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# Activity of α-Secretase as the Common Final Effector of Protein Kinase C-Dependent and -Independent Modulation of Amyloid Precursor Protein Metabolism

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**Abstract:** The metabolic fate of the amyloid precursor protein (APP) is one of the key factors in the pathogenesis of Alzheimer's disease (AD). A complex cellular mechanism regulates the rate at which the precursor is cleaved by α-secretase and released as soluble protein in the extracellular space. We show here that α-secretase constitutes the common final effector of several independent means of stimulation of soluble APP (sAPP) release. The release of sAPP by α-secretase resembles that of several other membrane-bound proteins with soluble counterparts, a process that is sensitive to matrix metalloprotease inhibitors. The hydroxamic acid-based compound KD-IX-73-4 inhibits phorbol ester-mediated sAPP release from COS cells with an $IC_{50}$ of 8 μ*M,* consistent with previous data for the same compound against leukocyte L-selectin shedding. Beyond direct protein kinase C (PKC) activation we show that KD-IX-73-4 inhibits also receptor-mediated sAPP release induced by carbachol in SH-SY5Y cells and by bradykinin in human skin fibroblasts, with the latter being a PKC-independent mechanism. Altogether these data suggest that all pharmacological means of stimulating sAPP release converge to a hydroxamic acid-based inhibitor-sensitive proteolytic enzyme. Moreover, because KD-IX-73-4 was effective in the inhibition of stimulated but not constitutive sAPP release, these data suggest the existence of different enzymes regulating the two metabolic pathways leading to sAPP secretion. **Key Words:** Alzheimer's disease—α-Secretase—Protein kinase C—Amyloid precursor protein—Hydroxamic acids—Metalloprotease inhibitors.
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The hallmark of Alzheimer's disease (AD) brains is the deposition of neuritic plaques whose major component is the β-amyloid protein (Aβ), a self-aggregating peptide of 39–42 amino acids. The peptide is derived from a ubiquitous precursor protein named amyloid precursor protein (APP) (Tanzi et al., 1987; Weidemann et al., 1989).

APP is a type I integral membrane protein that follows the normal constitutive secretory pathway. Cleavage of APP at the N terminus of Aβ by β-secretase and at the C terminus by one or more γ-secretases constitutes the amyloidogenic pathway for processing of APP and leads to the production of Aβ and its release from the cells (Haass et al., 1992; Shoji et al., 1992; Seubert et al., 1993). In addition, APP is cleaved by a proteolytic activity known as α-secretase, releasing the ectodomain into the extracellular space. Proteolytic processing of APP by α-secretase occurs within the sequence of Aβ, thus precluding the formation of the amyloidogenic fragment (Esch et al., 1990; Sisodia et al., 1990; Anderson et al., 1991).

Several integral membrane proteins are known to be released from the lipid bilayer by proteolysis. These include cell adhesion molecules and leukocyte antigens such as leukocyte L-selectin, CD14, and CD43; receptors and ligands such as tumor necrosis factor-α (TNF-α) and its receptors; ectoenzymes such as angiotensin-converting enzyme (ACE); and protein whose function is yet unknown, such as APP (for review, see Hooper et al., 1997).

The enzymes responsible for the cleavage of these proteins are generally named membrane-protein secretases, and they have some features in common. In particular, these enzymes are sensitive to inhibition by hydroxamic acid-based compounds, originally designed as inhibitors of matrix metalloproteases (Spatola et al., 1992; Gearing et al., 1994). These compounds bind at the active site and coordinate the essential zinc ion, but

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*Abbreviations used:* Aβ, β-amyloid protein; ACE, angiotensin-converting enzyme; AD, Alzheimer's disease; APP, amyloid precursor protein; BK, bradykinin; CCh, carbachol; KD-IX-73-4, *N*-[L-[2-(hydroxyaminocarbonyl)methyl]-4-methylpentanoyl]-L-3-(2′-naphthyl)-alanyl-L-alanineamide; PBS, phosphate-buffered saline; PdBu, phorbol 12,13-dibutyrate; PKC, protein kinase C; sAPP, soluble amyloid precursor protein; TACE, tumor necrosis factor-α-converting enzyme; TNF-α, tumor necrosis factor-α.

marked differences exist between the inhibition of the matrix metalloproteases and these secretases. The use of such a class of inhibitors has recently allowed the isolation and purification of the enzyme that regulates TNF-α release [TNF-α-converting enzyme (TACE) (Black et al., 1997; Moss et al., 1997)]. The enzyme responsible for the nonamyloidogenic pathways in AD may also be a member of this family of secretases, and examples of inhibition of α-secretase by hydroxamic acid-based compounds have been described (Arribas et al., 1996; Parvathy et al., 1998*a*,*b*).

Our knowledge of the APP α-secretase pathway has expanded in the past 10 years to unravel a complex cellular mechanism coupled to various extra- and intracellular signals that regulates the rate at which APP is cleaved by α-secretase and released as soluble protein into the extracellular space (reviewed by Checler, 1995), and the activation of such a pathway constitutes, even with its limitations, a key target for the pharmacological modulation of β-amyloid metabolism.

Direct activation of protein kinase C (PKC) by phorbol esters can induce an increase in amount of soluble APP (sAPP) released by cells in culture (Buxbaum et al., 1992; Gillespie et al., 1992; Bergamaschi et al., 1995) and a decrease of Aβ release in the extracellular medium (Buxbaum et al., 1993; Hung et al., 1993; Jacobsen et al., 1994). The nonamyloidogenic pathway can also be stimulated by G protein-coupled cell surface receptor through the activation of complex signal transduction pathways that include PKC-dependent and -independent mechanisms (Nitsch and Growdon, 1994; Checler, 1995; Nitsch et al., 1995).

In the present study we have examined the effect of KD-IX-73-4, a hydroxamic acid-based inhibitor, described and characterized toward the activity of L-selectin shedding enzyme in activated human neutrophils (Feehan et al., 1996), on APP α-secretase processing in different cell lines and with different stimuli. Here we establish that both α-secretase and the L-selectin "sheddase" have similar sensitivity to inhibition by KD-IX-73-4, suggesting at least a mechanistic homology between the two enzymes. Moreover, we demonstrate that both PKC-dependent and -independent pathways leading to sAPP release converge on the same proteolytic enzyme, which therefore becomes the limiting step for different pharmacological means of stimulation of the nonamyloidogenic processing of APP.

## MATERIALS AND METHODS

### Materials

All culture media, fetal calf serum, and supplements were obtained from GIBCO Life Technologies (Paisley, U.K.). Electrophoresis reagents were obtained from Bio-Rad (Hercules, CA, U.S.A.). KD-IX-73-4 {*N*-[L-[2-(hydroxyaminocarbonyl)-methyl]-4-methylpentanoyl]-L-3-(2′-naphthyl)alanyl-L-alanine-amide}was a kind gift of Dr. Takashi Kei Kishimoto of Boehringer Ingelheim (U.S.A.). All other reagents were of the highest grade available and were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) unless otherwise specified.

### Cell cultures and experimental treatments

Primary human skin fibroblasts, obtained from the Cell Repository at the IRCCS Centro S. Giovanni di Dio-FBF, Brescia, Italy, and COS-1 cell lines were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum, penicillin/streptomycin, nonessential amino acids, and Tricine buffer (20 m*M*, pH 7.4) at 37°C in 5% $CO_2$/95% air. The neuronal cell line SH-SY-5Y was cultured in the same medium supplemented with 1 m*M* pyruvate. Confluent monolayers of cells were washed twice with phosphate-buffered saline (PBS) and once with serum-free culture medium before treatments. Phorbol 12,13-dibutyrate (PdBu) and KD-IX-73-4 were dissolved in dimethyl sulfoxide as stock solutions of, respectively, 5 m*M* and 20 mg/ml. Carbachol (CCh) was dissolved and diluted to the working concentration in serum-free Eagle's minimum essential medium at the time of use. Bradykinin (BK) was prepared as stock solution (1 m*M*) in PBS. Controls receiving vehicle alone were included in all experiments. Cells were preincubated for 10 min at room temperature with KD-IX-73-4 and then stimulated with PdBu, CCh, or BK for 2 h at 37°C.

### Harvesting of cells and preparation of conditioned medium

Conditioned medium was collected after 2 h of incubation and centrifuged at 13,000 *g* for 5 min to remove detached cells and debris. Proteins in the medium were quantitatively precipitated by the deoxycholate/trichloroacetic acid procedure as previously described (Bergamaschi et al., 1995). Cell monolayers were washed twice with ice-cold PBS, lysed on the tissue culture dish by addition of ice-cold lysis buffer [50 m*M* Tris-HCl (pH 7.5), 150 m*M* NaCl, 5 m*M* EDTA, and 1% Triton X-100], and scraped with a rubber spatula. An aliquot of the cell lysate was used for protein analysis with the Bio-Rad Bradford kit for protein quantification.

### Immunodetection of APP

Normalization of protein loading on each blot was obtained by loading an equal volume of samples of conditioned medium standardized to total cell lysate protein concentration. Proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10% gel) and then transferred to a polyvinylidene difluoride membrane (NEN-Du Pont). For the detection of the secreted APP, either monoclonal antibody 22C11 (Boehringer-Mannheim) or 6E10 (Senetek) was used, and the blots were incubated overnight at 4°C. Detection was carried out by incubation with alkaline phosphatase-conjugated goat anti-mouse IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD, U.S.A.) for 1 h. The blots were then washed extensively, and sAPP was visualized using an enhanced chemiluminescence method (NEN). The same immunoreactive bands detected by antibody 22C11 were detected in western blots by antibody 6E10 and were also immunoprecipitated by antiserum ER3β1–16 (M.R., unpublished data). Both 6E10 and ER3β1–16 recognize epitopes in the first 16 amino acids of Aβ, 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.

Cell lysates (10 μg of total protein per lane) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by western blot with antibody 22C11 for the identification of steady-state levels of cellular APP.

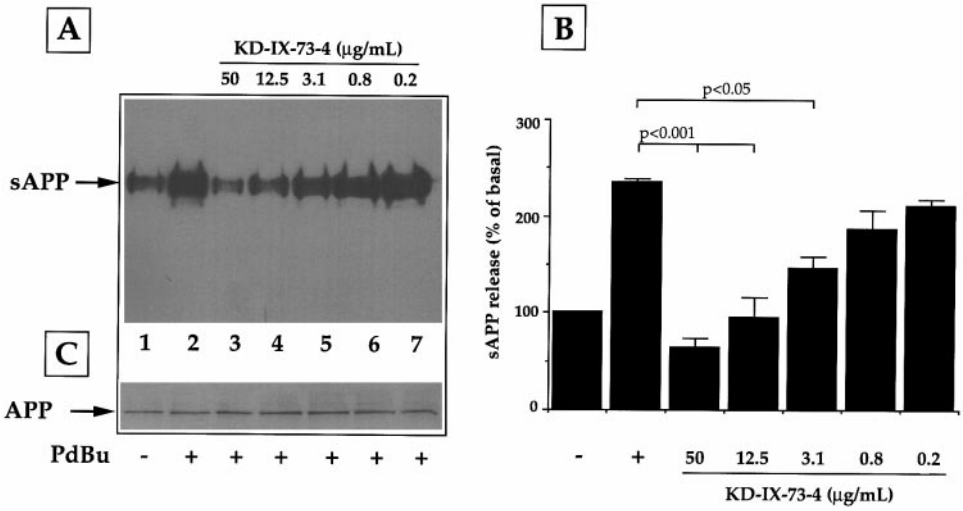


**FIG. 1.** Effect of KD-IX-73-4 on PdBu-induced sAPP release from COS cells. **A:** Cells were preincubated for 10 min with vehicle alone (lanes 1 and 2) or with the indicated concentrations of KD-IX-73-4. Following the preincubation time the cells were treated with 200 n*M* PdBu (except lane 1) for 2 h at 37°C. Proteins released into the conditioned medium were collected and subjected to western blot with sAPP-specific antibodies. **B:** Densitometric analysis of western blots expressed as percentages of basal release (vehicle treatment) and as mean ± SEM (bars) values of three independent experiments. **C:** Levels of intracellular steady-state APP determined with antibody 22C11 on 10 μg per lane of total lysate protein.

### Densitometry and statistics

Western blot analysis was performed by calculating the relative intensity of the immunoreactive bands after acquisition of the blot image through a Nikon CCD videocamera module and analysis by means of the Image 1.47 program (Wayne Rasband, Research Service Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, U.S.A.). The relative densities of the bands were expressed as arbitrary units and normalized to data obtained from the control sample assayed under the sample conditions. Controls were processed in parallel with stimulated samples and always included in the same blot. Preliminary experiments with serial dilutions of secreted protein allowed determination of the optimal linear range for the chemiluminescence reaction. Statistical analysis was done by one-way ANOVA followed, when significant, by two-tailed Student's $t$ test or multiple-comparison test, where appropriate; a value of $p < 0.05$ was considered significant.

## RESULTS

We examined the effect of KD-IX-73-4, a hydroxamic acid metalloprotease inhibitor, on the activity of α-secretase in three different cell types and following different secretory stimuli.

First we investigated the effect of the inhibitor on phorbol ester-mediated sAPP release from COS cells. Soluble α-secretase-derived APP is released into the conditioned medium of COS cells as a protein with an apparent molecular size of 120 kDa (Fig. 1A). The release of sAPP was stimulated in a concentration-dependent manner following treatment of COS cells with PdBu (data not shown). At a concentration of 200 n*M* PdBu the treatment elicited an increase of sAPP release of approximately twofold that of basal levels (135%). The same concentration was used to determine the inhibitory profile of KD-IX-73-4 on sAPP release. Cells were preincubated for 10 min with KD-IX-73-4 at concentrations ranging from 0.2 to 50 μg/ml (Fig. 1) and subsequently either with vehicle or with 200 n*M* PdBu. Western blot analysis demonstrated that the simultaneous treatment with KD-IX-73-4 produced an inhibition of sAPP release mediated by direct PKC activation by PdBu, inhibition that is dependent on the concentration of the compound. The maximal inhibition was obtained for a concentration of KD-IX-73-4 of 50 μg/ml, corresponding to ~100 μ*M*. At such a concentration the peptide inhibitor reduced the PdBu-mediated sAPP release to basal levels but, even at higher concentrations (data not shown), did not inhibit further the constitutive level of sAPP release from COS cells. We estimated the $IC_{50}$ of KD-IX-73-4 to be ~8 μ*M*, which is in the same order of magnitude of the $IC_{50}$ estimated in experiments on neutrophils versus the L-selectin sheddase (Feehan et al., 1996). We next turned to two other cellular models with the purpose of studying the effect of the inhibitor on receptor-mediated sAPP release.

Receptor binding studies have shown that SH-SY5Y cells express muscarinic receptors of the m1 and m3 subtype (Serra et al., 1988; Lambert et al., 1989). Treatment with increasing concentrations of CCh results in a concentration-dependent increase in sAPP release from these cells with a maximally effective concentration of 1 m*M* (data not shown). Western blots show that sAPP is released into the conditioned medium as a doublet with an apparent molecular mass of 100–120 kDa (Fig. 2A). The protein pattern is consistent with other previous reports (Lahiri et al., 1994; Parvathy et al., 1998*a*) and likely reflects the expression of the three major isoforms

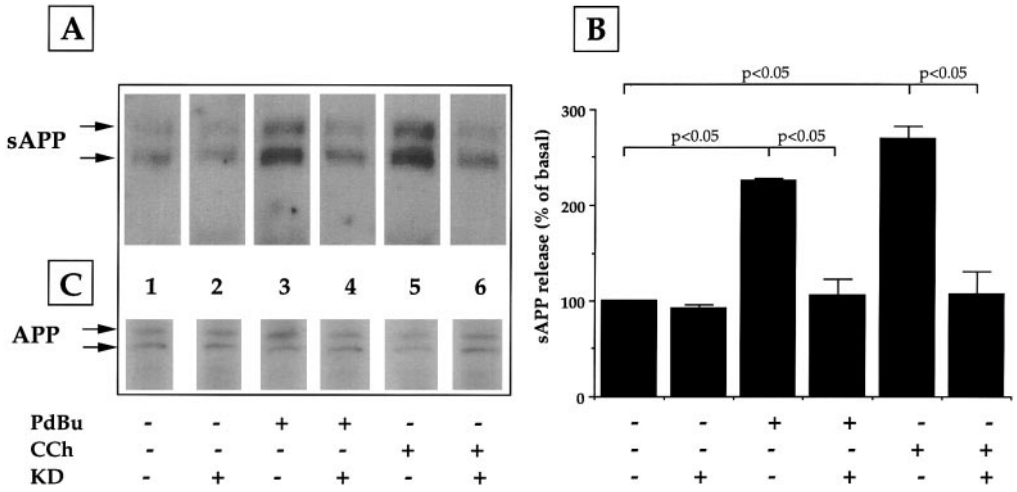


**FIG. 2.** Inhibition of carbachol-induced sAPP release by KD-IX-73-4 (KD) in SH-SY5Y neuroblastoma cells. **A:** Cells were preincubated for 10 min with vehicle alone (lanes 1, 3, and 5) or with 50 μg/ml KD. Following the preincubation time the cells were treated with 200 n*M* PdBu (lanes 3 and 4) or 1 m*M* CCh (lanes 5 and 6) for 2 h at 37°C. Proteins released into the conditioned medium were collected and subjected to western blot with sAPP-specific antibodies. **B:** Densitometric analysis of western blots expressed as percentages of basal release (vehicle treatment) and as mean ± SEM (bars) values of three independent experiments. **C:** Levels of intracellular steady-state APP determined with antibody 22C11 on 10 μg per lane of total lysate protein.

of APP, i.e., the two KPI-containing isoforms and the 695-amino acid isoform (M.R., unpublished data).

Cells were treated for 2 h with 200 n*M* PdBu in the presence or absence of KD-IX-73-4 at a concentration of 50 μg/ml, as inferred from the previous experiments. Following the same experimental scheme cells were treated with 1 m*M* CCh in the presence or absence of 50 μg/ml KD-IX-73-4. As shown before with COS cells the treatment with PdBu elicited an increase of sAPP release of approximately twofold over basal levels. Similar results were obtained with 1 m*M* CCh. The inhibitory effect of KD-IX-73-4 at the concentration of 50 μg/ml was confirmed also in the neuronal cellular model (Fig. 2), where the inhibitor was able to block completely the effect of CCh and PdBu, returning the level of sAPP release to basal values. As seen before with COS cells, constitutive release of sAPP was not affected by the treatment with KD-IX-73-4.

The third cellular model considered for our studies was human skin fibroblasts. These cells express endogenously BK receptors of the B2 subtype (Tippmer et al., 1994; Racchi et al., 1998). The treatment of these cells with BK results in a concentration-dependent increase of sAPP level with a maximal effect of BK obtained at a concentration of 1 μ*M* and resulting in an increase of sAPP level over basal values of 1.5 to twofold (Racchi et al., 1998). We have also shown that the effect of BK in this cellular system is not dependent on activation of PKC (Racchi et al., 1998). Therefore, this cellular model and secretory stimulus are an example of PKC-independent receptor-mediated activation of the α-secretase pathway. Using a treatment protocol similar to those detailed before, fibroblasts were treated for 2 h with 1 μ*M* BK. At the same time the cells were also treated with 200 n*M* PdBu as a positive control. The treatments were repeated also in the presence of KD-IX-73-4 at the concentration of 50 μg/ml. Figure 3 shows that, as found in COS cells and in SH-SY5Y cells, the treatment with the inhibitor restores to basal levels the release of sAPP following direct stimulation of PKC. It is interesting that the PKC-independent secretory effect of BK is blocked by addition of KD-IX-73-4, with a complete inhibition of stimulated sAPP release. Finally, and consistent with the results obtained with the other cell lines, we did not observe inhibition of constitutive release of sAPP from human skin fibroblasts following KD-IX-73-4 treatment.

Finally, in all cellular systems none of the treatments altered grossly the steady-state levels of cellular APP (Figs. 1C, 2C, and 3C).

## DISCUSSION

Increasing evidence suggests that α-secretase, possibly the limiting enzyme in the nonamyloidogenic processing of APP, may be part of a family of proteases, named generally secretases or sheddases (Hooper et al., 1997), that release soluble ectodomains of integral membrane proteins. We provide evidence here that the secretory processing of APP is sensitive to inhibition by the hydroxamic acid-based compound KD-IX-73-4, previously shown to be an effective inhibitor of L-selectin shedding in human neutrophils (Feehan et al., 1996). Our experiments with COS cells indicate that the inhibition of phorbol ester-mediated sAPP release obtained using KD-IX-73-4 is dependent on the concentration of the compound, and the results are remarkably similar to those obtained by Feehan et al. (1996) studying L-selectin shedding in human neutrophils. The $IC_{50}$ calculated in the latter experiments ranged from 4.5 to 3 μ*M*, depending on the experimental model used, whereas in

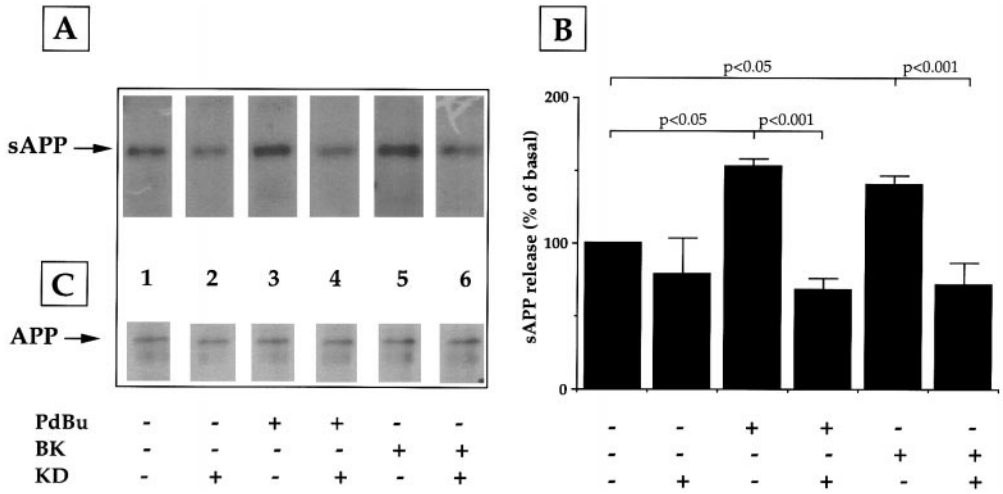


**FIG. 3.** Inhibition of bradykinin-induced sAPP release by KD-IX-73-4 (KD) in human skin fibroblasts. **A:** Cells were preincubated for 10 min with vehicle alone (lanes 1, 3, and 5) or with 50 $\mu$g/ml KD. Following the preincubation time the cells were treated with 200 n*M* PdBu (lanes 3 and 4) or 1 $\mu M$ BK (lanes 5 and 6) for 2 h at 37°C. Proteins released into the conditioned medium were collected and subjected to western blot with sAPP-specific antibodies. **B:** Densitometric analysis of western blots expressed as percentages of basal release (vehicle treatment) and as mean ± SEM (bars) values of three independent experiments. **C:** Levels of intracellular steady-state APP determined with antibody 22C11 on 10 $\mu$g per lane of total lysate protein.

our case we estimated an $IC_{50}$ of 8 $\mu M$, suggesting a high degree of similarity between $\alpha$-secretase and the L-selectin shedding enzyme. The use of other inhibitors in different experimental conditions has also suggested similarity between ACE secretase and $\alpha$-secretase. Similar potencies have been described for the effect of batimastat and other related compounds comparing ACE secretase inhibition and $\alpha$-secretase inhibition (Parvathy et al., 1998*a*,*b*). Thus, our data provide further compelling evidence that these proteolytic enzymes/activities are mechanistically related. It seems more likely that they are not the same protease but belong to a large family of related enzymes with distinct limited cleavage specificity. Recent data show that potencies of some hydroxamic acid-based compounds toward TACE differ 2 orders of magnitude compared with the activity toward $\alpha$-secretase, suggesting that the two enzymes may be distinct (Parvathy et al., 1998*b*). On the other hand, it has also been demonstrated that PKC-stimulated cleavage of APP is defective in knockout cells for the TACE gene (Buxbaum et al., 1998; Merlos-Suarez et al., 1998), thus suggesting that $\alpha$-secretase and TACE may be the same enzyme. It is, moreover, important to note that basal formation and release of sAPP are not affected by the TACE knockout, supporting the existence of distinct enzymes regulating constitutive and stimulated sAPP release, with the latter being possibly identical to TACE (Buxbaum et al., 1998).

In our experimental system we never observed inhibition by KD-IX-73-4 below the basal levels for all three cell lines studied and for all of the treatments. These observations are partially in disagreement with data obtained with batimastat and related compounds (Parvathy et al., 1998*a*); however, they are consistent with the interpretation of the data obtained by Buxbaum et al. (1998). Two different (although possibly related) enzymes may therefore play roles in the constitutive and regulated secretion of sAPP, and the lack of an inhibitory effect of KD-IX-73-4 on sAPP constitutive secretion may derive either from a differential access to the site(s) of activity of the constitutive and activated enzyme or a difference of specificity of the compound for the two related enzymes. The existence of different enzymes or different subcellular localization of the same enzyme was inferred by the studies of Marambaud et al. (1997) that demonstrated the differential effect of proteasome inhibitors on the constitutive and phorbol ester-stimulated sAPP release. We indirectly proposed a distinction between constitutive and regulated sAPP release following the demonstration that a defective PKC activity in fibroblasts from AD patients (Govoni et al., 1993) was correlated with a reduced constitutive release of sAPP (Bergamaschi et al., 1995). We subsequently demonstrated that specific inhibition of PKC$\alpha$ could result in complete abolishment of PdBu-mediated sAPP release but that only 30–40% of constitutive secretion was affected by the treatment, suggesting therefore that the constitutive secretion was partially independent from PKC$\alpha$ (Benussi et al., 1998). Our current results contribute to the demonstration that different enzyme activities (with different sensitivity to inhibitors) are involved in the constitutive and regulated secretion of sAPP.

One of the common features of the activity of secretases is that it can be up-regulated by phorbol esters, and this is true concerning the processing of many of the protein substrates used as examples, including APP (Buxbaum et al., 1990; Arribas et al., 1996; Hooper et al., 1997). In addition, concerning specifically APP, large numbers of experiments on cells in culture have demonstrated that cell surface receptors for neurotransmitters

(Nitsch et al., 1992) and peptides (Nitsch et al., 1998; Racchi et al., 1998) can accelerate the processing and release of sAPP and that the rate of formation of Aβ appears to be inversely related to the release of sAPP (Hung et al., 1993; Jacobsen et al., 1994). Assuming the excess of β-amyloid production is a major player in the pathogenesis of AD, then the pharmacological modulation of APP metabolism becomes one of the key targets in the search for a therapeutic tool. All of these strategies have the ultimate goal to modulate APP metabolism toward an increased production of soluble α-secretase-derived APP and a consequent reduction of β-amyloid production.

Modulation by muscarinc agonists is one of the examples of receptor-mediated sAPP release (Nitsch et al., 1992; Farber et al., 1995; Salvietti et al., 1996). We have chosen to study the effect of CCh in a neuronal model expressing endogenously muscarinic receptors. The effect of CCh stimulation in the SH-SY5Y cells was similar to that of direct activation of PKC by phorbol esters, and KD-IX-73-4 was able to inhibit equally the effect of PdBu and the effect of CCh. These observations demonstrate that a pharmacological stimulus such as CCh acting through a receptor and activating a cascade of second messengers ultimately leads to a pathway that involves cleavage of the APP substrate by α-secretase. It is important to note that although PdBu is a direct activator of PKC, the effect of CCh is not completely explained by the sole activation of PKC. In fact, it is known that PKC is necessary but not sufficient to sustain the secretory activity of cholinergic compounds (Buxbaum et al., 1994; Slack et al., 1995). Because of these complex interactions between intracellular signaling systems and APP secretion, we investigated another cellular system/receptor-coupled APP metabolism, that is, BK-induced sAPP release from human skin fibroblasts. We have previously shown that the effect of BK on sAPP secretion from fibroblasts is independent of PKC stimulation (Racchi et al., 1998). In our current study it is clear also that PKC-independent pathways converge on an enzyme sensitive to the hydroxamic acid-based inhibitors KD-IX-73-4, most likely α-secretase. Discussing the PKC-mediated sAPP release investigators have always included the α-secretase as one of the possible target substrates. Our data suggest that the actual proteolytic enzyme may be more downstream than the initial kinase substrates, as it would appear from the complex and diverse signal transduction messengers involved.

Although in most cell types the activation of sAPP secretion is often associated with a reduction in Aβ release, we were not able to measure the release of Aβ from the cell lines used in the same experimental paradigm for the detection of sAPP. As an example, the detection of Aβ released from cultured skin fibroblasts requires a completely different experimental design with incubation intervals of >24 h (M.R., unpublished data). Prolonged treatments with agonists such as PdBu, CCh, or BK may also have effects on APP expression that need to be controlled following the design of the appropriate experimental approach.

In summary, our study clearly identifies the α-secretase activity sensitive to KD-IX-73-4 as the common final effector of PKC-dependent and -independent pharmacological stimulation of sAPP release. The differential effect on constitutive and stimulated sAPP release awaits a more detailed characterization, yet it could be envisaged that normal levels of constitutive sAPP release may be sufficient to maintain the putative neurotrophic and/or neuroprotective functions of APP. Therefore, the development of a selective compound with lack of activity against the constitutive pathway may reduce concerns raised for its chronic therapeutic use.

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