Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme involved in DNA repair and transcription regulation. The aim of this study was to investigate the role of PARP-1 in muscarinic cholinergic receptor signaling. Our data indicate that activation of muscarinic cholinergic receptors by carbachol (1mM) in the presence of GTP$\gamma$S evoked a significant enhancement of PARP activity in the adult rat hippocampus. Moreover, TMB-8 (10µM), an antagonist of inositol 1, 4, 5 trisphosphate (IP$_3$) receptor prevented the activation of PARP-1, which indicates that IP$_3$/Ca$^{2+}$ signaling is involved in this pathway. The diacylglycerol (DAG)-regulated protein kinase C (PKC) inhibitor (GF109203X) (1µM) only slightly enhanced PARP activity in hippocampal nuclear fractions, which suggests that DAG/ PKC is not involved in PARP activation.

**Key words**: DAG, IP$_3$, muscarinic receptor signaling, PARP-1, PKC

**INTRODUCTION**

Poly(ADP-ribose) polymerase-1 (PARP-1 EC 2.4.2.30) is a highly conserved enzyme localized mainly to the cell nucleus and responsible for over 90% of poly(ADP-ribosyl)ation in the brain (1). PARP-1 activated by single and double DNA strand breaks is the earliest and most sensitive sensor of DNA damage. The overactivation of PARP-1 by massive DNA damage may lead to depletion of intracellular βNAD$^+$ and ATP and to cell death (2-6). Recent studies demonstrate that neuronal PARP-1 can also be stimulated in physiologic conditions, by a fast signal evoked in the cell by membrane depolarization (7, 8). It has been documented that DNA damage is not involved in this pathway. Upon activation,
the enzyme hydrolyzes nicotinamide adenine dinucleotide (βNAD⁺) and catalyzes extensive polymerization of transferred ADP ribose units to over 40 nuclear proteins including histones and PARP-1 itself (9). PARP-1 through poly(ADP-ribosyl)ation regulates the function of several transcription factors including the p53 and nuclear factor kappaB). It also regulates gene expression by modification of transcription factors in three different ways: poly(ADP-ribosyl)ation, protein-protein, or protein-ADP-ribose interaction (10-12). Pieper at al (1) have shown that poly(ADP-ribosyl)ation reflects glutamate-nitric oxide neurotransmission, activated by DNA strand breaks, which suggests that basal glutamate-PARP activity regulates neuronal energy dynamics. In a previous work, we have shown the involvement of PARP in NMDA glutamate receptor-evoked processes in the hippocampus, indicating that the enzyme could be a nuclear target for this receptor mediated signaling (13). A recent study of Cohen-Armon et al (7) has shown that PARP-1 mediates several forms of long-term memory in Aplysia. The authors suggest that a transient decondensation of the chromatin structure by poly(ADP-ribosyl)ation enables the transcriptions needed to form long term memory without strand breaks in DNA.

The involvement of PARP-1 in cholinergic signaling, which is essential for learning and memory, remains as yet unexplored. Activation of muscarinic cholinergic receptors leads to the degradation of phosphatidylinositol 4, 5 bisphosphate (PIP₂) and liberation of the potent second messengers inositol 1, 4, 5 trisphosphate (IP₃) and diacylglycerol (DAG). The role of these messengers in information transduction into the nucleus is unclear. Therefore, in the present study we set out to investigate the role of PARP-1 in muscarinic cholinergic receptor signaling and the involvement of IP₃ and DAG in the process.

MATERIAL AND METHODS

Chemicals

Inorganic chemicals were obtained from Merck (Darmstadt, Germany) and from POCh (Gliwice, Poland). p-APMSF, Carbachol, DTT, GTPγS, βNAD⁺, TCA, TMB-8 (8-(Diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride) and GF109203X (3-[1-(Dimethylaminopropyl)indol-3-yl]-4-(indol-3-yl)maleimide hydrochloride) was obtained from Sigma (St. Louis, MO) [adenine-¹⁴C]NAD was obtained from Amersham (Little Chalfont, Buckinghamshire, U.K.)

Animals

A local Ethics Committee that follows the European Communities Council Directive of November 1986 accepted the use of animals for this study. Male Wistar rats aged 4 mo were used. The animals were supplied from the Medical Research Center Farm, Warsaw, Poland.

Preparation of brain slices

The hippocampus was isolated and cut at 0.35 mm intervals in both sagittal and coronal planes using an McIlwain tissue chopper. The slices were preincubated in Krebs-Henseleit buffer solution
pH 7.4, without CaCl₂, for 30 min at 37°C, followed by another 30-min incubation under carbogen (95% O₂ and 5% CO₂) in the presence of CaCl₂ (2mM), carbachol (1mM), a nonhydrolysable analog of acetylcholine, and with GTPγS (100 µM), a non-hydrolyzable analog of guanosine 5’-trisphosphate, an activator of G protein. The IP₃ receptor antagonist 8-(Diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8) (10 µM) was added to selected samples 5 min before incubation. After incubation, the slices were homogenized and nuclear fractions were isolated, as described by Strosznajder et al (13). In the experiments with a protein kinase C inhibitor, nuclear fractions were incubated for 15 min with or without GF 109203X at a final concentration of 1 µM and the PARP-1 activity was determined using a radiochemical method.

**Determination of PARP activity**

PARP activity was determined using [adenine-14C]NAD as substrate. The incubation mixture in a final volume of 100 ml contained 200 µM βNAD and 2 x 10⁶ dpm [adenine-14C]NAD, 100 mM Tris-HCl buffer (pH 8.0), 10 mM MgCl₂, 5 mM DTT, 50 µM p-APMSF, and 100 µg protein. The mixture was incubated for 1 min at 37°C and the reaction was stopped by adding 0.8 ml of ice-cold 25% trichloroacetic acid (TCA). Precipitates were collected on Whatman GF/B filters, washed three times with 5% TCA and left overnight for drying. Afterwards, after adding Bray liquid scintillation cocktail, radioactivity was measured in a 1409 Wallac scintillation counter (Wallac Oy, Turku, Finland).

Differences in PARP activity were compared with one-way ANOVA followed by the Newman-Keuls post hoc test. A value of P<0.05 was deemed to indicate significant differences.

**RESULTS**

Stimulation of cholinergic receptors in the adult hippocampal slices by carbachol and GTPγS enhanced the activity of PARP-1 by about 100% comparing with the basal, unstimulated condition (*Fig. 1*). The enhancement was eliminated

![Fig. 1. Effects on PARP-1 activity of cholinergic receptor stimulation in the hippocampal tissue. Stimulation of PARP by carbachol + GTPγS was eliminated by the addition of the IP₃ receptor antagonist TMB-8. Data show percentage changes from control, taken as 100% (marked by the horizontal dotted line). *Different from control at P<0.01; #different from carbachol + GTPγS at P<0.05.](image)
by the IP₃ receptor antagonist TMB-8, which suggests that IP₃/Ca²⁺ signaling was involved in the PARP stimulation.

To investigate the possible role of DAG, acting via protein kinase C (PKC), in PARP-1-related muscarinic cholinergic receptor signaling, we used the PKC inhibitor GF109203X. Preincubation of the P1 nuclear fraction from rat hippocampal slices with the inhibitor resulted in the activity of PARP of 25.5 ±2.1 pmol/mg protein/min, as compared with the 22.5 ±3.9 pmol/mg protein/min in the control condition. A meager increase in PARP activity of 15% suggests that the DAG/PKC phosphorylation was not appreciably involved in PARP-1 activation.

**DISCUSSION**

The findings of this study indicate that the IP₃-mediated release of Ca²⁺ ions is involved in transduction of muscarinic cholinergic receptor signaling to the nuclear enzyme PARP-1. The results also speak against the possibility that PKC could be responsible for PARP-1 activation.

The IP₃ receptor that regulates intracellular Ca²⁺ signaling in cells takes part in shaping a host of physiological processes (14, 15). A study of Homburg et al (8) has demonstrated that fast activation of PARP evoked by depolarization is mediated by IP₃/Ca²⁺ mobilization. Our present data indicate that PARP-1 is a novel nuclear target for muscarinic cholinergic receptor signaling. This receptor-regulated phospholipase C (PLC) is responsible for the degradation of PIP₂ and liberation of the second messengers IP₃ and DAG that further transduce the signal through the PKC pathway. There are some indications that PARP activity might also be regulated by phosphorylation-dephosphorylation processes. It has been shown in vitro that PKC phosphorylates a highly purified PARP-1 (16). The phosphorylation of PARP-1 by PKC results in PARP inhibition (17). An inhibitor of PKC, GF109203X, used in our study only slightly enhanced the PARP-1 activity in the hippocampus, which supports the notion that phosphorylation of PARP-1 may lead to its inhibition.

The receptor-mediated PARP-1 response and the alteration of enzyme by covalent modification may affect the function of several nuclear proteins. PARP-1 by poly(ADP-ribosylation) regulates RNA polymerase II, topoisomerases, and high mobility proteins (2, 9, 11). Modulation of PARP-1 activity in the hippocampal muscarinic cholinergic receptor signaling may be of consequence for gene expression or for learning and memory.

**Acknowledgements:** This study was supported in part by grant No. 2P05A07926 from the Ministry of Scientific Research and Information Technology and by statutory budget of the Polish Academy of Science Medical Research Center (Theme No. 32).
REFERENCES


Author's address: R.P. Strosznajder, Department of Respiratory Research, Medical Research Center, Polish Academy of Sciences, 5 Pawińskiego St, 02-106 Warsaw, Poland; phone/fax: +48 22 6685416.
E-mail: roberts@cmdik.pan.pl