

Aging and β -amyloid peptides decrease cholinergic receptor-mediated calcium increase in brain cortex synaptoneurosomes

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Abstract. In this study, the muscarinic cholinergic receptor (MACHR)-evoked inositol 1,4,5-trisphosphate (IP₃)-mediated increase of cytosolic calcium concentration ([Ca]_i) in synaptoneurosomes from brain cortex of adult and aged rats was investigated. In addition, the effect of two β -amyloid (A β) peptides, 1-28 and 25-35, on the resting and MACHR-induced increase of [Ca]_i in brain cortex synaptoneurosomes of adult rats was evaluated. Release of IP₃ was measured after prelabeling of synaptoneurosomal phosphoinositides with *myo*-[2-³H]inositol. Changes in [Ca]_i were monitored by using fura-2 indicator. The effect of A β peptides was evaluated following their preincubation with synaptoneurosomal protein for 1, 5, 30 and 60 min. It was observed that in brain cortex synaptoneurosomes from aged rats, Ca²⁺-dependent and MACHR-mediated IP₃ production was not changed in comparison with that estimated in adult brain, over 60 min of incubation. Activation of MACHR in synaptoneurosomes from brain cortex of adult rats for 10 min increased [Ca]_i by about 60% over its resting level (240 nM). This increase was completely blocked by muscarinic antagonists, atropine and pirenzepine, as well as by the antagonist of IP₃ receptor, 8-(diethylamino)-octyl-3,4,5-trimethoxybenzoate (TMB-8). In aged brain, there was no detectable change in resting [Ca]_i (165 nM) due to MACHR stimulation. The 25-35 A β peptide caused a time-dependent significant increase of resting [Ca]_i in synaptoneurosomes from brain cortex of adult rats, which was almost five-fold after 60 min. In the same conditions, the action of 1-28 A β peptide was statistically insignificant up to 30 min, then a rapid increase of resting [Ca]_i by two-fold was observed up to 60 min. Both A β peptides decreased markedly the MACHR-dependent elevation of [Ca]_i in respect to control (resting [Ca]_i) in synaptoneurosomes from brain cortex of adult rats. These results indicate that β -amyloid 1-28 and 25-35 peptides may be involved in alteration of muscarinic receptor-mediated signal transduction during brain aging.

Key words: aging, β -amyloid peptides, muscarinic receptor, inositol

INTRODUCTION

In neurons, Ca^{2+} regulates numerous physiological processes, including neurotransmitter release and uptake. Calcium activates several enzymes, such as calmodulin-dependent protein kinase (CaMK) and protein kinase C (PKC), proteases and phospholipases. It is involved in liberation of arachidonic acid, nitric oxide, and other second messengers and in activation of gene expression (Gibson and Peterson 1987, Meldolesi and Pozzan 1987, Hadcock and Malbon 1993). Calcium is likely to play a determining role in brain plasticity and in mechanisms of learning and memory (Lynch and Voss 1991). Disturbances of Ca^{2+} homeostasis contribute to brain aging, memory impairments, or even severe dementia and Alzheimer's disease. The permissive role of cytosolic free Ca^{2+} in a variety of cellular processes requires its precise regulation. Disturbances in control mechanisms of calcium homeostasis alter neuronal function. It is suggested that changes in the cytosolic free calcium concentration ($[\text{Ca}]_i$) contribute to functional impairment, and eventually to cell death (Hartmann et al. 1993, Weiss et al. 1994).

During cell stimulation the increase of $[\text{Ca}]_i$ may result from extracellular Ca^{2+} influx through voltage- and receptor-operated calcium channels and/or it may be liberated from endoplasmic reticulum, by the action of inositol 1,4,5-trisphosphate (IP_3) on its receptor (Martinez et al. 1987). At least a few subtypes of muscarinic cholinergic receptors are now recognized to be coupled to phospholipase C (PLC) that, in response to the extracellular signals, liberates IP_3 , leading to a release of Ca^{2+} and to increase of $[\text{Ca}]_i$ (Jope et al. 1997).

Recently, it was observed that the number of IP_3 receptor recognition sites are reduced in Alzheimer's disease (Garlind et al. 1995, Shimohama et al. 1995). Moreover, it was found that β -amyloid ($\text{A}\beta$), accumulated in Alzheimer's brain, may affect L-type Ca^{2+} channels or may itself form a Ca^{2+} channel (Mattson et al. 1993). Multiple neurotransmitter systems, including cholinergic, serotonergic, noradrenergic and glutamatergic, are known to be affected in aging and Alzheimer's disease (for review see Greenamyre and Maragos 1993). It was hypothesized that, in Alzheimer's brain, the postsynaptically localized signal transduction pathways may be disrupted. In fact, there is evidence for reduction of agonist binding to muscarinic cholinergic M_1 receptors (M_1AChR), as well as for alteration of M_1AChR -G protein coupling and M_1AChR -dependent

PLC-mediated phosphoinositides degradation (Ladner et al. 1995, Kelly et al. 1996, Claus et al. 1997). Several studies indicated that $\text{A}\beta$ peptides are toxic toward central neurons, having their neurotoxicity residing within amino acids 25-35 of the $\text{A}\beta$ (Mattson et al. 1992). The mechanism of this toxicity remains unclear, but recent evidence suggests that disturbances of Ca^{2+} homeostasis and activation of free radical formation and action may play an important role. β -amyloid can aggregate to form Ca^{2+} -permeable channels in membranes or it may potentiate glutamate-mediated Ca^{2+} influx. Calcium is suggested to be a significant initiating signal for $\text{A}\beta$ -enhanced excitotoxicity and cell apoptosis (Kelly et al. 1996).

There is a lot of controversy concerning the disturbances of mechanism of Ca^{2+} homeostasis in aged brain. Many factors that modulate Ca^{2+} signal transduction cascade may contribute to brain aging. One of the most important is the progressive increase of $\text{A}\beta$ level, its deposition, fibrilization and formation of senile plaques in aged brain. The aim of this study was to investigate the MACHR-evoked cytosolic Ca^{2+} increase in synaptoneurosomes from brain cortex of adult and aged rats. Moreover, the effect of neurotoxic β -amyloid 25-35 peptide and non-neurotoxic β -amyloid 1-28 peptide on the resting $[\text{Ca}]_i$ and on the MACHR-dependent increase of $[\text{Ca}]_i$ in synaptoneurosomes from adult brain cortex was evaluated.

METHODS

Materials

Myo-2- $[\text{}^3\text{H}]$ inositol (15 Ci/mmol) was purchased from Amersham, Buckinghamshire, UK. Fura-2/AM and 8-(diethylamino)-octyl-3,4,5-trimethoxy benzoate (TMB-8) were delivered by Research Biochemicals Inc., Natick, MA, USA. 1-28 and 25-35 β -amyloid peptides, carbachol, CTP, EGTA, LiCl and TRITON X-100 were purchased from Sigma, St Louis, MO, USA. Male Wistar rats were supplied by the animal farm, Lomna, Poland.

Synaptoneurosomes preparation from brain cortex

Adult (4-months old, 200 g b.wt.) and aged (24-months old, 400 g b.wt.) rats were decapitated and the brains were rapidly removed. The cerebral cortex was

dissected manually with a cooled razor blade on an ice-cooled Petri dish. The synaptoneurosome-enriched preparation was obtained according to the method of Hollingsworth et al. (1985). This fraction was previously investigated morphologically under electron microscopy (Strosznajder and Samochocki 1991). In brief, the brain cortex slices were homogenized by hand (5 strokes) in 7 ml of Krebs-Henseleit (KRBS) buffer, pH 7.4, using a Dounce-type glass homogenizer. The KRBS buffer contained 120 mM NaCl, 5 mM KCl, 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 25 mM NaHCO_3 and 10 mM glucose and was equilibrated with O_2/CO_2 (95/5) to adjust the pH to 7.4. The homogenate was diluted with 28 ml of KRBS buffer and centrifuged at $1,100 \times g$ for 15 min. The supernatant fraction was decanted and the pellet was resuspended in 5 ml of KRBS buffer and incubated at 37°C for 30 min under O_2/CO_2 atmosphere, and subsequently used for the assay of *myo*-2- $[\text{}^3\text{H}]$ inositol incorporation or $[\text{Ca}]_i$ measurement. Protein was estimated according to the method of Lowry et al. (1951).

Assay of *myo*-2- $[\text{}^3\text{H}]$ inositol incorporation

A 50 μCi of *myo*-2- $[\text{}^3\text{H}]$ inositol was incubated 45 min at 37°C in the assay medium, consisting of 5 mM LiCl, 1.5 mM CTP, and 15 mg of synaptoneurosomal protein in a total volume of 5 ml, with continuous flow of O_2/CO_2 (95/5). Subsequently, the suspension was diluted with 20 ml of cold KRBS buffer, containing 20 mM unlabeled *myo*-inositol, and was centrifuged twice at $1,100 \times g$ for 15 min. Finally, the pellet was resuspended in 7 ml of KRBS buffer and used for experiments on inositol phosphates (IP_n) production.

Assay of MACHR-mediated IP_n production

Synaptoneurosomal fraction (1 mg protein), prelabeled with *myo*-2- $[\text{}^3\text{H}]$ inositol, was preincubated at 37°C for 10 min in KRBS buffer, containing 5 mM LiCl, after which 2 mM CaCl_2 with buffer or with 1 mM carbachol was added and the incubation was prolonged by a further 60 min. The reaction was stopped by addition of a chloroform/methanol mixture (1:2, v/v) and total lipids were extracted according to the method of Bligh and Dyer (1959). Radioactive water-soluble inositol metabolites were separated in increasing concentrations of ammonium formate in formic acid by column chromatography on Dowex-1, according to methods of Berridge

et al. (1983) and Strosznajder et al. (1987). Radioactivity of IP_n was measured using Bray's fluid in LKB Wallac 1409 liquid scintillation counter.

Assay of resting $[\text{Ca}]_i$

The procedure was carried out as described by Chandler and Crews (1990). The synaptoneurosomal protein was resuspended in 5 ml of Krebs-HEPES buffer, containing 0.1% bovine serum albumin and 1 μM fura-2/AM, to provide protein concentration of 6–8 mg/ml. The suspension was then incubated under O_2/CO_2 (95/5) at 37°C for 45 min in a Dubnoff shaking water bath. Subsequently, the suspension was centrifuged at $1,100 \times g$ for 15 min. The resulting pellet was washed twice with 5 ml of ice-cold buffer, without bovine serum albumin, by centrifugation at $1,100 \times g$ for 10 min, and then was resuspended in 3 ml of buffer, and placed on ice. A 0.5 ml aliquot of fura-2-loaded synaptoneurosomal fraction and 0.5 ml of warm buffer (37°C) was transferred to a quartz cuvet, containing a magnetic stirring bar, and was preincubated for 10 min at 37°C . Fluorescence was measured at excitation wavelengths of 340 and 380 nm (emission wavelength was set at 500 nm). Fluorescence measurements were made in a water-jacketed (37°C) Hitachi F-2000 spectrofluorophotometer, equipped with magnetic stirrer. Time-based data acquisitions were performed using an internal program for calcium determination, supported by the manufacturer, which automatically subtracts tissue autofluorescence at each wavelength, and reports initial data as the ratio of fluorescence intensity at 340/380 nm. To determine the maximal fluorescence intensity (F_{max}), 20 μl of TRITON X-100 (0.1%) and 20 μl of CaCl_2 (5 mM) were added. To determine the minimal fluorescence (F_{min}), 20 μl of TRITON X-100 (0.1%) and 20 μl of EGTA (6 mM) were added. Correction factors for fura-2 leakage were determined by measurement of fluorescence immediately after addition of 4 μM MnCl_2 at the end of the measurement. The $[\text{Ca}]_i$ was calculated by the following equation according to the method of Grynkiewicz et al. (1985):

$$[\text{Ca}]_i = 224 \times \frac{(R - R_{\text{min}})}{(R_{\text{max}} - R)} \times \frac{Sf_2}{Sb_2}$$
 where 224 is the effective fura-2- Ca^{2+} dissociation constant (K_d), R is the sample ratio (sample fluorescence at 340 nm / 380 nm), R_{min} is the ratio of (F_{min} at 340 nm / 380 nm), R_{max} is the ratio of (F_{max} at 340 nm / 380 nm), Sf_2 is the signal of free fura-2 at 380 nm under minimal Ca^{2+} binding con-

ditions (Fmin), and Sb₂ is the signal of free fura-2 at 380 nm under maximal Ca²⁺ binding conditions (Fmax).

Assay of mAChR-mediated increase of [Ca]_i

After 10 min equilibration at 37°C of fura-2-loaded synaptoneurosomal fraction, measurement of [Ca]_i was started for a further 20 min. Carbachol (1 mM) was applied directly in a 20 µl volume, using a syringe inserted through a small hole fitted with a rubber septum located in the lid above the cuvet. When the specific antagonists, such as 10 µM atropine, 10 µM pirenzepine or 10 µM TMB-8 were used, they were added 5 min prior the application of carbachol.

Assay of the effect of Aβ peptides on [Ca]_i

After 10 min equilibration at 37°C of fura-2-loaded synaptoneurosomal fraction, a 1-28 Aβ or 25-35 Aβ peptide was added to the suspension in a final concentration of 25 µM, and incubation was continued for 1, 5, 30 or 60 min at 37°C. Following addition of buffer or 1 mM carbachol, the measurement of resting or mAChR-stimulated increase of [Ca]_i, respectively, was started and continued for a further 20 min.

RESULTS

Muscarinic cholinergic receptor-mediated [Ca]_i increase in adult and aged brain cortex

We have found that in synaptoneurosomes from brain cortex of adult rats, the cholinergic agonist, 1 mM carbachol, increases resting [Ca]_i (240 ± 35.5 nM) by about 60%, 10 min after its addition. This carbachol-mediated enhancement of [Ca]_i is almost completely reduced by 10 µM atropine, the mAChR(s) antagonist. Application of a specific antagonist for M₁AChR, pirenzepine, at 10 µM concentration, also potently decreased the effect of carbachol. In addition, the observed carbachol-evoked enhancement of [Ca]_i was abolished by 10 µM TMB-8, a specific antagonist of endoplasmic reticulum IP₃ receptor (Fig. 1).

In contrast to the significant effect of carbachol in adult brain (Fig. 2A), there was no significant increase of [Ca]_i due to stimulation of mAChR by carbachol in synaptoneurosomes isolated from brain cortex of aged rats (Fig. 2B). The [Ca]_i was maintained at resting level

(165 ± 21.9 nM) during the whole 20 min of incubation with carbachol.

Muscarinic cholinergic receptor-mediated IP_n production in adult and aged brain cortex

A detailed analysis of the effect of Ca²⁺ on IP_n production has shown no statistically significant variations in accumulation of IP₁, IP₂ and IP₃ in aged brain cortex synaptoneurosomes (Fig. 2B) as compared with those from adult brain (Fig. 2A). Carbachol at 1 mM concentration increased Ca²⁺-dependent IP_n production both in adult and aged brain to a similar extent. This enhancement was confined mainly, if not exclusively, to IP₁ formation. In brain cortex synaptoneurosomes from both adult (Fig. 2A) and aged (Fig. 2B) rats no statistically significant changes in the radioactivity of accumulated IP₂ and IP₃ after carbachol stimulation were observed.

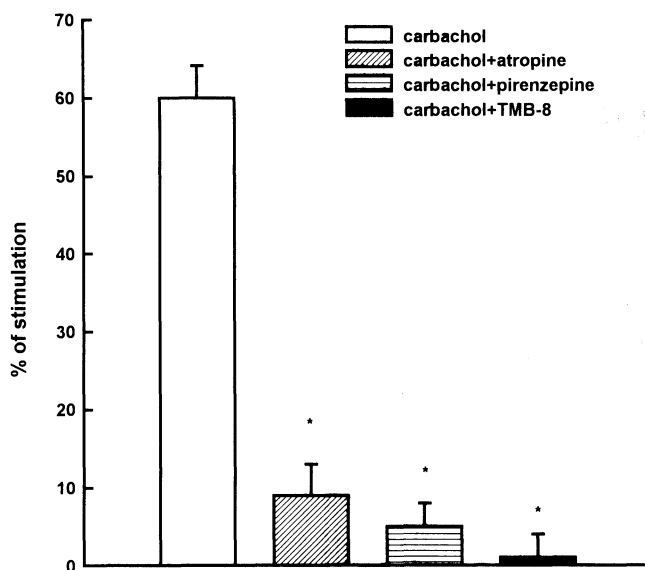


Fig. 1. Muscarinic cholinergic receptor-evoked cytosolic calcium increase in synaptoneurosomes from brain cortex of adult rats. Fura-2-loaded synaptoneurosomes were stimulated by 1 mM carbachol in the presence of 1 mM CaCl₂ for 10 min at 37°C. The cholinergic antagonists, atropine and pirenzepine, as well as IP₃ receptor antagonist, TMB-8, were all applied at 10 µM concentration 5 min prior to the carbachol. Results are expressed as a % of stimulation of resting [Ca]_i (240 ± 35.5 nM) in the presence of 1 mM CaCl₂ and absence of carbachol and other drugs. Data are means ± SD from 5 experiments made in triplicate. Statistical significance was evaluated using Student *t*-test. **P* < 0.05 in respect to the effect of carbachol.

Effect of 25-35 A β and 1-28 A β peptides on muscarinic cholinergic receptor-mediated $[Ca]_i$ increase

Incubation of synaptoneurosomes from adult brain cortex with neurotoxic 25-35 A β peptide at concentration of 25 μ M for 1, 5, 30 and 60 min caused a time-dependent rapid increase of resting $[Ca]_i$. A statistically significant effect of 25-35 A β peptide was observed after only 1 min of incubation. At this time point 25-35 A β

peptide enhanced resting $[Ca]_i$ by about 46%. After 60 min incubation 25-35 A β peptide resulted in an almost 5-fold increase of resting $[Ca]_i$ compared to its value observed in the absence of 25-35 A β peptide (Table I).

The effect of non-neurotoxic β -amyloid peptide, 1-28 A β peptide, at a concentration of 25 μ M, on resting $[Ca]_i$ in synaptoneurosomes from brain cortex of adult rats was apparently dependent on the time of incubation. In the case of incubation shorter than 30 min, the action of 1-28 A β peptide caused a small and statistically insignificant elevation of resting $[Ca]_i$ by about 15%. After 60 min its action on synaptoneurosomes produced a 2-fold enhancement of resting $[Ca]_i$ compared to its value found in the absence of 1-28 A β peptide (Table II).

Stimulation of MACHR with 1 mM carbachol increased resting $[Ca]_i$ by about 60% in synaptoneurosomes from brain cortex of adult rats. When they were treated with 25-35 A β peptide at concentration of 25 μ M for 1 min the ability of carbachol to further increase resting $[Ca]_i$ was potently reduced, as compared to its effect in the absence of 25-35 A β peptide. In the case of longer treatment of brain cortex synaptoneurosomes from adult rats with 25-35 A β peptide, carbachol was unable to evoke further elevation of resting $[Ca]_i$ (Table I).

The treatment of synaptoneurosomes from brain cortex of adult rats with 1-28 A β peptide at concentration of 25 μ M for 1, 5, 30 and 60 min significantly and to a similar extent decreased the stimulatory effect of carbachol on resting $[Ca]_i$ comparing to its effect observed in

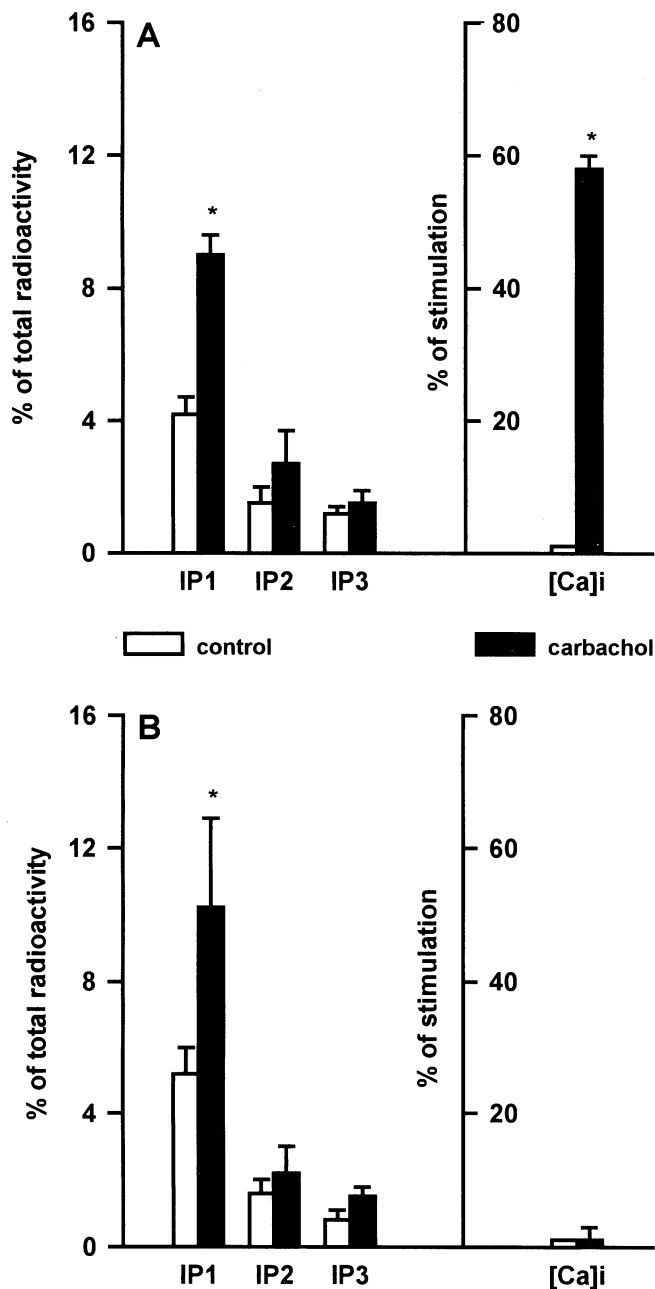


Fig. 2. Calcium- and muscarinic cholinergic receptor-mediated inositol phosphates (IP_n) production and cytosolic calcium ($[Ca]_i$) increase in synaptoneurosomes from brain cortex of adult and aged rats. [3 H]Inositol-labeled or fura-2-loaded synaptoneurosomes were incubated in the presence of 1 mM CaCl₂ with or without addition of 1 mM carbachol. In the case of determination of [3 H]IP_n production 5 mM LiCl was included into the medium and incubation was performed for 60 min at 37°C. Results are expressed as % of a particular IP_n radioactivity in total radioactivity of water-soluble inositol metabolites. Empty bars correspond to the calcium-dependent IP_n formation and solid bars correspond to the carbachol-stimulated IP_n production. Measurement of $[Ca]_i$ was made for 10 min at 37°C. Results are expressed as % of stimulation of resting $[Ca]_i$ (240 \pm 35.5 nM and 165 \pm 21.9 nM, in adult and aged brain respectively). Open bars indicate the absence, whereas solid bars the presence of 1 mM carbachol in the incubation medium. Results are means \pm SD from 4 experiments made in triplicate. Statistical significance was evaluated using Student *t*-test. **P* < 0.05 when compared to the value in the absence of carbachol. A, adult rats; B, aged rats.

TABLE I

Time-dependent effect of amyloid β 25-35 peptide on the resting and carbachol-evoked increase of cytosolic calcium concentration in synaptoneurosomes from brain cortex of adult rats

Condition	Preincubation (min)	% of control	
		Resting	Carbachol-activated
Control	0	100 \pm 14.8	161 \pm 8.8 [^]
A β 25-35	1	146 \pm 6.3*	168 \pm 6.9 [^]
A β 25-35	5	219 \pm 12.6*	210 \pm 15.5#
A β 25-35	30	274 \pm 19.1*	252 \pm 15.8#
A β 25-35	60	454 \pm 40.2*	420 \pm 31.0#

Synaptoneurosomes from brain cortex of adult rats were loaded with fura-2 and then they were incubated in the presence of 1 mM CaCl₂. In selected experiments, amyloid β (A β) 25-35 peptide was included into incubation medium for 1, 5, 30 and 60 min at 37°C. Thereafter, the measurement of cytosolic calcium concentration ([Ca]_i) was started with or without addition of 1 mM carbachol. The effect of carbachol and A β 25-35 peptide was expressed as a % of control (resting [Ca]_i in the absence of carbachol and A β 25-35 peptide). Control value, 240 \pm 35.5 nM, was set as a 100%. Data are means \pm SD from 5 experiments made in triplicate. **P* < 0.05 as compared to control. [^]*P* < 0.05 as compared to corresponding value of resting [Ca]_i. #*P* < 0.05 as compared to effect of carbachol alone.

the absence of 1-28 A β peptide. This 30% reduction of the effect of carbachol on resting [Ca]_i persisted during the whole time of pretreatment of synaptoneurosomes with 1-28 A β peptide (Table II).

DISCUSSION

Our results indicate that β -amyloid peptides, 1-28 A β and 25-35 A β , acting on synaptoneurosomes from brain cortex of adult rats, both increase [Ca]_i. 25-35 A β was found to have a much more potent effect on resting [Ca]_i than that of 1-28 A β . Preincubation of synaptoneurosomes with 1-28 A β peptide decreases progressively, depending on the incubation time, the ability of the MACHR agonist, carbachol, to increase [Ca]_i. A more pronounced effect was observed in the case of 25-35 A β peptide action, which almost eliminates carbachol-

TABLE II

Time-dependent effect of amyloid β 1-28 peptide on the resting and carbachol-evoked increase of cytosolic calcium concentration in synaptoneurosomes from brain cortex of adult rats

Condition	Preincubation (min)	% of control	
		Resting	Carbachol-activated
Control	0	100 \pm 14.8	157 \pm 9.1 [^]
A β 1-28	1	113 \pm 14.0	161 \pm 10.1 [^]
A β 1-28	5	117 \pm 15.6	156 \pm 12.0 [^]
A β 1-28	30	138 \pm 17.3*	180 \pm 18.5 [^]
A β 1-28	60	210 \pm 16.5*	248 \pm 13.9 [^] #

Synaptoneurosomes from brain cortex of adult rats were loaded with fura-2 and then they were incubated in the presence of 1 mM CaCl₂. In selected experiments, amyloid β (A β) 1-28 peptide was included into incubation medium for 1, 5, 30 and 60 min at 37°C. Thereafter, the measurement of cytosolic calcium concentration ([Ca]_i) was started with or without addition of 1 mM carbachol. The effect of carbachol and A β 1-28 peptide was expressed as a % of control (resting [Ca]_i in the absence of carbachol and A β 1-28 peptide). The control value, 240 \pm 35.5 nM, was set as a 100%. Data are means \pm SD from 5 experiments made in triplicate. **P* < 0.05 as compared to control. [^]*P* < 0.05 as compared to corresponding value of [Ca]_i. #*P* < 0.05 as compared to effect of carbachol alone.

-evoked Ca²⁺ signal. Our study indicates that in synaptoneurosomes from brain cortex of aged rats the MACHR-mediated IP₃-dependent [Ca]_i elevation is markedly reduced. In spite of this IP₃ production was unchanged. These data suggest that A β peptides may be involved in alteration of MACHR-mediated calcium signaling found in aged brain.

The cytosolic compartment of the cell with its intracellular organelles plays a crucial role in calcium homeostasis-related events owing to its dual ability to sense and transduce Ca²⁺-mediated signals and, on the other hand, to contribute to Ca²⁺ sequestration by its intrinsic Ca²⁺ buffering power. A normal cytosolic Ca²⁺ balance is essential for neuronal plasticity and synaptic transmission (Carvalho 1982). Impaired Ca²⁺ homeostasis is believed to play a major role in neurodegeneration and has been implicated in the mechanism of

action of β -amyloid on neurons, making them more vulnerable to excitatory amino acid-mediated neurotoxicity (Mattson et al. 1992). It was recently reported that A23817 calcium ionophore-induced $[Ca]_i$ elevations directly influence the processing of amyloid precursor protein (APP), leading to an approximate three-fold increase in soluble β -amyloid production (Querfurth and Selkoe 1994). The A β peptides, such as 1-40 and 25-35, were found to be neurotoxic in cortical and hippocampal cultures (Koh et al. 1990). It was observed that these peptides all increase resting $[Ca]_i$ and enhance glutamate-mediated Ca^{2+} entry into neurons (Cowburn et al. 1995, Jope et al. 1997). Our results indicate the involvement of 1-28 A β and 25-35 A β peptides in enhancement of $[Ca]_i$ and in alteration of MACHR-dependent calcium signaling in synaptoneurosomes from brain cortex of adult rats.

Although the mechanism of A β action on calcium homeostasis is not clearly understood, the present data indicate that the neuronal systems controlling calcium homeostasis may be a direct target for the actions of A β peptides (Joslin et al. 1991). The effect includes an enhancement of Ca^{2+} influx *via* voltage-gated and receptor-operated Ca^{2+} channels or changing of cytosolic Ca^{2+} buffering systems (Colvin et al. 1991, Mattson et al. 1992). Neurons possess several mechanisms to reduce $[Ca]_i$, such as Ca^{2+} -ATPase, Na^+/Ca^{2+} exchanger, and Ca^{2+} -binding proteins (Carafoli 1987). Concerning physiological aging it is suggested that cytosolic Ca^{2+} buffering system is impaired in older brains. A pronounced decrease of the activity of plasma membrane Ca^{2+} -ATPase and Na^+/Ca^{2+} exchanger has been observed in aged brain (Martinez-Serrano et al. 1992). On the other hand, Vitorica and Satrustequi (1986) reported a decrease in Ca^{2+} transport through voltage-sensitive Ca^{2+} channels in aged brain.

The alterations of MACHR-regulated $[Ca]_i$ in synaptoneurosomes from brain cortex of aged rats may result from modifications of receptor recognition sites, or receptor-G-protein coupling, or receptor-stimulated IP_3 formation. Recently, a selective loss of M_1 AChR-G-protein coupling was observed in Alzheimer's brain (Garlind et al. 1995, Jope et al. 1997). Moreover, an increased level of PLC with concomitant reduction of its specific activity was also found (Shimohama et al. 1995). PLC is a key enzyme in signal transduction between the extracellular and intracellular compartments. Our unpublished results showed that the action of 1-40 A β peptide has an inhibitory effect on PLC activity in brain cortex mem-

brane. In addition, Magnusson et al. (1993) demonstrated that the cytoplasmic ligand binding site of the IP_3 receptor can be degraded by the Ca^{2+} -activated neutral protease, calpain, in Alzheimer's brain. Some or all of these events may contribute to the disruption of calcium homeostasis in Alzheimer's brain.

Our data suggest an alteration of MACHR-dependent calcium signaling in aged brain cortex. It appears possible that the action of A β peptides on synaptoneurosomes from brain cortex is responsible for abolishment of M_1 AChR-evoked calcium signal. Such a hypothesis was recently confirmed using cortical neurons in culture by Kelly et al. (1996). On the other hand, Kurian et al. (1992) reported that the inositol 1,3,4,5-tetrakisphosphate formation and this molecule-dependent intracellular Ca^{2+} mobilization also decreases in synaptoneurosomes from aged brain cortex, compared to adult brain. However, in our study we found no differences in IP_n formation in brain cortex synaptoneurosomes between adult and aged rats. M_1 AChR has been implicated in regulation of APP release and processing. It is possible that this receptor may be more applicable and sensitive to modifications induced by subsequently formed A β peptides (Nitsch et al. 1992).

We conclude from this study that changes in muscarinic cholinergic receptor-mediated calcium signaling in aged brain cortex may, at least in part, contribute to the action of β -amyloid peptides on mechanisms responsible for regulation of calcium homeostasis.

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