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Protein kinase C and amyloid precursor protein processing in skin fibroblasts from sporadic and familial Alzheimer's disease cases

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Abstract

Non-amyloidogenic α-secretase processing of amyloid precursor protein (APP) is stimulated by protein kinase C (PKC). Levels and activity of PKC are decreased in sporadic Alzheimer's disease skin fibroblasts. We investigated whether alterations in PKC and PKC-mediated APP processing occur also in fibroblasts established from individuals with familial Alzheimer's disease APP KM670/671NL, PS1 M146V and H163Y mutations. These pathogenic mutations are known to alter APP metabolism to increase Aβ. PKC activities, but not levels, were decreased by 50% in soluble fractions from sporadic Alzheimer's disease cases. In contrast, familial Alzheimer's disease fibroblasts showed no significant changes in PKC enzyme activity. Fibroblasts bearing the APP KM670/671NL mutation showed no significant differences in either PKC levels or PKC-mediated soluble APP (APPs) secretion, compared to controls. Fibroblasts bearing PS1 M146V and H163Y mutations showed a 30% increase in soluble PKC levels and a 40% decrease in PKC-mediated APPs secretion. These results indicate that PKC deficits are unlikely to contribute to increased Aβ seen with APP and PS1 mutations, and also that PS1 mutations decrease α-secretase derived APPs production independently of altered PKC activity.

Keywords: Alzheimer's disease; Protein kinase C; Fibroblast; Amyloid precursor protein; Presenilin

1. Introduction

Alzheimer's disease is characterised neuropathologically by the deposition of extracellular amyloid plaques containing aggregates of the Aβ peptide [1], as well as intracellular neurofibrillary tangles composed primarily of abnormally phosphorylated tau protein [2]. Aβ is a 39–43-amino-acid, self-aggregating peptide that is cleaved from the amyloid precursor protein (APP). APP is a transmembrane glycoprotein with a long extracellular N-terminus and a short intracellular C-terminus that is processed by different pathways to yield both secreted and cell-associated derivatives. The α-secretase processing of APP involves cleavage within the Aβ region to yield a large N-terminal ectodomain (APPs) [3–5] and a 10 kDa C-terminal product which can undergo an additional cleavage. This leads to the formation of a 3 kDa Aβ fragment, known as p3 [6] and its complementary product p7. These α-secretase proteolytic

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cleavage products are thought to be non-amyloidogenic [7]. Aβ is generated when APP is cleaved by β-secretase at the N-terminus and by γ-secretase at the C-terminus of the Aβ domain. APP can also be degraded in an endosomal–lysosomal compartment resulting in multiple fragments some of which contain the intact Aβ domain and are therefore potentially amyloidogenic [5,8]. α-Secretase processing of APP can be regulated by receptor-coupled protein kinase C (PKC)-dependent, as well as PKC-independent mechanisms [9–14]. However, increased production of APPs does not always result in decreased production of Aβ, suggesting that the regulation of APP metabolism is cell type-specific [15]. Decreased levels of Aβ production following phorbol ester-stimulated release of APPs has been shown to occur in APP-transfected COS-, CHO-, and HEK293-cells [16–19] as well as in fibroblasts [20]. In contrast, Aβ and APPs generation appear to be independent of each other in human neuroblastoma SH-SY5Y cells [21].

Molecular genetic studies have identified Alzheimer's disease causing mutations in the APP gene on chromosome 21 and in the Presenilin 1 (PS1) and Presenilin 2 genes on chromosome 14 and chromosome 1, respectively. APP gene mutations include a double mutation at codons 670 and 671 which was found in a large Swedish family with dominantly inherited, early onset Alzheimer's disease [22]. Studies of cultured skin fibroblasts from members of the Swedish APP KM670/671NL family have shown that cellular production of the Aβ peptide is increased approximately three fold in mutation carrying cell lines [20,23]. Mutations in the PS1 gene have been found in approximately 45 families including one Swedish family (Swed2) at codon H163Y and one Swedish/Finnish family at codon M146V [24]. Mutations in the PS1 gene have been reported to cause a specific increase in the cellular production of more fibrillogenic forms of the Aβ peptide ending at amino acids 42/43 [25–28].

Increased Aβ peptide production due to APP and PS1 mutations has also been shown in primary skin fibroblasts from affected individuals [29,30]. Skin fibroblasts have also been used to look at a range of other biological changes in Alzheimer's disease including that of an altered PKC level and activity [31,32]. Decreased cytosolic PKC activity has been shown in cultured fibroblasts from both sporadic and familial Alzheimer's disease cases [33–35]. After characterising the different PKC isoforms in fibroblasts [36], it was shown that reduced PKC activity in sporadic Alzheimer's disease fibroblasts could be explained by a specific 30% reduction of the cytosolic PKC_{α} isoform [37].

Since both the Swedish APP KM670/671NL mutation and the PS1 H163Y and PS1 M146V mutations show an altered APP metabolism, we felt it important to investigate whether these phenotypes included PKC deficits that would contribute to altered α-secretase APP processing and the reported increased Aβ peptide production. In this study we have looked at PKC levels and activity in soluble and particulate fractions from mutation-bearing and control skin fibroblasts. PKC activities were determined as the phosphorylation of endogenous histone H III-SS substrate. A binding assay with [^{3}H]phorbol-12,13-dibutyrate (PdBu) was also used to estimate the amount of total PKC. Basal and PKC-stimulated APPs release were determined by immunoblotting of cell culture media with the monoclonal antibody 22C11.

2. Materials and methods

2.1. Cell culture

Skin biopsies taken from the upper arm of members of the Swedish family with the APP KM670/671NL mutation, the Swedish family with the PS1 H163Y mutation as well as the Swedish/Finnish family with the PS1 M146V mutation were explanted and grown in minimum essential medium with Earle's salts (EMEM), supplemented with 10% foetal bovine serum (FBS), 2 mM L-Glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 60 μg/ml Tylocine (Anti-PPLO agent), 20 mM Tricine buffer (pH 7.4) and 1% v/v non-essential amino acid solution (Gibco BRL, Life Technologies, European Division) at 37°C in humidified air with 5% CO_2. Cell lines originated from sporadic Alzheimer's disease (AD) cases and age-matched controls were obtained either from the Cell Repository at the IRCCS 'Centro S. Giovanni di Dio-FBF', Brescia, or from the NIA Aging Cell Repository, USA, and grown under the same conditions. Case and cell line details are given

in Table 1. Sporadic AD refers to individuals without a known family history of AD. Familial controls refers to non-mutation-bearing individuals within a mutation-bearing family.

2.2. Preparation of soluble and particulate fractions

The cells were treated for 5 min with EDTA-solution (1 mM EDTA in phosphate-buffered saline (PBS), pH 7.2) and then harvested with 0.025% trypsin in Ca^{2+} and Mg^{2+} free PBS (Gibco BRL) at confluence 5–7 days after previous subculture. Harvested cells were washed twice in PBS and thereafter frozen as pellets and stored at −70°C. The pellets were thawed and re-suspended in 20 mM Tris–HCl (pH 7.4) containing 0.32 M sucrose, 2 mM EDTA, 50 mM β-mercaptoethanol, 50 μg/ml phenylmethylsulfonylfluoride, 20 μg/ml leupeptin and 25 μg/ml aprotinin (buffer A), and homogenised using a glass Teflon homogenizer. Cell homogenates were centrifuged at $100\,000 \times g$ for 30 min. Pellets were re-suspended in the same volume of buffer A and centrifuged for 30 min at $100\,000 \times g$. The pooled supernatants constituted the soluble PKC fraction. The pellets were re-suspended in buffer A and divided into two parts, one was used as the membrane suspension in the binding experiments. The other was treated with 0.5% Triton X-100 on ice for 45 min and after sonication centrifuged at $100\,000 \times g$ for 30 min. The supernatant represented the solubilised particulate PKC fraction. Protein levels were determined according to Bradford [38] and homogenates then aliquoted and frozen at −70°C.

2.3. Protein kinase C enzyme activities

To determine PKC enzyme activities, 7–10 μg of soluble or solubilised particulate cell homogenate were incubated at 37°C in 250 μl buffer containing 20 mM Tris–HCl, 5 mM $Mg(CH_3COO)_2$ and 1.25 μg histone H III-SS. The phosphorylation was started after 5 min pre-incubation by adding 5 nmol [γ-^{33}P]ATP ([γ-^{33}P]ATP, Du Pont NEN, 2000 Ci/mmol; 0.24 pmol [γ-^{33}P]ATP diluted with 5 nmol cold ATP). Basal activity was measured in the presence of 2 mM ethylene glycol tetraacetic acid. Stimulated activity was measured in the presence of 2 mM $CaCl_2$, 10 μg phosphatidylserine and 1 μg diolein. These conditions have been reported to fully stimulate PKC [39,40]. Reactions were stopped by 25% trichloroacetic acid precipitation and filtering onto Millipore HA 0.45-mm filters. Filter bound radioactivity was determined by scintillation spectroscopy and enzyme activities were expressed as pmol phosphorylated histone/mg protein per minute. Experiments were repeated 2–4 times for each cell line.

2.4. [^{3}H]Phorbol-12,13-dibutyrate binding

PKC levels were determined by performing single concentration binding assays with [^{3}H]phorbol-12,13-dibutyrate ([^{3}H]PdBu, Du Pont NEN, 20 Ci/mmol). Seven to 14 μg protein of either soluble or particulate fractions was incubated with 10 nM [^{3}H]PdBu in a final volume of 500 μl reaction mix containing 20 mM Tris–HCl (pH 7.5), 2 mM $CaCl_2$, 1 mg/ml BSA, 50 mM β-mercaptoethanol, 100 μg/ml phosphatidylserine. 100μM PdBu was used to define non-specific binding. Soluble fractions were incubated for 15 min at 23°C after which 400 μl of 10% DEAE was added, and samples then incubated for 15 min 0°C. Particulate fractions were incubated for 60 min at 23°C. After incubations the mixtures were filtered through Whatman GF/C filters that had been pre-soaked in 0.05% polyethyleneimine. Filter-bound radioactivity was determined by scintillation spectroscopy. Each cell line was assayed in triplicate.

2.5. APPs Western blotting

APPs levels were determined by Western-blot analysis. Confluent cells in T25 flasks were rinsed twice with 2 ml of fresh culturing medium and then stimulated with 1 μM PdBu in 1 ml EMEM supplemented with 10% FBS, 2 mM L-Glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin (Gibco BRL) for 2 h at 37°C. Media were thereafter transferred to Eppendorf tubes, snap frozen and stored at −70°C until assayed. Protein quantifications were performed by BCA Protein Assay (Pierce, USA) and the proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis [41] using 10% acrylamide gels. Proteins were transferred electrophoretically onto Protran nitrocellulose membrane (Schleicher and Schuell, Germany). After blocking for 1 h using 5% (w/v) dried milk in Tris-buffered

Table 1
Genotype, clinical status, age of patient when biopsy was taken, family and gender of individuals from whom cell lines were used

Patient/genotype	Disease status	Age (years)	Gender
Sporadic cases (mean age (±S.D.) 76±11 years)			
ITA 4	AD	82	F
ITA 5	AD	74	F
ITA 8	AD	86	F
AG06265	AD	61	M
Controls (mean age (±S.D.) 57±9 years)			
ITA 9	Control	45	M
ITA 17	Control	67	M
AG09878	Control	61	F
AG11362	Control	63	F
AG07123	Control	62	M
AG11154	Control	47	M
AG07139	Control	53	F
APP KM670/671NL (mean age (±S.D.): +/− 55±8 years; −/− 64±15 years)			
+/−	AD	66	F
+/−	AD	58	F
+/−	AD	64	M
+/−	Pre-symptomatic	42	F
+/−	Early symptomatic	53	M
+/−*	Pre-symptomatic	49	F
+/−	AD	54	M
−/−	Non-mutant	70	M
−/−	Non-mutant	62	M
−/−	Non-mutant	59	M
−/−	Non-mutant	52	F
−/−	Non-mutant	75	F
−/−	Non-mutant	88	F
−/−	Non-mutant	44	F
PS1 H163Y (mean age (±S.D.): +/− 46±11 years; −/− 45±16 years)			
+/−	AD	55	F
+/−	AD	59	M
+/−	Pre-symptomatic	31	M
+/−	Pre-symptomatic	44	M
+/−	Pre-symptomatic	42	M
−/−	Non-mutant	64	F
−/−	Non-mutant	35	M
−/−	Non-mutant	37	M
PS1 M146V (mean age (±S.D.): +/− 42 years; −/−55±9 years)			
+/−	AD	43	F
+/−	AD	41	F
−/−	Non-mutant	37	F
−/−	Non-mutant	39	M
−/−	Non-mutant	36	F
−/−	Non-mutant	30	F

Mean age (±S.D.) PS1 pooled: +/− 45±9 years; −/− 40±11 years. AD, Alzheimer's disease.
*See Fig. 2.

solution containing 0.1% Tween-20 (TBS-t), the membranes were incubated for 3 h at room temperature (RT) with a 2500-fold dilution of the primary monoclonal antibody 22C11 (Boehringer Mannheim, Germany). Nitrocellulose membranes were rinsed in TBS-t for 15 min and then three times for 5 min, prior to a 1-h incubation at RT with a 2000-fold dilution of the secondary antibody (anti-mouse horseradish peroxidase linked). Thereafter, the membranes were washed overnight and bands detected by the ECL method (Amersham, UK). The relative density of immunoreactive bands on Western blots was calculated from the area multiplied by the optical density of selected bands, following acquisition of the blot image through Image Master (Pharmacia Biotech). The Western blotting for APPs were performed once for the APP mutation-bearing cell lines and as three separate repeat experiments for the PS1 mutation-bearing cell lines.

3. Results

3.1. PKC activity

PKC activities were significantly decreased by ca. 50% in soluble fractions from sporadic Alzheimer's disease patients, compared to age-matched controls (Table 2). No significant differences were seen in soluble PKC activity between mutation-bearing and control cell lines established from individuals of the Swedish APP KM670/671NL double mutation family (Table 2). Similarly PKC activities in soluble fractions from cell lines with and without PS1 mutations were not significantly different (Table 2). PKC activities in the particulate fractions were below the assay detection limit.

Table 2
PKC activity in soluble fractions from sporadic, APP and PS1 mutation-bearing fibroblasts

Group	AD/Mut	Controls
Sporadics	86 ± 55*	174 ± 64
APP KM670/671NL	210 ± 97	206 ± 53
PS1 mutations	145 ± 50	129 ± 45

The mean values (± S.D.) are shown as pmol/min per mg protein. Significance was determined using Student's unpaired *t*-test; $*P < 0.05$ compared to non-mutation carriers or controls.

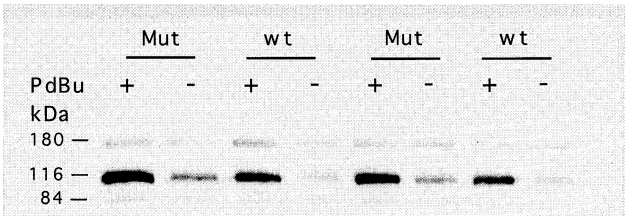

Fig. 1. A typical immunoblot showing the effect of PdBu (1 μM) on APPs secretion in human skin fibroblasts. The different lanes show stimulated (+) and control (−) levels of APPs from two individuals with (Mut) and two individuals without (wt) the APP KM670/671NL mutation.

3.2. PKC levels

[^{3}H]Phorbol-12,13-dibutyrate binding studies revealed no significant differences in PKC levels between sporadic Alzheimer's disease cases and controls in either soluble or particulate fractions (Table 3). Similarly, neither soluble or particulate fractions from APP KM670/671NL mutation-bearing cell lines showed differences in PKC levels, compared to controls (Table 3). In contrast, levels of PKC in soluble, but not in particulate, fractions from fibroblast cell lines with PS1 mutations were significantly increased by ca. 30% when compared with controls (Table 3).

3.3. PKC-stimulated APPs release

Fig. 1 shows a representative immunoblot from an experiment of basal and PdBu induced APPs secre-

Table 3
PKC levels in soluble and particulate fractions from sporadic, APP and PS1 mutation-bearing fibroblasts

Group	AD/Mut	Controls
Sporadics		
Soluble	4.69 ± 2.7	5.18 ± 1.5
Particulate	1.34 ± 0.7	2.76 ± 3.2
APP KM670/671NL		
Soluble	7.05 ± 2.4	6.56 ± 1.9
Particulate	2.21 ± 0.7	2.21 ± 0.4
PS1 mutations		
Soluble	9.80 ± 1.6*	7.45 ± 1.6
Particulate	2.22 ± 0.7	2.34 ± 0.7

The mean values (± S.D.) are shown as pmol/mg protein. Significance was determined using Student's unpaired *t*-test; $*P < 0.05$ compared to non-mutation carriers or controls.

tion. Western blotting of conditioned media from cultured skin fibroblasts using the monoclonal antibody 22C11 revealed the presence of a strong band of ~116 kDa. The intensity of this band increased after PdBu treatment, consistent with a PKC-mediated increase in α-secretase derived APPs.

Fig. 2 shows the optical density values for basal and PdBu stimulated APPs secretion from fibroblast cell lines established from family members with and without the Swedish APP KM670/671NL double mutation. The mean values (±S.D.) of basal APPs were 1.84±0.7 in mutation-bearing cell lines and 1.32±0.5 in control cells lines. Mean values (±S.D.) for secreted APPs following PKC stimulation with 1 μM PdBu were 6.36±5.2 and 7.00±3.1, respectively. The mean values (±S.D.) for the relative increases of APPs release after PdBu stimulation (following subtraction of basal levels) were 4.52±4.5 in mutation-bearing cell lines and 5.68±3.1 in the controls. No significant group differences were seen.

The mean values (±S.D.) of PKC-stimulated APPs release appeared to be lower in fibroblasts carrying PS1 mutations (3.63±0.9), compared to control cell lines (5.81±2.7) when data from both PS1 M146V and PS1 H163Y mutation families were pooled (Fig. 3). This result did not reach accepted levels of statistical significance. However, the relative increase of APPs after PdBu stimulation and following subtraction of basal release was shown to be significantly less in mutation-bearing cell lines (2.53±0.8), compared to controls (4.12±1.8, $P<0.05$, Mann–Whitney U-test). The mean values (±S.D.) for basal APPs release were 1.10±0.5 in cell lines carrying PS1 mutations, compared to 1.69±1.0 in cells lines from controls.

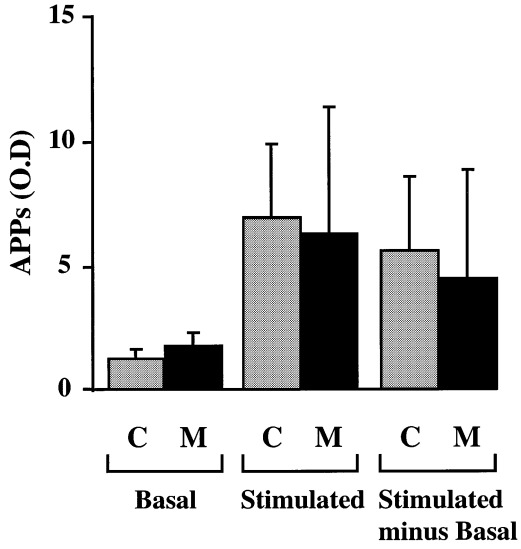

Fig. 2. Optical density (O.D) values for basal and PdBu (1 μM)-stimulated APPs secretion from fibroblasts carrying the APP KM670/671NL mutation ($n=6$; the cell line marked with an asterisk in Table 1 was not included in this study) and non-mutation carrying cell lines ($n=7$). Data are mean±S.D. (bars) values. No significant group differences were seen.

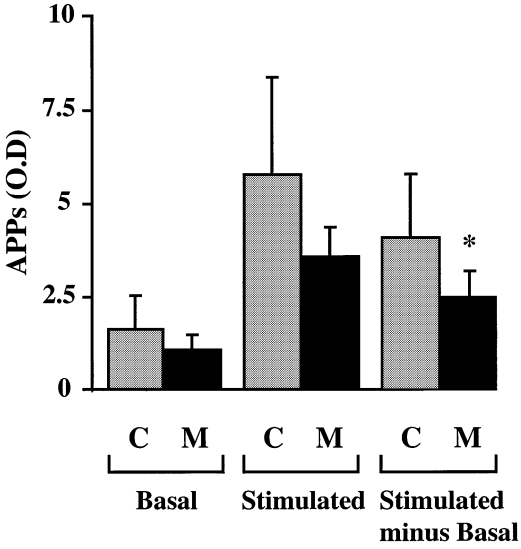

Fig. 3. Optical density (O.D) values for basal and PdBu (1 μM)-stimulated APPs secretion from fibroblasts carrying the PS1 M146V and PS1 H163Y mutations ($n=7$) and non-mutation carrying cell lines ($n=7$). Data are mean±S.D. (bars) values. Significance was determined using the Mann–Whitney U-test; $^*P<0.05$ compared to non-mutation carriers when data from both PS1 M146V and PS1 H163Y mutation families were pooled.

Data for PKC activities and levels, as well as APPs secretion were also analysed with respect to whether cell lines from APP and PS1 mutation carriers had been derived from pre-symptomatic or symptomatic individuals. No obvious differences were seen between pre-symptomatic and symptomatic individuals for any of the parameters assayed.

4. Discussion

One of the most robust findings of abnormal signalling in Alzheimer's disease is that of decreased PKC activity. This deficit has been shown by a num-

ber of techniques in post-mortem Alzheimer's disease brain [42], as well as in peripheral skin fibroblasts [33–35]. In the present study, we confirm the results from previous studies showing a 50% decrease of the PKC activity in fibroblasts established from patients with sporadic Alzheimer's disease, when compared to those from healthy controls [33,34]. This decreased PKC activity did not appear to be due to a loss of the protein per se, since the PdBu binding assays failed to show a decreased amount of PKC in sporadic Alzheimer's disease cytosolic and particulate fractions. In contrast, others have shown decreased amounts of PKC, as determined by PdBu binding, in sporadic Alzheimer's disease cell lines [34,35]. Govoni et al. reported no significant differences of the maximal binding capacities for PdBu binding between Alzheimer's disease and control groups in either soluble or particulate fractions [33]. However, these authors did show a significantly lower affinity for PdBu binding to PKC in the soluble fraction of sporadic AD fibroblasts ($K_d = 6.3$) when compared to controls ($K_d = 3.3$). Bruel et al. found that the B_{max} for PdBu binding to total PKC was reduced in sporadic Alzheimer's disease fibroblasts, using a saturating PdBu concentration of 75 nM [34]. In the present study we could find no difference in PdBu binding using a ligand concentration (10 nM) close to K_d. The use of different experimental methods might explain the discrepancies seen between the reports of Bruel et al., Govoni et al., Van Huynh et al. [35] and the data of the present study. Another explanation could be that even though skin fibroblasts are considered as a fairly robust cell type, there is a possibility that differences between laboratories in culturing conditions could influence the final result. One should also consider the importance of appropriate controls in these kind of studies. This is exemplified by the data presented in Table 2 which shows that soluble fraction PKC activities show considerable variation in control cell lines derived from different sources. For this reason, the familial skin fibroblasts we have used may provide more meaningful data, since the control and mutation-bearing cell lines were derived from within the same family.

In addition to confirming data from previous studies on sporadic Alzheimer's disease cell lines, we wanted to examine if both altered PKC activity and PKC mediated secretory processing of APP occurs in fibroblast cell lines bearing familial Alzheimer's disease causing mutations. Previous studies of PKC activity in familial Alzheimer's disease fibroblasts have used cell lines established from individuals with a history of Alzheimer's disease but in whom the chromosomal linkage and specific gene defect were unknown. In the present study we used cell lines with well defined APP and PS1 mutations. In addition, all familial cell lines used in this study were established by a single investigator (Dr. Nikolaos Venizelos) under similar conditions.

No differences in either PKC activity or levels were seen in fibroblasts carrying the Swedish APP KM670/671NL double mutation. We and others have shown that other aspects of cellular signalling in these fibroblasts, including β-adrenoceptor-stimulated adenylyl cyclase activity, internal calcium regulation and levels of the large Gsα subunit, are less affected than in fibroblasts established from sporadic Alzheimer's disease cases or fibroblasts carrying PS1 mutations [43–45]. Fibroblasts with the Swedish APP KM670/671NL mutation did not show an apparent change in PKC-mediated α-secretase APP processing which is in agreement with the findings of Citron et al [20]. Since there are no reports of an altered signalling in the family carrying the APP KM670/671NL mutation, it is likely that the increased amounts of Aβ seen in this family are due solely to the effects of the mutation on APP processing. The APP KM670/671NL mutation is situated at a site just N-terminal of the β-amyloid peptide were APP is cleaved by β-secretase. Haass and colleagues have hypothesised that the increased production and secretion of Aβ originated from Swedish APP KM670/671NL mutation is due to a competition between the α- and β-secretases that occurs in the Golgi-derived vesicles [46].

Pooling of data from both the PS1 M146V and PS1 H163Y mutation families revealed a significant 30% increase of PKC levels in mutation-bearing, compared to control cell lines. These changes in PKC levels did not appear to be accompanied by altered PKC activities. This observation of increased PKC levels without a change in enzyme activity would suggest an increased production of inactive PKC protein in PS1 mutation-bearing fibroblasts.

The mechanism by which PS1 mutations shift APP

processing into the more amyloidogenic way resulting in increased Aβ 1–42 levels remains to be resolved. It was shown recently that PS1 is involved in the γ-secretase-mediated proteolytic cleavage of APP, and it has been hypothesised that PS1 might function in some way as a regulatory cofactor of the γ-secretase [47].

The relative increase of APPs secretion after PdBu stimulation was shown to be significantly lower in PS1 mutation-bearing cell lines, compared to controls, when data was controlled for basal release. This indicates that PS1 mutations result in a decreased PKC-mediated α-secretase APP processing, independently of gross alterations in PKC levels and activity. Ancolio et al. recently showed that HEK293 cells transfected with mutated PS1 secreted lower amounts of α-secretase derived APPs [48]. The results of the present study provide an important confirmation that decreased α-secretase derived APP due to PS1 mutations does not represent an artefact due to PS overexpression. Together, these results imply that PS1 mutations limit the amount of APP available for PKC-mediated α-secretase APP processing and therefore shift the APP processing into an amyloidogenic pathway leading to increased amount of Aβ.

In summary, previous studies of PKC-regulated APP processing in Alzheimer's disease have been carried out in cell lines established from sporadic Alzheimer's disease cases or in transfected cell lines of different origin. In the present study we demonstrate that the levels and activity of PKC as well as the PKC-mediated α-secretase processing of APP are differentially affected in skin fibroblasts established from sporadic Alzheimer's disease cases when compared to cell lines established from individuals with familial Alzheimer's disease APP KM670/671NL, PS1 M146V and PS1 H163Y mutations. When taken together with results from other studies, our data suggest that in sporadic cases a decreased PKC activity is likely to limit non-amyloidogenic APP processing leading to increased Aβ production. In the APP KM670/671NL mutation bearing cell lines it is the mutation itself, rather than a deficit in PKC, that gives rise to increased levels of Aβ seen in affected family members. Similarly, the reported increase in Aβ production from PS1 mutation-bearing lines [25–28] occurs due to an effect of the mutation on APP processing [47] rather than due to a gross PKC deficit. Therefore, many different mechanisms could lead to a common pathology of increased Aβ in sporadic and familial forms of Alzheimer's disease.

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