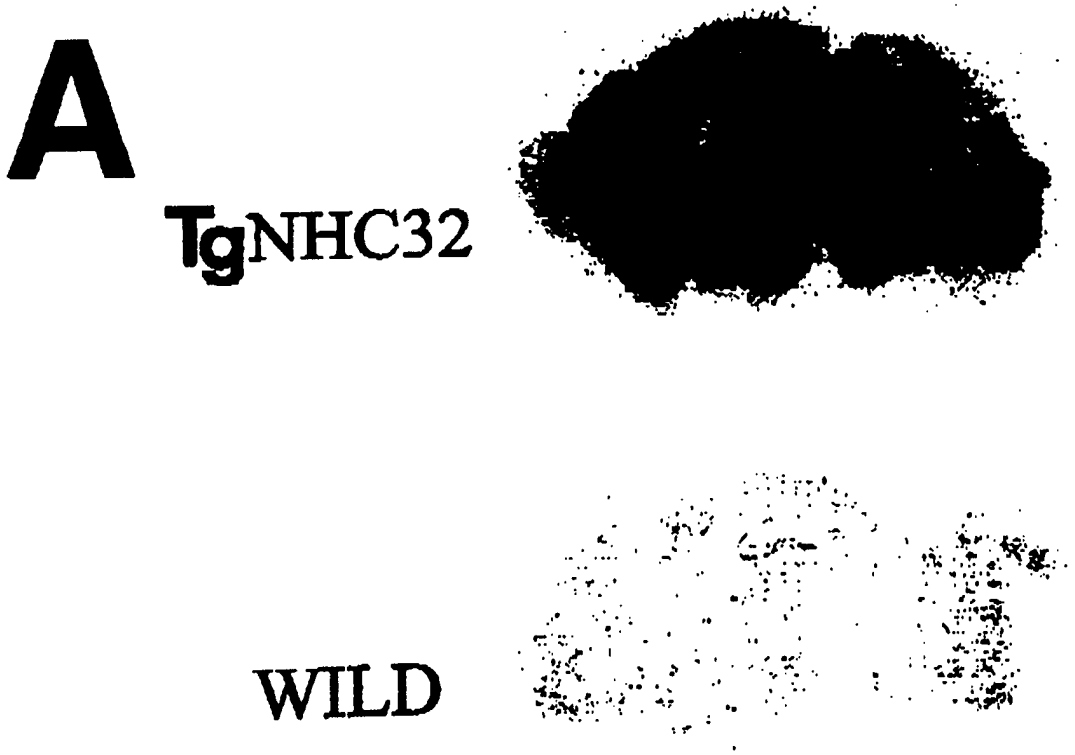

A
COX-2
MTT (% of Control)
100
75
50
*
* *
CTL
Aβ
Aβ+COX-2 transfection
Transfection with control vector
* P<0.001 vs ctl
* * P<0.01 vs ctl
B
COX-1
MTT (% of Control)
100
75
50
CTL
Aβ
Aβ+COX-1 transfection
Transfection with control vector

FIG. 19


A
TgNHC32
WILD

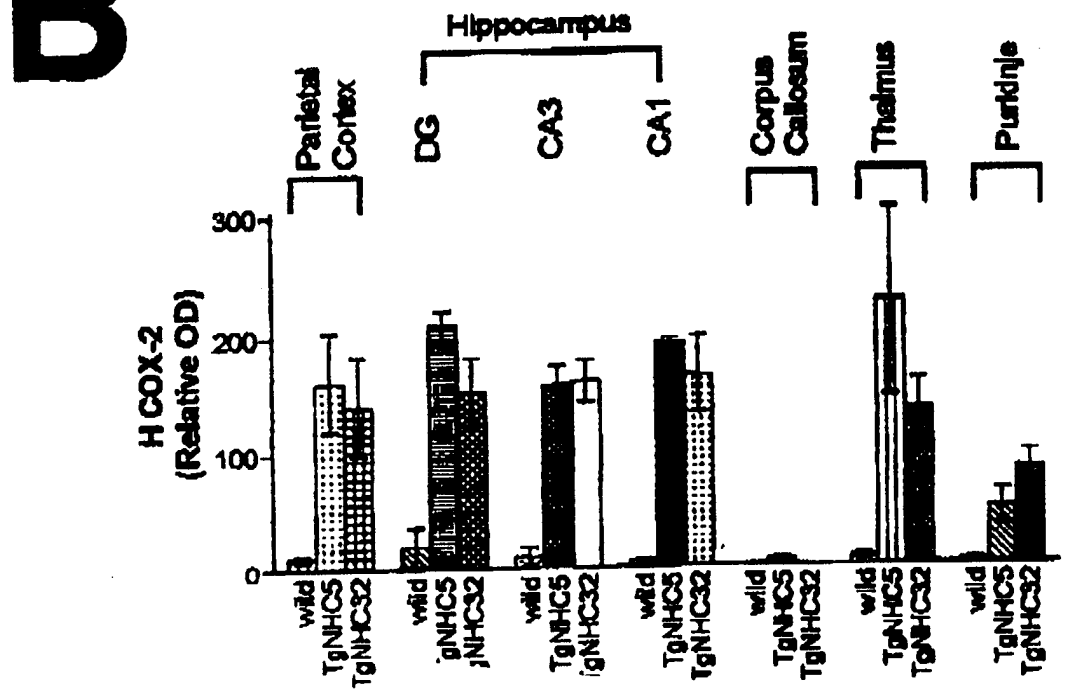

B
Hippocampus
Parietal Cortex
DG
CA3
CA1
Corpus Callosum
Thalmus
Purkinje
HCOX-2 (Relative OD)
300
200
100
0
wild
TgNHC5
TgNHC32

Fig. 20

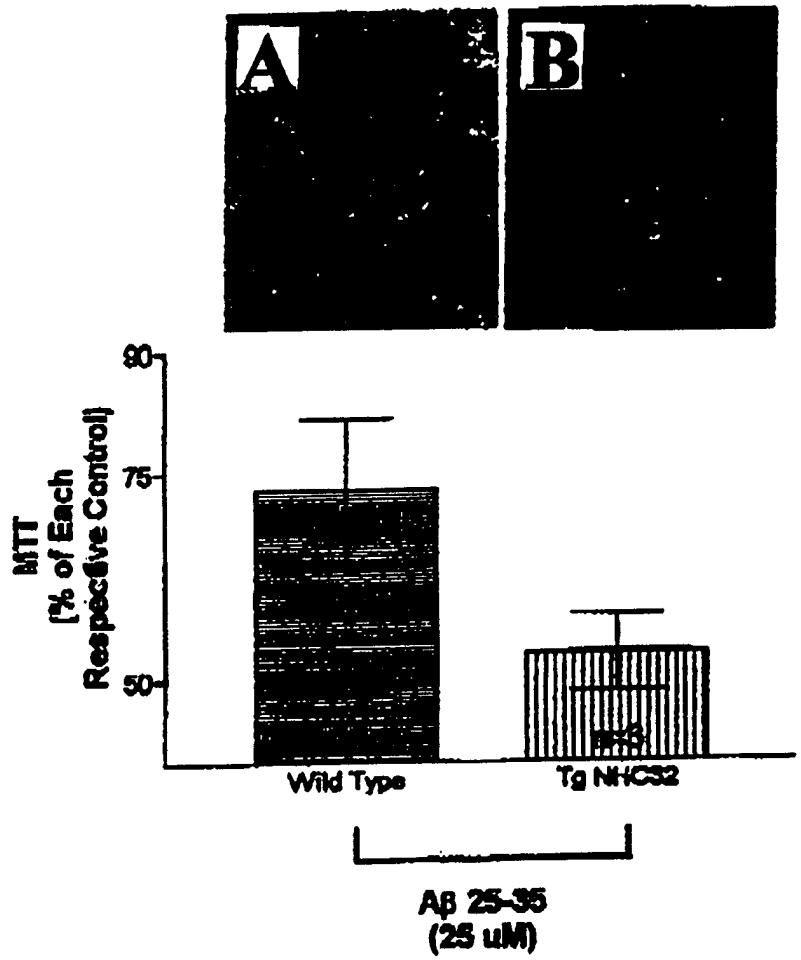

A
B
MTT
[% of Each
Respective Control]
100
75
50
Wild Type
Tg NHC32
Aβ 25-35
(25 uM)

FIG. 21

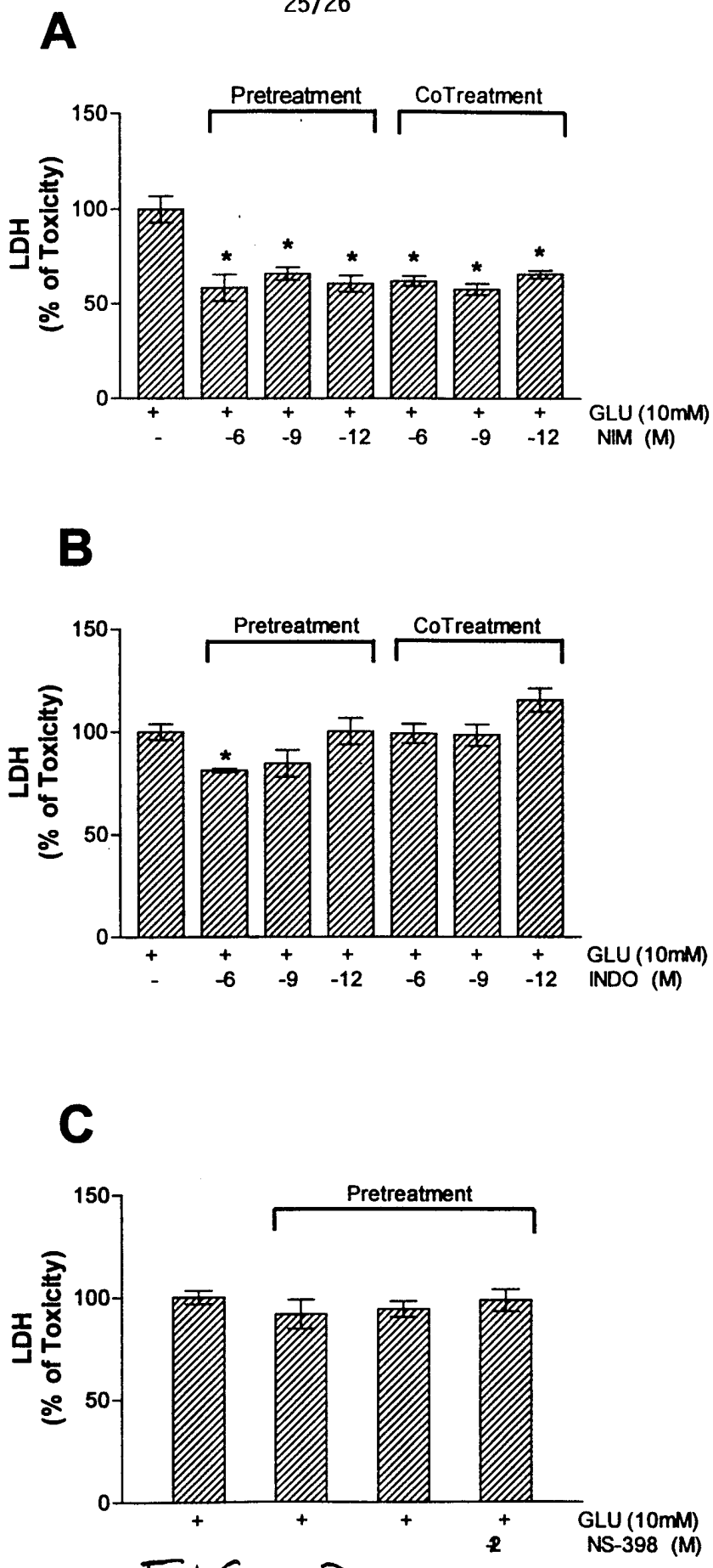

A
Pretreatment
CoTreatment
LDH (% of Toxicity)
150
100
50
0
+ + + + + + +
- -6 -9 -12 -6 -9 -12
GLU (10mM)
NIM (M)
B
Pretreatment
CoTreatment
LDH (% of Toxicity)
150
100
50
0
+ + + + + + +
- -6 -9 -12 -6 -9 -12
GLU (10mM)
INDO (M)
C
Pretreatment
LDH (% of Toxicity)
150
100
50
0
+ + + +
-2
GLU (10mM)
NS-398 (M)

FIG. 22

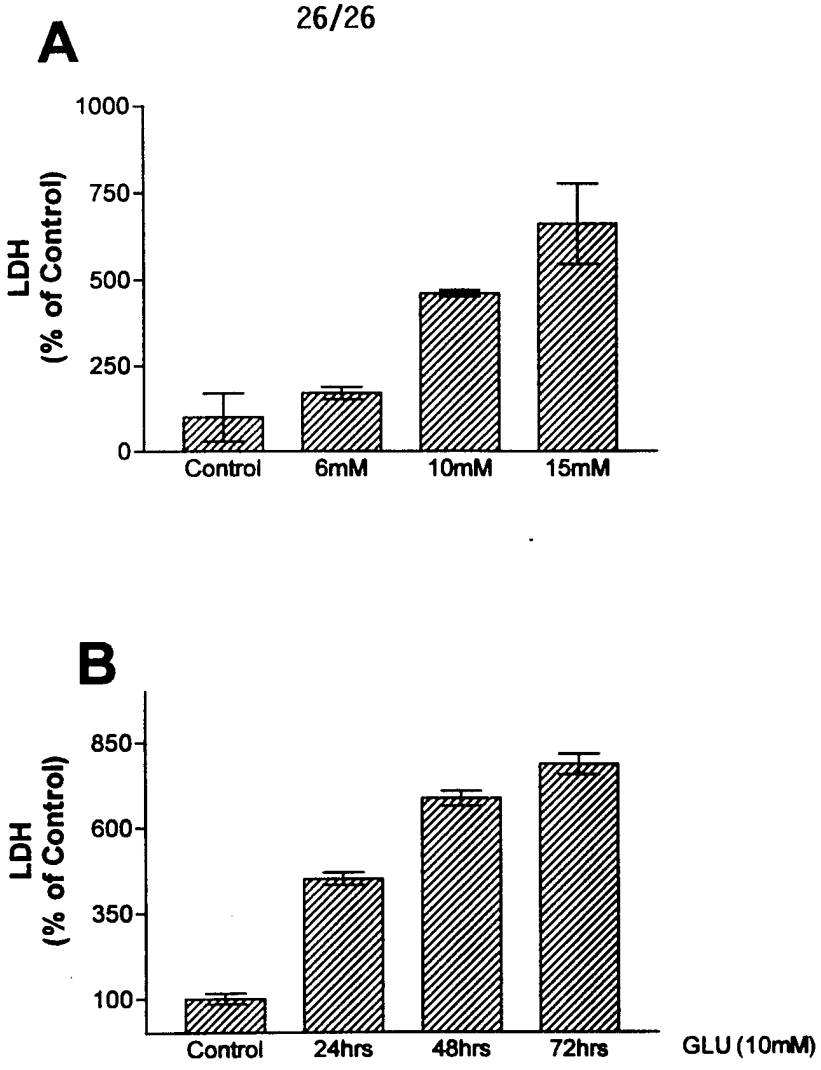

A
LDH
(% of Control)
1000
750
500
250
0
Control
6mM
10mM
15mM
B
LDH
(% of Control)
850
600
350
100
Control
24hrs
48hrs
72hrs
GLU (10mM)
C
LDH
(% of Control)
150
100
50
0
Control
NIM 10-6

FIG. 23