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# Morphine via nitric oxide modulates β-amyloid metabolism: a novel protective mechanism for Alzheimer's disease

**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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## Summary

**Background:** The deposition of intracellular and extracellular β-amyloid peptide (Aβ) in the brain is a pathologic feature of Alzheimer's disease (AD), a prevalent neurodegenerative disorder. However, the exact role of the Aβ peptide in causing AD's symptoms is unclear.

**Material/Methods:** CRL-2266 SH-SY5Y human neuroblastoma cells (ATCC, USA) and HTB-11 human neuroblastoma cells (ATCC, USA) were cultured. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to analyze the effects of β25-35, morphine, and SNAP treatments upon BACE-1 and BACE-2 mRNA expression semi-quantitative RT-PCR. The production of NO in SH-SY5Y cells was detected using the Apollo 4000 Free Radical Analyzer (World Precision Instruments).

**Results:** Untreated HTB-11 neuroblastoma cells constitutively express BACE-1 and BACE-2 mRNA. Morphine down regulates the expression of BACE-1 and up regulates the expression of BACE-2 in a naloxone antagonizable manner. When HTB-11 cells were treated with L-NAME, a cNOS inhibitor; the effects of morphine were blocked. SNAP (a NO donor) mimicked the effect of morphine. In SH-SY5Y cells, Aβ treated cells show a dose-dependent decrease in NO release, demonstrating that Aβ is dose-dependently inhibiting the release of constitutive NO.

**Conclusions:** Aβ and morphine/NO each inhibit the production of the other. This suggests that a deficiency of basal NO or endogenous morphine may trigger drastically reduced levels of basal NO. The outcome is chronic vasoconstriction and brain hypoperfusion and eventual neuronal death. This novel theorized mechanism for AD supports an increasingly-accepted vascular pathological hypothesis for the disease.

**key words:** **morphine • β-amyloid • Nitric Oxide • Alzheimer's disease • BACE-1 • BACE-2 • neuroblastoma cells**

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## BACKGROUND

Alzheimer's disease (AD) afflicts more than 4.5 million people in the United States, which may grow to 11.3 to 60 million by the year 2050 [1]. AD gradually destroys a person's memory, learning/reasoning ability, and capability of carrying out daily tasks [2]. There is currently no known cure for the disease, nor is there a known single cause; however, recent research has uncovered potential factors in the pathology of AD. At the microscopic level, AD is neuropathologically characterized by senile plaques, neurofibrillary tangles (NFTs), intracellular aggregation of the protein tau, and extensive neuronal loss [3]. The exact relationship between the plaques, NFTs, and the pathophysiological mechanisms underlying AD is still debated [4]. Evidence strongly suggests that neuritic plaques containing β-amyloid (Aβ) peptide, the main pathohistological feature of AD is critically involved at an early stage in its pathogenesis [3].

Aβ is a 39- to 43-amino acid β-sheet peptide derived from endoproteolytic processing at the *N*-terminus of the amyloid precursor protein (APP) [3]. A significant genetic link has been found between overproduction of the Aβ peptide and early-onset forms of familial AD [5]. Mutations in the genes for APP, presenilin 1 (PS1), and presenilin 2 (PS2) all increase production of Aβ42 presymptomatically and cause familial AD in a highly penetrant fashion [5]. Overproduction of Aβ occurs years before symptoms arise, suggesting that it plays a role in the early etiology of the disease [3,5].

Recent research has focused on how Aβ and/or its fragments exert neurotoxic effects on cells at the plasma membrane level [6]. This assumption of membrane-based neurotoxicity stems from the observation that amyloid deposits are mostly extracellular. There are two deficiencies in assuming membrane-level neurotoxicity to be the primary reason for neuronal loss in AD. Firstly, intracellular Aβ accumulation has been observed in neurons and endothelial cells [7,8]; intraneuronal Aβ appears to precede NFT and Aβ plaque formation, representing an earlier event in AD progression and demonstrating the possibility of effects unrelated to membrane interaction. Secondly, AD has a significant vascular component which has been verified by epidemiologic, neuroimaging, pathological, pharmacotherapeutic, and clinical studies [6,9]. Changes in cerebral circulation have been linked to AD [10], and a number of factors that compromise blood circulation are associated with prevalence to AD: non-insulin-dependent diabetes mellitus [11], atherosclerosis [12], smoking [13], and atrial fibrillation [14]. Chronic brain hypoperfusion may well be the reason for AD-related neuronal degradation and apoptosis [10].

AD's vascular component may involve nitric oxide (NO) [15]. Nitric oxide is a compound with important physiological functions, such as neurotransmission required for memory ability, and notably the relaxation of smooth muscles in arterial walls; consequently it is important in controlling blood flow and pressure [16]. While a relationship between Aβ and NO has been investigated, most reports deal with the neurotoxic effects of NO derived from inducible nitric oxide synthase (iNOS, NOS-2) as enhanced by Aβ [15]. Although this process might be involved in some neurodegeneration, more important is the NO released by constitutive NO synthase (cNOS, NOS-1/NOS-3), which demonstrates neuroprotective abilities and regulates critical neuronal/endothelial functions and vasorelaxation [15].

An alternate hypothesis, coupling the amyloid hypothesis to NO, is that an underlying Aβ-driven process may promote AD through a lack of basal NO from cNOS, which would result in loss of neurotransmittive function, brain hypoperfusion and decreased neuroprotectivity [2]. In this regard, NO's effects on Aβ production has not been investigated.

Morphine can have a neuroprotective effect in the presence of neurotoxic agents, and is associated with anti-inflammatory agents and the down regulation of physiological responses, especially those of the immune system [17]. Morphine has endogenous signaling functions and releases NO after binding to the mu-3 opiate receptor [17]. We now show that NO regulates Aβ production by altering the expression of BACE-1 (β-secretase) and BACE-2, which are degradative enzymes of APP, with the latter having a protective role. Simultaneously, Aβ down regulates cNOS derived NO production, again limiting its normal protective function. We surmise this proposed downward cycle ultimately results in the over production of Aβ, and a significant loss of NO required for normal neuronal function and vasoregulation within the brain.

## MATERIAL AND METHODS

### Cell culturing and treatment

SH-SY5Y human neuroblastoma cells (ATCC, USA) were cultured in Dulbecco's modified Eagle's medium/Ham's nutrient mixture (DMEM-F12) (Invitrogen, USA) and HTB-11 human neuroblastoma cells (ATCC, USA) were cultured in Minimum Essential Medium Alpha Medium (MEM-α) (Invitrogen, USA). Cells were kept in a 37°C incubator (Napco) gassed with 5% $CO_2$/95% air. All treatments were performed under a sterile hood.

### mRNA expression analysis

Reverse transcription-polymerase chain reaction (RT-PCR) was performed to analyze the effects of Aβ25-35, morphine, and SNAP treatments upon BACE-1 and BACE-2 mRNA expression in SH-SY5Y and HTB-11 cells. After the treatment time-period cells were harvested and total RNA was extracted using RNeasy RNA Isolation kit (Qiagen) following the manufacturer's procedures. Total RNA yield was determined using a RNA/DNA calculator (Pharmacia Biotech). Total RNA concentration was then standardized for semi-quantitative RT-PCR, which was carried out in a Geneamp Thermocycler PCR System 9700 (P.E. Applied Biosystems). Primers used for PCR were F: 5'-TGACTGGGAACACCCCATAACT-3' R: 5'-CGAGCGCCTC AGTGTTACTCT-3' for BACE-1 and F: 5'-AGCCATCCTCCTTGTCTTAATCG-3' R: 5'-TCTGGCGGAAAATAACCTCAA-3' for BACE-2, expected product length 556 bp for both primer sets. PCR products and a 100 BP DNA marker were then loaded in a 2% agarose gel stained with ethidium bromide. Gel electrophoresis was performed using a power-supply (E-C Apparatus Corp.) set at 110V with constant amperage for 1.5 hours. Gels were then photographed using a Gel Documentation System (UVP) and bands analyzed using Gel-Pro Analyzer (MediaCybernetics) on a P4 Windows machine.

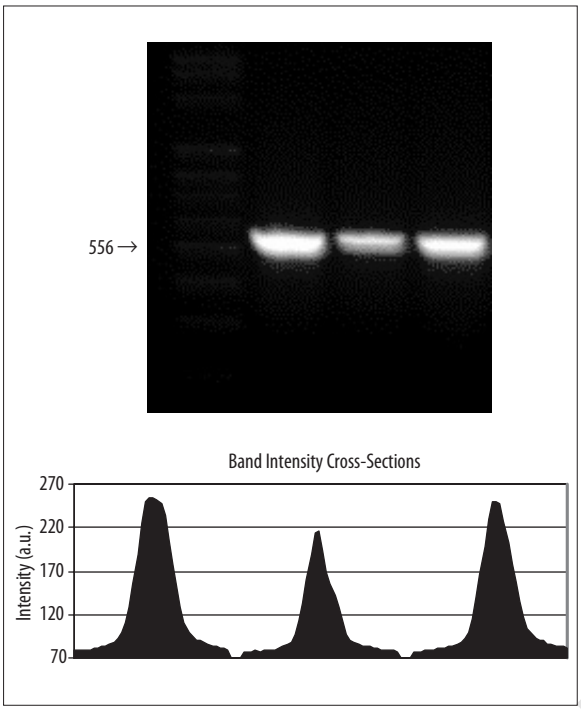


**Figure 1.** RT-PCR analysis of BACE-1 gene expression in HTB-11 neuroblastoma cells. Lane 1: untreated cells, lane 2: 24-h treatment with 1 μM morphine, lane 3: 24-h treatment with 5 μM morphine.

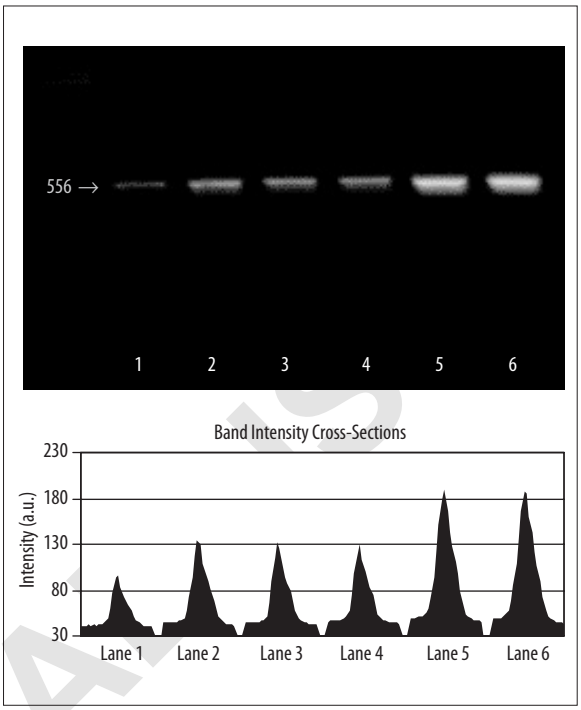


**Figure 2.** RT-PCR analysis of BACE-2 gene expression in HTB-11 neuroblastoma cells, 24-h treatments. Lane 1: untreated, lane 2: 1 μM Mo, lanes 3 and 4: 10 μM and 25 μM Aβ, lanes 5 and 6: 1 μM Mo with 10 μM and 25 μM Aβ.

### Nitric oxide detection apparatus

The production of NO in SH-SY5Y cells was detected using the Apollo 4000 Free Radical Analyzer (World Precision Instruments). SH-SY5Y cells were trypsinized and cultured in a 6-well plate for 48 hours. An L-shaped amperometric NO-specific probe was connected to the Apollo and calibrated using a SNAP + $CuCl_2$ solution, which releases calculable amounts of NO. Cells were pretreated with 10 and 25 μM Aβ for 30 m and 24 h. At the end of the treatment time-point the media was removed and replaced with PBS warmed in a 37°C bath, which is non-reactive with the probe; the probe was inserted ~1.5mm above the cells and allowed to equilibrate for 5 min, and then morphine-6-gluconuride (M6G) was added to each plate at a concentration of 1 μM. M6G attaches to G-protein-coupled Mu-3 receptors on SH-SY5Y cells, stimulating release of $Ca^{+2}$ ions which activate cNOS [18], normally releasing constitutive NO from neuroblastoma cells within minutes. The probe was monitored in real-time for the production of NO "spikes"; NO data was recorded using Free Radical Analyzer (World Precision Instruments). Cells were then discarded.

### Reagents

Aβ25-35, nitro-L-arginine methyl ester (L-NAME), ethidium bromide, and Trypsin-EDTA were purchased from Sigma-Aldrich, USA. 0.1M dithiothreitol (DTT), 10X Polymerase Chain Reaction (PCR) buffer, Superscript reverse-transcription enzyme, TAQ polymerase, 50 μM $MgCl_2$, 5x First Strand Buffer, custom PCR primers and Random Primers were purchased from Invitrogen, USA, and stored at –20°C. Phosphate-buffered saline was also purchased from Invitrogen, USA, and stored at room temperature. Nucleotides (dNTPs) were purchased from Amershar Pharmacia Biotech, USA, and stored at 25 μM concentration at –20°C. RNeasy RNA Isolation reagents and columns were purchased from Qiagen, USA. Stock solution of the Aβ25-35 peptide was prepared at 1mM concentration and kept frozen at –20°C. Electrophoresis-grade agarose was purchased from Fisher Biotech, USA and stored at room temperature. S-Nitroso-N-acetyl-D, L-penicillamine (SNAP) used for both cell treatment and NO detector calibration was purchased from World Precision Instruments, USA.

## Results

### Modulation of BACE-1 and BACE-2 mRNA expression by morphine

Untreated HTB-11 cells constitutively express BACE-1 and BACE-2 mRNA. Morphine exposure to these cells down regulates the expression of BACE-1 after 24 hours in a concentration dependent manner (1 μM dosage having a greater effect than 5, 44% as compared to 18%; Figure 1). Simultaneously, morphine up regulates the expression of HTB-11 BACE-2 expression, an effect enhanced in the presence of Aβ (Figure 2). Since BACE-1 promotes production of Aβ and BACE-2 inhibits it, we surmise morphine is neuroprotective because such modulation of the BACE enzymes would decrease Aβ production [19]. Morphine's effects on both BACE-1 and BACE-2 expression were shown to be naloxone antagonizable (Figure 3), verifying that the neuroprotective action of morphine is directly related to its binding to the mu-3 opioid receptor since opioid peptides do alter the expression levels of these enzymes [18].

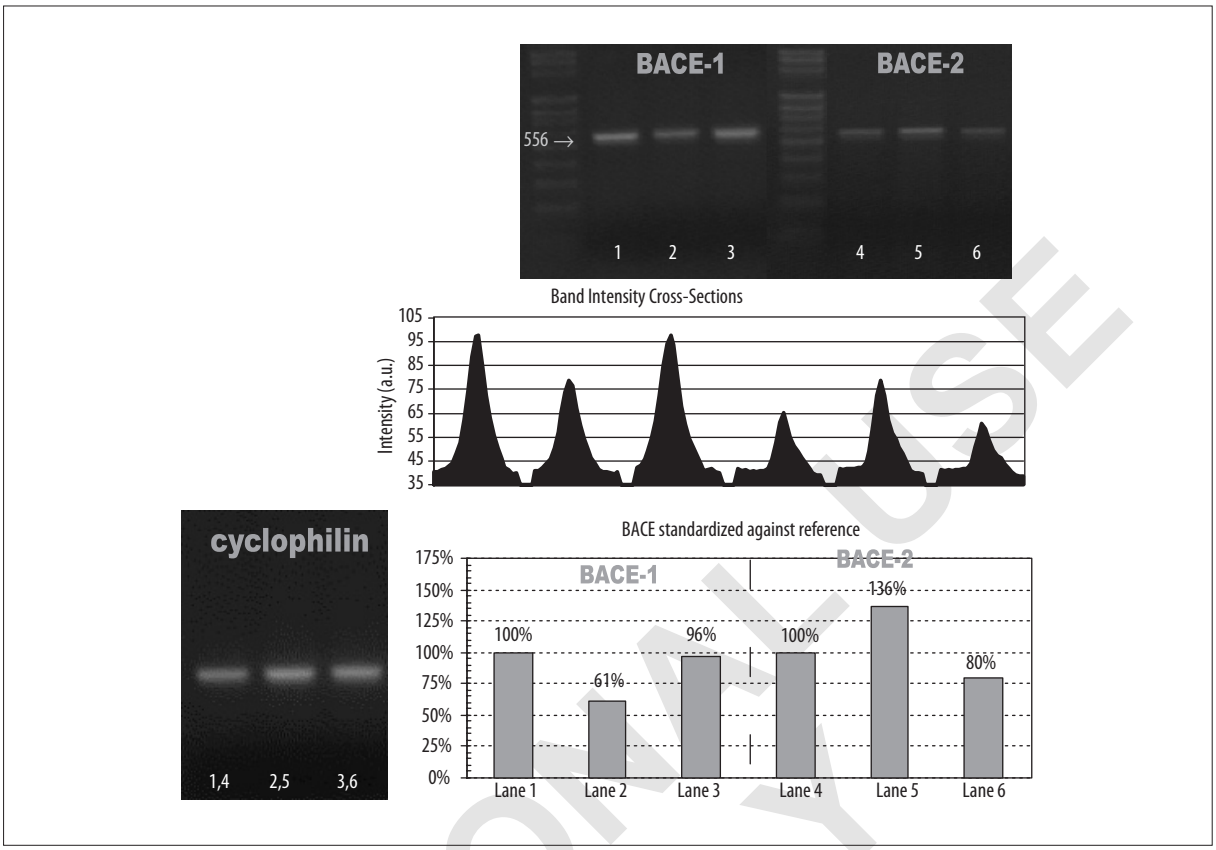


**Figure 3.** RT-PCR analysis of BACE-1 and BACE-2 gene expression in HTB-11 neuroblastoma cells, 24-h treatments. Lanes 1 and 4: untreated, lane 2 and 5: 1 μM Morphine, lane 3 and 6: 1 μM Morphine pretreated with 10 μM Naloxone for twenty minutes. Lanes 1–3 were analyzed for BACE-1, lanes 4–6 for BACE-2. At bottom left: cyclophilin reference gene expression, and the modulation of BACE standardized against the reference gene.

**Similar modulation of BACE-1 and BACE-2 mRNA expression by NO**

One of endogenous morphine's primary physiological effects is cNOS derived NO release via mu-3 opiate receptor subtype coupling [20]. To determine whether morphine's neuroprotective effects on the Aβ pathway were NO dependent, HTB-11 cells were treated with L-NAME, a cNOS inhibitor; L-NAME significantly antagonized the effects of morphine (Figure 4), indicating that NO release is critical to morphine's neuroprotective moderation of BACE-1 and -2. SNAP (a NO donor) exposure to HTB-11 for mRNA analysis was then performed. After 4 and 24 hour exposure, SNAP down regulated BACE-1 expression in a concentration dependent manner similar to morphine, which also was enhanced in the presence of Aβ (Figures 5,6). SNAP also up regulated in a concentration dependent manner BACE-2 at both the four 4 and 24 hour timepoints (Figures 7,8) as did morphine. In the presence of Aβ, SNAP dose-dependently increased BACE-2 expression (Figures 7,8).

To verify the semi-quantitative accuracy of the RT-PCR procedures and to explore whether the effects of SNAP on BACE expression occur earlier than four hours, BACE-1 and BACE-2 mRNA expression were analyzed in an additional experiment for two hours (Figure 9). BACE-1 and BACE-2 expression was altered by SNAP after two hours, with BACE-1 down regulated and BACE-2 up regulated. The expression of the reference genes β-actin, and cyclophilin were not affected.

**β-amyloid inhibits NO release in SH-SY5Y cells**

SH-SY5Y neuroblastoma cells normally release NO via cNOS in response to application of either morphine or its metabolite, morphine-6-glucuronide (M6G) [18,20]. Morphine/M6G binds to the G-protein coupled mu-3 receptor, stimulating intracellular $Ca^{+2}$ transients which activate cNOS, liberating NO [18]. To determine whether Aβ disrupts this process, SH-SY5Y cells were pretreated with varying concentrations of Aβ for 1 hour. Following the addition of M6G, the Aβ treated cells show a dose-dependent decrease in NO release, demonstrating that Aβ is dose-dependently inhibiting the release of constitutive NO (Figure 10). Pretreatment with L-NAME ($10^{-4}$ M), a cNOS inhibitor, for 4 minutes also prevented M6G-induced release of NO, verifying that M6G was indeed inducing release of NO through cNOS given the rapid time course of the coupling (Figure 10). The reduction of M6G-induced NO release after Aβ treatment suggests that Aβ is either a) directly inhibiting the activation of cNOS, b) interfering with the binding of M6G to the mu-3 receptor, both of which would potentially impact the level of basal NO in the AD-afflicted human brain. Furthermore, SH-SY5Y cells release NO at a low level compared to human immune and vascular tissues (3–4 nM compared to 26–29 nM morphine at $10^{-6}$ M).

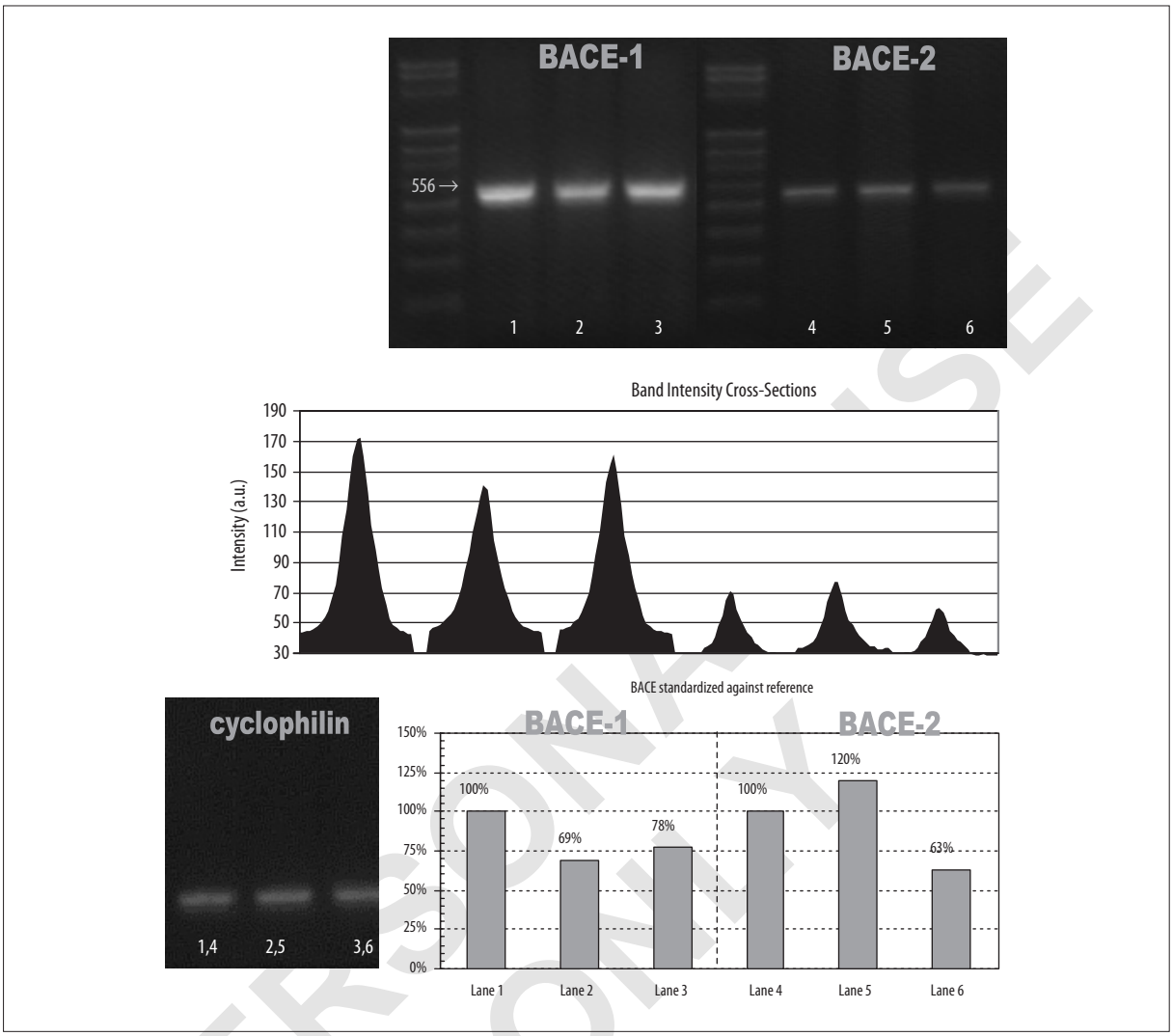


**Figure 4.** RT-PCR analysis of BACE-1 and BACE-2 gene expression in HTB-11 neuroblastoma cells, 24-h treatments. Lanes 1 and 4: untreated, lane 2 and 5: 1 μM Morphine, lane 3 and 6: 1 μM Morphine pretreated with 10 μM L-NAME for twenty minutes. Lanes 1–3 were analyzed for BACE-1, lanes 4–6 for BACE-2. At bottom left: cyclophilin reference gene expression, and the modulation of BACE standardized against the reference gene.

## Discussion

The results of the present study demonstrate that morphine, in a concentration and time dependent manner, up regulates BACE-2 expression while simultaneously down regulating BACE-1 expression. This phenomenon can be blocked by treating the cells with the opiate receptor antagonist naloxone. This morphine mediated process is coupled cNOS derived NO release, which was ascertained by treating the tissue with the NOS inhibitor L-NAME. NO alone can mediate this effect, further substantiating this observation. Additionally, in the presence of Aβ both the morphine and NO effects are enhanced. Aβ alone appears to have the ability to inhibit cNOS derived NO release at higher concentrations. We surmise a two-way relationship between Aβ and morphine/NO. Morphine/NO modifies the expression of two proteins critical to the production of Aβ, down regulating BACE-1 and up regulating the expression of BACE-2. In addition, after long term incubation Aβ seems to enhance the ability of NO to modify BACE expression. Taken together morphine, via its coupling to NO, appears to be neuroprotective since it promotes BACE-2 up regulation, which enhances Aβ catabolism, avoiding the effect of Aβ inhibiting NO production.

The production pathway of Aβ has been extensively studied. APP, a large type-I membrane-bound protein [21], is expressed ubiquitously throughout human cells, and Aβ is a normal product of APP metabolism in most cells. APP can be cleaved by β-secretase (BACE-1) to produce a secreted ectodomain of APP (sAPPβ) and C99, the membrane-bound C-terminal 99 amino acid chain of APP. γ-secretase, a protein that appears to require the presenilin proteins PS1 and PS2 to function proteolytically, then cleaves C99 to release Aβ, some of which is secreted from the cell. A third secretase, α-secretase, also has the ability to cleave APP but cuts the protein in the middle of the Aβ domain, precluding formation of Aβ. α-secretase cleavage produces the secreted sAPPα ectodomain, and the membrane-bound fragment C83, which is cleaved by γ-secretase to form a non-toxic 3-kDa

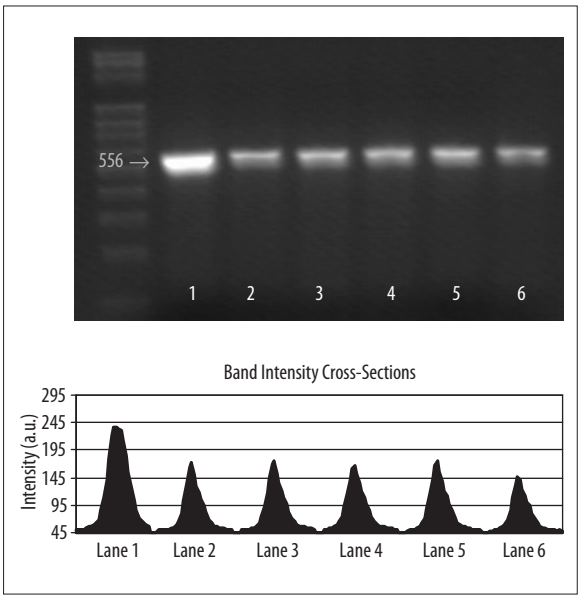


**Figure 5.** RT-PCR analysis of BACE-1 gene expression in HTB-11 neuroblastoma cells, 4-h treatments. Lane 1: untreated, lane 2, 3 and 4: 1 μM, 5 μM and 10μM SNAP, lane 5: 25 μM Aβ with 1 μM SNAP, lane 6: 25 μM Aβ with 10 μM SNAP.

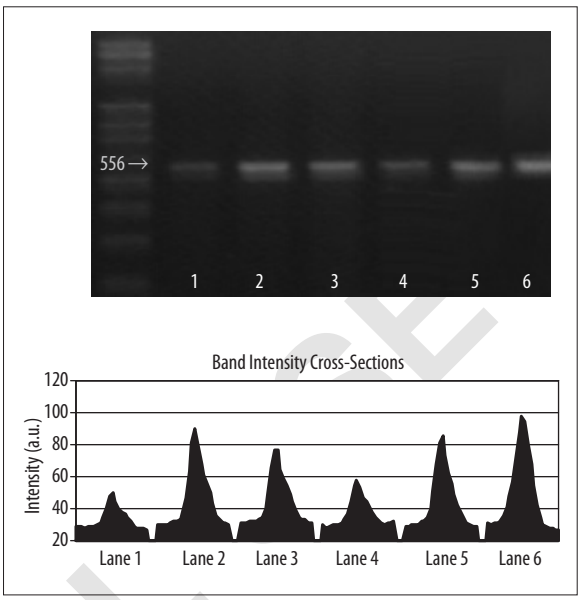


**Figure 7.** RT-PCR analysis of BACE-2 gene expression in HTB-11 neuroblastoma cells, 4-h treatments. Lane 1: untreated, lane 2, 3 and 4: 1 μM, 5 μM and 10 μM SNAP, lane 5: 25 μM Aβ with 1 μM SNAP, lane 6: 25 μM Aβ with 10 μM SNAP.

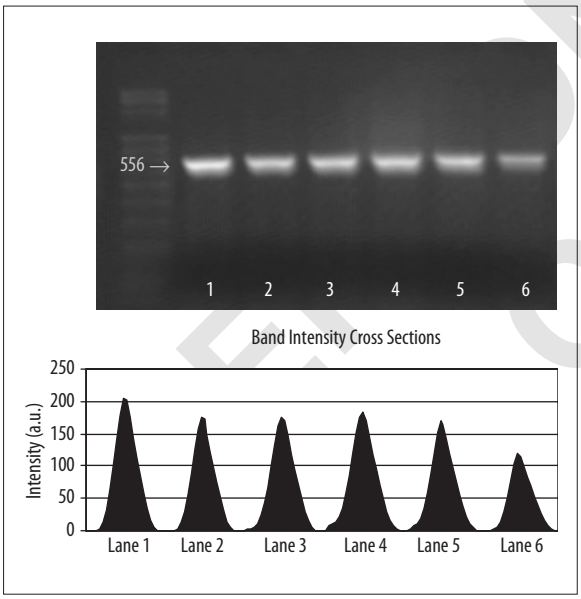


**Figure 6.** RT-PCR analysis of BACE-1 gene expression in HTB-11 neuroblastoma cells, 24-h treatments. Lane 1: untreated, lane 2, 3 and 4: 1 μM, 5 μM and 10 μM SNAP, lane 5: 25 μM Aβ with 1 μM SNAP, lane 6: 25 μM Aβ with 10 μM SNAP.

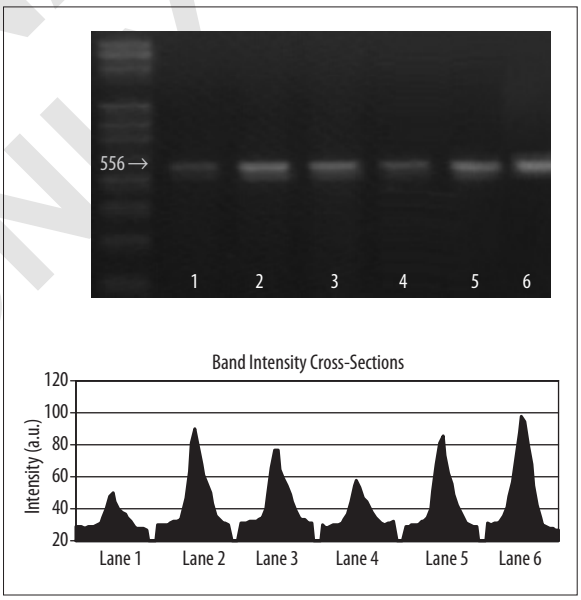


**Figure 8.** RT-PCR analysis of BACE-2 gene expression in HTB-11 neuroblastoma cells, 24-h treatments. Lane 1: untreated, lane 2, 3 and 4: 1 μM, 5 μM and 10 μM SNAP, lane 5: 25 μM Aβ with 1 μM SNAP, lane 6: 25 μM Aβ with 10 μM SNAP.

fragment (p3) whose function is unclear [19]. The BACE-1 homologue, and BACE-2, exhibits ~64% amino acid sequence identity [22]; while this homologue can cleave APP at the β-site, it cleaves with higher efficiency at two other positions within the Aβ domain near the α-secretase cleavage site, making it function as an alternative α-secretase. Thus, while BACE-1 promotes production of Aβ, BACE-2 helps to inhibit it, thereby exerting a protective role. We now demonstrate this protective role is extended to endogenous morphine and NO signaling.

Morphine's/NO's BACE 2's stimulatory activity, as demonstrated in this report, may be explained in several ways. Morphine via NO and Aβ could be interacting with transcriptional regulators for BACE-1 and BACE-2 and thus, the normal self-regulation of the Aβ production requires NO. On the other hand, it may be NO's antioxidant abilities that are at work, eliminating free radicals and superoxide anions that maintain or encourage the production of Aβ as an inflammatory signal [23]. Other studies have found a significant connection between AD and oxidative

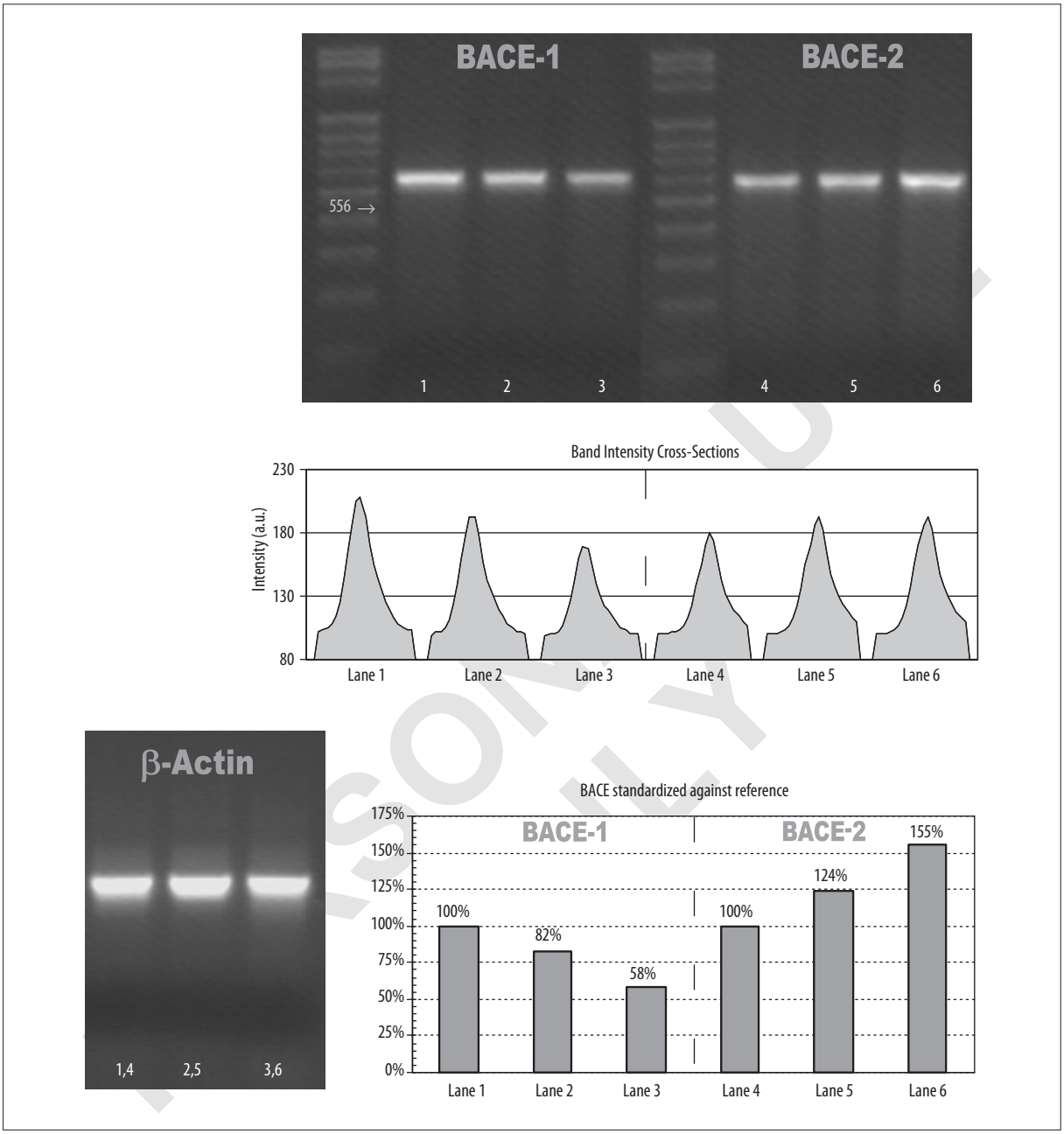


**Figure 9.** RT-PCR analysis of BACE-1 and BACE-2 gene expression in HTB-11 neuroblastoma cells, 2-h treatments. Lanes 1 and 4: untreated, lane 2 and 5: 1 μM SNAP, lane 3 and 6: 5 μM SNAP. Lanes 1–3 were analyzed for BACE-1, lanes 4–6 for BACE-2. At left: β-actin reference gene expression, and the moderation of BACE standardized against the reference gene.

stress [24]; the antioxidant properties of basal NO may be vital to combating the progression of AD [2].

The possibility that morphine may moderate key enzymes in the Aβ pathway alongside NO exists. Here, NO is not the primary regulator, and morphine is since it stimulates NO release. This relationship has been demonstrated in rat hippocampus [25]. This hypothesis is also based on the findings that morphine is found and can be made in vertebrate and invertebrate organisms [20,26] and functions as a neurotransmitter/neurohormone. Morphine also has been shown to modulate immune activities, including tumor growth [18]. Endogenous morphine appears also to be involved with memory retention [27], and nociceptive transmission [28]. A deficiency of endogenous morphine would likely correspond to a lowering of basal NO, which as shown above appears to have a role in moderating Aβ production.

Of equal importance is the observation that Aβ inhibits NO release in these neuronal cell lines, allowing a proinflammatory response to develop [29]. Indeed, in the past we speculated that various diseases may have a proinflammatory core, including AD [30]. Previous studies have attempted to implicate Aβ in neurotoxic mechanisms via the cell membrane, sometimes in connection with cell

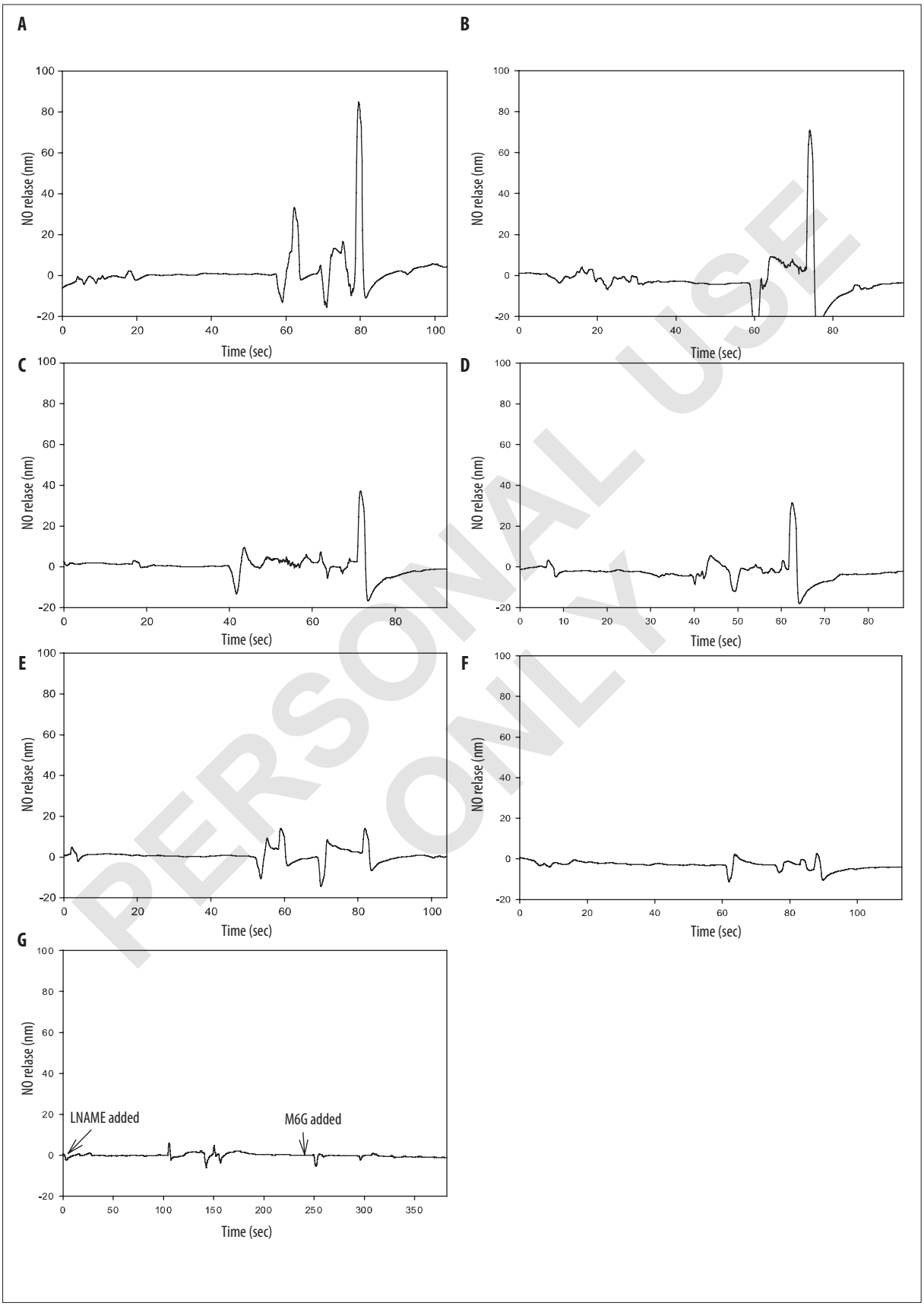


**Figure 10.** Representative real-time amperometric analysis of NO release from SH-SY5Y neuroblastoma cells pretreated with β-Amyloid, stimulated by 1 μM M6G added at t=0 on plots **A–F**. All pretreatments were for 1 hour. (**A**) control, (**B**) 1 μM β-Amyloid, (**C**) 5μM β-Amyloid, (**D**) 10 μM β-Amyloid; (**E**) 15 μM β-Amyloid, (**F**) 25mM β-Amyloid, (**G**) control with L-NAME added 4 minutes before M6G.

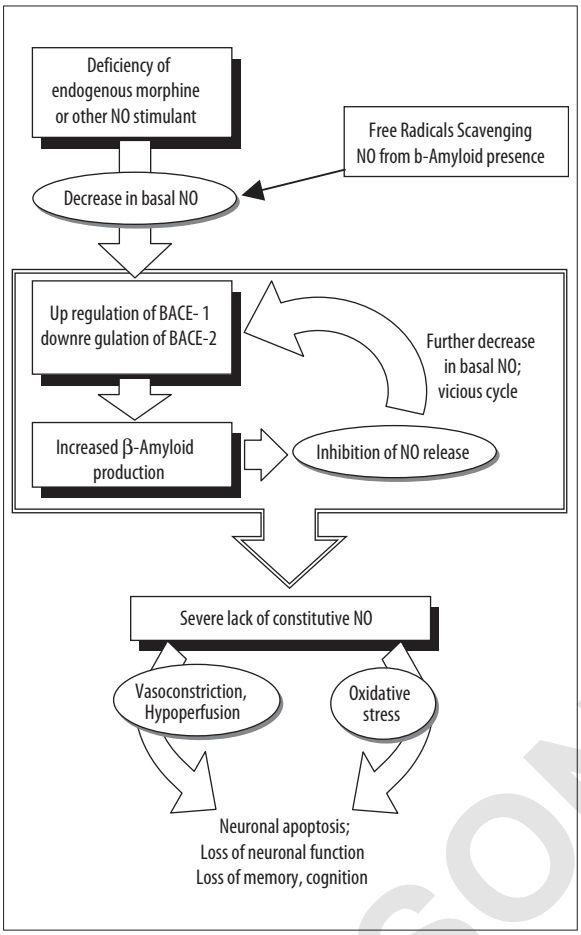


**Figure 11.** Diagram of theorized role of NO in Alzheimer's disease. Deficiency of endogenous morphine or other cNOS activator triggers a vicious cycle of reduced NO release and increased Aβ production. This causes a severe lack of basal NO, resulting in loss of neuronal function and symptoms of AD.

death genes or oxidative damage [6]. However, the most convincing and concrete explanation for AD based on the amyloid hypothesis would be one finding support in existing epidemiological/clinical data that AD and vascular diseases have significant coincidence [6,9–14], rather than rely solely on pharmacological experimentation. A vascular component to AD could be explained by Aβ's inhibition of NO, a chemical that controls vasodilation [16]. Inhibition of NO would require internalization of Aβ, which is a process that occurs well before the onset of AD [15]. Lack of adequate basal NO over an extended period of time, in addition to the presence of constrictive amyloid plaques throughout the brain, would produce or result from chronic hypoperfusion; the vascular endothelium in brain microvessels is under constant "shear stress" and any condition altering this state is likely to create hemodynamic abnormalities and reduce blood flow [31]. Hypoperfusion would impair neuronal function through the hindrance of neurono-glial energy metabolism, a result of decreased glucose and oxygen delivery from the circulatory system [31]. As well, chronic hypoperfusion has been linked to increases in oxidative stress and hypoperfusive brain lesions [32].

Assuming a deficiency of endogenous morphine or other cNOS activators/scavengers, a decrease in levels of basal NO will occur over time, allowing for a novel theory about the origin of AD. This scenario may work because NO is scavenged by excessive free radical generation. Moreover, since estrogen also stimulates cNOS derived NO production from neural and vascular tissues, a protective role for it too exists [33–37]. Thus, morphine metabolism alterations may also lead to a dynamic loss of control of blood vessel contractility, resulting in hypoperfusion. During this period NO's moderation of BACE-1 and BACE-2 gene expression decreases; BACE-1 expression rises and BACE-2 expression falls since this would be the proper signaling for proinflammatory signaling, i.e., Aβ is proinflammatory [2]. More BACE-1 then becomes available to cleave APP into Aβ, and Aβ levels increase; Aβ is secreted out of the cell to aggregate into amyloid plaques and soluble Aβ levels increase within the cell. Internalized Aβ inhibits NO release by the cell, which then creates a vicious cycle: NO levels are further decreased, lessening regulation of the BACE genes, which again increases the production of Aβ and the process repeats. Simultaneously, Aβ, would promote a chronic and progressively increasing inflammatory reaction, initiating both vascular and neural damage. As the pathology of AD continues, NO levels decrease to a point where hypoperfusion of the brain becomes chronically destructive. In brain cells oxidative stress rises; neurons undergo apoptosis and overall neuronal function decreases, producing memory loss, cognitive disorder, and other typical symptoms of AD.

Further evidence for the likelihood of the above scenario in a case of AD is seen in the potential of not only neurons but also glia and endothelia [15] to proteolitically process APP and create Aβ. Further examination of the existence of a two-way relationship of Aβ and NO in such cells is warranted: if such cells became involved in a process, the ability for NO deficiency to initiate AD pathology is greatly increased, with an exceedingly larger number of brain cells able to contribute to the disease's progression.

## Conclusions

A novel bidirectional relationship between Aβ and endogenous morphine/NO has been surmised, with NO regulating the expression of proteolytic enzymes involved in the synthesis and catabolism of Aβ and inhibiting the release of NO via cNOS activation. This relationship may help explain the pathological mechanism and progression of AD in accordance with the amyloid hypothesis; in addition, it offers support to the hypothesis of AD being a primarily vascular disease. Moreover, endogenous morphine via its down regulating abilities appears to have a protective role. Morphine's combined effect on BACE-1 and BACE-2 expression are neuroprotective; morphine is down regulating expression of BACE-1, the initial proteolytic enzyme for Aβ synthesis, and in the presence of Aβ, is up regulating BACE-2, which inhibits Aβ synthesis by cleaving APP in such a way that Aβ cannot be formed. Morphine's surmised role in this process also enhances its identity as an endogenous chemical messenger.

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logy Competition, Semifinalist – Intel Science Talent Search, Semifinalist – NYCSEF, Special Award (Ezra Levy Award) – American Association of Clinical Chemistry, Special Award – Honor Society for Women in Chemistry and First Team – USA Today All-Academic High School Team.

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