Effect of lithium on muscarinic cholinoreceptor-stimulated phosphoinositide-specific phospholipase C substrate selectivity in CHO-mI cells

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Phosphoinositide-specific phospholipase C (PI-PLC) activity is stimulated by a variety of hormones, neurotransmitters and growth factors to generate inositol 1,4,5-trisphosphate (Ins(1,4,5)P3) and sn-1,2-diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphosphate (PtdInsP2) [1]. In studies examining the activation of phospholipase C by receptors linked to PI-PLC activity via guanine nucleotide binding proteins, evidence has generally been obtained to support the view that PI-PLC preferentially hydrolyses PtdInsP2 over phosphatidylinositol 4-monophosphate (PtdInsP) and phosphatidylinositol (PtdIns) [2-4].

We have recently examined the effects of lithium on muscarinic receptor-stimulated phosphoinositide turnover in CHO cells expressing human recombinant m1 muscarinic receptors (CHO-m1 cells) [5]. In common with a number of other studies in a variety of cell systems [6-8], the presence of lithium resulted in marked increases in agonist-stimulated inositol monophosphate and CMP-phosphatidate accumulations, and time-dependent decreases in PtdInsP2 and Ins(1,4,5)P3 mass levels which occurred in parallel about 10 min after agonist-plus-lithium addition [5]. In the present study we have examined whether the lithium-dependent decrease in PtdInsP2 availability for agonist-stimulated PI-PLC has any effect on the phosphoinositide hydrolysis.

To achieve this we have examined time-courses of [3H]Ins(4)P and [3H]Ins(1,3,4,5)P4 accumulation in CHO-m1 cells labelled with myo-[3H]inositol for 48 h and challenged with methacholine (MCh; 1 mM) in the absence and presence of 5 mM LiCl [5]. At various times after agonist addition (0-30 min) incubations were terminated by acid addition and neutralized cell extracts prepared for h.p.l.c. analysis as described previously [5,9]. Using the h.p.l.c. protocol described by Batty et al. [9] it was possible to resolve nine InsP2-InsP4 isomers (identified using authentic [3H]InsP isomers and elution times in comparison to adenine and guanine nucleotide standards [9]), as Ins(1,3)P2, Ins(4)P, Ins(1,3,4,5)P4, Ins(1,4,5)P3, Ins(1,4)P3, Ins(3,4,5)P4, Ins(1,3,4)P3, Ins(1,3,4,5)P4 and Ins(1,3,4,5,6)P5.

Addition of MCh resulted in a similar time-course of Ins(1,4,5)P3 accumulation as previously observed when carbachol was used as the agonist [5]. Agonist-stimulated Ins(1,4,5)P3 accumulation reached a peak at 10 s, and following a partial decrease over the initial 5 min, increased again to achieve a new elevated steady-state level at 15-30 min. The initial changes in agonist-stimulated Ins(1,4,5)P3 and Ins(1,3,4,5)P4 levels were almost identical in the absence and presence of 5 mM LiCl, however, significantly lower levels of Ins(1,4,5)P3 and Ins(1,3,4,5)P4 were observed 15 min after agonist addition in the presence of Li+ (Fig. 1). This change in inositol polyphosphates was preceded by depletion of the cellular inositol pool, as indicated by dramatic increases in CMP-phosphatidate accumulation seen by 5 min after MCh-plus-Li+ addition, and occurred in parallel with a further MCh-stimulated decrease in PtdInsP2 level in the presence compared to the absence of Li+.

The time-courses of changes in [3H]Ins(4)P and [3H]Ins(1,3)P accumulations are shown in Fig. 1. In the absence of Li+, both Ins(1,3)P and Ins(4)P increased to new steady-state levels within 1 min of agonist addition. In the presence of Li+ Ins(4)P increased almost linearly over the first 5-10 min, but accumulation abruptly ceased at 10 min and no further change in the level of [3H]Ins(4)P was observed over the subsequent 20 min of MCh challenge. Ins(1,3)P also accumulated rapidly over the first 1-5 min of agonist-plus-Li+ addition. In contrast to the latter time-course for Ins(4)P, Ins(1,3)P continued to accumulate throughout the 30 min observation period (Fig. 1).

[Fig. 1] Time-course of changes in [3H]Ins(4)P and [3H]Ins(1,3)P accumulations in CHO-m1 cells stimulated with MCh (1 mM) in the absence (open triangles) or presence (filled squares) of 5 mM LiCl. CHO-m1 cells were labelled with myo-[3H]inositol (2 μCi/ml) for 48 h. Basal labelling of inositol monophosphates was: Ins(1,3)P, 1957 ± 393 and Ins(4)P, 891 ± 123 d.p.m./well. Values are shown ± SEM for at least 3 separate experiments.

[Ins(1,3)P] can arise as a product of two distinctly different metabolic routes. It is generally assumed that Ins(1,3)P accumulation occurs as a result of 3-kinase metabolism of Ins(1,4,5)P3 to Ins(1,3,4,5)P4 and its subsequent dephosphorylation; however, Ins(1,3)P can also be generated directly by PI-PLC hydrolysing PtdIns. The plateauing of MCh-stimulated Ins(4)P accumulation in the presence of Li+ correlated with the marked decrease in Ins(1,4,5)P3 (and Ins(1,3,4,5)P4) levels seen during this period. This suggests that in the presence of Li+, agonist-stimulated PI-PLC activity progressively is directed towards PtdIns hydrolysis (and the generation of Ins(1)P) as phosphatidial PtdInsP2 levels are increasingly depleted. We are currently employing the approach adopted by Cubitt et al. [10], which involves following the time-course of changes in [3H]phosphoinositide cycle intermediates in the presence of excess unlabelled myo-inositol to quantify the agonist-sensitive phosphoinositide pool in CHO-m1 cells and to provide an alternative approach to assessing the changes in substrate selectivity of PI-PLC.

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References