Non-steroidal Anti-inflammatory Drugs Stimulate Secretion of Non-amyloidogenic Precursor Protein*

Yael Avramovich‡§, Tamar Amit§, and Moussa B. H. Youdin¶

From the ‡Eve Topf and National Parkinson Foundation Centers of Excellence for Neurodegenerative Diseases Research and Department of Pharmacology, Technion – Faculty of Medicine, 31096 Haifa, Israel and §Intra-departmental Unit for Biotechnology, 31096 Haifa, Israel

Chronic inflammatory processes are associated with the pathophysiology of Alzheimer’s disease (AD), and it has been proposed that treatment with non-steroidal anti-inflammatory drugs (NSAIDs) reduces the risk for AD. Here we report that various NSAIDs, such as the cyclooxygenase inhibitors, nimesulide, ibuprofen and indomethacin, as well as thalidomide (Thal) and its non-teratogenic analogue, supidimide, significantly stimulated the secretion of the non-amyloidogenic α-secretase form of the soluble amyloid precursor protein (sAPPα) into the conditioned media of SH-SY5Y neuroblastoma and PC12 cells. These NSAIDs markedly reduced the levels of the cellular APP holoprotein, further accelerating non-amyloidogenic processes. sAPPα release, induced by nimesulide and Thal, was modulated by inhibitors of protein kinase C and Erk mitogen-activated protein (MAP) kinase. Furthermore, in results complementary to the inhibitor studies, we show for the first time that NSAIDs can activate the Erk MAP kinase signaling cascade, thus identifying a novel pharmacology mechanism of NSAIDs. Our findings suggest that NSAIDs and Thal might prove useful to favor non-amyloidogenic APP processing by enhancing α-secretase activity, thereby reducing the formation of amyloidogenic derivatives, and therefore are of potential therapeutic value in AD.

Alzheimer’s disease (AD) pathology is characterized by senile plaques containing β-amyloid peptide (Aβ), a protein with neurotoxic and glial immune-activating potential (for review, see Refs 1–3). Aβ, a 39–43 amino acid peptide, is derived from a larger transmembrane glycoprotein, the amyloid precursor protein (APP) (4, 5). APP can be processed proteolytically via alternative pathways; cleavages at the N and C termini of Aβ domain by β- and γ-secretases, respectively, lead to the formation of the Aβ peptide. In addition, in the α-secretase pathway, the cleavage occurs within the sequence of Aβ peptide and generates a large, secreted form of soluble APP (sAPP), thus precluding the formation of the amyloidogenic Aβ (for recent reviews of APP processing, see Refs. 6 and 7). Because the proportion of APP processed by β-secretase versus α-secretase may affect the amount of Aβ produced, the regulation of these two pathways may be critically important to the pathogenesis of AD. Previous studies have demonstrated that Aβ production could be enhanced by activation of various cell surface receptors, coupled to increased activation of second messenger cascades, including phosphatidylinositol hydrolysis, tyrosine phosphorylation, protein kinase C (PKC), protein kinase A, mitogen-activated protein kinase (MAPK), protein phosphatase 1 and 2B, and calcium (8).

In the search for the pathogenic mechanism of AD, much interest has been focused recently on the involvement of inflammatory reactions in AD. A chronic inflammatory response has been described in the brain of AD patients (9), characterized by the presence of activated astrocytes surrounding the senile plaque and activated microglia surrounding and extending processes into the plaque core (10–12). Proinflammatory proteins elevated in plaques include the cytokines tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, the acute phase protein α-1-antichymotrypsin, the complement protein Clq, the complement membrane attack complex C5b-9, and the chemokines MIP-1α and β (13–20). The activated glial cells surrounding the plaque are likely the major source of these proteins since Aβ can induce their expression in cultured microglia (21–23) and astrocytes (24, 25). In addition, it was shown that Aβ produced neurotoxins, such as nitric oxide, reactive oxygen species, and glutamate, in microglial cells and astrocytes (26–29).

Based on these studies, it was suggested that non-steroidal anti-inflammatory drugs (NSAIDs) might be effective in the prevention and treatment of AD. In support of this, several epidemiological and clinical studies have reported that the use of NSAIDs reduced the incidence and progression of AD (30–32). Moreover, it was reported that the NSAID, ibuprofen (Ibu), which has been associated with reduced AD risk in human epidemiological studies, significantly suppressed amyloid plaque pathology and inflammation in a transgenic mouse model of AD (33). Recently, Weggen et al. (34) demonstrated that a subset of NSAIDs preferentially decreased the highly amyloidogenic Aβ42 peptide produced from a variety of cultured cells, independently of cyclooxygenase (COX) activity.

Although the mechanism by which NSAIDs reduce the risk of AD is not entirely clear, they competitively inhibit COX catalytic activity, thereby reducing the production of inflam-

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‡ To whom correspondence should be addressed: Dept. of Pharmacology, Technion – Faculty of Medicine, P.O. Box 9697, 31096 Haifa, Israel, Tel.: 972-4-8295290; Fax: 972-4-8513145; E-mail: youdim@tx.technion.ac.il.

§ The abbreviations used are: AD, Alzheimer’s disease; APP, amyloid precursor protein; sAPP, soluble APP; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; Erk, extracellular signal-regulated kinase; MEK, MAPK/ERK; TACE, TNF-α converting enzyme; Aβ, β amyloid peptide; NSAID, non-steroidal anti-inflammatory drug; COX, cyclooxygenase; TNF-α, tumor necrosis factor-α; PG, prostaglandin; PLA2, phospholipase A2; Nim, nimesulide; Ibu, ibuprofen; Indo, indomethacin; Thal, thalidomide; Sup, supidimide; FCS, fetal calf serum; DMEM, Dulbecco’s modified Eagle’s medium; PMA, phorbol 12-myristate 13-acetate; ADAM, a disintegrin and metalloproteinase.

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matory prostaglandins (PGs) from membrane-derived arachidonate. COX thus represents a possible target of NSAIDs action in the neurodegenerative mechanism. Furthermore, studies showing that COX-2 expression was elevated in the AD brain and correlated with the total Aβ content (35–39), as well as the observation that COX-2 stimulated the production of Aβ1–42 in neuroblastoma transformed NG108-15 cells (40), strongly implicate Aβ in COX-2 activities. In addition, PGs produced by brain injury or inflammation have been shown to stimulate the synthesis of APP mRNA and APP holoprotein, whereas the immunosuppressants cyclosporin A and FK-506 inhibited PGE2-induced APP synthesis in primary culture of cortical astrocytes (41).

To further elucidate the complex action of anti-inflammatory drugs in the neurodegenerative processes of AD, we investigated the effects of these drugs on the regulation of APP processing and the signaling pathways that are involved by using cultured human SH-SY5Y neuroblastoma and rat PC12 cells. In the present study, we examined the effect of the following anti-inflammatory COX inhibitors: Ibu and indomethacin (Indo), which are non-selective COX inhibitors, and nimesulide (Nim), which is a preferential COX-2 inhibitor. In addition, we studied the effects of thalidomide (Thal) and its non-teratogenic analogue, supidimide (Sup), which have anti-inflammatory and immunosuppressive activities that inhibit TNF-α and may represent candidates for the treatment of inflammatory conditions in which TNF-α-induced toxicities are involved.

**EXPERIMENTAL PROCEDURES**

**Materials**—Nim, Ibu, and Indo were obtained from Sigma. Thal and Sup were a kind gift of Grunenthal GmbH (Aachen, Germany). Phorbol 12-myristate 13-acetate (PMA), GF109203X, calphostin C, PD98059, and USY, were obtained from Calbiochem, dissolved in dimethyl sulfoxide, and stored at −20 °C. Ro31-9790 was a kind gift from Roche Discovery Welwyn (Garden City, UK). Tissue culture reagents were obtained from Biological Industries (Beth-Haemeck, Israel). All other chemicals were of the highest grade available and were purchased from Sigma. The monoclonal antibody 22C11 was purchased from Cell Signaling Technology, Inc. (Beverly, MA).

**Cells and Cell Culture Procedures**—PC12 cells, originated from rat pheochromocytoma, were grown to confluence in T75 flasks containing DMEM (1000 mg/liter glucose) and supplemented with 5% FCS, 10% dimethyl sulfoxide, and stored at −20 °C. Ro31-9790 was a kind gift from Roche Discovery Welwyn (Garden City, UK). Tissue culture reagents were obtained from Biological Industries (Beth-Haemeck, Israel). All other chemicals were of the highest grade available and were purchased from Sigma. The monoclonal antibody 22C11 was purchased from Cell Signaling Technology, Inc. (Beverly, MA).

**Cells and Cell Culture Procedures**—PC12 cells, originated from rat pheochromocytoma, were grown to confluence in T75 flasks containing DMEM (1000 mg/liter glucose) and supplemented with 5% FCS, 10% horse serum, and 1% of a mixture of penicillin/streptomycin/nystatin. SH-SY5Y, obtained from Calbiochem, dissolved in dimethyl sulfoxide, and stored at −20 °C. Ro31-9790, at a concentration of 2 mM, was used to inhibit COX-2 activity.

**Cells and Cell Culture Procedures**—PC12 cells, originated from rat pheochromocytoma, were grown to confluence in T75 flasks containing DMEM (1000 mg/liter glucose) and supplemented with 5% FCS, 10% horse serum, and 1% of a mixture of penicillin/streptomycin/nystatin. Cell cultures were incubated at 37 °C in a humid 5% CO2, 95% air environment. At 18–24 h before the experiments, the medium was replaced with DMEM with 0.5% FCS. After incubation with the drugs for the indicated periods, conditioned media were collected, and cells were lysed for subsequent analyses. Collected media were centrifuged at 3500 × g for 10 min at 4 °C to remove the cellular debris, and the cleared supernatants were concentrated 10-fold by lyophilization. Cell monolayers were washed twice with ice-cold phosphate-buffered saline and lysed in lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1% Triton X-100, and protease and phosphatase inhibitor cocktails) for 10 min on ice. The cell lysates were centrifuged for 10 min at 14,000 × g, and the supernatants were saved at −20 °C until use. The protein amount in each sample was determined by the Bradford method (Bio-Rad).

**Determination of APP**—Normalization of protein loading on each blot was obtained by loading a sample of conditioned medium, standardized to the protein concentration in the cell lysate. sAPP in the medium was analyzed by SDS-PAGE on 4–12% gradient Tris-glycine-polyacrylamide gels (NOVEX Corporation, San Diego, CA) followed by immunoblotting on nitrocellulose membranes using either the monoclonal antibody 22C11 or the monoclonal antibody 6E10 in 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20, and 1% bovine serum albumin. Cell lysates (30 µg of total protein/lane) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blot with antibody 22C11 for the identification of the levels of cellular APP. Immunoreactivity was detected using alkaline phosphatase-conjugated goat anti-mouse IgG and an enhanced chemiluminescence method (Amersham Biosciences). Blots were developed for different times to be within a linear range of response, and the relative intensity of immunoreactive bands on the exposed films was quantified by a computer-assisted densitometry program (Quantity One, Bio-Rad). Statistical analysis was done by one-way analysis of variance followed by two-tailed Student’s t test; a value of p < 0.05 was considered significant. Each experiment was repeated three to five times, and results from a representative experiment are shown.

**Extracellular Signal-regulated Kinase (Erk) Activity**—The kinase activity of specific Erks was measured in cell lysates using the MAPK assay kit (Cell Signaling Technology, Inc.) according to the manufacturer’s protocol. Briefly, for kinase activity assays, PC12 or SH-SY5Y neuroblastoma cells were grown in 6-well plates (5 × 105 cells/well). Before each experiment, the cells were exposed to DMEM with 0.5% FCS for 24 h, and then experimental treatments were performed in 0.5% FCS/DMEM with the vehicle or test drugs at 37 °C, as described in the figure legends. After treatment, reactions were stopped by placing cells on ice and aspirating the medium. Cells were harvested in 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1% Triton X-100, and protease and phosphatase inhibitor cocktails. Protein concentration was determined by the method of Bradford. Each cell lysate, containing 30 µg of protein, was separated on 4–12% polyacrylamide electrophoresis gels, immunoblotted, and identified using phospho-p44/42 MAPK (Thr-202/Tyr-204) antibody or p44/p42 MAPK antibody. Data are representative of three to six independent experiments.

**RESULTS**

**NSAIDs Stimulate sAPPα Secretion**—To determine the action of NSAIDs on the secretion of sAPPα, we treated SH-SY5Y neuroblastoma cells with Nim, Ibu, Thal, or Sup and observed their dose dependence on sAPPα release into the medium. Fig. 1 shows that treatment of SH-SY5Y neuroblastoma cells for 20 h with increasing concentrations of these drugs resulted in a significant, dose-dependent increase in sAPPα release into the medium as compared with the level in control, untreated cells. The maximal effect was obtained at concentrations of 0.1 and 1 µM, which resulted in an ~2.5-fold increase in sAPPα secretion over the basal levels. However, the levels of sAPPα decreased upon treatment with high doses of the drugs (100 µM).

The stimulation of sAPPα secretion by drugs showed the same pattern, whether immunoblotting was performed with the monoclonal antibody 22C11, which recognizes the N terminus of APP, or with the monoclonal antibody 6E10, which recognizes an epitope within residues 1–17 of the Aβ domain of APP, a site that constitutes the C terminus of sAPP, cleaved at the α site. Therefore, the identified bands can be assumed to be the α-secretase-cleaved form of sAPP. Fig. 2 shows representative immunoblots, obtained for Nim and Thal, probed with the monoclonal antibody 6E10, and Table I summarizes the results of densitometric analysis, expressed as percentage of stimulated sAPPα release into the cultured medium. To exclude the possibility that this effect is specific to a particular cell type, we examined sAPPα secretion in response to NSAIDs in PC12 cells. Treatment of PC12 cells with increasing doses of Nim, Ibu, Thal, or Sup also resulted in a similar concentration-dependent increase in sAPPα release (data not shown). Fig. 3 demonstrates the maximal effect of these drugs, obtained at concentrations of 1 and 10 µM. Thus, this effect was observed in cell lines of human and rat and is not dependent on cell type.

Since α-secretase is a zinc metalloproteinase, susceptible to inhibition by hydroxamate-based compounds (42), we examined the effect of the hydroxamic acid-based metalloproteinase inhibitor, Ro31-9790, on Nim-, Thal-, or Sup-mediated sAPPα release from SH-SY5Y cells. As shown in Fig. 4, pretreatment of SH-SY5Y cells with 100 µM Ro31-9790 blocked Nim-, Thal-, or Sup-enhanced cleavage of sAPPα at both 1 and 10 µM concentrations of drugs. Thus, these findings demon-
strate that the anti-inflammatory drugs affect APP processing by activating Ro31-9790-sensitive metalloprotease(s), further suggesting that their effect was mediated via \( \text{H9251} \)-secretase activity.

The effects of NSAIDs on the levels of cellular APP were also determined. As can be seen in Fig. 5, Nim, Ibu, Indo, Thal, and Sup (1 or 10 \( \mu M \)) significantly reduced the levels of cellular APP holoprotein in SH-SY5Y neuroblastoma cells, relative to those in untreated cells (≈30% of control). No toxicity was detected by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay in SH-SY5Y cells treated with these NSAIDs up to 100 \( \mu M \) (data not shown).

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**TABLE I**

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<th>Drug</th>
<th>Concentration (( \mu M ))</th>
<th>% of control</th>
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<tr>
<td>Nim</td>
<td>0.1</td>
<td>261.4 ± 9.1**</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>213.6 ± 15.0*</td>
</tr>
<tr>
<td>Thal</td>
<td>0.1</td>
<td>248.1 ± 24.9*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>398.7 ± 18.3**</td>
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Fig. 4. Effect of Ro31-9790 on stimulated sAPP\( \alpha \) release. SH-SY5Y neuroblastoma cells were pretreated with vehicle or Ro31-9790 (100 \( \mu M \)) for 1 h and then treated without or with Nim, Thal, or Sup for 20 h at 37°C. Proteins released into the conditioned media were collected and subjected to Western blot analysis for sAPP\( \alpha \).
PKC and tyrosine kinase receptor regulation of APP processing (45, 46). We therefore assessed which, if any, of these kinases is involved in mediating the action of NSAIDs on sAPPα release using specific signaling inhibitors (Fig. 6). Initially, we tested the role of PKC in Nim- or Thal-induced sAPPα secretion by using the PKC inhibitors, GF109203X, and calphostin C. GF109203X (2.5 μM) and calphostin (1 μM) partially inhibited sAPPα secretion induced by 1 μM Nim (Fig. 6A), whereas they abolished almost completely Thal-induced sAPPα secretion (Fig. 6B).

As shown previously, 1 μM Nim or Thal significantly increased sAPPα release of sAPPα. To examine the possibility that the MAPK-dependent pathway may also be involved in Nim- and Thal-induced sAPPα secretion, we tested the effect of U0126, an inhibitor of MEK, which activates Erk kinase signaling. As shown in Fig. 6, inhibition of MEK by U0126 (5 μM) inhibited Nim-stimulated sAPPα secretion (35%) and significantly blocked (67%) the release of sAPPα, induced by Thal. These findings indicate that PKC- and MAPK-dependent pathways are involved in both Nim and Thal stimulation of sAPPα secretion.

**NSAIDs Activate MAPK**—To further investigate the observation that NSAID-induced release of sAPPα was mediated by the MAPK pathway, we examined whether the NSAIDs stimulate the MAPK cascade. In results complementary to the inhibitor studies, we found that all of the NSAIDs examined in this study activated the Erk MAPK signaling cascade.

Dual phosphorylation of Erk/MAPK on the threonine and tyrosine residues necessary for activation was evaluated using the anti-active MAPK antibody, which has been developed to correlate Erk1/2 MAPK activation with its phosphorylation state (47). Based on immunoblot analysis with anti-phospho-p44/p42, Nim dose dependently (1–100 μM) induced Erk phosphorylation in both PC12 cells (Fig. 7A and Table II) and SH-SY5Y neuroblastoma cells (Fig. 7B) but had no effect on total levels of the Erk proteins. As seen in Fig. 7C, Nim activated Erk in a time-dependent manner with peak Erk phosphorylation occurring after 15 min of stimulation (Fig. 7C). After 30 and 60 min, MAPK activation decreased and returned to basal levels (data not shown). To determine the inhibitory potential of noncompetitive inhibitors of MEK phosphorylation and activation, the effect of PD98059 and U0126 (48, 49) on Nim-induced phosphorylation of Erk1/2 was examined. Pre-treatment with PD98059 or U0126 blocked the Nim-induced increase of Erk1/2 phosphorylation (Fig. 7D). We also examined the role of the PKC signaling pathway in Nim-stimulated Erk activation by using the specific PKC inhibitor, GF109203X. Preincubation with GF109203X abolished the response to PMA and decreased the effect of Nim on Erk activation (Fig. 7E).

A similar time- and concentration-dependent increase in MAPK phosphorylation was observed by Ibu and Indo (Fig. 8). Furthermore, as shown in Fig. 9, exposure of PC12 cells to Thal and Sup also resulted in an increase in phosphorylated Erk1/2 with no change in total Erk1/2 level. Following the addition of Thal or Sup, Erk1/2 phosphorylation occurred within 15 min, and the effect was concentration-dependent in the range of 1–100 μM (Table II). Both Thal and Sup activated MAPK rapidly and transiently with peak MAPK phosphorylation occurring with 15 min of stimulation (Fig. 9A, part II, and B, part II). To examine the possibility that the activation of MAPK by Thal is caused by the action of MEK, PD98059 was added to the cell cultures. In the presence of 100 μM Thal, MAPK phosphorylation again increased within 15 min of Thal treatment, whereas PD98059 blocked Thal-induced Erk phosphorylation (Fig. 9A, part III). The effect of Thal on MAPK phosphorylation was also decreased by GF109203X (Fig. 9A, part III), suggesting a partial involvement of PKC in Thal-induced MAPK activation.

**DISCUSSION**

Inflammatory processes have been reported to be associated with the pathophysiology of AD, and it has been proposed that treatment with NSAIDs reduces the risk for AD (31, 32, 50–53). Since insights into the regulation of APP processing are thought to be crucial in understanding the pathogenesis of AD, we have investigated the action of anti-inflammatory drugs in APP processing and examined the signaling pathways that may mediate their effect. Our data demonstrate that various anti-inflammatory drugs, such as the COX inhibitors Nim, Ibu, or Indo, as well as Thal and its analogue Sup, can modulate the secretion of the non-amyloidogenic α-secretase form (sAPPα) into the conditioned media of SH-SY5Y neuroblastoma and PC12 cells.

Increased sAPPα release, induced by these drugs, was de-
tected by both the monoclonal antibody 22C11, which binds to the N-terminal region of the APP molecule, and the monoclonal antibody 6E10, which recognizes the N-terminal amino acids 1–16 of the /H9252-amyloid fragment of the APP molecule. Since /H9251-secretase cleavage of APP occurs at amino acid 16 within the /H9252-amyloid sequence, this antibody selectively detects the /H9251-secreted APP molecule, suggesting that the anti-inflammatory drugs indeed modulate the release of sAPP that was derived by /H9251-secretase processing. In agreement, a previous study by Kinouchi et al. (54) found that Indo induces sAPP secretion in human glioblastoma cells. These authors assumed the involvement of arachidonic acid, which may be accumulated upon treatment with COX inhibitors and affect PKC activation (54). Likewise, phospholipase A2 (PLA2), the enzyme that releases arachidonic acid from cellular stores, can also influence APP processing, as was demonstrated by increased sAPP secretion

**TABLE II**

<table>
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<th>Drug</th>
<th>Concentration</th>
<th>% of control</th>
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<tr>
<td>Nim</td>
<td>1</td>
<td>131.6 ± 10.4*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>238.3 ± 3.8***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>380.2 ± 5.1***</td>
</tr>
<tr>
<td>Thal</td>
<td>1</td>
<td>155.6 ± 12.6**</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>211.7 ± 6.5***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>247.5 ± 10.0***</td>
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**FIG. 7. Effect of Nim on MAPK activation.** In A and B, PC12 cells (A) or SH-SY5Y neuroblastoma cells (B) were treated for 15 min with various concentrations of Nim. In C, PC12 cells were treated with 100 μM Nim for various time intervals. In D, PC12 cells were preincubated for 15 min with vehicle alone or with 30 μM PD98059 or 1 μM U0126 and then incubated without or with 100 μM Nim for 15 min. In E, PC12 cells were preincubated for 15 min with vehicle alone or with 2.5 μM GF109203X and then incubated without or with 1 μM PMA or 100 μM Nim for 15 min. Phosphorylation of Erk was analyzed in cell lysates and detected with anti-phospho-MAPK (top blots) and anti-MAPK (bottom blots), as described under “Experimental Procedures.”
in response to the PLA₂-stimulating agent, melittin, in a variety of cell lines (8). Inhibiting PLA₂ activity antagonized serotonin- and glutamate-induced sAPP release (55, 56). In addition, Lee and Wurtman (57) recently reported in a preliminary study that neuroimmunophilin ligands and COX inhibitors stimulated sAPP secretion in cultured astrocytes and neurons, suggesting their effect on reducing PG levels and consequently cAMP formation.

The stimulatory effect of the NSAIDs on sAPPα secretion was completely blocked by the hydroxamic acid-based metalloprotease inhibitor, Ro31-9790, further suggesting the involvement of α-secretase in the drug-induced release of sAPP. Hydroxamic acid-based inhibitors, which bind to the essential zinc ion at the active site of the protease, were originally designed as inhibitors of zinc-dependent matrix metalloproteases (58, 59). Moreover, hydroxamate-based compounds, such as Immunex compound 3, batimastat, marimastat, BB2116, Ro31-9790, and KD-1X-73-4, have been shown to inhibit protein ectodomain shedding of several different types of membrane proteins, including APP (42–44, 60). Previously, the use of such a class of inhibitors has allowed the isolation and purification of the TNF-α converting enzyme (TACE), a member of the ADAM (a disintegrin and metalloprotease) subgroup of the metzincin family of proteases (61, 62), and recent reports have implicated TACE or ADAM-17 and ADAM-10 as candidate α-secretase(s) (63–66). Consistent with this, the inhibition or knockout of TACE was shown to decrease the regulated α-secretase cleavage of APP (64). In addition, overexpression of ADAM-10 increased α-secretase cleavage and dominant-negative form of ADAM-10 with point mutation in the zinc-binding site inhibited α-secretase activity (65). Thus, NSAIDs may affect APP metabolism by increasing the α-secretase processing pathway and thereby might be beneficial for the treatment of AD by shifting the balance of APP processing toward a presumably non-pathogenic process. Indeed, previous studies have shown that the proportion of APP processed by α-secretase versus β-secretase may affect the amount of the amyloid fragments; for example, mutations in APP, found in a Swedish familial AD pedigree, map to the β-secretase cleavage site in APP and favor β-secretase cleavage of APP (67, 68). Thus, cells expressing these mutations secrete increased amounts of Aβ as compared with cells expressing wild-type APP. In contrast, activation of PKC by PMA has been shown to favor α-secretase, non-amyloidogenic cleavage at the expense of β-secretase cleavage (69–71). Transgenic mice engineered to produce high levels of Aβ have decreased levels of brain Aβ following PMA treatment, suggesting that stimulation of α-secretase cleavage may be a useful intervention to influence the production of non-detrimental and even beneficial sAPPα and at the same time reduce the relative amounts of Aβ peptides (72).

Moreover, the physiological importance of sAPP as a paracrine neurotrophic/neuroprotective factor was described previously (for review, see Ref. 73). Thus, sAPP stimulates neurite outgrowth (74), regulates synaptogenesis (75), has trophic effects on cerebral neurons in culture (76), stabilizes neuronal calcium homeostasis, and protects hippocampal and cortical neurons against the toxic effects of glutamate and Aβ peptides (77). Therefore, it is more than possible that the protective effects of NSAIDs against AD, as suggested by epidemiological and experimental studies, are not mediated solely by their anti-inflammatory benefits but also by their action on APP processing.

In addition to increasing α-secretase cleavage of APP, treatment of SH-SY5Y cells with Nim, Ibu, Indo, Thal, or Sup also decreased the levels of cellular APP holoprotein relative to control, untreated cells. Consistent with the present study, overexpression of COX-2 stimulated APP mRNA expression and elevated secretion of Aβ1–42, whereas Indo suppressed the production of Aβ by inhibiting APP mRNA levels in transformed NG108-15 cells (40). Moreover, PGE2 stimulated overexpression of APP mRNA and APP holoprotein in primary cultures of cortical astrocytes, and this stimulating effect was inhibited by neuroimmunophilin ligands and NSAIDs (41). Thus, since APP overexpression in cell cultures and in transgenic mice is associated with disorders of the central nervous system and the production of neurotoxic or amyloidogenic APP fragments (78–80), it may be reasonable to suggest that anti-inflammatory agents, inhibitors of PLA₂, or inhibitors of PG synthase would prevent APP overexpression and possibly the pathophysiological processes underlying AD.

Fig. 8. The activation of MAPK by Ibu and Indo. PC12 cells were incubated with various concentrations of Ibu (A, part I) or Indo (B, part I) for 15 min or stimulated with 10 μM Ibu (A, part II) or 10 μM Indo (B, part II) for various time intervals. Phosphorylation of Erk was analyzed in cell lysates and detected with anti-phospho-MAPK (top blots) and anti-MAPK (bottom blots).
Among the mechanisms that regulate proteolytic APP processing, activation of PKC and PKC-coupled receptors was shown to increase the generation of sAPP derived by /H9251-secre-tase cleavage. In addition, it was suggested that the MAPK signaling pathway mediates both PKC and tyrosine kinase receptor regulation of APP catabolism (8). The data presented here demonstrate that sAPP/H9251 release, induced by Nim and Thal, was modulated by inhibitors of PKC and the Erk MAPK signaling pathway. Moreover, in results complementary to the inhibitor studies, we found that the NSAIDs dose dependently increased the immunoreactivity of the phosphorylated MAPK in PC12 and SH-SY5Y neuroblastoma cells. Thus, Western blot analysis, using a phosphospecific MAPK antibody, revealed a time-(maximal response at 15 min) and concentration- (1–100 μM) dependent increase in MAPK phosphorylation in cells stimulated with Nim, Ibu, and Indo. Moreover, the MAPK kinase (MEK) inhibitors, PD98059 or U0126, antagonized MAPK activation, indicating that MEK phosphorylates MAPK in the presence of Nim. Activation of MAPK was also effectively attenuated by the specific PKC inhibitor, GF109203X, which indicates the dependence on the PKC signaling pathway activity.

Thal and its analogue Sup also caused a rapid phosphorylation and activation of Erk1/2 that appears to involve upstream components of the signaling pathway. Taken together, these results indicate that the NSAIDs activate the Erk MAPK cascade and confirm the involvement of MAPK in the effect of NSAIDs on sAPPα release. Our findings are in line with previous data in relation to the involvement of MAPK signaling in sAPPα release. Indeed, PD98059 was shown to antagonize nerve growth factor stimulation of sAPPα release and Erk in PC12 cells. Moreover, exposure to PD98059 or overexpression of a kinase-inactive MEK mutant reduced PKC-mediated effects on APP processing in a variety of cell lines (45, 46).

In summary, our data indicate, for the first time, that the anti-inflammatory drugs Nim, Ibu, Indo, Thal, and Sup stimulate the non-amyloidogenic sAPPα processing and that this may result from their stimulatory effects on MAPK processing in a variety of cell lines. Furthermore, NSAIDs markedly reduce the levels of cellular APP holoprotein, further accelerating non-amyloidogenic processes. Thus, we suggest that NSAIDs and Thal might prove useful to favor non-amyloidogenic APP processing by enhancing α-secretase activity, thereby reducing the formation of amyloidogenic derivatives, and therefore are of potential therapeutic value in AD.
Non-steroidal Anti-inflammatory Drugs Stimulate Secretion of Non-amyloidogenic Precursor Protein
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