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Rapid communication

Dose-dependent and sequence-sensitive effects of amyloid-β peptide on neurosteroidogenesis in human neuroblastoma cells

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Abstract

Interactions between neurosteroidogenesis and proteins involved in age-related diseases are unknown. High concentrations of amyloid-β (Aβ) peptides induce plaques in Alzheimer's disease but several studies demonstrated that physiological or non-toxic doses are neuroprotective. We compared the effects of non-toxic and toxic concentrations of $A\beta_{1-42}$ and $A\beta_{25-35}$ on neurosteroidogenesis in human neuroblastoma SH-SY5Y cells. Viability assays revealed that nanomolar doses of Aβ are devoid of cytotoxicity while 12 μM induced cell death. Pulse-chase, high-performance liquid chromatography and flow-scintillation analyses showed that non-toxic $A\beta_{1-42}$ concentrations, acting selectively, decreased [^{3}H]progesterone but increased [^{3}H]estradiol production from the precursor [^{3}H]pregnenolone. Non-toxic $A\beta_{25-35}$ doses reduced [^{3}H]progesterone formation but had no effect on [^{3}H]estradiol biosynthesis. At 12 μM, both $A\beta_{1-42}$ and $A\beta_{25-35}$ inhibited [^{3}H]progesterone formation but only $A\beta_{1-42}$ reduced [^{3}H]estradiol production. The results demonstrate a selective and amino-acid sequence-dependent action of Aβ on neurosteroidogenesis. The fact that non-toxic $A\beta_{1-42}$ doses stimulated neuroprotective-neurosteroid estradiol synthesis, which is inhibited by high $A\beta_{1-42}$ doses, may explain $A\beta_{1-42}$ ability to exert either protective or deleterious effects on nerve cells.
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Keywords: Amyloid-β peptide; Cell survival; Cell viability; Neuroactive steroid; Neurodegenerative disease; Neuroprotection; Steroids and nervous system

Although neurosteroidogenesis was evidenced in humans, little is known on the role of endogenous neurosteroids in the regulation of cellular mechanisms involved in human nervous system pathophysiology (Baulieu et al., 1999; Stoffel-Wagner, 2001). In particular, post-mortem quantifications suggested a possible correlation of decreased brain levels of neurosteroids with Alzheimer's disease (AD)-related dementia (Weill-Engerer et al., 2002) but the direct interactions between neurosteroidogenesis and the activity of key etiological factors/proteins involved in age-related diseases had never been investigated. AD, the most frequent neurodegenerative disease in humans, is characterized by an amyloidogenic pathway responsible for β-amyloid plaques which constitute with neurofibrillary tangles the pivotal lesions (Kosik, 1992; Selkoe, 2001; Goedert and Spillantini, 2006). High concentrations of Aβ (≥5 μM) are well-known to generate cytotoxicity and cell death through mechanisms involving hydrogen peroxide (H_2O_2)-evoked oxidative stress which is considered as a pivotal causal factor in AD (Miranda et al., 2000). Moreover, we have recently observed that H_2O_2 kills human neuroblastoma SH-SY5Y cells by down-regulating the biosynthesis of the neuroprotective-neurosteroid estradiol, suggesting that toxic concentrations of Aβ may be a negative regulator of neurosteroidogenesis in human nerve cells (Schaeffer et al., 2008). However, several lines of evidence have shown that physiological concentrations of Aβ exhibit trophic, anti-oxidant, anti-apoptotic or neuroprotective effects (Whitson et al., 1989; Koo et al., 1993; Atwood et al., 2003). Therefore, we made the hypothesis that physiological or non-toxic concentrations of Aβ may induce trophic and/or protective effects on human nerve cells by stimulating the endogenous production of neuroprotective-neurosteroids which may be inhibited by Aβ toxic doses. To check our hypothesis, we

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decided to use the well-validated method combining pulse-chase experiments, high-performance liquid chromatography (HPLC) and flow-scintillation detection (Mensah-Nyagan et al., 1994, 1996; Patte-Mensah et al., 2003, 2005) to compare the effects of physiological and toxic concentrations of the full-length pathogenic peptide $A\beta_{1-42}$ and $A\beta_{25-35}$ fragments on neurosteroidogenesis in human neuroblastoma SH-SY5Y cells. This cell line, which displays neuronal phenotype and properties, is considered as a representative cellular model for studies on AD (Jämsä et al., 2004). Furthermore, SH-SY5Y cells were characterized as neurosteroid-producing cells containing key neurosteroidogenic enzymes (Melcangi et al., 1993; Wozniak et al., 1998; Guarneri et al., 2000). In order to identify accurately concentrations to be considered as non-toxic (physiological) or toxic for SH-SY5Y cells, we performed, prior to the comparative analysis of $A\beta_{1-42}$ and $A\beta_{25-35}$ effects on neurosteroid formation, cell viability assays in the presence or absence of physiological (100, 500 and 1000 nM) or high (12 μM) doses of $A\beta_{1-42}$ and $A\beta_{25-35}$. We have also tested the scrambled peptides $A\beta_{35-25}$ and $A\beta_{42-1}$ in control experiments to ensure the specificity of $A\beta_{25-35}$ or $A\beta_{1-42}$ effects on SH-SY5Y cell viability and neurosteroidogenic activity.

1. Experimental procedures

1.1. Chemicals and reagents

Dulbecco's-modified Eagle medium (DMEM), Glutamax, penicillin/streptomycin, propylene glycol and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) were from Sigma (St. Louis, MO). Fetal calf serum and horse serum were from Gibco (Basel, Switzerland). β-amyloid peptides 25–35 ($A\beta_{25-35}$), 1–42 ($A\beta_{1-42}$), 35–25 ($A\beta_{35-25}$) and 42–1 ($A\beta_{42-1}$) were from Bachem AG (Bubendorf, Switzerland). Synthetic non-radioactive steroids were from Steraloids (Newport, RI). Tritiated steroids such as 7-^{3}H(N)-pregnenolone ([^{3}H]PREG), 1,2,6,7-^{3}H(N)-progesterone, 1,2,6,7-^{3}H(N)-testosterone ([^{3}H]T) and 9,11-^{3}H(N)-3α-androstanediol were from PerkinElmer (Boston, MA). Letrozole was a gift from Dr. D. Evans of Novartis (Basel, Switzerland). Trilostane was generously provided by Dr. Y. Akwa (Le Kremlin-Bicêtre, France).

1.2. Cell culture

Human neuroblastoma SH-SY5Y cells were grown at 37 °C under an atmosphere of 5% CO_2 in DMEM supplemented with 10% (v/v) heat-inactivated fetal calf serum, 5% (v/v) heat-inactivated horse serum, 2 mM Glutamax and 1% (v/v) penicillin/streptomycin. Cells were passaged every 3–4 days and were used for pulse-chase experiments when they reached 80–90% confluence.

1.3. Aβ peptides preparation

The lyophilized Aβ peptides were solubilized in sterile deionized water to 1 mM and stored in small aliquots at −20 °C. The stock solution was diluted in Tris buffer (5 mM, pH 5.4) at 100 μM and incubated for 24 h at 37 °C to have aggregated Aβ.

1.4. MTT reduction assay

To assess cell viability, MTT reduction assays were performed. SH-SY5Y cells were seeded at 5×10^4 cells per well into 96-well plates and allowed to attach. After 48 h, cells were maintained in the culture medium (controls) or exposed for 24 h to preaggregated Aβ peptides diluted at different concentrations (100 nM, 500 nM, 1000 nM or 12 μM) in the medium. MTT (10 μl of a 3.6-mM stock solution) was added to all wells and allowed to incubate in the dark at 37 °C for 5 h. After cell lysis, spectrophotometric measurement was performed at 595 nm to determine the cell viability. All MTT assays were repeated four times.

1.5. Pulse-chase experiments

For each experiment, cells were incubated at 37 °C for 3 h with 3 ml of culture medium containing 240 nM [^{3}H]PREG supplemented with 0.66% propylene glycol in the presence or absence of test substances. The confluent cells were pretreated with 100 nM, 500 nM, 1000 nM or 12 μM aggregated $A\beta_{25-35}$ or $A\beta_{1-42}$ during 24 h and the peptide was maintained in the medium during the incubation with [^{3}H]PREG. Three different types of control experiments were performed: (i) replacement of aggregated $A\beta_{25-35}$ or $A\beta_{1-42}$ by the culture medium (untreated cells or control 1); (ii) replacement of aggregated $A\beta_{25-35}$ by the scrambled peptide $A\beta_{35-25}$ (control 2); (iii) replacement of aggregated $A\beta_{1-42}$ by the scrambled peptide $A\beta_{42-1}$ (control 3). To assess the effects of steroidogenic enzyme inhibitors on neurosteroid production, confluent SH-SY5Y cells were pretreated with trilostane (10 μM) or letrozole (4 μM) during 24 h and these selective inhibitors were also kept in the medium during the incubation with [^{3}H]PREG. In a series of experiments, [^{3}H]PREG was replaced by 240 nM [^{3}H]T to assess the effect of letrozole on [^{3}H]T conversion into [^{3}H]estradiol. In all series of experiments, the incubation with the radioactive precursor was performed in a water-saturated atmosphere (95% air, 5% CO_2) which made it possible to maintain the pH at 7.4. At the end of the incubation period, the reaction was stopped by adding 1 ml of ice-cold DMEM and transferring the incubation medium in tubes into a cold water bath (0 °C). Newly synthesized neurosteroids released by the cells were extracted from the incubation medium three times with 2 ml of dichloromethane and the organic phase was evaporated on ice under a stream of nitrogen. The dry extracts were redissolved in 2 ml of hexane and prepurified on Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA). Steroids were eluted with a solution made of 50% isopropanol and 50% hexane. The solvent was evaporated in a RC-10-10 Speed Vac Concentrator and the dry extracts were kept at −20 °C until HPLC analysis. The extraction efficiency was 89 ± 7%.

1.6. HPLC-Flo/One characterization of steroids

The newly synthesized steroids extracted from the incubation medium already purified on Sep-Pak cartridges were characterized using a previously validated method which combines HPLC analysis and flow-scintillation detection (Mensah-Nyagan et al., 1994, 1996; Patte-Mensah et al., 2003, 2005). Briefly, the prepurified extracts were analyzed by reversed-phase HPLC on a Gilson liquid chromatograph (322 pump, UV–vis 156 detector, Unipoint system, Gilson, Middleton, WI) equipped with a 4.6 mm × 250 mm SymetryShield C_{18} column (Waters Associates) equilibrated with 100% hexane. The radioactive steroids were eluted at a flow rate of 0.5 ml/min using a gradient of isopropanol (0–60% over 65 min) including five isocratic steps at 0% (0–10 min), 1% (30–35 min), 2% (40–45 min), 30% (50–55 min) and 60% (60–65 min). The tritiated steroids eluted from the HPLC column were directly quantified with a flow-scintillation analyzer (Radiomatic Flo/One-Beta A 500, Packard Instruments, Meriden, CT) equipped with a Pentium IV PC computer for measurement of the percentage of total radioactivity contained in each peak. Synthetic steroids used as reference standards were chromatographed under the same conditions as the extracts obtained from the cell incubation media and their elution positions were determined by ultraviolet absorption using a UV–vis 156 detector (Gilson).

The elution positions of steroids change on analytic columns after the purification of a certain number of tissue extracts. Therefore, to optimize the characterization of newly synthesized neurosteroids, synthetic tritiated neuroactive steroids including [^{3}H]PREG, [^{3}H]progesterone, [^{3}H]T, [^{3}H]estradiol and [^{3}H]3α-androstanediol were also used as reference standards, chromatographed under the same conditions as the extracts and identified by their elution times with the Flo/One computer system before and after each extract analytic run.

1.7. Protein assays and quantification of steroid biosynthesis

In a first step, the amount of radioactive steroids formed by the conversion of [^{3}H]PREG (or [^{3}H]T) was calculated as a percentage of the total radioactivity contained in all peaks resolved by the HPLC-Flo/One system, including [^{3}H]PREG (or [^{3}H]T) itself. Afterwards, the final amount or value used for

the tables or chartbars was determined after normalization to the quantity of proteins contained in the dish of confluent cells, which served for the pulse-chase experiment. The protein concentration was determined using a Bio-Rad Protein Assay kit (Bio-Rad, München, Germany) and bovine serum albumin as standard. Each value is the mean of four independent experiments.

1.8. Statistical analysis

All values presented are the mean ± S.E.M. of four different experiments. Statistical analysis was performed with the 5.1 version of Statistica software (Statsoft, Maison Alfort, France). Student's *t*-test was used for comparisons in pairs while one- or two-way ANOVAs followed by Tukey post hoc were applied for multi-parameter analyses.

2. Results

2.1. Effects of $A\beta_{1-42}$ and $A\beta_{25-35}$ on human neuroblastoma cell viability

These series of experiments were performed in order to determine physiological or non-toxic concentrations of $A\beta_{1-42}$ and $A\beta_{25-35}$ to be tested on neurosteroidogenesis in SH-SY5Y cells. Because it is well demonstrated that Aβ concentrations higher than 5 μM induce cell death (Selkoe, 2001), we tested on cell viability the action of $A\beta_{1-42}$ or $A\beta_{25-35}$ at 100 nM, 500 nM or 1000 nM (1 μM) to ensure that these concentrations are not toxic for SH-SY5Y cells. No cell death was revealed by MTT assays after 24 h treatment neither with the scrambled peptides ($A\beta_{42-1}$ and $A\beta_{35-25}$) nor with $A\beta_{1-42}$ or $A\beta_{25-35}$ at 100 nM, 500 nM or 1000 nM. At 12 μM, $A\beta_{1-42}$ and $A\beta_{25-35}$ killed 68% and 49% of SH-SY5Y cells, respectively (Fig. 1).

2.2. Comparative analysis of the effects of $A\beta_{1-42}$ and $A\beta_{25-35}$ on neurosteroid biosynthesis

Qualitative analysis performed with the HPLC-Flo/One system revealed that untreated (control 1) and treated cells with the scrambled peptides (controls 2 and 3) or with physiological/non-toxic (100 nM, 500 nM or 1000 nM) or toxic (12 μM) concentrations of $A\beta_{1-42}$ or $A\beta_{25-35}$ were all capable of converting the precursor [^{3}H]PREG into various [^{3}H]neurosteroids including [^{3}H]progesterone, [^{3}H]T, [^{3}H]3α-androstanediol and [^{3}H]estradiol (Fig. 2A–C). Quantitative assessments showed that only [^{3}H]progesterone and/or [^{3}H]estradiol formation was affected in SH-SY5Y cells by $A\beta_{1-42}$ or $A\beta_{25-35}$ which did not modify the amounts of [^{3}H]T and [^{3}H]3α-androstanediol produced from [^{3}H]PREG (Table 1, Figs. 3 and 4). At physiological/non-toxic doses, both $A\beta_{1-42}$ and $A\beta_{25-35}$ inhibited [^{3}H]progesterone synthesis from [^{3}H]PREG but only $A\beta_{1-42}$ was able to affect and stimulate [^{3}H]estradiol production (Figs. 3 and 4). At 12 μM (toxic), $A\beta_{1-42}$ effect inverted to inhibition of [^{3}H]estradiol production contrary to the stimulation observed with low doses (Fig. 4). Toxic $A\beta_{25-35}$ concentrations such as 12 μM and

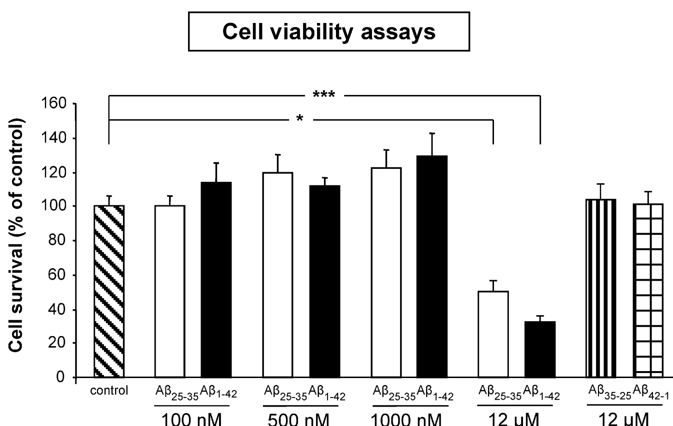

Fig. 1. Comparative analysis of the effects of $A\beta_{1-42}$, $A\beta_{25-35}$ and the scrambled peptides ($A\beta_{42-1}$ and $A\beta_{35-25}$) on SH-SY5Y cell viability. Neuroblastoma cells were exposed for 24 h to physiological (100 nM, 500 nM or 1000 nM) or high (12 μM) concentrations of $A\beta_{1-42}$ or $A\beta_{25-35}$. The scrambled peptides ($A\beta_{42-1}$ and $A\beta_{35-25}$) were used at 12 μM. MTT reduction assays were applied to determine the cell viability, which was expressed as percent of control (untreated) cells. Each value is the mean ± S.E.M. of four independent experiments.

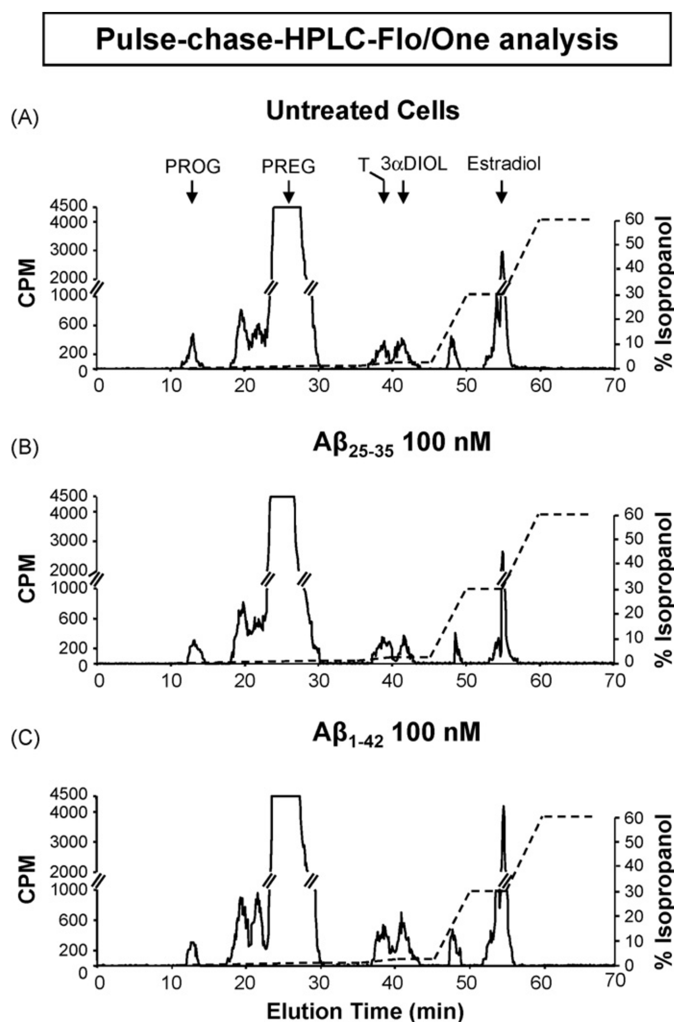

Fig. 2. HPLC-Flo/One characterization of [^{3}H]neurosteroids produced, after a 3-h incubation with [^{3}H]PREG, by controls (A) and SH-SY5Y cells treated with physiological concentrations of $A\beta_{25-35}$ (B) or $A\beta_{1-42}$ (C). The ordinate indicates the radioactivity measured in the HPLC eluent. The dashed line represents the gradient of secondary solvent (% isopropanol). The arrows indicate elution positions of standard steroids. PROG, progesterone; PREG, pregnenolone; T, testosterone; 3αDIOL, 3α-androstanediol.

Table 1
Effects of $A\beta_{25-35}$, $A\beta_{1-42}$ and the scrambled peptides ($A\beta_{42-1}$ and $A\beta_{35-25}$) on [^{3}H]PREG conversion into [^{3}H]progesterone (P) and [^{3}H]estradiol (E_2) in SH-SY5Y cells

	Untreated cells	$A\beta_{25-35}$					$A\beta_{35-25}$ (12 μM)	$A\beta_{1-42}$				$A\beta_{42-1}$ (12 μM)
	Control 1	100 nM	500 nM	1000 nM	12 μM	50 μM	Control 2	100 nM	500 nM	1000 nM	12 μM	Control 3
P (nM)												
Samples	1.01	0.84	0.71	0.42	0.45	0.82	0.98	1.77	0.47	0.73	0.35	0.91
	1.07	0.47	0.41	1.03	0.48	0.61	1.04	0.93	0.52	1.54	0.37	1.18
	1.32	0.50	0.81	0.62	0.59	0.78	1.28	1.17	0.50	0.99	0.46	0.98
	1.45	0.32	0.68	0.63	0.65	0.62	1.41	0.89	0.63	0.95	0.51	1.33
Mean ± S.E.M.	1.21 ± 0.1	0.53 ± 0.11	0.65 ± 0.08	0.68 ± 0.13	0.54 ± 0.05	0.71 ± 0.05	1.18± 0.10	1.19 ± 0.20	0.53 ± 0.03	1.05 ± 0.17	0.42 ± 0.04	1.10 ± 0.09
% effect vs. controls		−56.2%***	−46.3%**	−43.8%**	−55.4%**	−41.3%*	−2.48% NS	−1.6% NS	−56.2%*	−13.2% NS	−65.3%**	−9.09% NS
E_2 (nM)												
Samples	4.75	7.01	3.97	2.90	5.78	4.91	4.31	7.57	6.41	5.99	1.40	4.47
	5.54	5.55	7.20	5.53	6.75	4.73	4.87	7.21	6.49	7.29	1.64	5.46
	5.04	2.58	3.28	6.74	6.14	5.54	4.54	6.91	6.55	6.54	1.49	4.29
	5.87	4.71	5.70	3.07	7.15	3.81	5.41	6.63	8.17	7.01	1.73	4.97
Mean ± S.E.M.	5.30 ± 0.25	4.96 ± 0.93	5.04 ± 0.88	4.56 ± 0.94	6.45 ± 0.3	4.75 ± 0.36	4.78 ± 0.24	7.08 ± 0.20	6.91 ± 0.42	6.71 ± 0.28	1.56 ± 0.07	4.80 ± 0.26
% effect vs. controls		−6.4% NS	−4.9% NS	−14% NS	+21.7% NS	−10.4% NS	−9.8% NS	+33.6%**	+30.4%**	+26.6%*	−70.6%***	−9.43% NS

The values presented above were obtained from a complete series of experiments designated independent experiment 1. A total of four independent experiments were performed. In each independent experiment, four samples of control SH-SY5Y cells and four samples of cells treated with each tested $A\beta_{1-42}$ or $A\beta_{25-35}$ concentration were analyzed. The values were obtained from experiments similar to that presented in Fig. 2. Each value was calculated as the relative amount of [^{3}H]neurosteroid compared with the total [^{3}H]-labeled compounds resolved by HPLC-Flo/One characterization (100×) and was normalized to the protein concentration. Results were then expressed as the concentration of newly synthesized P or E_2. The percent of effect vs. control 1 expresses increase (+) or decrease (−) induced by Aβ on P or E_2 formation in SH-SY5Y cells. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS, not significant.

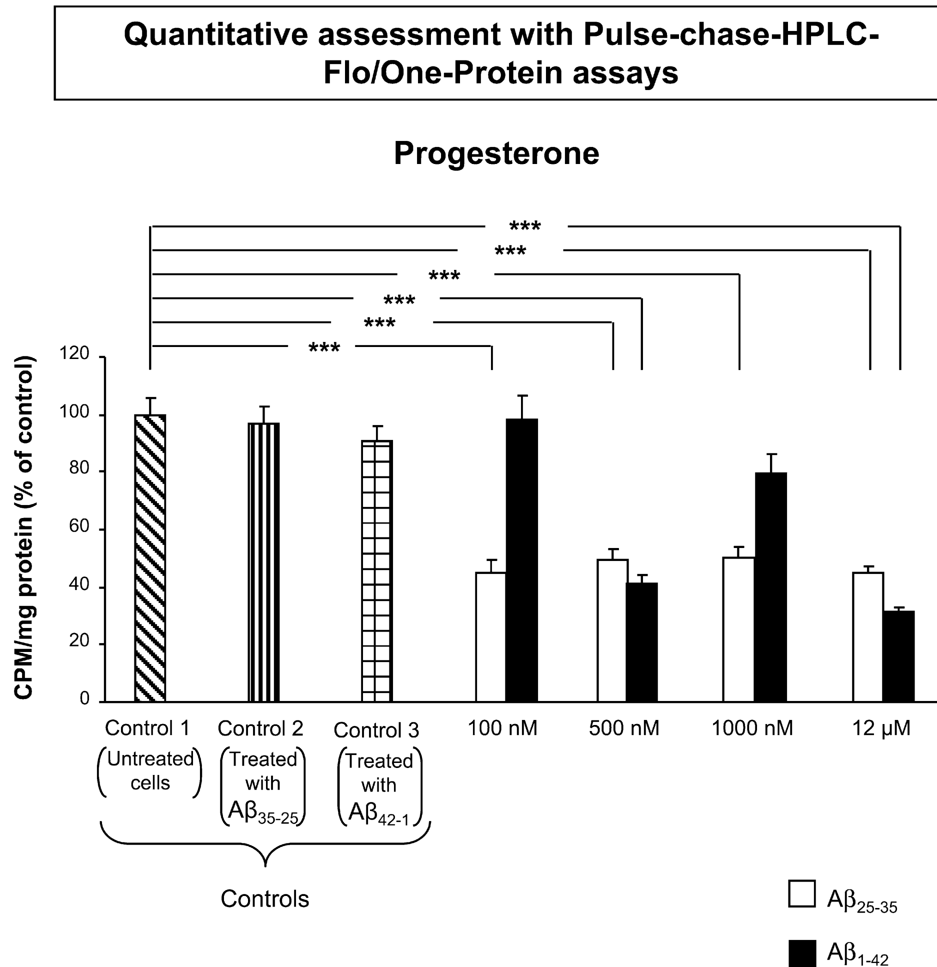

Fig. 3. Quantitative assessments of the effects of physiological (100 nM, 500 nM and 1000 nM) and toxic (12 μM) concentrations of $A\beta_{1-42}$ and $A\beta_{25-35}$ on [^{3}H]PREG conversion into [^{3}H]progesterone in SH-SY5Y cells. Control experiments were performed with untreated SH-SY5Y cells (control 1) and neuroblastoma cells incubated with the scrambled $A\beta_{35-25}$ (control 2) or $A\beta_{42-1}$ (control 3) peptide at 12 μM. The chartbars represent the mean values of four independent experiments including experiment 1 presented in Table 1. Results were expressed as percentages of the amount of progesterone formed in control cells. ***$p < 0.001$.

50 μM have remained ineffective in the modulation of [^{3}H]estradiol formation as previously observed with physiological doses (Table 1, Fig. 4). [^{3}H]progesterone production was inhibited by toxic $A\beta_{1-42}$ and $A\beta_{25-35}$ concentrations (Fig. 3). The scrambled peptides $A\beta_{35-25}$ (control 2) and $A\beta_{42-1}$ (control 3) did not modify neurosteroid biosynthesis in neuroblastoma SH-SY5Y cells (Table 1, Figs. 3 and 4).

2.3. *Effects of selective inhibitors of 3β-hydroxysteroid dehydrogenase and aromatase activities on neurosteroid formation*

Trilostane, the selective inhibitor of 3β-hydroxysteroid dehydrogenase activity, significantly reduced the conversion of [^{3}H]PREG into [^{3}H]PROG in neuroblastoma cells (−51%, $p < 0.05$) (Fig. 5A). In addition, the transformation of [^{3}H]PREG or [^{3}H]T into [^{3}H]estradiol significantly decreased in SH-SY5Y cells (−47% and −49%, respectively, $p < 0.05$) in the presence of letrozole, the non-steroidal inhibitor of aromatase activity (Fig. 5B and C).

3. Discussion

Thanks to MTT reduction assays, we observed that physiological Aβ concentrations (within nanomolar to 1 μM) do not induce SH-SY5Y cell death while high doses (12 μM) are cytotoxic. In agreement with this observation, estimated concentrations of endogenous Aβ intracellularly produced in wild-type APP-transfected SH-SY5Y cells are within nanomolar to 1 μM and no toxicity was observed in these cells as well as in PC12 cells overexpressing APP (Kogel et al., 2003; Schaeffer et al., 2006). The well-validated method combining pulse-chase experiments, HPLC and flow-scintillation detection (Mensah-Nyagan et al., 1994, 1996; Patte-Mensah et al., 2003, 2005) allowed us to observe that, among numerous neurosteroids produced from the precursor PREG in SH-SY5Y cells, only progesterone and estradiol formations were affected by Aβ. Interestingly, while progesterone production was inhibited non-selectively by physiological and toxic concentrations of both $A\beta_{1-42}$ and $A\beta_{25-35}$, estradiol formation was regulated only by the full-length pathogenic peptide $A\beta_{1-42}$. Indeed, physiological $A\beta_{1-42}$ doses induced a

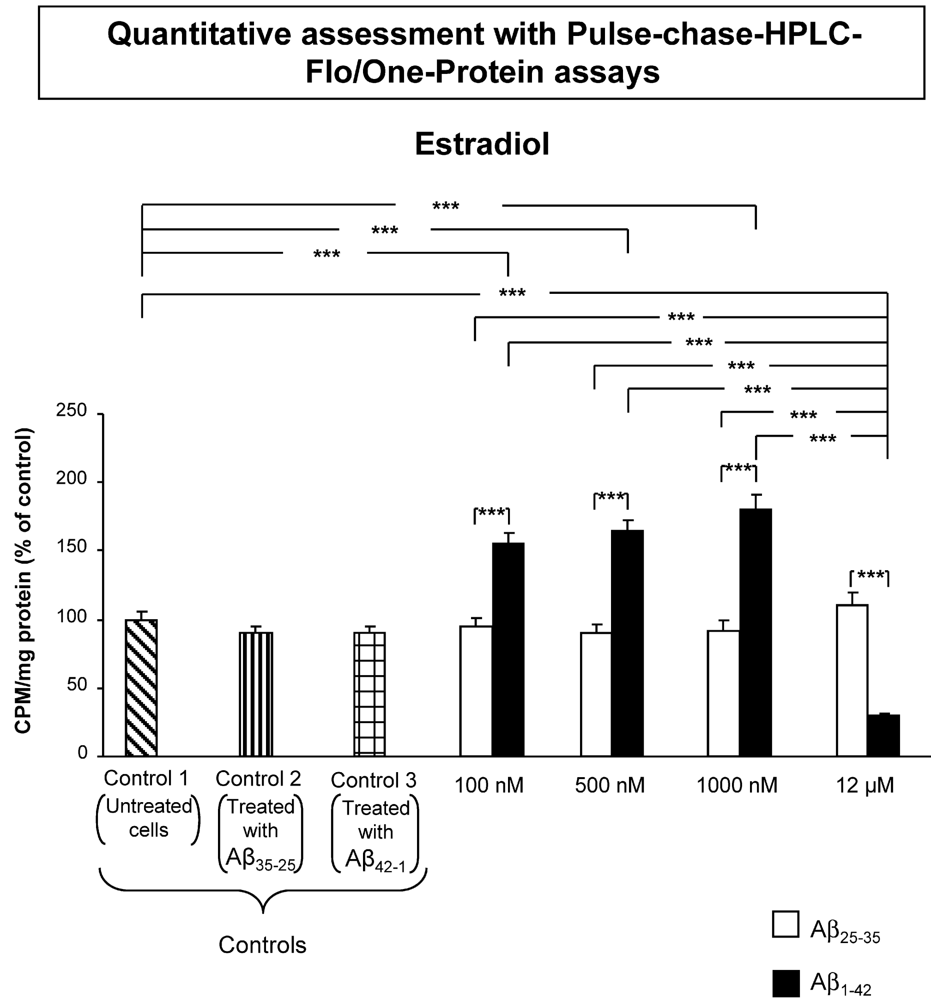

Fig. 4. Quantitative assessments of the effects of physiological (100 nM, 500 nM and 1000 nM) and toxic (12 μM) concentrations of $A\beta_{1-42}$ and $A\beta_{25-35}$ on [^{3}H]PREG conversion into [^{3}H]estradiol in SH-SY5Y cells. Control experiments were performed with untreated SH-SY5Y cells (control 1) and neuroblastoma cells incubated with the scrambled $A\beta_{35-25}$ (control 2) or $A\beta_{42-1}$ (control 3) peptide at 12 μM. The chartbars represent the mean values of four independent experiments including experiment 1 presented in Table 1. Results were expressed as percentages of the amount of estradiol formed in control cells. ***$p < 0.001$.

stimulatory effect on estradiol synthesis, which inverted to an inhibitory action at high $A\beta_{1-42}$ concentration. Together, these results reveal the existence of a selective and amino-acid sequence-dependent action of Aβ on neurosteroid biosynthesis in SH-SY5Y cells. In support of this hypothesis, several studies have shown that $A\beta_{25-35}$ induces different effects from those of $A\beta_{1-42}$ in various experimental models (Mattson et al., 1997; Yao et al., 2002). For instance, it has been demonstrated that $A\beta_{1-42}$, but not $A\beta_{25-35}$, reproduced pathological features of AD such as microglia activation or acetylcholine esterase stimulation (Casal et al., 2002; Saez-Valero et al., 2003). [^{3}H]PREG conversion into [^{3}H]estradiol requires complementary activities of various enzymes such as 3β-hydroxysteroid dehydrogenase (PREG into progesterone), cytochrome P450c17 (progesterone into androstenedione), 17β-hydroxysteroid dehydrogenase (androstenedione into testosterone) and aromatase (testosterone into estradiol). No difference was observed between $A\beta_{1-42}$ and $A\beta_{25-35}$ on progesterone synthesis from PREG, suggesting that Aβ may reduce 3β-hydroxysteroid dehydrogenase activity in SH-SY5Y cells. Moreover, the actions of $A\beta_{1-42}$ and $A\beta_{25-35}$ on cytochrome P450c17 and 17β-hydroxysteroid dehydrogenase seem to be similar because no discrepancy was detected in the production of testosterone from PREG in SH-SY5Y cells exposed to $A\beta_{1-42}$ or $A\beta_{25-35}$. In contrast, $A\beta_{1-42}$ regulated dose-dependently estradiol formation while $A\beta_{25-35}$ had no effect on this mechanism, suggesting that aromatase modulation depends on Aβ amino-acid sequence. In particular, the data suggest that aromatase activity may be controlled either by the N terminal sequence 1–24 or by the C terminal sequence 36–42 in agreement with the fact that the fragment 25–35 ($A\beta_{25-35}$) had no effect on estradiol synthesis while the full-length sequence 1–42 ($A\beta_{1-42}$) modulated this process. Furthermore, inversion of the stimulatory action of non-toxic $A\beta_{1-42}$ doses on estradiol production to inhibition at high concentration, suggests the existence of a bimodal effect of $A\beta_{1-42}$ on aromatase activity, which may be determinant in the mediation of protective or degenerative effects of $A\beta_{1-42}$ on nerve cells. Thus, increase of estradiol production in SH-SY5Y cells by physiological $A\beta_{1-42}$ concentrations may protect them against death since several

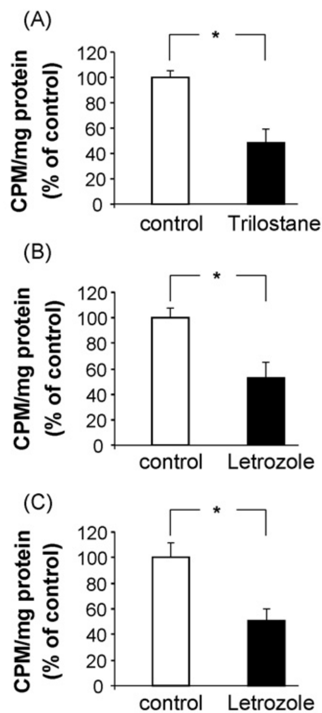

Fig. 5. Effect of trilostane (3β-hydroxysteroid dehydrogenase inhibitor) and letrozole (aromatase inhibitor) on neurosteroid biosynthesis in SH-SY5Y cells. (A) Inhibitory action of trilostane (10 μM) on [^{3}H]PREG conversion into [^{3}H]PROG. Inhibitory effects of letrozole (4 μM) on the conversion of [^{3}H]PREG (B) or [^{3}H]T (C) into [^{3}H]estradiol. *$p < 0.05$.

studies demonstrated neuroprotective effects of estrogens (Garcia-Segura et al., 2001). In contrast, decreased estradiol formation induced by high $A\beta_{1-42}$ doses may contribute to SH-SY5Y cell degeneration and death. Consistently with this suggestion, we have recently observed decreased estradiol production in SH-SY5Y cells 12 h after treatment with H_2O_2 a key mediator of Aβ accumulation-induced cell death (Schaeffer et al., 2008). Furthermore, the results provided herein show that the incubation of SH-SY5Y cells with letrozole or trilostane, the respective inhibitors for aromatase (Lamb and Adkins, 1998) and 3β-hydroxysteroid dehydrogenase (Komanicky et al., 1978; Mensah-Nyagan et al., 1994) decreased estradiol and progesterone biosynthesis from the precursor testosterone or PREG. These results strongly support the involvement of steroidogenic enzyme activities in the mediation of Aβ effects on neurosteroidogenesis in SH-SY5Y cells. Moreover, testosterone level did not increase in human neuroblastoma cells when toxic $A\beta_{1-42}$ doses decreased aromatase activity. It is possible that the amount of testosterone was maintained at its normal value thanks to another metabolic pathway. In support of this hypothesis, it has clearly been demonstrated that in SH-SY5Y cells testosterone is actively metabolized into dihydrotestosterone and 3α-androstanediol by 5α-reductase and 3α-hydroxysteroid oxido-reductase (Melcangi et al., 1993).

Although accumulation of fibrillar deposits of Aβ is considered as a key step in AD pathogenesis, interactions with cell membranes and signaling pathways coupled with Aβ binding to nerve cells are not well understood. Therefore, intracellular cascades leading to the modulation of neurosteroidogenic enzymes in SH-SY5Y cells after their activation by Aβ remain under the scope of speculation. However, a recent study, which investigated Aβ binding to neuronal cell membranes by fluorescence correlation spectroscopy, suggested that Aβ may kill cells via a receptor or target molecule (Hossain et al., 2007). Characterization of this target molecule will certainly help in the future to investigate intracellular cascades mediating the regulatory action exerted by Aβ on neurosteroidogenic enzymes localized in SH-SY5Y cells (Melcangi et al., 1993; Wozniak et al., 1998; Guarneri et al., 2000). Nevertheless, the series of results described herein suggest that the selective targeting of neurosteroidogenic pathways in nerve cells may be an interesting possibility to explore for novel strategies against neurodegenerative diseases.

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