Amino Acids (2006) 31: 449–456
DOI 10.1007/s00726-005-0272-z

Amino Acids
Printed in Austria

Brain G protein-dependent signaling pathways in Down syndrome and Alzheimer's disease

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Received October 31, 2005
Accepted November 2, 2005
Published online April 4, 2006;

Summary. Premature aging and neuropathological features of Alzheimer's disease (AD) are commonly observed in Down syndrome (DS). Based on previous findings in a DS mouse model, the function of signaling pathways associated with adenylyl cyclase (AC) and phospholipase C (PLC) was assessed in cerebral cortex and cerebellum of age-matched adults with DS, AD, and controls. Basal production of cAMP was reduced in DS but not in AD cortex, and in both, DS and AD cerebellum. Responses to GTPγS, noradrenaline, SKF 38393 and forskolin were more depressed in DS than in AD cortex and cerebellum. Although no differences in PLC activity among control, DS and AD cortex were observed under basal and GTPγS- or Ca-stimulated conditions, the response of DS cortex to serotonergic and cholinergic stimulation was depressed, and that of AD was only impaired at cholinergic stimulation. No differences were documented in cerebellum. Our results demonstrate that PLC and AC were severely disturbed in the aged DS and AD brains, but the alterations in DS were more severe, and differed to some extent from those observed in AD.

Keywords: Adenylyl cyclase – Phospholipase C – Cerebral cortex – Cerebellum – Down syndrome – Alzheimer's disease

Introduction

Down syndrome (DS) is the most common genetic form of mental retardation and occurs with an incidence of 1 in 700–1000 live births. It results from trisomy of chromosome 21, 95% of which are full trisomies being the remainder mosaics or partial trisomies (Antonarakis, 1997; Bar-Peled et al., 1991; Hassold et al., 2002). DS is thus, a model of complex and multigenic disorder due to chromosome imbalance.

One of the most obvious features of DS is premature aging, with many DS individuals often developing an early onset of Alzheimer's-type neuropathology by the fourth decade of life. These neurodegenerative changes are characterized by progressive accumulation of senile plaques and neurofibrillary tangles, and occur with similar regional distribution as in Alzheimer's disease (AD) (Wisniewski et al., 1985; Mann et al., 1985; Cork, 1990). As in AD, there is a correlation between the apolipoprotein E4 genotype (Alexander et al., 1997) oestrogen deficiency and high levels of Aβ1–42 and cognitive decline in DS subjects, while the apolipoprotein E epsilon2 allele was associated with reduced mortality and reduced risk of dementia (Menéndez, 2005). Moreover, in the DS brain, choline acetyl transferase activity is decreased in the same regions than in AD brain (Jope et al., 1997). However, although the genotype-phenotype correlation analysis show a quite clear relation between APP triplication and the occurrence of AD in DS, several works show marked differences in the pathogenesis of both diseases. In this regard, chronic overexpression of S100β present in DS may confer increased risk for later development of Alzheimer's disease (see Mrak and Griffin, 2004). Moreover, BACE2, the beta-amyloid cleaving enzyme-2, haplotype has shown association with AD in two independent datasets. These results provide further evidence for an AD susceptibility locus on chromosome 21q within or close to BACE2 that may not only contain APP (Myllykangas et al., 2005). Moreover, expression of mRNA molecular markers of AD are not found prior to neurofibrillary tan-

gle formation in aged DS brain, suggesting that changes in these messages are not required for tangle formation in DS (Goodison et al., 1993).

Numerous studies have revealed that neurotransmission is compromised in AD brains, due to disrupted post-receptor signal transduction, in particular that mediated by G-protein regulated adenylyl cyclase (AC) and phosphoinositide hydrolysis, linked to phospholipase C (PLC) pathways (Cowburn et al., 2001; Fernhall and Otterstetter, 2003). There are no data, however, on the functioning of these signaling systems in DS brains, although consistent changes found in myo-inositol and phospholipid composition of fetal and adult DS brain membranes (Brooksbank and Martinez, 1989; Mann et al., 1985; Murphy et al., 2000; Shetty et al., 1995) may predict disregulation of G-protein linked transduction processes.

In fact, we could determine in an animal model of DS, the Ts65Dn mouse (Davisson et al., 1997; Dierssen et al., 1999, 2005), a reduced ability of several brain areas to respond to the indirect (receptor-mediated) and direct stimulation of the AC (Dierssen et al., 1996, 1997) and PLC (Ruiz de Azúa et al., 2001) signaling systems. The present experiments were aimed, therefore, at elucidating the functioning of the AC and PLC signaling pathways in brain cortical and cerebellar membranes of DS adults, in comparison with AD and control samples of matched ages, in order to establish the functional status and similarities between these related pathologies. We have explored the changes induced in adenylyl cyclase activity by determining cAMP formation in isolated membranes under basal conditions and after stimulation with GTPγS (a non hydrolyzable analog of GTP), the adrenoceptor agonist noradrenaline, the D_1-agonist SKF38393, and forskolin. The phosphatidylinositol 4,5-bisphosphate (PIP_2) breakdown was studied after stimulation of neurotransmitter receptors associated to G-protein coupled to PLC using the muscarinic agonist carbachol and the 5-HT_2 agonist 5-methyltryptamine, GTPγS, and calcium.

Materials and methods

Materials

Adenosine 3′,5′-cyclic monophosphate, 3-isobutyl-1-methyl-xantine, noradrenaline, forskolin and bovine serum albumin were supplied by Sigma Chemical Co. (Spain). Phenoxybenzamine was obtained from Smith Kline & French Laboratories, Philadelphia (USA). (^{3}H)CGP12-177 and (^{3}H) cyclic AMP assay system was supplied by Amersham Biosciences (Spain) and liquid scintillation OptiPhase Hisafe II by LKB-FSA, Loughborough (England). Carbachol (Cch), 5-methyltryptamine (5-MT), phosphatidylinositol 4,5-bisphosphate (PIP_2), and ATP were obtained from Sigma Chemical Co. (Spain). Guanosine 5′-0-(3-thiotriphosphate) (GTPγS) from Boehringer Mannheim (Spain), and (^{3}H)PIP_2 (specific activity 6 Ci/mmol) from Amersham Biosciences (Spain). All other chemicals were obtained from Sigma Chemical Co. (Spain), and Bio-Rad (Spain).

Table 1. Description of tissues samples

Group	Characteristics	Control	Down syndrome	Alzheimer's disease
1	Sample	Cerebral cortex	Cerebral cortex	Cerebral cortex
	Age	56 years	54 years	54 years
	Sex	Male	Male	Male
	Postmortem time	51 hours	9 hours	70 hours
2	Sample	Cerebral cortex	Cerebral cortex	Cerebral cortex
	Age	57 years	57 years	60 years
	Sex	Male	Male	Male
	Postmortem time	21 hours	31 hours	25 hours
3	Samples	Cerebral cortex Cerebellum	Cerebral cortex Cerebellum	Cerebral cortex Cerebellum
	Age	49 years	46 years	50 years
	Sex	Male	Male	Male
	Postmortem time	44 hours	72 hours	10 hours
4	Samples	Cerebral cortex Cerebellum	Cerebral cortex Cerebellum	Cerebral cortex Cerebellum
	Age	69 years	69 years	68 years
	Sex	Male	Male	Male
	Postmortem time	24 hours	30 hours	27 hours

Cerebral samples

Twelve adult human brains were obtained from The London Neurodegenerative Disorders Brain Bank, Institute of Psychiatry (Denmark Hill, London, U.K.) (Table 1). Individuals with AD (4 males, 50–68 years old) satisfied the established standards of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA). Individuals with DS presented full trisomy 21, confirmed by karyotype (4 males, 46–69 years old). Control brains (4 males, 49–69 years old) were selected randomly and presented no history of neuropsychiatric pathology. The causes of death in controls, DS and AD were pneumonia or heart failure. Individuals with AD satisfied the established standards of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA). All procedures were performed with the consent of the hospital ethical committee and following dissection all samples were stored at −80 °C and the freezing chain was never interrupted.

Preparation of crude plasma membranes

Frozen brain samples were thawed in ice-cold 20 mM Tris-HCl buffer, pH 7.0, containing 1 mM EGTA (Tris/EGTA buffer and homogenized in 20 volumes of the same hypotonic buffer using a glass homogenizer with a Teflon pestle (20 strokes with a motor-driven pestle at maximum setting). A crude plasma membrane preparation was isolated by repeated centrifugations and re-homogenizations in the hypotonic buffer as described previously for human and rat cerebral cortex (Sallés et al., 1993; Wallace and Claro, 1993). Briefly, the homogenate was centrifuged for 15 min at 40,000 × *g*. The pellet was then re-suspended in Tris/EGTA

buffer, re-homogenized, and centrifuged again. This procedure was repeated twice more. We aliquot the membranes in micro centrifuge tubes and kept the pellets at −80 °C. Protein were measured using the Bio-Rad dye reagent.

Cyclic AMP assay

The method of Slotkin (1998) was modified by substituting GTP for GTPγS. The described membrane preparations were diluted 20-fold with 250 mM sucrose, 1 mM ethylene glycol bis (b-aminoethylether)-N,N′-tetracetic acid and 10 mM Tris (pH 7.4). Aliquots of membrane preparation containing 40 mg of protein were then incubated for 10 min at 30 °C with final concentrations of 100 mM Tris-HCl (pH 7.4), 1 mM adenosine 5′-triphosphate, 10 mM $MgCl_2$, 1 mg bovine serum albumin and a creatine phosphokinase-ATP-generating system consisting of 10 mM sodium Phosphocreatine, 8 I.U. of phosphocreatine kinase and 10 mM GTPγS in a total volume of 250 ml. The enzymatic reaction was stopped by placing the samples in a 90 °C water bath for 5 min, followed by sedimentation at 3000 × g for 15 min. The supernatant solution was assayed for cyclic AMP using (^{3}H) cAMP radioimmunoassay kits. 0.2 ml aliquots of the upper aqueous phases were mixed with 4 ml of Optiphase "Hi-Safe" for scintillation counting.

Preliminary experiments showed that the enzymatic reaction was linear with membrane protein concentration well beyond the 10 min time period. Concentrations of cofactors were optimal, and, in particular, addition of higher concentrations of GTPγS produced no further augmentation of activity. In addition to evaluating basal activity, the maximal total activity of the adenylyl cyclase catalytic unit was evaluated in response to forskolin (100 mM) plus Cl_2Mn (10 mM). Protein G stimulation of activity was determined by using GTPγS (10 mM); stimulation via Gs was induced by the β-adrenergic agonist noradrenaline (100 mM), and the D_1 dopaminergic agonist SKF 38393 (10 mM).

Phospholipase C assay

Membrane pellets were re-suspended at a concentration of 2.5 mg protein/ml in a cold buffer consisting of 25 mM Tris-maleate, 5 mM ATP, 15 mM $MgCl_2$, and 25 mM LiCl, pH 6.8 (adjusted with KOH). The reactions (100 ml total volume) were initiated by adding 40 ml of membrane preparations (100 mg protein) to tubes with 25 mM Tris-maleate, pH 6.8, containing (^{3}H) PIP2 (final concentration 30 mM), 1 mM sodium deoxycholate, 3 mM EGTA and $CaCl_2$ necessary to yield different free calcium concentrations (Harafugi and Ogawa, 1980). The function of PLC was assessed by measuring the hydrolysis of exogenous labeled phosphoinositides (^{3}H) PIP2 and the production of (^{3}H) inositol phosphates under basal conditions (50 nM calcium free) and after stimulation of neurotransmitter receptors and G-protein-coupling to PLC with the nonhydrolyzable analog of GTP, GTPγS (10 mM), the muscarinic agonist carbachol (1 mM), the 5-HT_2 agonist 5-methyltryptamine (300 mM), and calcium (10 mM).

The reactions were stopped with 1.2 ml chloroform/methanol (1:2, v/v), then 0.5 ml each of chloroform and 0.25 M HCl were added to create two phases, and were thoroughly vortexed. The phases were allowed to separate and then, after centrifugation, 1 ml aliquots of the upper aqueous phases containing (^{3}H) inositol phosphates were mixed with 4 ml of Optiphase "Hi-Safe" for scintillation counting.

Data analysis

PLC experiments were carried out, at least, two times while the adenylyl cyclase assay was carried out, at least, four times, each by triplicate and with the experimental groups in parallel (Table 1). Cyclic AMP levels were calculated as pmol of cAMP formed per mg of protein per minute, and the breakdown of (^{3}H) PIP2 by PLC was calculated as pmol of IP3 formed per mg of protein per minute. Data were expressed as means ± S.E.M. Significance of the overall effect of the drugs was determined by two-way ANOVA followed by the Duncan's multiple range tests and comparisons between groups were evaluated using Student's t-test (two-tailed). The level of significance was set at $P < 0.05$. Statistical analysis was performed with SPSS program.

Results

Regulation of adenylyl cyclase activity by GTPγS and agonists in brain cortical membranes

Basal production of cAMP in cortical membranes of control and AD brains was similar, whereas that of DS brains was significantly lower ($F(2,125) = 20.28$; $P < 0.0001$) (Fig. 1A). After stimulation by GTPγS, cAMP accumulation was significantly increased compared to the basal state in all groups (control: $F(3,160) = 48.12$; $P < 0.00001$. AD: $F(3,160) = 19.54$; $P < 0.00001$. DS: $F(3,160) = 42.79$; $P < 0.00001$). Significant differences among the three groups were detected ($F(2,125) = 38.65$; $P < 0.0001$), so that the percent increments in the AD and DS groups were

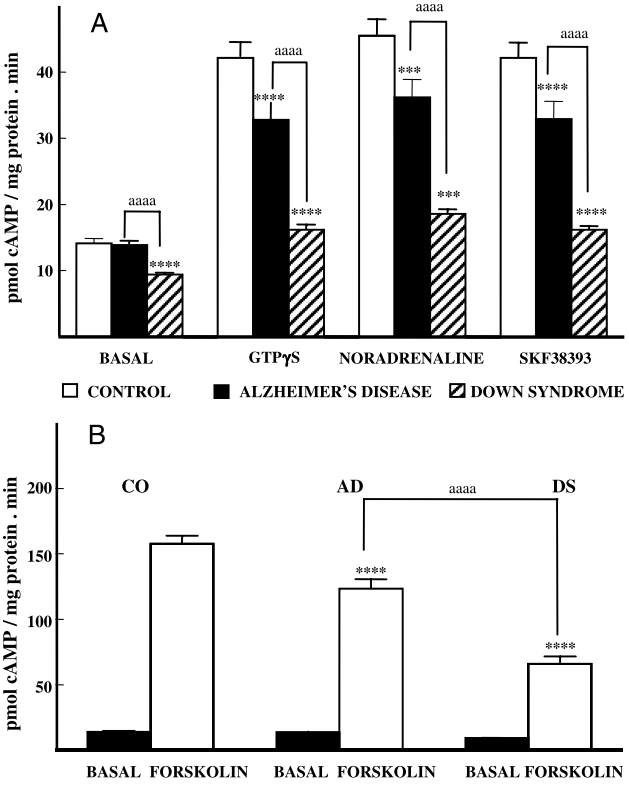

Fig. 1. Cyclic AMP production in cerebral cortex of control (CO), Alzheimer's disease (AD) and Down syndrome (DS) brains under basal conditions and after stimulation with different drugs. **A** Responses to GTPγS (10 mM), noradrenaline (100 mM), and SKF38393 (10 mM). $^{****}P < 0.0001$, AD and DS versus CO; $^{aaaa}P < 0.0001$, DS versus AD. **B** Responses to forskolin (100 mM). $^{****}P < 0.001$, AD and DS versus CO; $^{aaaa}P < 0.001$, DS versus AD

significantly smaller compared to control (139.9 ± 17% and 77.6 ± 9.3% vs. 208.9 ± 16.7%, respectively ($F(2,125) = 19.18$; $P < 0.0001$). The order of response values were DS < AD < control, DS values being significantly smaller than AD values.

Stimulation of the β-adrenergic and D_1 receptors by noradrenaline (100 μM) and SKF 38393 (10 μM), respectively, in GTPγS-treated membranes, did not elicit further accumulation of cAMP, but the significant differences in the responses among groups were maintained (Fig. 1A): noradrenaline ($F(2,125) = 41$; $P < 0.00001$); SKF 38393 ($F(2,125 = 40.31$; $P < 0.00001$).

The response to stimulation of the catalytic subunit of adenylyl cyclase with forskolin (10 μM) was also tested (Fig. 1B). Forskolin induced a significant increase in cAMP levels of cortical membranes in all groups, compared to basal levels: control ($F(4,205) = 293.1$; $P < 0.00001$), AD ($F(4,205) = 130.85$; $P < 0.00001$), and DS ($F(4,205) = 78.91$; $P < 0.00001$). Significant differences, however, were observed among the increments attained in each group ($F(2,125) = 54.05$; $P < 0.00001$), the order of responses being: SD < AD < control. SD values were significantly lower than AD values ($P < 0.001$).

Regulation of adenylyl cyclase activity by GTPγS and agonists in brain cerebellar membranes

In contrast to cortical membranes, basal production of cAMP was much lower in both AD and DS than in control cerebellar membranes ($F(2,62) = 20.56$; $P < 0.0001$) (Fig. 2A). GTPγS induced similar increments of cAMP production in the three cerebellar membranes of all groups (150.8 ± 22.71%, 119.5 ± 16.9% and 114.5 ± 13.8% in control, AD and DS, respectively, $P = 0.32$), although the actual values showed significant differences between the three groups ($F(2,62) = 68.54$; $P < 0.00001$). The agonists noradrenaline and SKF 38393 did not elicit further stimulation over that induced by GTPγS. In these conditions, cAMP accumulation was much lower for AD and DS than for control membranes: noradrenaline ($F(2,62) = 52,71$;

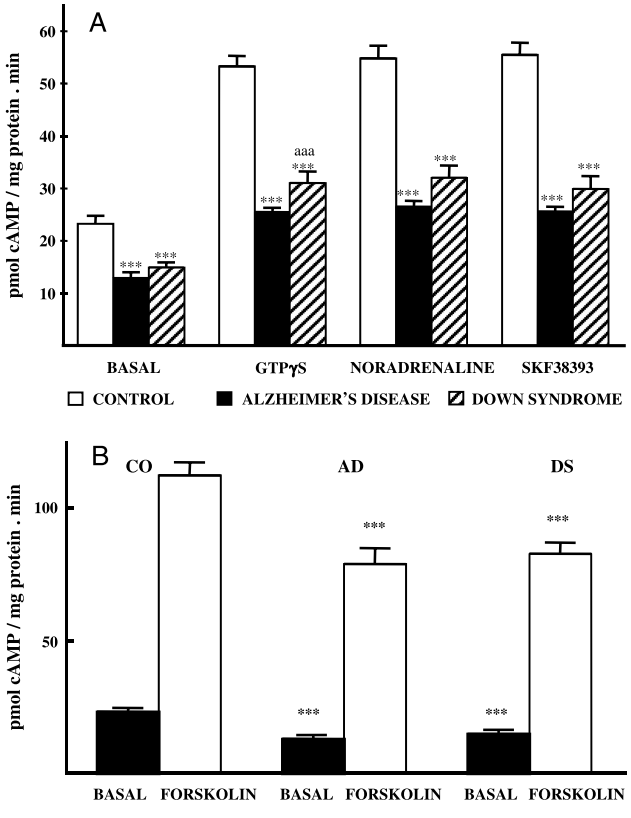

Fig. 2. Cyclic AMP production in cerebellum of control (CO), Alzheimer's disease (AD) and Down syndrome (DS) brains under basal conditions and after stimulation with different drugs. **A** Responses to GTPγS (10 mM), noradrenaline (100 mM), and SKF38393 (10 mM). ***P < 0.001, AD and DS versus CO; aaaP < 0.001, DS versus AD. **B** Responses to forskolin (100 mM). ***P < 0.001, AD and DS versus CO

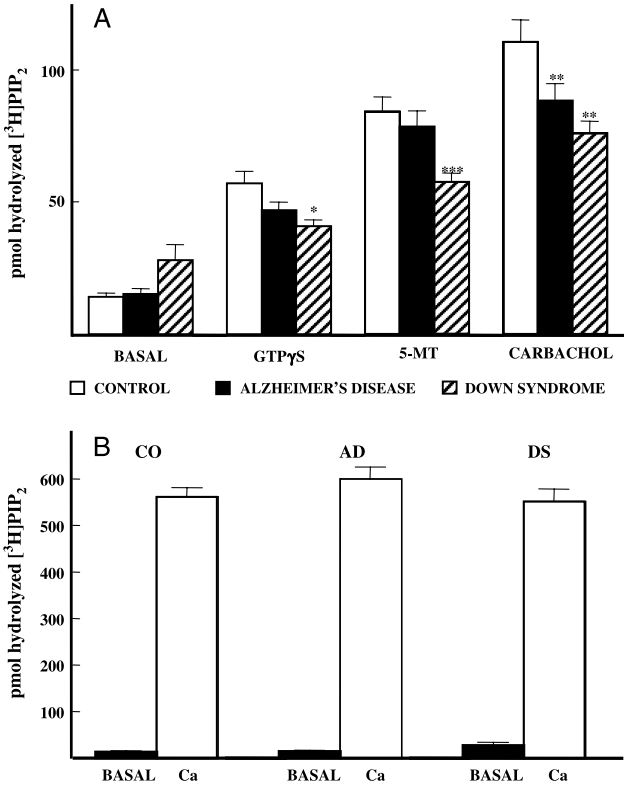

Fig. 3. PLC activity in cerebral cortex of control (CO), Alzheimer's disease (AD) and Down syndrome (DS) brains under basal conditions and after stimulation. **A** Responses to GTPγS (3 mM), 5-methyltryptamine (5-MT) (300 mM) and carbachol (1 mM). *P < 0.05, DS versus CO; **P < 0.01, DS and AD versus CO; ***P < 0,001 DS versus CO and AD. **B** Responses to calcium (10 mM). No significant differences were observed among the three groups

$P < 0.00001$), SKF 38393 ($F(2,62) = 63.83$; $P < 0.00001$). Direct stimulation of adenylyl cyclase with forskolin elicited marked increments of cAMP production in the three groups (382%, 507% and 450% in control, AD and DS cerebellum respectively ($F(2,62) = 12.8$, $P < 0.0001$) (Fig. 2B), but the attained actual values were significantly lower in AD and DS than in control brains ($P < 0.001$).

Regulation of PLC activity by GTPγS and agonists in brain cortical membranes

Values of basal breakdown of (^{3}H)-PIP_2 were similar in control, AD and DS brain cortical membranes ($P = 0.16$) (Fig. 3A). Based on previous concentration-response studies carried out in rat and human brain cortical crude membranes, a concentration of 3 μM GTPγS plus 300 μM 5-MT or plus 1 mM carbachol was selected to activate PLC activity. GTPγS (3 μM) clearly stimulated basal (^{3}H)-PIP_2 breakdown in the three groups, DS ($F(3,31) = 27.05$, $P < 0.00001$); AD ($F(3,31) = 12.62$, $P < 0.00001$) and CO ($F(3,31) = 27.05$, $P < 0.00001$) but the response of DS brains was significantly lower than that of control brains ($F(2,63) = 3.94$; $P = 0.025$). Further stimulation to that observed after GTPγS was elicited by both 5-MT and carbachol in the three groups of brains, the responses to carbachol being higher than to 5-MT in all groups. However, the responses to 5-MT were significantly lower in DS than in control and AD brains ($F(2,63) = 7.43$; $P = 0.001$), and those to carbachol were lower in both AD and DS than in control brains ($F(2,63) = 6.67$, $P = 0.002$) (Fig. 3A). DS brains showed consistently the lowest level of activation. To investigate if the variations of PLC activity among groups were due to alterations in the sensitivity to calcium or levels of PLC activities, the response of PLC to maximal concentration of calcium (10 μM) was examined. As shown in Fig. 3B, calcium produced significant increments in total PLC activity that, as occurred in basal conditions, were similar in control, AD and DS cortical membranes ($P = 0.338$).

Regulation of PLC activity by GTPγS and agonists in brain cerebellar membranes

Since our previous experiments had shown that cerebellum of Ts65Dn mice, a model for DS, had severe deficiencies in PLC activation after stimulation of G-protein coupled receptors, PLC activity was evaluated in cerebellar samples (Fig. 4). Basal activity of PLC in cerebellar membranes was higher than in cortical membranes for all groups (Student t-test, control: $P < 0.0001$; AD: $P = 0.001$; DS: $P = 0.003$); however, the activation of G-proteins with 3 μM GTPγS stimulated (^{3}H)-PIP_2 hydrolysis to a significantly lower extent in cerebellar than in cortical membranes ($P < 0.01$). 5-MT failed to stimulate further the (^{3}H)-PIP_2 hydrolysis over that induced by GTPγS in control and AD cerebellar membranes, but significantly stimulated in DS cerebellum ($F(4,42) = 32.41$; $P < 0.001$). On the other hand, carbachol stimulated (^{3}H)-PIP_2 hydrolysis by $156 \pm 33\%$ ($F(4,42) = 27.61$; $P < 0.00001$) in control and by $55.2 \pm 25\%$ ($F(4,42) = 21.6$; $P < 0.00001$) in AD cerebellar membranes over the GTPγS-stimulated values, whereas no further stimulation was elicited in DS cerebellum. Calcium (10 μM) produced a significant and similar increase in total PLC activity of the cerebellar membranes in the three groups ($P < 0.0001$). This increment, however, was much lower than that attained in cortical membranes.

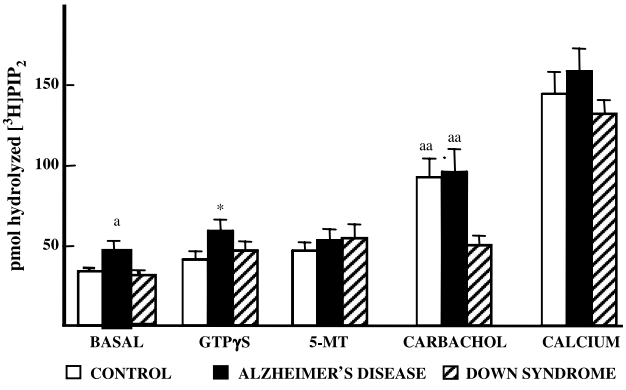

Fig. 4. PLC activity in cerebellum of control (CO), Alzheimer's disease (AD) and Down syndrome (DS) brains under basal conditions and after stimulation with GTPγS (3 mM), 5-methyltryptamine (5-MT) (300 mM), carbachol (1 mM) and calcium (10 mM). $^*P < 0.05$, AD versus CO; $^{aa}P < 0.01$, DS versus CO and AD

Discussion

The present experiments reveal consistent disturbances in the G protein-associated signal transduction processes in cerebral cortex and cerebellum of adults with DS and AD and delineate differences in these genetically-related neurodegenerative processes.

When analyzing the adenylyl cyclase system, we observed that basal production of cAMP was significantly reduced in DS but not in AD cerebral cortex, whereas it was decreased in both DS and AD cerebellum. The response to stimulation with GTPγS, noradrenaline, SKF 38393 and forskolin was also markedly depressed in DS and AD cerebral cortex and cerebellum, DS brains being

consistently less responsive than AD brains. Our results confirm previous studies reporting reduced stimulation by GTPγS and aluminum fluoride in several areas of the cerebral cortex and cerebellum and impaired β-adrenoceptor-G protein coupling in AD temporal and frontal cortices (Cowburn et al., 2001). These effects could be determined by the significant reduction of the adenylyl cyclase type I and II isoforms documented in AD parietal cortex (Yamamoto et al., 1997). Reduced forskolin-stimulated enzyme activity has been also shown in AD hippocampus (Ohm et al., 1991).

In DS we observed a severe deficiency in adenylyl cyclase activity in both cerebral cortex and cerebellum, showing a very poor response to various stimulatory signals, including forskolin. Preliminary data obtained in the cortex of children with DS have revealed normal levels of basal and GTPγS- and β-agonist-stimulated adenylyl cyclase activity, but reduction in the response to forskolin (unpublished observations). Moreover, the deficits observed are consistent with those we reported in the frontal and hippocampal cortices but not in cerebellum of young adult mice, in an animal model of DS, the Ts65Dn mouse (10, 11). Thus the impairment is observed at different ages, and both in human and in trisomic mouse models. The reduced adenylyl cyclase activity possibly participates in the decreased activities of downstream PKA and some of its substrates, such as synapsin I, CREB and ARPP-19 (cAMP-regulated phosphoprotein of Mr = 19,000), reported in hippocampus and temporal cortex of AD patients (Kim et al., 2001; Parks et al., 1991; Perdahl et al., 1984; Yamamoto-Sasaki et al., 1999). It is difficult to ascertain if this is a specific feature of aged DS brains or if AD-like neuropathological features are adding to the DS condition, since there are common genetic factors involved in AD and DS pathology, such as APP, BACE2, SOD1 or apolipoprotein E4. Our data provide first direct evidence of severe disturbances in the adenylyl cyclase-signaling pathway presumably leading to decreased expression of cAMP-dependent gene expression.

We have extended our studies to analyze the function of a phosphoinositide-specific PLC, the other major effector system by which neurotransmitter receptors trigger the formation of second messengers in mammalian brain. While phosphoinositide signaling has been widely studied in cortical regions of AD brains (Cowburn et al., 2001; Crews et al., 2002; Jope et al., 1997), there are no data in DS adult brains. Previous studies performed in Ts65Dn mice showed reduction in PLC activity (basal and GTPγS-stimulated conditions), specifically related to a lower level of expression of the PLC-β4 isoform (Ruiz de Azúa et al., 2001).

Our present studies showed that basal and maximal response to calcium stimulation of (^{3}H)-PIP_2 hydrolysis was preserved in cerebral cortex and cerebellum of DS and AD patients. However, in DS brains there was a significant reduction in the responses of cerebral cortex to stimulation by GTPγS and by serotonergic and cholinergic agonists, as well as in the response to carbachol in the cerebellum, compared to control. On the other hand, in AD there was a modest reduction in the response to 5-MT and a significant reduction in the response to carbachol. These results in AD brains confirm previous findings on agonist-stimulated phosphoinositide hydrolysis (Crews et al., 2002). The lower (^{3}H)-PIP_2 hydrolysis in response to carbachol that we have observed in both DS and AD brains, could be followed by reduction in diacylglycerol production and subsequent PKC activation, thus disturbing α-secretase-mediated cleavage of APP while favoring the Aβ production and subsequent apoptosis, which is a consistent brain feature in DS and AD pathology. Since activated PKC enhances Bcl-2 expression and may thus have a neuroprotective effect on AD-related apoptosis, disturbances in PLC system may increase apoptosis both increasing Aβ-related apoptosis and reducing neuroprotection.

The reduced response to 5-MT stimulation that we observe in DS brains may be related to the loss of serotonin observed in DS and AD postmortem brain (Mann et al., 1985). In DS patients with AD-like pathology, there is a region-specific change in serotonin neurotransmission, with higher amounts of serotonin transporter in frontal and occipital cortices (Gulesserian et al., 2000), while significant reductions occur in thalamus, caudate, cerebellum, and temporal cortex (Shemer et al., 1991). The serotonergic deficit in DS brains may not be related solely related to the AD-like neurodegenerative process, since developmental alterations are also present in DS serotonergic system. In this regard, the 5-HT_{1A} receptor has been shown to decrease below normal levels by birth in developing DS brains (Bar-Peled et al., 1991; Engidawork and Lubec, 2003). The present findings may contribute to delineate some differences in the neurotransmitter systems affected by DS and AD and to get insight into the underlying causes of serotonergic dysfunction in DS. Indeed, the concurrent reduction of responsiveness to cholinergic and serotonergic stimulation observed in DS brains reflects the impairment in the function of the PLC pathway, and must limit severely its contribution to the numerous and distinct signaling complexes and micro-

domains that ensure speed and specificity of signal transduction events (Delmas et al., 2004). In DS there is a significant and age-related loss of cholinergic and serotonergic neurons that project to some cortical areas (Mann et al., 1985; Seidl et al., 1999). Altogether, the impaired pre- and postsynaptic abilities to convey cholinergic and serotonergic information may explain some of the cognitive and behavioral features associated with aging in DS, such as the decline in speech and memory and the behavioral depression.

The finding of dysregulated G-protein-associated signal transduction in cerebral cortex (and cerebellum) of adults with DS and AD may be a crucial factor to determine the impairment of cognitive functions. It is well known and documented that PLC and adenylyl cyclase systems are main determinants for the generation of neuronal information storage. Derangement of these systems may therefore help to explain the cognitive deficits in aged DS and AD patients. Adenylyl cyclase activity has been proposed to be critical in the formation of memory. Therefore, genetic alterations that have consequences on the functioning of this second messenger system may lead to impairment of information processing. In this regard, it must be pointed out that specific learning deficits associated to hippocampal and cortical functions typically emerge in individuals with DS. The data also suggest the possibility that drugs or other manipulations that stimulate expression or activity of adenylyl cyclase and PLC may prove of therapeutic benefit in AD patients.

Acknowledgements

This study was supported by grants from the Spanish Ministry of Education (SAF-2002-02178), Fundación Marcelino Botín (Spain) and Fondation Jèrôme Lejeune (France). The technical assistance of María José Rozas is gratefully acknowledged.

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