Copper inhibits β -amyloid production and stimulates the non-amyloidogenic pathway of amyloid-precursor-protein secretion

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Previous studies have demonstrated that amyloid precursor protein (APP) can bind and reduce Cu(II) to Cu(I), leading to oxidative modification of APP. In the present study we show that adding copper to Chinese-hamster ovary (CHO) cells greatly reduced the levels of amyloid A β peptide (A β) both in parental CHO-K1 and in copper-resistant CHO-CUR3 cells, which have lower intracellular copper levels. Copper also caused an increase in the secretion of the APP ectodomain, indicating that the large decrease in A β release was not due to a general inhibition in protein secretion. There was an increase in intracellular full-length APP levels which paralleled the decrease in A β generation, suggesting the existence of two distinct regulating mechanisms, one acting on A β production and the other on APP synthesis.

Maximal inhibition of A β production and stimulation of APP secretion was achieved in CHO-K1 cells at about 10 μ M copper and in CHO-CUR3 cells at about 50 μ M copper. This dose 'window of opportunity' at which copper promoted the non-amyloidogenic pathway of APP was confirmed by an increase in the non-amyloidogenic p3 fragment produced by α -secretase cleavage. Our findings suggest that copper or copper agonists might be useful tools to discover novel targets for anti-Alzheimer drugs and may prove beneficial for the prevention of Alzheimer's disease.

Key words: Alzheimer's disease, APP processing, copper-resistance.

INTRODUCTION

Amyloid A β peptide (A β , β A4), the principal constituent of senile plaques and cerebrovascular amyloid [1,2] of Alzheimer's disease (AD), is proteolytically derived by consecutive cleavage of β -secretase and γ -secretase from the larger integral membrane amyloid precursor protein (APP), the major isoforms of which contain 695, 751 and 770 residues respectively. APP processing within the A β domain by α -secretase precludes amyloid form-

ation and leads to the release of the p3 peptide containing A β residues 17–40/42 (Figure 1). Cleavage of APP by α - or β -secretase generates soluble APP fragments (sAPP α and sAPP β) representing APP ectodomains which are released into the extracellular space [3–7]. Although the physiological function of APP ectodomains is unknown, APP has features in common with cell-adhesion molecules and molecules involved in wound-healing. This is exemplified by the binding sites of APP for heparin sulphate, collagen, laminin, proteases, lectins and metal

Figure 1 Processing of amyloid precursor protein (c-Myc tag APP695), A β , p3.5 and p3 (white letters for amino acid sequence of A β 1–40/42)

The approximate location of the epitopes of antibodies is indicated below the sequence (black bars). The α -, β - and γ -secretase protease activities cleave APP within its ectodomain. The remaining membrane-bound C-terminal fragments p3CT (C-terminal 83 residues after α -secretase cleavage) and A4CT (C-terminal 99 residues after β -secretase cleavage) are further cleaved by γ -secretase in the middle of their putative transmembrane domains, yielding p3 (α - and γ -secretase activity), p3.5 (β - and γ -secretase activity) and A β (β - and γ -secretase activity) respectively.

Abbreviations used: A β , amyloid A β peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; sAPP, soluble APP fragment; cAPP, cellular APP; PEN, D-(–)-penicillamine; BC, bathocuproine disulphonate; LDH, lactate dehydrogenase; DMEM, Dulbecco's modified Eagle's medium; BME, basal Eagle's medium; MEM, minimal essential medium; CM, conditioned medium.

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ions [8]. Regarding the latter, APP interacts specifically with Zn(II) and Cu(II) at two distinct sites [9,10].

Regulation of APP secretion has been characterized in a wide variety of cell lines [11,12] and, under some circumstances, the rate of A β formation was inversely coupled to the rate of sAPP secretion [12–20]. Activation of certain signal-transduction pathways increases α -secretase cleavage, and some factors can regulate A β production, including calcium, oestrogen and cholesterol [14,21–26]. Mutations within or flanking the A β region and mutations in the presenilin 1 and presenilin 2 genes increase the extracellular concentration of the longer more amyloidogenic A β peptide species [27,28].

As a possible treatment for AD it would be highly desirable to develop a drug with specific high-affinity binding to APP. One major requirement would be that these ligands should be able to interfere with amyloidogenic APP processing *in vivo*. Of the known APP ligands it has been shown that Zn(II) increases the levels of membrane-associated APP and decreases secretory APP forms *in vivo* [29]. APP also binds Cu(II), and this leads to oxidation of APP *in vitro* [30–32]. Furthermore, Cu(II) binding causes conformational changes in APP [10], and this can modulate APP–APP interactions [33]. This encouraged us to examine the regulation of APP processing by Cu(II). To study this we used normal Chinese-hamster ovary (CHO) cells and a Cu(II)-resistant variant of CHO cells (CUR3). The Cu(II) resistance of CUR3 cells is due to the maintenance of lower intracellular Cu(I) in the presence of elevated extracellular Cu(II) as a consequence of enhanced copper efflux resulting from overexpression of the Menkes P-type ATPase Cu-translocating pump [34]. Treatment of these cells with Cu(II) increased the secretion of C-terminal truncated APP and decreased A β release into the conditioned medium (CM) in a concentration-dependent manner. This suggests that Cu(II) binding of intracellular APP attenuates A β production and at the same time fosters the generation of secretory APP.

EXPERIMENTAL

Cell lines and transfections

CHO cells were stably transfected with a c-Myc-tagged APP695 vector [35] or the expression vector alone using a high-efficiency calcium phosphate transfection method [36]. The pSP65 N-tag APP695 DNA was cloned into the pcDNA3 vector (Invitrogen/ITC Biotechnology, Heidelberg, Germany) using the cloning sites *Sma*I and *Eco*RV respectively.

Transfection efficiency was assessed by immunoprecipitation of APP with rabbit polyclonal anti-c-Myc antibody (18/47, raised against EQKLISEEDL of the c-Myc sequence by Eurogentec, Seraing, Belgium) and immunoblotting using the anti-APP mouse monoclonal antibody 22C11 (Boehringer, Mannheim, Germany) in the ECL[®] (enhanced chemiluminescence) detection system (Amersham, Braunschweig, Germany).

Parental CHO-K1 and Cu(II)-resistant variant CUR3 cells [34] were grown at 37 °C in basal Eagle's Medium (BME) supplemented with 2 mM L-glutamine, 0.1 mM proline, 20 mM Hepes and 10% (v/v) fetal-calf serum (Boehringer, Ingelheim, Germany). The basal Cu(II) concentration of BME is 0.8 μ M [34]. CUR3 cells were grown in medium containing 200 μ M added Cu(II) until the cells reached a 70% confluent monolayer. This reflects the minimal Cu(II) level that is necessary for CUR3 cells to maintain the 70-fold elevated level of the Menkes P-type ATPase Cu-translocating pump and to ensure that the CUR3 cells are viable in the presence of elevated Cu(II) concentrations.

Metabolic labelling and immunoprecipitation

Stably transfected CHO cells of a 60 mm-diameter culture dish were washed with PBS and then treated with 2 ml of minimum essential medium (MEM) lacking methionine (Sigma, München, Germany) to allow a 'reset' of growing conditions regarding the various Cu(II) levels in media of CHO-K1 and CHO-CUR3 cells during culturing. Labelling was performed in 2 ml of MEM, supplemented with 220 μ Ci of [³⁵S]methionine (Amersham) and 5% CO₂ for 4 h. CM and cells were harvested and immunoprecipitated. Protein concentrations were assayed by using a Bio-Rad Protein Assay [37] or radiolabelled proteins were precipitated in 10% (w/v) trichloroacetic acid and the incorporated [³⁵S]methionine label (Beckman LS 6000IC) was measured.

Cells were lysed in an extraction buffer containing 50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 2% Triton X-100, 2% (v/v) Nonidet P40, 1 mM PMSF, 10 μ g/ml aprotinin and 10 μ g/ml leupeptin. The lysate and the medium were centrifuged for 10 min at 13 000 g to remove cellular debris and the medium was adjusted to 25 mM Tris/HCl (pH 8.5)/1 mM PMSF/10 μ g/ml aprotinin/10 μ g/ml leupeptin/0.5% Triton X-100/0.5% Nonidet P40. The solubilized proteins were diluted 1:2 in 100 mM Tris/HCl (pH 7.5)/300 mM NaCl/4 mM EDTA. The supernatants were incubated with anti-APP antibodies overnight by end-over-end rotation at 4 °C and, in some experiments, the resulting supernatant was subsequently analysed with anti-A β antiserum. Immune complexes were recovered with Protein A–Sepharose and subjected to the analysis described in [38].

Antibodies and electrophoresis

For the detection of APP, rabbit polyclonal antibodies that were raised against recombinant Fd-APP770 (antiserum 22734/8 against APP770 residues 18–687 or antiserum 23850/6 against APP residues 18–491 (Figure 1) were diluted 1:500 for precipitation. A β , p3.5 and p3 were recognized by the polyclonal antibody 730 (dilution 1:50) precipitating equally A β , p3.5 and p3 that was raised against a synthetic peptide corresponding to A β residues 1–40 (Figure 1). A β and its derivatives were separated by 10–20% polyacrylamide/Tris/Tricine, APP by 7% polyacrylamide/Tris/glycine or 7% polyacrylamide/Tris/Tricine gels. For immunoblotting, cell extracts and extracellular medium were precipitated in 10% trichloroacetic acid, washed with acetone and solubilized in sample buffer. After electrophoresis the gels were processed as described in [39] and bands quantified with the Fuji–Bas PhosphorImager system. Data are representative of at least two separate trials.

Drug treatments and lactate dehydrogenase (LDH) assay

During the time of labelling, Cu(II) or Zn(II) chloride was added to growth medium at concentrations of 5 μ M–200 μ M; D(–)-penicillamine (PEN; Sigma), bathocuproine disulphonate (BC; Aldrich) and 1,10-phenanthroline (Sigma) were added to medium to give final concentrations of 100 μ M (PEN and BC) and 200 μ M (1,10-phenanthroline) respectively. Cell viability was measured by assaying LDH leakage into the culture medium [40]. The leakage was measured as enzyme activity (units/litre) after 2 and 4 h in the medium of 70% confluent monolayers of CHO cells in Dulbecco's modified Eagle's medium (DMEM).

RESULTS

The parental CHO-K1 and the Cu(II)-resistant variant CUR3 cell lines were used to study the role of Cu(II) on APP processing

Figure 2 Immunoprecipitation of c-Myc-tagged APP695

APP was precipitated from the lysate (Myc-APP695) and the CM (Myc-sAPP695) of wild-type (a, CHO-K1) and Cu(II)-resistant CHO cells (b, CHO-CUR3) with polyclonal c-Myc antibodies 18/47. The precipitated samples were immunoblotted with APP monoclonal antibody 22C11 after transfer to nitrocellulose membrane.

under the conditions of Cu(II) depletion and increasing Cu(II) (0–200 μ M). CUR3 cells were used to assess the role of intracellular Cu(II)/(I) on APP processing. Compared with CHO-K1 cells, at elevated extracellular Cu(II) levels the CUR3 cells have a lower intracellular Cu(II)/(I) concentration, owing to enhanced Cu(II)/(I) efflux as a consequence of a 70-fold elevated level of the Menkes P-type ATPase Cu-translocating pump [34,41].

CHO-K1 and CUR3 cells were stably transfected with cDNAs encoding APP695 with an N-terminal c-Myc epitope (Figure 1). APP expression was detected by immunoprecipitation with polyclonal anti-c-Myc antiserum, followed by immunoblotting with monoclonal antibody 22C11 (Figures 2a and 2b). The polyclonal anti-c-Myc antibody immunoprecipitated c-Myc-tagged APP695 with an apparent molecular mass of 105 kDa from the cell lysate (cAPP) and 105 and 97.5 kDa from the CM (sAPP). As expected, c-Myc-APP695 was detected in neither the lysate nor the medium of cells stably transfected with pcDNA3 alone (Figures 2a and 2b).

To analyse the regulation of APP metabolism by Cu(II) ions, stably transfected CUR3-APP695 cells were labelled for 4 h with [35 S]methionine in the presence of different Cu(II) levels and the secretion of APP ectodomain, A β and p3 were analysed by immunoprecipitation and SDS/PAGE in a Tris/Tricine/polyacrylamide gel system. This allowed the quantification of APP and its derivatives on the same gel. Cell lysates of CUR3-APP695 cells revealed the 105 kDa immature cAPP695 holoprotein (Figure 3a). APP695 derivatives released into CM were detected as bands of 105 and 97.5 kDa (Figure 3b), most likely differing from each other by a higher content of sialic acids in the upper band [39]. The cell lines did not show a decrease in viability as measured by LDH release.

The levels of APP holoprotein and soluble APP increased significantly when CUR3-APP695 cells were incubated with

increasing concentrations of Cu(II). Compared with basal medium the maximum increase in sAPP occurred at 50 μ M Cu(II) ($265 \pm 15\%$ increase) before a general increase in total cellular secreted proteins occurred (Figures 3c, 3e and 3f). Immunoprecipitation of radiolabelled peptides derived from the A β region of APP was achieved with rabbit antiserum 730, which recognized A β (4.5 kDa), p3.5 (3.5 kDa) and p3 (3 kDa) (Figure 1). Secretion of p3, the C-terminal counterpart to secretory APP produced by α -secretase cleavage, increased by $204 \pm 14\%$ at 50 μ M Cu(II) (Figures 3d, 3e and 3f) in the medium, reaching a maximum of $270 \pm 14\%$ between 20 μ M and 50 μ M Cu(II) (Figures 3d and 3e). The percentage of cAPP was markedly increased by $205 \pm 3.5\%$ at 50 μ M Cu(II) (Figures 3a, 3c and 3f). When Cu(II) concentrations exceeded 50 μ M in the medium, the general protein metabolism of cells was affected and cAPP levels increased further (Figures 3a and 3c). In contrast, A β levels were greatly reduced at 50 μ M Cu(II) ($20 \pm 1.4\%$ of basal levels; Figures 3d, 3e and 3f) and A β was barely detectable above 100 μ M in CUR3-APP695 cells (Figures 3d and 3e). The p3.5 peptide showed a similar pattern (Figure 3d) and confirmed an earlier finding that p3.5 is the product of an alternative β -secretase activity [39]. The polyclonal A β antiserum 730 and a monoclonal antibody recognizing A β 40 specifically precipitated equal amounts of A β (results not shown).

To determine if cellular APP, sAPP and downstream catabolites are also modulated by a decrease in Cu(II) levels, CUR3-APP cells were incubated with the Cu(II) chelators PEN and BC, a cell non-permeable Cu(I) chelator. It was found that the production of A β and p3.5 was unaffected, whereas sAPP and p3 were reduced to $77 \pm 1.6\%$ and $50 \pm 1.3\%$ respectively (Figure 3, lane BC/PEN in d, data point '0' in e). Since APP has a Zn(II)-binding site, the effect of Zn(II) levels on APP processing was studied. In contrast with Cu(II), the addition of Zn(II) decreased APP secretion in CUR3-APP695 cells at concentrations of Zn(II) above 100 μ M. Levels of A β were unchanged for concentrations up to 20 μ M Zn(II) (results not shown). Interestingly, in the presence of the Zn(II) chelator 1,10-phenanthroline and in the absence of the Cu(I) chelator BC, APP secretion was increased in CHO-K1-APP695 cells (results not shown). These data indicate that the Cu(II)-induced changes are due to a specific modulation of APP metabolism by Cu(II).

In CHO-K1-APP695 cells the levels of APP [cAPP $295 \pm 9.9\%$ and sAPP695 $475 \pm 66.1\%$ at 10 μ M Cu(II)] and p3 [$154 \pm 7.0\%$ at 10 μ M Cu(II)] were significantly increased when incubated with increasing Cu(II) concentrations (Figures 4a–4f). However, the production of A β decreased to $33 \pm 1.3\%$ at 10 μ M Cu(II) (Figures 4d–4f) and was undetectable beyond 20 μ M (Figures 4d–4e). In the presence of BC/PEN, sAPP695 secretion was increased by $170 \pm 7.5\%$ (Figure 4, lane BC/PEN in b, data point '0' in c) and p3 secretion was $171 \pm 5.8\%$ (Figure 4, lane BC/PEN in d, data point '0' in e), but no change in the level of A β was observed (Figures 4d–4e).

In contrast with CUR3-APP695 cells, the maximum rate of APP secretion of CHO-K1-APP695 cells was reached at 10 μ M Cu(II) for sAPP695 (Figures 4b, 4c, 4e and 4f) and then decreased at Cu(II) concentrations between 20 μ M and 200 μ M (Figures 4b, 4c and 4e). There was a general increase in secreted proteins at concentrations greater than 20 μ M (Figure 4c). The 5-fold difference in Cu(II) concentrations required for maximum rate of APP secretion in CUR3-APP695 cells [50 μ M Cu(II)] as compared with CHO-K1-APP695 cells (10 μ M) is consistent with the 5-fold-increased Cu(II) resistance of CUR3 cells to maintain their reduced intracellular Cu(II)/(I) levels [34]. The stimulation of APP secretion following Cu(II) depletion in CHO-K1-APP695 cells contrasts with the effect in CUR3-APP695 cells (Figure 4,

Figure 3 Immunoprecipitation of APP and its derivatives

Holo-APP695 from (a) lysate, (b) soluble APP695, (d) A β and p3 were immunoprecipitated from the medium of metabolically labelled CUR3 cells with anti-APP (22734/8; a and b) or anti-A β (730; d) after 4 h incubation in the presence of the Cu(I) chelators BC and PEN, in normal medium [CM; containing basal media Cu(II)] or in medium containing Cu(II) concentrations as indicated. The doublet appearance of sAPP695 (b), restricted to higher Cu(II) concentrations is due to its different sialic acid content [39]. A fragment that migrates just above the A β band (d) is detected at low levels and is presumably generated by δ - and γ -secretase activity [39] (see Figure 1). (c) The relative amounts of cAPP (Δ), sAPP ($\square$) and total secreted protein ($\blacklozenge$) measured in this experiment are quantitated as a percentage of radioactivity obtained at basal Cu(II) medium concentration. (e) A β ($\blacksquare$), p3 ($\blacktriangle$) and sAPP ($\square$); data from the same experiments as in c, shown for better comparison). The data are the averages for three independent experiments. (f) The increase of sAPP695, cAPP695 and p3 and the decrease of A β at 50 μ M Cu(II) are relative to standard conditions, i.e. basal medium containing 0.8 μ M Cu(II). Bars represent the standard error of the estimate of mean value (S.E.M.). Note that '0' μ M and '1' μ M Cu(II) in (e) are the incubation conditions of Cu(II)/(I) depletion (PEN/BC) and basal medium Cu(II) (CM; 0.8 μ M). For characterization of the α -, β - and γ -secretase-cleaved fragments, see reference [39] and Figure 1 for cleavage sites. The intensity of signals for p3.5 and fragments generated by the δ -secretase [39] is too low for reliable analysis.

lane BC/PEN in b, data point '0' in c). This suggests that Cu(II) pools in untreated CHO-K1-APP695 cells may down-regulate APP expression, since CHO-K1 and Cu(II)-resistant CHO cells show different intracellular Cu(II) distribution (J. Camakaris, unpublished work).

Comparison of APP secretion with p3 secretion showed that increases in APP and p3 secretion occurred concomitantly, suggesting that APP is released by α -secretase activity as sAPP α (Figures 5a and 5b). The decrease in A β production from CHO-K1-APP695 and CHO-CUR3-APP695 cells did not appear to be related to the increase in APP secretion (Figures 5a and 5b), leading to the conclusion that different mechanisms are involved.

DISCUSSION

The CHO model system was chosen to study the effects of Cu(II) on APP processing because of the ability of modulating extracellular Cu(II) levels to influence intracellular Cu(I) [34,42]. Previous studies revealed a reduced intracellular Cu(II)/(I) accumulation due to enhanced Cu(I) efflux as the molecular basis of

Cu(II) resistance in CHO-CUR3 cells [34,42]. In contrast with the CHO-CUR3 cells, the parental line CHO-K1 has normal intracellular Cu(II)/(I) levels, since it was not selected for Cu(II) resistance or overexpression of Menkes active P-type ATPase, which is a transmembrane Cu-translocating pump [34]. We measured a specific effect of Cu(II) on the secretion of sAPP, the production of APP and A β release that was dependent on the concentration of Cu(II) and the degree of cellular Cu(II) resistance. Maximal inhibition of A β production and stimulation of APP secretion was achieved in CHO-K1-APP695 cells at about 10 μ M copper and in CHO-CUR3-APP695 cells at about 50 μ M copper. The addition of Cu(II) to the medium results in an increase of the release of the APP products sAPP and p3 of the α -secretory pathway and a decrease in the release of the β -secretase products A β and p3.5. Cleavage by γ -secretase, which is required to generate the C-terminus of p3, did not appear to be inhibited by increasing Cu(II) concentrations. Unfortunately we were unable to determine if the ratio of A β 42 to A β 40 is influenced by increasing Cu(II) concentrations, owing to two superimposing factors: the total amount of A β produced decreases under the given conditions and the affinity of the monoclonal antibody recognizing A β 42 is insufficient.

Figure 4 Immunoprecipitation of holo-APP695, soluble APP695, A β and p3

Proteins were immunoprecipitated (a) from lysate, (b) from the medium of metabolically labelled K1-APP695 cells with anti-APP (22734/8) or anti-A β (730) after 4 h incubation time in the presence of the Cu(I) chelators BC and PEN, in normal medium [CM; containing basal media Cu(II)] or in medium containing Cu(II) concentrations as indicated and quantified as in Figure 3. [Note that the endogenous level of sAPP751/770 in K1 cells is influenced by the Cu(II) concentration in the same way as c-Myc-tagged APP695.] (c) The relative amounts of cAPP (Δ), sAPP695 (□) and total secreted protein (◆) measured in this experiment are quantified by the PhosphorImager system. (e) A β (■), p3 (▲) and sAPP695 (□); data from the same experiments as shown in (c). The data are the averages for three independent experiments. (f) The increase in sAPP695, cAPP695 and p3 and the decrease in A β at 10 μ M Cu(II) are relative to standard conditions, i.e. basal medium containing 0.8 μ M Cu(II). A fragment that migrates just above the A β band (d) is detected at low levels and is presumably generated by δ - and γ -secretase activity [39] (see Figure 1). Note that '0' μ M and '1' μ M Cu(II) in (e) are the incubation conditions of Cu(II)/(I) depletion (PEN/BC) and basal medium Cu(II) (CM; 0.8 μ M). The results are the means for three independent experiments. Bars in (f) represent the S.E.M.

The increase in sAPP and p3 secretion by elevated Cu(II) concentrations in both cell lines is accompanied by a concomitant increase in cell-associated full-length APP. This APP pool appears to consist of mainly N-glycosylated endoplasmic-reticulum forms of APP [38], since the N- and O-glycosylated mature forms of APP represent, if at all present, a minor fraction. The presence of this APP pool could indicate that only mature APP is diverted into the pathway leading to constitutive secretion of APP and p3. Alternatively, inhibition of the β -secretase pathway, which leads to A β production by Cu(II), may occur in a cellular compartment from which APP cannot reach the constitutive α -secretory-vesicle system. This suggests that two pools of APP could exist: one for secretory APP and p3 and another for A β production.

The recent association of an α -secretase activity with a Zn(II) disintegrin metalloprotease [43] does not explain our results. First, Zn(II) did not give the same results as found for Cu(II). Secondly, at least in CHO-K1-APP695 cells, APP and p3 were found to be slightly increased in the presence of Cu(I) and Zn(II) chelators (Figure 4). Thirdly, a general increase in ectodomain shedding by activation of any potential metalloprotease due to

elevated Cu(II) concentration has not been observed when the specific increase of sAPP and p3 in the dose 'window of opportunity' was recorded.

On the basis of the different Cu(II) concentrations required to inhibit completely A β production in the two cell lines used in the present study, we conclude that Cu(II) has to be internalized before it can exert its effect on APP metabolism. The arguments are as follows. The Cu(II)-resistant CHO cell line CHO-CUR3 requires five times higher extracellular Cu(II) concentrations to achieve the same diminution of A β secretion as the CHO-K1 cells. This is due to the 70-fold elevated expression of the Menkes P-type ATPase [34], leading to significantly reduced intracellular Cu(I) levels in CUR3 cells. Therefore a 5-fold higher extracellular Cu(II) concentration is required to reach the same intracellular Cu(II) levels as in CHO-K1 cells. Otherwise it is difficult to conceive how a 5-fold difference could influence APP metabolism, since the binding constant for Cu(II) of APP exposed to the cell surface should be identical for APP in K1-APP695 and CUR3-APP695 cells.

How could intracellular Cu(II)/(I) interfere with APP processing? Since Cu(II) binding to APP is known to cause a

Figure 5 Quantification of the relative level of sAPP695, p3 and Aβ

Proteins were immunoprecipitated from CM of (a) CHO-K1-APP695 cells and (b) CHO-CUR3-APP695 cells. The increase in sAPP695 (□) reached a peak in K1 cells at 10 μM Cu(II) (a) and in CUR3 cells at 50 μM Cu(II) (b) and then began to decline. The change in level of p3 (▲) lagged behind the changes seen with the sAPP (□), which reached the maximum level at slightly higher Cu(II) doses, most likely because of its prolonged half-life time compared with sAPP. By contrast, in both cell lines the levels of Aβ (■) dramatically decreased at Cu(II) concentrations below the basal level. Note that the first data point was obtained at basal Cu(II) concentrations [0.8 μM Cu(II)].

conformational change, this could facilitate α-cleavage and attenuate β-cleavage. It was recently shown that β-cleavage depends on the transport of APP to lipid rafts enriched in cholesterol [25]. Therefore APP-Cu(II) complexes could be compromised in this transport and, as a consequence, not reach the compartment in which the β-secretase resides.

Our findings of the inhibition of the amyloidogenic pathway by Cu(II) is potentially important for the Alzheimer's-disease treatments aimed at the inhibition of Aβ production. The current approaches are directed at interfering with Aβ generation by compounds that inhibit the hitherto-unidentified β-secretase or γ-secretase [44]. Since these proteases are likely to be involved in processing several transmembrane proteins, their inhibition may have unforeseen consequences for cellular viability. On the basis of our findings, we propose an alternative and more specific approach for the design of Cu(II) agonists which promote the formation of APP complexes and a subsequent attenuation of Aβ metabolism. Since Cu(II) binding by APP also generates highly cytotoxic Cu(I) ions, these agonists would have the additional potential to protect cells from damage by blocking Cu(II) binding of APP and thus preventing the formation of reactive oxygen species produced by Cu(II) and hydrogen peroxide.

In conclusion, the CHO model has proved to be powerful in elucidating the modulating effect of Cu(II) on APP processing. Further studies will be directed to investigating this effect in neuronal cells to see whether our results can be generalized.

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