MUSCARINIC RECEPTOR - MEDIATED PHOSPHOINOSITIDE HYDROLYSIS IN RAT BRAIN

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ABSTRACT

Objective: Activation of cholinergic muscarinic receptors leads to activation of membrane - bound phospholipase C, leading to generation of IP_3 and diacylglycerol, The present study aimed at measuring muscarinic receptor-mediated phosphoinositide breakdown in three different regions of rat brain.

Methods: Cortical, hippocampal and striatal slices labeled with [^{3}H] myo-inositol were incubated with muscarinic agonist carbachol (Cch) and LiCl. Reaction was terminated by the addition of chloroform/methanol and labeled inositol phosphates were extracted from tissue. Individual [^{3}H]- inositol phosphates were separated using anion exchange chromatography.

Results: Cch caused a rise in IP_1 formation, where the increase over basal in the cortex, hippocampus and striatum were 98%, 73% and 45%, respectively.

Conclusion: Phosphoinositide turnover was stimulated by carbachol in all brain structures studied, with the most pronounced effects observed in the cortex and hippocampus.

Key Words: Muscarinic receptors, inositol phosphates, carbachol, rat brain

INTRODUCTION

Muscarinic cholinergic receptors mediate a wide array of physiological effects both in the central and peripheral nervous system. Four muscarinic receptor subtypes designated as M_1, M_2, M_3 and M_4 have been distinguished based on receptor binding studies (1). Through molecular cloning, five distinct muscarinic receptor genes (m_1-m_5) have been identified to be expressed in the brain and peripheral tissues (2-5). m_1-m_4 muscarinic acetylcholine receptor subtypes are expressed to similar extents in whole rat brain (4). Only low levels of m_5 receptors are detected in several brain regions (6). The tissue distribution of m_1-m_4 mRNA has been studied using *in situ* hybridization and Northern blot analysis (7,8). The concentration of m_1 and m_3 mRNA were highest the cerebral cortex and hippocampus, while m_4 mRNA was present at low levels in these regions and at high levels in the striatum (7). Subtype -specific antisera have been employed to measure the relative levels of five muscarinic receptor subtypes in rat and rabbit brain (6,9,10). The largest numbers of m_4 receptors were found in the striatum while the largest percentage of m_2 receptors were located in the cerebellum. On the other hand, the percentage of m_3 receptors did not change dramatically across the brain.

The expression of each receptor subtype in mammalian cells has established that m_1, m_3 and m_5 receptors are preferentially coupled to phosphoinositide metabolism via a pertussis toxin independent G-protein (G_q), while m_2 and m_4 receptors inhibit cAMP production through G_i and possibly through G_z (2,11-13). Brain muscarinic receptors have been found to activate PI metabolism (14-16). The present study was aimed to investigate coupling of muscarinic receptors to inositol phospholipid hydrolysis in three different regions of the brain: cortex, hippocampus and striatum.

MATERIALS AND METHODS

Myo-[2-^{3}H] inositol (19.1Ci/mmol) was obtained from Amersham, U.K. Dowex AG1-X8, 200-400 mesh resin (formate form) was purchased from Bio-Rad. All other reagents and drugs were from Sigma Chemical Co.

Albino rats of both sexes (200-250 g) were decapitated to obtain the cerebral cortex, hippocampus and striatum. Each brain region was chopped into slices of 0.5x0.5 mm, resuspended in Krebs buffer containing

120 mM NaCl, 5.5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 11 mM glucose and 20 mM $NaHCO_3$, pH 7.4 and preincubated in the same buffer gassed with a mixture of 95% O_2 and 5% CO_2 for 30 min at 37°C. Inositol phosphates were determined using the method described by Berridge (18) with minor modifications. Tissue slices distributed into 12x75 mm polypropylene tubes containing 0.3 ml Krebs buffer and 10 mM LiCl were labeled with 1 µCi Myo-[^{3}H] inositol for one hour with continuous oxygenation and gentle agitation. Subsequently, Cch (10^{-3} M) was added and the mixture was incubated for one hour after which reactions were terminated by the addition of 0.94 ml of cold chloroform/methanol (1:2, v/v). The samples were homogenized in an Ultraturrax tissue homogenizer and 0.31 ml of chloroform and 0.3 ml of dH_2O were added. The samples were mixed and centrifuged at 3000xg for 5 min. The upper aqueous phase was applied to polypropylene columns packed with Dowex AG1-X8 formate resin and the [^{3}H]-inositol phosphates formed were separated by anion exchange chromatography as described by Berridge (18). Free inositol was eluted with 20 ml of water and glycerophosphoinositol was eluted with 20 ml of 5 mM sodium tetraborate / 60 mM sodium formate. IP_1, IP_2, IP_3 were eluted sequentially with 5 ml of 0.1 M formic acid and 0.2 M ammonium formate (IP_1); 16 ml of 0.1 M formic acid and 0.5 M ammonium formate, (IP_2); 10 ml of 0.1 M formic acid and 1 M ammonium formate (IP_3). One milliliter of each fraction was added to vials containing 10 ml of scintillation fluid. The vials were vortexed and the radioactivity was counted two hr later by liquid scintillation spectrometry. The lower organic phase was evaporated and used to determine the protein content according to Lowry et al.(19)

RESULTS

Carbachol-induced phosphoinositide accumulation was determined in rat hippocampal slices. Incubation of slices with Cch increased the formation of [^{3}H] IP_1 in a dose-dependent manner (Figure 1). The relative accumulation of [^{3}H] IP_1, [^{3}H] IP_2 and [^{3}H] IP_3 induced by 10^{-3} M Cch were 74.5 ± 8.65, 16.49 ± 6.31 and 8.96 ± 2.46 %, respectively (mean of 3 experiments performed in duplicate, Table I). Subsequent experiments were performed by incubating rat hippocampal, striatal and cerebral cortical slices with 10^{-3} M Cch for 60 min. Under these conditions, Cch caused accumulation of IP_1 formation, where the increases over basal values in the cortex, hippocampus and striatum were 98%, 73% and 45%, respectively (Figure 2).

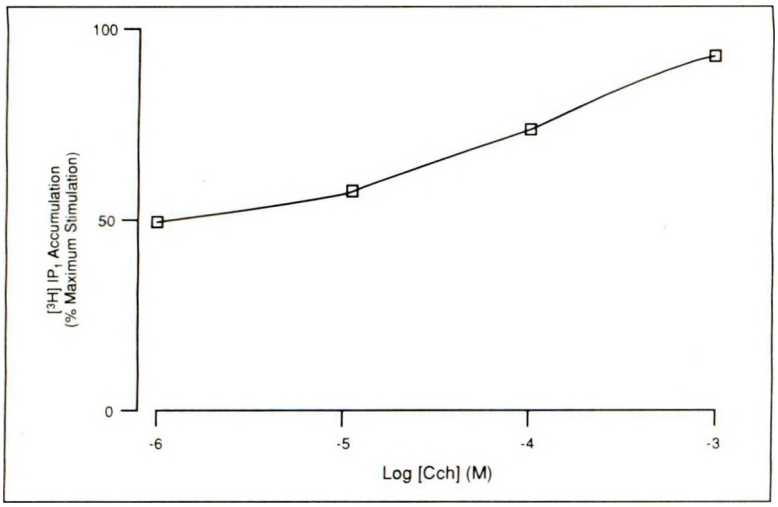

Fig. 1.: Carbachol-induced PI hydrolysis in rat hippocampus. Tissue slices were incubated with 1µCi [^{3}H] myo-inositol and increasing concentrations of Cch. Labeled inositol phosphates extracted with chloroform/methanol were separated on Dowex AG1-X8 resin as described under Materials and Methods. Data represent one experiment performed in duplicate.

Table I. Relative levels of inositol phosphates formed in response to carbachol in rat hippocampus. Rat hippocampal slices were labeled with [^{3}H] myo-inositol and carbachol (10^{-3} M). [^{3}H]-inositol phosphates formed were separated as described under Materials and Methods. Data are expressed as dpm/mg protein and relative percentage of each inositol phosphate.

Experiment	IP_1		IP_2		IP_3		Total $IP_1 + IP_2 + IP_3$	
	dpm/mg	%	dpm/mg	%	dpm/mg	%	dpm/mg	%
1	21036.6	75.7	3986.5	14.35	2745	9.9	27767.5	100
2	20722.0	58.95	9962.4	28.34	4465	12.7	35149.4	100
3	8371.4	88.87	641.4	6.8	406.9	4.3	9419.7	100
Mean	16709.9	74.5	4863.4	16.49	2538.9	8.96	24122.4	100

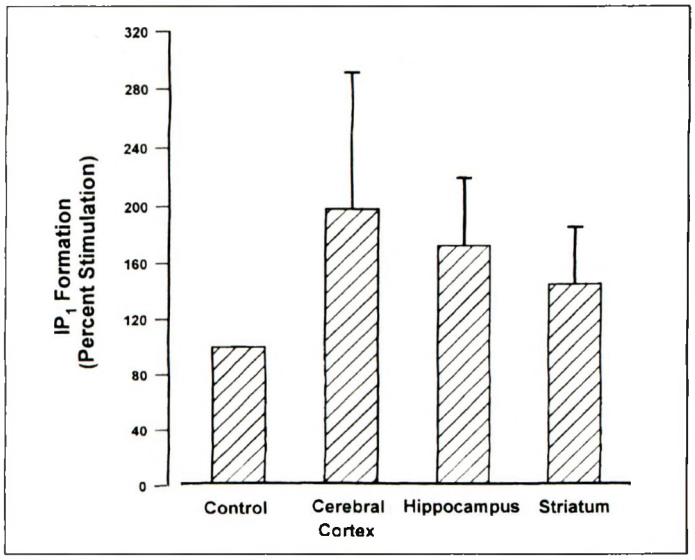

Fig.2.:

PI hydrolysis in rat cerebral cortex, hippocampus and striatum. Tissue slices were incubated with 1 μCi[^{3}H] myo-inositol and 10^{-3} M Cch for 60 min. Data represent the mean ± S.E.M. of three independent experiments performed in duplicate. IP_1 formation in each tissue was significantly different from the control value ($P<0.05$).

DISCUSSION

Muscarinic receptor-mediated activation of membrane-bound phospholipase C results in hydrolysis of the membrane phospholipid, phosphatidylinositol-4,5-biphosphate (PIP_2) to generate the second messengers IP_3 and diacylglycerol. Molecular cloning studies have established the presence of five muscarinic receptor subytpes, all of which are expressed in the brain (2-5). The five receptors have distinct distribution in brain (5), implying possible functional differences among the subtypes. m_1 and m_3 receptors are found to be highest within the cortex and hippocampus, while the striatum is rich in m_4 subtype (7). The present study aimed to determine regional differences in phosphoinositide hydrolysis evoked by muscarinic agonist, carbachol, in rat brain. Phosphoinositide turnover was stimulated by carbachol in all brain structures studied, with the most pronounced effects observed in the cortex and hippocampus. These findings are in agreement with those of Tonnaer et al. (20) and consistent with the presence of significant amounts of m_1 and m_3 receptors, subtypes which are preferentially coupled to PI hydrolysis in these regions. The increase in PI hydrolysis was 45% over basal value in striatum, compared to a 98% increase in cerebral cortex. This finding is not surprising in view of the presence of low levels of m_1 and m_3 receptors in striatum in contrast to high levels of m_4 receptors which are preferentially coupled to adenylyl cylase.

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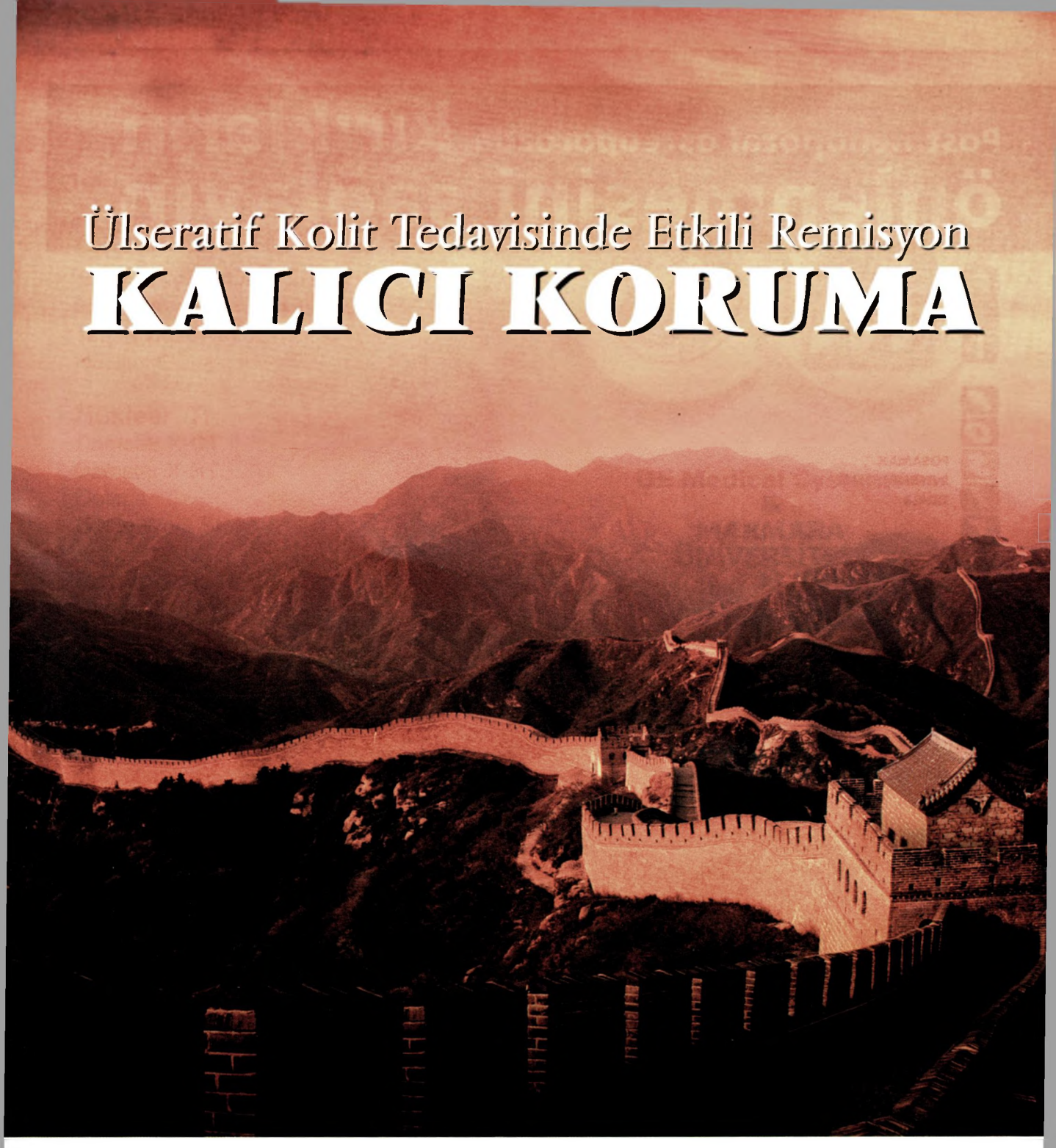

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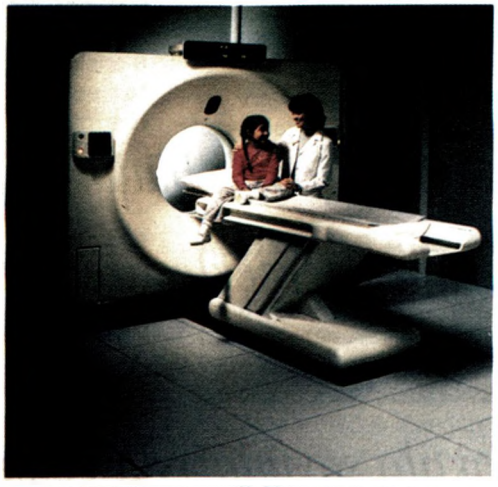

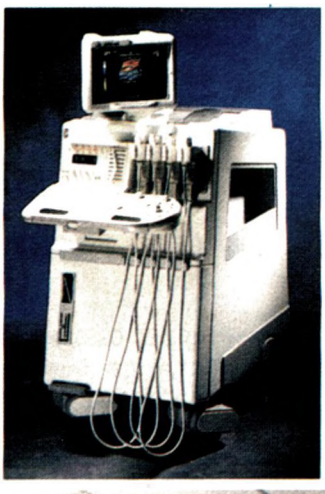

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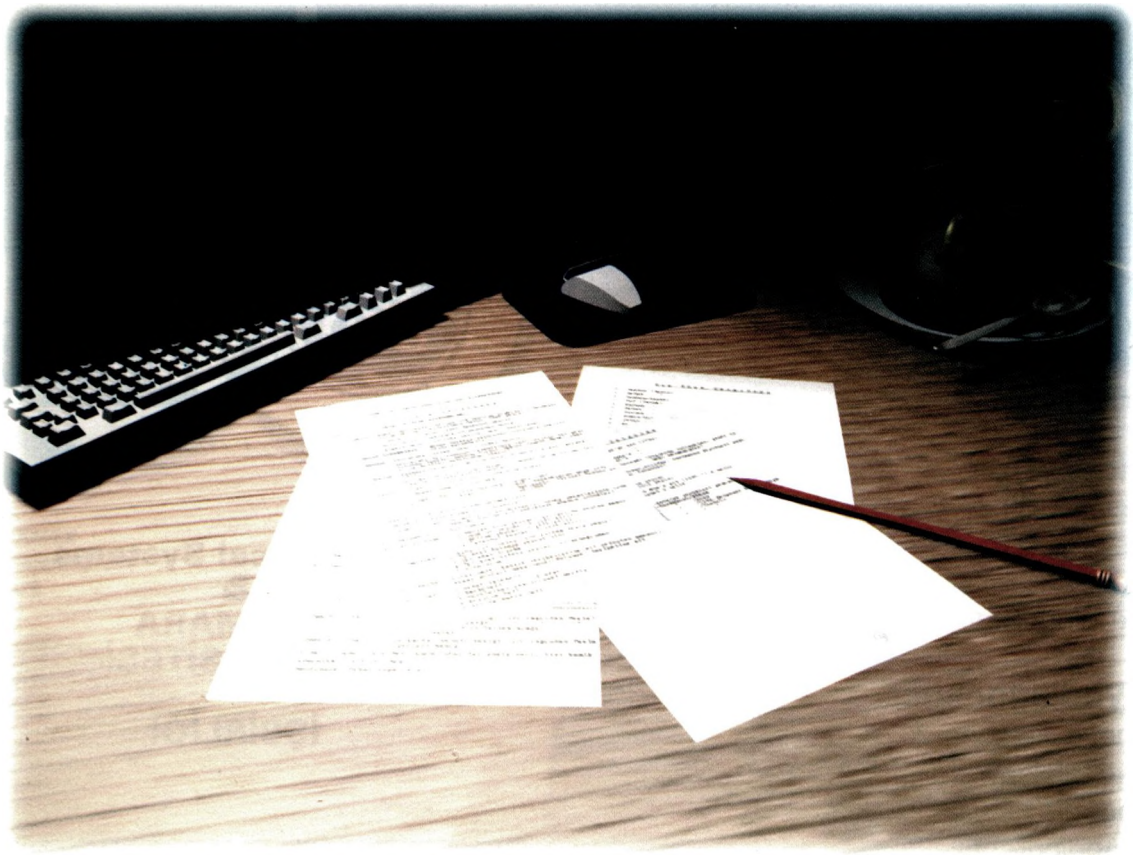

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