Neuroscience 135 (2005) 181–190

EFFECTS OF SINGLE AND CONTINUOUS ADMINISTRATION OF AMYLOID β-PEPTIDE (25–35) ON ADENYLYL CYCLASE ACTIVITY AND THE SOMATOSTATINERGIC SYSTEM IN THE RAT FRONTAL AND PARIETAL CORTEX

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Abstract—It is unknown whether the amyloid β-peptide (Aβ), a principal component found in extracellular neuritic plaques in the brain of patients with Alzheimer's disease (AD), is capable of altering adenylyl cyclase (AC) activity and the somatostatin (SRIF) receptor-effector system in the cerebral cortex of the patients. Therefore, the objective of this study was to investigate the effect of the β fragment, β (25–35), on AC activity and the somatostatinergic system in the rat frontoparietal cortex. A single dose of β (25–35) (10 μg) injected intracerebroventricularly significantly decreased the density of SRIF receptors (27.4%) and increased their affinity (32.2%) in the frontoparietal cortex. The inhibitory effect of SRIF on basal and forskolin (FK)-stimulated AC activity was significantly lower in the β (25–35)-treated rats when compared with controls. β (25–35) did not modify Giα1, Giα2 nor Giα3 levels in membranes from the frontoparietal cortex. Continuous infusion of the peptide induced a decrease in the SRIF receptor density in this brain area to a similar extent as that observed 14 days after the single administration of the peptide. Likewise, this treatment decreased the SRIF receptor density in the frontal cortex (15.3%) and parietal cortex (27.2%). This effect was accompanied by a decrease in the SRIF-mediated inhibition of FK-stimulated AC activity (from 41.6% to 25.6%) in the frontal cortex as well by a decrease in basal AC activity (from 36.9% to 31.6%) and FK-stimulated AC activity (from 35.6% to 27.1%) in the parietal cortex. Continuous infusion of Aβ (25–35) had no effect on Giα1, Giα2 or Giα3 levels in membranes from frontal and parietal cortex. However, this treatment caused a decrease in SRIF-like immunoreactivity content in the parietal (38.9%) and frontal (20.4%) cortex. These results suggest that Aβ might be involved in the alterations of somatostatinergic system reported in AD. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: brain, Alzheimer's disease, somatostatin receptors, cerebral cortex.

Alzheimer's disease (AD) is the most common form of senile dementia, accounting for more than 50% of the cases reported. The prevalence increases logarithmically with age (Katzman and Saitoh, 1991). The main pathological features of AD are extracellular deposits of amyloid β-peptide (Aβ), intraneuronal cytoskeletal abnormalities and neuronal degeneration (Armstrong et al., 1991). The neurotoxicity of Aβ was first demonstrated by Yankner et al. (1990). In addition to a direct neurotoxic effect, Aβ activates microglia to produce neurotoxins, such as proteolytic enzymes, cytokines, free radicals and nitric oxide (Meda et al., 1995). Neurochemical studies on postmortem brains from AD patients have demonstrated changes in several neurotransmitter systems (Cowburn et al., 1996). The most consistent finding is a deficit in choline acetyltransferase activity in the cerebral cortex (Koshimura et al., 1986). Recent reports suggest that neurotransmitter receptors are affected in AD patients, more profoundly in younger patients (Greenamyre et al., 1985; Pavia et al., 2000). Noradrenergic, serotonergic and possibly dopaminergic neurons are also affected, although to a lesser extent (Reinikainen et al., 1990). The GABAergic interneurons have also been shown to be involved in AD (Cagnin et al., 2001). Among the neuropeptides, the most consistently reported deficit in AD is the reduction in the cortical somatostatin (SRIF) concentration (Davies et al., 1980). Regarding the SRIF receptors, several authors (Beal et al., 1985; Bergström et al., 1991; Krantic et al., 1992) have reported a marked loss in the SRIF receptor density in various cortical areas of the AD brain. Cowburn et al. (1991) found a reduced SRIF-mediated inhibition of adenylyl cyclase (AC) activity in the superior temporal cortex of AD brains as compared with controls.

SRIF is widely distributed in the CNS and peripheral tissues and is considered as a hormone, neurohormone and neuromodulator (Epelbaum, 1986). In addition to its multiple neuroendocrine effects, SRIF has been also suggested to modulate cognitive processes (Vécsei et al., 1984; Cacabelos et al., 1988; Dournaud et al., 1996). The somatostatinergic innervation of the cerebral cortex is of intrinsic origen (Epelbaum, 1986). In fact, SRIF mRNAs and immunoreactive SRIF are present in nonpyramidal neurons mainly localized in layers II–III and V–VI. These neurons display vertical axonal arborization that often terminates in layer I (Garrett et al., 1994). Autoradiographic studies conducted in the adult neocortex have demonstrated that SRIF receptors are located primarily in layers

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Abbreviations: Aβ, amyloid β-peptide; Aβ (25–35), amyloid β-peptide fragment (25–35); AC, adenylyl cyclase; AD, Alzheimer's disease; BSA, bovine serum albumin; cAMP, cyclic AMP; CRE, cyclic AMP response element; CREB, cyclic AMP-response element-binding protein; FK, forskolin; IBMX, 3-isobutyl-1-methylxanthine; PMSF, phenylmethylsulfonyl fluoride; SRIF, somatostatin; SRIF-LI, somatostatin-like immunoreactivity.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.02.017

V–VI and to a lesser extent in layers I–IV. Several studies have demonstrated that not only neurons but also astrocytes and microglial cells express SRIF receptors (Feindt et al., 1995, 1998). The frontoparietal cortex contains high levels of SRIF receptors (Srikant et al., 1981; Epelbaum et al., 1982). Five SRIF receptors have been cloned to date (Bell and Reisine, 1993), which are all coupled to AC via the guanine nucleotide-binding inhibitory protein Gi (Sakamoto et al., 1988; Schettini et al., 1989).

To date, it is unknown whether Aβ is capable of altering AC activity and the SRIF receptor-effector system in the cerebral cortex. Numerous laboratories have used the smaller 11 amino acid fragment of the full-length peptide, amyloid β-peptide fragment (25–35) (Aβ (25–35)), as a convenient alternative in AD research since the smaller peptide mimics several of the toxicological and oxidative stress properties of the native full-length peptide. Aβ (25–35) is more rapidly toxic and causes more oxidative damage than the parent peptide Aβ (1–42) (Varadarajan et al., 2001).

The aim of this study, therefore, was to investigate the effect of Aβ (25–35) on AC and the somatostatinergic system in the rat frontoparietal cortex. Consequently, we examined the binding of ^{125}I-Tyr^{11}-SRIF to SRIF receptors, basal and forskolin (FK)-stimulated AC activity and SRIF-mediated inhibition of AC activity in membranes from the rat frontoparietal cortex as well as from the frontal or parietal cortex alone. somatostatin-like immunoreactivity (SRIF-LI) levels and Gi protein levels (Giα$_1$, Giα$_2$ and Giα$_3$) in these brain areas were also determined after single or continuous administration of Aβ (25–35).

EXPERIMENTAL PROCEDURES

Synthetic Tyr^{11}-SRIF and SRIF-14 were purchased from Universal Biologicals Ltd (Cambridge, UK); carrier-free Na ^{125} I (IMS 100 mCi/ml) was purchased from the Radiochemical Center (PerkinElmer, Boston, Massachusetts, USA); bacitracin, bovine serum albumin (BSA), forskolin (FK), Aβ (25–35), Aβ (35-25), phenylmethylsulfonyl fluoride (PMSF), guanosine triphosphate, and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma (Madrid, Spain). Specific antisera against the αi1 (MAB 3075) and αi2 (MAB 3077) G protein subunits were obtained from Chemicon International (Temecula, California, USA) whereas antiserum against the αi3 (sc-262) G protein subunit was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, California, USA). Nitrocellulose membranes as well as the chemiluminescence Western blotting detection system were purchased from Amersham (Buckinghamshire, UK). The rabbit antibody used in the radioimmunoassay technique was raised in rabbits against SRIF-14 conjugated to BSA and is specific for SRIF, but since SRIF-14 constitutes the C-terminal portions of both SRIF-25 and SRIF-28, the antiserum does not distinguish between these three forms. The binding of SRIF-14 to this antibody does not depend on an intact disulfide bond in the molecule as breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change the immunoreactivity of the peptide.

Cross-reactivity with other peptides was less than 0.5%. Cross-reaction with several SRIF analogs demonstrated that neither the N-terminal glycine nor the C-terminal cysteine residue is required for antibody binding, suggesting that the antigen site is directed toward the central part of the molecule containing the tryptophan residue.

Experimental animals

The animal experiments performed in the present study conform to the guidelines set by the Animal Care Committee of Alcalá University and were performed in accordance with the European Communities Council Directive for the care of laboratory animals. Great care was taken to avoid or minimize discomfort to the animals. The animals used in this study were 50 male Wistar rats weighing between 200 and 250 g. Rats were maintained on a 12-h light/dark cycle (07:00–19:00 h) and allowed free access to food and water. The Aβ (25–35) peptide as well as the scrambled peptide Aβ (35-25) were dissolved in distilled water, which favors aggregation (Pike et al., 1995), and administered i.c.v. to the rats in a single dose (10 μg; Giovannelli et al., 1995) or via an osmotic minipump (Alzet) connected to a cannula. On the day of surgery, the cannula attached to the osmotic minipump was implanted in the right cerebral ventricle of the rat as previously described (Nitta et al.1994) and Aβ (25–35) was continuously infused at doses of 300 pmol/day for 14 days (Nitta et al., 1994; Nag et al., 1999). Control animals received vehicle alone. The rats were killed by decapitation 7 or 14 days after the single injection or 14 days after the minipump implantation. All solutions were freshly prepared prior to administration. In all experimental groups, the brains were rapidly removed and the frontoparietal cortex, frontal cortex and parietal cortex were dissected over ice according to the method of Glowinski and Iversen (1966).

Tissue extraction and SRIF radioimmunoassay

For SRIF-LI measurements, the frontoparietal cortex was rapidly homogenized in 1 ml of 2 M acetic acid using a Brinkman polytron (setting 5, 30 s). The extracts were boiled for 5 min in a water bath, chilled in ice, and aliquots (100 μl) were removed for protein determination (Patel and Reichlin, 1978). The homogenates were subsequently centrifuged at 15,000×*g* for 15 min at 4 °C and the supernatant was neutralized with 2 M NaOH. The extracts were then stored at −70 °C until assay. The tissue concentration of SRIF-LI was analyzed in the extracts by a competitive radioimmunoassay, as previously reported (Patel and Reichlin 1978), with a sensitivity limit of 10 pg/ml. All samples from a given brain region were assayed on the same radioimmunoassay run. Incubation tubes prepared in duplicate contained 100 μl samples of unknown or standard solutions of 0–500 pg cyclic SRIF tetradecapeptide diluted in phosphate buffer (0.1 M, pH 7.5 containing 0.2% BSA, 0.1% sodium azide), 200 μl of appropriately diluted anti-SRIF serum, 100 μl of freshly prepared ^{125}I-Tyr^{11}-SRIF diluted in buffer to give 6000 cpm/assay tube (equivalent to 5–10 pg), and enough buffer to give a final volume of 0.8 ml. All reagents, as well as the assay tubes, were kept chilled in ice before their incubation for 24 h at 4 °C. Separation of bound and free hormone was accomplished by the addition of 1 ml of dextran-coated charcoal (dextran T-70: 0.2% w/v; Pharmacia, Uppsala, Sweden; charcoal Norit A: 2% w/v; Serva; Feinbiochemica, Heidelberg, Germany). Serial dilution curves for the samples were parallel to the standard curve.

Binding assay

Tyr^{11}-SRIF was radioiodinated by the chloramine-T method (Greenwood et al., 1963). The tracer was purified in a Sephadex G-25 fine column (1×100 cm) equilibrated with 0.1 M acetic acid containing BSA 0.1% (W/V). The specific activity of the purified labeled peptide was about 600 Ci/mmol.

Membranes from the rat frontoparietal cortex, frontal cortex and parietal cortex were prepared as previously described by Reubi et al. (1981). Membrane protein was determined by the method of Lowry et al. (1951) using BSA as a standard. Specific SRIF binding was measured according to the modified method of Czernik and Petrack (1983). Briefly, brain membranes (0.15 mg protein/ml) were incubated in 250 μl of a medium containing

50 mM Tris–HCL buffer (pH 7.5), 5 mM $MgCl_2$, 0.2% (w/v) BSA and 0.1 mg/ml bacitracin with 160 pM ^{125}I-Tyr^{11}-SRIF either in the absence or presence of 0.01–10 nM unlabeled SRIF. After incubation for 60 min at 30 °C, the free radioligand was separated from the bound radioligand by centrifugation at 11,000×*g* for 2 min and the resultant pellet was counted in a Kontron gamma counter. Nonspecific binding was obtained from the amount of radioactivity bound in the presence of 10^{-7}M unlabeled SRIF. This non-specific component was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding.

Evaluation of radiolabeled peptide degradation

To determine the extent of tracer degradation during incubation, we measured the ability of pre-incubated peptide to bind to fresh membranes as previously described (Aguilera et al., 1982). Briefly, ^{125}I-Tyr^{11}-SRIF (250 pM) was incubated with membranes from the rat frontoparietal cortex, frontal cortex or parietal cortex (0.15 mg protein/ml) for 60 min at 30 °C. After this pre-incubation, aliquots of the medium were added to fresh membranes and incubated for an additional 60 min at 30 °C. The fraction of added radiolabeled peptide which was specifically bound during the second incubation was measured and expressed as a percentage of the binding that had been obtained in control experiments performed in the absence of membranes during the pre-incubation period.

AC assay

AC activity was measured as previously reported (Houslay et al., 1976) with minor modifications. Briefly, rat membranes from the frontoparietal, frontal or parietal cortex (0.06 mg/ml) were incubated with 1.5 mM ATP, 5 mM $MgSO_4$, 10 μM GTP, an ATP-regenerating system (7.5 mg/ml creatin phosphate and 1 mg/ml of creatin kinase), 1 mM IBMX, 0.1 mM PMSF, 1 mg/ml bacitracin, 1 mM EDTA, and test substances (10^{-4} M SRIF or 10^{-5} M FK) in 0.1 ml of 0.025 M triethanolamine/HCl buffer (pH 7.4). After a 15-min incubation at 30 °C, the reaction was stopped by heating the mixture for 3 min. After letting the tubes cool, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) were added and the suspension was centrifuged. The supernatant was taken for assay of cyclic AMP (cAMP) by the method of Gilman (1970). The SRIF concentration used was that necessary to achieve inhibition of rat (Schettini et al., 1989) and human (Bergström et al., 1991; Garlind et al., 1992) brain AC. FK was used at a concentration that could effectively stimulate the catalytic subunit of the enzyme (Seamon and Daly, 1986).

Immunodetection of the αi subunits of G proteins

Membranes from the parietal, frontal or frontoparietal cortex were solubilized in SDS-sample buffer. The proteins were then run on a 12% SDS–polyacrylamide gel as described previously (Laemmli, 1970). The transfer of proteins to nitrocellulose membranes and the immunodetection of the αi1, αi2 and αi3 subunits of the G proteins using the specific mouse anti-Gi monoclonal antibodies MAB3075, MAB3077 and sc-262, respectively, were carried out as described elsewhere (Mumby et al., 1986). Briefly, after protein transfer, the nitrocellulose membranes were pre-incubated with blotting buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.05% (v/v) Tween-20 and 3% (w/v) non-fat dry milk). Antisera were diluted in buffer (dilution 1:1000) and were then added to the nitrocellulose membranes. Incubation was carried out overnight at 4 °C. Subsequently, excess antibody was removed and three 15-min washes with blotting buffer were carried out. After washing, the bound immunoreactive proteins were incubated with horseradish–peroxidase-conjugated goat antibody to mouse IgG for 1 h at 4 °C. After eliminating unbound antibody, three 5-min washes with HEPES 20 mM pH 7.5 were carried out and proteins were detected by chemiluminescence using an ECL Western blotting analysis system (Amersham Pharmacia Biotech) according to the manufacturer's instructions. The optical density of the bands obtained was analyzed using the Scion Image program (Scion Corporation). X-ray films (Hyperfilm ECL) were exposed for 30 s–1 min.

Data analysis

The computer program LIGAND (Munson and Rodbard, 1980) was used to analyze the binding data. The use of this program enables models of receptors that best fit the given sets of data to be selected. The same program was also used to present the data in the form of Scatchard plots (Scatchard, 1949) and to compute values for receptor affinity (K_d) and density (B_{max}) that best fit the sets of binding data for each rat. Statistical comparisons of all the data were carried out with one way analysis of variance and the Student-Newman-Keuls test. Means among groups were considered significantly different when the *P* value was less than 0.05. Each individual experiment was performed in duplicate.

RESULTS

Frontoparietal cortical membranes from control and Aβ (25–35)-treated rats bound ^{125}I-Tyr^{11}-SRIF in a time-dependent manner, with an apparent equilibrium being observed between 50 and 180 min at 30 °C. Hence, all subsequent experiments were conducted at 30 °C for 60 min. The injection of a single dose of Aβ (25–35) did not modify the SRIF receptor density after 7 days of its administration. However, after 14 days of its administration, Aβ (25–35) significantly decreased the SRIF receptor density and increased their affinity in the rat frontoparietal cortex (Fig. 1; Table 1). Frontoparietal cortical membranes from control and Aβ (25–35)-treated rats showed a similar tracer degradation capacity and the values varied by no more than 10% in all experimental groups (data not shown).

The scrambled peptide Aβ (35-25) was used to examine the effects of reversing the sequence of the active region of the peptide on the binding of ^{125}I-Tyr^{11}-SRIF to its receptors. A single administration of Aβ (35-25) exerted no effect on the SRIF binding parameters at 14 days of its administration in all brain areas studied (Tables 1 and 3).

Because SRIF receptors have been shown to be negatively coupled to AC, we examined basal and FK-stimulated AC activity as well as SRIF-mediated inhibition of the enzyme in membranes from the frontoparietal cortex. Basal and FK-stimulated AC activity were inhibited by SRIF in all the experimental groups studied. No significant differences in either basal or FK-stimulated AC activity or in SRIF-mediated inhibition of both activities were detected in the frontoparietal cortical membranes from rats treated with a single dose of Aβ (25–35) after 7 days of its administration. However, after 14 days of its single administration, the inhibitory effect of SRIF on basal and FK-stimulated AC activity was significantly lower in the Aβ (25–35)-treated rats as compared with controls (Table 2).

Since SRIF receptors are coupled to AC via Gi proteins, we next analyzed whether the levels of the inhibitory G protein subunits αi1, αi2 or αi3 were affected by Aβ (25–35). To this aim, Western blot analyses were performed. A single dose of Aβ (25–35) did not modify the

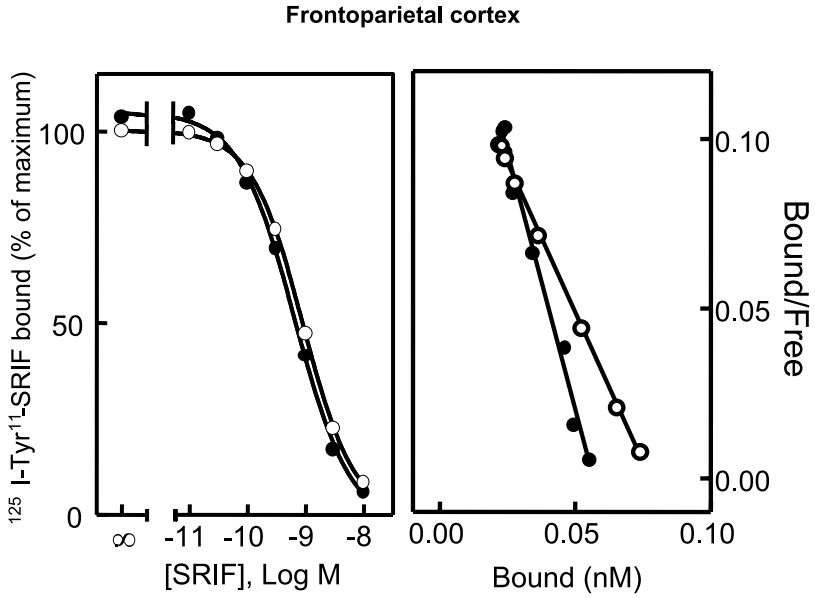

Fig. 1. Effect of a single dose (10 μg) of Aβ (25–35) after 14 days of its administration on SRIF binding to rat frontoparietal cortical membranes. Left panel: competitive inhibition of specific ^{125}I-Tyr^{11}-SRIF binding by unlabeled SRIF to frontoparietal cortical membranes. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30 °C in the presence of 250 pM ^{125}I-Tyr^{11}-SRIF and increasing concentrations of native peptide. Points correspond to values from the control rats (○) and rats treated with Aβ (25–35) (●). Each point represents the mean±S.E.M. of five rats. Each experiment was performed in duplicate. Right panel: Scatchard analysis of the binding data. The corresponding equilibrium parameters are included in Table 1.

levels of Giα1, Giα2 or Giα3 in membranes from the frontoparietal cortex at 7 and 14 days of its administration (Fig. 2).

Since the neurodegenerative changes detected in AD are caused by a continuous deposition of Aβ at the cerebral level, we examined the effects of a continuous i.c.v. infusion of Aβ (25–35) during 14 days. Continuous infusion of the peptide induced a decrease in the SRIF receptor density in the frontoparietal cortex to a similar extent as that observed at 14 days of the single administration of the peptide (data not shown). In order to further our knowledge on these effects, the same parameters were analyzed separately in the frontal cortex and parietal cortex of rats that received a continuous i.c.v. infusion of Aβ (25–35) during 14 days. This treatment decreased the SRIF receptor density in the frontal cortex and parietal cortex (Figs. 3 and 4). This effect was accompanied by a decrease in SRIF-mediated inhibition of FK-stimulated AC activity in the frontal cortex as well a decrease in basal AC activity and FK-stimulated AC activity in the parietal cortex (Tables 3 and 4).

Continuous infusion of Aβ (25–35) had no effect on the protein levels of Giα1, Giα2 or Giα3 in membranes from the frontal and parietal cortex (Fig. 2). However, it caused a decrease in SRIF-LI content in the frontal cortex (from 13.8±0.3 to 8.4±0.1 ng SRIF per mg of protein, *** $P<0.001$) and parietal cortex (from 12.1±0.4 to 9.6±0.1 ng SRIF per mg of protein, *** $P<0.001$).

DISCUSSION

Recent studies carried out by Kubo et al. (2002) reinforce the hypothesis that soluble (D-ser-26)-Aβ (1–40), possibly produced during aging, is released from plaques and converted by proteolysis to toxic (D-Ser-26)-Aβ (25–35/40), which damages the neurons by enhancing exitotoxicity in

Table 1. Effect of a single dose of Aβ (25–35) (10 μg) after 7 and 14 days of its administration or of Aβ (35-25) (10 μg) at 14 days of its administration on the equilibrium parameters for SRIF binding in rat frontoparietal cortical membranes

Groups	SRIF receptors			
	7 Days		14 Days	
	B_{max}	K_d	B_{max}	K_d
Control	591±37	0.67±0.06	523±27	0.59±0.03
Aβ (25–35)	583±35	0.62±0.06	380±26**	0.40±0.03**
Control			522±8	0.58±0.05
Aβ (35-25)			503±8	0.53±0.04

Binding parameters were calculated from Scatchard plots by linear regression. Units for K_d are nM and units for B_{max} are fmol SRIF bound/mg protein. The results are the mean±S.E.M. of five separate experiments performed in duplicate. Statistical comparison vs. control: ** $P<0.01$.

Table 2. Effect of a single dose of Aβ (25–35) (10 μg) after 7 and 14 days of its administration on basal and FK-stimulated AC activity (pmol cAMP/min/mg protein) in frontoparietal cortical membranes from control ($n=10$) and Aβ (25–35)-treated rats ($n=10$)

	7 Days		14 Days	
	Control	Aβ (25–35)	Control	Aβ (25–35)
Basal	328±11	298±14	415±29	372±19
10^{-4} M SRIF	228±20	198±9	269±18	263±17
10^{-5} M FK	1229±89	1242±66	1949±60	1658±92
10^{-5} M FK+10^{-4} M SRIF	869±53	856±39	1298±68	1358±91
Fold FK stimulation over basal	3.8±0.1	4.2±0.3	4.4±0.5	4.4±0.3
% SRIF inhibition of basal activity	30±3	33±3	37±2	29±1*
% SRIF inhibition of FK-stimulated activity	31±4	32±1	33±4	18±1**

Membrane preparations were incubated with or without SRIF (10^{-4} M) in the absence or presence of FK (10^{-5} M). Values represent the mean±S.E.M. of five separate experiments each performed in duplicate. Statistical comparison vs. control: * $P<0.05$; ** $P<0.01$.

AD. Thus, the short peptide Aβ (25–35) has been proposed to be a functional domain of Aβ responsible for its neurotoxic properties (Yankner et al., 1990; Pike et al., 1993). Damaged cells and neuronal loss in the neocortex as well as in the hippocampal subfields CA1 and CA3 and basal ganglia were observed after i.c.v. administration of Aβ (25–35) to aged rats (Stepanichev et al., 2000). The dose of Aβ (25–35) and the time period used in the present study were selected according to previous studies (Nitta et al., 1994). The Aβ-infused rat model is, at best, a partial model of AD. Maurice et al. (1996)) have demonstrated that i.c.v. administration of Aβ (25–35) leads to Aβ deposits widely disseminated throughout the frontoparietal cortex, which illustrates the relative facility with which Aβ (25–35) diffuses throughout the brain after its administration into the lateral ventricle. The frontoparietal cortex was chosen for our study since moderate/severe AD pathology and reductions in SRIF receptor density have been detected in this brain area (Beal et al., 1985; Krantic et al., 1992).

The SRIF-LI levels in the control animals, as determined by radioimmunoassay, were similar to those previously reported by our group and other authors (Patel and Reichlin, 1978; Puebla and Arilla, 2003). Continuous infusion of Aβ (25–35) led to a reduction in the SRIF-LI content in the frontoparietal cortex as well as in the frontal and parietal cortex when studied separately. The alteration in the SRIF levels demonstrated here parallels that detected in brains of patients with AD (Davies et al., 1980). Although Aβ is thought by many researchers to be central to the pathogenesis of AD, it was unknown whether the peptide acts on somatostatinergic neurons. However, it is known that a SRIF deficiency is not due to a loss of SRIF-synthesizing interneurons (Dournaud et al., 1994) but rather to a reduction of the SRIF precursor molecule in senile dementia of the Alzheimer type (Pierotti et al., 1985). This could be due to a significant reduction in the expression of SRIF mRNA (Dournaud et al., 1994). Alterations in posttranslational processing could be due to alterations in proteolytic metabolism and peptidase activity, leading to changes in the metabolic half-life (Weber et al., 1992). More recently, Strittmatter et al. (1997) suggested the decrease of SRIF content is due to complex alterations of dysregulated synthesis and/or postranslational processing of SRIF. Recently, Tran et al. (2001) reported an impairment of cyclic AMP-response element-binding protein (CREB) signaling in neurons by Aβ (25–35) at a concentration in which cell survival is not compromised. Since the cyclic AMP response element (CRE) of the SRIF promotor contains binding sites for CREB that are essential for cAMP-regulated transcription (Montminy and Bilezikjian, 1987; Andrisany and Dixon, 1990; Montminy et al., 1996), this mechanism might explain the decrease in SRIF-LI content seen in the present study.

The equilibrium parameters of the SRIF receptors in the frontoparietal cortex of control rats were similar to

Table 3. Effect of continuous infusion of Aβ (25-35) (300 pmol/day) or Aβ (35–25) during 14 days on the equilibrium parameters for SRIF binding in membranes from the rat frontal cortex and parietal cortex

Groups	SRIF receptors			
	Frontal cortex		Parietal cortex	
	B_{max}	K_d	B_{max}	K_d
Control	512±33	0.55±0.1	539±61	0.60±0.01
Aβ (25–35)	431±21*	0.43±0.06	393±11*	0.50±0.01
Control	482±25	0.62±0.03	452±28	0.58±0.03
Aβ (35-25)	466±24	0.64±0.04	508±20	0.58±0.01

Binding parameters were calculated from Scatchard plots by linear regression. Units for K_d are nM and units for B_{max} are fmol SRIF bound/mg protein. The results are the mean±S.E.M. of five separate experiments performed in duplicate. Statistical comparison vs. control: * $P<0.05$.

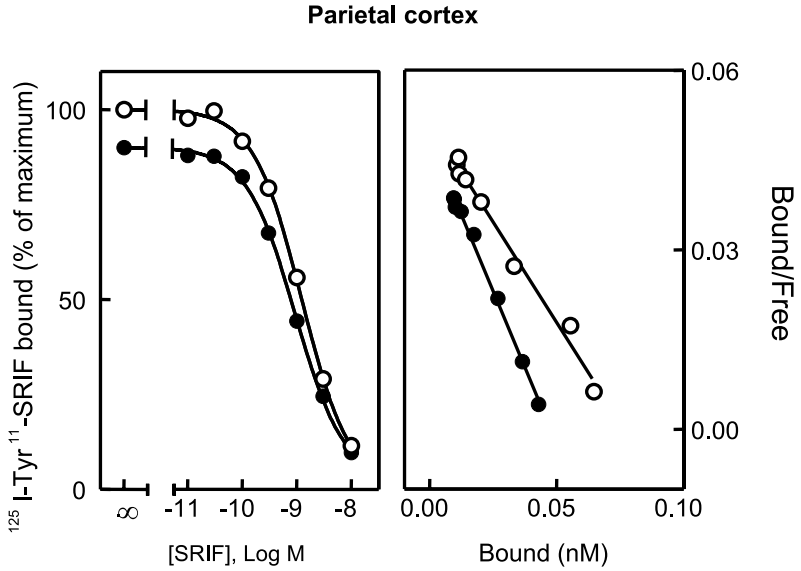

Fig. 2. Effect of continuous i.c.v infusion of Aβ (25–35) (300 pmol/day) during 14 days on SRIF binding to membranes from the rat parietal cortex. Left panel: competitive inhibition of specific ^{125}I-Tyr^{11}-SRIF binding by unlabeled SRIF to membranes from the rat parietal cortex. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30 °C in the presence of 250 pM ^{125}I-Tyr^{11}-SRIF and increasing concentrations of native peptide. Points correspond to values from the control rats (○) and rats treated with Aβ (25–35) (●). Each point represents the mean±S.E.M. of five rats. Each experiment was performed in duplicate. Right panel: Scatchard analysis of the binding data. The corresponding equilibrium parameters are included in Table 2.

those previously reported by other authors (Srikant and Patel, 1981; Epelbaum et al., 1982). After 14 days of the single administration of Aβ (25–35) and after continuous Aβ (25–35) infusion, a decrease in the SRIF receptor density was observed in the rat frontoparietal cortex. These results are in agreement with those previously published in the postmortem frontoparietal cortex of AD patients (Krantic et al., 1992). The mechanisms underlying the Aβ (25–35)-induced decrease in SRIF receptors is presently unknown. However, if the decrease in SRIF-LI observed in our study after Aβ injection is due to an increased SRIF release, then this increased release could lead to a down-regulation of SRIF receptors. On the other hand, the CRE located in the promoter of the sst2 and sst3

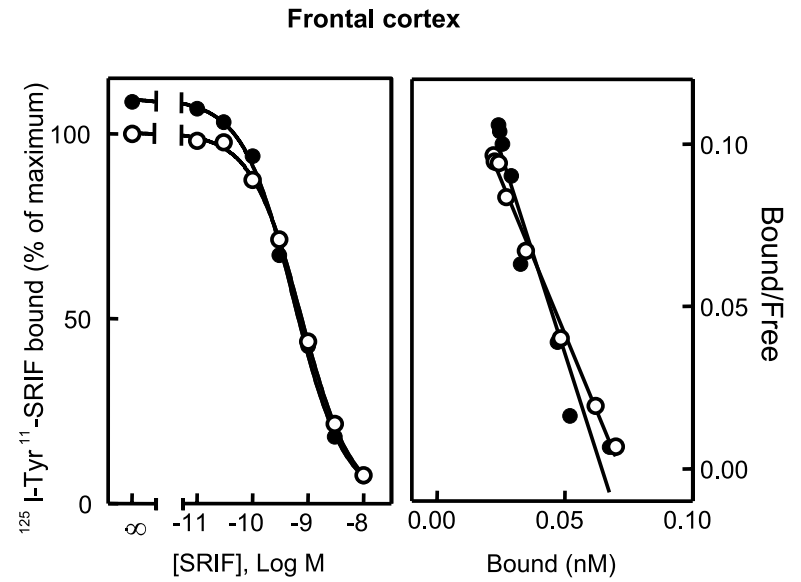

Fig. 3. - Effect of continuous i.c.v. infusion of Aβ (25–35) (300 pmol/day) during 14 days on SRIF binding to membranes from the rat frontal cortex. Left panel: competitive inhibition of specific ^{125}I-Tyr^{11}-SRIF binding by unlabeled SRIF to membranes from the frontal cortex. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30 °C in the presence of 250 pM of ^{125}I-Tyr^{11}-SRIF and increasing concentrations of native peptide. Points correspond to values from the control rats (○) and rats treated with Aβ (25–35) (●). Each point represents the mean±S.E.M. of five rats. Each experiment was performed in duplicate. Right panel: Scatchard analysis of the binding data. The corresponding equilibrium parameters are included in Table 2.

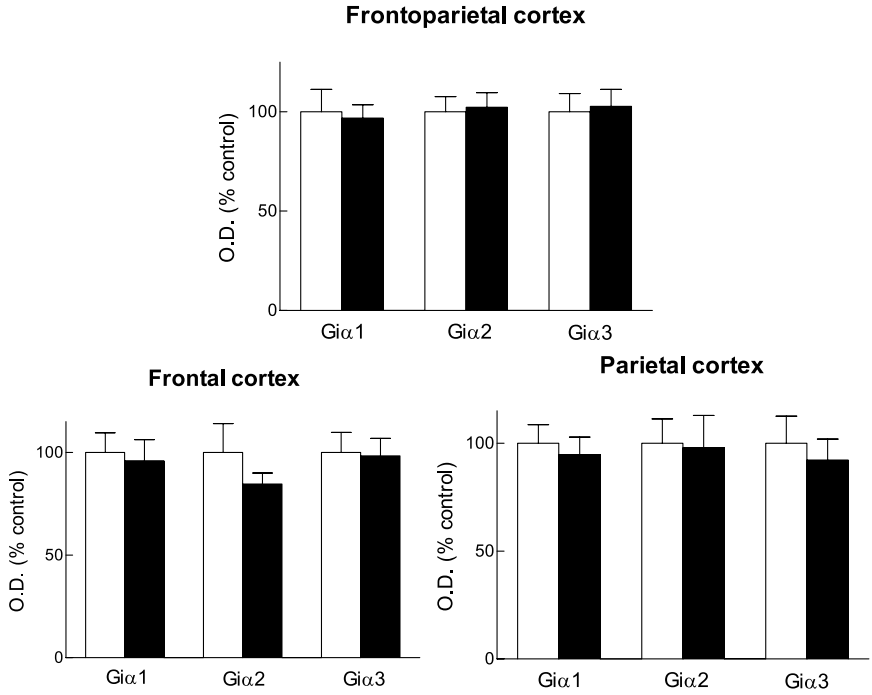

Fig. 4. Densitometric analysis of the autoradiographs derived from the immunoblots of the αi1, αi2 and αi3 subunits of G proteins. Frontoparietal, frontal and parietal cortical membranes from control and Aβ (25–35)-treated rats were resolved on SDS-PAGE as described in the Experimental Procedures section. Proteins were transferred to nitrocellulose membranes and the immunodetection was achieved using mouse anti-Giα1 or anti-Giα2 monoclonal antibodies or a rabbit anti-Giα3 policlonal antibody. Each experiment is representative of five others. For details, see the Experimental Procedures section. Integrated optical densities for control autoradiographs were assigned an arbitrary value of 100. Integrated optical densities for autoradiographs from frontoparietal, frontal and parietal cortical membranes following a single or continuous administration of Aβ (25–35) are presented as a percent of the control value. Data represent the mean±S.E.M.

genes (Glos et al., 1998; Woltje et al., 1998), prominently expressed across the rat brain (Patel et al., 1993), is a prototype of a highly cAMP-responsive element regulated by the transcription factor CREB (Montminy et al., 1996). Since Aβ (25–35) leads to an impairment of CREB signaling in neurons, it is possible, at least in part, that the mechanism underlying the Aβ (25–35)-induced decrease in the number of SRIF receptors observed in the present study may involve transcriptional downregulation.

Finally, damaged cells and neuronal loss in the neocortex were observed after i.c.v. administration of Aβ (25–35) to rats (Stepanichev et al., 2000). Since SRIF receptors are present in different cell types in the rat brain, these findings may explain the decrease in the SRIF receptor density found in the present study. Moreover, due to the continuous infusion of the peptide into the brain, an increased concentration of Aβ could alter the phosphoinositide metabolism of the membranes leading to a disorganization of biochemical structures (Prasad et al., 1998; Wells et al., 1995), and thus, to a decrease in the binding of SRIF to its receptors.

The scrambled peptide Aβ (35-25) did not alter the SRIF binding parameters at any of the time-periods studied, which indicates that the Aβ (25–35)-induced decrease

Table 4. Effect of the continuous infusion of Aβ (25–35) (300 pmol/day) during 14 days on basal and FK-stimulated AC activity (pmol cAMP/min/mg protein) in frontoparietal cortical membranes from control (n=10) and Aβ (25–35)-treated rats (n=10)

	Frontal cortex		Parietal cortex	
	Control	Aβ (25–35)	Control	Aβ (25–35)
Basal	241.4±15.2	248±42.2	393.6±11.25	319.15±18.7**
10^{-4} M SRIF	172.3±9.7	162.8±34.2	254.8±13.3	215.6±12.94
10^{-5} M FK	678.1±50.1	690.6±173	1199.39±128.43	1099.7±108.7
10^{-5} M FK+10^{-4} M SRIF	392.6±24	473.4±145.7	779.79±90.91	773±82.86
Fold FK stimulation over basal	2.8±0.1	3±0.3	3.19±0.35	3.61±0.19
% SRIF inhibition of basal activity	28.5±0.9	31.1±0.8	36.97±1.2	31.68±1.28*
% SRIF inhibition of FK-stimulated activity	41.9±1.1	30±3.68*	35.64±1.4	28.17±0.66**

Membrane preparations were incubated with or without SRIF (10^{-4} M) in the absence or presence of FK (10^{-5} M). Values represent the mean±S.E.M. of five separate experiments each performed in duplicate. Statistical comparison vs. control: * $P<0.05$; ** $P<0.01$.

in SRIF receptor density is a specific effect of the peptide. The reversal of the amino acid sequence in Aβ (35-25) has been shown to alter the aggregation properties of the peptide from β-sheet to random coil formation, thereby reducing the neurotoxic effects on cultured cells (Buchet et al., 1996).

Basal and FK-stimulated AC activity was inhibited by SRIF in all the experimental groups, which is in agreement with the literature (Bergström et al., 1991; Garlind et al., 1992; Schettini et al., 1989). A high concentration of SRIF (10^{-4}M) was required to produce this inhibition although the same concentration was used by other researchers in their studies on SRIF-mediated inhibition of AC activity in the rat brain (Bergström et al., 1991; Garlind et al., 1992; Schettini et al., 1989). In a previous study from our group (Puebla and Arilla, 1995), the inhibitory effect of increasing SRIF concentrations on basal and FK (10^{-5} M)-stimulated AC activity in control rats was analyzed. SRIF-mediated inhibition of AC activity was only significant at the maximal concentration tested (10^{-4} M). Thus, this concentration was chosen for subsequent studies on AC activity. It should also be noted that abundant studies on the SRIF effect on AC activity were performed in cell lines expressing higher levels of SRIF receptors than in animal tissues. In addition, it is tempting to speculate that because the frontoparietal cortex is very rich in SRIF-containing neurons, the amount of SRIF released may be sufficiently great as to justify the high SRIF concentration used to inhibit AC activity.

Aβ (25–35) administration led to a decrease in SRIF-mediated inhibition of basal and FK-stimulated AC activity at 14 days of the single administration and after continuous i.c.v. infusion. However, no changes were detected in either basal or FK-stimulated AC activity, suggesting that the decreased sensitivity of AC to SRIF inhibition was not due to an alteration in the AC catalytic subunit. In addition, Western blot analyses of Giα1, Giα2 and Giα3 proteins indicate that none of these proteins were modified by Aβ (25–35). Taken together, the present results suggest that the decrease in SRIF-mediated inhibition of AC activity is most probably due to the decrease in the SRIF receptor density. Cowburn et al. (1992)) have shown a preservation of Gi-protein inhibited AC activity in the brains of patients with AD. O'Neill et al. (1994) also found that Gi-protein-inhibited AC activity was unaltered in the frontoparietal cortex of AD patients. Kato et al. (1991)) showed no difference in the relative abundance of Giα2 in the AD temporal cortex as compared with control values.

In relation to the specificity of Aβ (25–35) on the SRIFergic system, other authors have shown that Aβ (25–35) also exerts effects on radioligand binding to excitatory amino acid receptors and voltage-dependent calcium channels, evidenced by a selective affinity for the glutamate and glycine recognition sites of the NMDA receptor (Cowburn et al., 1997). In addition, Aβ (25–35) has been reported to alter the number and affinity of bradykinin, muscarinic and nicotinic receptors (Huang et al., 1998; Guan et al., 2003). All these findings, therefore, concur to indicate that the SRIFergic system is vulnerable to the toxicity of Aβ (25–35) together with other neuronal systems. Thus, the Aβ (25–35) infusions performed in our study might produce a general toxic effect that causes nonspecific effects on neurotransmitter systems.

The significance of our results is, at present, difficult to assess. However, potent amnesic properties were reported for the 11-amino acid fragment of Aβ, Aβ (25–35) (Olariu et al., 2001; Yamaguchi and Kawashima, 2001). Several findings suggest a role for SRIF in cognitive processes such as learning and memory (Vécsei et al., 1984; Cacabelos et al., 1988). Recently, Dournaud and colleagues (1996) suggested that frontal and parietal somatostatinergic interneurons are likely to participate in learning behavior and in the regulation of spatial amnesic processes. Therefore, the reduction in SRIF receptors in both brain areas after infusion of Aβ (25–35) might be involved in the impairment of cognitive function described after administration of Aβ (25–35). In conclusion, the present results suggest that Aβ might be involved in the alterations of the somatostatinergic system reported in AD. Nevertheless, it must be pointed out that there are some subtle differences between Aβ (25–35) and the full-length peptide Aβ (1–42). Aβ (25–35) has often been studied as the potent component of the full-length peptide following the observation that it produces toxic effects similar to those caused by Aβ1–42 such as neuronal death, protein oxidation, and lipid peroxidation in cell cultures (Varadarajan et al., 2001). However, the shorter peptide is more rapidly toxic than the full-length peptide and, in addition, often causes more oxidative damage. Furthermore, Aβ1–42 has been shown to strongly bind Cu(II) ions and reduce them to Cu(I), whereas Aβ25–35 lacks the Cu(II) binding motif that was identified in Aβ1–42 (Atwood et al., 1998). Therefore, great care must be taken when interpreting our results since there might be important differences in the mechanism of action of Aβ25–35 and Aβ1–42 in the pathogenesis of AD. Further studies will be necessary to explore these possible differences and compare the effects of the two peptides. Moreover, the possibility that the impairment of cognitive function associated with Aβ (25–35) administration may be due, at least in part, to damage in some other neuronal system cannot be ruled out.

Acknowledgments—This work was supported by a grant (PM99-0129) from the Dirección General de Investigación del Ministerio de Ciencia y Tecnología of Spain.

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(Accepted 12 February 2005)
(Available online 5 August 2005)