INSULIN-DEGRADING ENZYME RAPIDLY REMOVES THE
THE β-AMYLOID PRECURSOR PROTEIN INTRACELLULAR
DOMAIN (AICD)

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Running title: IDE degrades the APP intracellular domain

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Abbrevations: Aβ, amyloid β-peptide; AD, Alzheimer’s disease; APP, β-amyloid precursor protein; AICD, APP intracellular domain; insulin-degrading enzyme, IDE; NICD, Notch intracellular domain; PS, presenilin

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The intramembraneous $\gamma$-secretase cleavage of the $\beta$-amyloid precursor protein (APP) is dependent on biologically active presenilins (PS). Notch also undergoes a similar PS dependent $\gamma$-secretase-like cleavage resulting in the liberation of the Notch intracellular domain (NICD), which is critically required for developmental signal transduction. $\gamma$-Secretase processing of APP results in the production of a similar fragment called AICD (APP intracellular domain), which may function in nuclear signaling as well. AICD like NICD is rapidly removed. By using a battery of protease inhibitors we demonstrate that AICD, in contrast to NICD, is degraded by a cytoplasmic metalloprotease. In vitro degradation of AICD can be reconstituted with cytoplasmic fractions obtained from neuronal and non-neuronal cells. Taking into account the inhibition profile and the cytoplasmic localization we identified three candidate enzymes (neurolysin, thimet oligopeptidase, and insulin-degrading enzyme (IDE), also known as insulysin), which all are involved in the degradation of bioactive peptides in the brain. When insulin, a well-characterized substrate of IDE was added to the in vitro degradation assay removal of AICD was efficiently blocked. Moreover, overexpression of IDE resulted in enhanced degradation of AICD, whereas overexpression of the inactive IDE E111Q mutant did not affect AICD degradation. Finally, immunodepletion of IDE significantly reduced the AICD degrading activity. Therefore our data demonstrate that IDE, which is one of the proteases implicated in the removal of extracellular Aβ also removes the cytoplasmic product of $\gamma$-secretase cleaved APP.
INTRODUCTION

Current evidence strongly implicates that aggregation and deposition of Amyloid β-peptide (Aβ) in the brains of Alzheimer's disease (AD) patients is an invariant pathological feature (1). Aβ is generated from the β-amyloid precursor protein (APP) by endoproteolytic processing. Two sequential cleavages, first by β-secretase and then followed by γ-secretase, are required to liberate Aβ (for review see (2,3)). The intramembrane γ-secretase cleavage is dependent on biologically active presenilins (PS) that may be unusual aspartyl proteases (4,5), that probably constitute the active site of γ-secretase (for review see (2,3)). PSs are not only involved in proteolytic processing of APP but also in a similar intramembranous cut of Notch (6). Notch, like APP, is a type I transmembrane protein that undergoes very similar endoproteolytic processing pathways (for review see (2,3)). After ectodomain shedding, Notch is cleaved within or close to its TM domain by the γ-secretase-like S3 protease activity. The S3 cut finally liberates the Notch intracellular domain (NICD), a key molecule in developmental signal transduction (7). Cleavage of Notch at S3 is PS dependent and can be blocked by γ-secretase inhibitors (6). Consistent with a role of PSs in Notch signaling, the PS1/PS2 double knockout in mice has a phenotype similar to that of Notch1-/- mice (8), and Notch cleavage is completely inhibited in PS deficient cells (9,10). The requirement of PSs in both, NICD formation and γ-secretase cleavage of APP provided the basis for the hypothesis that the γ-secretase generated cytoplasmic domain of APP may also carry out an important function in nuclear signaling. Recently, several reports (11-15) described the γ-secretase
generated cytoplasmic fragment of APP, which we termed AICD (APP intracellular domain) (13) in analogy to NICD. AICD is generated by a PS dependent mechanism, since a PS1 gene knock out as well as a "dominant negative" PS1 mutations (D385N) inhibited AICD generation (13). Moreover, γ-secretase inhibitors, including ones that are known to bind to PSs, also blocked AICD generation in vivo and in vitro (12,13,15). Interestingly, the N-terminus of AICD is predominantly generated by a cut between amino acids 49 and 50 of the β-amyloid domain and not by the expected γ-secretase cut after amino acids 40 or 42 (13-15). This cleavage releases in vivo a 50 amino acid long AICD fragment into the cytoplasm but not the predicted 57 or 59 amino acid fragment. Sequence comparison revealed that this cleavage occurs at a very similar position as the S3 cleavage of Notch (13-15). Taken together, these analogies between APP and Notch processing may favor the idea that AICD, like NICD has a physiological function in nuclear signal transduction. Indeed, recent findings with highly sensitive reporter gene assays supported the hypothesis that AICD may be involved in gene transcription (16). However, AICD alone was not sufficient to allow the detection of a significant transcriptional activity in the reporter gene assays. Only upon co-transfection of Fe65, a nuclear adaptor protein that has been shown to bind to the APP cytoplasmic tail (17-19), a significant transcriptional activity was observed (16). This may be related to the observation that the recombinant 59 amino acid AICD like molecules are stabilized by the co-expression of Fe65 (20), although the latter has not been confirmed by others (21).

If AICD plays a role in signal transduction, its activity may be regulated. In the case of NICD, a ubiquitin dependent proteasomal degradation pathway prevents NICD
accumulation and constitutive signaling (6,22,23). AICD is also rapidly degraded but apparently by a protease activity different from the proteasome (12,21). We therefore searched for the protease activity involved in AICD degradation. Surprisingly, we found that AICD can be efficiently degraded by insulin-degrading enzyme (IDE), a thiol-dependent metalloprotease known to degrade insulin, glucagon and other peptide hormones (24). Interestingly, IDE is also implicated in the clearance of extracellular Aβ (for review see (25)). Our data therefore suggest that therapeutic stimulation of IDE activity to remove Aβ will also affect AICD metabolism and its potential function in nuclear signal transduction.
EXPERIMENTAL PROCEDURES

Cell lines and cell culture

Human embryonic kidney 293 cells (HEK293) were cultured in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycine, 200 µg/ml G418 (to select for APP expression) and 200 µg/ml zeocin (to select for IDE expression). HEK293 cells stably expressing rat IDE variants were generated by transfection of HEK293 cells stably expressing APP<sub>695</sub> containing the Swedish mutation (swAPP) (26). Mouse neuroblastoma N2a cells stably expressing swAPP were cultured as described (27).

Protease inhibitors

Protease inhibitors were used at the following final concentrations in the<em> in vitro</em> assays for AICD degradation: protease inhibitor mix (PI-mix) with or without EDTA (1x Complete, Roche), DAPT (1 µM, gift from Boehringer Ingelheim KG), EDTA (5 mM, Sigma), 1,10-<em>o</em>-phenanthroline (PNT, 5 mM, Sigma), phosphoramidon (100 µM, Calbiochem), bestatin (200 µM, Calbiochem),<em> clasto</em>-lactacystin β-lactone (10 µM, Sigma), MG132 (50 µM, Calbiochem), N-ethylmaleimide (NEM, 0.1-10 mM, Sigma), dynorphin A-(1-13) (0.5 mM, Bachem), Pro-Ile (5 mM, Bachem), cFP-AAY-pAB (cFP, 100 µM, gift from Ian Smith), insulin (2-250 µg/ml, Sigma), zincov (100 µM, Calbiochem), E64 (50 µM, Roche), PMSF (1 mM, Sigma), leupeptin (1 µg/ml, Roche), aprotinin (2 µg/ml, Roche), antipain (50 µg/ml, Roche), and pepstatin (2 µg/ml, Calbiochem).
cDNA constructs

A rat IDE cDNA (gift from R. Roth) was cloned into pcDNA3.1/zeo(+) (Invitrogen). The nonfunctional IDE E111Q mutant was generated by PCR mediated mutagenesis using appropriate primers.

Antibodies

The polyclonal antibody 6687 to the 20 C-terminal amino acids of APP (5) and the monoclonal antibody 9B12 to human IDE (28) have been described. Antibody 9B12 also recognizes rat IDE.

AICD generation/degradation in vitro

To characterize the protease activity involved in AICD degradation we used a previously established in vitro assay (13). HEK293 cells were resuspended (0.5 ml/10 cm dish) in ice-cold hypotonic homogenization buffer (10 mM MOPS pH 7.0, 10 mM KCl, without protease inhibitors) and incubated on ice for 10 min. Following homogenization on ice with a tight fitting douncer (30 strokes) a post nuclear supernatant (PNS) was prepared by centrifugation at 1000g for 15 min at 4°C. Crude membranes were isolated from the post-nuclear supernatant (PNS) by centrifugation at 16, 000g for 40 min at 4°C. The membranes were then resuspended (50 µl/10 cm dish) in assay buffer (150 mM sodium citrate pH 6.4, supplemented with the indicated protease inhibitors) and AICD was generated by incubation of samples at 37°C for 2 h in a volume of 25 µl/assay. After termination of the assay reactions on ice, samples were separated into pellet (P100) and supernatant (S100) fractions by ultracentrifugation for 1 h at 100, 000g at 4°C. The S100 fraction was separated by SDS-
PAGE on 10-20% Tris-Tricine gels (Invitrogen) or 16.5% Tris-Tricine gels (29) and analyzed by immunoblotting with antibody 6687 and detection using enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech).

**Alkaline extraction of membranes**

Crude membranes prepared as above were resuspended (0.5 ml/10 cm dish) in 100 mM Na$_2$CO$_3$ pH 11.0 (30) and incubated on ice for 15 min. After ultracentrifugation for 1 h at 100,000g at 4°C and two consecutive washes in assay buffer, carbonate extracted membranes were resuspended (50 µl/10 cm dish) in assay buffer and analyzed for AICD degradation as above.

**Preparation of cytosol**

Cytosol was prepared from the indicated cell lines by ultracentrifugation of the PNS fraction at 100,000g for 1 h at 4°C. The soluble S100 fraction was used as cytosol.

**Reconstitution of AICD degradation in vitro**

Cytosol-free membranes were generated by three consecutive washes of membranes with assay buffer and centrifugation at 100,000g for 30 min at 4°C. Cytosol-free membranes were then resuspended in assay buffer (20 µl/assay) and mixed with aliquots (5 µl) of cytosol fractions containing the indicated protein amounts. AICD was then generated as described above. In assays with insulin, AICD was first generated from cytosol-free membranes as described above. After ultracentrifugation, membrane-free S100 fractions containing preformed AICD were mixed with cytosol preparations supplemented with the indicated amounts of insulin and assayed for AICD degradation as above.
Immunodepletion of IDE

IDE depleted cytosol preparations were generated by immunoprecipitation for 2 h at 4°C using excess amounts of antibody 9B12 that was prebound with rabbit anti-mouse antibody (Sigma) to protein A sepharose. As a control a mock immunoprecipitation lacking antibody 9B12 was carried out with rabbit anti-mouse antibody prebound to protein A sepharose.
RESULTS

AICD is rapidly degraded by a metalloprotease activity

We and others have previously demonstrated that AICD can efficiently be generated in vitro in a γ-secretase and PS-dependent manner from crude membrane fractions prepared by centrifugation at 10,000g - 16,000g (11-14). However, the in vitro generated AICD is extremely rapidly degraded and the addition of a protease inhibitor mix containing EDTA is necessary to stabilize AICD (11,12). To identify the protease involved in this extremely rapid degradation pathway, the in vitro assay was carried out in the presence of a variety of protease inhibitors (Fig. 1). In the absence of any inhibitor, no AICD could be detected, demonstrating that at 37°C AICD degradation is even faster than de novo synthesis. Consistent with previous results a general protease inhibitor mix including EDTA inhibited AICD degradation. The additional presence of the γ-secretase inhibitor DAPT inhibited AICD generation as expected (13). Interestingly, the protease inhibitor mix without EDTA did not block AICD degradation suggesting the involvement of a divalent metal ion-dependent proteolytic activity in AICD generation. Indeed, the metal-chelators EDTA and 1,10 o-phenanthroline (PNT) allowed robust accumulation of AICD when used either alone or in combination. However, the more specific metalloprotease inhibitors phosphoramidon and bestatin, an inhibitor of aminopeptidases, did not inhibit degradation of AICD. AICD degradation was not affected by inhibitors of cysteine proteases (E64, leupeptin), serine proteases (PMSF) and aspartyl proteases (pepstatin) (data not shown). Interestingly, the
potent proteasome inhibitors clasto-lactacystin β-lactone and MG132 did also not block AICD degradation (Fig. 1). This is in contrast to NICD that has been shown to be rapidly degraded by a ubiquitin dependent proteasomal degradation pathway (6,22,23). Thus, although AICD and NICD are both generated by a PS-dependent protease, they are degraded by different proteolytic activities. Taken together, we conclude that most likely a metalloprotease is involved in AICD degradation.

**AICD is degraded by a cytoplasmic protease**

We next investigated whether the metalloprotease activity involved in AICD degradation is cytosolic or membrane bound. We first assayed crude membranes for AICD degradation after alkaline extraction with Na\(_2\)CO\(_3\) that separates peripheral from integral membrane proteins (30). As shown in Fig. 2a, AICD could be recovered in the absence of PNT/EDTA from Na\(_2\)CO\(_3\) extracted membranes (Fig. 2a) suggesting that the AICD degrading activity is either peripherally attached to the membrane or soluble. In addition, these data confirm previous findings that γ-secretase is integrally membrane bound (11). We next separated crude membranes from residual cytosol by additional centrifugation. As shown above, when crude membranes were used in the *in vitro* assay, AICD could not be recovered in the absence of PNT/EDTA (Fig. 2b) due to its rapid degradation. In contrast, when crude membranes were used after additional re-centrifugation at 100, 000g, robust accumulation of AICD was observed in the absence of PNT/EDTA (Fig. 2b). Thus, these data suggest that the AICD degrading activity is a soluble protease that is primarily located within the cytoplasm.
To further prove the cytoplasmic localization of the AICD degrading activity the *in vitro* degradation assay was performed with washed cytosol-free membranes, which allowed robust accumulation of AICD in the absence of PNT/EDTA. The degrading activity could then be reconstituted by the addition of increasing amounts of cytosol from HEK293 or mouse neuroblastoma N2a cells (Fig. 2c). From these results we conclude that a cytoplasmic metalloprotease present in peripheral as well as in neuronal cells is involved in AICD degradation.

**Identification of candidate enzymes responsible for AICD degradation**

Based on the inhibition profile and the cytoplasmic localization of the AICD degrading activity we searched the MEROPS protease database (http://www.merops.co.uk) for candidate AICD degrading enzymes. This search revealed that three cytoplasmic candidate proteases from two metalloprotease families (M3A and M16A families) could be responsible for the degradation of AICD: thimet oligopeptidase (TOP, M3A family), neurolysin (M3A family) and insulin-degrading enzyme (IDE, insulysin, M16A family). Consistent with these candidate proteases being thiol-group dependent (31,32), the SH-alkylating agents N-ethylmaleimide (NEM) strongly inhibited AICD degradation (Fig. 3a). Next, to discriminate which of these proteases could be involved in AICD degradation, the *in vitro* assay was carried out in the presence or absence of dynorphin A-(1-13), a known inhibitor of TOP and neurolysin (31) and potentially also of IDE. The dipeptide Pro-Ile, a highly specific neurolysin inhibitor (31), cFP-AAY-pAB (cFP), a potent TOP and less potent
neurolysin inhibitor (33) and insulin, a well-characterized substrate of IDE, that functions as competitive inhibitor, were investigated as well (24). As shown in Fig. 3b, weak inhibition of AICD degradation by dynorphin A-(1-13) suggests either TOP or neurolysin as a candidate protease without excluding IDE. However, Pro-Ile did not block AICD degradation ruling out neurolysin as a candidate protease (Fig. 3b). Since cFP did also not block AICD degradation (Fig. 3b), IDE remained the only candidate for AICD degradation. Indeed, insulin competed for the AICD degrading activity present in cytosol derived from N2a cells in a dose-dependent manner (Fig. 3c). BSA used to control for unspecific inhibition of degradation had no effect (Fig. 3c). Similar results were obtained with cytosol from HEK293 cells (data not shown). Thus, these data indicate that IDE may be one of the AICD degrading enzymes in N2a and HEK293 cells.

**IDE degrades AICD**

In order to prove if IDE is an AICD degrading enzyme we generated HEK293 cell lines stably overexpressing rat IDE or an inactive IDE mutant (IDE E111Q) in which the glutamate of the HXXEH active site motif was changed to glutamine (34,35). As shown in Fig. 4a, immunoblotting of cytosol fractions confirmed overexpression of wt and mutant IDE proteins. Cytosol fractions derived from cells overexpressing wt IDE contained significantly higher AICD degrading activity than cytosol fractions from IDE E111Q overexpressing cells (Fig. 4b). Finally, cytosol of HEK293 cells was immunodepleted of endogenous IDE by immunoprecipitation with excess amounts of an anti-IDE antibody (Fig. 4c). AICD
generation was then assayed from cytosol-free membranes in the presence of these fractions. As shown in Fig. 4d, a dose dependent AICD degradation was observed with non-depleted control fractions in contrast to a markedly reduced AICD degrading activity in IDE depleted cytosol fractions. Thus, these data strongly suggest that IDE is a major AICD degrading enzyme.
DISCUSSION

PSs may function in facilitating nuclear signaling. Such a function is well proven for the role of PS in NICD generation. Very recently the corresponding cytoplasmic fragment of APP, AICD has been identified (13-15). AICD is generated by a remarkably similar molecular mechanism like NICD (Fig. 5). If AICD functions in nuclear signaling as proposed by Cao and Südhof (16), one may expect a rapid clearance of this fragment, since constitutive uncontrolled signaling must be avoided. Indeed this is the case for NICD, which is rapidly removed by an ubiquitin-dependent proteasomal degradation pathway (6,22,23). Here we show that AICD is not degraded by the proteasome but by a completely different protease, namely IDE (Fig. 5). The biochemical properties of IDE make it an ideal candidate for AICD degradation. It is highly expressed in the same cellular compartment as AICD; it is a common enzyme in brain tissue, where abundant APP expression is observed and it preferentially degrades small cytoplasmic peptides of about 20-50 amino acids in length (for review see (24)).

We found an extremely rapid turnover of AICD, which even exceeds the kinetics of de novo production in vitro. This is consistent with the high levels of IDE in the cytoplasm of many cells and tissues. Although we have identified IDE as a major AICD degrading enzyme in non-neuronal and neuronal cell lysates, we can not exclude the possibility that other enzymes may degrade AICD in cells where IDE activity or expression is low (24).

Interestingly, overexpressed artificial 59 amino acid AICD variants have been localized to the nucleus to some extend (20,21). However, this is in contrast to endogenous
AICD with the correct N-terminus that has so far not been detected within the nucleus. Due to the rather rapid turnover of endogenous AICD and the apparent lack of its nuclear localization, one may question a function of this small peptide in nuclear signaling. However, one needs to point out that NICD could not be detected in the nucleus for a long time because sensitive techniques have been lacking. Indeed using hypersensitive reporter gene assays Cao and Südhof (16) could provide indirect evidence that a cytoplasmic fragment of APP (which was not identical to the in vivo existing AICD) complexed with Fe65 and the histone acetyltransferase Tip60 could mediate gene transcription. However, nuclear transport of authentic AICD and the formation of a nuclear Tip60/Fe65/AICD complex remains to be shown. The identification of IDE as an AICD degrading enzyme may facilitate research on the putative role of AICD in nuclear signaling, since this function could be strongly enhanced by the inhibition of AICD degradation.

Interestingly, IDE not only degrades AICD, but is also involved in the clearance of secreted Aβ in the brain (36-40), although the major Aβ degrading activity appears to be neprilysin (41-43). Surprisingly, Aβ degrading IDE appears to be released from living cells (36,37) in very small amounts by so far unknown cellular mechanisms. In contrast to the secreted Aβ, AICD is released into the cytoplasm where it is rapidly degraded by IDE. Since IDE is one of the Aβ clearing enzymes it is tempting to increase its activity to lower the amyloid burden in AD patients. Indeed this was the major goal behind the identification of the Aβ degrading enzymes. The finding that IDE is not only involved in Aβ degradation but also in the removal of AICD may have important implications for therapeutic strategies.
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involving the manipulation of IDE activity. If one assumes that AICD is required for nuclear signaling, its enhanced removal will interfere with AICD mediated functions. Moreover, other therapeutic strategies such as the inhibition of γ-secretase activity will also interfere with AICD production. In that case a major reduction in ACID mediated signaling may occur, which could also have detrimental implications. Careful analysis of AICD function and metabolism is therefore required not only to understand the biological function of APP in nuclear signaling but also for the safety of ongoing therapeutic trials.
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FIGURE LEGENDS

**Fig. 1: Chelators of divalent metal ions block AICD degradation.** Membrane preparations were incubated at 37°C for 2 h in the presence or absence of the indicated protease inhibitors. Membranes were pelleted by ultracentrifugation and AICD was analyzed from the soluble fraction by immunoblotting with antibody 6687. Note that AICD degradation is blocked by a protease inhibitor mix (PI-mix) as well as the metal chelators EDTA and 1,10-phenanthroline (PNT), but not by inhibitors of the proteasome (*clasto*-lactacystin β-lactone and MG132).

**Fig. 2: AICD is degraded by a cytosolic metalloprotease.** (A) Alkaline extraction separates the AICD degrading activity from the AICD generating activity. Crude membrane preparations were subjected to alkaline extraction with Na₂CO₃. Following centrifugation at 100,000 g and two subsequent washes, membranes were incubated at 37°C for 2 h in the presence or absence of PNT/EDTA and AICD was analyzed as in Fig. 1. Note that AICD can be recovered in the absence of PNT/EDTA upon removal of the degrading activity by alkaline extraction with Na₂CO₃ without affecting the AICD generating γ-secretase activity. (B) The AICD degrading activity can be removed by ultracentrifugation. Crude membrane preparations were incubated directly after preparation (P16 fractions) or after additional centrifugation at 100,000 g (P100 fractions) at 37°C for 2 h in the presence or absence of PNT/EDTA. AICD was then analyzed as in Fig. 1. Note that AICD can be recovered even in
the absence of PNT/EDTA after additional ultracentrifugation of P16 fractions. (C) The AICD degrading activity is present in the cytosol. Cytosol-free membrane preparations were incubated at 37°C for 2 h in the presence or absence of PNT/EDTA together with indicated amounts cytosol fractions from HEK293 or mouse N2a cells. AICD was then analyzed as in Fig. 1. Note the dose dependent degradation of AICD in the presence of cytosol derived from kidney and neuronal cells.

Fig. 3: Identification of candidate metalloproteases involved in AICD degradation. (A) The SH-alkylating agent N-ethylmaleimide (NEM) inhibits AICD degradation. Crude membrane preparations were incubated at 37°C for 2 h in the presence or absence of the indicated concentrations of NEM and AICD was analyzed as in Fig. 1. (B) AICD degradation is reduced by dynorphin A-(1-13) but not by the specific neurolysin inhibitor Pro-Ile or the specific TOP inhibitor cFP. Crude membrane preparations were assayed for AICD degradation as in Fig. 1. (C) Dose dependent inhibition of AICD degradation by insulin. AICD was generated by incubation of cytosol-free membranes for 2 h at 37°C and subsequently separated from the membranes by ultracentrifugation. The S100 fractions containing preformed AICD were mixed with cytosol (8 µg) derived from N2a cells and the indicated amounts of insulin or BSA and assayed for AICD degradation by incubation for 2 h at 37°C as in Fig. 1. Note the dose dependent inhibition of AICD degradation by insulin, but not by BSA.
Fig. 4: **IDE is an AICD degrading enzyme** (A) Cytosol fractions of HEK293 cells stably co-expressing Swedish mutant APP and rat wt IDE or the inactive rat IDE E111Q mutant were analyzed for IDE expression by immunoblotting with antibody 9B12. Note the increased levels of both IDE variants in the cytosol fraction upon overexpression. (B) Overexpressed rat IDE, but not inactive IDE E111Q efficiently degrades AICD. Cytosol-free membrane preparations were incubated at 37°C for 2 h as in Fig. 2c in the presence or absence of PNT/EDTA with the indicated amounts of cytosol fractions from HEK293 cells stably overexpressing IDE or the inactive IDE E111Q mutant. Note that overexpressed wt IDE strongly enhances AICD degradation compared to inactive IDE. (C) Immunodepletion of IDE. Cytosol fractions of HEK293 cells were immunodepleted of endogenous IDE by immunoprecipitation with antibody 9B12. As a control, a mock immunoprecipitation lacking antibody 9B12 was carried out. Note the significant depletion of IDE with antibody 9B12. (D) Cytosol fractions immunodepleted of IDE were assayed for AICD degradation as in Fig. 2c. Note that immunodepletion of IDE blocks degradation of AICD.

Fig. 5: **Distinct pathways are involved in the degradation of AICD and NICD.** AICD and NICD are generated by PS-dependent γ-secretase and S3 protease cleavages at similar sites within the membrane. Following liberation from the membrane, NICD and probably also AICD translocate to the nucleus. In the nucleus NICD activates the transcription of Notch target genes. Proteasomal degradation of NICD prevents its accumulation and constitutive
signaling. In contrast to NICD, AICD is degraded by IDE that like neprilysin is also involved in the extracellular degradation of secreted Aβ.
A

| 0°C | 37°C |
|-----|------|
| -   | -    |
| +   | +    |

PNT/EDTA

100 mM Na₂CO₃

AICD

B

| 0°C | 37°C | 0°C | 37°C |
|-----|------|-----|------|
| +   | -    | +   | -    |
| +   | -    | +   | +    |

PNT/EDTA

AICD

P16

P100

C

| 293 | N2a |
|-----|-----|
| 8   | 4   |
| 4   | 2   |
| 2   | 1   |

Cytosol (µg)

PNT/EDTA

AICD

Edbauer et al., Fig. 2
A

| endog. IDE | wt | E111Q | IDE |
|------------|----|-------|-----|

B

| wt | E111Q | Cytosol (µg) | PNT/EDTA |
|----|-------|--------------|----------|
| -  | -     | 6            | -        |
| -  | +     | 3            | -        |
| -  | -     | 1.5          | -        |
| -  | -     | 0.75         | -        |

C

| input | control | anti-IDE | IDE |
|-------|---------|----------|-----|

D

| control | anti-IDE | Cytosol (µg) | PNT/EDTA |
|---------|----------|--------------|----------|
| -       | -        | 8            | -        |
| -       | +        | 4            | -        |
| -       | -        | 2            | -        |
| -       | -        | 1            | -        |

Edbauer et al., Fig. 4
degradation via the ubiquitin proteasome pathway

Edbauer et al., Fig. 5
Insulin-degrading enzyme rapidly removes the β-amyloid precursor protein intracellular domain (AICD)

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