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Alzheimer's disease related peptides affected cholinergic receptor mediated poly(ADP-ribose) polymerase activity in the hippocampus

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Abstract

Our previous studies indicated that Alzheimer's disease (AD) related amyloid beta peptide (Aβ) significantly altered muscarinic cholinergic receptor (mChR) signaling on the level of G protein regulated phospholipase C (PLC) leading to the lower formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). Recent studies indicated that poly (ADP-ribose) polymerase-1 (PARP-1) is a new nuclear target in signal transduction pathway in the brain. In this study the effect of Aβ 25-35 (25 μM) and non-Aβ component of Alzheimer's disease amyloid (NAC, 10 μM) on mChR-dependent signaling to PARP-1 was determined. PARP-1 activity was estimated radiochemically using egzogenous substrate adenine[^{14}C]NAD. The results showed that the non hydrolysable agonist of mChR, carbachol (1 mM) together with GTP(γ)S (100 μM) stimulated PARP-1 activity in the hippocampus by about 100%. TMB-8, inhibitor of IP_3 receptor decreased PARP-1 activation evoked by carbachol/GTP(γ)S. Stimulation of mChR did not lead to free radicals generation but activate PARP-1 through IP_3/Ca^{2+} regulated processes. This cholinergic receptor dependent PARP-1 activation was abolished by Aβ and NAC peptide. These toxic peptides themselves significantly stimulated PARP-1 activity by free radicals mediated DNA damage. These data indicated that Aβ and NAC peptide affected mChR-dependent signal transduction to PARP-1 probably through free radicals evoked inhibition of IP_3 formation by phospholipase C.

Key words: *PARP-1, IP_3 receptor, Aβ, NAC peptide*

Introduction

Our previous study presented that activation of the muscarinic cholinergic receptor (mChR) by non hydrolysable analog of acetylcholine, carbachol and activator of G protein, GTP(γ)S significantly enhanced inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) formation and that this signaling events are significantly altered by amyloid beta peptide (Aβ) [27]. In this study, the mChR evoked signals were investigated on the level of DNA-bound enzyme poly(ADP-ribose) polymerase (PARP-1, EC 2.4.2.30). The recent data suggested that PARP-1 is the new nuclear target for signal transduction processes evoked by receptor(s) stimulation or membrane depolarization [12,21,23]. Activated PARP-1 cleaves NAD into

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Table I. Inhibition of IP_3 receptor decreases PARP-1 activation evoked by mChR stimulation

PARP-1 activity [pmol x mg of protein^{-1} x min^{-1}]		
control	carbachol + GTP(γ)S	carbachol + GTP(γ)S + TMB-8
18.06±4.32	32.76±5.44 (188) (**)	27.04±5.83 (81) (#)

*The data are mean ±SEM from 3 experiments carried out in triplicate. Statistical analysis of the results was carried out using ANOVA followed by Newman-Keuls post-hoc test. **p<0.01 compared to slices incubated without treatment (in parenthesis percent of control), #p<0.05 compared to slices incubated with carbachol and GTP(γ)S (in parenthesis percent of carbachol/GTP(γ)S).*

nicotinamide and forms long and branched polymers of ADP-ribose on over forty nuclear proteins and PARP-1 itself. Poly (ADP-ribosyl) ation has been implicated in the regulation of a diverse array of cellular processes ranging from DNA repair, chromatin organization, transcription, to replication [2,7,9,15,21,25]. However, oxidative stress-induced DNA strand breaks that stimulates PARP-1 lead to βNAD^+ and ATP depletion and to necrotic or apoptotic processes [10,13,26]. The pathophysiological significance of PARP-1 over-activation is suggested in brain ischemia, diabetes, inflammation and cancer [4,11,21]. Increased expression of this enzyme and accumulation of poly(ADP-ribose) was observed in Alzheimer's and Parkinson's brain [14,17,19]. Our resent data indicated the involvement of mChR in the regulation of PARP-1 activity through IP_3 receptor pathway [23]. In the present study, we investigated the effect of Alzheimer's disease (AD) related Aβ and NAC peptide on mChR-dependent signal transduction to PARP-1.

Material and methods

Animals

Male Wistar rats, 4-month-old (250-300 g), were supplied from the Animal Breeding House of the Medical Research Centre (Warsaw). The Institutional Ethics Committee accepted the research project.

Materials

Adenine[C^{14}]NAD (sp. activity: 252 mCi/mmol), was obtained from Amersham, Buckinghamshire, UK, Aβ 25-35, carbachol, GTPγS, and the all other reagents were from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Preparation of Aβ and NAC peptides

Aβ 25-35 was dissolved in bidistilled deionized (BDD) water at 2.5 mM concentration and incubated at room temperature to obtain the aggregated form as described previously [20]. Aβ was used at 25 µM final concentration. NAC peptide was stored lyophilized, and 100 µM stock solution was prepared in BDD water and then used at final 10 µM concentration. To obtain the aggregated form NAC was incubated for 3 days at 37°C.

Preparation of brain slices

Animals were killed by decapitation, the brain was quickly removed, hippocampi were dissected and cross-chopped into slices (350x350 µm) using a McIlwain tissue chopper. The slices were placed in ice-cold Krebs buffer (in mM: NaCl 124, KCl 5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 26 and glucose 10) equilibrated with 5% CO_2 in 95% O_2 to maintain a pH of 7.4 and pre-incubated for 30 min at 37°C in Krebs buffer. Then, $CaCl_2$ was added to final 2 mM concentration and incubation was prolonged for 30 min at 37°C in the presence or absence of the following compounds: Aβ 25-35 (25 µM), NAC (10 µM), carbachol (1 mM) together with GTP(γ)S (100 µM), TMB-8 (10 µM). After incubation, the slices were homogenized in glass-glass homogenizer and used for determination of the PARP-1 activity.

Determination of the PARP-1 activity

The PARP-1 activity was assayed as described previously [22]. The total reaction mixture in the final volume of 100 µl contained 400µM [adenine-^{14}C]NAD as a substrate ($2x10^5$ d.p.m., sp. activity: 252 mCi/mmol), 100 mM Tris-HCl buffer pH 8.0, 10 mM $MgCl_2$, 1mM dithiothreitol (DTT) and 200 µg of protein. The reaction was carried out for 1 min at 37°C and stopped with 800 µl of ice-cold 25% trichloroacetic acid (TCA). The precipitate was collected on Whatman GF/B filters, washed five times with ice-cold 5% TCA and processed for determination of radioactivity in Bray's scintillation fluid using scintilator counter Wallac 1409 LKB.

Results

In the present study, we examined the role of mChR on PARP-1 activation in the brain cortex and hippocampus. Moreover, the effect of both AD related peptide Aβ and NAC on this receptor mediated signaling to PARP-1 was evaluated. The data showed that carbachol at 1mM concentration together with GTP(γ)S at 100 μM significantly enhanced the PARP-1 activity by about 100% in the hippocampus with no effect on this enzyme in the brain cortex (Fig. 1). Inhibition of IP_3 receptor through TMB-8 (10 μM) decreased PARP-1 activation evoked by carbachol/GTP(γ)S in the hippocampus (Table I). MChR stimulation by carbachol/GTP(γ)S had no effect on free radicals generation and macromolecules oxidation (data not shown). In addition, this receptor stimulation had no effect on the PARP-1 activity in the presence of Aβ and NAC peptide (Fig. 2). However, both peptides, Aβ and NAC significantly stimulated this enzyme by 88% and 23%, respectively by free radicals evoked DNA strand breaks (Fig. 2).

Discussion

The major finding of this study is that AD related peptide Aβ and NAC disturbed phosphoinositide signaling to PARP-1 in the hippocampus. These and our recent results presented PARP-1 as a nuclear target for mChR pathway [23] are in agreement with the previous data of Homburg et al [12]. They showed that depolarisation of neurons activated PARP via IP_3-induced Ca^{2+} signaling. In addition, it was shown that *N*-methyl-D-aspartate (NMDA) receptors stimulation could mediate PARP-1 activation [18,22]. Moreover, the present data indicated that Aβ and NAC peptide enhanced basal PARP-1 activity. These results supported our earlier data that both peptides induced reactive oxygen species (ROS) generation and DNA degradation [1]. It is well documented that PARP-1 is the earliest and the most sensitive indicator of DNA strand breaks evoked by oxidative stress [8,28]. Over-activation of PARP-1 leads to cellular βNAD+ depletion, apoptotic inducing factor (AIF) release and cell death [13,26]. Aβ together with NAC peptide is a major component of senile plaques in AD and through ROS generation could participate in neuronal cell death [3,5,6,16,24]. Our previous results showed that mChR-dependent signal transduction in the rat brain is damaged by Aβ peptide that significantly inhibited phosphatidylinositol-4,5-bisphosphate phospholipase C (PIP_2-PLC) activity through ROS formation and

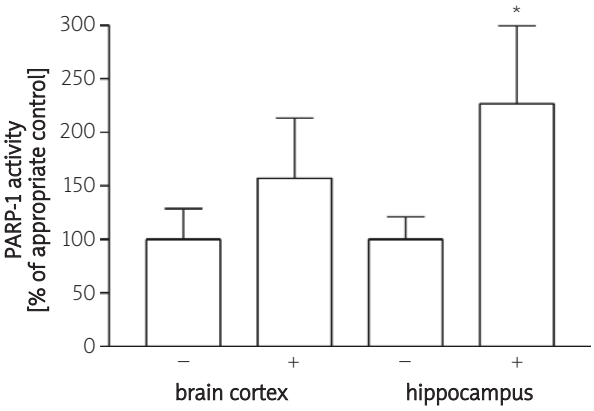

Fig. 1. MChR stimulation on PARP-1 activity in the rat brain cortex and hippocampus.

The PARP-1 activity was determined as described in Material and Methods, (–) slices incubated without tratment, (+) – slices incubated with 1 mM carbachol and 100 μM GTP(γ)S. The data are mean ±SEM from 3 experiments carried out in triplicate. Control value of the PARP-1 activity in the brain cortex and hippocampus evaluated 15.52±3.94 and 18.06±4.32 pmol x mg of $protein^{-1}$ x min^{-1}, respectively. Statistical analysis of the results was carried out using ANOVA followed by Newman-Keuls post-hoc test, *p<0.05 compared to slices incubated without carbachol and GTP(γ)S

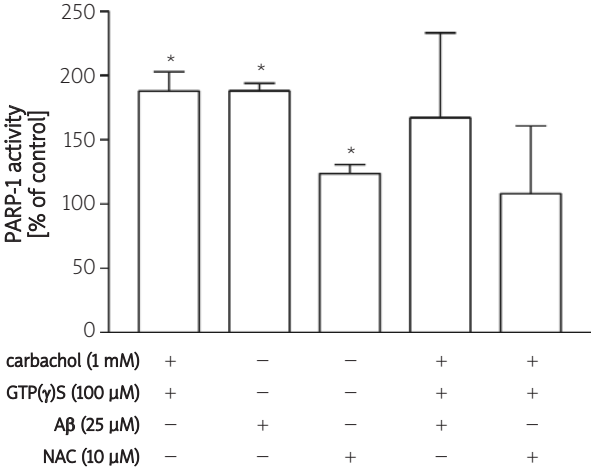

Fig. 2. Efecct of Aβ and NAC peptide on mChR signaling to PARP-1 in the hippocampus.

The data are mean ±SEM from 3 experiments carried out in triplicate, presented as percentage of appropriate control. Statistical analysis of the results was carried out using ANOVA followed by Newman-Keuls post-hoc test. *p<0.05 compared to slices incubated without treatment

membrane lipids peroxidation [20,27]. The present data indicated that Aβ and NAC peptide decreased PARP-1 activation evoked by mChR stimulation. We therefore consider that both peptides through the free radicals mechanism inhibited PIP_2-PLC activity and IP_3 liberation and disturbed signal transduction from mChR to PARP-1.

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