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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.

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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