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Accumulation of inositol phosphates in human lymphocytes after cholinergic stimulation

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Activation of muscarinic acetylcholine receptors leads to at least two distinct biochemical events: inhibition of adenylate cyclase activity and stimulation of phosphoinositide-specific phospholipase C [1 - 3]. Receptor coupled activation of phospholipase C is an event induced also by many other neurotransmitters, neuromodulators and hormones which use calcium as a messenger [4]. The key reaction of this transducing system is the hydrolysis of phosphatidylinositol-4,5-bisphosphate yielding two metabolites, diacylglycerol and inositol trisphosphate, either of which can play the role of a second messenger. Diacylglycerol stimulates the calcium- and phospholipid-dependent protein kinase C, whereas inositol trisphosphate acts by mobilising intracellular calcium [5, 6].

The rapid breakdown of polyphosphoinositides after cholinergic stimulation has been described for several tissues, e.g. brain and parotid gland [7, 8]. This reports concerned mainly cholinergic stimulation of the muscarinic receptor whereas activation of phospholipase C after stimulation of the nicotinic receptor has not been confirmed so far. No evidence has been presented on polyphosphoinositide breakdown in human lymphocytes stimulated with cholinergic agonists.

In our previous studies we have reported the presence of cholinergic: muscarinic and nicotinic receptors on the surface of human peripheral blood lymphocytes and leukemic B lymphocytes from Raji line [9]. These two kinds of receptors were also found on the surface of leukemic T lymphocytes from Jurkat line (not shown).

The aim of our study was to investigate whether the cholinergic stimulation of human lymphocytes affects the turnover of polyphosphoinositides.

In our experiments we used human blood lymphocytes and lymphocytes from two leukemic lines, Jurkat and Raji. Lymphocytes were stimulated with suboptimal concentrations of a specific mitogen, i.e. phytohemagglutinin or bacterial lipopolysaccharide before stimulation with the agonists (carbachol, nicotine). To control the specificity of stimulation, the cells were treated with specific antagonists (0.5 mM atropine sulfate or 1.0 mM D-tubocurarine) prior to the addition of the agonist.

To examine the turnover of polyphosphoinositides we determined the amount of inositol phosphates using the method described by Berridge *et al.* [5] in the modification of Nalepa & Vetulani [10]. Inositol-1,4,5-trisphosphate (IP_3) measurement was done using the D-myo-inositol-1,4,5-trisphosphate assay system (Amersham). Statistical analysis was performed using Student's *t*-test. As a control we considered the level of inositol phosphates or IP_3 after stimulation of lymphocytes with mitogen only.

Figure 1 illustrates the effect of carbachol(10^{-5} M and 10^{-4} M) and nicotine (10^{-5} M and 10^{-4} M) on accumulation of [^{3}H]inositol phosphates in lymphocytes. In the peripheral blood lymphocytes we observed a maximal increase of inositol phosphates level after stimulation with carbachol at a concentration of 10^{-5} M. In this concentration carbachol increased the accumulation of inositol phosphates by 54% over the control level. On the other hand, nicotine at a concentration of 10^{-5} M evoked an increase of 28%. In lymphocytes T from the Jurkat cell line the largest increase, by 72% over the control level was observed after stimulation with 10^{-4} M nicotine. In lymphocytes B from the Raji cell

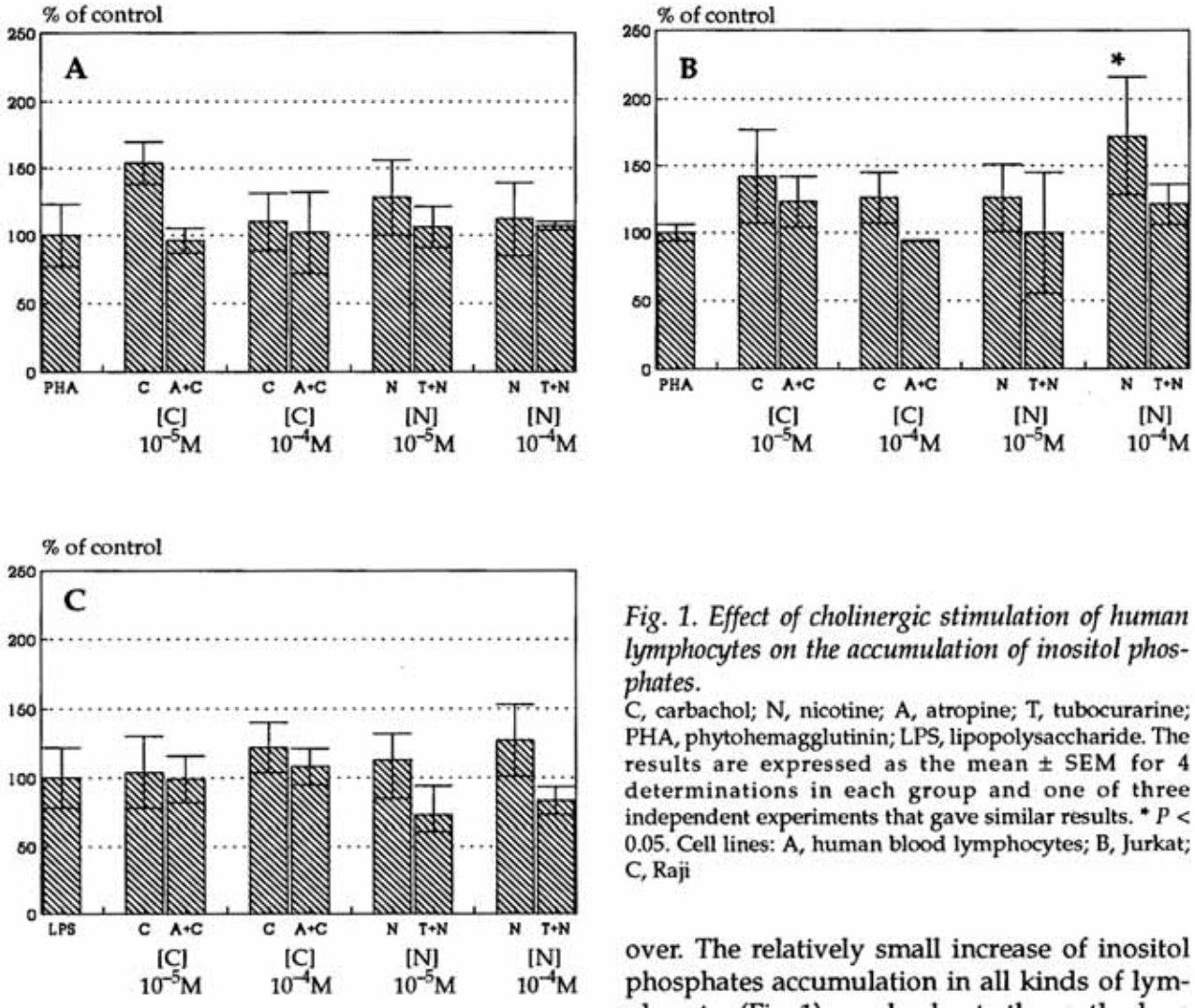
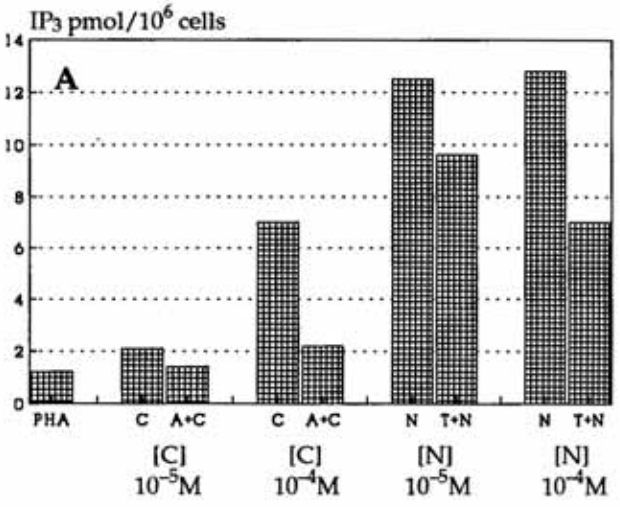
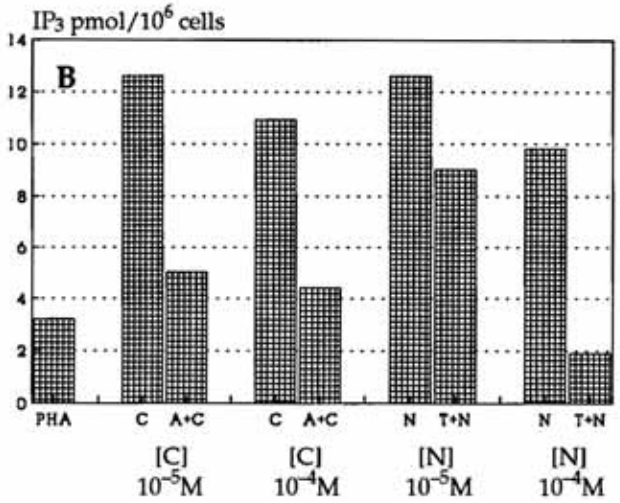


Fig. 1. Effect of cholinergic stimulation of human lymphocytes on the accumulation of inositol phosphates.
C, carbachol; N, nicotine; A, atropine; T, tubocurarine; PHA, phytohemagglutinin; LPS, lipopolysaccharide. The results are expressed as the mean ± SEM for 4 determinations in each group and one of three independent experiments that gave similar results. * $P < 0.05$. Cell lines: A, human blood lymphocytes; B, Jurkat; C, Raji

line, 10^{-4} M nicotine increased accumulation of inositol phosphates by 27%.

The data shown in Fig. 2 indicate the carbachol and nicotine stimulated IP_3 production in lymphocytes. Maximal level of IP_3 was observed in peripheral blood lymphocytes after stimulation with nicotine, both at concentrations of 10^{-5} and 10^{-4} M. In lymphocytes T from the Jurkat cell line the increase in the amount of IP_3 was similar (about 4 fold above the control level) after stimulation with carbachol and nicotine. In contrast, in lymphocytes B from the Raji cell line we observed a significant increase of the level of IP_3 after stimulation with carbachol alone, especially at a concentration of 10^{-5} M, but not after stimulation with nicotine. These results provide the first evidence that the cholinergic receptors on the human lymphocytes can be linked to phosphoinositide turnover. The relatively small increase of inositol phosphates accumulation in all kinds of lymphocytes (Fig. 1) may be due to the method we have used. As it was shown by Taylor *et al.* [11] the column method does not assure full recovery of the total amount of inositol phosphates. Therefore, to measure the amount of IP_3 we have applied the radioreceptor assay system. The data obtained (Fig. 2) suggest that cholinergic stimulation leading to an increase in polyphosphoinositide turnover may be effected through different receptors present in different types of human lymphocytes. We can hypothesize that this cholinergic stimulation of human blood lymphocytes is linked mostly to stimulation of nicotinic receptors, whereas in B lymphocytes from the Raji line this stimulation proceeds mostly through the muscarinic receptors. Cholinergic stimulation of T lymphocytes from Jurkat line seems to be associated with both muscarinic and nicotinic receptors. Whether these various stimulatory effects of





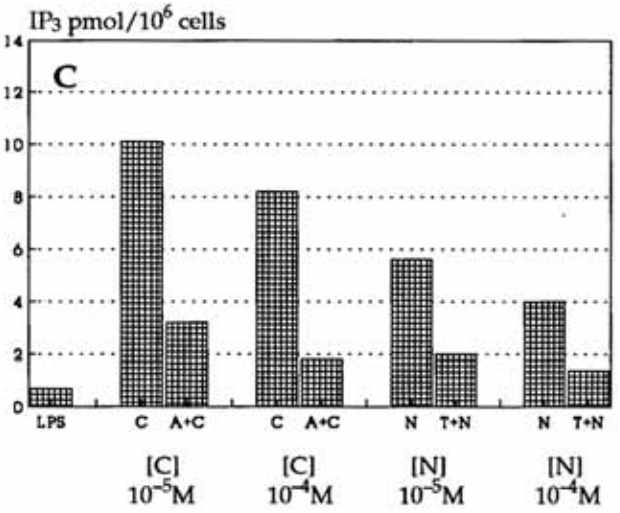


Fig. 2. Effect of cholinergic stimulation of human lymphocytes on inositol-1,4,5-trisphosphate (IP_3) accumulation.
C, carbachol; N, nicotine; A, atropine; T, tubocurarine; PHA, phytohemagglutinin; LPS, lipopolysaccharide. Values are means of duplicate determinations in representative experiment. Cell lines: A, human blood lymphocytes; B, Jurkat; C, Raji

the cholinergic agonist are due to different muscarinic and nicotinic receptor subtypes present on the human lymphocytes, is still a matter of debate and needs further experiments.

Our results obtained after stimulation of nicotinic receptor are quite surprising. It is known that nicotinic acetylcholine receptor controls a sodium ion channel and elicits membrane depolarisation when activated. Our findings suggest that activation of phospholipase C after nicotinic receptor stimulation could also result from another transduction mechanism in which G proteins do not participate.

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