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Chronic but Not Acute Intracerebroventricular Administration of Amyloid β-Peptide(25–35) Decreases Somatostatin Content, Adenylate Cyclase Activity, Somatostatin-Induced Inhibition of Adenylate Cyclase Activity, and Adenylate Cyclase I Levels in the Rat Hippocampus

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Although alterations in adenylate cyclase (AC) activity and somatostatin (SRIF) receptor density have been reported in Alzheimer's disease, the effects of amyloid β-peptide (Aβ) on these parameters in the hippocampus are unknown. Our aim was to investigate whether the peptide fragment Aβ(25–35) can affect the somatostatinergic system in the rat hippocampus. Hence, Aβ(25–35) was injected intracerebroventricularly (i.c.v.) to Wistar rats in a single dose or infused via an osmotic minipump connected to a cannula implanted in the right lateral ventricle during 14 days. The animals were decapitated 7 or 14 days after the single injection and 14 days after chronic infusion of the peptide. Chronic i.c.v. infusion of Aβ(25–35) decreased SRIF-like immunoreactive content without modifying the SRIF receptor density, SRIF receptor expression, or the $Gi\alpha_1$, $Gi\alpha_2$, and $Gi\alpha_3$ protein levels in the hippocampus. This treatment, however, caused a decrease in basal and forskolin-stimulated AC activity as well as in the capacity of SRIF to inhibit AC activity. Furthermore, the protein levels of the neural-specific AC type I were significantly decreased in the hippocampus of the treated rats, whereas an increase in the levels of AC V/VI was found, with no alterations in type VIII AC. A single i.c.v. dose of Aβ(25–35) exerted no effect on SRIF content or SRIF receptors but induced a slight decrease in forskolin-stimulated AC activity and its inhibition by SRIF. Because chronic Aβ(25–35) infusion impairs learning and memory whereas SRIF facilitates these functions, the alterations described here might be physiologically important given the decreased cognitive behavior previously reported in Aβ-treated rats.

Key words: rat; brain; hippocampus; amyloid β peptide; adenylate cyclase; Gi proteins

The neuropeptide somatostatin-14 (SRIF-14) is a cyclic tetradecapeptide, which is widely distributed in the peripheral and central nervous systems (Epelbaum, 1986). In the hippocampus, immunohistochemical studies have revealed many SRIF-containing interneurons and a profuse network of intrinsic and extrinsic SRIF-containing fibers that appears to project to pyramidal and granule neurons (Bakst et al., 1986; Jöels et al., 1990). Behavioral and clinical findings suggest an important role for SRIF in cognitive functions (Epelbaum, 1986). Specific SRIF receptors are present in the hippocampus (Reubi and Maurer, 1985), suggesting that SRIF may be a neurotransmitter or neuromodulator in the hippocampal formation. The actions of SRIF are mediated by specific high-affinity, membrane receptors, which are sensitive to guanine nucleotides. Five SRIF receptor subtypes, termed *sst1–sst5*, have thus far been cloned (Bell and Reisine,

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1993). These receptors are expressed in different amounts in the CA1 region of the hippocampus (Csaba and Dournaud, 2001) and are negatively coupled to adenylate cyclase (AC) via inhibitory guanine nucleotide-binding proteins (Gi proteins). Among the numerous neuropeptides produced in intrinsic hippocampal and cortical neurons, SRIF has been found to be the most consistently reduced in Alzheimer's disease (AD) (Davies et al., 1980; Rossor et al., 1980; Wood et al., 1982; Reinikainen et al., 1987; Atack et al., 1988).

AD is an irreversible neurodegenerative disorder that is characterized clinically by progressive dementia and histopathologically by the presence of extracellular deposits of amyloid fibrils in the core of senile plaques, intracellular neurofibrillar tangles, and neuronal cell loss (Braak and Braak, 1991). One of the principal components of senile plaques, amyloid β-peptide (Aβ), is considered to be involved in the pathogenesis of AD (Hsiao et al., 1996; Selkoe, 2001). An accumulation of Aβ has been associated with progressive neuronal death, cognitive deficits, and neuropsychiatric disorders such as agitation, apathy, and increased anxiety (Hardy and Higgins, 1992; Selkoe, 1996; Weiner et al., 1997; Sheuner et al., 2004; Stepanichev et al., 2004). Recent studies suggest that neurotransmitter receptor function in AD may be compromised because of disrupted postreceptor signal transduction, in particular, the phosphoinositide and AC pathways (Cowburn et al., 2001). AC activity has been reported to be reduced in different brain regions of patients with AD (Ohm et al., 1991; Cowburn et al., 1992; Ross et al., 1993). Ohm et al. (1991) observed a significant reduction in basal and stimulated AC activity in the AD hippocampus, a brain area that typically shows severe histopathological changes in this disorder. No significant changes in the levels of any of the G-proteins, however, were detected in the AD frontal cortex or hippocampus (Ross et al., 1993). Bergström et al. (1991) reported a reduction in the inhibitory effect of SRIF on AC activity in the superior temporal cortex of a group of AD cases compared with a group of matched controls, with no changes in other brain regions. Studies on the SRIF receptor binding sites in the AD brain have given conflicting results (Beal et al., 1985; Cowburn et al., 1991). It is presently unknown, however, whether Aβ can alter the inhibitory effect of SRIF on AC activity in the rat hippocampus.

In view of these facts, we sought to determine whether Aβ(25–35), an 11-amino-acid fragment of Aβ, can affect the rat hippocampal somatostatinergic system. Hence, we measured basal and forskolin (FK)-stimulated AC activity, the capacity of SRIF to inhibit both activities, and the protein levels of the AC isoforms I, V/VI and VIII in rat hippocampal membranes. We also tested whether Aβ(25–35) can alter the binding of SRIF to its receptors, the expression of the SRIF receptor subtypes sst1–sst4, the SRIF-like immunoreactive (SRIF-LI) content, or $Gi\alpha_1$, $Gi\alpha_2$, or $Gi\alpha_3$ protein levels in the rat hippocampus after either acute or chronic intracerebroventricular (i.c.v.) administration. The hippocampus was chosen for this study because of its involvement in cognitive functions (Kesner and Hopkins, 2006), in which SRIF plays a pivotal role.

MATERIALS AND METHODS

Chemicals

Synthetic Tyr^{11}-SRIF and SRIF-14 were purchased from Universal Biologicals Ltd. (Cambridge, United Kingdom). Aβ(25–35), Aβ(35–25), bacitracin, phenylmethylsulfonyl fluoride (PMSF), 3-isobutyl-1-methylxanthine (IBMX), bovine serum albumin (fraction V; BSA), guanosine triphosphate (GTP), and forskolin (FK) were purchased from Sigma (Madrid, Spain). Specific antisera against the αi_1 (MAB3075) or αi_2 (MAB3077) G protein subunits were obtained from Chemicon International (Temecula, CA). Specific antisera against the G protein subunit αi_3 (sc-262), the AC isoforms AC I (sc-25743), AC V/VI (sc-590), and AC VIII (sc-1967), the somatostatin receptor subtypes sst1 (sc-11604), sst2 (sc-11606), sst3 (sc-11614), and sst4 (sc-11619), and the secondary antibody (mouse IgG-peroxidase conjugate) were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The nitrocellulose membranes and the chemiluminescence Western blotting detection system were purchased from Amersham (Buckinghamshire, United Kingdom). Dextran was obtained from Serva Feinbiochemica (Heidelberg, Germany) and carrier-free $Na^{125}I$ (IMS 30; 100 mCi/ml) from the Radiochemical Centre (Amersham). The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham). This antiserum was raised in rabbits against SRIF-14 conjugated to BSA and is specific for SRIF, but, because SRIF-14 constitutes the C-terminal portions of both SRIF-25 and SRIF-28, the antiserum does not distinguish among these three forms.

Experimental Animals

All procedures conform to the guidelines set by our Animal Care and Use Committee and were approved by this committee before implementation. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Thirty male Wistar rats weighing 200–250 g were used in this study. All animals received food and tap water ad libitum. Room temperature was kept at 22°C, and a 12-hr day-night cycle was maintained. Aβ(25–35) was dissolved in 1% acetic acid according to the manufacturer's instructions and was incubated at 37°C on the day before its administration. Aβ(25–35) was administered i.c.v. to the rats in a single dose (10 μg) or via an osmotic minipump (Alzet) connected to a cannula implanted in the rat right lateral ventricle, as previously described (Nitta et al., 1994; Ping Tang et al., 2000; Fukuta et al., 2001; Nag and Tang, 2001). Chronic Aβ(25–35) or Aβ(35–25) infusion was carried out at a dose of 300 pmol/day for 14 days (Nag and Tang, 2001). Control animals received equivalent volumes of the vehicle, 1% acetic acid, to control whether acetic acid per se could lead to pathogenic changes. The animals were decapitated 7 or 14 days after the single injection of Aβ(25–35) and 14 days after chronic infusion of the peptide. The brains were quickly removed from the

skull, and the hippocampus was immediately dissected over ice as described by Glowinski and Iversen (1966).

Tissue Extraction and Somatostatin Radioimmunoassay

For measurements of SRIF-LI content, the hippocampus was homogenized in 1 ml of 2 M acetic acid using a Brinkman polytron (setting 5, 30s). Extracts were boiled for 5 min in a water bath and chilled in ice. Subsequently, homogenates were centrifuged at 15,000*g* for 15 min at 4°C. The pellet was discarded, and 25 μl of the supernatant was taken for determination of protein concentration (Lowry et al., 1951). The extracts were immediately stored at −80°C until the time of assay. The SRIF-LI content was determined in tissue extracts by a modified radioimmunoassay method (Patel and Reichlin, 1978). Briefly, the assay tubes contained 100 μl of unknown or standard solutions (0–500 pg) of cyclic SRIF-14, diluted in 0.1 M phosphate buffer, pH 7.2, containing 0.2% BSA and 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 yield 6,000–10,000 cpm (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 at 4°C for 24 hr. The separation of bound and free hormone was accomplished by addition of 1 ml dextran-coated charcoal (dextran: 0.2% w/v). The coefficients for intra- and interassay variation were 6.3% and 8.1%, respectively.

Binding Assay

Tyr^{11}-SRIF was radioiodinated by the chloramine-T method according to 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. Hippocampal membranes were prepared as previously described by Reubi et al. (1981). The protein concentration was assayed by the method of Lowry et al. (1951), with BSA as a standard. Specific SRIF binding was measured according to the modified method of Czernik and Petrack (1983). Briefly, the 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 250 pM of ^{125}I-Tyr^{11}-SRIF either in the absence or in the presence of 0.01–10 nM unlabelled SRIF. After a 60 min incubation at 30°C, the bound and free ligand were separated by centrifugation at 11,000*g* for 2 min, and the radioactivity in the resultant pellet was measured. Nonspecific binding was obtained from the amount of radioactivity bound in the presence of 10^{-7} M SRIF and represented about 20% of the binding observed in the absence of unlabelled peptide. This nonspecific component was subtracted from the total bound radioactivity to obtain the corresponding specific binding.

Evaluation of Radiolabeled Peptide Degradation

The inactivation of ^{125}I-Tyr^{11}-SRIF in the incubation medium after exposure to membranes was studied by measuring the ability of the preincubated peptide to rebind to fresh membranes (Aguilera et al., 1982). Briefly, ^{125}I-Tyr^{11}-SRIF (250 pM) was incubated with rat hippocampal membranes (0.15 mg protein/ml) for 60 min at 30°C. After this preincubation, aliquots of the medium were added to fresh membranes and incubated for an additional 60 min at 30°C. The fraction of the added radiolabeled peptide that 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 preincubation period.

AC Assay

AC activity was measured as previously reported (Houslay et al., 1976), with minor modifications. Briefly, hippocampal membranes (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 creatine phosphate and 1 mg/ml creatine 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 cooling, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) was added, and the suspension was centrifuged. The supernatant was taken for assay of cAMP by the method of Gilman (1970).

Immunodetection of αi Subunits of G Proteins, AC Isoforms, and SRIF Receptor Subtypes

Membranes (100 μg) were solubilized in SDS-sample buffer. The proteins were then run on a 12% SDS-polyacrylamide gel, as described by Laemmli (1970). After separation, the proteins were transferred onto nitrocellulose membranes in a buffer consisting of 25 mM Tris/HCl, pH 8.3, 192 mM glycine, 20% methanol, and 0.05% SDS. The nitrocellulose membranes were then blocked with TTBS (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween-20) containing 5% (w/v) nonfat dry milk for 1.5 hr at 4°C. The nitrocellulose membranes were subsequently incubated with the corresponding antibodies: mouse anti-Giα_1 or anti-Giα_2 monoclonal antibody (1:1,000 dilution), rabbit anti-Giα_3 polyclonal antibody (1:1,000 dilution), rabbit anti-AC I, AC V/VI, or AC VIII polyclonal antibody (1:1,000 dilution) or goat anti-sst1, anti-sst2, anti-sst3, or anti-sst4 polyclonal antibody (1:1,000 dilution) in TTBS overnight at 4°C. After incubation, three 5-min washes in TTBS containing 5% (w/v) nonfat dry milk were carried out. A goat or mouse IgG-peroxidase conjugate (1:2,000 dilution) in TTBS was then added to the membranes and incubated for 1 hr at 4°C. After washing, the bound immunoreactive proteins were detected by a chemiluminescent (ECL) Western blotting detection system. Quantification of the bands was carried out by densitometric analysis in Scion Image (Scion Inc.).

Data Analysis

The computer program Ligand (Munson and Rodbard, 1980) was used to analyze the binding data. The use of this

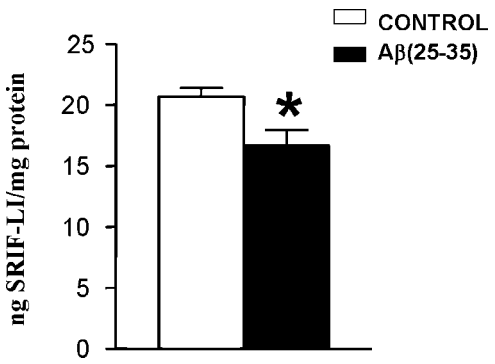

Fig. 1. Effect of chronic (14 days) i.c.v. infusion of Aβ(25–35) on somatostatin-like immunoreactivity (SRIF-LI) concentration in the rat hippocampus. Open bars correspond to control rats and solid bars to Aβ(25–35)-treated rats. Results are expressed as the mean ± SEM of five rats in each group. Statistical comparisons of the data were carried out by one-way ANOVA and Student's Newman-Keuls test. Statistical significance vs. control: $\star P < 0.05$.

TABLE I. Equilibrium Parameters for ^{125}I-Tyr^{11}-Somatostatin (^{125}I-Tyr^{11}-SRIF) Binding to Hippocampal Membranes From Control Rats, Rats Treated With a Single i.c.v. Dose of Aβ(25–35) (10 μg) at 7 or 14 Days of Administration and Rats Treated for 14 Days With Aβ(25–35) (300 pmol/day) or Aβ(35–25) (300 pmol/day)⋆

	Somatostatin receptors	
Groups	B_{max}	K_d
Control	537 ± 6	0.52 ± 0.03
Aβ(25–35) (single dose, 7 days)	472 ± 31	0.54 ± 0.04
Control	415 ± 40	0.53 ± 0.04
Aβ(25–35) (single dose, 14 days)	476 ± 36	0.58 ± 0.06
Control	523 ± 22	0.51 ± 0.03
Aβ(25–35) (chronic infusion, 14 days)	492 ± 27	0.50 ± 0.05
Aβ(35–25) (chronic infusion, 14 days)	440 ± 37	0.46 ± 0.02

⋆Binding parameters were calculated from Scatchard plots by linear regression. Units for K_d are nM, and units for B_{max} are fmoles SRIF bound/mg protein. The results are represented as the mean ± SEM of five separate experiments, each performed in duplicate.

program allows models of receptors that best fit a given set of binding data to be selected. The same program was also used to present data in the form of Scatchard plots (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 data were carried out by one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test. Means among groups were considered significantly different when the P values were less than 0.05. Each individual experiment was performed in duplicate.

RESULTS

SRIF-Like-Immunoreactive Content Is Reduced in Aβ(25–35)-Treated Rats

Chronic i.c.v. infusion of Aβ(25–35) during 14 days induced a significant decrease in SRIF-LI content in the rat hippocampus compared with controls (Fig. 1).

Effect of Aβ(25–35) on Hippocampal SRIF Receptors

Hippocampal membranes from control and Aβ(25–35)-treated rats bound ^{125}I-Tyr^{11}-SRIF in a time-dependent manner, an apparent equilibrium being observed between 50 and 180 min at 30°C. All subsequent experiments were therefore conducted at 30°C for 60 min.

The results show a lack of effect of Aβ(25–35) or the reverse Aβ(35–25) on SRIF binding to its receptors in rat hippocampal membranes (Table I) following either a single i.c.v. administration or chronic infusion of the peptide. Hippocampal membranes from control and Aβ(25–35)-treated rats showed a similar tracer degradation capacity, the values varying by no more than 10% in all the experimental groups. Because the tracer used in the binding assay, Tyr^{11}-SRIF-14, binds with similar affinity to all the SRIF receptor subtypes, we next analyzed the expression of sst1, sst2, sst3, and sst4 to evaluate the possibility that one of these subtypes is altered. Western blot analyses with subtype-specific antibodies revealed no alterations in the protein levels of any of these receptors in hippocampal membranes from rats treated chronically with Aβ(25–35) compared with control rats (Fig. 2).

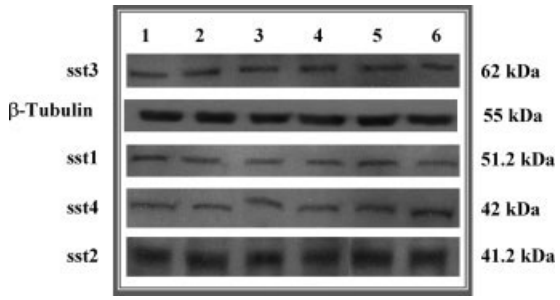

Fig. 2. Effect of chronic (14 days) i.c.v. infusion of Aβ(25–35) on protein levels of the somatostatin receptor subtypes sst1–sst4. Hippocampal membranes from control and Aβ(25–35)-treated rats were resolved by SDS-PAGE as described in Materials and Methods. Proteins were transferred to nitrocellulose membranes, and the immunodetection was achieved using a goat anti-sst1, anti-sst2, anti-sst3, or anti-sst4 polyclonal antibody. In each case, β-tubulin was used as a loading control. Each experiment is representative of five others. Lanes 1, 3, and 5 correspond to control rats; lanes 2, 4, and 6 correspond to Aβ(25–35)-treated rats.

Reduction of AC Activity and AC I Levels by Aβ(25–35)

We next sought to examine some of the components of the SRIF signalling pathway in control and Aβ(25–35)-treated rats. Because SRIF receptors are negatively coupled to the effector AC, we measured basal and FK-stimulated AC activity as well as SRIF-mediated inhibition of the enzyme in hippocampal membranes

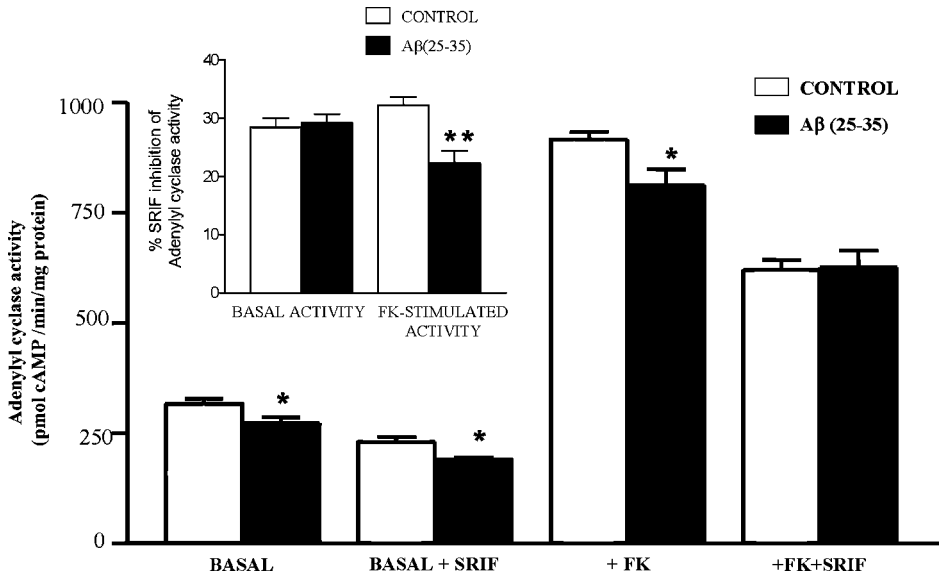

Fig. 3. Effect of chronic i.c.v. infusion of Aβ(25–35) (300 pmol/day) during 14 days on basal and forskolin (FK; 10^{-5} M)-stimulated adenylate cyclase (AC) activity as well as on somatostatin (SRIF)-mediated inhibition of AC activity in rat hippocampal membranes. Membrane preparations from control (open bars) or Aβ(25–35)-treated rats (solid bars) were incubated with or without SRIF (10^{-4} M) in the absence (basal) or presence of 10^{-5} M FK. Data are expressed as the mean ± SEM of five rats. Each experiment was performed in duplicate. Statistical comparison vs. the control group: $\star P < 0.05$. **Inset:** Percentage of SRIF-mediated inhibition of basal or stimulated (+FK) AC activity. Statistical comparison vs. the control group: $\star\star P < 0.01$.

TABLE II. Effect of Somatostatin (SRIF; 10^{-4} M) and Forskolin (FK; 10^{-5} M) on Adenylyl Cyclase Activity (pmol cAMP/min/mg Protein) in Hippocampal Membranes From Control Rats (n = 5) and Rats Treated With a Single Dose (10 μg) of Aβ(25–35) (n = 5) at 7 or 14 Days of Administration[†]

	Control	Aβ(25–35) (7 days)	Control	Aβ(25–35) (14 days)
Basal activity	332 ± 22	304 ± 38	267 ± 27	253 ± 35
$+10^{-4}$ M SRIF	236 ± 14	212 ± 31	189 ± 10	173 ± 7
SRIF inhibition of basal activity (%)	28.6 ± 0.8	31.6 ± 3.3	30.4 ± 2.1	30.3 ± 3.2
$+10^{-5}$ M FK	851 ± 63	818 ± 75	889 ± 28	796 ± 24⋆
$+10^{-5}$ M FK + 10^{-4} M SRIF	591 ± 75	563 ± 38	589 ± 68	574 ± 44
SRIF inhibition of FK-stimulated activity (%)	30.8 ± 2.3	29.3 ± 7	32.2 ± 1.6	27.9 ± 1.1⋆⋆
-Fold FK stimulation	2.6 ± 0.3	2.7 ± 0.4	3.2 ± 0.3	3.1 ± 0.4

[†]Data are the mean ± SEM of five separate experiments, each performed in duplicate.
⋆$P < 0.05$ vs. control.
⋆⋆$P < 0.01$ vs. control.

from control and Aβ(25–35)-treated rats. As shown in Figure 3, chronic infusion of Aβ(25–35) during 14 days caused a decrease in both basal and FK-stimulated AC activity. Although basal and FK-stimulated AC activities were inhibited by SRIF in all the experimental groups studied, the capacity of SRIF to inhibit FK-stimulated AC activity was significantly lower in the rats treated chronically with Aβ(25–35) compared with controls (Fig. 3). No significant differences in basal and FK-stimulated AC activity or in SRIF-induced inhibition of these activities were detected in the hippocampal membranes of rats treated acutely with a single dose of Aβ(25–35) at 7 days of administration (Table II). There was a slight decrease, however, in FK-stimulated AC activity and its inhibition by SRIF at 14 days of administration (Table II). These results led us to investigate further the cause of the reduction observed after chronic infusion of the peptide. We therefore analyzed the expression of the AC isoforms AC I, AC V/VI, and AC VIII at the protein level by means of Western blot. Type I and type VIII AC are Ca^{2+}/calmodulin-sensitive enzymes that are expressed exclusively in brain. AC V and VI, present in brain and heart, are two isoforms that are sensitive to inhibition by Gi proteins, including $Gi\alpha_1$, $Gi\alpha_2$, and $Gi\alpha_3$. Representative immunoblots of these AC isoforms are shown in Figure 4. The results revealed a significant decrease in the protein levels of type I AC, whereas an increase in AC V/VI levels was found in the Aβ-treated group compared with the control group (Fig. 4). No differences in type VIII AC

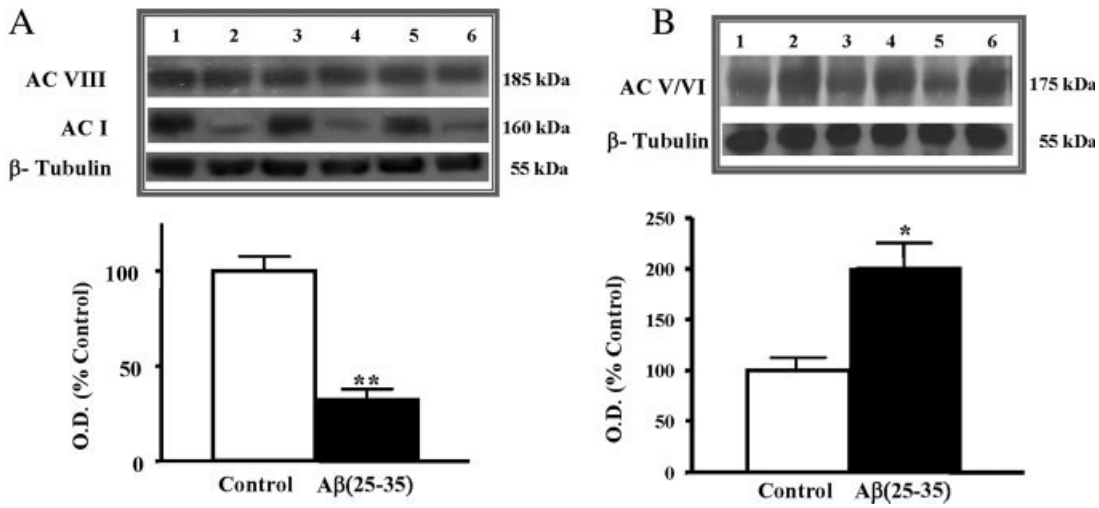

Fig. 4. Effect of chronic i.c.v. infusion of Aβ(25–35) during 14 days on the protein levels of the adenylate cyclase isoforms AC I, AC V/VI, and AC VIII in the rat hippocampus. Hippocampal membranes from control and Aβ(25–35)-treated rats were resolved on SDS-PAGE as described in Materials and Methods. **Top:** Proteins were transferred to nitrocellulose membranes, and the immunodetection was achieved by using a rabbit anti-AC I (**A**), anti-AC VIII (A), or anti-AC V/VI (**B**) polyclonal antibody. Lanes 1, 3, and 5 correspond to control rats; lanes 2, 4, and 6 correspond to Aβ(25–35)-treated rats. In each case, β-tubulin was used as a loading control. Each experiment is representative of five others. For details see Materials and Methods. **Bottom:** Densitometric analysis of the bands obtained in the immunoblots corresponding to AC I (**A**) or AC V/VI (**B**). Integrated optical densities for bands were assigned an arbitrary value of 100. Integrated optical densities for bands corresponding to chronic infusion of Aβ(25–35) are represented as a percentage of the control value. Data represent the mean ± SEM. Statistical comparison vs. control: $\star P < 0.05$, $\star\star P < 0.01$.

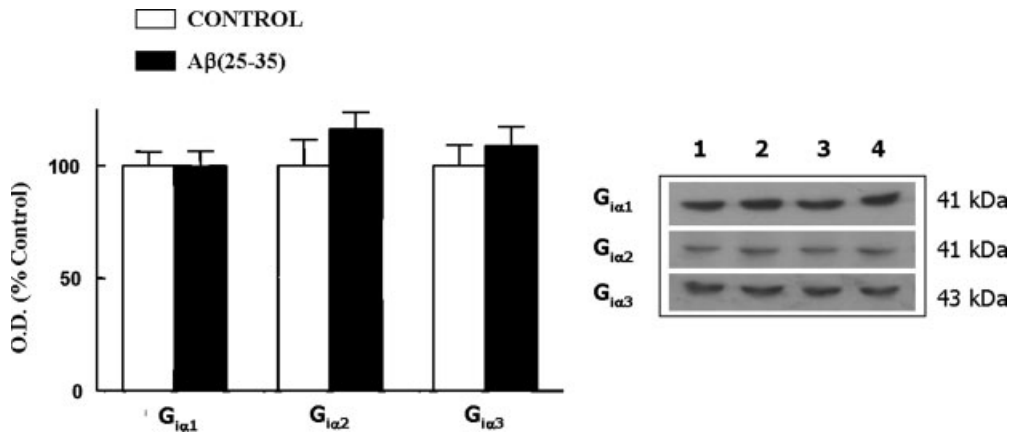

Fig. 5. **Right:** Immunodetection of the αi_1, αi_2, and αi_3 subunits of G proteins. Hippocampal membranes from control and Aβ(25–35)-treated rats were resolved by SDS-PAGE as described in Materials and Methods. Proteins were transferred to nitrocellulose membranes, and the immunodetection was achieved by using mouse anti-$Gi\alpha_1$ or anti-$Gi\alpha_2$ monoclonal antibodies or an anti-$Gi\alpha_3$ polyclonal antibody. Lanes 1 and 3 correspond to control rats; lanes 2 and 4 correspond to Aβ(25–35)-treated rats. Each experiment is representative of five others. For details see Materials and Methods. **Left:** Densitometric analysis of the bands obtained. Integrated optical densities for control bands were assigned an arbitrary value of 100. Integrated optical densities for bands corresponding to chronic infusion of Aβ(25–35) are presented as a percentage of the control value. Data represent the mean ± SEM.

content, however, were detected among these experimental groups.

Giα Protein Levels Are Unaltered in Hippocampal Membranes From Aβ(25–35)-Treated Rats

The inhibitory G proteins $Gi\alpha_1$, $Gi\alpha_2$, and $Gi\alpha_3$ all mediate the effects of SRIF signals on AC. Hence, our next objective was to determine whether the levels of the α subunits of these proteins were affected by Aβ(25–35). Western blot analyses revealed that chronic i.c.v. infusion of Aβ(25–35) during 14 days does not significantly modify αi_1, αi_2, or αi_3 protein levels in rat hippocampal membranes compared with controls (Fig. 5). Likewise, a single dose of Aβ(25–35) exerted

no effect on this parameter at 7 or 14 days of injection (data not shown).

DISCUSSION

In this study we have used an 11-amino-acid fragment of the Aβ peptide, Aβ(25–35), which is located at the C-terminal end of Aβ(1–42) in the hydrophobic domain. 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 hippocampal CA1 neurons by enhancing excitotoxicity in AD. By using specific anti-(D-Ser^{26})-Aβ(25/26–35/40) antibodies, Kubo et al. (2002) demonstrated clearly that the truncated fragments (D-Ser^{26})-Aβ(25/26–35/40) were present in the brains of AD patients, but not in the age-matched control subjects.

The experimental paradigm, i.c.v. infusion of Aβ (25–35), has been demonstrated by several authors relevantly to mimic some of the pathological processes in AD brain. A large body of experimental data has demonstrated that Aβ injected i.c.v. to rodents significantly influences their cognitive behavior. The peptide fragment Aβ(25–35), in particular, has been demonstrated to induce impairment of spontaneous alternation behavior (Stepanichev et al., 2000), water maze and radial maze learning (Yamaguchi and Kawashima, 2001; Sun and Alkon, 2002), passive avoidance (Yamaguchi and Kawashima, 2001), and social recognition (Stepanichev et al., 2000) in rats. In addition, damaged cells and neuronal loss in the hippocampal subfields CA1 and CA3 as well as in the neocortex and basal ganglia were observed after i.c.v. administration of Aβ(25–35) to aged rats (Stepanichev et al., 2000).

The effects of Aβ(25–35) on different components of the SRIF signalling pathway in the rat hippocampus were more pronounced after chronic infusion than after a single administration of the peptide, even though the dose administered acutely was slightly higher. Dornan et al. (1993) failed to show any focal deposit of Aβ(25–35) in the rat hippocampus when the peptide was administered acutely. Studies by Nag et al. (1999), however, have revealed a clear and widespread presence of Aβ immunoreactivity in rats that received a chronic i.c.v. infusion of Aβ during 14 days, achieving a final concentration of 4.2 nmol, the same as that used in our study. Furthermore, there was abundant Aβ immunoreactivity in the hippocampal formation. Thus, the temporal feature observed in our study implies a chronic pathological process in the hippocampus triggered by the gradual accumulation of Aβ(25–35).

The SRIF-LI content in the hippocampus of control rats was similar to that previously reported by our group (Puebla and Arrila-Ferreiro, 2003) and by others (Srikant and Patel, 1981). The present study demonstrates that chronic i.c.v. infusion of Aβ(25–35) leads to a reduction in hippocampal SRIF-LI levels. Notably, the alteration in SRIF content demonstrated here parallels that seen in brains of AD patients (Davies et al., 1980; Slama et al., 1991). To date, however, it is unknown whether these changes are caused directly by the toxic effect of Aβ(25–35). There is evidence suggesting that Aβ(25–35) induces the opening of mitochondrial pores, which is an important aspect in the mechanism of neurotoxicity (Bachurin et al., 2003). The changes in SRIF-LI content observed might also be, to a certain extent, secondary to the cholinergic dysfunction demonstrated in AD and in Aβ protein-infused rats (Itoh et al., 1996).

Alternatively, Aβ(1–42) has been reported to impair cAMP response element binding protein (CREB) signalling in neurons (Tong et al., 2001). It is well established that the gene encoding for SRIF contains a cAMP response element designated *CRE* (Montminy and Bilezikjian, 1987). The second messenger cAMP can induce transcription of the SRIF gene via PKA-induced phosphorylation of the protein CREB, which is a transcription factor that subsequently binds to CRE (Montminy and Bilezikjian, 1987). Hence, a decrease in SRIF gene expression resulting from decreased CREB signalling might explain, partially or wholly, the decrease in hippocampal SRIF content observed in our study.

Chronic i.c.v. infusion of Aβ(25–35) reduced basal and FK-stimulated AC activity in the rat hippocampal membranes. This finding is in line with a post-mortem study carried out by Ohm et al. (1991), which showed reduced FK-stimulated enzyme activities in the AD hippocampus. This suggested a loss of AC enzyme units in AD, which has been supported by more recent immunoblotting studies using isoform-specific antibodies. Molecular cloning techniques have thus far identified nine mammalian genes that encode membrane-bound ACs and one gene encoding a soluble isoform (Sunahara and Taussig, 2002). In our study, we focused on four AC isoforms, namely, AC I, AC V/VI, and AC VIII. Types I and VIII, which are expressed exclusively in the brain, have been postulated to play a pivotal role in learning and memory. The results obtained demonstrate a selective reduction in the protein levels of AC I as a result of chronic Aβ(25–35) infusion in the rat hippocampus. These results are partially concordant with studies by Yamamoto et al., who showed a significant loss of AC type I in the AD hippocampus (Yamamoto et al., 2000) and AD parietal cortex (Yamamoto et al., 1996, 1997). The decrease we observe might in part explain the attenuation of AC activity under both basal and stimulated conditions in the Aβ(25–35)-treated rats. Furthermore, it is plausible that this reduction might contribute to a decreased phosphorylation of CREB in somatostatinergic neurons, which in turn would lead to the decrease in the rat hippocampal SRIF-LI content. This is feasible given the fact that SRIF is also involved in cognitive processes. Type VIII AC, on the other hand, was unaffected by chronic Aβ(25–35) infusion, in contrast to human AD hippocampus, in which a decrease was found (Yamamoto et al., 2000). We also tested AC V/VI, both of which are abundant in heart

and brain. SRIF receptors couple negatively to AC via the inhibitory G proteins $Gi\alpha_1$, $Gi\alpha_2$, and $Gi\alpha_3$, which have all been demonstrated to inhibit these two AC isoforms selectively (Sunahara and Taussig, 2002). Interestingly, an increase in AC V/VI protein levels was detected in the hippocampal membranes derived from rats treated chronically with Aβ. Although the functional significance of this increase is as yet unknown, it does seem to argue against the possibility that the lower expression of AC I in the Aβ(25–35)-treated group might be due to a loss of neurons, taking into account that damaged cells and neuronal loss have been reported in the hippocampal subfields CA1 and CA3 of AD patients; if this were the case, all isoforms studied would be decreased.

The levels of the α subunits of the Gi proteins were not significantly altered after either acute or chronic i.c.v. administration of Aβ(25–35). However, preservation of G protein levels is not necessarily indicative of the integrity of signal transduction in the disease state. Thus, whereas the numbers of SRIF receptors are unaltered in the present study as well as in the AD hippocampus, the ability of SRIF to inhibit AC is reduced. This result is in agreement with that obtained by Cowburn et al. (1991) in the temporal cortex of AD patients. The loss of AC activity, therefore, cannot be attributed to an altered Gi expression.

Although the underlying mechanism for the Aβ(25–35)-induced effects is not fully understood, it might be related to the ability of Aβ(25–35) to initiate membrane lipid peroxidation and to enhance oxidative stress (Mark et al., 1997; Stepanichev et al., 2004). In this regard, it has been demonstrated that direct infusion of Aβ peptides into rat brain leads to a significant increase in the production of reactive oxygen species (Parks et al., 2001; Stepanichev et al., 2004). Furthermore, Aβ(25–35) administration has been reported to lead to a gradually developing oxidative stress in the rat hippocampus.

Our results indicate that there are no significant changes in the SRIF binding capacity in the hippocampus of rats infused with Aβ(25–35) compared with controls. This result is in agreement with some (Whitford et al., 1988; Slama et al., 1991) but not all (Beal et al., 1985) studies carried out in the AD hippocampus. Slama et al. (1991) observed no significant changes in the total SRIF binding capacity in the hippocampus of patients with dementia compared with controls. Because the SRIF analog used in the binding assay, however, has affinity for all five SRIF receptor subtypes, we measured the protein levels of each subtype except for sst5, owing to its very low expression in the rat hippocampus. Western blot analysis revealed no differences in sst1–sst4 protein levels between the control and the Aβ(25–35)-treated rats, which agrees with the binding data. Thus, the possibility that the reduction of AC activity is due to a decreased sst density can be discarded.

Together, the present data demonstrate that, although the hippocampal SRIF receptors are fairly well preserved after chronic Aβ(25–35) infusion, their functionality seems to be compromised at the signal transduction level, as evidenced by the decrease in basal and FK-stimulated AC activity as well as in the inhibitory effect of SRIF on these activities. In addition, Aβ(25–35) induces a decrease in SRIF-LI content in the rat hippocampus. Hence, these findings seem to implicate Aβ in the impairment of somatostatinergic transmission that occurs in AD. Because chronic i.c.v. infusion of Aβ(25–35) impairs learning and memory whereas SRIF facilitates these functions, the alterations described in the present study might be correlated with the decreased cognitive behavior previously reported for Aβ-treated rats.

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